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THE EFFECT OF SUBCHRONIC EXPOSURE TO ACRYLONITRILE ON RESPIRATORY FUNCTION IN RAT

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อภินันทนาการ
จาก

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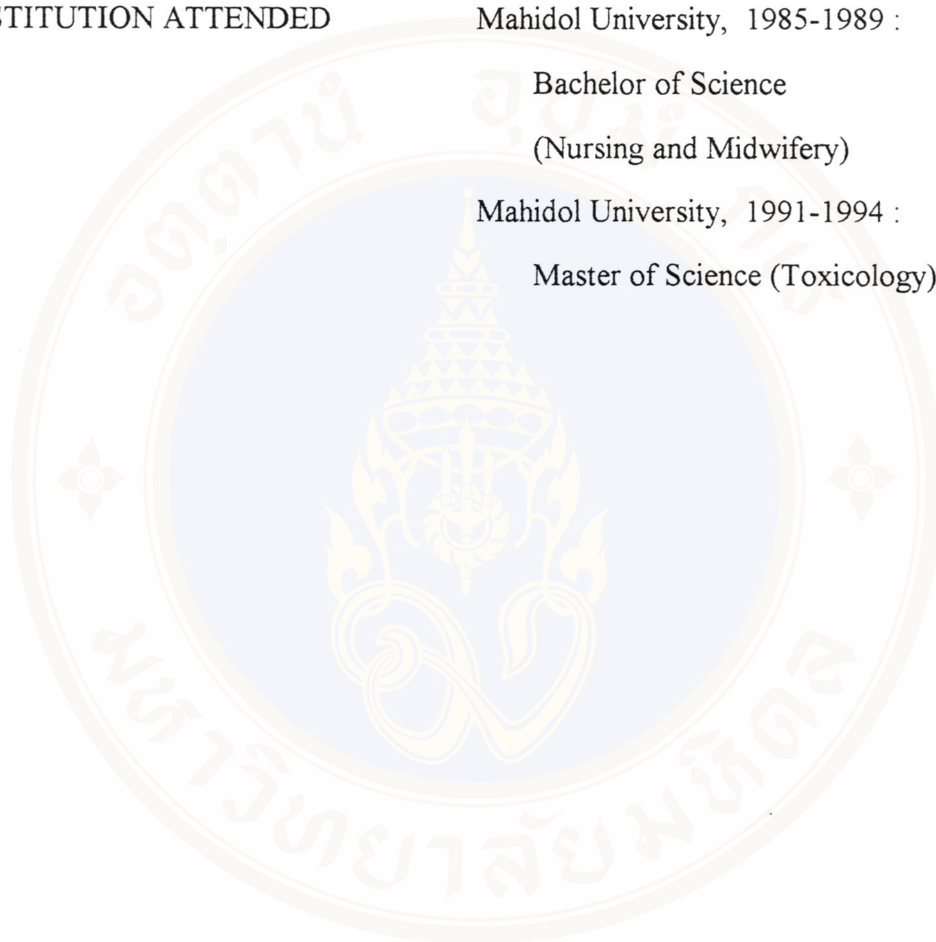

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ชื่อวิทยานิพนธ์ ผลต่อการทำงานของระบบหายใจของหนูขาวเมื่อได้รับสารอะไครโลไนไตรล์เป็นเวลานาน

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บทคัดย่อ

การศึกษาผลกระทบต่อการทำงานของระบบหายใจของอะไครโลไนไตรล์และการเปลี่ยนแปลงซึ่งเกี่ยวเนื่องกับระบบประสาทโคลิเนอร์จิกซึ่งควบคุมระบบหายใจ ได้ทำการศึกษาในหนูขาวซึ่งได้รับอะไครโลไนไตรล์โดยการฉีดเข้าใต้ชั้นผิวหนังในปริมาณ 1 และ 25 มิลลิกรัมต่อกิโลกรัม วันละ1 ครั้ง, 5 วันต่อสัปดาห์ เป็นเวลา 8 สัปดาห์ ส่วนในหนูทดลองกลุ่มควบคุมจะได้รับน้ำเกลือแทนในปริมาณที่เท่ากัน การทำงานของระบบหายใจ (อัตราหายใจออกสูงสุด, อัตราการหายใจ, ปริมาตรอากาศที่หายใจเข้าปอดในเวลา 1 นาที, ปริมาตรหายใจปกติ, เวลาที่ใช้หายใจเข้า, เวลาที่ใช้หายใจออก, เวลาช่วงคลายตัวของกล้ามเนื้อหายใจ, ความต้านทานของทางเดินอากาศ, แรงขับดันในการระบายอากาศ) จะถูกบันทึกโดย เครื่องมือวิเคราะห์การทำงานของระบบหายใจ ในสัปดาห์ที่ 1, 2 4, 6,และ 8 ของกำหนดการทดลอง

หนูขาวที่ได้รับอะไครโลไนไตรล์ 1 มิลลิกรัมต่อกิโลกรัม เป็นระยะเวลานาน 8 สัปดาห์ ไม่พบว่ามีการเปลี่ยนแปลงการทำงานของระบบหายใจเมื่อเทียบกับกลุ่มควบคุม ในขณะที่กลุ่มซึ่งได้รับอะไครโลไนไตรล์ 25 มิลลิกรัมต่อกิโลกรัมมีการเปลี่ยนแปลงในการทำงานของระบบหายใจ การเปลี่ยนแปลงนี้ขึ้นกับระยะเวลาที่ได้รับสาร โดยจะเห็นผลการเปลี่ยนแปลงชัดเจนในสัปดาห์ที่ 1 และ 2 ของการทดลอง เช่นมีอัตราหายใจออกสูงสุดลดลง มีความต้านทานของทางเดินอากาศเพิ่มขึ้นเป็นต้น อย่างไรก็ตามพบว่าค่าเหล่านี้จะปรับมาเข้าใกล้กับในกลุ่มควบคุม โดยเฉพาะอย่างยิ่งที่ 1 สัปดาห์หลังจากให้อะไครโลไนไตรล์มาจนครบ 8 สัปดาห์แล้ว นอกจากนี้หนูขาวที่ได้รับอะไคร-

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โลไนไตรล์ในขนาด 25 มิลลิกรัมต่อกิโลกรัมมีอาการต่อระบบโคลิเนอร์จิกเช่น น้ำลายไหล, น้ำตาไหล, ท้องเดิน ซึ่งเป็นผลจากการกระตุ้นระบบโคลิเนอร์จิกอีกด้วย

ในการศึกษาเกี่ยวกับการเปลี่ยนแปลงของระบบโคลิเนอร์จิก หนูขาวจะได้รับอะโทรปีน (10 มิลลิกรัมต่อกิโลกรัม, ฉีดเข้าชั้นกล้ามเนื้อ) หรือ ไฟโสสติกมีน (0.5 มิลลิกรัมต่อกิโลกรัม, ฉีดเข้าชั้นกล้ามเนื้อ) ในวันที่ 5 ของสัปดาห์ที่ 8 ของการทดลอง หลังจากการให้อะโทรปีนพบว่า ในหนูขาวกลุ่มที่ได้รับอะไครโลไนไตรล์ 25 มิลลิกรัมต่อกิโลกรัมไม่มีการเปลี่ยนแปลงอย่างมีนัยสำคัญต่อการทำงานของระบบหายใจ ผลการทดลองเช่นนี้ก็จะพบได้เช่นกันในหนูขาวกลุ่ม 1 มิลิกรัมต่อกิโลกรัมเมื่อได้รับอะโทรปีน แต่ไฟโสสติกมีนจะเสริมให้เกิดผลโคลิเนอร์จิกของอะไครโล-ไนไตรล์น้อยกว่า ในหนูที่ได้รับ 25 มิลลิกรัมต่อกิโลกรัม, กลุ่มที่ได้รับอะไครโลไนไตรล์ 25 มิลลิกรัมต่อกิโลกรัมจะแสดงผลตอบสนองต่อระบบหายใจเช่นเดียวกับในกลุ่ม 1 มิลลิกรัมต่อกิโลกรัม แต่จะเป็นไปในทางที่น้อยกว่ามากเมื่อเทียบกับกลุ่มที่ได้รับสารนี้ 1 มิลลิกรัมต่อกิโลกรัมและกลุ่มเปรียบเทียบ

จากผลการทดลองแสดงให้เห็นว่า หลังจากได้รับอะไครโลไนไตรล์ ผลกระทบต่อการทำงานของระบบหายใจจะขึ้นอยู่กับปริมาณของอะไครโลไนไตรล์ที่สัตว์ทดลองได้รับและระยะเวลาที่ทำการทดลอง ในกลุ่มที่ได้รับอะไครโลไนไตรล์ 1 มิลลิกรัมต่อกิโลกรัมไม่พบว่ามีผลกระทบต่อการทำงานของระบบหายใจและการตอบสนองของตัวรับมัสคารินิคต่ออะเซ็ทติลโคลีน อย่างไรก็ตามในขนาดของอะไครโลไนไตรล์ที่สูงขึ้น คือที่ 25 มิลลิกรัมต่อกิโลกรัมหลังจากได้รับเป็นเวลา 8 สัปดาห์ ผลการทดลองแสดงถึงความเป็นไปได้ในการเกี่ยวเนื่องกับการทำงานของระบบหายใจกับการลดการตอบสนองของตัวรับมัสคารินิคต่ออะเซ็ทติลโคลีน ซึ่งผลที่ได้นี้ไม่สามารถที่จะแสดงได้ชัดเจนจากการให้อะโทรปีนแต่จะพบเมื่อให้ไฟโสสติกมีน ในขณะนี้ไม่สามารถอธิบายได้อย่างแน่ชัดถึงกลไกในการลดการตอบสนองของไฟโสสติกมีนในหนูขาวที่ได้รับอะไครโลไนไตรล์ จึงจำเป็นต้องมีการศึกษาต่อไปถึงตำแหน่งและกลไกในการเหนี่ยวนำให้เกิดการลดลงของการตอบสนองของตัวรับโคลิเนอร์จิกหลังจากที่ได้รับไฟโสสติกมีน

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Thesis Title	The Effect of Subchronic Exposure to Acrylonitrile on Respiratory Function in Rat.
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Abstract

The effect of subchronic exposure to low doses of acrylonitrile on respiratory functions and the alterations of the cholinergic responses controlling this system of the male Wistar rats had been investigated. Acrylonitrile at the doses 1 and 25 mg/kg body weight were administered subcutaneously once daily, 5 days per week for 8 weeks. Normal saline in comparable amount was administered to the control group. Respiratory functions (peak expiratory flow, respiratory rate, minute volume, tidal volume, inspiratory time, expiratory time, relaxation time, specific airway resistance, specific airway conductance) were recorded by using Noninvasive Respiratory Analyzer at the 1st, 2nd, 4th, 6th, and 8th week of the treatment schedule.

The subchronic exposure to 1 mg/kg dose of acrylonitrole showed no significant alterations in respiratory functions when compared with control group. Whereas, the administration of acrylonitrile 25 mg/kg showed time-dependent alterations. The significant effects in the respiratory functions, for example decreased peak expiratory flow, increased specific airway resistance, were observed markedly at the first and second week of acrylonitrile 25 mg/kg treatment, however, the adaptive changes to nearly the values of control group especially at one week following the termination of

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8 weeks of acrylonitrile treatment were also detected. The other signs of acrylonitrile on cholinergic functions including salivation, lacrimation, and diarrhea were also observed in acrylonitrile 25 mg/kg treated group.

The acrylonitrile-treated rats were challenged with atropine sulfate(10 mg/kg,Im.) or physostigmine (0.5 mg/kg,Im.)at day 5 of the 8th week of the treatment schedule. No significant alterations in respiratory functions of acrylonitrile 25 mg/kg treatment were observed after atropine challenged. Similar results were found at the dose of acrylonitrile 1 mg/kg after challenge with atropine in almost all of respiratory functions.

After challenge with physostigmine, significant hyperactivity of cholinergic functions were observed in control and acrylonitrile 1 mg/kg treated group. In acrylonitrile 25 mg/kg treated group, the similar pattern of response to physostigmine on respiratory functions were observed but mostly to the lesser extent than acrylonitrile 1 mg/kg treatement.

The results of this study revealed that, the effect of acrylonitrile on respiratory functions after subchronic exposure were dose and time dependent. At the dose of 1mg/kg acrylonitrile treatment, there was no effect on respiratory functions and muscarinic response to acetylcholine. However, at higher dose 25 mg/kg, the results obtained after subchronic exposure for 8 weeks suggested that there was a subtle decrease in muscarinic response to acetylcholine. These observations could not be detected clearly by the administration of muscarinic antagonist, atropine, but it would be revealed upon a marked activation of muscarinic receptors induced by physostigmine. At present, it is not possible to explain what is the exact mechanism of reduced physostigmine response in acryloniyrile-treated rat. Further studies are needed to elucidate the specific sites and mechanisms of acrylonitrile-induced reduction in cholinergic responses following the administration of physostigmine.

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LIST OF ABBREVIATIONS

ACh	=	Acetylchoine
ACN	=	Acrylonitrile
ATROP	=	Atropine
COND	=	Specific airway conductance
DG	=	Diacylglycerol
HCN	=	Hydrocyanic acid
GST	=	Glutathione-S-Transferase
LD_{50}	=	Median lethal dose
M_1	=	Muscarinic subtype 1 receptor
M_2	=	Muscarinic subtype 2 receptor
M_3	=	Muscarinic subtype 3 receptor
mACh	=	Muscarinic acetylcholine receptor
mg	=	Milligram
mg/kgBW	=	Milligram per kilogram body weight
mg/m^3	=	Milligram per cubic meter
min.	=	Minute
MV	=	Minute volume
MW	=	Molecular weight
PEF	=	Peak expiratory flow
PHYSO	=	Physostigmine
RES	=	Specific airway resistance
RR	=	Respiratory rate
sec.	=	Second
TE	=	Expiratory time
TI	=	Inspiratory time
TV	=	Tidal volume

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CHAPTER I

INTRODUCTION

Acrylonitrile (CH_2=CH-CN; VCN) monomer is a major industrial chemical with a wide range commercial applications including the production of synthetic fibers and plastics. Its principal uses are the manufacture of acrylic and modacrylic fibers, as a constituent of acrylonitrile-butadiene-styrene(ABS) and acrylonitrile styrene(AS) resins and as an intermediate in a number of chemical processes(1). Acrylonitrile may also enter the environment during storage, transport, transfer and end-use (2).

Acrylonitrile has an extremely wide range of uses, so that there is a strong likelihood of occupational and general human exposure through air, water and food. In addition, there is widespread direct physical contact from manufactured products, such as packaging, including that of food and beverages, textile fibers for clothing and furnishing. There is evidence that acrylonitrile has persisted in soil for a long period following accidental spillage(2,3). Acrylonitrile has been reported to cause human poisoning and deaths from occupational (mostly resulting from exposure to acrylonitrile-contaminated air in industrial production and processing sites) and accidental exposure.

In in vitro preparation acute exposure to high concentrations of acrylonitrile has been reported to cause irritation of respiratory tract, eye, nasal cavity, and adverse effects on central nervous system and gastrointestinal tract, dysfunction of liver and kidney (1,2,4,5).

There are many studies reported that long term exposure to acrylonitrile caused mild liver dysfunction, cancer (particulary of the lung), lability of autonomic function (1,2,3,6,7). From epidemiological studies, there was a report indicated a significant decrese in an epinephrine-like substance and the increase in acetylcholine level in acrylonitrile-exposure workers (7). In addition, symptoms of irritation to the respiratory tract such as stuff up and running nose, constant coughing, feeling of pressure on chest,

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and chocking lump in the throat were reported significantly more often in worker involved in acrylonitrile production. These adverse effects may be regarded as a temporary and reversible symptoms(8). At present, there are few studies about the effects of low dose and chronic exposure to acrylonitrile on respiratory functions. The preliminary studies from our laboratory indicated that chronic exposure to acrylonitrile could induce alterations in tracheal muscarinic receptors. In this study attempts have been made to gain more informations about the influence of acrylonitrile related to the respiratory functions in intact test animals .

The objectives of this study are as follows :

1. To investigate the effects of long-term exposure to acrylonitrile on body weight and respiratory functions (peak expiratory flow, tidal volume, respiratory rate, minute volume, relaxation time, inspiratory and expiratory time, specific airway resistance, specific airway conductance).

2. To detect whether long-term exposure to acrylonitrile can induce the alterations in respiratory function related to cholinergic nervous system.

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CHAPTER II

LITERATURE REVIEWS

1. ACRYLONITRILE

1. INTRODUCTION

Acrylonitrile is also known as ; 2-Propenenitrile; AN; Cyanoethylene; VCN; Ventox; Vinyl cyanide; Acrylon; Carbacryl ; Fumigrain. (2,9,10)

1.1. Physical and chemical properties :

Acrylonitrile (CH_2=CH-CN) is a volatile,colourless, flammable liquid with a sweet characteristic odour (2). It is soluble in water (at 25 °C, 7.4 percent by weight), and miscible in all proportions in acetone, benzene, carbon tetrachloride, ethyl alcohol and a number of other organic solvents(10).

Important physical properties of acrylonitrile are as follows: boiling point 77.5-79°C, freezing point -83.5 °C, relative molecular weight 53.1, conversion factor : 1 ppm in air = 2.2 mg/m^3(3).

The structural formula as shown in figure 1 :

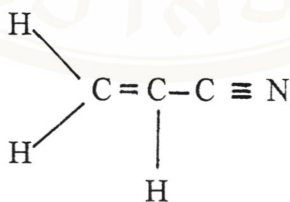

FIGURE 1. Structural formula of acrylonitrile.

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The reaction of acrylonitrile involves the double bond (C=C) and/or the cyano group (CN). It polymerizes to polyacrylonitrile and copolymerizes with,e.g, styrene, butadiene, esters of acrylic or methacrylic acid to form acrylonitrile-butadiene-styrene-resins (ABS), acrylonitrile-styrene(AS) resins, nitrile rubber, acrylic and modacrylic fibers. Hydration of acrylonitrile produces acrylamide or acrylic acid(2). Acrylonitrile is used in the manufacture of synthetic fibers, rubbers, and plastics and as an important chemical intermediate(11).

1.2 Industrial and environmental sources, and level of exposure :

Acrylonitrile does not occur as a natural product(2,3). The major sources of contamination of the general environment are acrylonitrile producing and polymerizing plants. The occurence of acrylonitrile in air, water and soil near industrial plants has also been described. The production and use of acrylonitrile at the workplace provide the greatest potential for exposure. Airborne exposure to acrylonitrile near industrial sites appears to pose the highest risk for the general population; the potential for exposure through water and food appear to be low by comparison(2).

Acrylonitrile is among the 50 most extensively synthesized chemicals in the United State. Commercial production of acrylonitrile was first reported in 1940 (3). The annual production for 1974-1976 was only 1/4 of that of vinyl chrolide, but about 10 times more than that of DDT (2).

In the U.S.A. in 1976 (3) acrylonitrile was used for the manufacture of acrylic and modacrylic fibers (48%), acrylonitrile-butadiene-stylene resins (21%), adiponitrile (12%) and other products (mainly the production of polyether polymer, polyols, acrylamide) (19%). Acrylic and modacrylic fibers are used primarily in clothing and home furnishings (82%) and the remainder (18%) was exported.

The major applications of acrylonitrile-butadiene-styrene and acrylonitrile-styrene resins are the pipe fitting, automotive vehicle components, automobile instrument panels,household items.

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Acrylonitrile may enter the environment during the various stages of storage, transport, transfer, and end-use. Of the estimated total emission of 3,856 mg per year, monomer acrylonitrile-butadiene-styrene(ABS), styrene-acrylonitrile resins(SAN) and acrylic fiber production facilities emit acrylonitrile 802, 1,424 and 1,276 mg, respectively, to the atmosphere per year (1).

Half-life of acrylonitrile in air is estimated to be 13 hours which is long enough to allow transport of acrylonitrile from emission sourses to nearby pollution areas. It would appear that only minimum amounts will remain in the environment; thus acrylonitrile dose not represent a long-term environment hazard(12). In water, the half-life, as estimated by the BOD test, is 5-7days. Acrylonitrile has also been detected in drinking water, although the lelvels were not quantified. There are insufficient data with which to determine the human intake of acrylonitrile through food and drinking water(2). Acrylonitrile polymers and copolymers are components of products intends for uses in contact with food. The migration of acrylonitrile from these containers into food and beverages have been considered(2,13). The use of copolymers of acrylonitrile for making beverage bottles was banned in the U.S.A in September 1977 (2).

In Thailand, Hazardous Substance Office, Ministry of Industries stated that acrylonitrile was imported approximately 15,452.71 MT in 1990, 11,109.12 MT in 1991, and 23,811.49 MT in 1992 (14).

Prior to 17 January 1978, the US Ocupational Safety and Health Administration's health standards for exposure to air contaminants required that an employee's exposure to acrylonitrile not exceeding an eight-hour time-weighted average of 20 ppm (45 mg/m^3) in the workplace air in any eight-hour work shift of a forty-hour work. On 17 January 1978 the US Ocupational Safety and Health Administration announced an emergency standard for acrylonitrile, which limits employee exposure to an eight-hour time-weight average of 2 ppm(4.5 mg/m^3)acrylonitrile in air (3).

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2. TOXICOKINETICS :

2.1 ABSORPTION : Acrylonitrile is emitted in the form of vapor and in aqueous effluents (2). It is absorbed through skin, stomach and lung with most acute exposure resulting from inhalation (15). Acrylonitrile can readily absorbed from respiratory tract (9). Rogaczewska and Piotrowski in 1968 (16) reported that in human studies, the retention of acrylonitrile in the respiratory tract in 3 volunteers exposed to a concentration of 20 mg/m^3 for up to 4 hours was about 46 % and did not change throughout the inhalation period.

The uptake by lung tissue was extremely fast, having a maximum at 0.5 hour after oral administration (17).

Acrylonitrile is readily absorbed through the skin, although systemic intoxication is rarely seen, probably because it evaporated before toxic quantities are absorbed. It is readily absorbed by leather gloves and footwear, causing skin absorpion and blistering (9). The average absorption rate of liquid acrylonitrile through skin was 0.6 mg/cm^2 per hour (16). However, dermal absorption is poor and occur at about 1% of that of the lung. Following absorption of radiolabled acrylonitrile, the radioactivity disappears in a biphasic manner, with the half-life for the first phase of 3.5-3.8 hours and for second phase of 50-70 hours (1). Acrylonitrile is only slightly absorbed from the stomach (18).

The absorption rate of acrylonitrile was lower in rats after oral administration than after subcutaneous or intraperitoneal administration. After intraperitoneal administration, the blood concentration of acrylonitrile reached a maximum in several minutes and then decreased rapidly (19,20).

2.2 DISTRIBUTION : After a single intraperitoneal and oral administration of radiolabled acrylonitrile to rats. Most of ^{14}C found in the tissues were associated with erythrocytes,liver, kidney, intestine, skeletal muscle, lower levels being found in lung and brain(21). Loss of ^{14}C from liver and kidney occured fairly rapidly, but ^{14}C in the erythrocytes was still mostly retained 48 hour after treatment(18). Up to maximum of

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94% of ^{14}C acrylonitrile in erythrocytes were found to be covalently bound to cytoplasmic and membrane proteins(22). Radioactivity in lung tissue declined gradually as a function of time and was still detected at 72 hours after single oral dose (17).

The evidence available at present on the distribution of acrylonitrile in the body and on tissue damage following expossure does not indicate increased accumulation in any particular tissue or organ, except erythrocytes, and there is no indication from animal studies of tissue accumulation following long-term exposure (2).

There are no data on the distribution to the brain of acrylonitrile or its metabolites, although brain lesions have been associated with acrylonitrile exposure (12). However, from the studies of Ahmed et al. in 1982, and Sato et al. in 1982 about the distribution and accumulation of 2,3-^{14}C acrylonitrile in the rat, they observed the longer retention of acrylonitrile in brain and muscle. The cytosol fractions of brain, liver, and kidney showed a relatively high specific radioactivity (23,24).

2.3 BIOTRANSFORMATION: The oxidative pathway of acrylonitrile biotransformation includes a number of consecutive enzyme-catalyzed or spontaneous reactions. The first step, oxidation of acrylonitrile to glycidonitrile, is catalyzed by hepatic microsomal mono-oxygenases(2,25,26). Glycidonitrile is a reactive intermediate; in in vitro experiments it is transformed by epoxide hydrolase to glycoaldehyde cyanohydrin, which decomposes spontaneously to hydrocyanic acid (cyanide) and glycolaldehyde (2,25,26). The results of animal studies have shown that cyanide formed in vivo is subsequently converted by rhodanese to thiocyanate and eliminated in urine (2,27).

The proposed routes of oxidative pathway are shown diagrammaticaly in figure2.

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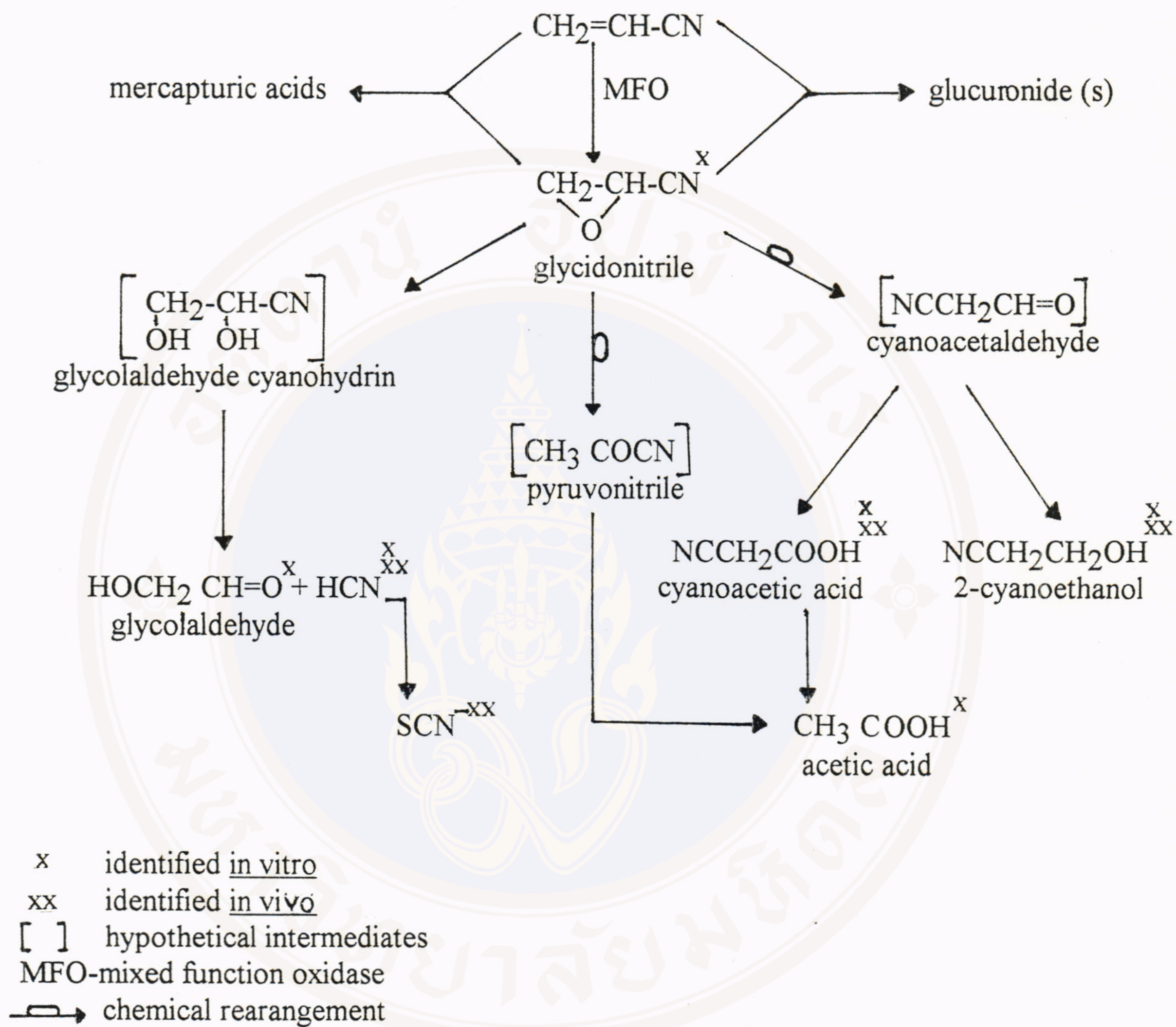

FIGURE 2. The main possible pathway of acrylonitrile biotransformation

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Acrylonitrile forms stable conjugates with L-cysteine and L-glutathione in vitro. Depressed levels of sulfhydryl compounds have been reported following acrylonitrile administration (28,29). The spontaneous conjugation of glutathione with acrylonitrile or glycidonitrile proceeds very slowly. Since glutathione conjugates are precursors of mercapturic acids (N-acetyl-S-(2-hydroxyethyl)-L-cysteine), the occurrence of mercapturic acids derived from acrylonitrile and glycidonitrile may be expected in the urine of animals exposed to acrylonitrile (2).

Acrylonitrile-derived glucuronide might be the alternative substance to conjugate metabolites (23).

Gut et al. in 1975 (18) reported that after a single dose of acrylonitrile 0.5 or 0.75 mM/kg BW., the main metabolite of acrylonitrile is thiocyanate and found that acrylonitrile was more effectively metabolized to thiocyanate in mice than in rats after oral,intraperitoneal and intravenous administration. A greater response of acrylonitrile to thiocyanate metabolism and a large decrese in its acute toxicity after thiosulfate treatment in mice than in rats indicate possible difference mechanism of acrylonitrile toxicity in these animals. Cyanide apparently plays a minor role in the acrylonitrile toxicity in rats, but may play quite an important one in mice .

Acrylonitrile-derived glucuronide migth be the alternative substance to conjugate metabolites (30).

In general, all metabolic systems appear lesss active in human than in rats (31).

2.4 ELIMINATION : Studies, particularly animal studies, on the absorption, distribution, biotransformation, and elimination of acrylonitrile have shown that a small fraction of the acrylonitrile absorbed is rapidly eliminated in the urine without biotransformation, while the remainder is biotransformed via several pathways, a number of metabolites being excreted in urine ; some of this metabolites are unique to acrylonitrile(2,12,18).

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The two major urinary metabolites were identified as thiocyanate and N-acetyl-S-(2-cyanoetyl) cysteine (26).

Dose-dependent urinary excretion of acrylonitrile metabolites was studied by Kedderis et al. in 1993 (32). They found that urine was the major route of excretion of acrylonitrile metabolites (77-104 % of the dose), with less than 8 % of the dose excreted in the feces. The results also demonstrate that GSH conjugation is the major disposition pathway of acrylonitrile and the ratio of acrylonitrile epoxidation to glutathione conjugation was 0.5 in rats and 0.67 in mice. This species difference in acrylonitrile oxidation could have important toxicological implications (26).

In Wistar rats the elimination of the sum of radioactive metabolites of acrylonitrile-^{14}C was not markedly influenced by the route of acrylonitrile administration. The elimination of thiocyanate, however, was significantly higher after oral (23% of the dose) than after intraperitoneal (4%), subcutaneous (4.6%) or intravenous (1.2%) administrations. The elimination of the sum of radioactive metabolites was highest in the first 4 hours after acrylonitrile administration and rapidly decreased, whereas the excretion of thiocyanate reached maximum between hour 8 and 14 after oral or intraperitoneal administration (33).

In the rabbit, acrylonitrile has been detected in urine 72 hours and in expired air for 1 hour after dosing, strongly suggesting that it storage in adipose tissue (34).

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3. ACRYLONITRILE TOXICITY :

3.1 Animal toxicity:

There seems to be little consistancy in the effect of route or vehicle of administration or of sex on the lethal dose. From the toxicity studies, the mean lethal doses (LD_{50}) of acrylonitrile in rat (male) are as follows :

Route (administration)	LD_{50} (mg/kg BW)	Vehicle	Reference
Oral	78	Physiol. saline	(6)
Subcutaneous	80	-	(4)
	96	water	(108)
Intraperitoneal	100	-	(4)

The acute LC_{50} (lethal concentration in air for 4-hour inhalation) for male, Wistar rat is 470 mg/m^3 (4).

Acrylonitrile induces a variety of toxic effects. Effects due to over exposure are non specific and mainly relate to the gastrointestinal tract, respiratory tract, the central nervous system, and the kidney (1,2,4,5,15).

Previous studies identified the acute adrenocorticolytic action of acrylonitrile (35) as well as a chronic adrenal atrophy and insufficiency caused by acrylonitrile (36). Liver damage has also been reported (37).

Death following convulsions occured between 4 and 6 hours after administration of high dose of acrylonitrile to both female rats (100 or 150 mk/kg BW) and male rats (150 mg/kg BW) (38).

The causes of death seem to be related to acute adrenocortical insufficiency, toxicity to the central nervous system (e.g.excitation and convulsion followed by paralysis which probably involves the respiratory center as well), and congestive lung

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edema (29).

Acrylonitrile is readily absorbed through the skin and application of 0.8 to 1.6 g/kg to the shaved rabbit skin gave severe irritation and immediate signs of systemic toxicity, with one of six animal dying, but the others making a complete recovery (39).

It was found that the toxicity of acrylonitrile was due not only to the hydrogen cyanide liberated from it but also to acrylonitrile itself (1,40). There is evidence to suggest that there is a slow release of cyanide. A small amount of hydrogen cyanide liberate in blood of animals (rabbits, guinea pig and rats) after a single injection of acrylonitrile. This deduction is supported by the claimed therapeutic effect of sulfhydryl compounds which react readily with acrylonitrile and cause neither acute or delay symptoms when injected into animals (40).

In experimental animals, there is considerable species variation in susceptibility to acrylonitrile intoxiction. Dogs appear to be the most sensitive species tested and the sensitivity decreased in the following order : mice, rabbits, cats, rats, guinea pig (1,2,10).

The pathological changes in subchronic exposure of rats to acrylonitrile belived to be included hyperplasia and hyperkeratosis of the squamous epithelium of the non glandular portion of stomach, proliferation of grain cells in brain, and mammary gland hyperplasia in females (1).

The hemorrhagic gastritis was found in rats necropsied 24 hours after oral administration of 150 mg/kg acrylonitrile(22). Ghanayem and Ahmed in 1983 (11) had reported that after oral or subcutaneous adminnistration of a single acrylonitrile 50 mg/kg in rats, the metabolic activation of the acrylonitrile molecule to the epoxide intermediate (2- cyanoethylene oxide) , in a reaction catalyzed by the cytochrome P_{450} enzymes, is strongly suspected to be the cause of the acrylonitrile-induced gastrointestinal hemorrhage.

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The effect of inhalation exposure to acrylonitrile on blood glucose, total-SH (T-SH) and non protein-SH (NP-SH) in tissue were investigated using male Wistar rats. T-SH levels in liver significantly decreased after the 6 hour inhalation of acrylonitrile. The most pronounced NP-SH depletion was detected in the liver, less in blood and moderate in brain and lung. Significantly increased blood glucose levels were found following the inhalation of acrylonitrile 2.83, 5.65, 11.3 and 17 mM/m^3(41).

The acute experiments in rats shown a rapid time and dose dependent decrease in reduced glutathione (GSH) in the liver, lung, kidney and adrenal gland. The diminution of GSH concentration seemed to correlate with the occurrence of mortality in an acute experiment(29).

Acrylonitrile has an interesting toxicological profile. When it is given by difference routes of administration, it causes tumors at the site of first contact. Thus, by inhalation it causes respiratory and brain tumors, by gavage it causes gastric and brain tumors (42).

Acrylonitrile was tested for carcinogenicity in rats by oral administration and by inhalation. Following its oral administration, it induced neoplasm of the brain, squamous cell papillomas of the stomach, tumor of tonge, small intestine and mammary gland. Following its inhalation, neoplasms of the central nervous system, mammary gland, forestomach were observed (43). The oral route of administration was chosen because trace amounts of unreacted acrylonitrile monomer may migrate into food that pakaged in container made of acrylonitrile copolymers. The inhalation route was selected because it is a likely route of exposure in the workplaces (44).

Acrylonitrile is also embryotoxic (3) and mutagenic (3,34,45,46,47).

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3.2 Human toxicity.

Acrylonitrile can enter the body by inhalation, ingestion, or through the skin (48). Inhaled acrylonitrile vapour is readily absorbed. Acute systemic effects following absorption of vapour have been described. Symptoms were non-specific and referable to the gastrointestinal tract, respiratory tract, liver, kidney, the central nervous system (1,12,48,49). If exposure continues, nausea, vomiting, weakness and occasionally headache and diarrhea are other complaints (50). The threshold limit value of acrylonitrile is 4.5 mg/m^3 for human (1).

Liquid acrylonitrile may cause irritation, erythema, and severe burn (1,12). In serious cases it may cause unconcious and death (48).

Several causes of mild jaundice accompanied by mild anemia and leucocytosis have been reported. Acrylonitrile closely resembles hydrocyanic acid in its toxic action, by inhibiting the respiratory enzymes of tissue, it renders the tissue cells incapable of oxygen absorption. There is little evidence of cumulative action on repeated exposure (50).

Several cases of acrylonitrile intoxication resulting from exposure to fumes in factories of synthetic rubber and polymerization have been reported (8). The highest exposure occur in the workplace (acrylonitrile - producing and - polymerizing plants).

In the manufacture of synthetic rubber, acrylonitrile presents definite hazards of vapor toxicity and of toxic absorption. However, there were no fatal causes occured (10).

Muto et al. in 1992(51) studied the health effects of acrylonitrile in seven Japanese acrylic fiber manufacturing factories. The results of this study provided a no-observe effect level for worker who had been exposed to acrylonitrile for 17 years on the average.

There are many studies which do not support the hypothesis that acrylonitrile is a human carcinogen(52). However, the concern from low-level exposure to acrylonitrile is

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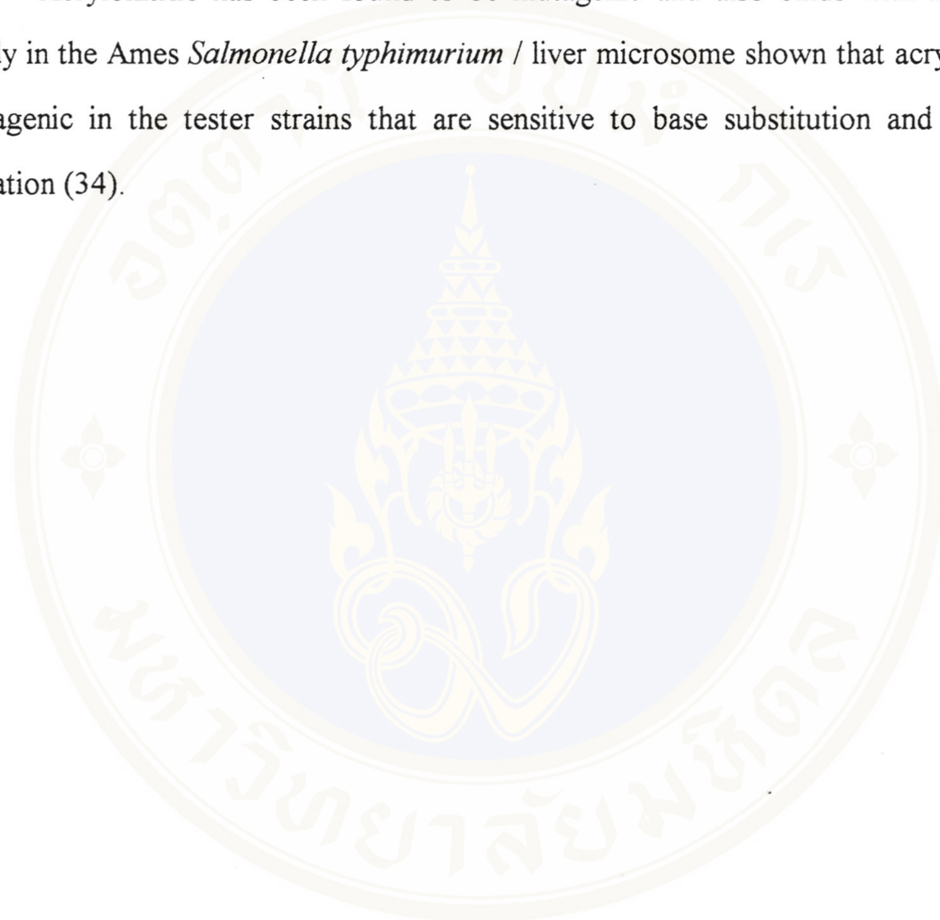
primary due to its potential for carcinogenicity (12). Several epidemiology studies provided suggestive evidence for an association of lung cancer in worker exposed to acrylonitrile. It has been speculated that acrylonitrile is metabolized to 2-cyanoethylene oxide, which is proximate carcinogen (12).

Acrylonitrile has been found to be mutagenic and also binds with DNA (17). Study in the Ames *Salmonella typhimurium* / liver microsome shown that acrylonitrile is mutagenic in the tester strains that are sensitive to base substitution and frameshift mutation (34).



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4. TOXIC EFFECT ON NERVOUS SYSTEM

Kaneko and Ome in 1992 (49) has designed the cross-sectional study to clarify the relationship between long-term exposure to acrylonitrile and its effects on subjective symptom in 7 acrylic fiber manufacturing factories. They found that long-term exposure to acrylonitrile up to an average level of 14.4 ppm, did not induce neurotoxic effects. The symptoms of irritation to the respiratory tract and mucous membranes, heavy sweating even in winter were complained significantly more often in acrylonitrile workers.

Ageeva(7) reported a significant decrease of epinephrine-like substance in blood and an increase of acetylcholine in worker producing acrylonitrile, and suggested that increase sweating in worker exposed to acrylonitrile was a symptom related to the effect of acrylonitrile on the autonomic nervous system. Krysiak and Knobloch in 1971(6) found that rats receiving acrylonitrile 20 mg/kg body weight intraperitoneally, daily for 6 weeks or subcutaneously 40 mg/kg body weight daily for 4 weeks, exhibited a significant lengthening of time to correctly perform condition food reflex test, and a significant decrese in a number of correction reactions compared with pretreatment observations or controls. Performance improved when the treatment was discontinued .

Acrylonitrile is a highly toxic chemical affecting the central nervous system (1,2,3,4,29,40). The signs of neuron lesions in the central nervous system were observed in rats and rabbits breathing acrylonitrile 50 mg/m^3 air for 6 months (5). Daily intraperitoneal administration of acrylonitrile to rats at the dose of 50 mg/kg body weight for 3 weeks caused a vacuolization of neuronal cells of the cortex and brain stem (4).

The toxic action is not mediated in the same fashion as HCN. Although acrylonitrile is a very reactive chemical, its toxicity might be partially due to the liberation of cyanide radical,since the amount of release cyanide is not enough to cause cyanide poisoning (53).

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Walum and Peterson in 1984 (54) reported that no information exist about biotransformation of acrylonitrile in neurons .

Recently, Cova et al. in 1992 (55) studied the toxicity of acrylonitrile in a human neuroblastoma cell line and its effect on glutathione and glutathione-s-transferase (GST). They found that the exposure of the cells to several concentrations of acrylonitrile did not produce any significant change in this enzymatic activity with respect to control activity and lack of stoichiometry between cyanide release and the concentration of acrylonitrile. They suggested that, these finding might be due to one or several enzymes deficiency such as GST in the human neuroblastoma cell cultures.

The mechanism of acrylonitrile toxicity has been studied by Abreu and Ahmed in 1979 (123). Two phases of acute toxicity were observed in fasted rats given an oral LD_{50} of acrylonitrile (90 mg/kg). The first phase was cholinomimetic in nature and the second phase appeared to be central nervous system (CNS) dysfunction. From this finding, they suggest that the cholinomimetic phase of acrylonitrile toxicity could be mediated by the entired molecule while the CNS phase was a function of cyanide liberation.

Acrylonitrile is metabolized to cyanide in rats and mice. The study of Ahmed and Patal in 1981 (27) showed that the signs of acrylonitrile toxicity observed in rats and mice were different. In rats, early signs of toxicity were cholinomimetic (salivation, diarrhea, lacrimation, peripheral vasodilatation, excessive gastric secretion). In mice, the signs of acrylonitrile toxicity were related to central nervous system effects (depression, convulsion, asphyxia) which are the characteristics of cyanide toxicity. The cholinomimetic signs observed following acrylonitrile exposure suggested that acrylonitrile or one of its metabolites, other than cyanide, might alter the autonomic balance in rats. They also suggested that mice metabolized acrylonitrile faster or detoxified cynanide more slowly than rats, so that in rat, acrylonitrile metabolism to cyanide might play a minor role in acrylonitrile toxicity whereas in the mouse, cyanide

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liberation appeared to be the major mechanism for the acrylonitrile effect.

Recently, Ghanayem et al. in 1991 (56) reported that the cholinomimetic toxicity induced by acrylonitrle was dose related regardless of the route of administration. They also reported that, atropine could protect against acrylonitrile-induced cholinomimetic neurotoxicity in rats which suggested the possible involvement of cholinergic system in acute acrylonitrile-induced neurotoxicity.

Satayavivad et al. in1991 (57) found that the industrial chemical,acrylonitrile, exhibited strong influence on cholinergic responses and apparenently this effect is not related to the acetylcholinesterase activity.

The effects of varying doses and duration of pretreated schedule of acrylonitrile on rat tracheal and cardiac muscarinic receptors in vitro were also studied by Satayavivad et al. in 1991 (58). The results from this study indicated that high dose of acute exposure to acrylonitrile (25, 50 and 100 mg/kg body weight, subcutaneously) had no effect on the tracheal and cardiac muscarinic responses to acetylcholine. However, low doses and chronic exposure of acrylonitrile (subcutaneous administration of 0.05 and 5 mg/kg body weight, once daily for 2, 4, 6 and 9 weeks) induced the alterations of tracheal and cardiac muscarinic response to acetylcholine, both increased and decreased responses were observed depending on the time of exposure.

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5. PULMONARY TOXICITY :

Acrylonitrile is readily absorbed from the respiratory tract and exposure to low concentrations causes flushing of face and salivation, further exposure results in irritation of eyes and nose, deepened respiration and oppressive feeling of the chest (50). Respiratory distress, lethargy, convulsions and coma occur with lethal or near lethal exposures (7500 mg/m^3, inhalation)(2).

It was stressed by Paulet et al. (59) that, after a lethal intravenous dose of acrylonitrile (120 mg/kg body weight), the respiratory rate in rabbits did not increase as observed in cyanide poisoning. However, respiratory disturbance was observed in anesthetized dogs given 100 mg/kg body weight intravenously(60). Pulmonary edema was also detected.

In the study of Dudley et al.(61), it was found that lungs were affected by subacute bronchopneumonia, congestion and edema of alveolar walls, extravasation of erythrocytes and serum into the alveoli, focal collection of lymphocytes and polymorphonuclear leukocytes , in most guinea pigs, rabbits, the monkey, and 1 out of 3 rats .

Symptoms of irritation to the respiratory tract and mucous membranes such as chocking lump in the throat, stuff nose, runny nose and coughing were complained of significantly more often in acrylonitrile workers(5.6-8.6 years exposure) (8). Except for chocking lump in the throat, these symptoms had no consistent relationship to either the length of exposure to acrylonitrile or the mean acrylonitrile levels in workplaces. Thus, these effects may be regarded as temporary, reversible irritative symptoms (49).

Acrylonitrile induced lung toxicity was studied following a single oral dose (46.5mg/kg) in Male-Sprague-Dawley rats. The mechanism of toxic injury was investigated by assessing the covalent interaction of [2,3-^{14}C]acrylonitrile with pulmonary DNA. Histologic examination revealed that lungs of acrylonitrile treated animals showed moderate to marked hyperplasia of the clara cells lining the bronchioles.

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The cytoplasm of the clara cells appeared dense and eosinophilic, the nuclei were hyperchromatic. Focal perivascular edema was present in some lungs ,however, there was no evidence of vasculitis or pulmonary inflammation. [^{14}C] Lung tissue uptake was extremely fast, having a maximum at 0.5 hours after treatment (150 DPM/mg tissue). The total radioactivity in lung tissues declined gradully as a function of time, but was still detected at 72 hours after treatment (59 DPM /mg tissue). This study also provided evidence for the acute genetic toxicity of acrylonitrile (and/or its metabolites) in lungs tissue following a single oral dose of acrylonitrile (17).

Gut et al.(62) studied the effect of inhalation exposure to acrylonitrile 280mg/m^3, 8 hours a day for 5 days in male, Wistar rats. A microscopic examination of the lungs, liver, kidneys and adrenals did not show structural changes ; the number and enzymes activities of alveolar macrophages were also unaffected .

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2.CHOLINERGIC CONTROL OF RESPIRATORY FUNCTION

2.1 INTRODUCTION

2.1.1 SRUCTURE OF THE RESPIRATORY SYSTEM :

The respiratory system is divided into three major divisions (63,64).

2.1.1.1 Nasopharyngeal region

This region extends from the anterior nares to the level of the larynx. It is lined with a mucous membranes composed primarily of ciliated columnar epithelial cells that are interpersed with mucous- secreting cells and glands. The primary function in this region is to filter large airborne particles out of the inspired air (all particles larger than 10 μm in diameter are affected), moistened, humidified and warmed in coming air.

2.1.1.2 Tracheobronchial region

This region encompasses of the trachea, bronchi and bronchioles which serve as major conduction airways between nosopharynx and the peripheral lung bronchioles,and regulate the regional and generalized distribution of air in the lung. Moving distally along the tracheobronchial region, the frequency of cartilagenous ring decrease. Bronchi, therefore contain only a few terminal plates of cartilage, while bronchioles contain none. As cartilage decreases, there is an inversely proportional increase in smooth muscle. The presence of smooth muscle is the most important determinant factor of airway diameter. Respiratory smooth muscle tone is under autonomic control, principally parasympathetic nervous system.

2.1.1.3 Pulmonary region

The pulmonary region is the area of the lung where gas exchange takes place. It includes the respiratory bronchioles, alveolar ducts, alveolar sacs, alveoli and their associated capillaries,lymphatic tissues and supportive tissues. Alveoli are thin-walled polyhedral-shaped pouches which one side open to respiratory bronchiole, alveolar duct or alveolar sac. Gas exchange, the primary function of the pulmonary

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system, occurs between the lumen of this thin-walled alveoli and a dense pulmonary capillary network that cover about 85 to 95 percent of the alveolar surface.

2.1.2 MECHANIC OF RESPIRATION:

The lung and the chest wall are elastic structures (65). Inspiration is an active process. During inspiration the volume of thoracic cavity increases and air is drawn into the lung, the increase is brought about partly by the action of the external intercostal muscles which raise the ribs, thus increasing the cross-sectional area of the thorax (66). At the end of inspiration, the lung recoil pull the chest back to the expiratory position, where the recoil pressure of the lungs and chest wall balance. Expiration during quiet breathing is passive in the sense that no muscles with decerased intrathoracic volume contract (65).

The most important muscle of inspiration is the diaphragm, it is supplied by the phrenic nerves from cervical segments 3, 4, and 5. When it contracts,the abdominal contents are force downward and forward and the vertical dimension of the chest cavity is increased. The external intercostal muscles connect adjacent ribs and slope downward and forward. When they contract, the ribs are pull upward and forward causing an increase in diameter of thorax. The internal intercostal muscles assist active expiration by pulling the ribs downward and inward. The intercostal muscles are supplied by intercostal nerves which come off the spinal cord at the same level (67). Thus, transection of the spinal cord at or below the lower cervical region does not affect respiration because the phrenic nerves are spared and the diaphragm can sustain normal respiration on its own (68).

Zhang and Hui in 1992 (69) have studied the phrenic nerve firing activity, they suggested that the phrenic nucleus could be excited by intrathecal injection of acetylcholine and its excitatory effect was mediated by muscarinic receptors. It was assumed that the cholinergic system does not showed any tonic excitation action on the phrenic nucleus under normal physiological conditions.

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The mechanical behavior of the lung is affected by airway caliber, elastic properties of lung tissue, and the surfactant properties of the alveoli as well as the mechanical properties of the chest wall (64). Ohrui et al. in 1992 (70) suggested that in chronic ostructive airway disease the decrease in maximum expiratory flow was more often caused by the combination of airway narrowing, loss of elastic recoil and alter airway collapsibility.

The significant changes in lung volumes or in the dynamic flow of gas in the lung are used to interpret functional changes in pulmonary system and detect the presence of pulmonary disease (64).

2.1.3 CONTROL OF VENTILATION

The remarkable regulation of gas exchange is possible because the level of ventilation is also carefully controlled.

The three basic elements of the respiratory control system are (71) :

1. **Sensors** (chemoreceptors, lung and other receptors) which gather information and feed it to the central nervous system.

2. **Central controllers** (pons, medulla, other part of brain) in the brain which coordinates the information and, in turn, send impulses to the effectors.

3. **Effectors**(respiratory muscles)which cause ventilation.

SENSORS :

Central chemoreceptors : This central chemoreceptors are located on ventral surface of the medulla and separated from blood by the blood-brain barrier (68). This area is highly sensitive to change in either blood CO_2 or hydrogen ion concentration. It has especially potent effect on increasing the degree of activity of inspiratory center, increasing both the rate of the inspiratory signal and also the intensity of the signal (72).

When the blood CO_2 rises, CO_2 diffuses into the CSF from cerebral blood vessels, liberate hydrogen ions which stimulate chemoreceptors. The resulting hyperventilation reduces the P_{CO_2} in blood and therefore in the CSF (71).

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Peripheral chemoreceptors : These are located in the carotid bodies at the bifurication of the common carotid arteries, and the aortic bodies above and below the aortic arch (71). The peripheral chemoreceptors response to decrease arterial PO_2 or hypoxia and thereby stimulate the firing of impulses of peripheral chemoreceptors and produced hyperventilation (68). The increase in either pH or aterial PO_2 also excites these chemoreceptors, however, the direct effects of both factors on the respiratory center itself are much more powerful than their effects mediated through these chemoreceptors (72).

The sensitivity of this system to low aterial PO_2 is less than the sensitivity of central chemoreceptors to hypercapnia (68). Low blood PO_2 normally will not increase alveolar ventilation significantly until the alveolar PO_2 fall almost to one-half of the normal value (72).

CENTRAL CONTROLLERS: The normal automatic process of breathing originated in impulses that come from the brain stem. The motor outflows from this system to the respiratory motor neurons are located in the lateral and ventral portion of spinal cord. The cortex can overide these centers if voluntary control is desired, this system sends impulses to the respiratory motor neurons via the corticospinal tracts (65). In brain stem, there are at least three major parts of the respiratory center :

a. A medullary center : It is capable of initiating and maintaining the rhythmic sequence of inspiration and expiration (68). The cells of the inspiratory area in the medullary center have the property of intrinsic periodic firing, and they are responsible for the basic rhythm of ventilation. These inspiratory cells generate repetitive bursts of action potentials that result in nervous impulses going to the diaphragm and the other inspiratory muscles. The output of inspiratory cells is further modulated by impulses from the vagus and grossopharyngeal nerves (71).

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The expiratory area remains dormant during most normal quiet respiration, because quiet respiration is acheived by the contraction of the inspiratory muscles only while expiration results from passive recoil of the elastic structures of the lung and surrounding chest cage. On the other hand, when the respiratory drive for increased pulmonary ventilation become greater than normal, signals then spill over into the expiratory area as the consequence the expiratory muscles then contribute their powerful contractile to the pulmonary ventilatory process (72).

When pontine tissue is separated from the medulla, respiration continued whether or not the vagi are intact. This respiration is somewhat irregular and gasping, but it is rhythmic.Its occurrence demonstrated that the respiratory center neurons are capable of spontaneous rhythmic discharge (65).

b. An apneustic center in the lower pons. This center provides the inspiratory drive and these neurons are intermittently inhibited by impulses in afferents from the pneumotaxic center and vagal afferents. There are stretch receptors in the lung parenchyma that relay to the medulla via afferents in the vagi, and rapid inflation of the lung inspiratory discharge (Hering-Breuer reflex) thus, stretching of the lung during inspiration reflexly inhibits inspiratory drive. This is why the depth of inspiration is increased after vagotomy in otherwise intact experimental animals (65).

c. A pneumotaxic center in the upper third of the pons. This area appears to switch off or inhibit inspiration and thus regulate inspiratory volume and respiratory rate (71). St. John in 1977 (73) reported that the pontile pneumotaxic center exerts a function in regulating both hypercapnia and hypoxia induced frequency responses.

EFFECTORS : This basic element represents respiratory muscles include the diaphragm, intercostal muscles, and accessory muscles such as the sternomastoids. It is crucially important that these various muscle groups work in a coordinated manner and this is the responsibility of the central controllers (71).

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2.2 CHOLINERGIC CONTROL OF RESPIRATORY FUNCTION

The parasympathetic nervous system is the dominant neural bronchoconstrictor mechanism in all animals including humans and plays an important role in the regulation of airway tone (74,75,76).

Mammalian airways receive a rich cholinergic innervation(77). Cholinergic efferent nerves arise in the vagal nuclei of the brainstem and pass down the vagus nerve to synapse in ganglia that situated in the airway wall. From this ganglia, relatively short postganglionic fibers pass to target cells, such as airway smooth muscle and submucosal gland (63,74,76,78).

Stimulation of the vagus nerve causes constriction of the airway, increases glandular or globlet-cell secretion (77). The location of the airway constriction corresponds to the distribution of cholinergic fibers. Tracheal and bronchial smooth muscle are most affected, whereas alveolar ducts and terminal bronchioles are unaffected (63).

In comparison to the parasympathetic nervous system, the sympathetic nerve supply to the airway appears to be a minor importance (63,79). Activation of the sympathetic innervation to the lung smooth muscle results in airway dilation through the release of norepinephrine. The effects of this innervation are to produce bronchodilation and diminish mucous production (79).

Another aspect of parasympathetic function is its role in the modulation of the breathing patterns. In animals, blocking vagal transmission produces a slow, deep pattern of breathing, whereas stimulation of afferent vagal pathways causes rapid shallow ventilation(80,81,82,83). In addition, parasympathetic effects on breathing pattern may have clinical implication in patients with sleep apnea since drugs with anticholinergic properties improve sleep disordered breathing (84).

In animal studies,it has been suggested that inflammatory mediators and

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particularly prostaglandins, may enhance cholinergic responsiveness of airway smooth muscle(97). In vivo prostaglandin D_2 enchanced the bronchoconstriction effect of cholinergic agonists in asthmatic patients (85).

Efferent cholinergic stimulation causes release of acetylcholine from the glandular vesicles in cholinergic nerve terminals, which rapidly diffuses relatively short distance to cholinergic receptor on the target cells. Acetylcholine is rapidly broken down by the acetylcholinesterase enzyme which is concentrated in synaptic cleft (78).

The onset of bronchoconstriction is rapid and readily reversible, suggesting contraction of airway smooth muscle rather than bronchial wall edema or luminal obstruction with mucous. Rapid freezing of the airways in cats after vagal stimulation has confirmed that muscle contraction is responsible for the airway narrowing (76).

Two distinct types of cholinergic receptors are muscarinic and nicotinic receptors (86). Nicotinic cholinergic receptors are located on autonomic ganglion cells and the membranes of skeletal muscle at the neuromuscular junction (87). The activation of nicotinic receptor always causes a rapid increase in cellular permeability to Na^+ and K^+, depolarization and excitation (86). By contrast, muscarinic receptors are glycoproteins with molecular weights of approximately 80,000 ,whose functions are mediated by interaction with G proteins (86).

The bronchoconstrictor response is potentiated by cholinesterase inhibitor (which prevents the degradation of acetylcholine) and blocked by muscarinic receptor agonist, atropine (78).

Activation of muscarinic receptors in airway smooth muscle causes contraction by stimulating the breakdown of certain membrane phospholipids (phosphoinositides), which results in the release of calcium ions from intracellular stores, leading to contraction (88). Autoradiographic mapping studies have shown that muscarinic receptors are localized in airway smooth muscle, particularly in large airways and to submucosal glands (76,89).

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Muscarinic receptors subtypes in the airways appear to subserve different physiological functions. By current convention, receptors that have been defined pharmacologically are designated as M_1, M_2 and M_3 while those that have been revealed by molecular cloning are termed m_1, m_2, m_3, m_4 and m_5 (90).

Fortunately m_1,m_2 and m_3 appear to corresponse to M_1, M_2 and M_3, respectively. There is less information about the nature and cellular location of m_4 and m_5 receptors (86).

M_1 receptors, which are pirenzepine sensitive (76,78,86,89)are found in cerebral cortex (89), parasympathetic ganglion (76,78,86,89,91). Recent evidence suggests that M_1 receptors also present in alveolar walls (91). M_1 receptors facilitate neurotransmission through ganglion and enhance cholinergic reflexes. M_2 receptors act as autoreceptors on post-ganglionic cholinergic nerve and inhibit acetylcholine release (91). These receptors are sensitive to gallamine, AF-DX 116 and methoctramine (76,89). M_2 receptors are found predominately in the myocardium and also appear to be found in smooth muscle (86). Prejunctional M_2 receptors have been demonstrated in human bronchi in vitro , which have a potent inhibitory effect on cholinergic neurotransmission (92). M_3 receptors mediate contractile responses in airway smooth muscle via phosphoinositide hydrolysis, and are predominant receptors on submucosal glands and airway vascular endothelium (91). All these subtypes are also found in CNS (86).

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2.3 ACUTE AND CHRONIC EFFECTS OF ACETYLCHOLINESTERASE INHIBITORS ON REGULATION OF MUSCARINIC CHOLINERGIC RECEPTORS.

The effects of acute intoxication by anticholinesterase (antiChE) agents are manifested by muscarinic and nicotinic signs and symptoms (93). Organophosphate (OP) anticholinesterases are toxic primarily because of their ability to irreversibly phosphorylate and inactivate acetylcholinesterase, which results in accumulation of acetylcholine in cholinergic synaptic clefts. This prolonged activation of nicotinic, and muscarinic receptors, resulting in the initial potentiation, followed by receptor desensitized (94).

It is well established that repeated exposure of laboratory rats or mice to sublethal dose of organophosphates results in tolerance to many of their parasympathetic effects(95,96).

The regulation by synaptic activity of the macromolecules which are important for neural transmission represents a mechanism for the modulation of the activity and function of nerve cells. For example, prolonged exposure of receptors to a neurotransmitter can lead to a decrease in a number of receptors remaining on the cell surface and thus decrease the sensitivity of the cell to further stimulation. Such agonist-induced decreases (down-regulation)in receptor number (97). Muscarinic acetylcholine receptor (mAChR) number in vivo can be altered by a variety of pharmacological treatments. Chronic administration of acetylcholinesterase inhibitor leads to decrease in a numbers of mAchR present in brain, persumably because the anticholinesterase increases the concentration of endogenous ACh available for receptor binding (98).

Administration of paraoxon(organophosphorous compound) causes symptoms of exessive cholinergic stimulation which diminish with chronic administration. Binding assays were performed on tissue homogenates from rats chronically treated with

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paraoxon for 7 days. The reduction of muscarinic recepters was detected and the decrease in agonist binding was greater than the decrease in antagonist binding, demonstrating the mechanism of tolerance development through muscarinic receptor modulation(99).

Conversely, prolonged treatment with specific muscarinic acetylcholine receptor antagonist resulted in an increase in muscarinic acetylcholine receptors (up regulation) (100).

The kinetic of receptors loss suggested that it is due to an increase in the rate of receptor break down or due to the inhibition of protein synthesis(60). Jett et al. in 1983 reported that,there is the reduction in M_3 subtype of mAChR, without the changing of its affinity, this finding is consistent with the reduced mRNA expression of the M_3 subtype observed in 14 days parathion-exposed mice. They suggested that, this down regulation resulted from the reduction of receptor synthesis during transcription, rather than increase degradation and there is a significant though incomplete recovery in acetylcholinesterase and muscarinic receptors occurs in 7 days following the termination of exposure (94).

The study of Balduini et al. in 1993 (101) found that after chronic treatment with sublethal dose of organophosphate pesticide, diisopropylfluorophosphate (DFP) caused a marked inhibition of acetylcholinesterase activity and a significant down regulation of muscarinic receptor number either after administration during post-natal or in adult rats. In adult animals muscarinic receptor down regulation and reduction of receptor stimulated phosphoinositide hydrolysis may represent a functional adaptation to hyperstimulatory conditions induced by acetylcholinesterase inhibition and may play an important role in the development of tolerance to organophosphate toxicity (102).

Chronic inhibition of acetylcholinesterase in developing rats also resulted in significant alteration in muscarinic neurotransmission which may delay the maturation of cholinergic system and therefore may account for some of long lasting neurotoxic effects

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(101).

Changes in affinity states during down regulation of muscarinic receptors in tracheal smooth muscle of acetylcholinesterase inhibitor - treated swine were studied by Yang et al.in 1988 (103). They found that subchronic administration of DFP caused not only a decrease in the number of receptors but also change to the low affinity of the receptors for agonist which was related to the interaction of the guanine nucleotide binding protein and muscarinic receptor.

Direct administration of muscarinic agonist in in vivo led to a decrease in receptor number and physiological responsiveness in the heart in a dose and time dependent maner(104).

Conversely, after administration of muscarinic antagonist, the increase in the level of muscarinic receptors were found in both various development stages and at maturity (105).

The regulation of individual muscarinic receptor suptypes in rats cerebral cortex/dorsal hippocampus was examined following 14 days administration of the non selective antagonist, atropine by Wall et al. in 1992 (106). They found the increase in total muscarinic receptor density (24%), and reported that receptors linked preferentially to PI turnover (m_1 and m_3) are up regulated to a greater extent than receptors linked to adenylyl cyclase inhibition (m_2 and m_4).

Receptor number gradually returned to control values if the further receptor-agonist interactions were blocked by the administration of a muscarinic antagonist. This recovery of receptor was also blocked by administration of the protein synthesis inhibitor. The recovery of receptor number was dependent on new protein synthesis this finding suggested that the reappeared receptors were newly synthesized (107).

In neuroblastoma cells (109), heart cells (110), and smooth muscles (111) after continuing presence of muscarinic agonist for several hours, the decrease in muscarinic acetylcholine receptors and the decrease in maximum contractile response to

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acetylcholine were observed.

Considerable evidences have been accumulated from these investigations, which indicated that a reduction in the sensitivity of muscarinic receptors may be the major mechanism of tolerance development to reduced acetylcholinesterase activity. While Weckerl et al.in 1977 (112) provided information which suggested that changed in the presynaptic mobilization and storage of acetylcholine may be an alternative mechanism for the development of tolerance to chronically reduced acetylcholinesterase activity.

Siman and Klein in 1981(113) reported that loss of muscarinic receptors caused by long-term receptor stimulation was not a consequence of fundamental changes evoked in overall cellular physiology but reflects a specific regulation of cholinoceptive cell responsiveness.

The decline in muscarinic receptor sites caused by chronic treatment with cholinomimetics was accompanied by a decrease in physiological sensitivity to cholinergic stimulation such as a decrease in magnitude of receptor-mediated increase in phosphatidylinositol (PI) turnover(113) and a decrease in receptor-mediated inhibition of adenylate cyclase (114).

From this literature review, it could be noticed clearly that several studies have been performed in order to elucidate the mechanism of tolerance or desensitization following chronic or continue exposure to acetylcholine. A lot of information have been obtained, however, the precise mechanism(s) are still unknown.

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CHAPTER III

MATERIALS AND METHODS

1. ANIMALS :

The animals used in this study were male, Wistar rats, 4 weeks old, obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakorn Pathom, Thailand. Before these animals were used in this study, they were acclimatized in animal housing for 1 week. Animals were selected at random and four of them were kept per one hanging cage in a temperature controlled room (22 $\pm$ 2°C) under a 12 hours : 12 hours light : dark cycle with light on at 7:00 a.m. They were fed with standard rat chaw obtained commercially and tap water was given ad libitum except during the testing periods.

One day before the experiment, rats were weighed and randomly divided into 3 groups (7-10 rats per group) as one control and two treated groups. Different groups of rats were housed together (4 rats per cage).

2. DRUGS AND CHEMICALS :

Acrylonitrile (MW 56.03)was purchased from Merck. It was dissolved in 0.9% normal saline before giving to the test animals by the subcutaneous injection at the dose of 1 and 25 mg/kg body weight. Control rats were subcutaneously injected with comparable volume of 0.9% normal saline. Acrylonitrile was freshly prepared every week during the treatment schedule.

Pentobarbital sodium was purchased from Abbott Laboratory, while atropine sulfate and physostigmine were perchased from Sigma Chemical Company. All drugs were dissolved in 0.9% normal saline before the drug administration.

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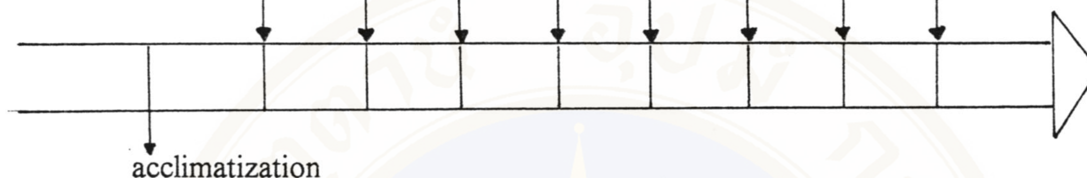
3. EXPERIMENTAL PROTOCOLS :

3.1 THE ACRYLONITRILE TREATMENT SCHEDULE :

Acrylonitrile- treated group

(5 days/week,

for 8 week) 1 2 3 4 5 6 7 8(weeks)



Rats were treated subcutaneously with acrylonitrile 1 and 25 mg/kg body weight/day, 5 days/week, for 8 weeks. Control groups were received normal saline in comparable amounts.

All animal treatments were perfomed between 8:30 a.m. and 10:00 a.m.

Body weight of rats were recorded at day 5 of each week.

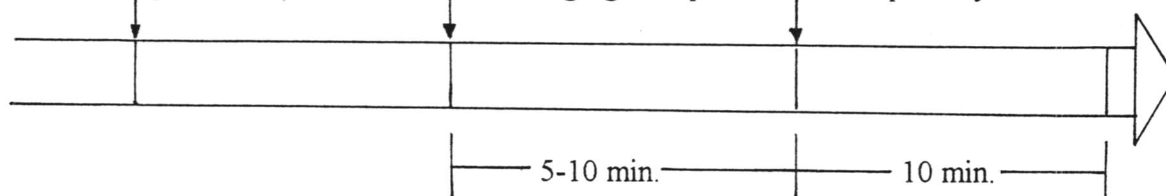
The rats at the 8 th week were divided into three groups as follows : the first group used for measuring the respiratory function at day 5 of the 8 th week of acrylonitrile treatment without any drug challenges. The second and the third group used for measuring the respiratory function at day 5 of the 8 th week of acrylonitrile treatment after challenged with the intramuscular administration of atropine sulfate 10 mg/kg and physostigmine 0.5 mg/kg , respectively.

3.2 RESPIRATORY FUNCTION STUDIES :

The rats at day 5 of

1st,2nd,4th,6th,8th,9^{th}week Pentobarbital sodium Measurement of

were weighed and treated. 20-30mg/kgBW,Ip. the respiratory function



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Respiratory function studies were performed at day 5 of the 1st, 2nd, 4th, 6th and 8th week of the treatment and at the 9th week (one week after the termination of acrylonitrile treatment).

The respiratory functions studies were done between10:30a.m.and 4:00 p.m.

Three groups of rats (one control and two acrylonitrile treated-rats) were studied in parallel.

After the treatment with normal saline in control group and acrylonitrile 1 and 25 mg/kg body weight in treated groups at day 5 of the week as described earlier, each rat was slightly anesthetized by intraperitoneal injection of pentobarbital sodium 20- 30 mg/kg body weight to let the animal calm down before starting the experiment.

The rat was gently put into the double chamber of the Noninvasive Respiratory Analyzer (Buxco Electronics, Inc). The rat's head was inserted through the hole which has the latex ring sealed around the neck of the rat to avoid air leakage. Place rat on the thoracic portion of the box. The test animal was allowed to rest for 5-10 minutes. The respiratory functions measurement began when the signal waveforms of plethysmograph were stable in the same pattern and no fluctuations. This experiment procedure was done during spontaneous breathing of the rat.

Computation of the respiratory function values : peak expiratory flow (PEF), respiratory rate (RR), minute volume (MV), tidal volume (TV), inspiratory time (TI), expiratory time (TE), relaxation time (RT), specific airway resistance (RES) and specific airway conductance (COND) were performed by the analyzer and then recorded and printed out by L-12 logging system.

Duration of the measurement in each rat was taken for 10 minutes. This 10 minutes observation was suitable because in preliminary experiments, this duration was adequately provided the difference in respiratory functions pattern between control and ACN-treated rats. At the end of the experiment in each week, rats would be sacrificed.

The details of Noninvasive Respiratory Analyzer are shown as follows :

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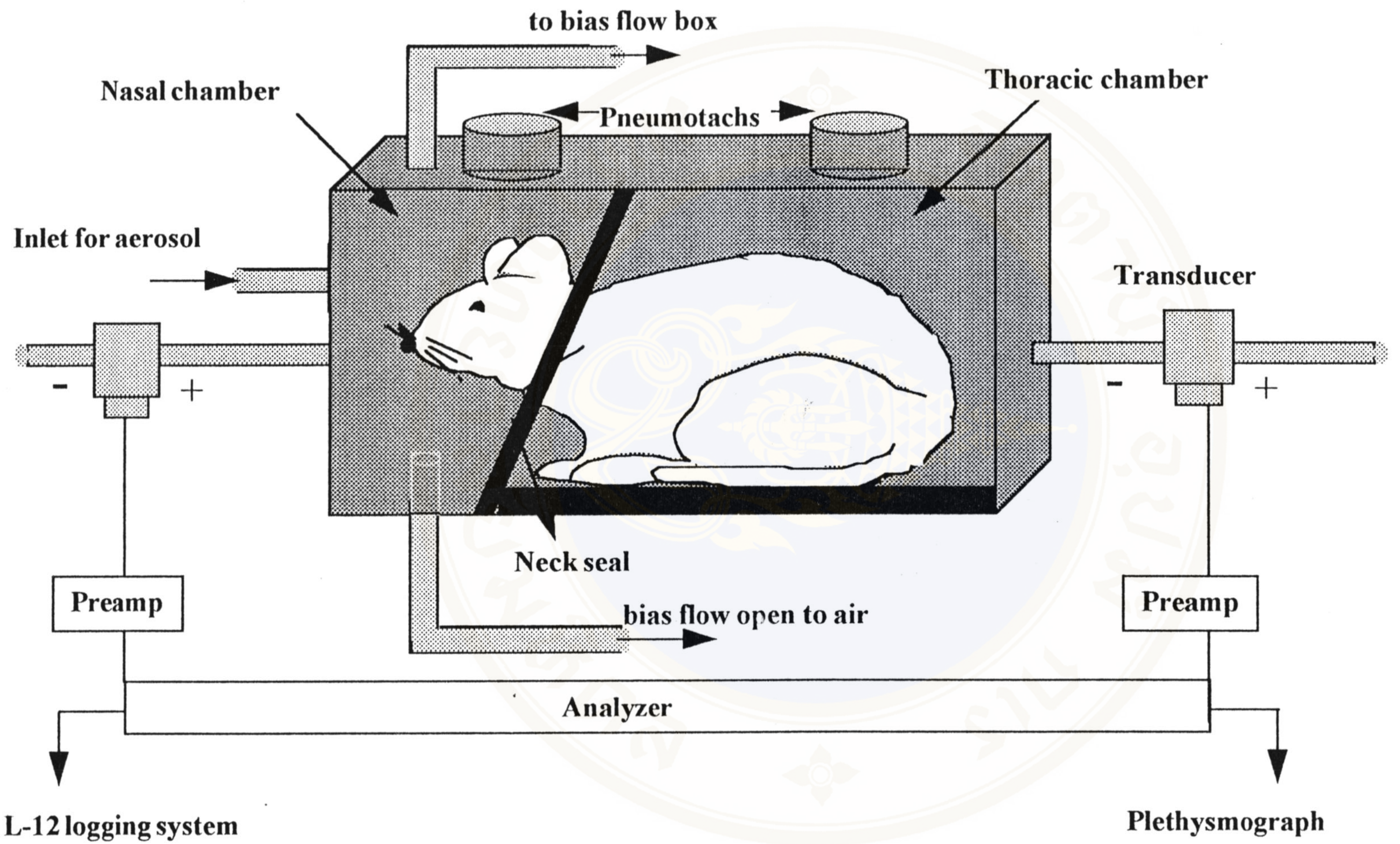

Figure 3 : Connection from body box to analyzer.

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The double chamber was connected to the transducer that further connected to preamplifiers, analyzer, and plethsmograph(or L12-logging system) as shown in figure3.

The double chamber consisted of a head (nasal) chamber and a body (thoracic) chamber. Each chamber has it own pneumotachograph. The respiratory flow go predominantly through this pneumotachograph.

The bias flow box introduced into the nasal chamber to remove CO_2 that builded up excessively with breathing and provide a constance smooth flow out of the nasal chamber. The primary signal out of the preamplifier was flow signal (thoracic flow and nasal flow) that was integrated to generate the volume and the other signal waveforms.

The thoracic flow was integrated into tidal volume waveform. Computations of rate, minute volume, peak expiratory flow, inspiratory time, expiratory time, and relaxation time were performed on this signal.

The flow from the nasal or head chamber was used to compute the specific airway resistance and specific airway conductance.

All values of respiratory functions were monitored and recorded by the Noninvasive Respiratory Analyzer and then the results were printed out by the L-12 logging system.

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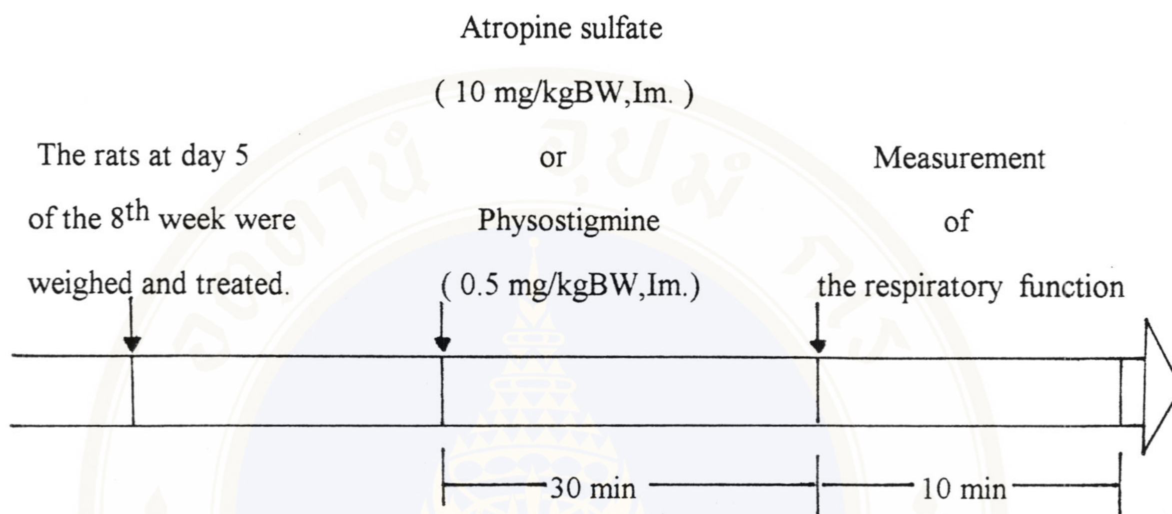
3.3 THE STUDY OF THE EFFECTS OF SUBCHRONIC EXPOSURE TO ACRYLONITRILE ON THE CHOLINERGIC RESPONSE OF THE RESPIRATORY SYSTEM :



In order to study the effects of subchronic exposure of acrylonitrile on the cholinergic response of the respiratory system, three groups of rats (one control group and two treated groups) were injected intramuscularly with atropine sulfate 10 mg/kg.BW or physostigmine 0.5 mg/kgBW (these two chemicals were dissolved in 0.9% normal saline and calculated to the requried concentrations).

At day 5 of the 8th week of acrylonitrile treatment, the measurement of respiratory functions began at 30 minutes after the test animal was challenged with atropine sulfate or physostigmine.

The 30 minutes interval is a suitable period because it was found in preliminary experiment that this period can provide optimal times for detecting the influence of atropine and physostigmine on the respiratory function of rat subchronically exposed to low dose of acrylonitrile. This time schedule also used by Ghanayen et al in 1991 (56) for the study of assessment of the acute acrylonitrile-induced neurotoxicity in rats.

The respiratory function measurement was performed by the method as described before.

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Table 1 : The description of the parameters recorded by "Noninvasive Respiratory Analyzer"

Peak expiratory flow : PEF The maximum flow rate maintained for at least 0.1 second the force expiration. This value updates in every breath. **Tidal volume : TV** The amount of air that moved into the lungs with each inspiration (or the amount that moved out with each expiration). This value updates in every breath. **Respiratory rate : RR** The detection of respiratory frequency per minute **Minute volume : MV** The amount of air inspired per minute. This is the product of the respiratory rate and the tidal volume peak. **Inspiratory time : TI** The period computed by measuring the flow signal from the start of one inspiration to the start of the next expiration. **Expiratory time : TE** The period computed by measuring the flow signal from the start of one expiration to the start of the next inspiration. **Relaxation time : RT** The time for the volume to return from its peak level to approximately one third of the peak level. **Specific airway resistance : RES** Resistance to airflow through an airway divided by the volume of thoracic gas flow. **Specific airway conductance : COND** The reciprocal of the RES. Its level is two time the reciprocal of the level of the resistance.

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ANALYSIS OF DATA

Student's t-test was used to compare the significant difference between means of the control and treated groups.

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CHAPTER IV

RESULTS

Part 1. The effect of subchronic exposure to acrylonitrile on the body weight and respiratory functions.

1. The effect of acrylonitrile on the body weight :

The results showed that subcutaneous administration of acrylonitrile 1 mg/kg, 5 days per week for 8 weeks did not significantly alter the body weight of the treated-rats. Whereas in the acrylonitrile (25 mg/kg)-treated group, the significantly lower body weight than control group was detected at the 4th, 6th and 8th week. One week after the termination of acrylonitrile treatment (at the 9th week), in the acrylonitrile (25 mg/kg)-treated group, the significantly lower body weight than control group have also been detected as showed in table 2 and figure 4.

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Table 2: The effect of subchronic exposure to acrylonitrile on **the body weight.**

Duration of treatment	Body weight(gm.)					
	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
week 1	9	202.72 ± 4.02	8	210.86 ± 3.34	7	200.5 ± 3.50
week 2	8	242.75 ± 5.26	9	250.78 ± 2.78	10	244.35 ± 6.22
week 4	9	333.72 ± 8.81	9	325.06 ± 6.82	9	283.94 ± 5.42 [aaa,bbb]
week 6	9	380.72 ± 8.05	9	390.78 ± 8.22	9	360.67 ± 7.76 [b]
week 8	24	395.63 ± 5.20	24	401.05 ± 7.16	23	375.61 ± 6.16 [a,b]
week 9	8	429.63 ±12.11	8	448.38 ± 8.44	8	415.56 ± 8.66 [b]

Rats were administered subcutaneously with saline solution in control group and acrylonitrile 1 and 25 mg/kg.BW in the treated-groups, 5 days per week, for 8 weeks.

The body weight was recorded at day 5 of each week.

The rats at the 8th week were divided into three groups : the first group used for measuring respiratory function at day 5 of the 8 th of acrylonitrile treatment without any challenges, the second and the third group used for measuring respiratory function at day 5 of the 8th week of acrylonitrile treatment after challenged with atropine sulfate 10 mg/kg,im. and physostigmine 0.5 mg/kg,im., respectively at that week.

The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.

a,aaa represent significant differences from control on the same period of testing at $p < 0.05$, 0.001, respectively.

b,bbb represent significant differences from acrylonitrile 1 mg/kg on the same period of testing at $p < 0.05$, 0.001, respectively.

Each value represents the mean ± S.E.

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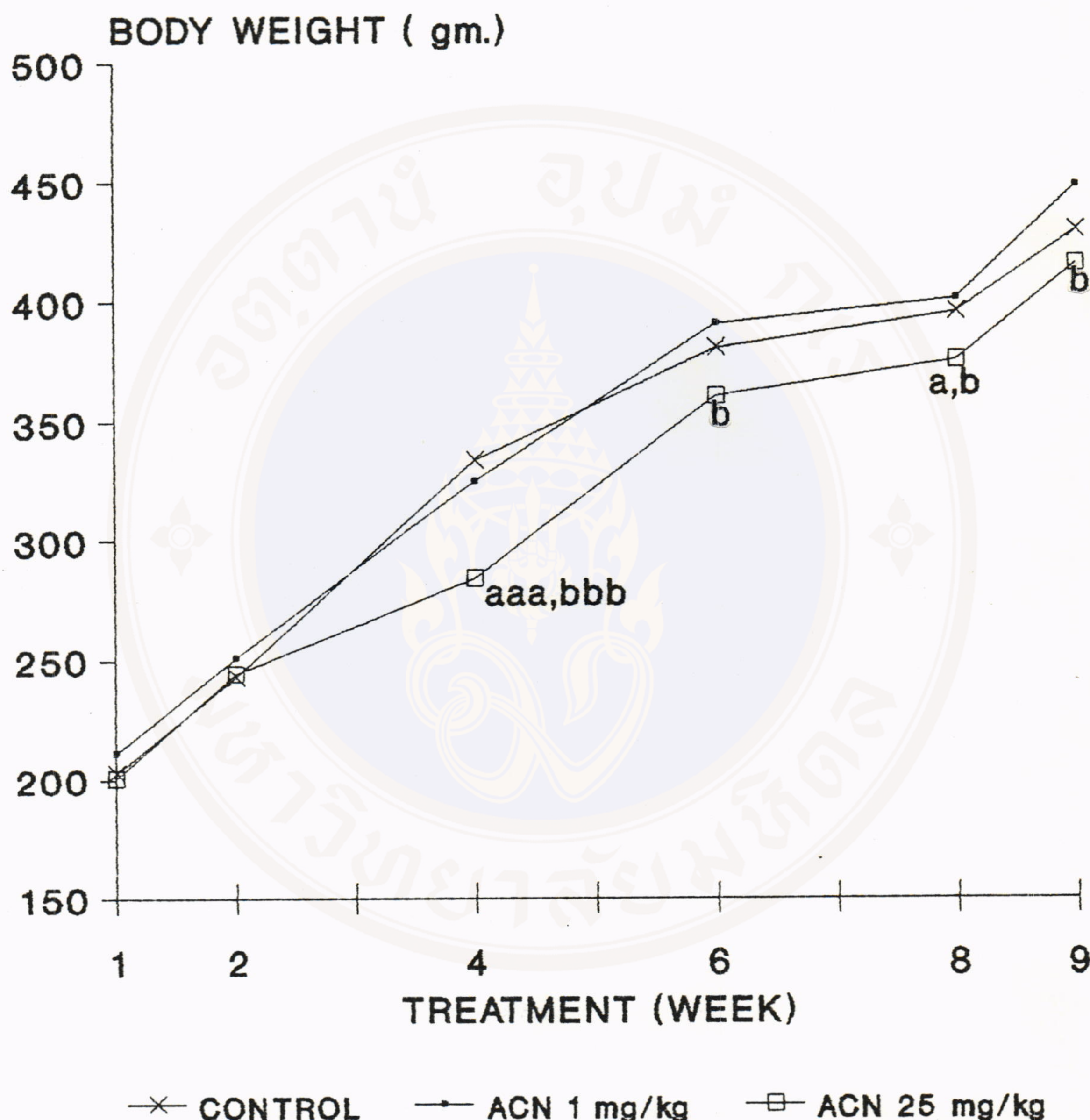

Figure 4: The effect of subchronic exposure to acrylonitrile on **the actual body weight.**
The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.
a,aaa represent significant differences from control on the same period of testing at $p < 0.05, 0.001$, respectively.
b,bbb represent significant differences from acrylonitrile 1 mg/kg on the same period of testing at $p < 0.05, 0.001$, respectively.
Each point represents the mean of actual body weight.

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2. The effects of subchronic exposure of acrylonitrile on respiratory functions :

This study was carried out in intact animals. The quantitative effects of sub-chronic exposure of acrylonitrile on respiratory functions were measured. The ventilatory response to acrylonitrile was studied without the additional influence of surgical alteration of the respiratory passage. The respiratory parameters ; peak expiratory flow (PEF), respiratory rate (RR), minute volume (MV), tidal volume (MV), inspiratory time (TI), expiratory time (TE), relaxation time (RT), specific airway resistance (RES), specific airway conductance (cond) were measured by using Noninvasive Respiratory Analyzer. The results were showed table 3 to 11 and figure 5 to 13.

2.1 <u>The effects of subchronic exposure of acrylonitrile on peak expiratory flow(PEF)</u>.

The acrylonitrile(1mg/kg)-treated group showed no significant differences from control group of the same period of treatment throughout the entire experiment. Whereas in acrylonitrile (25mg/kg)-treated group, the peak expiratory flow was lower than control and acrylonitrile (1mg/kg)- treated group as showed in table 3 and figure 5. The significant decrease in peak expiratory flow was detected at the 4th week of the treatment.

Furthermore, at the 8th week of acrylonitrile treatment, peak expiratory flow of control and ACN- treated group showed significantly higher than that at week 1 of the same dose as showed in table 3 and figure 5.

At the 9th week (one week following the termination of 8 weeks of acrylonitrile treatment), the peak expiratory flow of the rats in acrylonitrile (25 mg/kg)-treated group were increased to nearly those of control and acrylonitrile (1 mg/kg)-treated rats, and these values at the 9th week were significant differences from the 1st week of the treatment.as showed in table 3 and figure 5.

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Table 3: The effect of subchronic exposure to acrylonitrile(ACN) on **peak expiratory flow (PEF).**

Duration of treatment	Peak expiratory flow (ml/sec)					
	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
week 1	9	3.01 $\pm$ 0.07	8	3.04 $\pm$ 0.13	7	3.00 $\pm$ 0.05
week 2	8	3.34 $\pm$ 0.14	9	3.32 $\pm$ 0.12	10	3.22 $\pm$ 0.14
week 4	9	4.11 $\pm$ 0.24^{cc}	9	3.63 $\pm$ 0.24^{c}	9	3.37 $\pm$ 0.15^{a,c}
week 6	9	4.29 $\pm$ 0.24^{c}	9	4.52 $\pm$ 0.31^{cc}	9	3.99 $\pm$ 0.25^{cc}
week 8	8	3.99 $\pm$ 0.20^{cc}	8	4.06 $\pm$ 0.21^{cc}	8	3.60 $\pm$ 0.23^{c}
week 9	8	4.48 $\pm$ 0.43^{cc}	8	4.61 $\pm$ 0.38^{cc}	8	4.50 $\pm$ 0.33^{cc}

Acrylonitrile 1 and 25 mg/kg were administered subcutaneously once daily for 5 days per week. Control groups were received saline solution in comparable amount instead of acrylonitrile. The duration of experiment is 10 minutes.

The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.

a represents a significant difference from control on the same period of testing at $p< 0.05$

c,cc represent significant differences from week 1 of the same dose at $p<0.05$, 0.01, respectiveley.

Each value represents as mean $\pm$ S.E.

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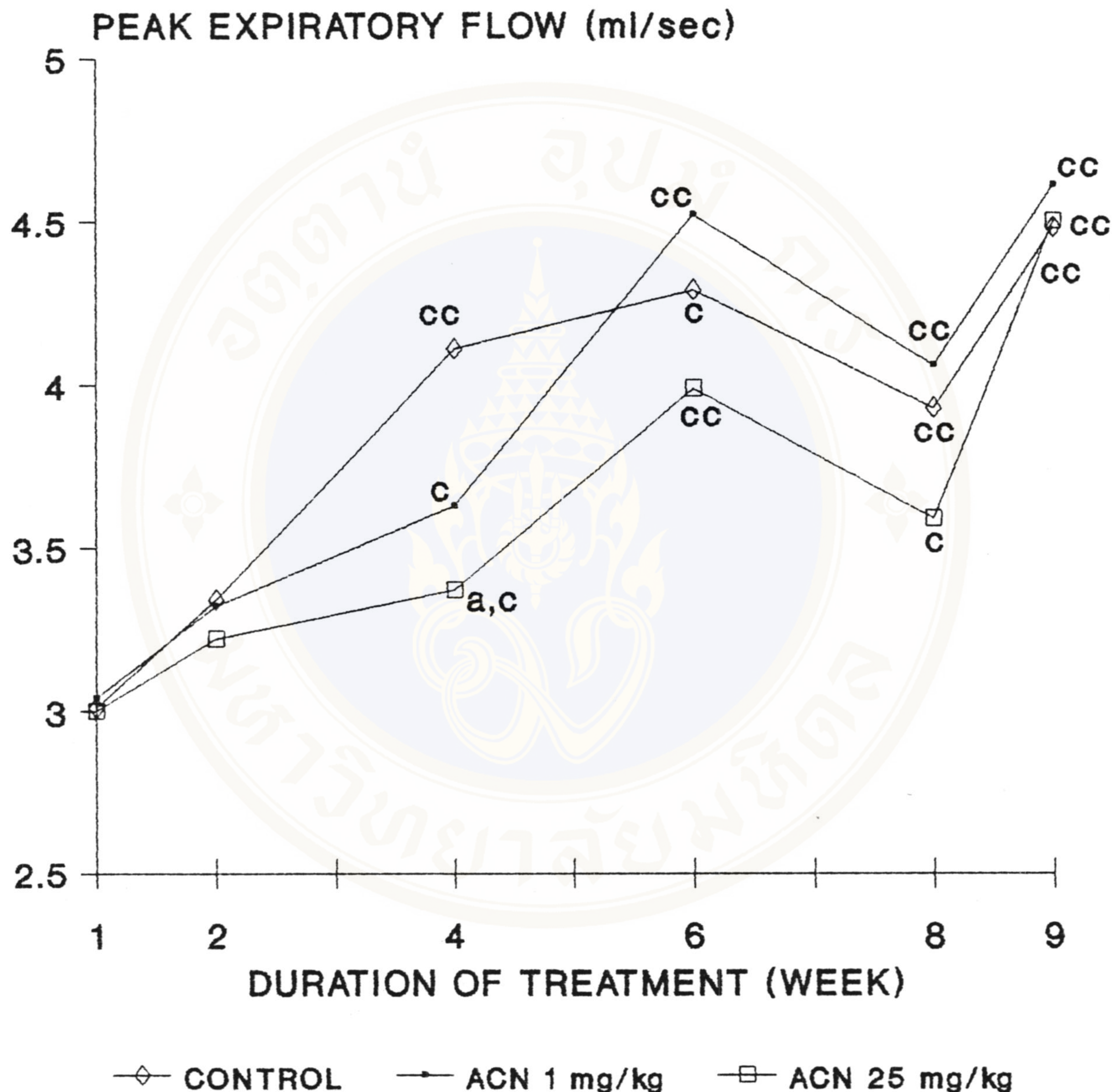

Figure 5: The effect of subchronic exposure to acrylonitrile (ACN) on **peak expiratory flow (PEF)**. The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.
a represents a significant difference from control on the same period of testing at $p < 0.05$
c,cc represent significant differences from week 1 of the same dose at $p < 0.05, 0.01$, respectively.
Each point represents the mean of PEF.

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2.2 The effects of subchronic exposure of acrylonitrile on tidal volume (TV), respiratory rate (RR), and minute volume (MV).

At the 9th week of the treatment the tidal volume of acrylonitrile (25 mg/kg)-treated group showed significantly lower when compared with control and acrylonitrile (1 mg/kg)-treated group. In addition, the tidal volume of control group and acrylonitrile-treated groups at the 9th week and tidal volume of control and acrylonitrile(25mg/kg)-treated group at the 8th also showed significant differences from the 1st week as showed in table 4 and figure 6.

In acrylonitrile (25 mg/kg)-treated group, the respiratory rate and minute volume were significantly lower than those in control and acrylonitrile (1 mg/kg)-treated group at the 1st, 4th,$6^{th}$and 8th week of the treatment (for respiratory rate) and at the 1st, 2nd, 4th,6th and the 8th week of the treatment (for minute volume). It was noticed that after the termination of acrylonitrile treatment for one week(at the 9^{th}week), both respiratory rate and minute volume showed no significant differences from control group and acrylonitrile (1 mg/kg)-treated group as showed in table 5,6 and figure 7,8.

At the 8th week of acrylonitrile treatment minute volume of control and acrylonitrile (25 mg/kg)-treated group showed significant differences when compared with the first week of the same dose showed in table 6 and figure 8.

Furthermore, the respiratory rate of acrylonitrile (25 mg/kg)-treated group and minute volume of all three groups (one control and two acrylonitrile-treated groups) after the termination of acrylonitrile for one week showed significant differences when compared with the 1st week at the same dose showed in table 5,6 and figure 7,8.

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Table 4: The effect of subchronic exposure to acrylonitrile(ACN) on **tidal volume (TV)**.

Duration of treatment	Tidal volume (ml)					
	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
week 1	9	0.75 ± 0.02	8	0.78 ± 0.05	7	0.68 ± 0.02^{a}
week 2	8	0.89 ± 0.04^{c}	9	0.88 ± 0.04	10	0.83 ± 0.05^{c}
week 4	9	0.99 ± 0.10^{c}	9	0.95 ± 0.05^{c}	9	0.97 ± 0.07^{cc}
week 6	9	1.13 ± 0.10^{cc}	9	1.20 ± 0.04^{ccc}	9	1.12 ± 0.08^{ccc}
week 8	8	1.06 ± 0.07^{cc}	8	0.94 ± 0.06	8	$0.87 \pm 0.03^{a,ccc}$
week 9	8	1.19 ± 0.04^{cc}	8	1.04 ± 0.07^{cc}	8	$0.96 \pm 0.06^{a,cc}$

Acrylonitrile 1 and 25 mg/kg were administered subcutaneously once daily for 5 days per week. Control groups were received saline solution in comparable amount instead of acrylonitrile. The duration of experiment is 10 minutes.

The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.

a represents a significant difference from control on the same period of testing at $p < 0.05$

c,cc,ccc represent significant differences from week 1 of the same dose at $p<0.05$, 0.01,0.001, respectively.

Each value represents as mean $\pm$ S.E.

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Table 5: The effect of subchronic exposure to acrylonitrile(ACN) on **respiratory rate (RR).**

Duration of treatment	Respiratory rate (breaths/min)					
	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
week 1	9	85.96 ± 3.22	8	83.78 ± 2.14	7	60.32 ± 5.02 aa,bb
week 2	8	80.54 ± 3.20	9	81.86 ± 1.95	10	73.27 ± 4.21
week 4	9	94.18 ± 6.55	9	78.54 ± 4.35	9	70.46 ± 3.48 aa
week 6	9	84.79 ± 6.05	9	82.33 ± 4.23	9	66.42 ± 4.64 a,b
week 8	8	82.61 ± 2.83	8	83.94 ± 3.15	8	71.81 ± 2.98 a
week 9	8	83.38 ± 3.71	8	92.89 ± 3.74	8	91.99± 6.06 cc

Acrylonitrile 1 and 25 mg/kg were administered subcutaneously once daily for 5 days per week. Control groups were received saline solution in comparable amount instead of acrylonitrile. The duration of experiment is 10 minutes.

The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment

a,aa represent significant differences from control on the same period of testing at $p< 0.05$, 0.01, respectively.

b,bb represent significant differences from acrylonitrile 1 mg/kg on the same period of testing at $p< 0.05$, 0.01, respectively.

cc represent a significant difference from week 1 of the same dose at $p< 0.01$

Each value represents as the mean ± S.E.

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Table 6 : The effect of subchronic exposure to acrylonitrile (ACN) on **minute volume(MV).**

Duration of treatment	Minute volume (ml/min)					
	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
week 1	9	69.74 ± 2.25	8	70.15 ± 4.37	7	43.97 ± 3.70 aaa,bbb
week 2	8	77.33 ± 4.08	9	77.87 ± 2.48	10	5.01 ± 4.48 a,b,cc
week 4	9	93.24 ± 5.26 cc	9	79.29 ± 5.04	9	72.86 ± 4.99 a,ccc
week 6	9	101.26 ± 9.98 c	9	106.28 ± 7.01 ccc	9	78.44 ± 6.10 bb,ccc
week 8	8	93.85 ± 5.16 cc	8	85.38 ± 6.78	8	66.44 ± 4.90 aa,b,cc
week 9	8	101.79 ± 7.00 cc	8	101.36± 6.68 cc	8	96.30 ± 8.16 cc

Acrylonitrile 1 and 25 mg/kg were administered subcutaneously once daily for 5 days per week. Control groups were received saline solution in comparable amount instead of acrylonitrile. The duration of experiment is 10 minutes.

The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.

a,aa,aaa represent significant differences from control on the same period of testing at $p < 0.05, 0.01, 0.001$, respectively.

b,bb,bbb represent significant differences from acrylonitrile 1 mg/kg on the same period of testing at $p < 0.05, 0.01, 0.001$, respectively.

c,cc,ccc represent significant differences from week 1 of the same dose at $p<0.05, 0.01, 0.001$, respectively

Each value represents as the mean ± S.E.

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Figure 6 : The effect of subchronic exposure to acrylonitrile on tidal volume (TV)

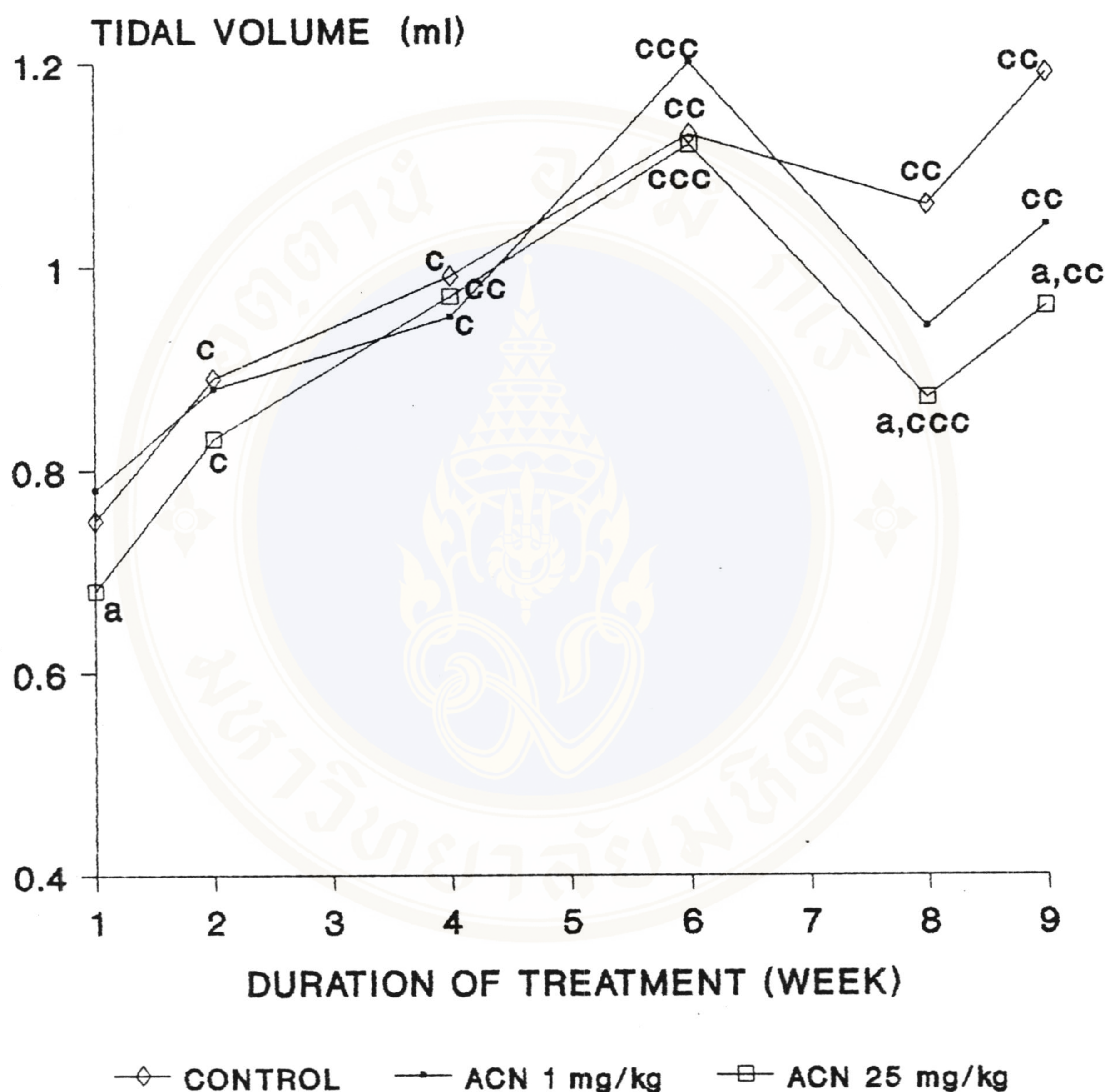

Figure 6: The effect of subchronic exposure to acrylonitrile (ACN) on **tidal volume (TV).**
The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.
a represents a significant difference from control on the same period of testing at $p < 0.05$
c,cc,ccc represent significant differences from week 1 of the same dose at $p < 0.05$, 0.01, 0.001, respectively.
Each point represents the mean of TV.

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Figure 7: The effect of subchronic exposure to acrylonitrile on respiratory rate (RR)

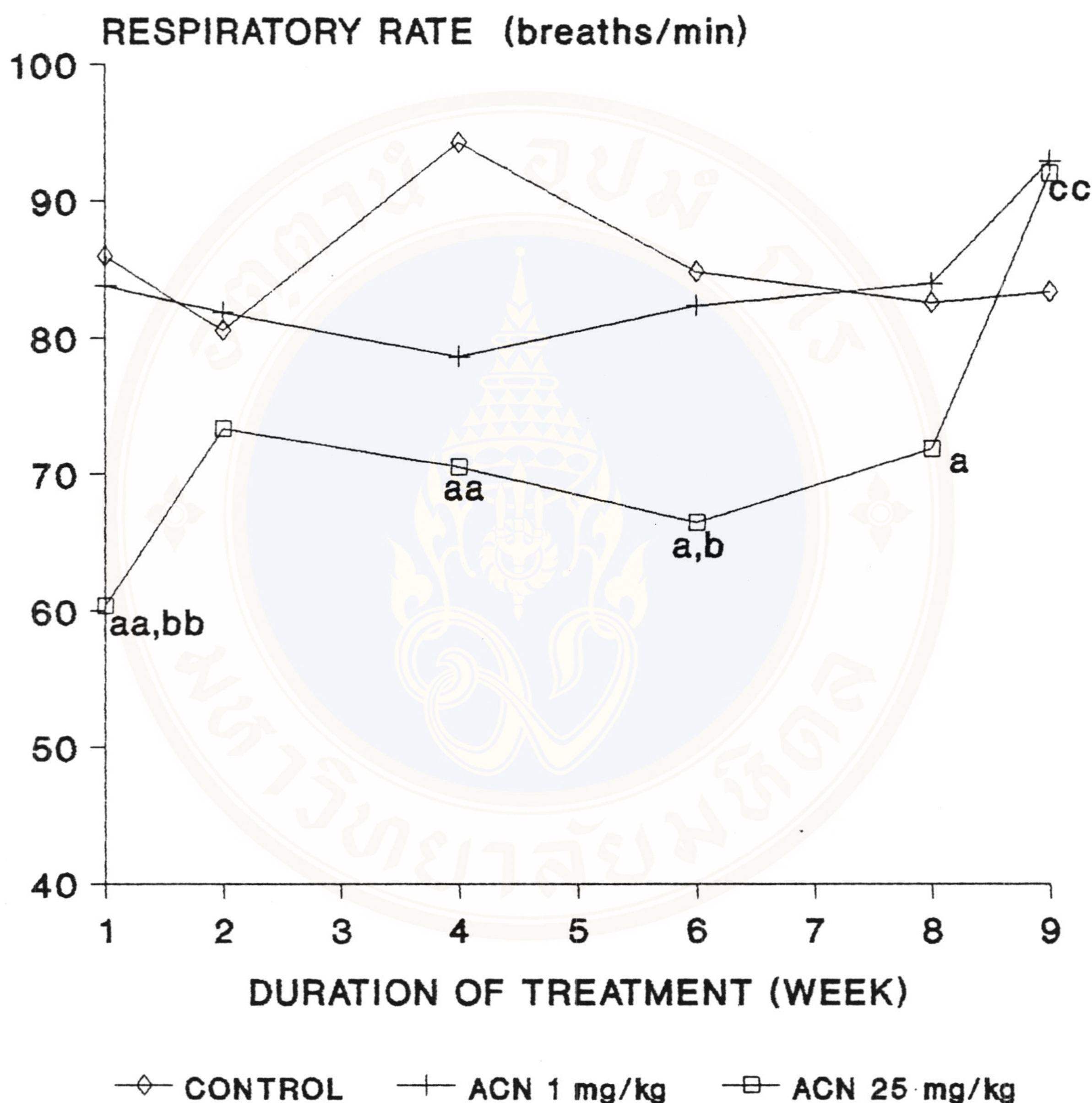

Figure 7: The effect of subchronic exposure to acrylonitrile (ACN) on **respiratory rate (RR)**.
The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.

a,aa represent significant differences from control on the same period of testing at $p< 0.05$, 0.01, respectively.

b,bb represent significant differences from acrylonitrile 1 mg/kg on the same period of testing at $p < 0.05$, 0.01, respectively.

cc represents a significant difference from week 1 of the same dose at $p<0.01$

Each point represents the mean of RR.

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Figure 8 : The effect of subchronic exposure to acrylonitrile on minute volume (MV)

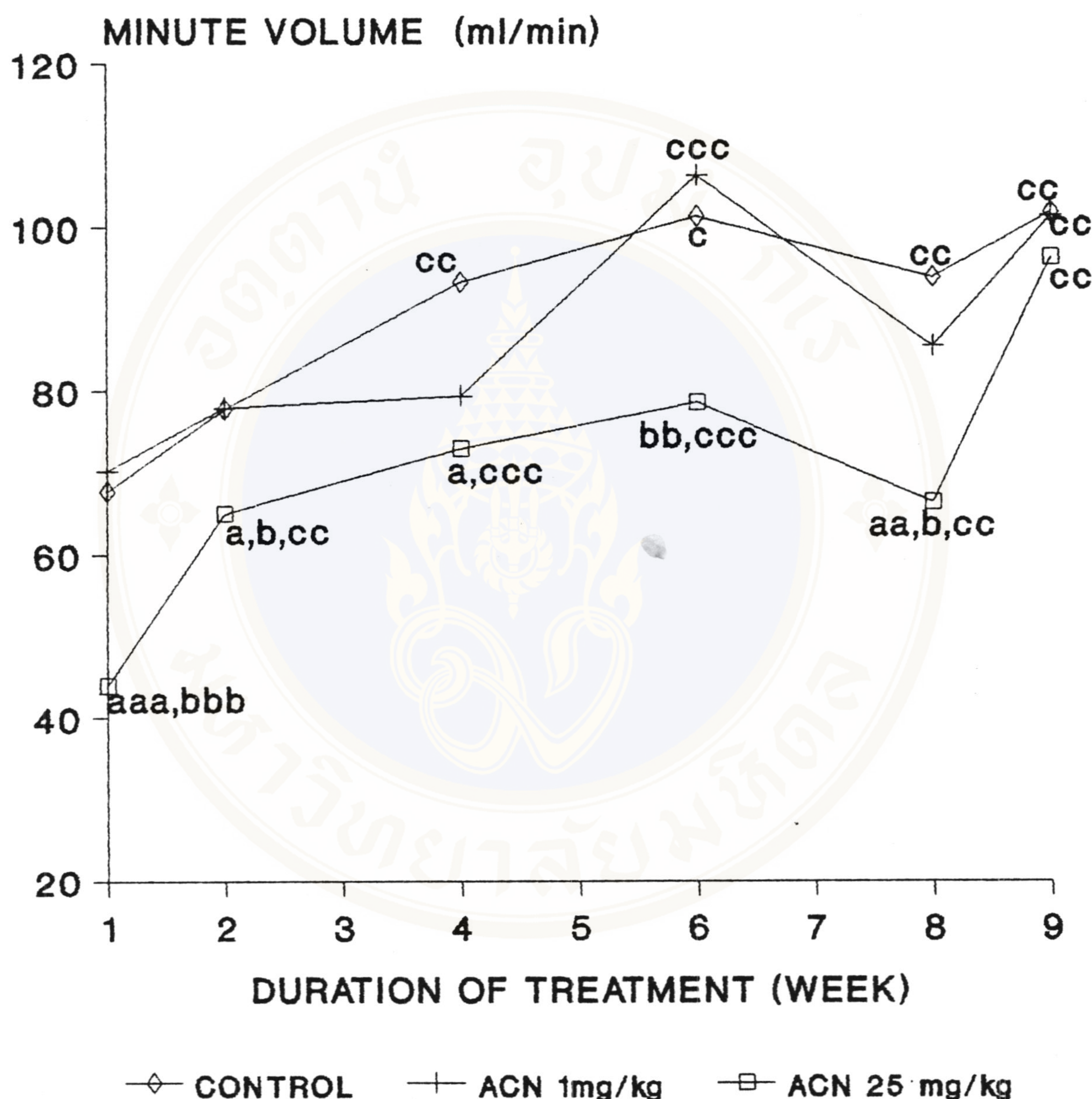

Figure 8: The effect of subchronic exposure to acrylonitrile (ACN) on **minute volume (MV)**.
The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.

a,aa,aaa represent significant differences from control on the same period of testing at $p < 0.05$, 0.01,0.001, respectively.

b,bb,bbb represent significant differences from acrylonitrile 1 mg/kg on the same period of testing at $p < 0.05$, 0.01, 0.001, respectively.

c,cc,ccc represents significant differences from week 1 of the same dose at $p < 0.05$, 0.01, 0.001, respectively.

Each point represents the mean of MV.

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2.3 The effects of subchronic exposure of acrylonitrile on inspitratory time (TI), expiratory time (TE), and relaxation time (RT).

Acrylonitrile (25 mg/kg)-treated group showed significantly higher in inspiratory time than that of control and acrylonitrile (1 mg/kg)-treated group, began at the first week and throughout the acrylonitrile-treated experiment for 8 weeks. It was noticed that, it has the time-related reduction of inspiratory time in acrylonitrile (25 mg/kg)-treated group. As showed in table7 and figure 9, at the 9th week (one week after the termination of acrylonitrile treatment) the inspiratory time of acrylonitrile (25 mg/kg)-treated group was reduced to nearly that of control and acrylonitrile (1 mg/kg)-treated group.

At the 8th week of acrylonitrile treatment, it was found that inspiratory time of ACN-treated groups showed significant differences when compared with the same dose at the first week of acrylonitrile treatment as showed in table 7 and figure 9.

In addition, it was found that at the 9th week, the values of inspiratory time of control and 1, 25 mg/kg acrylonitrile treated groups showed significant differences from the first week of acrylonitrile treatment as showed in table 7 and figure 9.

During the entired period of acrylonitrile treatment, there were no alterations in both expiratory time (except at the 2nd week) and relaxation time, except that at the 2nd week, expiratory time of acrylonitrile (25 mg/kg)-treated group showed significantly lower than that of control group as showed in table 8, 9 and figure 10, 11, and at the 8th week, it was found that expiratory time of acrylonitrile (25 mg/kg)-treated group showed significant difference from that of the first week as showed in table 8 and figure 10.

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Table 7 : The effect of subchronic exposure to acrylonitrile (ACN) on **inspiratory time (TI).**

Duration of treatment	Inspiratory time (sec)					
	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
week 1	9	0.29 ± 0.01	8	0.29 ± 0.01	7	0.75 ± 0.07 [aa,bb]
week 2	8	0.26 ± 0.00 [c]	9	0.31 ± 0.04	10	0.51 ± 0.07 [aa,b,c]
week 4	9	0.23 ± 0.02 [cc]	9	0.31 ± 0.03 [a]	9	0.43 ± 0.05 [aa,cc]
week 6	9	0.26 ± 0.06	9	0.28 ± 0.05	9	0.41 ± 0.05 [a,b,cc]
week 8	8	0.27 ± 0.01	8	0.24 ± 0.01 [a,cc]	8	0.39 ± 0.02 [aaa,bbb,cc]
week 9	8	0.26 ± 0.01 [c]	8	0.23± 0.01 [cc]	8	0.24± 0.01 [ccc]

Acrylonitrile 1 and 25 mg/kg were administered subcutaneously once daily for 5 days per week. Control groups were received saline solution in comparable amount instead of acrylonitrile. The duration of experiment is 10 minutes.

The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.

a,aa,aaa represent significant differences from control on the same period of testing at $p< 0.05, 0.01, 0.001$, respectively.

b,bb,bbb represent significant differences from acrylonitrile 1 mg/kg on the same period of testing at $p< 0.05, 0.01, 0.001$, respectively.

c,cc,ccc represent significant differences from week 1 of the same dose at $p<0.05$, 0.01, 0.001, respectively..

Each value represents as the mean ± S.E.

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Table 8 : The effect of subchronic exposure to acrylonitrile (ACN) on **expiratory time (TE).**

Duration of treatment	Expiratory time (sec)					
	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
week 1	9	0.41 ± 0.02	8	0.41 ± 0.01	7	0.38 ± 0.02
week 2	8	0.46 ± 0.02	9	0.40 ± 0.02	10	0.34 ± 0.04 [a]
week 4	9	0.41 ± 0.02	9	0.45 ± 0.02	9	0.43 ± 0.06
week 6	9	0.45 ± 0.03	9	0.44 ± 0.03	9	0.50 ± 0.03 [cc]
week 8	8	0.44 ± 0.02	8	0.46 ± 0.03	8	0.47 ± 0.03 [c]
week 9	8	0.46 ± 0.03	8	0.40 ± 0.02	8	0.41 ± 0.02

Acrylonitrile 1 and 25 mg/kg were administered subcutaneously once daily for 5 days per week. Control groups were received saline solution in comparable amount instead of acrylonitrile. The duration of experiment is 10 minutes.

The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.

a represents a significant difference from control on the same period of testing at $p < 0.05$

c,cc represent significant differences from week 1 of the same dose at $p<0.05$, 0.01, respectively.

Each value represents as the mean ± S.E.

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Table 9 : The effect of subchronic exposure to acrylonitrile (ACN) on **relaxation time (RT).**

Duration of treatment	Relaxation time(sec)					
	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
week 1	9	0.19 ± 0.01	8	0.19 ± 0.01	7	0.20 ± 0.01
week 2	8	0.22 ± 0.01 c	9	0.21 ± 0.01	10	0.18 ± 0.02
week 4	9	0.20 ± 0.02	9	0.23 ± 0.02	9	0.22 ± 0.03
week 6	9	0.23 ± 0.02	9	0.24 ± 0.01 cc	9	0.27 ± 0.03 c
week 8	8	0.22 ± 0.02	8	0.21 ± 0.01	8	0.22 ± 0.01
week 9	8	0.23 ± 0.02 c	8	0.21 ± 0.01	8	0.19 ± 0.01

Acrylonitrile 1 and 25 mg/kg were administered subcutaneously once daily for 5 days per week. Control groups were received saline solution in comparable amount instead of acrylonitrile. The duration of experiment is 10 minutes.

The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.

c,cc represent significant differences from week 1 of the same dose at $p<0.05$, 0.01, respectively.

Each value represents as the mean ± S.E.

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Figure 9: The effect of subchronic exposure to acrylonitrile on inspiratory time (TI)

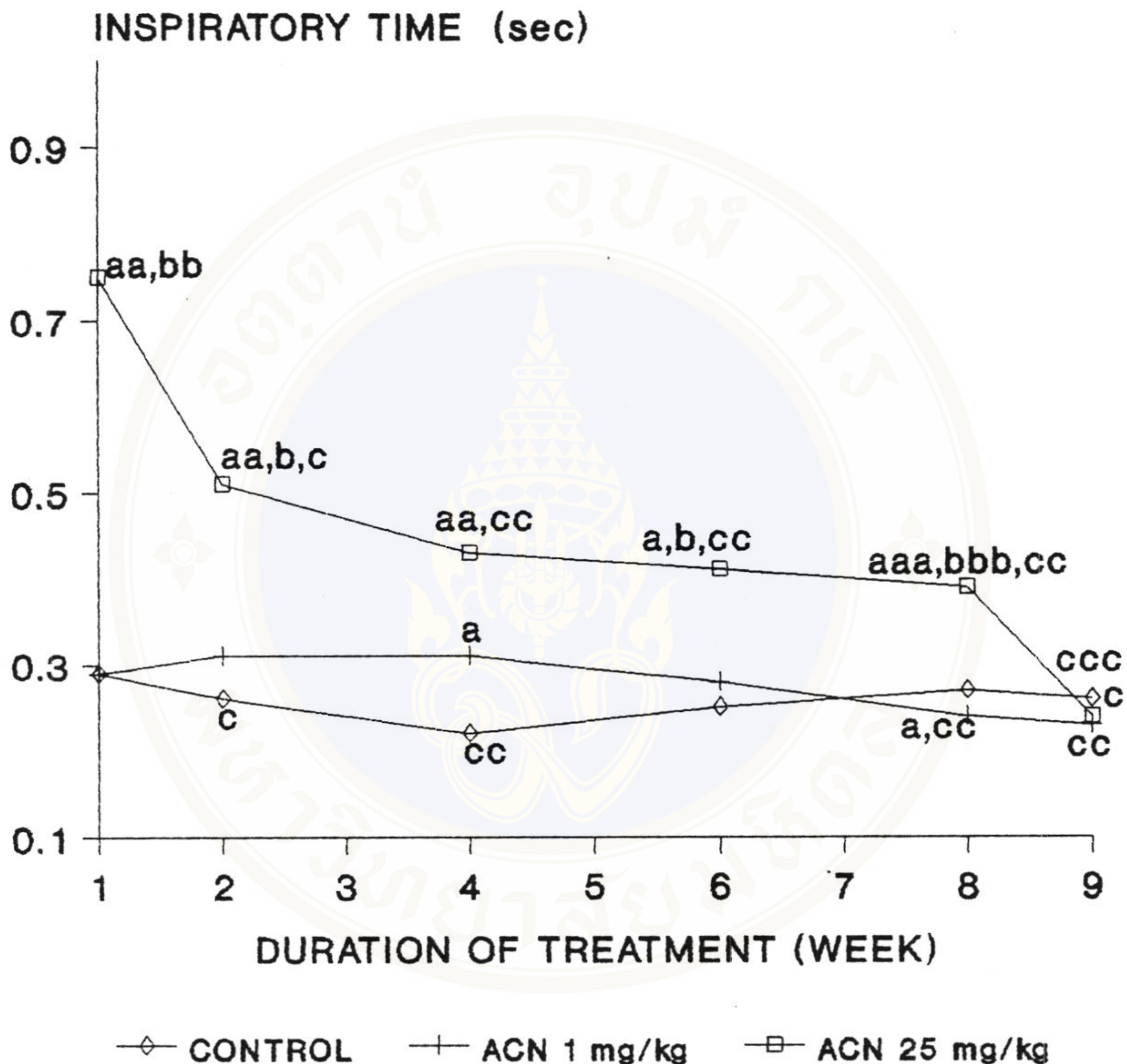

Figure 9: The effect of subchronic exposure to acrylonitrile (ACN) on **inspiratory time (TI)**.
The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.

a,aa,aaa represent significant differences from control on the same period of testing at $p < 0.05$, 0.01,0.001, respectively.

b,bb,bbb represent significant differences from acrylonitrile 1 mg/kg on the same period of testing at $p < 0.05$, 0.01, 0.001, respectively.

c,cc,ccc represent significant differences from week 1 of the same dose at $p < 0.05$, 0.01, 0.001, respectively.

Each point represents the mean of TI.

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Figure 10 : The effect of subchronic exposure to acrylonitrile on expiratory time (TE)

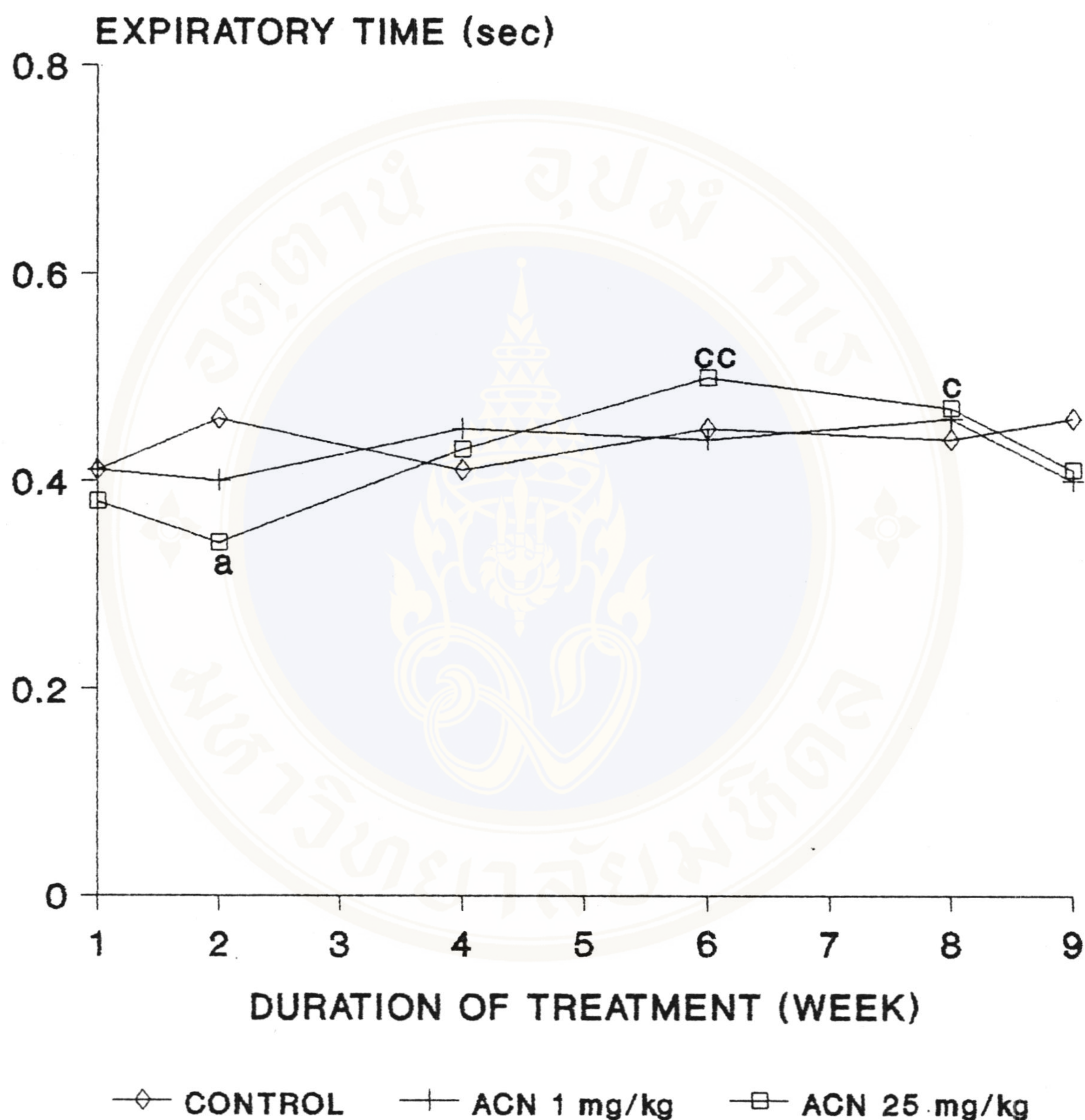

Figure 10: The effect of subchronic exposure to acrylonitrile (ACN) on **expiratory time (TE)**.
The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.
a represents a significant difference from control on the same period of testing at $p < 0.05$.
c,cc represent significant differences from week 1 of the same dose at $p < 0.05$, 0.01, respectively.
Each point represents the mean of TE.

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Figure 11 : The effect of subchronic exposure to acrylonitrile on relaxation time (RT)

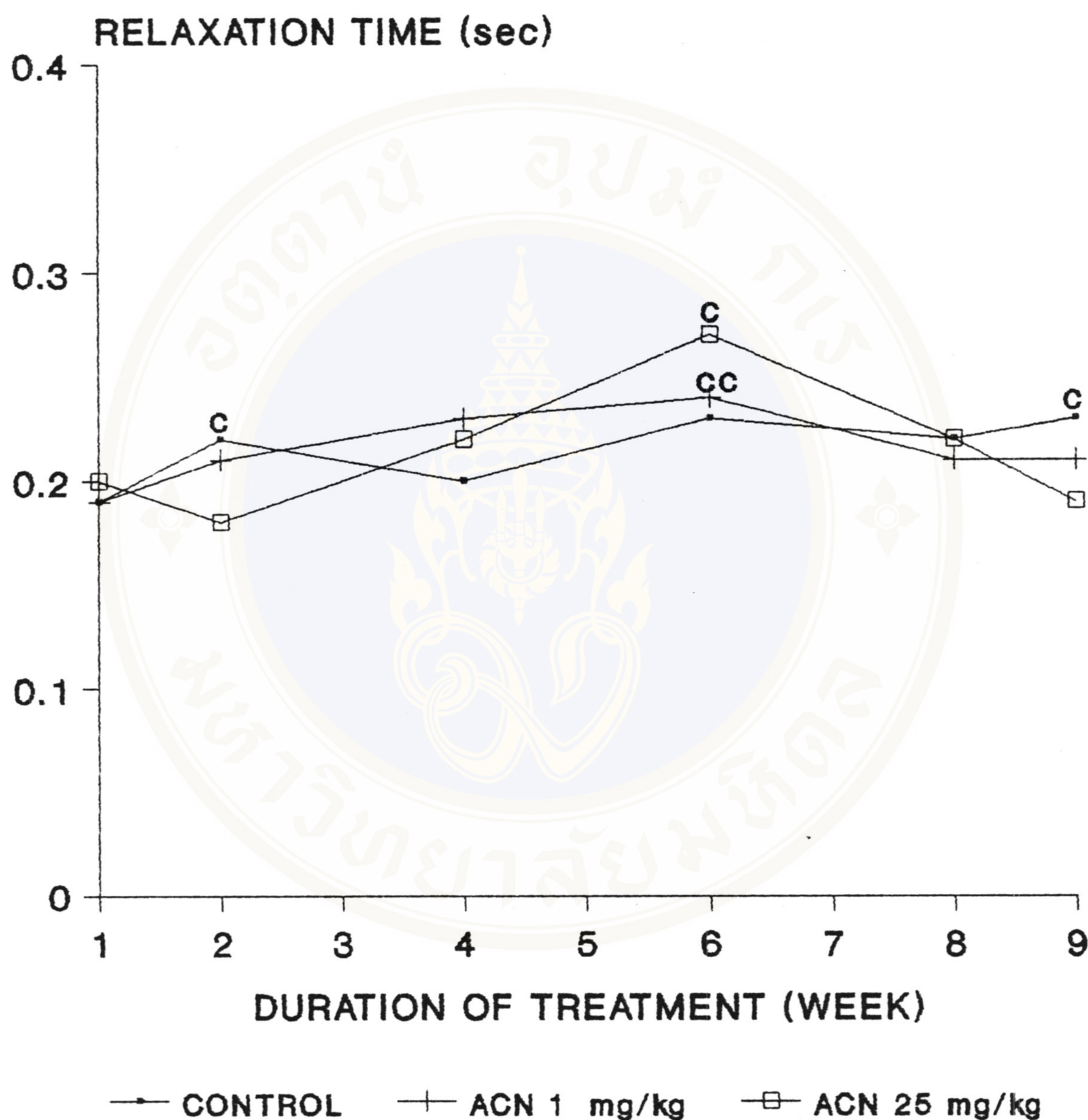

Figure 11: The effect of subchronic exposure to acrylonitrile (ACN) on **relaxation time (RT)**.
The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.
c,cc represent significant differences from week 1 of the same dose at $p < 0.05$, 0.01, respectively.
Each point represents the mean of RT.

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2.4 The effects of subchronic exposure of acrylonitrile on specific airway resistance (RES) and specific airway conductance (COND).

There was no significant difference between control group and acrylonitrile (1 mg/kg)-treated group in both specific airway resistance and specific airway conductance throughout the entired experiment as showed in table 10, 11 and figure 12,13.

At the 1st and 2nd week, acrylonitrile (25 mg/kg)-treated group showed the significant increased in specific airway resistance and showed the significant decreased in specific airway conductance when compared whih control and acrylonitrile (1 mg/kg)-treated group. It should be noticed that, this specific airway resistance and specific airway conductance of acrylonitrile (25 mg/kg)-treated group returned to nearly the values of control group during the 4th, 6th, 8th week especially at the 9th week of the experiment as showed table 10, 11 and figure 12,13.

In table 10 and figure12 it was found that control and acrylonitrile (25 mg/kg)-treated group at the 8th week and acrylonitrile(25mg/kg)-treated group at the 9th week showed significant differences from that of the 1st week of the treatment. And in table 11 and figure13 at the 8th and 9th week of the treatment, control group showed significant difference in specific airway conductance from that of the first week, in other hand, 1 and 25 mg/kg acrylonitrile treated groups did not showed significant differences.

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Table10 : The effect of subchronic exposure to acrylonitrile (ACN) on **specific airway resistance (RES).**

Duration of treatment	Specific airway resistance(cm.H_2O/sec)					
	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
week 1	9	3.55 ± 0.38	8	2.71 ± 0.58	7	8.06 ± 1.03 ^{aa,bb}
week 2	8	3.13 ± 0.56	9	3.94 ± 0.78	10	11.12 ± 1.81 ^{aa,bb}
week 4	9	3.33 ± 0.63	9	4.53 ± 0.54 ^{c}	9	5.77 ± 1.15
week 6	9	3.71 ± 0.37	9	4.29 ± 0.50 ^{c}	9	7.10 ± 1.85
week 8	8	5.51 ± 0.61 ^{c}	8	4.09 ± 0.75	8	5.85 ± 1.46
week 9	8	4.78 ± 0.47 ^{c}	8	4.49 ± 0.80	8	4.90 ± 0.97 ^{c}

Acrylonitrile 1 and 25 mg/kg were administered subcutaneously once daily for 5 days per week. Control groups were received saline solution in comparable amount instead of acrylonitrile. The duration of experiment is 10 minutes.

The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.

aa represents a significant difference from control on the same period of testing at $p < 0.01$

bb represents a significant difference from acrylonitrile 1 mg/kg on the same period of testing at $p < 0.01$

c represents a significant difference from week 1 of the same dose at $p < 0.05$

Each value represents as the mean ± S.E.

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Table 11 : The effect of subchronic exposure to acrylonitrile (ACN) on **specific airway conductance (COND).**

Duration of treatment	Specific airway conductance(sec/cm.H_2O)					
	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
week 1	9	0.321 ± 0.030	8	0.379 ± 0.039	7	0.191 ± 0.030 ^{aa,bb}
week 2	8	0.344 ± 0.044	9	0.320 ± 0.046	10	0.146 ± 0.024 ^{aa,bb}
week 4	9	0.340 ± 0.014	9	0.264 ± 0.036 ^{c}	9	0.264 ± 0.044
week 6	9	0.296 ± 0.032	9	0.274 ± 0.029 ^{c}	9	0.248 ± 0.045
week 8	8	0.206 ± 0.025 ^{cc}	8	0.303 ± 0.039	8	0.309 ± 0.042 ^{c}
week 9	8	0.237 ± 0.020 ^{c}	8	0.280 ± 0.046	8	0.283 ± 0.051

Acrylonitrile 1 and 25 mg/kg were administered subcutaneously once daily for 5 days per week. Control groups were received saline solution in comparable amount instead of acrylonitrile. The duration of experiment is 10 minutes.

The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.

aa represents a significant difference from control on the same period of testing at $p < 0.01$

bb represents a significant difference from acrylonitrile 1 mg/kg on the same period of testing at $p < 0.01$

c,cc represent significant differences from week 1 of the same dose at $p<0.05$, 0.01 respectively.

Each value represents as the mean ± S.E.

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Figure 12 :The effect of subchronic exposure to acrylonitrile on specific airway resistance(RES)

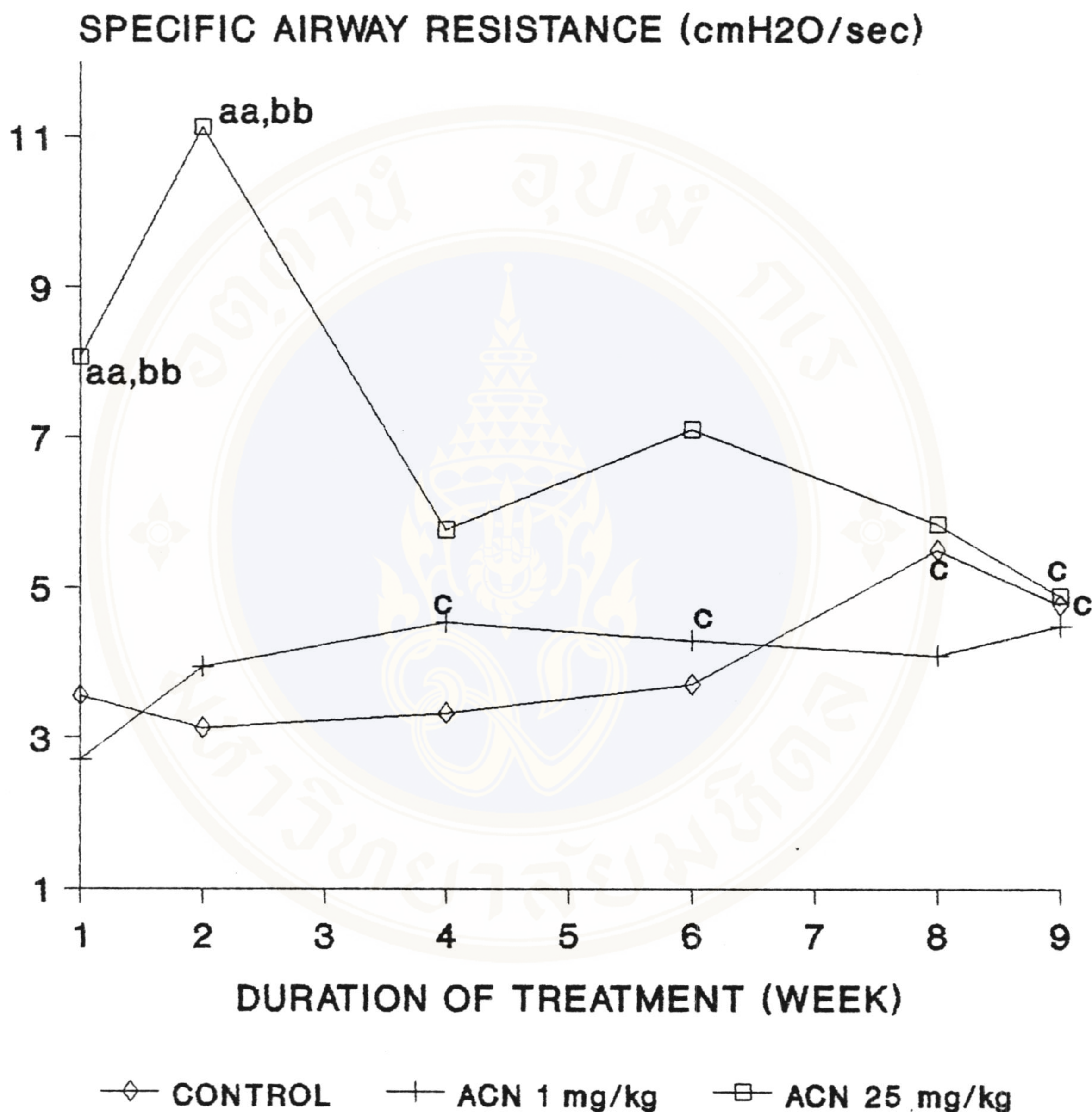

Figure 12: The effect of subchronic exposure to acrylonitrile (ACN) on **specific airway resistance (RES).** The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.
aa represents a significant difference from control on the same period of testing at $p < 0.01$
bb represents a significant difference from acrylonitrile 1 mg/kg on the same period of testing at $p < 0.01$
c represents a significant difference from week 1 of the same dose at $p < 0.05$
Each point represents the mean of RES.

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Figure13: The effect of subchronic exposure to acrylonitrile on specific airway conductance (COND)

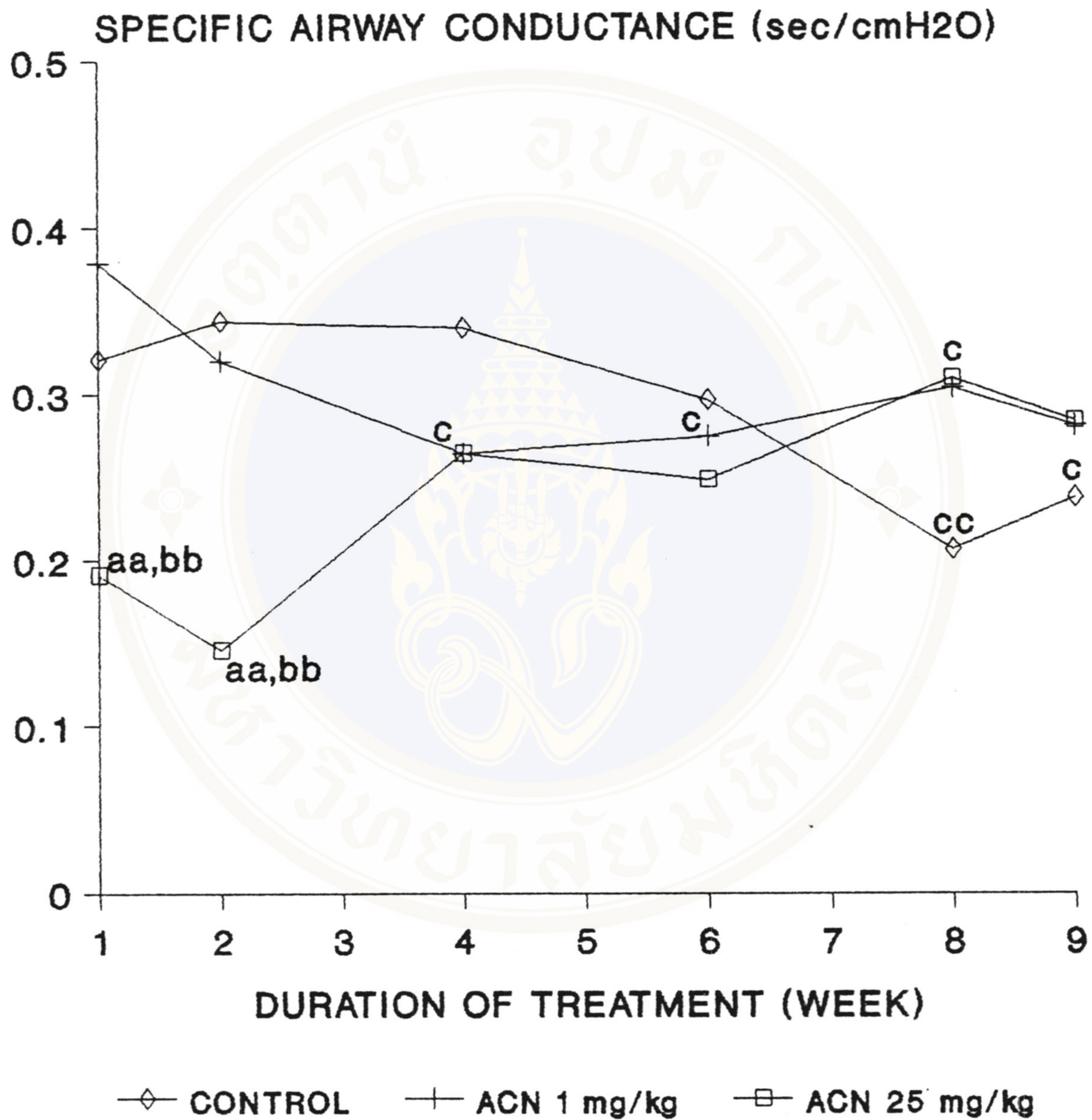

Figure 13: The effect of subchronic exposure to acrylonitrile (ACN) on **specific airway conductance (COND).**
The 9th week is the measurement one week following the termination of 8 weeks of acrylonitrile treatment.
aa represents a significant difference from control on the same period of testing at $p < 0.01$
bb represents a significant difference from acrylonitrile 1 mg/kg on the same period of testing at $p < 0.01$
c,cc represent significant differences from week 1 of the same dose at $p < 0.05, 0.01$, respectively.
Each point represents the mean of COND.

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Part 2. The effect of subchronic exposure to acrylonitrile on the cholinergic response of the respiratory system.

In order to study the effect of subchronic exposure to acrylonitrile on the cholinergic response of the respiratory system, the rats were injected with atropine sulfate (muscarinic recepter antagonist) 10 mg/kgBW., or physostigmine (a reversible acetylcholinesterase inhibitor) 0.5 mg/kg BW.,at day 5 of the 8th week of acrylonitrile treatment. 30 minutes after the test animals were challenged, the respiratory functions were measured.

The other signs of acrylonitrile on cholinergic functions for examples, hypersalivation, lacrimation and diarrhea were observed in all acrylonitrile-treated animals.

The rats treated with acrylonitrile 25 mg/kg, subcutaneously 5 days per week for 8 weeks hypersalivation and lacrimation were observe for half an hour after 10 minutes of acrylonitrile treatment. These observations were found at the second week and throughout the 8th week of acrylonitrile treatment. Diarrhea was also observed in some rats in this group. However, these effects were not found at week 9 of the treatment (one week after termination of the acrylonitrile treatment).

2.1 Atropine sulfate

The rats challenged with atropine sulfate 10 mg/kg,im. at day 5 of the 8th week of acrylonitrile treatment showed no significant differences in peak expiratory flow, minute volume, inspiratory time, expiratory time, relaxation time, specific airway resistance, and specific airway conductance when compared between control group and acrylonitrile (1mg/kg)-treated group as showed in table 12 and figure 14, 17 to 22.

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It was found that the respiratory rate of acrylonitrile (1 mg/kg)-treated group which challenged with atropine was significantly higher than control group. Thus, despite the significantly lower tidal volume observed in atropine challenged group, the minute volume did not altered significantly as showed in table 12 and figure15,16,17. These results suggest that minute volume is governed by two factors, respiratory rate and tidal volume.

There were no significant differences in respiratory functions after challenged with atropine sulfate, compared between control group and acrylonitrile (25 mg/kg)-treated group in the same period of testing as showed in table 12 and figure 14 to 22. Furthermore, there were no significant differences in respiratory functions of control and ACN-treated rats after challenged with atropine sulfate when compared with those of control group at the 8th and 9th week of the treatment as showed in table 12 and figure 14 to 22.

2.2 Physostigmine

The effects of physostigmine, a reversible acetylcholinesterase inhibitor which can enter the central nervous system, provided the following results :

After administered intramuscularly with physostigmine 0.5 mg/kg at day 5 of the 8th week of acrylonitrile treatment, it was found that in control group and acrylonitrile (1 mg/kg)-treated group showed significantly increased in almost all of respiratory functions (peak expiratory flow, tidal volume, minute volume, relaxation time and specific airway resistance) as showed in table 12 and figure 14;15,17,20,21. Acrylonitrile (25mg/kg)-treated group also exhibited the similar pattern of influences on respiratory functions but mostly to a lesser extent than acrylonitrile (1mg/kg)-treated group.

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In addition, control group and acrylonitrile (1 mg/kg)-treated group were also showed the significant reduction in specific airway conductance as showed in table12and figure 22, when compared with acrylonitrile(25 mg/kg)-treated group.

Respiratory rate of control and ACN-treated rats were decreased after challenged with physostigmine as showed in table12 and figure 16.

From the results,it can be noticed that in control and acrylonitrile (1 mg/kg)-treated group showed significantly alterations in respiratory function after challenged with physostigmine. Whereas in acrylonitrile(25 mg/kg)-treated group,after challenged with physostigmine, almost all of the respiratory functions showed no significant differences from control group at the 8th and 9th week of the treatment.

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Table 12 : Respiratory functions during spontaneous breathing in atropine and physostigmine treatments.

Respiratory parameters	Treatment	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
Peak expiratory flow (ml/sec)	week 1	9	3.01 ± 0.07	8	3.04 ± 0.13	7	3.00 ± 0.05
	week 8	8	3.99 ± 0.20 [cc]	8	4.06 ± 0.21 [cc]	8	3.60 ± 0.23 [c]
	week 8 + atropine	8	4.39 ± 0.58	8	3.83 ± 0.19 [cc]	8	4.04 ± 0.21 [cc]
	week 8 + physostigmine	8	7.08 ± 0.83 [cc,dd]	8	5.93 ± 0.40 [ccc,dd]	7	4.26 ± 0.29 [aa,bb,cc]
Tidal volume (ml)	week 1	9	0.75 ± 0.02	8	0.78 ± 0.05	7	0.68 ± 0.02 [a]
	week 8	8	1.06 ± 0.07 [cc]	8	0.94 ± 0.06	8	0.87 ± 0.03 [a,ccc]
	week 8 + atropine	8	1.10± 0.06 [ccc]	8	0.94 ± 0.04 [a,c]	8	1.08 ± 0.05 [ccc]
	week 8 + physostigmine	8	1.68 ± 0.11 [ccc,ddd]	8	1.73 ± 0.12 [ccc,ddd]	7	1.06 ± 0.06 [aaa,bbb,ccc]

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Table 12:(Cont.)

Respiratory parameters	Treatment	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
Respiratory rate (breaths/min)	week 1	9	85.96 ± 3.22	8	83.78 ± 2.14	7	60.32 ± 5.02 [aa,bb]
	week 8	8	82.61 ± 2.83	8	83.94 ± 3.15	8	71.81 ± 2.98 [a]
	week 8 + atropine	8	79.66 ± 3.34	8	86.49 ± 2.00 [a]	8	77.89 ± 2.91 [c]
	week 8 + physostigmine	8	67.08 ± 3.72 [cc,dd]	8	67.09 ± 4.18 [cc,dd]	7	62.80 ± 2.41 [aaa,bbb,ddd]
Minute volume (ml/min)	week 1	9	69.74 ± 2.25	8	70.15 ± 4.37	7	43.97 ± 3.70 [aaa,bbb]
	week 8	8	93.85 ± 5.16 [cc]	8	85.38 ± 6.78	8	66.44 ± 4.90 [aa,b,cc]
	week 8 + atropine	8	93.50 ± 3.99 [ccc]	8	89.56 ± 4.70 [cc]	8	89.48 ± 3.51 [ccc]
	week 8 + physostigmine	8	121.70 ±12.31 [cc]	8	122.75 ±10.67 [ccc,d]	7	72.33 ± 2.41 [aa,bb,ccc,dd]

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Table12:(Cont.)

Respiratory parameters	Treatment	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
Inspiratory time(sec)	week 1	9	0.29 ± 0.01	8	0.29 ± 0.01	7	0.75 ± 0.07 [aa,bb]
	week 8	8	0.27 ± 0.01	8	0.24 ± 0.01 [a,cc]	8	0.39 ± 0.02 [aaa,bbb,cc]
	week 8 + atropine	8	0.35 ± 0.06	8	0.25 ± 0.00 [c]	8	0.25 ± 0.00 [ccc]
	week 8 + physostigmine	8	0.38 ± 0.04 [d]	8	0.36± 0.03 [d]	7	0.37 ± 0.03 [cc]
Expiratory time(sec)	week 1	9	0.41 ± 0.02	8	0.41 ± 0.01	7	0.38 ± 0.02
	week 8	8	0.44 ± 0.02	8	0.46 ± 0.03	8	0.47 ± 0.03 [c]
	week 8 + atropine	8	0.38 ± 0.04	8	0.40 ± 0.01	8	0.43 ± 0.02
	week 8 + physostigmine	8	0.51 ± 0.03 [cc]	8	0.52 ± 0.03 [c]	7	0.55 ± 0.05 [c]

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Table 12:(Cont.)

Respiratory parameters	Treatment	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
Relaxation time(sec)	week 1	9	0.19 ± 0.01	8	0.19 ± 0.01	7	0.20 ± 0.01
	week 8	8	0.22 ± 0.02	8	0.21 ± 0.01	8	0.22 ± 0.01
	week 8 + atropine	8	0.22 ± 0.01	8	0.20 ± 0.01	8	0.22 ± 0.01
	week 8 + physostigmine	8	0.27 ± 0.01 [ccc,d]	8	0.30 ± 0.03 [cc,d]	7	0.21 ± 0.02 [aa,bb]
Specific airway resistance (cm.H_2O/sec)	week 1	9	3.55 ± 0.38	8	2.71 ± 0.58	7	8.06 ± 1.03 [aa,bb]
	week 8	8	5.51 ± 0.61 [c]	8	4.09 ± 0.75	8	5.85 ± 1.46 [c]
	week 8 + atropine	8	6.85 ± 1.06 [c]	8	5.29 ± 0.46 [cc]	8	4.39 ± 0.80 [c]
	week 8 + physostigmine	8	11.13 ± 1.44 [cc,dd]	8	11.23 ± 1.09 [ccc,ddd]	7	6.13 ± 1.62 [a,bb]

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Table 12:(Cont.)

Respiratory parameters	Treatment	n	Control	n	ACN 1mg/kg	n	ACN 25mg/kg
Specific airway conductance (sec/cm.H_2O)	week 1	9	0.321 ± 0.030	8	0.379 ± 0.039	7	0.191 ± 0.030 aa,bb
	week 8	8	0.206 ± 0.025 cc	8	0.303 ± 0.039	8	0.309 ± 0.042
	week 8 + atropine	8	0.185 ± 0.025 cc	8	0.202 ± 0.017 cc	8	0.287 ± 0.046
	week 8 + physostigmine	8	0.118 ± 0.012 ccc,dd	8	0.110 ± 0.011 ccc,dd	7	0.295 ± 0.062 a,bb

"week 8+atropine" represents challenged with atropine 10 mg/kg,im.30 minutes before the treatment at the 8 th week.

"week 8+physostigmine" represents challenged with physostigmine 0.5 mg/kg,im.30 minutes before the treatment at the 8 th week.

The duration of experiment is 10 minutes.

a,aa,aaa represent significant differences from control on the same period of testing at p< 0.05, 0.01, 0.001, respectively.

b,bb,bbb represent significant differences from acrylonitrile 1 mg/kg on the same period of testing at p< 0.05, 0.01, 0.001, respectively.

c,cc,ccc represent significant differences from week 1 of the same dose at p<0.05, 0.01, 0.001, respectively..

d,dd,ddd represent significant differences from control group at week 8 at p< 0.05, 0.01,0.001, respectively.

Each value represents as the mean ± S.E.

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Figure 14: Peak expiratory flow (PEF) during spontaneous breathing in atropine and physostigmine treatments

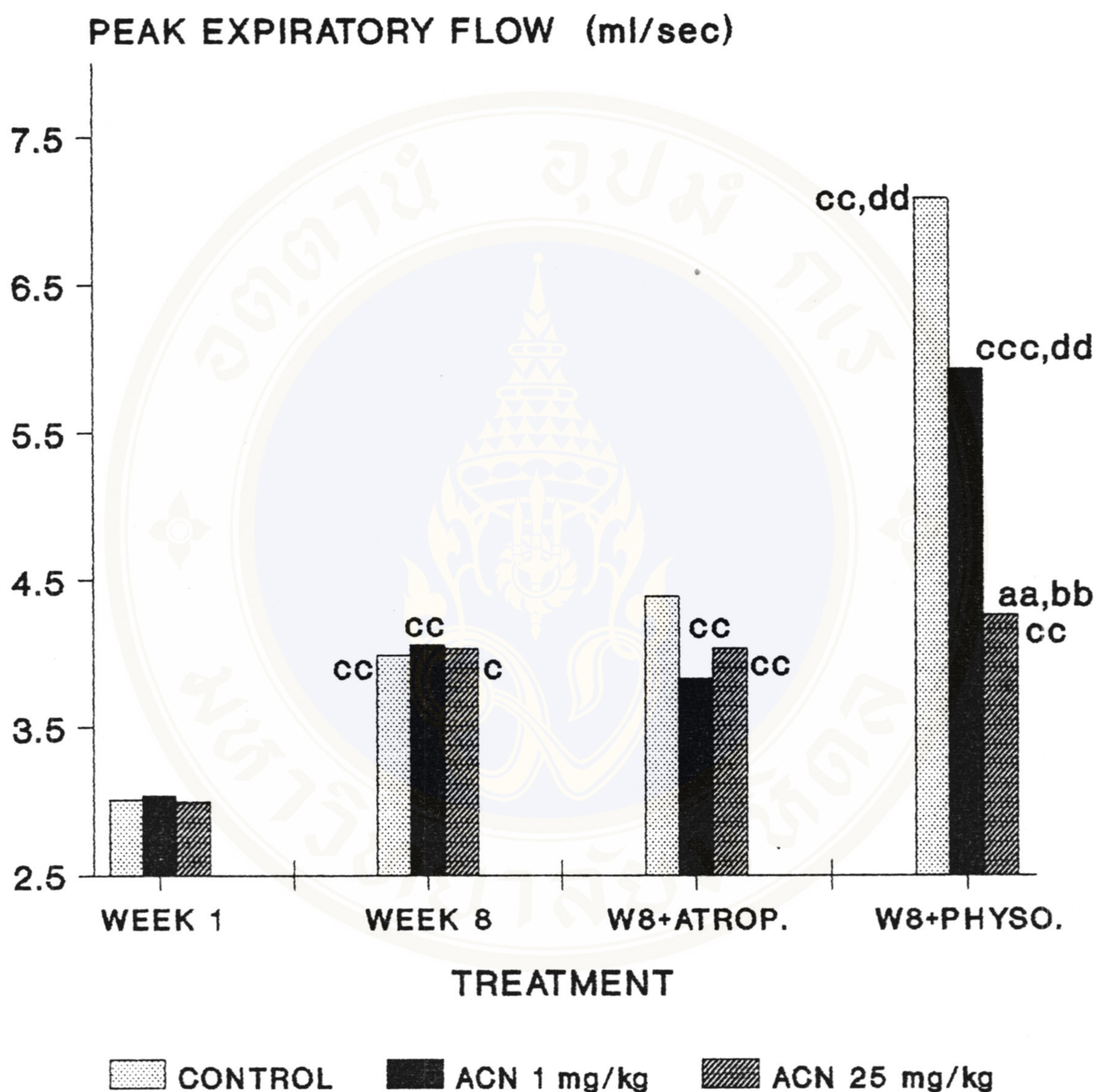

Figure 14 : Peak expiratory flow (PEF) during spontaneous breathing in atropine and physostigmine treatments.

WA+ATROP. represents challenged with atropine 10 mg/kg,im.30 minutes before the treatment at the 8 th week.

W8+PHYSO. represents challenged with physostigmine 0.5 mg/kg,im.30 minutes before the treatment at the 8th week.

aa represents a significant difference from control on the same period of testing at $p< 0.01$

bb represents a significant difference from acrylonitrile 1 mg/kg on the same period of testing at $p< 0.01$

c,cc,ccc represent significant differences from week 1 of the same dose at $p< 0.05, 0.01, 0.001$, respectively.

dd represents a significant difference from control group of week 8 at $p< 0.01$

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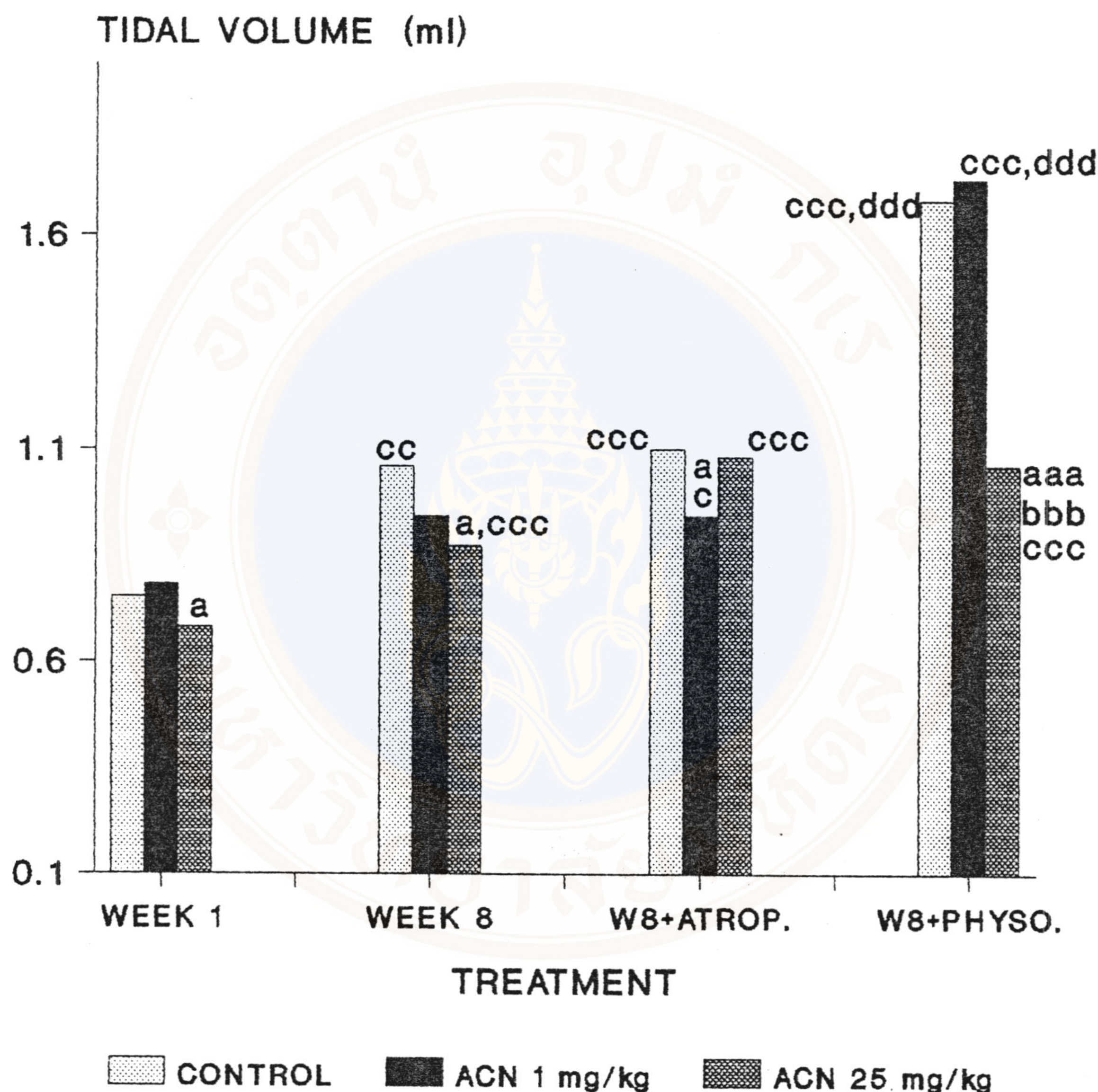

Figure 15: Tidal volume (TV) during spontaneous breathing in atropine and physostigmine treatments.
WA+ATROP. represents challenged with atropine 10 mg/kg,im.30 minutes before the treatment at the 8 th week.

W8+PHYSO. represents challenged with physostigmine 0.5 mg/kg,im.30 minutes before the treatment at the 8thweek.

a,aaa represent significant differences from control on the same period of testing at $p< 0.05$, 0.001, respectively.

bbb represents a significant difference from acrylonitrile 1 mg/kg on the same period of testing at $p< 0.001$

c,cc,ccc represent significant differences from week 1 of the same dose at $p<0.05$, 0.01, 0.001, respectively.

ddd represents a significant difference from control group of week 8 at $p< 0.001$

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Figure 16: Respiratory rate (RR) during spontaneous breathing in atropine and physostigmine treatments

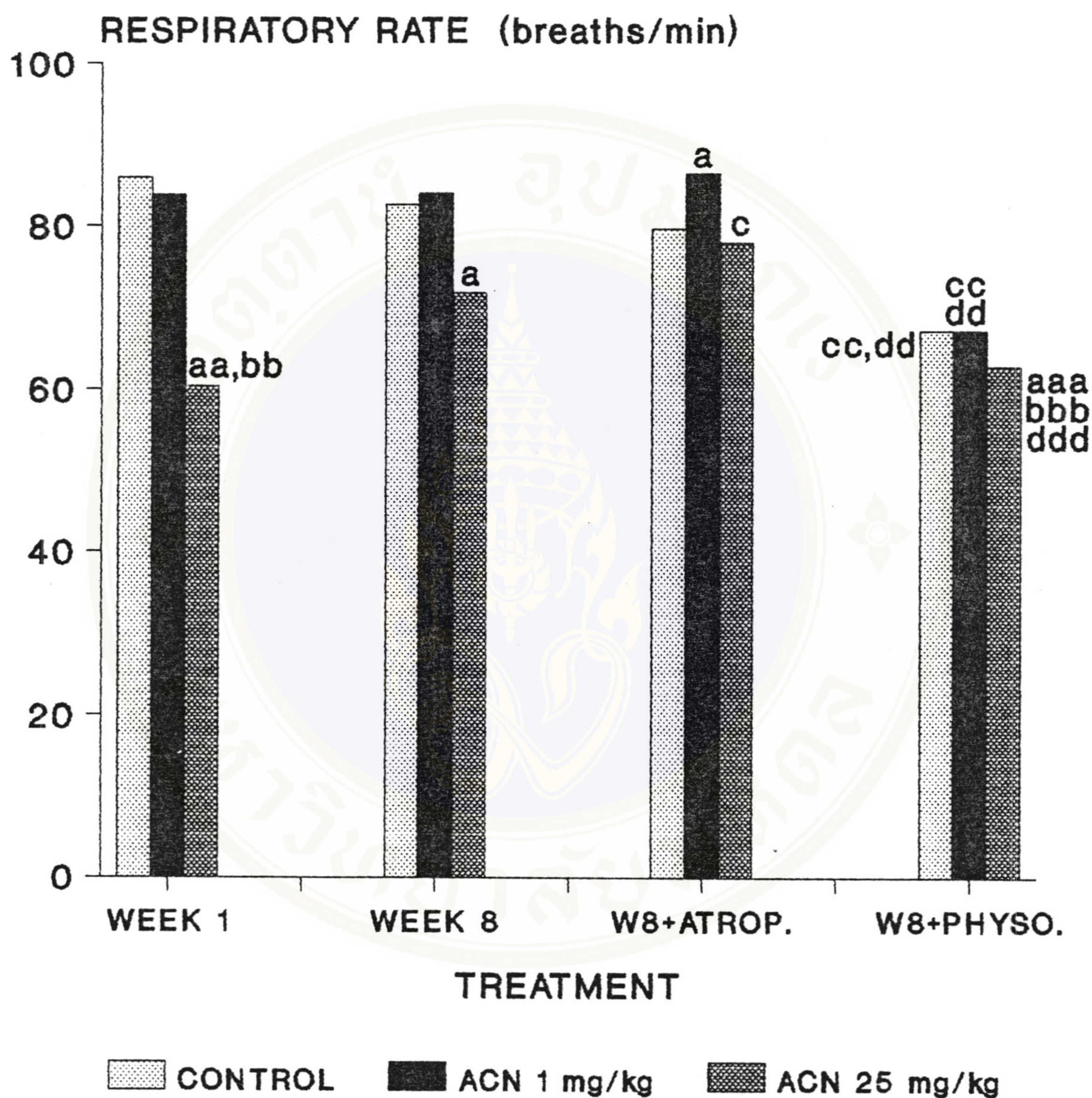

Figure 16 : Respiratory rate (RR) during spontaneous breathing in atropine and physostigmine treatments.

WA+ATROP. represents challenged with atropine 10 mg/kg,im.30 minutes before the treatment at the 8 th week.

W8+PHYSO. represents challenged with physostigmine 0.5 mg/kg,im.30 minutes before the treatment at the 8 th week.

a,aa,aaa represent significant differences from control on the same period of testing at $p< 0.05, 0.01, 0.001$, respectively.

bb,bbb represent significant differences from acrylonitrile 1 mg/kg on the same period of testing at $p< 0.01, 0.001$, respectively.

c,cc represent significant differences from week 1 of the same dose at $p< 0.05, 0.01$, respectively.

dd,ddd represent significant differences from control group of week 8 at $p< 0.01, 0.001$, respectively.

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Figure 17: Minute volume (MV) during spontaneous breathing in atropine and physostigmine treatments

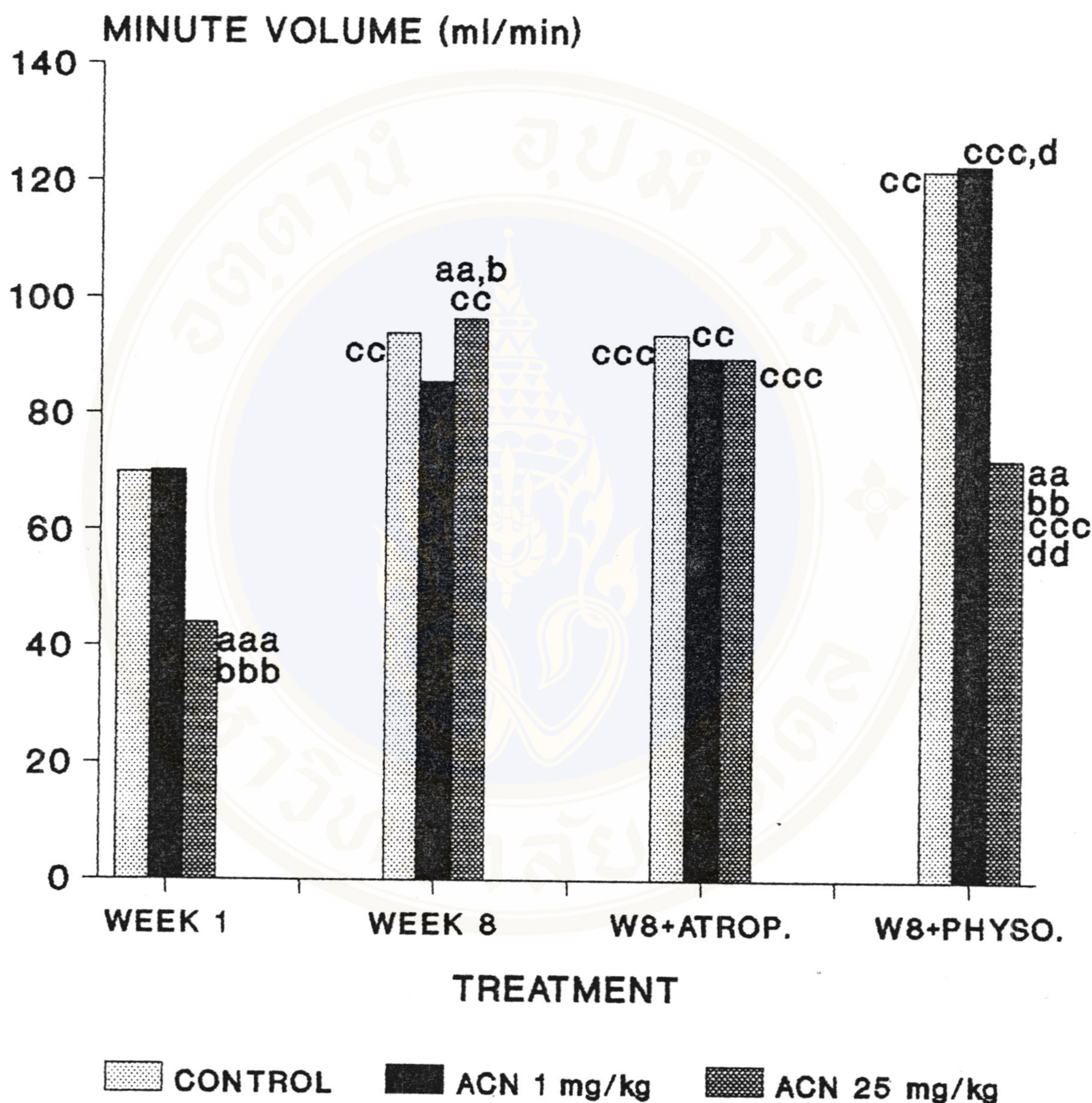

Figure 17 :Minute volume (MV) during spontaneous breathing in atropine and physostigmine treatments.
WA+ATROP. represents challenged with atropine 10 mg/kg,im.30 minutes before the treatment at the 8 th week.
W8+PHYSO. represents challenged with physostigmine 0.5 mg/kg,im.30 minutes before the treatment at the 8 th week.

aa,aaa represent significant differences from control on the same period of testing at $p< 0.01$, 0.001, respectively.

b,bb,bbb represent significant differences from acrylonitrile 1 mg/kg on the same period of testing at $p< 0.05$, 0.01,0.001, respectively.

cc,ccc represent significant differences from week 1 of the same dose at $p< 0.01$, 0.001, respectively.

d,dd represent significant differences from control group of week 8 at $p< 0.05$, 0.01, respectively.

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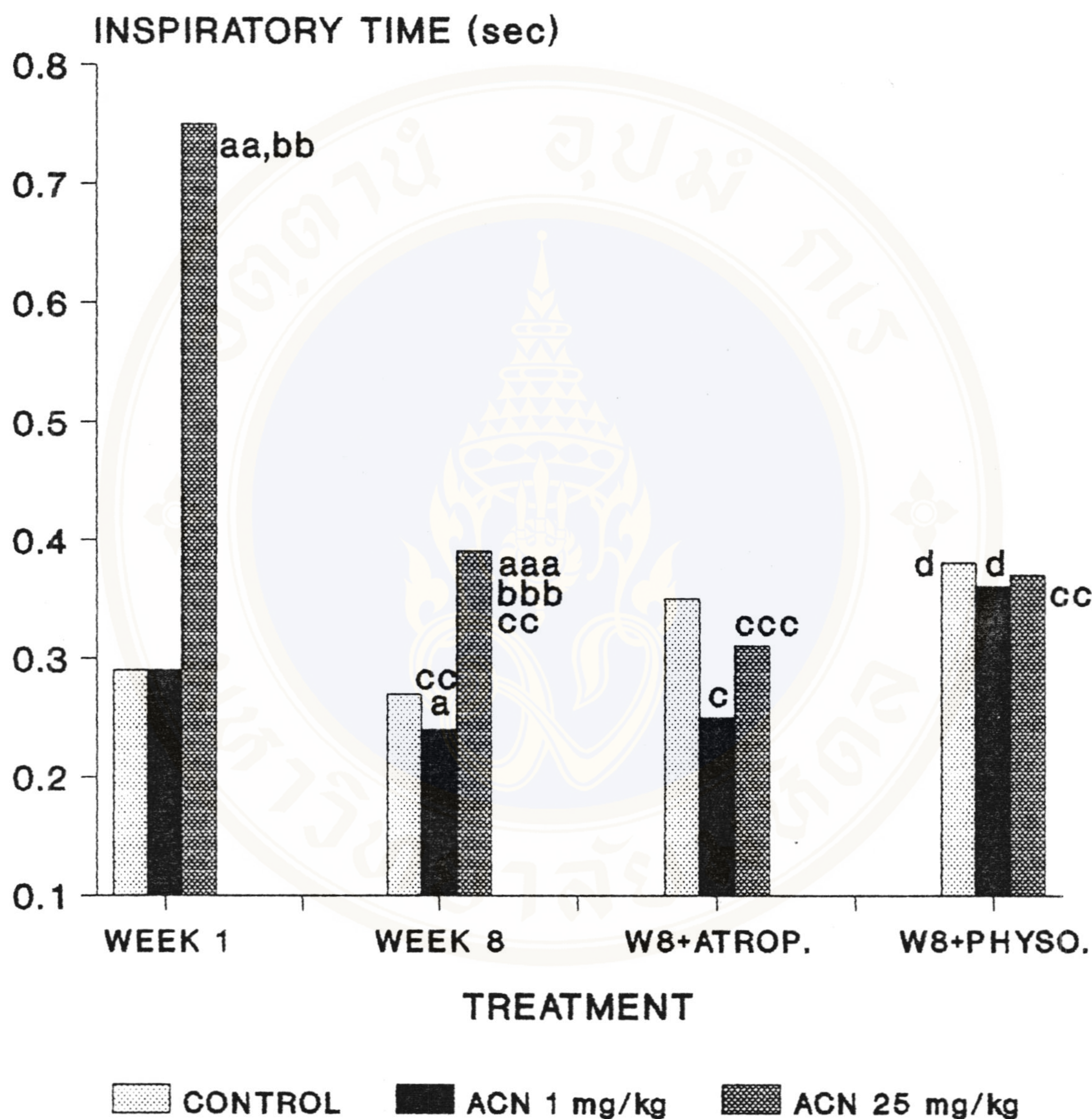

Figure 18 : Inspiratory timew (TI) during spontaneous breathing in atropine and physostigmine treatments.

WA+ATROP. represents challenged with atropine 10 mg/kg,im.30 minutes before the treatment at the 8 th week.

W8+PHYSO. represents challenged with physostigmine 0.5 mg/kg,im.30 minutes before the treatment at the 8th week.

a,aa,aaa represent significant differences from control on the same period of testing at $p < 0.05$, 0.01, 0.001, respectively.

bb,bbb represent significant differences from acrylonitrile 1 mg/kg on the same period of testing at $p < 0.01$, 0.001, respectively.

c,cc,ccc represent significant differences from week 1 of the same dose at $p < 0.05$,0.01, 0.001, respectively.

d represents a significant difference from control group of week 8 at $p < 0.05$

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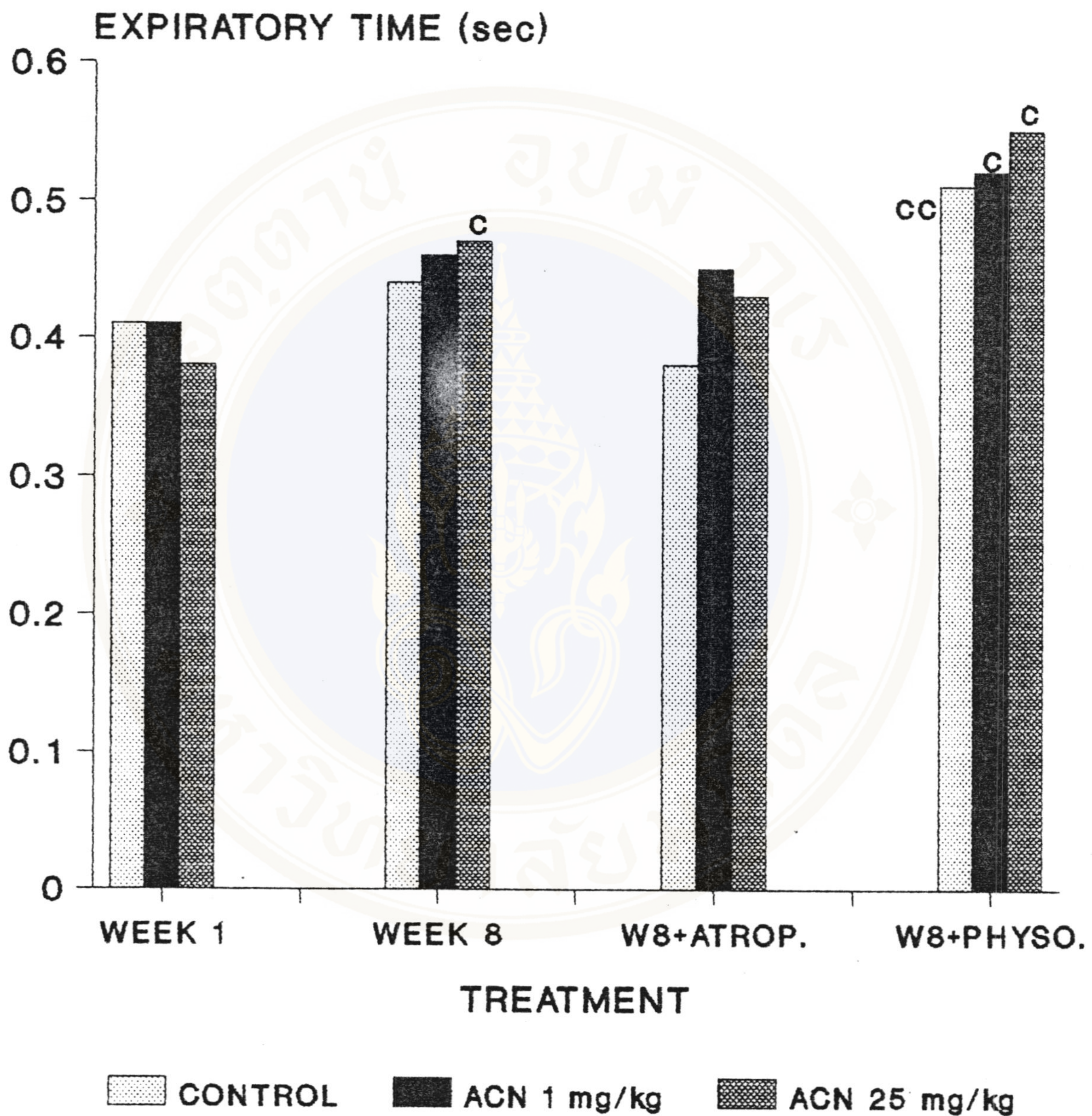

Figure 19 : Expiratory time (TI) during spontaneous breathing in atropine and physostigmine treatments.
WA+ATROP. represents challenged with atropine 10 mg/kg,im.30 minutes before the treatment at the 8 th week.
W8+PHYSO. represents challenged with physostigmine 0.5 mg/kg,im.30 minutes before the treatment at the 8th week.
c,cc represent significant differences from week 1 of the same dose at $p < 0.05$, 0.01, respectively.

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Figure 20 : Relaxation time (RT) during spontaneous breathing in atropine and physostigmine treatments

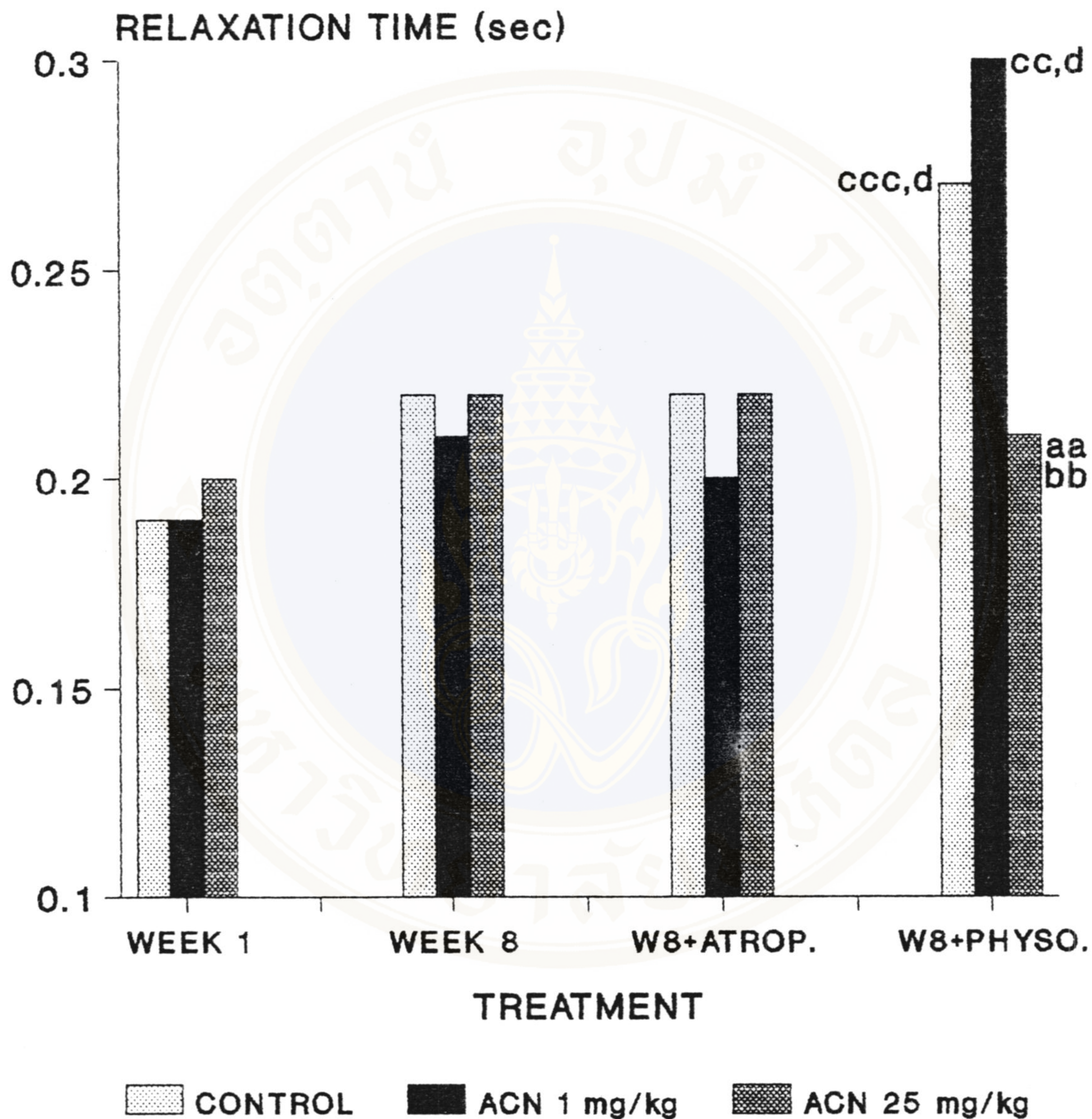

Figure20 : Relaxation time (RT) during spontaneous breathing in atropine and physostigmine treatments.
WA+ATROP. represents challenged with atropine 10 mg/kg,im.30 minutes before the treatment at the 8th week.
W8+PHYSO. represents challenged with physostigmine 0.5 mg/kg,im.30 minutes before the treatment at the 8th week.
aa represents a significant difference from control on the same period of testing at $p < 0.01$
bb represents a significant difference from acrylonitrile 1 mg/kg on the same period of testing at $p < 0.01$
cc,ccc represent significant differences from week 1 of the same dose at $p < 0.01$, 0.001, respectively.
d represents a significant difference from control group of week 8 at $p < 0.05$

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Figure21:Specific airway resistance(RES) during spontaneous breathing in atropine and physostigmine treatments

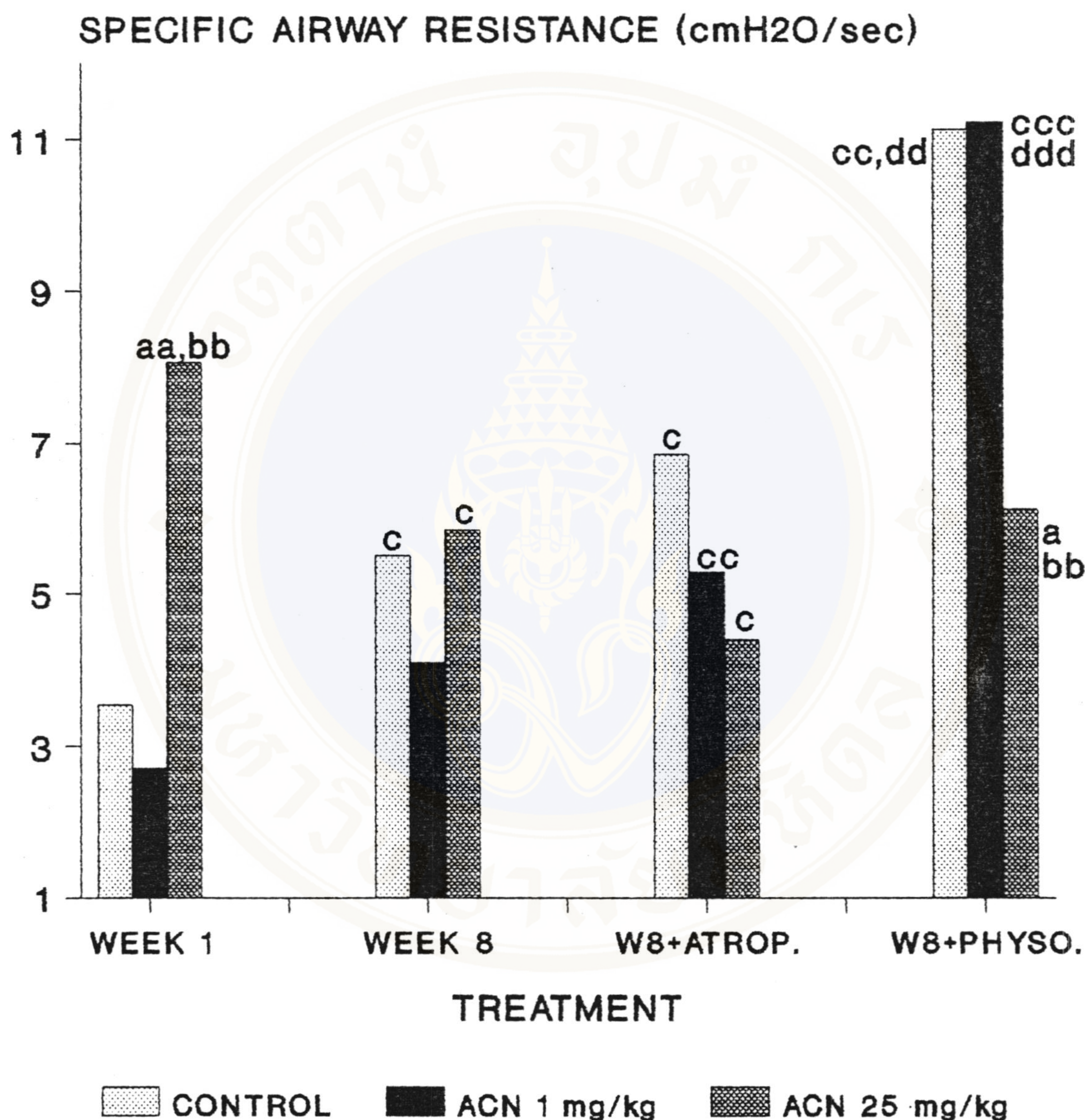

Figure 21: Specific airway resistance (RES) during spontaneous breathing in atropine and physostigmine treatments.

WA+ATROP. represents challenged with atropine 10 mg/kg,im.30 minutes before the treatment at the 8th week.

W8+PHYSO. represents challenged with physostigmine 0.5 mg/kg,im.30 minutes before the treatment at the 8th week.

a,aa represent significant differences from control on the same period of testing at $p< 0.05$, 0.01, respectively.

bb represents a significant difference from acrylonitrile 1 mg/kg on the same period of testing at $p< 0.01$.

c,cc,ccc represent significant differences from week 1 of the same dose at $p< 0.05$, 0.01, 0.001, respectively.

dd,ddd represent significant differences from control group of week 8 at $p< 0.01$, 0.001, respectively

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Fig.22:Specific airway conductance(COND) during spontaneous breathing in atropine and physostigmine treatments

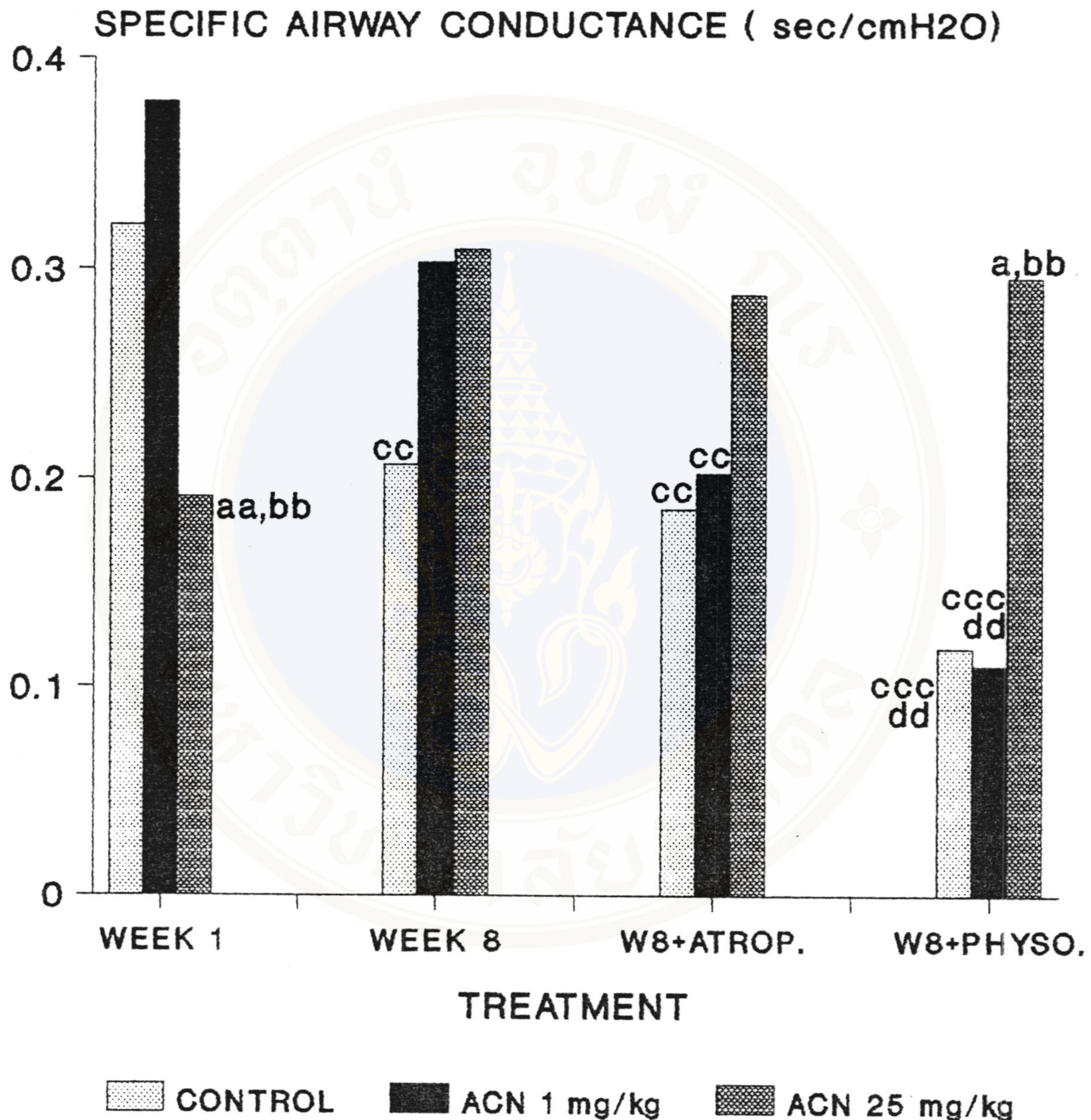

Figure 22 : Specific airway conductance (COND)during spontaneous breathing in atropine and physostigmine treatments.

WA+ATROP. represents challenged with atropine 10 mg/kg,im.30 minutes before the treatment at the 8 thweek.

W8+PHYSO. represents challenged with physostigmine 0.5 mg/kg,im.30 minutes before the treatment at the 8 th week.

a,aa represent significant differences from control on the same period of testing at $p < 0.05$, 0.01, respectively.

bb represents a significant difference from acrylonitrile 1 mg/kg on the same period of testing at $p < 0.01$

cc,ccc represent significant differences from week 1 of the same dose at $p < 0.01$, 0.001, respectively.

dd represents a significant difference from control group of week 8 at $p < 0.01$

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CHAPTER V

DISCUSSION

Acrylonitrile is a major industrial chemical which has an extremely wide range of uses as commercial applications. There is a strong likelihood of occupational and general human exposure through air, water and food (1,2,3). The respiratory and neural effects of acrylonitrile in human were noted on exposure to 16-100 ppm for 20-45 minutes (9). Since autonomic nervous system controlling respiratory tract is very important for the well being of human, the modification of this autonomic function, therefore may lead to pathological state (57). It is generally recognized that cholinergic nerves are predominant neural pathway in the airway and cholinergic bronchoconstriction may be important factor in airawy obstruction (74,75,76). The preliminary experiments and results from our laboratory study revealed that chronic exposure to acrylonitrile induced alterations in tracheal muscarinic receptors both decreased and increased muscarinic responses to acetylcholine depending on time of exposure (58). However, there are few reports show the effects of low dose and chronic exposure to acrylonitrile on respiratory functions in intact animals that emphasized the relation with the cholinergic nervous system ,therefore in this present study by using the intact test animals, attempts have been made to evaluate the effects of subchronic exposure of acrylonitrile on the respiratory functions and to detect whether long-term exposure of acryloniytile can alter cholinergic functions controlling respiratory functions. Furthermore, the effect of subchronic exposure of acrylonitrile on the body weight of the treated rats were also detected.

It was found that the subcutaneous administration of acrylonitrile 25 mg/kgBW, 5 days per week, for 8 weeks induced the significant reduction in body weight than control group and this reduction was still observed at the 9 th week (after termination of acrylonitrile for one week). Loss of body weight or failure to gain body weight, loss of

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appetite, gastro-intestinal disturbance have been reported long time ago in rat exposed to acrylonitrile at 330 mg/m^3 for 4 hours a day, 5 days a week for 13 weeks (61). This effect was also found by the intraperitoneal injection of 50 mg/kg daily for 3 weeks in adult rat (4). Our result conformed that body weight loss could occur and this effect was not related to route of administration. At present, it is notknown how long-term treatment of acrylonitrile can induce the decrease in body weight.

There was a study reported a decrease in body weight in male Wistat rats after exposure to acrylonitrile 280 mg/m^3,8 hours a day, for 5 days, the lower body weight observed was correlated with the decrease in serum concentration of cholesterol and triglyceride. The effect of acrylonitrile on lipid, protein and carbohydrate metabolism may be responsible for the loss of body weight induced by acrylonitrile(62).

Furthermore, in the present study, it was observed that acrylonitrile (25mg/kg)-treated rats were slightly sedative especially after acrylonitrile administration, and during the repeated exposures by subcutaneous route, rats in this group have skin irritation and became agressive when handling during the treatment. It may be possible that acrylonitrile induced alterations in mood may involve in the eating behaviors of the treated-rats resulting in loss of body weight.

The study of the effect of subchronic exposure to acrylonitrile on respiratory functions were measured in the test animals during spontaneous breathing. It was found that respiratory functions measured by using the Noninvasive Respiratory Analyzer in rats exposed to acrylonitrile 1 mg/kg BW, 5 days per week for 8 weeks did not showed the significant differences in peak expiratory flow, tidal volume, respiratory rate, minute volume, expiratory time, inspiratory time (except at the 4th, 8th week), relaxation time, specific airway resistance when compared with control group throughout the entire experiment.

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In addition, after termination of acrylonitrile treatment for one week all values of respiratory functions of these 1 mg/kg acrylonitrile treated group and control group also showed no significant difference.

The results of this study showed that at relatively low dose (1 mg/kg) of acrylonitrile did not showed alterations of the respiratory functions.

The influences of subchronic administration of acrylonitrile 25 mg/kgBW, 5 days per week for 8 weeks on the respiratory functions can be divided into two parts the first part (at 1st, 2nd, 4th, 6th, and 8th week of acrylonitrile treatment) and the second part (after the termination of acrylonitrile treatment for one week).

The subchronic exposure to acrylonitrile 25 mg/kg resulted in lowering of peak expiratory flow when compared with control and acrylonitrile 1 mg/kg treated group. The significant decrease were detected at the 4th week of the treatment. However, longer the time of exposure to acrylonitrile these values were increased, furthermore at the 9th week (one week after the termination of acrylonitrile treatment) the peak expiratory flow of the rats in this group were increased to nearly that of control and acrylonitrile (1mg/kg)-treated group, indicated that repeated exposure to acrylonitrile resulted in adaptive changes of the peak expiratory flow values. The peak expiratory flow rate represented the maximum flow rate during the force expiration (68). It demonstrated the interaction of the compression force of the bronchi, bronchioles and the driving force that expelled the air out of the lung. The peak expiratory flow rate is useful in clinical assessment of airway resistance. The increase in airway resistance resulted in decreasing in speed of expiration. In fixed obstruction of the major airway especially intrathoracic obstruction as in tracheal stenosis, laryngeal stenosis or in intrathoracic airway obstruction such as a chronic bronchitis, bronchial asthma, the peak expiratory flows were reduced (68).

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In the present study, the gradually increased in peak expiratory flow values following the administration of acrylonitrile may be occurred as the results of the simultaneously decreased of specific airway resistance.

The increase in specific airway resistance which observed during the first and the second week of the treatment may be resulted from the overactivity of cholinergic function.

The activation of muscarinic receptor in airway smooth muscle caused the bronchoconstriction (88). The cholinergic nerves are predominant neural pathway in the airway, therefore, overactivity of this system may cause death due to airway obstruction resulted from respiratory secretions and bronchoconstriction.

It is interesting to find that the specific airway resistance of acrylonitrile (25mg/kg)-treated group was significantly higher than control and acrylonitrile (1mg/kg)-treated group, but later on it decreased and returned to nearly control group especially at the nineth week of the treatment (after termination of acrylonitrile treatment for 1 week). It was reported that in vitro study acrylonitrile may induce the release of acetylcholine and this effect may be responsible for the increase airway resistance in the acute phase, repeated treatment of acrylonitrile resulted in desensitization due to loss of its indirect cholinergic effect mediating by the release of ACh, and/or the adaptive changes of tracheal muscarinic receptors following repeated exposure to the released ACh.

The effects of subchronic exposure to acrylonitrile on tidal volume, respiratory rate, minute volume, inspiratory time and specific airway conductance were reversible.

The reciprocal of specific airway resistance is specific airway conductance (115). The effect of acrylonitrile 25 mg/kg on specific airway conductance during the first and second week of treatment as showed in table 11 was correlated with the increase in specific airway resistance. When the later parameter gradually returned to normal, the former parameter also returned to normal.

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Tidal volume of acrylonitrile (1mg/kg)-treated group seemed to be unaltered throughout the entired experiment. At the early treatment of acrylonitrile (25 mg/kg)-treated group, rats showed significantly lower tidal volumes than control and acrylonitrile (1mg/kg)-treated group (table 4). The tidal volume is amount of air that moves into lungs in each inspiration (65). It is not clear at present how acrylonitrile decreased this value at the first week. It may be due to the increase specific airway resistance.

It has also been noticed that even in the control group tidal volume values at the 8th,and 9th week were higher than the value at the1^{st}week, and the increased tidal volume were attenuated when the test animals were treated with acrylonitrile 25 mg/kg. It seems that as age of the animal increased the tidal volume values increased but how these values were increased remained unclear.

At present it was not known for certain how acrylonitrile induced the decrease in respiratory rate, however, there was a possibility that this effect may due to the effect of hydrocyanic acid which is one of the metabolites of acrylonitrile. Goldfrank et al. in 1990 (116) reported that after acute exposure to cyanide, the rapid slowing of respiratory rate with subsequent profound respiratory depression was occurred. The another possibility is, in the situation of airway obstruction, compensatory response to this effect is to increase the inspiration and expiration, then the respiratory frequency woud be very slow (68). In this study, the inspiratory time of acrylonitrile (25mg/kg)-treated group was significantly higher than that of control and acrylonitrile (1mg/kg)-treated group, and the specific airway resistance was also increased in acrylonitrile (25mg/kg)-treated group at the same period of treatment. In addition, Richardson et al. in 1984 (117) reported that asphyxia or hypercapnia all caused increased inspiratory drive and thus and increased in airway resistance.

The amount of air inspired per minute, minute volume, is the product of respiratory rate and tidal volume (64). The decrease in minute volume in the rats treated

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with acrylonitrile 25 mg/kg (as showed in table 6) may be the results of the decrease in respiratory rate because the tidal volume did not altered significantly.

The significant alterations induced by acrylonitrile 25 mg/kg are decreased respiratory rate, increased specific airway resistance, decreased minute volume. How these alterations occurred are not clearly understood. However, there are some possibilities, for examples, it was reported that acrylonitrile closely resembles hydrocyanic acid in its toxic action, by inhibiting the respiratory enzymes of tissue, it renders the tissue cells incapable of oxygen absorption (86). In addition, it initially induced tachypnea and dyspnea follows by slowing the respiratory rate ; pulmonary edema may be involved in the later stage (116). However, several studies have suggested that toxic effects of acrylonitrile may be partially due to the liberation of cyanide radical, since the amount of released cyanide is not enough to cause cyanide poisoning (9,35,40).

Another possible mechanism by which acrylonitrile induced early signs of toxicity was the induction of cholinomimetic effects. Ghanayem et al. in 1991 (36) reported that after single oral or subcutaneous dose of acrylonitrile 20, 40 or 80 mg/kgBW, the results showed the cholinomimetic overstimulation, such as salivation, lacrimation , increase gastric secretion and diarrhea. In our preliminary experiments, after intraperitoneal administration of acrylonitrile 5, 25, 50,and 75 mg/kg BW, the increasing in serum cholinesterase activity was detected following acute administration of acrylonitrile. Ageeva in 1970 (7) reported the significant increase of acetylcholine in worker producing acrylonitrile and suggested that sweating in worker exposed to acrylonitrile was an effect of acrylonitrile on the autonomic nervous system. In addition, symptoms of irritation to the respiratory tract such as stuff nose, runny nose, coughing which complained significantly more often in acrylonitrile workers may be regarded as temporary, reversible symptoms induced by short-term exposure to high concentration of acrylonitrile (49). Furthermore, the early acrylonitrile toxicity signs involving

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cholinomimetic effects were suggested to be caused by acrylonitrile or its metabolites, other than cyanide (27,29).

The results from animal studies suggested that inflammatory mediators might enhance cholinergic responsiveness resulted in the bronchoconstriction (70). However, in the study of Ahmed in 1992 (17) about the pulmonary toxicity of acrylonitrile, he reported that after single oral dose of 46.5 mg/kg in rats, there were no evidence of vasculitis or pulmonary inflammation.

The adaptive changes of respiratory functions such as gradually decreased inspiratory time, specific airway resistance or gradually increased of peak expiratory flow, respiratory rate, minute volume and specific airway conductance to nearly the values of control group (and also showed differ from the first week of the treatment) after long-term treated with acrylonitrile 25 mg/kg, may be due to the depletion of endogeneous acetylcholine which caused the reduction of cholinergic overstimulation (reduction of constriction of airway, decreased glandular or globlet cell secretion). In our preliminary experiment, it was found that after treated with acrylonitrile 25 mg/kg, intraperitoneally, the specific activity of serum acetylcholinesterase was significantly lower from control group. However, the effects of low dose and chronic exposure of acrylonitrile may result in increased or decreased muscarinic response to acetylcholine depending on time and dose of exposure (58).

In order to study the effects of subchronic exposure of acrylonitrile on the cholinergic response of the respiratory system, at day 5 of the 8^{th}week of acrylonitrile treatment, test animals were challenged with atropine sulfate 10 mg/kg BW (muscarinic receptor antagonist) or physostigmine 0.5 mg/kg BW (a reversible acetylcholinesterase inhibitor), intramuscularly.

The results obtained from this study showed that subchronic exposure to acrylonitrile 1 mg/kg had no significant effects in almost all of respiratory functions compared with control group after challenged with atropine, furthermore, all values of

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respiratory functions of control and ACN-treated rats showed no significant differences from control group of the 8 thweek and the 9th week of the treatment. Respiratory functions of acrylonitrile (25 mg/kg)-treated group showed significant differences when compared with the control group.

These results indicated that at this selected dose of atropine, it did not alter the effects of subchronic exposure to acrylonitrile 1 and 25 mg/kg on cholinergic responses of the respiratory system. However, this finding cannot be concluded that this subchronic exposure to acrylonitrile has no effect on cholinergic response of respiratory system because only one dose was selected.

The respiratory rate of acrylonitrile(1 mg/kg)-treated group after challenged with atropine was significantly higher than control group. Thus, despite the significantly lower tidal volume, minute volume did not alter significantly (figure15,16,17). These results suggested that minute volume is governed by two factors, respiratory rate and tidal volume.

In addition, respiratory rate of control and acrylonitrile-treated groups after challenged with atropine were decreased when compared with those of the 9th week (table 12). The study of Richardson et al. in 1984 (117), found that atropine can block the inspiratory related modulation of slowly adaptive firing rate of phrenic activity. At persent, the exact mechanism of reduction of respiratory rate is still unknown.

Furthermore, control and acrylonitrile(1mg/kg)-treated group showed the slightly higher in the specific airway resistance and slightly lower in the specific airway conductance when compared which acrylonitrile(25mg/kg)-treated group. Atropine is non-specific muscarinic antagonist (118). It compete with acetylcholine for a common binding site on muscarinic receptor, it was used as bronchodilator and can decrease the airway resistance (74,119). The study of Gross and Skorodin in 1984 (120) reported that 10 minutes after administration of atropine it can increase the specific airway conductance in bronchospasm in asthma.

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M_1-receptors facilitated neurotransmission through parasympathetic ganglion and enhanced cholinergic reflex on bronchoconstriction, M_3-receptors mediated contractile response in airway smooth muscle and are predominant receptors on submucosal gland. Contraction of airway smooth muscle is primarily the results of stimulation of M_3-receptors by acetylcholine release from the vagus nerve (91). The activations of M_1 and M_3 can be blocked by nonspecifically by atropine.

By using atropine, it is anticipated that if the cholinergic functions are unaltered by subchronic treatment of acrylonitrile, the respiratory functions in these three test groups should not show any significant differences from each other.

In this study, the specific airway resistance decreased following the treatment with atropine in 25 mg/kg acrylonitrile treated groups. This result suggested that the bronchoconsrtiction mediated by the activation of tracheal muscarinic receptors may be involved.

The effects of physostigmine is due primarily to the prevention of hydrolysis of acetylcholine at site of cholinergic transmission (93). Stimulation of the vagus nerve caused release of acetylcholine which activated muscarinic receptors on smooth muscle and submucosal gland cells, which resulted in bronchoconstriction and mucous secretion, respectively (78). The results of this study showed that in control and acrylonitrile(1mg/kg)-treated groups, specific airway resistance, inspiratory time, expiratory time, relaxation time were increased while respiratory rate and specific airway conductance were decreased. At present, the exact mechanism of reduction in respiratory rate is still unknown.

The respiratory minute volume is proportionated to the metabolic rate and link between ventilation of CO_2 (65). To increase minute ventilation, either tidal volume or respiratory rate must be increased (121). In this study, physostigmine decreased the respiratory rate but this effect did not cause a reduction in minute volume because there

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was a significant increase in tidal volume in all test groups as compared to control groups.

In control and acrylonitrile(1mg/kg)-treated group, the interesting results were observed following physostigmine challenge, instead of lowering of the peak expiratory flow due to the increase in specific airway resistance, it was found that peak expiratory flow were increased (table 12).

A significant increased in the peak expiratory flow rate indicated that the airway obstruction is at least partially reversible (68). The possible mechanism which may be involved in the increased in peak expiratory flow are as follows : In airway obstruction, there is an increase in residual volume and functional residual capacity because of air trapping (68), and in the enlarged lung the bronchial are held open partially via elastic pull on the outsides by lung structural elements (87), therefore, the increase in peak expiratory flow rate through the airway may increase easier.

The another possible mechanism may be due to the consequences of enhanced concentrations of acetylcholine at motor endplate of skeletal muscle which innervated by nicotinic cholinergic fibers (122). The accumulation of acetylcholine caused contraction of expiratory muscle which pushed the diaphragm upward and pulled the rib cage downward (65). The force of muscle contraction is required to create a pressure to push air out of the lung. With increase the airway resistance, the elastic recoil force may not be great enough to expel all of the air available for expiration. At the start of the next inspiration the funtional residual capacity will be greater. By increasing the functional residual capacity and has greater elastic recoil, this increased recoil force can provide enough force to expel the tidal volume (121). So the increase in peak expiratory flow is a complex parameter and several factors are involved.

The gross behavioral observations revealed that in acrylonitrile (25mg/kg)-treated group, the signs of salivation, lacrimation, diarrhea(in some rats) were present about 10 minutes after acrylonitrile treatmenrt. These effects began at the second week and

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throughout 8 weeks of the treatment. Ghanayem et al. in 1991(56) also found these signs occur early after acute acrylonitrile subcutaneously administration and they suggested that these signs are acetylcholine-like toxicity. Furthermore, the preliminary experiments of this study found the increased acetylcholinesterase activity after acute acrylonitrile treatment and reduced at the 8th week of the treatment. These observations suggested that the increase in enzyme activity may be the consequence of increased endogenous acetylcholine level during early acrylonitrile treatment, and in the longer treatment the adaptive change occurred.

It is interesting to find that the respiratory functions of acrylonitrile(25mg/kg)-treated group also showed adaptive changes which reached nearly control values after 8 weeks of the treatment.

The mechanism involved in adaptation has been studied by Wecker et al. in 1977 (112). They suggested that, changes in the presynaptic mobilization and storage of acetylcholine were the alternative mechanism for the development of the tolerance to the chronically reduced acetylcholinesterase activity.

At the 8th week of the treatment, the results showed that rats in acrylonitrile (25mg/kg)-treated group have only slightly altered in the respiratory functions after challenged with physostigmine which is quite different from values of control and acrylonitrile(1mg/kg)-treated group. Except the respiratory rate, minute volume and inspiratory time, all values of respiratory functions of acrylonitrile(25mg/kg)-treated group after challenged with physostigmine showed no significant differences when compared with that of control group at the 8th and 9th week of acrylonitrile treatment.

Physostigmine produced accumulation of acetylcholine at the site of cholinergic transmission resulting in overstimulation of muscarinic responses at autonomic effector organs (93). The activation of muscarinic receptors in the respiratory system caused bronchoconstriction and increase bronchial secretion. These effects were found as expected in control and acrylonitrile(1mg/kg)-treated groups. By contrast,

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acrylonitrile(25mg/kg)-treated group showed only slightly alteration in respiratory functions which indicated the decrease response of muscarinic effect on airway smooth muscle.

Ghanayem et al in 1991 (56) reported that acrylonitrile may produced the effects on the cholinergic system at the receptor level. The preliminary experiments have been conducted and found that chronic exposure to acrylonitrile induced alterations in tracheal muscarinic receptors both increased and decreased muscarinic response to acetylcholine may be observed depending on time of exposure (58). In this study subchronic exposure to acryloniyrile 25mg/kg BW for 8 weeks resulted in the decrease in muscarinic responses.

The possible mechanism of the decrease muscarinic response or the desensitization of muscarinic receptor may be due to the prolonged-exposure of receptors to a released neurotransmitter which can lead to a decrease in the number of receptors on the cell surface and thus decrease the sensitivity of the cell to further stimulation (100). The decrease in receptor number afters long-term agonist exposure have been reported in neural cell line (109), heart cells (110), and smooth muscle (111).

Halvorsen and Nathanson in 1981 (104) have suggested that direct administration of muscarinic agonist in vivo could lead to the decrease in receptor numbers and physiological responsivenes in dose and time dependent manner. The decline in muscarinic receptor sites caused by chronic treatment of cholinomimetics is accompanied by a decrease in physiological sensitivity to cholinergic stimulation (113,114).

In smooth muscle after continuing presence of muscarinic agonist for several hours, the decrease in muscarinic acetylcholine receptor and the decrease in maximum contractile response to acetylcholine were obtained (111).

Jett et al in 1993 (94) has reported that, the reduction of m3 subtype of mACh receptor without change in its affinity is consistant with the reduced mRNA expression of the m3 subtype observed in 14 days parathion exposed mice.

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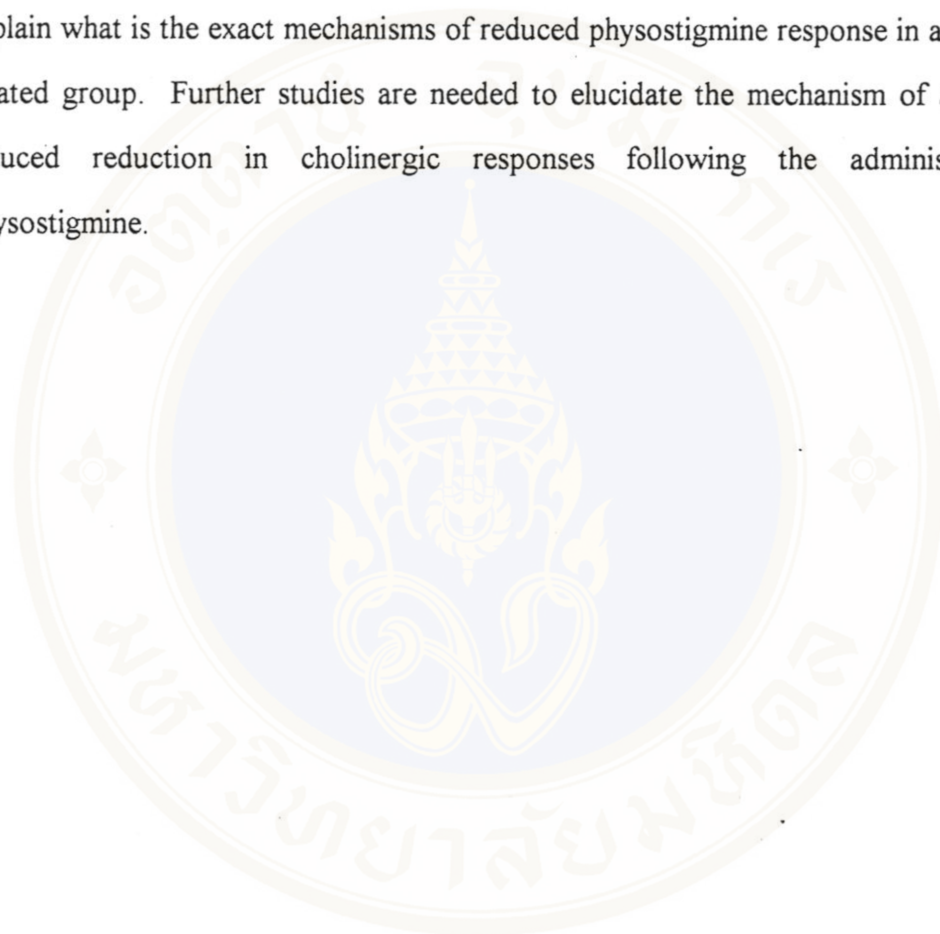
In this study, the physostigmine challenge in acrylonitrile (25 mg/kg) group suggested that the cholinergic function in this acrylonitrile treated group is subtlely decreased which could not be detected clearly by muscarinic antagonist, but it would be revealed upon full activation induced by physostigmine. At present, it is not possible to explain what is the exact mechanisms of reduced physostigmine response in acrylonitrile-treated group. Further studies are needed to elucidate the mechanism of acrylonitrile induced reduction in cholinergic responses following the administration of physostigmine.



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CHAPTER VI

SUMMARY

The subchronic exposure to acrylonitrile 1 mg/kg, once daily, 5 days per week, for 8 weeks had no significant effects on respiratory functions when compared with control group. The subchronic exposure to acrylonitrile 25 mg/kg altered the body weight of the rats this observation may be due to acrylonitrile-induced alterations in mood which directly or indirectly affecting eating behaviors. In addition, at the dose of 25 mg/kg, the results showed the significant alterations in all the respiratory functions except expiratory time and relaxation time, which observed markedly at the first and second week of the treatment, however, these values gradually change to nearly those of control groups.

The other effects of acrylonitrile are related to cholinergic overstimulation including salivation, lacrimation and diarrhea. In rats treated with acrylonitrile 25 mg/kg, these effects were detected about ten minutes following acrylonitrile treatments, began at the second week and could be observed throughout the 8 weeks of acrylonitrile treatment. The alterations in respiratory functions of acrylonitrile 25 mg/kg at the first and the second week may due to the release of acetylcholine which resulting in cholinergic overstimulation. The adaptive change following the repeated treatment of acrylonitrile may due to loss of its indirect cholinergic effect (mediating by the release of acetylcholine).

To detect whether long-term exposure to acrylonitrile can induce the alterations in respiratory functions related to cholinergic nervous system, rats at day 5 of the 8th week of acrylonitrile treatment were challenged with atropine sulfate (10 mg/kg, Im.) or physostigmine (0.5 mg/kg, Im.) 30 minutes before measurement of respiratory functions.

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After atropine challengd, acrylonitrile (25 mg/kg)-treated group showed no significant difference in respiratory functions as compared to the control group, except for the specific airway resistance. Similar results were found in the acrylonitrile (1mg/kg)-treated group and challenged with atropine, except that there was a significant decrese in tidal voume. However, there was no alteration in minute volume due to the significantly increase in respiratory rate. These results show that minute volume is governed by two factors, respiratory rate and tidal volume.

After physostigmine challenged, acrylonitrile 1 mg/kg treatment showed the decrease in specific airway conductance and increase in almost all of respiratory functions except the respiratory rate. Physostigmine also exhibited the similar pattern of influences on respiratpry functions in acrylonitrile (25 mg/kg)-treated group but mostly to the lesser extent than acrylonitrile 1 mg/kg treatment. In addition at the dose of acrylonitrile 1 mg/kg treatment after physostigmine challenged, peak expiratory flow showed significant increase while the specific airway resistance also significant increase at the same time. The mechanisms which are responsible for these observations are not known at present, further studies are needed.

The results obtained after physostigmine challenged in acrylonitrile 25 mg/kg suggest that the cholinergic functions in this acrylonitrile treated group is subtlely decreased which could not be detected by muscarinic antagonist.

The results of this study indicated that the alterations on respiratory functions after subchronic exposure to acrylonitrile is dose and time dependent. Acrylonitrile 1 mg/kg treatment had no effect on respiratory functions and muscarinic response to acetylcholine as tested by challenge with atropine and physostigmine, on the other hand, the acrylonitrile (25mg/kg)-treated group showed the possible involvement of the decrease in cholinergic function. Further studies are needed to elucidate the mechanisms of acrylonitrile induced reduction in cholinergic responses following the administration of physostigmine.

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