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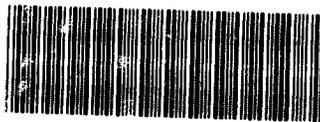
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8EHQ-0595-13439

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In accordance with TSCA section 8(e), the International Isocyanate Institute, Inc. (III) on behalf of its members (BASF Corporation, Bayer Corporation, The Dow Chemical Company, ICI Americas, Inc., and Olin Corporation) hereby submits portions of the following Ph.D. thesis which recently came to our attention. The III did not sponsor this research.

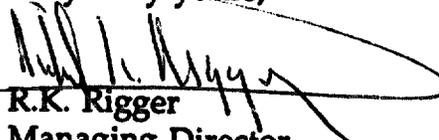
Ugis Bickis: *Investigations of dermally induced airway hyperreactivity to toluene diisocyanate in guinea pigs.* (Queen's University, Kingston, Canada, November, 1994). Pages ii - iii and 163 - 167.

We have been orally informed that an 80:20 mixture of TDI isomers (2,4-TDI; 2,6-TDI, CAS # 26471-62-5), was used. We expect to receive a full copy of Bickis' thesis, and will forward a copy to EPA.

Dr. Bickis indicates that the application of TDI to the intact skin of guinea pigs results in TDI-specific systemic immune response and airway hyperreactivity that persist for a year after the application. These findings are consistent with the 1981 publication by M.H. Karol (*Dermal contact with toluene diisocyanate (TDI) produces respiratory tract hypersensitivity in guinea pigs*, Toxicol. Appl. Pharmacol. 58:221-230). Bickis may have found a consistent response at a slightly lower dose than Karol. Immunologic sensitization tends to persist; other investigators have published studies on the persistence of TDI sensitization in humans, so that the research results on guinea pigs can be interpreted in light of the information already available on humans.

Although we don't believe that this represents new substantial risk information, we are providing this to EPA to assure compliance.

Very truly yours,


R.K. Rigger
Managing Director

ABSTRACT

Ugis Bickis: *Investigation of dermally induced airway hyperreactivity to toluene diisocyanate in guinea pigs*. PhD thesis. Department of Pharmacology and Toxicology, Queen's University, Kingston, Canada. November, 1994.

Hyperreactivity to environmental factors, with asthma as one manifestation, is becoming increasingly prevalent. An understanding of etiological mechanisms is of interest from both preventive and therapeutic standpoints. Isocyanates are responsible for more cases of occupational asthma than any other industrial chemical; toluene diisocyanate (TDI) has been most commonly involved. Isocyanate sensitization remains enigmatic: degree of chronic airborne exposure, genetic allergic predisposition ("atopy"), blood level of TDI-specific antibodies, and nonspecific bronchial hyperreactivity (methacholine sensitivity) do not appear to be directly related to the development of airway obstruction in response to airborne TDI challenge.

This thesis posits that worker skin contact with TDI may be a critical event in the development of the hypersensitive state, culminating for some in bronchospasm upon subsequent TDI inhalation.

By means of a guinea pig (gp) model involving sensitization/humoral immunity determination, *in vivo* plethysmographic challenge and *in vitro* tissue bath studies, four hypotheses were tested:

1. persistent immune response ("sensitization") to TDI can be induced by a single dermal exposure to dilute TDI;
2. the 'early' response to TDI challenge, while associated with sensitization, is not directly related to the level of TDI-specific antibody; response to methacholine inhalation challenge is independent of sensitization status;
3. *in vivo* sensitivity to airborne challenge correlates with *in vitro* airway tissue responsiveness to TDI;
4. *in vitro*, airways from sensitized animals can be differentiated pharmacologically from control airways.

Immune response was assessed by ELISA: within two weeks of the application of 12 mg of TDI, as a 5 % solution in acetone to the animals' skin, all developed a high level of IgG directed against TDI; 63-71 weeks post-sensitization the mean IgG effect in sensitized animals was six times that of the control animals. No immune response was observed after TDI inhalation.

Airway hyperreactivity *in vivo* was assessed in awake, unrestrained animals situated in flow-through whole-body plethysmographs. Animals were challenged (as groups of 3 sensitized and 1 control gp) with both methacholine and TDI in step-wise dose increments; the challenge atmosphere was actively generated for 60 seconds and the animals' respiratory pattern was monitored for 5 or 10 minutes (depending on the specific protocol). Methacholine challenges were conducted on two consecutive days, and two or more weeks prior to the TDI challenges, which were also conducted on two

consecutive days. A third methacholine challenge was conducted immediately following the second TDI challenge. During the methacholine challenges, animals were removed from their plethysmograph chamber if they progressed to severe bronchospasm, from which they then recovered spontaneously within seconds; endpoint was defined as the methacholine concentration at which the animal's ΔP (plethysmograph pressure amplitude) doubled from the pre-exposure ΔP . The respiratory response to TDI was quite different, and the animals did not progress to the paroxysmal breathing characteristic of methacholine endpoint. Ultimately, endpoint was defined in terms of a characteristic pattern of flattened waveforms associated with a retardation of the exhalation phase. With both challenge agents, when comparing by animal the consecutive day concentrations required to achieve endpoint, there was a significant correlation; also, there was no difference between the group mean concentrations required on the two days. Following the TDI inhalation challenge, the animals' sensitivity to methacholine challenge had decreased (by a factor > 2). There was no difference between control and sensitized animals in their methacholine sensitivity; the TDI 'sensitivity' of sensitized animals was seven times greater than that of control animals, when assessed almost a year after a single dermal sensitizing event; only 2 of 14 were not airway-hypersensitive (i.e. at least $1.96 \text{ sd} > \text{mean control sensitivity}$) to TDI. There was no association between TDI sensitivity and TDI-IgG level in sensitized animals.

Tracheal ring responsiveness was evaluated in conventional tissue baths, over a year following the single, dermal sensitization. Tissues from sensitized animals contracted when TDI was added to the baths; control tissues relaxed. A 'confounding' control animal (with baseline levels of TDI-IgG, yet in several respects more characteristic of sensitized animal response *in vivo* and *in vitro*) is discussed. Atropine, diphenhydramine and indomethacin did not inhibit the TDI response. Removal of the tracheal epithellum had no effect on this response, nor that to methacholine; conversely it increased the maximal tension (g_{max}) produced in response to histamine (in both control and sensitized tissues), and in sensitized tissues it decreased the degree of relaxation produced by isoproterenol. Epithelium removal also rendered tissues from control gps more sensitive to histamine than the corresponding intact sensitized tissue; otherwise, control and sensitized tissues showed no difference in g_{max} or EC_{50} to histamine or methacholine on their own. However, when methacholine response was assessed after the addition of TDI to the baths, the g_{max} demonstrated a change in opposite directions, for control and sensitized gps. The extent of TDI-induced contraction *in vitro* correlated, by animal, with its *in vivo* TDI sensitivity ($p < 0.01$) and, TDI-IgG level at the time of death ($p = 0.02$).

In summary, a single dermal contact with a small quantity of isocyanate in dilute form was sufficient to cause a specific airway hyperreactivity that could be determined *in vivo* and *in vitro* a year later. Occupational exposure limits do not currently recognize the dermal route as a potential for airway sensitization to isocyanates; the TLV should be assigned a "skin" notation, and appropriate warnings issued to all users.

6. CONCLUSIONS; FUTURE DIRECTION

When this project was first conceived, one of the most intriguing and challenging hypotheses was that the immune system manifestations of isocyanate exposure were not a necessary component of the overall sensitization response, and specifically, that there were fundamental pharmacological differences between the "sensitized" and the "exposed-but-unsensitized", perhaps on a hereditary basis. Quite recently Bignon *et al*³³ examined HLA Class II genetic markers in isocyanate exposed individuals, and found some that were associated with susceptibility to isocyanate-induced asthma, and some that conferred protection; this avenue is of itself a broad pathway for future research. This particular finding is somewhat redeeming to the writer: the initially-envisaged step of identifying and characterizing genetically-determined "non-responders" (i.e. those who were exposed but did not become sensitized, as determined from immunological and/or plethysmographic determinations) was quantitatively unsuccessful in the present study; all exposed animals responded immunologically, and 12 of the 14 exposed animals in the final complement that were assessed plethysmographically were defined as airway-hypersensitive to TDI. Accordingly, one of the original objectives, to pharmacologically differentiate "responders" from "nonresponders", was abandoned.

On the other hand, the sensitization process was eminently successful, and of itself may have provided a rationale for this work, which was precipitated by an interest in disease caused by environmental agents. This research project involved the sensitization of animals by the dermal application of a single dose of TDI, and then monitoring, a year later:

- the immune response (TDI-specific IgG)
- the *in vivo* sensitivity to airborne TDI (plethysmographic endpoint)
- the *in vitro* reactivity to TDI (tracheal muscle contraction due to TDI administration)

In these three respects, the exposed animals showed a significant increase, compared to the control animals. Especially when taken together with other findings, this is a compelling indication that dermal exposure to isocyanates in humans may also cause long-term airway hyperreactivity. Although direct evidence is not available (and the

ethics of any project intending to test this hypothesis would have to be closely scrutinized), there is sufficient basis for an immediate increase in human caution in this respect. As a minimum, appropriate written warnings should be provided by the manufacturers/suppliers of isocyanates, and the various occupational exposure indices should be assigned a "skin" notation.

The research hypotheses (articulated in the Introduction) are re-stated below, together with the appropriate conclusion, based on the data provided in this thesis:

1. That persistent systemic immune response to TDI ("sensitization") can be induced in guinea pigs by a single dermal exposure to diluted TDI, without dermal abrasion, occlusion or the use of chemical depilating agents. This was clearly shown to be the case : see Figs. 2-4, 2-5.

2 a) That the airway response to TDI challenge in vivo relates to the sensitization state of the animal. Table 3-7 illustrates that sensitized animals respond to a lower level of challenge.

b) That the response of TDI-sensitized guinea pigs to airborne methacholine challenge is independent of immune status. As evidenced by Fig. 3-13, this is clearly the case.

c) That the "early" response to TDI challenge in sensitized animals is not directly associated with antibody level. Fig. 5-3 does not demonstrate a correlation in this respect.

3. That in vivo sensitivity to TDI challenge correlates with in vitro airway tissue responsiveness to TDI. In Fig. 5-1 this is clearly shown to be the case.

4. a) That there are differences in response to pharmacological agonists between tracheal tissues from control and sensitized animals; The evidence in support of this was less clear, at least in part due to the low number of animals involved in any one trial set; nevertheless, as detailed in Chapter 4, some significant differences were found (in association with rubbing or TDI pre-treatment of the rings) with histamine, methacholine

and atropine. TDI is arguably a pharmacological agent; there were categorical differences in the response of the tracheae from control and sensitized guinea pigs.

b) *That the in vitro TDI response of tissues from sensitized animals can be modulated pharmacologically.* There was no evidence in support of this hypothesis.

c) *That the tracheal epithellum has a protective role in this response.* There was no evidence in support of this hypothesis

The pursuit of the determination of whether this same phenomenon occurs in humans is warranted, and could be conducted with a combination of occupational hygiene, medical surveillance and prospective epidemiological study: the work force in question would be thoroughly apprised of the potential risks associated with dermal contact with isocyanates, and would be provided with the necessary equipment and work procedures to preclude exposure by this route. No doubt there would nevertheless be some individuals who had experienced dermal contact; the hypothesis predicts that they would show a higher prevalence and/or severity of isocyanate-related airway dysfunction. There are many other avenues of investigation, some of which are listed, below.

Sensitization

•to determine the lower threshold (dose and concentration) below which dermal TDI contact does not cause immune system activation and airway hyperreactivity; can intermediate levels of immune response be evoked; what is the role (if any) of the specific vehicle used (acetone)?

Immunotoxicology

•to develop an understanding of the *in vivo* persistence of the level of IgG directed against TDI-albumin; is this IgG inherently different from "typical" IgG (e.g. as perhaps evidenced by its *in vitro* stability)?

•to (further) characterize the specificity of the IgG-TDI interaction; would other proteins be equally effective as the macromolecular constituents of the conjugate; can other (non-TDI bound) forms of albumin be produced chemically, in a manner analogous with 'senescence', to test the hypothesis of Cartier *et al*¹⁰⁴ that IgG against "TDI" normally

serves to remove damaged albumin; would these modified albumins interact with this antibody? Accordingly, does TDI evoke an autoimmune condition?

•to determine whether there are TDI-specific immunoglobulins resident in the tracheal tissue of sensitized animals; if so, are the levels of these associated with the *in vivo* or *in vitro* hyperreactivity of the airways to TDI?

Plethysmography

•to further characterize the methacholine and TDI dosing and endpoints; can the delayed exhalation endpoint for TDI exposures be monitored electronically?; does an immediate increase in guinea pig respiratory rate upon methacholine challenge serve as prodromal indication of impending bronchospasm?

•to systematically assess the apparent phenomenon of abortion of an incipient methacholine-induced bronchospasm by 'distraction' of the guinea pig; is it reproducible, and can it be characterized pharmacologically?

•to determine whether tachykinin antagonists prevent the hyperreactivity of sensitized guinea pigs to TDI challenge; to elucidate the relationship between TDI challenge and decreased methacholine sensitivity

Tissue bath studies

•to characterize with a larger number of animals some of the relationships that were suggestive; what agents (e.g. tachykinin antagonists?) prevent the contraction otherwise caused by TDI in sensitized tissues?

•to understand and develop further the use of refrigerated tissues in tissue bath work; would this increase the amount of useful information that could be gleaned from an individual animal?

•to determine the pharmacological / immunological balance in the tissue response to TDI

The insightful research of Dr. Meryl Karol's lab (developing an initial animal model for isocyanate hypersensitivity) was a fundamental base for the work reported here. It is perhaps fitting to finish with the closing lines from her 1994 review paper:

"It is certain that continued advances in molecular immunology will result in further understanding of the aetiology of early- and late-onset respiratory

hypersensitivity reactions. Development of effective in vitro screening methodologies and preventive programmes are envisaged. Until then, prudent employment of animal models will continue to guide us in recognition, treatment, and prevention of occupationally-based asthma."

Part of the challenge that remains is the effective communication of the scientific findings to the appropriate arena. An increased awareness amongst clinicians and government officials, of the association between exposure to specific environmental factors and the development of disease, may be beneficial. In this instance, the achievement of a general recognition of airway sensitization by dermal exposure as a real phenomenon, and further study to determine if this occurs uniquely in guinea pigs or is also of direct human relevance, would constitute reasonable goals.

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