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Arun Leelaprute

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## Immunoglobulins in toluene abusers

Ratana Sindhuphak\*

Vilai Chinveschakitvanich\* Arun Leelaprute\*\*

Sindhuphak R, Chinveschakitvanich V, Leelaprute A. Immunoglobulins in toluene abusers. *Chula Med J* 1995 May; 39(5): 351-359

*Toluene is a volatile organic solvent that causes a variety of acute and chronic toxicities, including the depression of bone marrow activity. It may also affect the synthesis of immunoglobulins. The examination of IgG, IgA and IgM in the sera of paint manufacture workers, solvent abuse patients and in a control group revealed that the levels of IgG and IgA of the patients were lower than those found in the control group. There was no correlation between the blood toluene and serum immunoglobulin levels.*

**Key words :** *Toluene, Toluene abusers, Immunoglobulins.*

Reprint request : Sindhuphak R. Institute of Health Research, Chulalongkorn University,  
Bangkok 10330, Thailand.

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\*Institute of Health Research, Chulalongkorn University.

\*\*Department of Microbiology, Faculty of Medicine, Chulalongkorn University.

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โทลูอิน. จุฬาลงกรณ์เวชสาร 2538 พฤษภาคม; 39(5): 351-359

โทลูอินจัดอยู่ในจำพวกสารระเหยชนิดหนึ่ง มีพิษต่อร่างกายทั้ง พิษเฉียบพลัน และพิษเรื้อรัง  
จากพิษเรื้อรังประการหนึ่ง คือ กัดการทำงานของไขกระดูก ซึ่งอาจมีผลต่อการสร้างภูมิคุ้มกัน  
ของร่างกายได้ จากการศึกษาในกลุ่มตัวอย่าง 3 กลุ่ม คือ กลุ่มพนักงาน โรงงานผลิตสี คนใช้  
(รพ.ธัญญารักษ์) และกลุ่มควบคุม (ผู้บริจาคโลหิต ณ ศูนย์บริการโลหิต สภากาชาดไทย)  
พบว่าปริมาณ IgG และ IgA ในกลุ่มคนใช้ต่ำกว่ากลุ่มควบคุมส่วนความสัมพันธ์ของปริมาณโทลูอิน  
ในเลือด และปริมาณภูมิคุ้มกันในน้ำเหลือง เกือบไม่มีความสัมพันธ์กันเลย

Toluene is a volatile organic solvent which evaporates at room temperature. It is a colorless, highly refractive inflammable liquid obtainable from tolu and other resins and from coal tar. Toluene constitutes 2 to 10 per cent of commercial benzene. It is easily obtained on the consumer market in the form of solvents and cleaners such as paints, thinners, varnishes, paint removers, dry cleaning solvents, and so on.<sup>(1)</sup>

Inhalation induces euphoria and is rapidly transferred to the brain via the lungs and blood-stream so the effects are experienced rapidly. Initially, the effect may be somewhat similar to

mild alcohol intoxication but, at a deeper level of intoxication, auditory and visual hallucinations can occur<sup>(2,3)</sup>

Toluene can enter the body by vapour inhalation, ingestion of the solvent, or adsorption through the skin.<sup>(4)</sup> Normal breathing eliminates 60 per cent and the remaining 40 per cent will be adsorbed.<sup>(5)</sup> More than 80 per cent of adsorbed toluene is oxidized to benzoic and hippuric acids before excretion in the urine.<sup>(6)</sup> Some adsorbed toluene (0.4-1.1%) is hydroxylated, and excreted as a mixture of cresols.<sup>(7)</sup> The metabolic pathway of toluene is shown in figure 1.

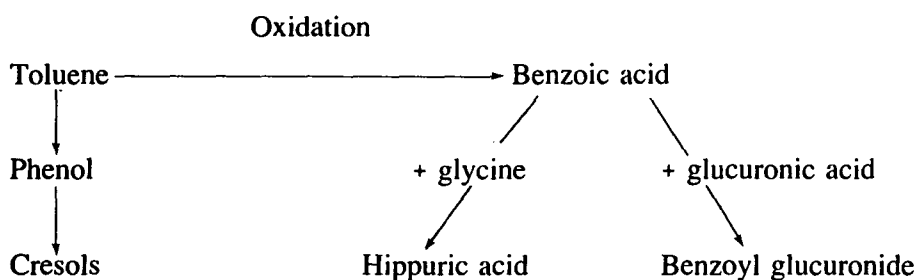


Figure 1. The metabolic pathway of toluene.

Acute toluene abusers experience euphoria and disinhibition but this may be followed by nausea and vomiting, dizziness, coughing and increased salivation, cardiac arrhythmias, convulsion, coma and death in severe cases.<sup>(8-12)</sup> The main chronic neurological toxicity causes permanent damage to the central nervous system.<sup>(10,13)</sup> Chronic non-neurological toxicity causes damage to the lungs<sup>(14)</sup> and heart,<sup>(15)</sup> kidney disorders and haemato toxicity,<sup>(16)</sup> abnormal liver function,<sup>(17)</sup> and progressive depressant action on the bone marrow.<sup>(18,19)</sup> The bone marrow in adults serves not only as the source of all blood cells, but it also serves to remove particulate antigens from circu-

lating blood. In addition, it functions as a major source of antibodies.<sup>(20)</sup> The purpose of the present investigation was to examine whether the effect of toluene causes immunoglobulins production among paint manufacture workers and solvent abuse patients.

## Materials and methods

### Subjects

1. **Workers** One hundred paint manufacture workers, seventy one men (aged 21-49 years) and twenty nine women (aged 23-55 years), who were regularly exposed to organic solvents for 8 hours a day.

**2. Patients** Fifty solvent abuse patients from Thanyarak Hospital, forty four men (aged 12-34 years) and six women (aged 14-25 years). Blood was collected before treatment.

**3. Controls** Twenty nine male donors (aged 24-43 years) from the Thai Red Cross Blood Bank were used as referents. None of these were exposed to organic solvents.

#### **Blood collection**

About ten milliliter blood samples were collected at room temperature. Two to three milliliters of whole blood was divided for toluene assay. Serum was used for immunoglobulin determinations.

#### **Analysis of serum immunoglobulins (IgG, IgA, IgM)**

The concentrations of IgG, IgA and IgM were measured by the single radial immunodiffusion method of Mancini.<sup>(21)</sup> In this technique, the immunoglobulin containing sample is loaded into wells bored in an agar gel (1% agarose A, Sigma) which contains a fixed concentration of antibody (goat anti-human IgG, IgA, IgM : Kallested, USA). The antigen diffuses into the gel and combines with the antibody until equivalence is reached. At this point an immunoprecipitin "halo" is formed round the well, and the diameter of the halo at equivalence is related to the immunoglobulin concentrations. The values were converted to mg. per cent of protein from a standard curve established with immunoglobulin reference valued (Kallested, USA).

The precisions (interassay) in determination of IgG, IgA and IgM were 2.80, 2.90 and 1.66% CV respectively.

#### **Analysis of blood toluene**

The technique of head space gas chromatography was used in accordance with Bassette,<sup>(22)</sup> Sato<sup>(4)</sup> and Chinveschakitvanich.<sup>(23)</sup> Basically one, milliliter of whole blood was pipetted into a 24 milliliters vial. Cyclohexane, 2.5 ul (0.648 ug) was added as an internal standard and a rubber septum was used for sealing. A Varian 3700 gas chromatography with FID detector was used with a head space technique. This allowed equal heating time for all samples (30 minutes at 50°C).<sup>(23)</sup> A glass column (2 m. x 2 mm.) packed with Porapak Q 80-100 mesh was employed (Waters Associates). Column temperature was 200°C and gas flow (N<sub>2</sub>) 30 ml per minute.

By this method, the linearity of toluene was 0-6.48 ug/ml blood. The sensitivity was 0.68 ug/ml blood. The precision (interassay) was 2.90 and 8.29% CV, (interassay) was 3.94 and 6.88% CV at toluene concentrations of 2.16 and 4.32 ug/ml respectively.

#### **Results**

The mean serum immunoglobulin levels in workers, patients and controls were 1838, 1591, 1869 mg. per cent for IgG; 312, 222 and 293 mg. per cent for IgA; and 182, 176 and 196 mg. per cent for IgM, respectively (Table 1).

Table 1. The mean serum IgG, IgA and IgM (mg%) levels in workers, patients and controls.

	IgG			IgA			IgM		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
Workers	1838	353	35	312	103	10	182	77	8
Patients	1591**	457	65	222**	86	12	176	77	11
Controls	1869	416	77	293	95	18	196	92	17
F Prob	0.0007			0.0000			0.5494		

\*\* = Significant (p>0.001)

The comparative study of immunoglobulins in 3 groups by analysis of variance (Table 1) showed that only the serum IgG and IgA levels in patients were lower than in the control group (p=0.007 and 0.0000 respectively).

The distribution of blood toluene levels is shown in figure 2. From the distribution, most of blood toluene found in workers, 60 per cent, was concentrated between 1.6-2.5 ug/ml. In patients there were 14 per cent having blood toluene

over 6.5 ug/ml blood which was not found in workers.

Table 2 shows mean serum IgG, IgA and IgM (mg%) levels in workers, patients and controls with low, medium and high blood toluene levels. In patients with medium and high blood toluene levels, IgG and IgA, respectively, the levels were lower than in the controls (p<0.05). However, with low blood toluene levels, IgG and IgA were also lower than in the controls (p<0.05).

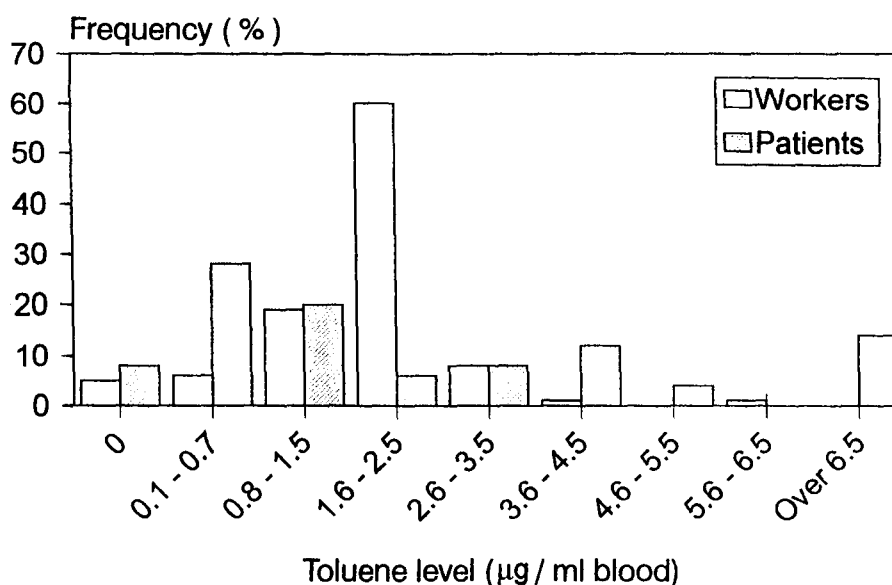


Figure 2. Distribution of blood toluene level in workers and patients.

**Table 2.** Mean serum IgG, IgA and IgM (mg%) levels in workers, patients and controls with low, medium and high blood toluene levels.

		Mean	SD	SE	t-value	DF	2-Tail Prob
<b>Workers</b>							
Low n=11	IgG	1785	473	142	-.55	38	0.588
	IgA	300	121	36	.19	38	0.850
	IgM	212	84	25	.50	38	0.617
Medium n=79	IgG	1869	330	37	-.00	106	1.000
	IgA	323	97	11	1.44	106	0.154
	IgM	175	75	8	-1.23	106	0.222
High n=10	IgG	1648	354	112	-1.5	37	0.142
	IgA	235	108	34	-1.61	37	0.116
	IgM	209	79	25	.40	37	0.688
<b>Patients</b>							
Low n=18	IgG	1559*	374	91	-2.53	44	0.015
	IgA	190**	85	21	-3.69	44	0.001
	IgM	179	62	15	-.65	44	0.521
Medium n=13	IgG	1403**	434	116	-3.39	41	0.002
	IgA	249	72	19	-1.51	41	0.140
	IgM	168	69	18	-1.01	41	0.320
High n=19	IgG	1756	501	115	-.85	46	0.402
	IgA	230*	91	21	-2.27	46	0.028
	IgM	178	97	22	-.66	46	0.515
<b>Controls</b>							
n=29	IgG	1869	416	77			
	IgA	293	95	18			
	IgM	196	92	17			

\* = significant ( $p < 0.05$ ), \*\* = significant ( $p < 0.005$ )

## Discussion

Solvent sniffing is a major social problem among adolescents in many countries and toluene is the most commonly misused volatile solvent. In our study on immunoglobulins in paint manu-

facture workers, solvent abuse patients and in a control group, it was found that only the serum IgG and IgA levels in patients were lower than found in the control group ( $p < 0.001$ ). This result may be associated with bone marrow deterioration

according to toluene or toluene contaminated with benzene.<sup>(18,19)</sup> The bone marrow in adults serves not only as the source of all blood cells, but it also serves as a major source of antibodies.<sup>(20)</sup> Some patients with toluene diisocyanate sniffing presented an elevated total IgE,<sup>(24)</sup> and this increased significantly in percentage of CD<sub>8</sub> positive lymphocytes and suppressor cytotoxic T lymphocytes.<sup>(25)</sup> In mice ingesting toluene showed immunotoxic effects and changes in immune function have been seen.<sup>(26,27)</sup> Nevertheless, in people chronically addicted to sniffing substances containing toluene, ocular motor abnormalities,<sup>(28)</sup> increased total creatine kinase activity,<sup>(29)</sup> and abnormal results of liver function tests have been noted.<sup>(30)</sup> Toxic vapour abuse during pregnancy was found to be associated with increased maternal and fetal morbidity.<sup>(31)</sup>

From this study, there was no correlation between blood toluene and serum immunoglobulins because the change in serum immunoglobulins had been spread in all blood toluene levels (Table 2). Miyazaki found that the neuropsychiatric effects did not depend on the concentration of blood toluene collected on admission.<sup>(32)</sup> There is only a weak correlation between blood toluene and the clinical features of toxicity, possibly because of rapid initial tissue distribution and elimination.<sup>(9,10,12)</sup>

There was no change of immunoglobulins in workers. This may be due to the lower toluene levels persisting in the bodies. Most of the workers (90 per cent) had blood toluene levels below 2.5 ug/ml and none had over 6.5 ug/ml such as occurred in the patients. Several symptoms, body weight loss, dimmed vision, dizziness, drunken feelings, sore throat, headache and tightness in the chest occurred in workers depending on the intensity of exposure to the toluene.<sup>(33,34)</sup>

Although there was no noted effect on immunoglobulins in the paint manufacture workers, there were some signs or symptoms as mentioned above. Extensive health examination programs should be carried out in all such industries and be evaluated more critically.

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