

**A 01**

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<b>Chemical Category</b>	ACRYLONITRILE		

A 03

# AN GROUP

8EHQ-0900-14711

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September 15, 2000

Document Processing Center (7407)  
Attention: 8(e) Coordinator  
Office of Pollution Prevention and Toxics  
U. S. Environmental Protection Agency  
401 M Street, S.W.  
Washington, Dc 20460

Dear 8(e) Coordinator,

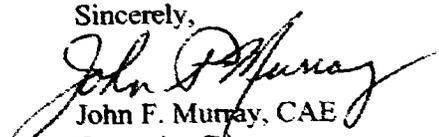
RE: File [8EHQ-400-14711]

On April 26, 2000, the Acrylonitrile (AN) Group, Inc., a trade association representing producers and users of acrylonitrile, submitted four unpublished surveys of Chinese workers to the EPA 8(e) coordinator.

We have recently received English translations of additional Chinese epidemiology surveys and toxicology studies on acrylonitrile. Neither the AN Group or any individual member of the AN Group has made a determination as to whether a significant risk of injury to health or the environment is actually presented by the findings. However, the information in these translated studies and surveys may meet EPA's guidance for reporting under TSCA 8(e) and accordingly the AN Group is submitting this material for inclusion in file/docket # [8EHQ-0400-14711].

The US acrylonitrile producers are committed to pursuing a better understanding of the quality and meaning of these studies and surveys and to incorporating any scientifically sound information into management of acrylonitrile health risks. Please contact me if you have any questions or comments about these reports.

Sincerely,

  
John F. Murray, CAE  
Executive Director



2000 SEP 27 AM 10:34

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**A 04**

**PUBLISHED AND NON-PUBLISHED ARTICLES  
AND DATA**

**RELATED ACRYLONITRILE**

**EXPOSURE TO CHINESE WORKERS**

**A 04**

**PUBLISHED AND NON-PUBLISHED ARTICLES  
AND DATA**

**RELATED ACRYLONITRILE**

**EXPOSURE TO CHINESE WORKERS**

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**Investigation of Acrylonitrile Impact on Sex and  
Reproductive Potency of Male Workers**

**An Abstract**

**By Dong Dinglong, Tao Deyan, Man Zhishan**

**Industrial Health Dept. of Safety and Technology Division,  
Daqing Petrochemical General Plant (DAQI 163714)**

## Investigation of Acrylonitrile Impact on Sex and Reproductive Potency of Male Workers

### An Abstract

By Dong Dinglong, Tao Deyan, Man Zhishan

Acrylonitrile is a high toxic substance. Animal Experiment Industry has already approved that it has embryonic toxicity and causes deformity. But so far, in China there is no report of its impact on male workers' sex and reproductive potency. This article will talk about a preliminary survey result on 42 married male workers (exposed group) with over five years of contact in a Chemical Fiber Plant and 22 patients who had an acute poisoning history (poisoning group). The survey was done in accordance with Luo's Sex Function Survey Form and Occupational Reproductive Epidemic Disease Survey Requirements regulated by the National Female Labor and Health Group. The result shows that when the average AN density in the air reached between 1.25% - 16.88mg/m<sup>3</sup>, there was a sex function imbalance. Abnormal sex function people in both poisoning group and exposed group had statistics significance. Overall judgment of several data shows there was as high as 22.7% people in the poisoning group, but there was only 11.9% people in the exposed group. The main problems were impotence and premature ejaculation. Regardless of what group, poisoning or exposed, impotence occurrence was obviously higher than that of control group ( $P < 0.01$ ). In addition, premature ejaculation was more than that of the control group. Poisoning group had more of the above changes, plus it showed an increase tendency. AN had an impact on sex function by means of disturbing nerve system. Exposed group had more premature deliveries and threatened abortion of male workers' wives than that of the control group and  $P$  was smaller than 0.05. Although occurrences of overdue deliveries, natural miscarriage, sterility in the poisoning group and exposed group had no statistics significance, they were higher than that of the control group. It means AN is harmful to the reproductive system. This survey will be a good basis for future research and prevention.

**INVESTIGATION OF PREVALENCE RATE IN WORKERS  
EXPOSED TO ACRYLONITRILE**

**BY**

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**Published on**

**Chinese Journal of Industrial Hygiene**

**April 2000**

## INVESTIGATION OF PREVALENCE RATE IN WORKERS EXPOSED TO ACRYLONITRILE

By WANG Weiqun, XIA Zhaolin, JIN Fusheng, et al

### Abstract:

**Objective:** To investigate the prevalence rate in workers exposed to acrylonitrile (AN).

**Method:** The health examination registration forms of 1121 worker exposed to AN and 489 worker not exposed to any poison were selected. Exposure group included 888 male and 233 female workers, and control group included 290 male and 199 female workers. All of the forms were registered in 1998. According to the standard of disease categories ICD-9, the data was analyzed.

**Result:** The chronic effect of AN workers was mainly digestive system, especially hepatic disease. The prevalence rate of hepatic disease was the highest (12.5%) in workers exposed to AN over 20 years. With advancing of exposure time, the prevalence rate of chronic disease was tending to increase. AN also could induce chronic and accumulative toxicity to circulatory system.

**Conclusion:** There was chronic harmful effect of AN on digestive and circulatory system, especially on liver of human bodies.

**Key words:** Acrylonitrile; Prevalence rate

In a petrochemical corporation, workers were exposed to different kinds of toxicity. The most toxic and effective substance to workers was acrylonitrile (AN). AN is an important monomer to produce acrylic fiber, acrylonitrile-butadiene rubber, acrylonitrile and butadiene-styrene (polystyrene) ABS engineering plastics. It is also an environmental hazardous substance in the petrochemical industry. Yearly AN production output had already reached 230,000 ton in 1993. It is still increasing every year. In recent years, its acute intoxication has been understood deeply, but its chronic toxicity is not understood greatly. We have done analysis to AN workers exposed, in order to have a better understanding of its chronic effects to human bodies.

### **Target and method**

1. Survey target and information resource:

- (1) exposed group: 1121 ACN workers, from the acrylonitrile plant and acrylic fiber plant in a petrochemical corporation, were selected to be in the exposed group. There were 888 male workers and 233 female workers. Their age was between 20 to 59 years old. The average age was 36.24 years old. Their exposure years were between 1 to 38 years. The average year of exposure was 13.87 years. All the information was from 1998 Occupational Health Exam Registration Forms.
- (2) Control group: 489 people who were professors, administrative workers and researchers in a university of the same area and who had no exposure to AN were selected as the control group. There were 290 males and 199 females. Their age was between 25 to 60 years old. The average age was 40.75 years old. The length of service was between 3 to 30 years. The average length of service was 10.96 years. All the information was from 1998 University Health Exam Forms. The general gynecology surveys of that year were used as women information.

2. Method: All the information collected was first numbered and then put into the computer. Samples of workers with occupational exposure or who got sick after the contact (based on ICD-9) were selected for prevalent analysis.

## Result

### A. Disease disposition of people surveyed

1. Comparisons of prevalence rate of people with different length of service between exposed group and control group: Table 1 shows the facts. Prevalence rates increased as the length of service increased. Prevalence rate of people with under 10 years of service was lower than that of control group, while prevalence rate of people with service years between 10 to 19 was higher than that of control group. But there was no significant difference ( $P>0.05$ ).

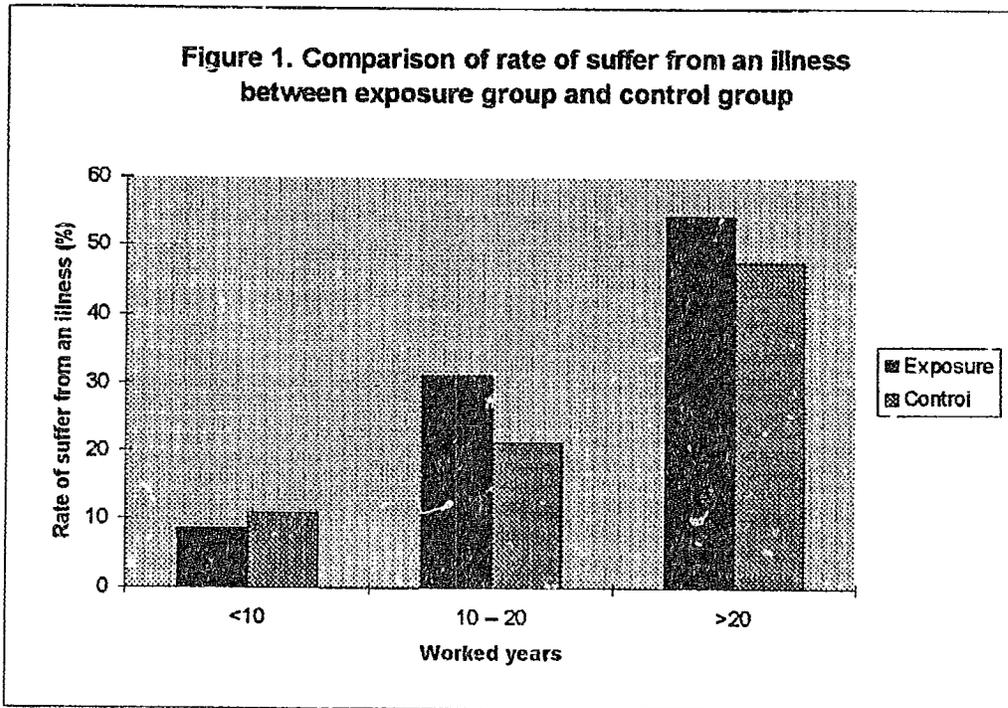
2. Analysis of prevalence rate of people in the two groups: Table 1 shows in the exposed group the no. one disease was digestive system among all systems, next was circulatory system, and no. three was respiratory system. They were followed by urinary system, reproductive system, endocrine system, nervous system, blood system, skeleton and joint system. In the control group, no. one and no. two were also digestive system and circulatory system. No. three was endocrine system. They were followed by urinary system, reproductive system, respiratory system, skeleton and joint system, nervous system and blood system. The prevalence rates of respiratory system and digestive system in the exposed group were higher than control group. Their difference was significant ( $P<0.05$ ,  $P<0.01$ ).

**Table 1. Comparison of illness between exposure group and control group**

Type of illness	Exposure group (n=1121)			Control group (n=489)		
	Case of illness	Rate of suffer from an illness	Order	Case of illness	Rate of suffer from an illness	Order
<b>Respiratory Apparatus</b>	23	2.05	5	2	0.41	10
Bronchitis	3	0.27		1	0.20	
Other's disease	20	1.78		1	0.20	
<b>Circulatory system</b>	57	5.08		32	6.54	
Hypertension	34	3.03	3	21	4.29	1
heart disease	9	0.80	8	7	1.43	5
Other's disease	14	1.25		4	0.82	
<b>Digestive system</b>	190	16.95**		40	8.18	
Stomach disease	49	4.37	2	14	2.86	2
Intestines disease	17	1.52	6	8	1.64	5
Liver disease	91	8.12**	1	9	1.84	3
Gallbladder disease	33	2.94	4	9	1.84	3
<b>Urological and reproductive system</b>	17	1.52		10	2.04	
Kidney disease	10	0.89	7	1	0.20	
Gynaecology	3	0.27		7	1.43	5
Ovary cyst	0	0.00		2		
Other's disease	4	0.34		0	0.00	
<b>Nervous system</b>	6	0.54		1	0.20	
Apoplexy	2	0.18		0	0.00	
Other's disease		0.34		1	0.20	
<b>Endocrine system</b>	14	1.25		1	2.25	
Thyroid Gland	7	0.62	10	6	1.23	8
Diabetes	4	0.34		1	0.20	
Other's disease	3	0.27		4	0.82	
<b>Blood system</b>	6	0.54		1	0.20	
Anaemia	1	0.09		1	0.20	
Thrombocytopathy	4	0.34		0	0.00	
Other's disease	1	0.09		0	0.00	
<b>Skeleton and joint system</b>	5	0.45		2	0.41	10
Five sense organs disease	8	0.71	9	4	0.82	9
Dermatology disease	4	0.34		0	0.00	
Other's disease	6	0.54		1	0.20	
<b>Total</b>	336	29.97		104	21.27	

\* P<0.05, \*\* P<0.01;

Except intestines disease, organ disease prevalence rate was different between the two groups. The order in the two groups was different. There was a significant difference in hepatic disease occurrence ( $P < 0.01$ ). The prevalence rate of gynecologic tumor was higher in the control group than exposed group.



### B. Stratification analysis of prevalence rates in each system of the two groups

Sex stratification analysis: In comparison of diseases in each system, digestive system disease prevalence rate (17.68%) in the male workers of exposed group was higher than that of control group (6.55%). Its difference was apparent ( $P < 0.01$ ). Age stratification analysis: Digestive system disease prevalence rate (18.21%, 26.03%, 17.86%) among the age groups of 30-39 years old, 40-49 years old and over 50 years old in the exposed group was higher than those of control group (9.95%, 9.09%, 6.67%). Its difference was apparent ( $P < 0.01$ ). Length of service stratification analysis: Digestive system prevalence rate (28.75%) in the groups of people with over 20 years of service was higher than that of control group (2.5%). The difference was apparent. Circulatory system disease prevalence rate (1.10%, 3.30%) in the two groups of less than 20 years of service was lower than that of control group (4.06%, 10.85%). The difference was apparent ( $P < 0.05$ ,  $P < 0.01$ ). But circulatory system prevalence occurrence rate (10.94%) in the groups of more than 20 years of service was higher than that of control group (1.25%). The difference was apparent ( $P < 0.01$ ).

### C. Analysis of major prevalence rates between exposed group and control group

Table 2 shows hepatic disease prevalence rate, in the exposed group of male workers, in the age groups of 30-39 years old (9.29%) and 40-49 years old (8.45%), in the length of service groups of 10-19 years (9.89%) and over 20 years (8.91%) was higher than that of control group. Its difference was apparent ( $P < 0.01$ ). In the control group, hypertension prevalence rate in the groups of less than 10 years of exposure and 10-19 years of exposure was higher than that of exposed group. Hypertension in the exposed group was mainly in the groups of people with over 20 years of service (7.63%). The prevalence rate in the control group was only 1.25% in the same level of groups.

### Discussion

A large number of researches indicate that AN can be harmful to organism in many ways. AN acute intoxication shows similar manifestation of cyanide acid intoxication<sup>[1]</sup>. People with serious intoxication would die of breath failure.

AN chronic toxicity effect research is mainly from animal experiments. Usually their emaciation, weight loss and some abnormal conditions of nervous system are observed. Whether AN has chronic toxic effects during metabolic process in human bodies is still not confirmed by any reliable source. In our comparison of prevalence rate of people in the different length of services between exposed group and control group, exposure to AN for under 10 years, prevalence rate in the control group was higher than exposed group, but exposure to AN for over 10 years, rate of the exposed group was higher than control group. May be new workers' health was generally better than professors and staff in the university, that's why prevalence rate was higher than exposed group. As the exposure time of AN increased, prevalence rate had an increase tendency in the exposed group. And it surpassed control group. That pointed out that AN has chronic toxic effects to human bodies. However, this issue needs further research and approve.

Overall analysis of resources for this article indicate that the chronic effects of AN to human beings health is mainly the effects to the digestive system and liver. In the male workers, abnormal liver function rate was higher. The prevalence rate of people in their 30-49 years of age and with over 10 years of service was higher. There was a significant difference in comparison with the control group ( $P < 0.05$ ,  $P < 0.01$ ). And prevalence rate of occupational exposure of AN for over 20 years was the highest rate (12.5%). There was a rate increase tendency for AN workers as their years of service increased. This pointed out that AN has chronic effects to liver in human bodies. Disputes of AN toxicity and whether it has accumulative chronic effects during metabolic process still exist at present. When most of AN is oxidized to metabolism, AN might affect mitochondria function and biochemical oxidation process and create toxicity to liver cells<sup>[2]</sup>. Mitochondria function is easily affected<sup>[3]</sup> and causes harm to the liver. Although HbsAg

Table 2. Analysis of worker's main illness who exposed to acrylonitrile

	Exposure group							Control group						
	No. Of people	Hypertension	Rate of illness %	Hepatitis	Rate of illness %	Tbp	Rate of illness %	No. Of people	Hypertension	Rate of illness %	Hepatitis	Rate of illness %	Tbp	Rate of illness %
Sex														
Male	888	32	3.60	57	6.42**	2	0.23	290	13	4.48	1	0.34	0	0.00
Female	233	2	0.86	10	4.29	2	0.86	199	8	4.02	6	3.02	0	0.00
Age (years)														
<30	347	0	0.00	3	0.86	1	0.29	50	1	2.00	0	0.00	0	0.00
30 - 39	280	3	1.07	26	9.29**	3	1.07	191	4	2.09	1	2.09	0	0.00
40 - 49	438	21	4.79	37	8.45*	0	0.00	143	5	3.50	3	2.10	0	0.00
50 -	56	10	17.80	1	1.79	0	0.00	105	11	10.48	0	0.00	0	0.00
Worked years														
<10	455	2	0.44	5	1.10*	2	0.44	197	6	3.05	1	0.51	0	0.00
10 - 19	273	2	0.73	27	9.89**	2	0.73**	212	14	6.60	6	2.83	0	0.00
20 -	393	30	7.63	35	8.91**	0	0.00	80	1	1.25	0	0.00	0	0.00

Note:

Only two main type of illness are list

Tbp:

Thrombocytopathy

\*  $P < 0.05$ ; \*\*  $P < 0.01$

analysis is not done in this survey, comparison of abnormal rate of liver function between the two groups showed the significant difference ( $P < 0.01$ ). It implies that AN may be harmful to liver.

The information data of this article shows prevalence rate of the respiratory system in the exposed group was higher than that of control group. The difference was apparent. But there was no much difference in stratification analysis. The main harm of AN to respiratory system is acute effect. It causes pulmonary edema and respiratory irritation symptom. But whether it has chronic effects to respiratory system is still unknown. There are no similar reports from overseas either. Prevalence rate of circulatory system in the two groups of workers with less than 20 years of service within the control group was higher than that of exposed group. The difference was apparent. Prevalence rate of circulatory system in the two groups of workers with more than 20 years of service within the exposed group was higher than that of control group. The difference was apparent. It implies AN may have chronic and accumulative effects to the circulatory system. But the toxicity effects are shown after a certain time of exposure.

After the survey and analysis of this prevalence rate are done, it is concluded that chronic intoxication of AN is mainly to digestive system, especially to liver, and to circulatory system at the same time. As AN production output increases every year, more and more people will be exposed to AN. Therefore, further research and study need to be done, in order to understand AN's chronic toxicity and its toxic mechanism to human bodies. Then proper measurements can be implemented to ensure occupational health of the people exposed.

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**STUDY ON THE EFFECTS OF OCCUPATIONAL  
EXPOSURE TO ACRYLONITRILE IN WORKERS**

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**Published by China Occupational Medical Journal**

**February, 2000**

## STUDY ON THE EFFECTS OF OCCUPATIONAL EXPOSURE TO ACRYLONITRILE IN WORKERS

CHEN Yuqing, CHEN Changfa, ZHU Ping et al

### Abstract:

#### Objective:

To look for the health effects on workers exposed to concentration (1.040 mg/m<sup>3</sup>) acrylonitrile (AN).

#### Methods:

An epidemiological study with 234 workers exposed to AN (subjects) and same number of non-exposed controls were carried out.

#### Results:

The detecting rates of dizziness, hypernesia, chest choking, anorexia in the subject were significantly higher than those in control ( $P < 0.05$ ). The levels of serum Y-GT and urine SCN, compared with that of controls, were significantly increased ( $P < 0.05$ ). The average rate of mIro-nucleus tested in peripheral blood lymphocytes showed significant increase, and smoking might exert synergetic effect on it.

#### Conclusion:

It seemed that the slight adverse effects on the workers exposed to low concentration of AN (less than or equal to 2 mg/m<sup>3</sup> MAC) should not be ignored.

{Key words} Acrylonitrile; health effect; epidemiology study

Acrylonitrile (AN) is a monomer which is widely used in the organic and synthetic industry. Its acute toxicity is understood by the society. It can cause acute toxicity to human beings, and is mainly harmful to CNS system [1,2]. However, whether it can cause slow toxicity if exposed under low concentration for a long time is not certain yet [3]. In order to discuss its slow toxicity to AN workers, we have done studies of a production plant and occupational epidemiology to its AN workers. The following is our report.

### I. Material and Method

#### 1.1 Target:

224 workers who made and used AN in a plant were the exposed group (180 male workers, 44 female workers). Their average age was 38.6 ( 8.2 years old (19-57 years old), average years of service was 13.00 ( 6.54 (1-23 years). There was 79.41% of male workers who smoked. Another 224 workers in another plant who were not exposed to any toxicity were selected as control group. They were about the same age, (difference was smaller than (2 years of age), had similar years of service and smoking ratio and living condition. Research was done on these two groups.

1.2 General health exam.

Physical exam was done to all people involved, through interrogation, internal medicine, neurology, five sense organs and dermatology departments.

1.3 Laboratory examination

Lab tests were done to check blood count, blood protein, white cell, platelet, biology index, ALT,  $\gamma$ -GT, ALP, BUN, biochemical exam index, USCN, GSH, genetic toxicity index and to do analysis of peripheral blood lymphocytes.

1.4 AN test in the air of work place was done.

According to Labor and Health Regulations, a sampling point was set up at the gate of reactor base, refine, polymerization and control rooms to test AN in the air.

II. Study results

2.1 Occupational Health Study

2.1.1 The unit used acrylo plus ammonia oxidation to manufacture AN, then polymer AN to acrylic fibres. The complete production line was imported from overseas, production process was all through pipeline that was sealed very well in an open air frame with good ventilation.

2.1.2 AN test result of the workshop air

Samples were collected. At least 3-4 samplings were made in every sampling point. Test was done every week. Average density was 1.040mg/m<sup>3</sup>.

2.2 AN impact to workers' health

2.2.1 Self-awareness symptoms

See Table 1. The detecting rates of dizziness, falling memory, chest choking, anorexia in the exposed group were significantly higher than those of the control group. ( $P > 0.05$ ). For the physical exam, rate of throat hyperaemia was as high as 48.7%. But it did not show any significant difference from that of the control group.

Table 1. Self awareness and physical symptom between exposure and control

Main symptom	Exposure group		Control group	
	No.of people	%	No.of people	%
Total people	224	100.00	224	100.00
headache & Dizzy	92	41.07*	48	21.43
Lack of physical strength	24	10.71	22	9.82
Insomnia	44	19.64	40	17.86
Failing memory	68	30.36*	30	13.39
Chest choking	30	13.39**	18	8.04
Jaded appetite	30	13.39**	17	7.59
Quiver	8	3.57	6	2.68
Pharynx hyperaemia	82	36.61	78	34.82

\* $P < 0.01$ , \*\* $P < 0.05$

## 2.2.2 Blood count results

Blood protein, white cell and platelet between the two groups had no significant difference ( $P>0.05$ ). See Table 2.

**Table 2. Comparison of blood test between exposure group and control group**

	No. of people	Heamoglobin (g/L)	Leukocyte counts ( $\times 10^9$ /L)	Platelet counts ( $\times 10^9$ /L)
Exposure group	224	139 $\pm$ 12.00	4.16 $\pm$ 1.15	118 $\pm$ 13.90
Control group	224	136 $\pm$ 12.20	4.20 $\pm$ 0.79	120 $\pm$ 14.10

## 2.2.3 Biochemistry and biology index test results:

The results of ALT,  $\gamma$ -GT, ALP, BUN GSH and USCN are listed in Table 3.

**Table 3. Comparison of biochemicals test between exposure group and control group**

	No. of people	ALT (IU)	$\gamma$ -GT (IU/L)	ALP (IU/L)	GSH (mmol/L)	BUN (mmol/L)	USCN (mmol/L)
Exposure group	224	28.23 $\pm$ 20.17	44.32 $\pm$ 32.21*	70.65 $\pm$ 25.12	0.64 $\pm$ 0.12	4.06 $\pm$ 1.10	47.18 $\pm$ 20.66*
Control group	224	26.42 $\pm$ 21.32	40.22 $\pm$ 31.06	76.32 $\pm$ 24.56	0.65 $\pm$ 0.13	3.96 $\pm$ 0.84	43.38 $\pm$ 11.88

\* $P<0.05$

2.2.4 In the above indexes of biochemistry and biology, for the average value of  $\gamma$ -GT and USCN, exposed group was higher than that of control group. After it was processed by statistics, it showed significant meanings ( $P<0.05$ ). There was no big difference in the rest of indexes ( $P>0.05$ ). The above indexes did not show any obvious connection. Peripheral blood lymphocytes study results: Average particle ratio for exposed group was 3.78 ( 2.69% which was higher than that of control group (2.60 ( 0.62%). Their difference was significant ( $P<0.05$ ). Average particle ratio of male smokers in the exposed group (4.05 ( 3.01%) was higher than those of non-smokers (3.45 ( 2.70%) and those smokers in the control group (3.13 ( 1.84%). Their difference was significant ( $P<0.05$ ).

### III. Discussion

AN is a colorless and volatile liquid. It has special almond smell. Its water solubility is 0.073mg. It is easily dissolved in the organic solvent. It is highly toxic, just like hydrocyanic acid[3]. Its vapor or liquid can enter human's body through respiratory tract, skin and digestive system causing acute toxicity and doing harm to CNS. The symptoms are weakness, dizziness, headache, nausea, vomiting or mucous membrane irritation, even twitch, unconsciousness and death [2.3]. Whether long time exposure to AN in a low concentration area can cause slow toxicity is still uncertain. But its effect

on human bodies still exist. 224 workers who were exposed to low AN concentration (1.040mg/m<sup>3</sup>) were surveyed for their health. Nervous system problems were found more in the exposed group (See Table 1). Among them, the rates of dizziness, falling memory, chest choking and poor appetite were significantly higher than those of the control group who had no exposure to toxicity ( $P < 0.05$ ). It complies with the existing reports [3,4]. But except throat hyperaemia, there were no positive symptoms of falling blood pressure and reflex hyperfunction. This might be related to a better work environment and low AN concentration (smaller than the maximum allowable concentration of 2 mg/m<sup>3</sup> by the State).

Positive rate of throat hyperaemia in the exposed group was as higher as 36.61%, but it did not show it was related to years of service, and did not have big difference from that of control group (34.82%) ( $P > 0.05$ ). AN irritation could not be regarded totally as the cause of higher rate of throat hyperaemia. It is also related to geographical conditions and local climate.

Results of studies also showed that AN exposure did not have clear effects on human's blood white cells, platelet, blood protein, ALT, ALP, BUN and GSH, because the data was similar to those in the control group ( $P > 0.05$ ). However,  $\gamma$ -CT and USCN level in the exposed group were higher than that of control group (See Table 3). This difference was significant after statistics was done ( $P < 0.05$ ).

USCN is an index of cyanide exposure. AN is an organic cyanide. Therefore USCN for an exposed person will be higher. In addition,  $\gamma$ -GT is the data to reflect liver function.  $\gamma$ -GT level in the exposed group was higher than that of control group. This means to be exposed to low AN concentration for a long time may have a little toxicity effect on liver. This also conforms to some related research reports [5-7].

Some reports indicate AN can cause GSH be reduced in the red cell [8,9]. But we did not find any GSH reducing in the exposed group. The reason might be because they were exposed to low AN concentration and their working environment was different. Besides, AN mainly causes reduction of GSH in liver and brain, but not GSH in blood<sup>[10]</sup>.

Under the research of the groups, micro particle test result indicated that there were more average micro particles in the exposed group (3.78%) than those of control group (2.60%), in addition, there were more in workers who smoked (4.05%) than those of non-smokers (3.45%). This means long time low concentration of AN exposure may cause a little genetic toxicity effect on human bodies, and there may be a certain coordination between smoking and AN genetic effect. Again, this conforms to some genetic toxicity research reports<sup>[11]</sup>.

In summary, although AN can have a lightly covalent binding in the human bodies and its effect can be weak, it still can cause genetic toxicity and be a suspicious carcinogen. But there are very few reports stating its chronic poisoning fact<sup>[12]</sup>. When this survey was done to long-time low concentration of AN exposed workers (1.040 mg/m<sup>3</sup>), there were no AN chronic poisoning patients found. Therefore, we can say workers who work under the maximum allowable AN concentration that the State allows are safe. However, this survey also indicates that AN is a little bit harmful to human's CNS, liver and genetic toxicological effect. Therefore, health protection, as well as development observation research, is still needed.

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**Study of Occupational Harmfulness to AN Workers**

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## Study of Occupational Harmfulness to AN Workers

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Acrylonitrile (AN) is an important monomer and a chemical intermediate in the organic synthesis chemistry. As petrochemical industry develops, more and more workers will be exposed to AN every year. People have started to pay more attention to its effects. This article will talk about the labor and health study done on an AN workshop of a chemical plant to discuss AN's effects to its workers. The following is the report.

### I. Target and method

#### 1. Survey target:

1.1.1 Exposed group: 93 workers in the AN plant of a chemical plant were selected as the exposed group. Among them, there were 68 male workers and 25 female workers. Average age was 27.94 (±2.67). Years of service: the shortest was 1 year, and the longest was 15 years. There were 9 workers with over 5 years of service, 56 workers with 5-9 years of service, and 28 workers with 10 years of service.

1.1.2 Control group: 96 administrators and office workers with similar labor and living condition, interests including smoking and alcohol, but no exposure to AN or other toxicity were selected as the control group. Among them, there were 58 men and 38 women. Average age was 32.34 (plus or minus 4.92).

#### 1.2 Survey contents

1.2.1 AN unit flow process and workshop sanitary facility: The AN unit with yearly production capacity of  $5 \times 10^7$  kg was put into production in August, 1988. Production method was propylene ammonia oxidation. Propylene, ammonia and air were formed into AN under the temperature of 440°C and catalyst action. See Diagram 1 for its flow process. Material feeding, oxidation, recovery, refine, finished products and material out were all done automatically in the sealed pipeline. There were several rooms in the workshop, such as control room, synthetic room, refine room, cyanide acid room and finished product room. Workers examined each room on a regular basis and they worked mostly in the control room.

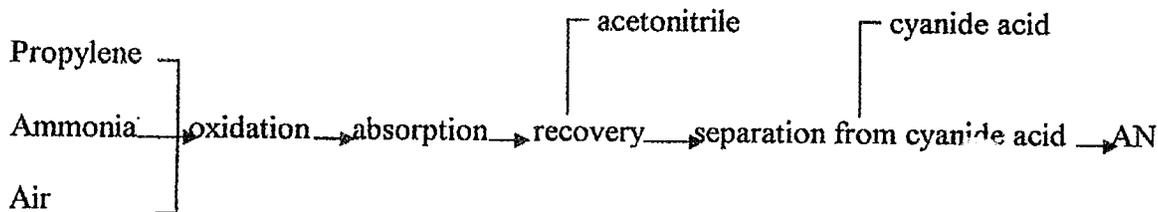


Diagram 1 AN production process flow chart

1.2.2 Air test of AN concentration in the plant: According to the requirements of Petrochemical Health Guardianship Technology Specifications issued by China Petrochemical Corporation (temporary implementation) and the flow process, regular tests of ammonia, cyanide acid, AN and acetonitrile in the air were done at the stop points or fixed operating positions of synthesis room, refine room, cyanide acid room and finished products room.

1.2.3 Health exams of the workers: medical history, occupational history, smoking and drinking history and self-awareness symptoms were systematically asked. Clinical exams, chest X-rays, liver and gallbladder ultra-sound, EKG, biochemical index exam, blood and urine test were done through internal medicine department, surgical department and five sense department.

## II. Results

2.1 Test result of toxic concentration in the air: ammonia, cyanide acid, AN and acetonitrile in the AN unit were tested continuously for three years. Table 1 shows some samples of ammonia, cyanide acid and acetonitrile were a little bit over the national health limit. But average concentration was all under the health standards. 9 ammonia samples were collected within three years. The passing rate was 100%. 126 cyanide acid samples were collected. 98.41% passed. The 2 samples that did not pass the test were from cyanide acid room monitored by computers and worker did not have to go in. 144 acetonitrile samples were collected, and passing rate was 95.83%. 6 samples were a little bit over the limit. The possibility of leakage couldn't be eliminated. The passing rate of 144 AN samples collected for three years was only 37.5%. This meant it had been over the national health limit of 2mg/m<sup>3</sup> all the time. The highest concentration reached 22.79mg/m<sup>3</sup>. Therefore, the main harmfulness of AN unit is considered as a basic occupational hazard to the AN workers.

Table 1. Test of acrylonitrile concentration in the workshop air (mg/m<sup>3</sup>)

		1995			1996			1997		
		No. of Sample	Range	Annual Average	No. of Sample	Range	Annual Average	No. of Sample	Range	Annual Average
Pump room of reaction	Ammonia	1	0.08	0.08	4	0.33-11.18	3.57	4	0.85-7.07	3.17
Pump room of purification	HCN	3	0.02-0.048	0.032	33	0.003-0.018	0.008	36	0.003-0.16	0.035
	Ammonia	36	0.71-50.35	2.05	36	0.01-22.79	7.75	36	0.19-16.57	4.76
	Aceto	36	0.01	0.01	36	0.01-3.98	0.64	36	0.01	0.01
Pump room of HCN	HCN	8	0.007-0.215	0.057	22	0.003-0.184	0.026	24	0.005-1.078	0.155
Pump room of products	Ammonia	12	0.28-3.95	1.09	12	0.01-19	5.59	12	0.01-4.82	2.03
	Aceto	12	0.01	1.01	12	0.01-4.3	0.89	12	0.01-0.13	0.03

### 2.2 Physical exam results

2.2.1 Clinical and physical symptoms: Comparison of self-awareness and physical symptoms between AN plant workers and control group can be found in Table 2. Neurasthenia symptom was highlighted. Headache, dizziness, sleeping disorder, chest choking were significantly higher than those of control group ( $P<0.05$ ). There were also more of trembling of eyes, face and fingers than those of control group. These data were useful for statistics study ( $P<0.05$ ).

Table 2. Self awareness and physical symptom of workers in acrylonitrile workshop

Main symptom	Exposure group n= 93	Control group n=96	X <sup>2</sup>
headache & Dizzy	34	19	6.58*
Insomnia	16	7	4.34*
Lack of physical strength	5	5	0.003
hobby of sleepy	1	2	0.31
Too much dream	10	10	0.006
Failing memory	2	0	2.09
Chest choking	15	6	4.67*
Palpitation	11	5	2.67
Sweater	9	7	0.35
Quiver	3	0	3.15
Pharynx thrusting	2	0	2.09
Trembling of eyelid	38	25	4.67*
Trembling of tongue	6	2	2.22
Trembling of fingers	22	10	5.89

\* <0.05

2.2.2 Blood and biochemical exam results: The ALT abnormal rate of AN workers was 12.90% (12 people) which was 7.29% higher than the control group (7 people). This had no meaning for statistics study. There were 11 people with positive HBsAg (11.85%) which was higher than control group (8 people) (8.33%). Rest of blood and biochemical index results can be found in Table 3. Average value of red cell, hemoglobin, white cell, blood sugar, total cholesterol, triglyceride were all within the normal range which had no significant differences from the control group.

**Table 3. Blood and bio-chemicals testing index of workers exposed acrylonitrile**

		Exposure group (Average ± Error)	Control group (Average ± Error)	t
Erythrocyte	Male	4.8 ± 0.1683	4.8 ± 0.1250	0
	Female	4.3 ± 0.2189	4.2 ± 0.3255	0.83
Hemotoglobin	Male	140.1 ± 3.0249	39.7 ± 3.7440	0.67
	Female	124.4 ± 5.4620	23.2 ± 5.9710	0.8
	Total ( X 10 <sup>9</sup> /L)	5.60 ± 0.4067	5.60 ± 0.4146	0
Leukocyte	Neutrophils %	0.68 ± 0.0067	0.68 ± 0.0071	0
	Lymphocyte %	0.31 ± 0.0066	0.31 ± 0.0074	0
Blood Sugar	(mmol/L)	5.04 ± 1.72	4.77 ± 0.81	1.38
Total Cholesterol	(mmol/L)	4.5 ± 0.69	4.5 ± 1.12	0
Triglyceride	(mmol/L)	1.30 ± 1.10	1.37 ± 1.56	0.35

2.2.3 Urine carbon cyanate test: 93 people were tested. Average value was 4.57 ( plus/minus 2.44mg/L. 96 people from the control group were tested. Their average value was 3.89 ( plus/minus 2.25mg/L (t=2.2286). Comparison of the two groups showed significant differences (P<0.05).

2.2.4 Blood pressure test and other tests: liver and galbladder ultra-sound tests were performed. Tests of 6 people in the exposed group with no history of liver problem, showed their liver diffuse changes (6.45%). This number was a lot higher than that of control group (0 people) (P<0.05). The average value of systolic pressure and diastolic pressure was all within the normal range (See Table 4). In addition, EKG, chest X-ray and clinical exam did not show any positive results.

**Table 4. Blood pressure test of workers in acrylonitrile workshop**

	Systolic pressure (kPa)			Diastolic pressure (kPa)			Pulse pressure (kPa)		
	Average	Error	Range	Average	Error	Range	Average	Error	Range
Exposure	15.7	1.58	12.0-19.3	10.17	1.11	7.4-13.5	5.57	1.36	2.1-8.8
Control	15.7	2.01	11.5-22.7	10.19	1.26	7.1-13.1	5.51	1.42	2.0-9.6
t	0			0.1121			0.3185		
P	>0.05			>0.05			>0.05		

2.2.5 Plant sanitary facility: AN was from propylene and ammonia in the oxidation reactor and then was shipped directly from the pipeline of the plant. The whole process of production, feeding and output were all done in the sealed pipeline. Workers mainly worked in the control room. They went to exam other different rooms according to flow process for several minutes or several tens of minutes.

### III. Discussion

- 3.1 The whole production flow process in the AN workshop of a chemical plant that we did a study on was all done through the sealed pipeline, from material feeding, oxidation, recovery, refine to finished products going out of the plant. Although in each shift, workers were exposed to AN for a very short time, (between several minutes to several tens of minutes), for three consecutive years of testing AN concentration, only 37.5% out of 144 samples passed the test. In general, it had been over the national health limit of 2mg/m<sup>3</sup> all the time. The highest concentration of the sample reached 22.79%mg/m<sup>3</sup>. This working environment was harmful to workers' health. Pipeline needed to be checked out for leakage and spill.
- 3.2 Whether AN is a chronic toxic substance is still uncertain. Physical exam documents indicated there was more neurasthenia in the workers exposed to AN for a long time than that of control group ( $P < 0.05$ ). Although ALT and HBsAg could not be used for statistics purpose, the number in the exposed group was higher which had the same results as before [1]. Ultra sound test was done to workers with no history of liver problem, but the tests showed liver diffuse changes. The rate of this change was significantly higher than that of control group. This meant that long-time exposure to AN might cause liver ultrasound morphological changes of the workers [2]. There were more urine carbon cyanate in the exposed group than that of control group ( $P < 0.05$ ). In summary, the above results indicate that AN has chronic effects to the health of workers. Therefore, workers protection must be enhanced to ensure their safety and good health.
- 3.3 It is very meaningful to protect AN workers' safety and health. This study indicates that HBsAg ultrasound test, along with liver biochemical function test, is meaningful, in terms of monitoring AN workers occupational health. Some reports said AN workers had more serum glycocholate than those in the control group. It is a sensitive data to show liver function changes at an early stage. Therefore, its can be used as a liver index when giving exams to AN workers [3]. AN can be metabolized to sulphur cyanate and be discharged through urine. So sulphur cyanate amount was used as a reference index to see if workers were AN poisoning [4,5]. Overall, selecting sensitive and meaningful exam indexes can reach the purpose of protecting workers' health.

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**Study of Acrylonitrile Hazardous Effects on Workers'  
Reproductive System**

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## Study of Acrylonitrile Hazardous Effects on Workers' Reproductive System

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Acrylonitrile (AN) is an important monomer in the organic synthesis industry. It is highly toxic. It is reported that AN has strong embryonic toxicity and deformity effects on rodents<sup>[1,2]</sup>. But there are few reports on its effects to human reproductive system. Based on our past report<sup>[3]</sup>, we expanded our survey to an epidemiological study of AN workers. This article provides a scientific base for prevention and control of occupational diseases, as well as for health protection of workers and their children.

### 1 Target and method

#### 1.1 Survey target

The exposed group included 939 married workers with at least 1 year of exposure from an acrylic fiber plant, a chemical fiber plant and a chemical plant. There were 548 male workers with an average age of  $33.8 \pm 4.6$ , and average years of service were  $11.0 \pm 4.5$ . There were 391 female workers with an average age of  $32.4 \pm 3.9$  and average years of service were  $10.4 \pm 3.8$ . The control group included 496 male workers and 427 female workers. Their ages, length of service, working condition, life style (smoking and drinking), and schooling were similar, but there was no AN and other toxic substances to affect their reproductive system. Average age of male workers was  $33.2 \pm 4.7$ , and average years of service were  $10.8 \pm 4.5$ . Average age of female workers was  $33.4 \pm 4.3$ , and average years of service were  $11.7 \pm 4.1$ .

#### 2.1 Survey method

Same survey forms were used. Specialists asked and filled in each worker's and their spouses' health condition, their smoking and drinking history, medical history, menstrual period history, family history, occupational history, birth history, as well as dangerous factors of sickness, medicine-taking, X-rays of female workers or wives of male workers. Statistical analysis<sup>[4]</sup> was done in accordance with reproductive occupational epidemiology survey methods issued by National Woman Labor and Health Department, with the exception if husband and wife both were exposed to hazardous substances which were harmful to their reproductive system.

## 2 Result and analysis

### 2.1 AN concentration in the shop air

The chemical plant, acrylic fiber plant and chemical fiber plant were all tested AN concentration in the shop air periodically. See Table 1 for yearly average AN concentration for the recent five years. Table 1 shows that except control room and drawing room, all other rooms often exceeded the maximum allowable concentration of AN ( $2\text{mg}/\text{m}^3$ ). Among them, pump room for finished products, room for raw material and polymerization had the highest concentration.

Table 1. Average acrylonitrile concentration in the workshop air

Working place	mg/m <sup>2</sup>									
	1991		1992		1993		1994		1995	
	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$
Control room	82	0.96								
Pump room for polymerization	30	3.11	20	2.51	20	2.75	20	3.55	24	0.86
Pump room for purification	115	10.22	46	11.41	10	3.29	46	8.67	46	1.67
Pump room for products	12	2.06	12	4.11	12	10.35	12	10.01	12	1.35
Raw materials	33	15.52	26	8.79	22	6.56	24	8.53	24	6.11
Polymerization	123	11.20	84	10.78	82	8.40	82	14.01	84	9.13
Filtering	90	3.73	24	4.86	22	3.00	24	9.54	24	7.83
Spanning	100	2.52	60	3.16	54	2.70	60	6.49	60	9.36
Water washing	76	2.21	36	2.52	34	1.98	36	1.97	36	2.87
Drawing	102	0.53	42	1.24	48	1.92	48	0.53	48	0.11

### 2.1 Reproductive outcome of spouses of male workers in the exposed group

Table 2 shows the statistic analysis results of reproductive system for the wives of male workers in the exposed group and wives of male workers in the control group. The valid pregnancy times of the wives of male workers in the exposed group were 614, and 510 in the control group. The rates of vomiting during early pregnancy, premature delivery, spontaneous abortion, threatened abortion, stillborn fetus and sterility, birth defects were all higher than those of control group.

Table 2. Comparison of reproductive result of worker's wife between exposure and control group

	Exposure group	Control group
Total people	548	496
Times of pregnancy	614	510
Alive newborn	574	494
Normal born	532	466
Vomiting during early pregnancy (%)	29 (4.72)	18 (3.53)
Premature delivery (%)	35 (5.70)	17 (3.33)
Later born (%)	18 (2.93)	13 (2.55)
Spontaneous abortion (%)	29 (4.72)	14 (2.75)
Threatened abortion (%)	12 (1.95)	4 (0.78)
Stillborn fetus (%)	13 (2.12)	3 (0.59)
Birth defects (%)	9 (15.68)	3 (6.07)
Low weight of newborn (%)	22 (3.83)	20 (4.05)
Sterility (%)	21 (3.83)	10 (2.02)

## 2.1 Results of the reproductive system of female workers in the exposed group

There was a significant difference for the rate of vomiting during early pregnancy and premature delivery of female works in the exposed group ( $P < 0.01$ ). Besides, exposed group had more birth defects and stillborn fetus. This was meaningful for statistics ( $P < 0.05$ ). Although occurrence rates of hypertension, anemia, spontaneous abortion, stillborn fetus and sterility had no statistic meaning, they were still higher than control group (See Table 3).

Table 3. Comparison of female's reproductive result between exposure and control group

	Exposure group	Control group
Total people	391	427
Times of pregnancy	413	439
Alive newborn	375	416
Normal born	338	380
Vomiting during early pregnancy (%)	48 (11.62)**	28 (6.38)
Hypertension during pregnancy	13 (3.15)	9 (2.05)
Anaemia during pregnancy	19 (4.60)	10 (2.28)
Premature delivery (%)	34 (8.23)**	17 (3.87)
Later born (%)	13 (3.15)	11 (2.51)
Spontaneous abortion (%)	28 (6.78)	21 (4.78)
Threatened abortion (%)	12 (2.91)	10 (2.28)
Stillborn fetus (%)	11 (2.66)*	3 (0.68)
Birth defects (%)	8 (21.33)*	2 (4.81)
Mortality of newborn (%)	4 (10.67)	2 (4.81)
Low weight of newborn (%)	13 (3.47)*	20 (4.81)
Sterility (%)	11 (2.81)	5 (1.17)

### 3 Discussion

In recent years, more and more people are concerned about the issue of occupational effects on the reproductive system. In terms of AN's toxicity to reproductive system, there were reports stating that AN was embryo toxic and caused deformation. But in China, there are very few reports on its effects of reproductive system to AN workers. We did surveys of the reproductive system on 939 workers exposed from a chemical plant, acrylic fiber plant and chemical fiber plant and 923 workers in a control group.

The results showed when average AN concentration in the air was 0.11-15.52mg/m<sup>3</sup>, it was harmful to the reproductive system of female workers. The rates of vomiting during early pregnancy, premature delivery, birth defects and stillborn fetus in the exposed group were obviously much higher than those of control group. It was also meaningful for statistic purpose, because it was pretty much similar to some reports <sup>[5, 6]</sup>. Although the rates of vomiting during early pregnancy, premature delivery, spontaneous abortion, threatened abortion, stillborn fetus, sterility and birth defects of the wives of the male workers in the exposed group could not be compared with those in the control group, their numbers were all higher. This conforms to a same conclusion of a report <sup>[7]</sup>. This points out AN does damages to the reproductive system.

AN not only is harmful to female reproductive system, but also is harmful to male reproductive system. Husbands exposed to AN from work causes high rate of increasingly dangerous child deliveries of their wives. One reason might be AN directly affects reproductive system of male workers. Another reason might be wives had contacts with their husbands' clothes, skin and hair which had AN on it. The causes of harmfulness to reproductive system need to be further discussed.

Since the shop air had exceeded AN concentration and workers were working under such environment, the danger to affect their reproductive system increased. In order to ensure the good health of workers and their children, AN concentration in the shop air must be reduced greatly.

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**AN OVERVIEW OF THE STUDY OF ACRYLONITRILE  
ON REPRODUCTIVE TOXICOLOGY**

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## AN OVERVIEW OF THE STUDY OF ACRYLONITRILE ON REPRODUCTIVE TOXICOLOGY

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**Keywords:** Acrylonitrile; Reproductive Toxicology

Acrylonitrile (ACN) is an important monomer used extensively in organic synthetic industry for synthesis of acrylic fibers, acrylonitrile-butadiene rubber, ABS engineering plastics and certain types of resins. In 1994, world production of ACN exceeded 3 billion pounds. In 1995, the production capacity had reached 10 billion pounds<sup>(1)</sup>. Currently, besides occupation-related exposure, potential ACN exposure to average people via the use of everyday consumable goods, such as food containers, has attracted increasing attention. There are many studies about ACN toxicity, but up to recently, they are focused on its common toxicity, mutagenicity, and potential carcinogenicity. Studies have discovered potential toxicity of ACN to reproductive system. Reproductive toxicity study of ACN has become an important subject in this field. This article provides an overview of the progress of studies on reproductive toxicology of ACN in recent years.

### 2 Epidemiological Studies on Human Beings

There are very few studies on ACN toxic effects on the human reproduction system. In our country, Wu Weikai, et al.<sup>(2,3)</sup> conducted studies on 534 male workers from heavily contaminated synthetic rubber factories who were exposed to ACN. The study categorized workers based on departments they worked, and analyzed data distribution of the gestational outcomes of their spouses. The data showed that the rate of still birth in the RO department, the rate of newborn defects in the ABS-2 department, and the rate of spontaneous abortion in the acrylic fiber department increased. The relative risks were 4.73 (95% CI, 1.35 - 16.53), 3.89 (95% CI, 1.32 - 11.51), and 2.64 (95% CI, 1.23 - 4.90). There were substantial differences between the exposed group and the control group. For spouses of male workers from other departments and exposed to ACN, their rates of gestational still birth and newborn defect did not differ substantially from the control group.

Similar method was utilized to study 477 female workers exposed to ACN. The results showed that among gestational complications, rates of pernicious vomiting and anemia increased; and among gestational outcomes, rates of premature delivery and birth defects of exposed female workers were significantly higher than those in the control group. The relative risks were 1.637 (95% CI, 1.166 - 2.298), 2.790 (95% CI, 1.661 - 4.687), 1.605 (95% CI, 1.116 - 2.307), and 3.631 (95% CI, 1.237 - 10.664). Among

gestational complications, rates of miscarriage and pregnancy-induced hypertension were also higher than the control group, but the differences were not substantial. Regression analysis of the data showed that exposure to ACN was a risk factor for premature delivery and birth defects. Although other toxic chemical contaminants may exist in the rubber industry, studies have established the reproductive risks of workers exposed to ACN. Moreover, Li Zhilan <sup>(4)</sup> conducted a study comparing working couples who were both exposed to ACN (double-exposed) and those with only female spouses were exposed (single-exposed). Study found that among abnormal gestational outcomes, the rate of newborn death, still birth, gestational complications, premature delivery, post-mature delivery and birth defects were all higher for the double-exposed group than for the single-exposed group.

## 2. Animal Studies

### 2.2 Influences on Reproduction of Female Animals

In the late 1970's, Murrar et al.<sup>(3)</sup> had already found that SD female rats intoxicated during gestational days between 6 to 15 exhibited significant embryo-toxicity (gavage tube feeding 65 mg/kg, inhalation 189.5 mg/m<sup>3</sup>), manifested as increased fetus malformation (short tail, short trunk, missing vertebrae and right-curved aorta). Maternal toxicity was also detected. This study identified a dosage of 25 mg/kg (gavage) or 189.5 mg/m<sup>3</sup> (inhalation) as being teratogenic to rats. Saillenfait et al.<sup>(6, 7, 8)</sup> found that administration of 59.2 mg/m<sup>3</sup> (inhalation) could cause fetotoxicity, manifested as fetus weight reduction, but apparent embryo-toxicity was not observed.

The administration was during days between 6 to 20 of gestation, and maternal toxicity was apparent. However, whole-embryo culture proved that ACN could cause apparent embryo-toxicity, with decreases of yolk sac diameter, crown-rump length, and head length. Levels of embryonic and yolk sac proteins were also reduced.

The chemical also caused dysmorphogenesis of the brain and of the caudal extremity. According to reports from *Environmental Health Criteria* published by IPCS <sup>(9)</sup>, single intraperitoneal injection of 32 mg/kg ACN to animals during 5-7 days of gestation induced embryo-toxicity in AB Jena-hall inbred strain mice, but not in DBA and C57C1 strain mice.

Teratogenicity was not detected when chicken embryos were studied. SD rats intoxicated with 500 mg/kg ACN in drinking water exhibited reduced reproductively and reduced survival rate for the second generation. The female rats of the second generation also exhibited progressive weakness in the muscle of the right arms. Intoxication of pregnant hamster with 80 mg/kg ACN induced fetal skeletal malformation. Pathological studies of the fetuses showed mesodermic changes, including reduced cell counts, cytoplasmic atrophy, increased intercellular space, decreased mitosis and pathological necrosis. Affected fetuses were relatively small in size and developmentally retarded compared to the control group.

## 2.2 Effects on Reproduction of Male Animals

### 2.2.1 Changes of Testicular Morphology and Biochemical Indexes in Male Animals

Abdel-Naim et al. <sup>(10)</sup> administered daily doses of 0, 10.5, 23.46 mg/kg ACN to rats by gavage tube feeding for 4 weeks, and found that their body and testicular weights decreased in a concentration-related fashion. Sperm counts and activity reduced substantially. Light microscopic examination of the tests showed progressive decrease of spermatocyte counts for groups administered more than 23 mg/kg. Long-term intoxication via inhalation and drinking water did not seem to show similar effects. Tandon et al. <sup>(11)</sup> similarly administered 10 mg/kg daily to CD-1 mice for a period of 60 days and found decreases in the sperm counts, degeneration of the somniferous tubules, and decrease of the spermatocytes.

This was accompanied by decreases of testicular sorbitol dehydrogenase (SDH) and acid phosphates (ACP), and an increase in the activities of lactate dehydrogenase (LDH) and beta-glucuronidase ( $\beta$ -G). Recently in our country, Zhang Yumei, et al. <sup>(12)</sup> administered the chemical through subcutaneous injection, and measured the levels of various stimulating hormones in serum and testicular homogenates of rats by RIA. The results showed the serum and testicular T, LH, FSH, E3 levels of rats (intoxicated for 11 weeks) have changed substantially, and the changes correlated well with administered concentration. Light microscopy and electron microscopy also observed different degrees of pathological changes between different dosage groups.

### 2.2.2 Influences on Genetic Materials

Ahmed et al. <sup>(13)</sup> administered to rats an oral dose (46.5 mg/kg) of [2,3-<sup>14</sup>C] labeled ACN to observe its effects on reproductive genetic materials. Covalent binding of radioactivity to testicular tissue DNA was examined for a period of 72 hours. The result showed maximum covalent binding at 0.5 hour (8.9  $\mu$ mol ACN equivalent/mol nucleotide). Binding decreased gradually thereafter, but was still detected after 72 hours of ACN administration (2.5  $\mu$ mol ACN equivalent/mol nucleotide).

They further examined the effects of ACN on DNA synthesis and repair in the tests of rats following an oral dose (46.5 mg/kg) of ACN. A significant decrease in testicular DNA synthesis (80% of control) was observed at 0.5 hour after ACN treatment, while testicular DNA repair was increased 1.5-fold. At 4 hours following administration, testicular DNA synthesis was severely inhibited (which was 38% of the control group), while DNA repair increased more than 3.3-fold at 24 hour post treatment.

These results indicated that ACN might affect the male reproductive function by alkylation DNA in testicular tissue and by interfering with testicular DNA synthesis and repair processes. However, Bjorge <sup>(14)</sup> used a variety of compounds to induce DNA

damage in testicular cells from human organ transplant donors and Wistar rats, and found ACN did not cause statistically important increase in the level of single-strand DNA (ssDNA) breaks.

## 2 Possible Mechanism of Toxicity

Besides the above point of view that ACN affects the male reproductive function by affecting the genetic materials in testicular tissues, Ahmed and Willhite<sup>(15, 16)</sup> postulated that aliphatic nitriles exercised their toxicity through cyanide (CN<sup>-</sup>) release and based on their own unsaturated degree. Since simultaneous administration of thiosulfate, an ionic antagonist of CN<sup>-</sup>, protected hamsters from ACN-caused malformation, they deduced that cyanide release which was implicated in the teratogenic effect of ACN. Abdel-Aziz et al.<sup>(17)</sup> used testicular centrifugal fractions from SD rats to examine the mechanism of ACN metabolism in the testes.

The result showed that testicular ACN metabolism and liberation of CN<sup>-</sup> was through cytochrome P450-dependent mixed function oxidize system. However, depending on different routes of administration, duration of intoxication and other factors, release of CN<sup>-</sup> can differs greatly<sup>(18)</sup>, thus this mechanism awaits further studies. In addition, the toxic effects of ACN on reproduction can be exercised through (1) affecting testicular enzymes: Activities of certain specific enzymes in the testes are regarded as markers for the process of spermatogenesis. Among which, LDH and SDH are implicated in maturation of seminiferous epithelium.

Testes of newborn rats contain high concentration of LDH, which decreases during differentiation of spermatocytes and spermatids. SDH is present in the tail portion of the sperm in the mitochondrion of spermatocytes and spermatozoa. Its concentration increases rapidly during spermatogenesis. The activity of SDH is related to the pachytene stage of spermatocytes.  $\beta$ -G and ACP, both present in lysosomes, are also markers of various differentiation stages of spermatogenesis. During maturation of the testes, the activity of ACP present in different stages of sperm cells increases, and the concentration of  $\beta$ -G in Sertoli cells decreases. Based upon this, Tandon et al.<sup>(11)</sup> concluded that their observations of degenerated seminiferous tubules and decreased sperm counts in intoxicated animals were closely related to the increase of testicular LDH activity, the decrease of SDH activity, the increase in  $\beta$ -G concentration and the decrease in ACP activity; and (2) (the toxic effects of ACN) was related to glutathione (GSH) functions: ACN is an  $\alpha$ ,  $\beta$  unsaturated electrophilic compound, and could react *in vivo* or *in vitro* with GSH non-enzymatically.

ACN could also be oxidized by cytochrome P450 to produce its epoxide, 2-cyanoethylene oxide (CEO). CEO could also react with GSH, and can bind to DNA better than does ACN<sup>(19)</sup>. The increase of urinary thiocyanate, an ACN metabolite, after hamsters were pretreated with BSO, an inhibitor of GSH synthesis, suggests that decreased GSH leads to increased CEO, which alkylates and binds DNA in body cells.

Alkylating pathway could play a critical role in the embryo-toxicity of ACN. Therefore, Saillenfait et al. <sup>(7)</sup> postulated that ACN embryo-toxicity increased after GSH depletion in both *in vitro* embryo and yolk sac were due to increased alkylating capacity of ACN, or increased CEO synthesis, or both. It is likely that testicular GSH level was insufficient to bind ACN and its oxidized metabolite CEO. And the subsequent yolk sac defects and blood vessel formation (which damage the embryonic synthesis and transport of GSH and its precursor) could further aggravate ACN embryo-toxicity.

With fast developments of the chemical industry in recent years, ACN has gained increasingly extensive usage as an important chemical material. Occupation-related and -unrelated exposures also have increased drastically. As a summary, ACN poses potential and severe reproductive hazards to human beings. Currently, studies on ACN toxicity to the reproductive system are far less than sufficient. Forceful epidemiological data are inadequate, animal studies have been limited to a few species and strains, while studies on combined effects of ACN with other compounds are scarce. Therefore, the study of ACN on reproductive toxicity awaits more in-depth efforts.

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**Medical Hygiene Technology Project  
Investigation and Consulting Report**

by

**Xiao Wei**

**Medical Hygiene Technology Project**

**Investigation and Consulting Report**

**Project Name** Study on the Combined Toxicity Effects on Acrylonitrile and Alcohol

**Inspection Purpose** Report on Achievement Award

**Inspection Organization Entrusted** Lanzhou Medical College

**Inspection Requested by** XIAO Wei Title: Associate Professor

**Mailing Address** 7 Dong Gang Road, West, Lanzhou

**Telephone** 8617418 Zip Code: 730000

**Date** December 28, 1998

Inspection organization: Medical Information Research Institute of Gansu Province (seal)

Date: December 28, 1998

**Main Inspection Contents:**

Acrylonitrile (AN) is an organic acrylic substance. It is widely used to synthesize plastics, medicine and dye as an important organic synthetic monomer and chemical intermediate. AN can go through multiple ways to enter the organism and does injuries to it. In recent years, AN toxicity studies are widely done at home and abroad. Researches are conducted on the effects of AN to liver, kidney, brain, thyroid gland, circulatory and reproductive system, as well as effects of carcinogen, teratogenesis and mutagenesis. AN toxicity is better understood now. This research is to use mixed liquid of AN and alcohol to Wistar rats by gavage feeding on an empty stomach. Hepatic toxicity is considered as the main point.

To observe (1) mixed contamination to the toxicity of liver, powerfulness, weakness and characteristics of toxicity; (2) mixed contamination to the effects of the white cells of peripheral blood system, red cells, serum protein, etc. of the experimental animals; (3) mixed contamination to the effects of enzyme activities, such as cholinesterase, GOT, GPT, etc. Through experiments and research and exploration of the combined toxicity, we can provide bases for guiding AN workers to prevent AN chronic intoxication and implement effective protection measurements.

**Key Words**

- #1 Acrylonitrile
- #2 Alcohol
- #3 Toxicity

**Classification (Subject Classification of the National Standards)**

306 Occupational Medicine

**Inspection Scope, Time Limit, etc.**

1982-12/1998 Chinese Documents  
1986-12/1998 Foreign Language Documents

**Data Base Name**

- MEDLINE on CD-ROM
- China Biomedical Documents Data Base (CD-ROM)
- China Technology Journal Titles Data Base (CD-ROM)
- China Biomedical Journal Contents Data Base (CD-ROM)
- China Technology Achievements Encyclopedia
- China Technology and Economic News Data Base
- China Labor and Health and Occupational Medicine Magazine

**Examination Tactic and Formula**

- #1 and #3
- #1 and #2 and #3

**Examination Results**

1. Table of examination results

Total Types of Tools used	Time Limit	Total Documents	Documents picked	Closely-Related do.
7	82-12/98	5,000,000	50	3

**List of related documents**

- (1) Study on Combined Effects of Acrylonitrile and Alcohol on Peripheral Blood In Mouse / XIAO Wei...//China Public Hygiene School, 1997; 16(6): 368-369
- (2) Study on Acrylonitrile to Teratogenesis of Rats Sperm /WANG Zhengquan, et al.// Lanzhou Medical College School Journal, 1995; 21(3): 130-131
- (3) Study on Mixed Effects of Acrylonitrile and Alcohol to Mouse Liver/ XIAO Wei...//Lanzhou Medical College School Journal, 1994; 20(2): 80-81
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- (7) Investigation of AN to the Reproductive System of Female AN Workers and the Health of Their Children / LIAN Suqing, et al. Gansu Scientific Information 1995; (2): 11-14

(The above are articles written by authors in this research project)

- (1) Experimental Research of AN on Early Injuries of Buccal Mucosa in Rats / FU Ping, et al. // Shanghai Oral Medicine 1998; 7(2):63-66
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- (12) Toxicity of Acrylonitrile on Human KB Cells in Culture.
- (13) Comparison of Cancer Risks Projected from Animal Bioassays to Epidemiologic Studies of Acrylonitrile -Exposed Workers.
- (14) Assessment of the Acute Acrylonitrile-Induced Neurotoxicity in Rats.

### **Inspection conclusions:**

According to the key words and requirements provided by the applicant, the following references were checked:

“China Biomedical Documents Data Base on CD-ROM (CBM-DISC, 1982-10/1998)”, “Chinese Language Biomedical Journal Contents Data Base (CMCC) Volume 1-8 of Issue 6 of 1998”, “Biomedical Medicine Data Base 1989-1998” of “Chinese Language Technological Journal Titles Data Base on CD-ROM”, “China Technology and Economic News Data Base, No. 1-11 of 1997”, “MEDLINE on CD-ROM 1996-12/1998”, “China Technological Achievements Encyclopedia 1992-1994, and “China Labor Hygiene Occupational Disease Magazine” issues 1-5 of 1998.

The related 50 articles and closely related 3 articles were selected. The main contents of these articles are in the following:

- (1) Reduction type of GSH in blood, GPT activity, measurement of serum abnormal citric acid dehydrogenase, study of chronic effects on liver and white cells, observation of cell micronucleus rate and chromosome breakage rate and study of reproductive epidemiological survey.
- (2) Animal experiments on AN effects of humoral immunity function, oxidative injuries of DNA, early injuries of buccal mucosa, toxicity effects to cell membrane, teratogenesis of sperm and carcinogenesis.
- (3) Research of the combined toxic effects of acrylonitrile and alcohol (Two articles were written by the applicant).

Based on the overall analysis of documents checked, comparison with the project of the applicant is done in the following:

- (1) There are reports both domestically and abroad in the studies of toxicity on liver, nervous system, teratogenesis, mutagen and carcinogenesis.
- (2) There are reports both domestically and abroad in the studies of GPT activity, measurement of serum abnormal citric acid dehydrogenase and reduction type of GSH in blood, study of chronic effects on liver and blood system, observation of cell micronucleus rate and chromosome breakage rate and study of reproductive epidemiological survey.
- (3) The authors of this project wrote eleven articles. They included: effects on blood cholinesterase activity on AN workers, measurements of GPT activity and four blood biochemical indexes, study on acrylonitrile to teratogenesis of rats sperm and mouse liver, the combined toxicity effects of acrylonitrile and alcohol.
- (4) Except this project, there are no similar reports available on the systematically research of AN toxicity on liver, blood, nervous system and reproductive system.
- (5) Except the articles written by the authors in this project, there are no similar reports of the following three aspects done both domestically and abroad.

The combined toxicity effects: (A) whether mixed intoxication to experimental animals are toxic and the characteristics of powerfulness, weakness and injuries of the toxicity; (B) effects to peripheral blood system; (C) effects of cholinesterase activity, enzyme activities of GPT and GOT.

Reporter: ZHANG Zhongyong      Title: Physician      Signature:

Examiner: YAN, Youlu      Title: Assistant Director of Physician      Signature:

Inspection Organization: Gansu Medical Information Research Institute (seal)

Date: December 25, 1998

**STUDY ON REPRODUCTIVE ORGANS IN FEMALE  
WORKERS EXPOSED TO ACRYLONITRILE**

**By Li Zhilan**

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Lanzhou, Gansu 730000  
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# STUDY ON REPRODUCTIVE ORGANS IN FEMALE WORKERS EXPOSED TO ACRYLONITRILE

By Li Zhilan

Acrylonitrile is a common toxicant in the organic synthetic industry. People are very much concerned with its toxicity research. But there are only a few reports on its damages to human reproductive organs. Therefore, from March to April in 1991, we did a survey of review on reproductive organs of female workers exposed to AN. Our purpose was to protect and control occupational hazards, and to provide scientific evidence to protect the health of workers and their children.

## 1. Survey Target and Method

We chose 379 female workers as the exposed group in a Lanzhou Chemical Industry Company. They were exposed to AN manufacturing, and all married more than a year. Their average age was 33.95 years old (22.75-54.83). Average length of service was 14.10 years (3.25-34.42). In addition, we chose 511 female married workers as the control group in a Bed Sheet Factory and Biological Research Institute. They had no exposure to AN and all married more than a year. The two groups had similar ages and length of service.

## 1.2 Method

Survey forms were filled by professionals. Survey of review was done in accordance with the "Judgment Standards of Observation Data of Child-Bearing of Workers" and "Explanation of Filling the Survey Form" written by the Labor Health & Occupational Disease Research Institute of China, Protective Medical Scientific College.

## 1.3 Statistic Analysis

The statistics did not include people who had a history of marriage of close relatives, family genetic disease, deformity and sterility. When counting times of pregnancy, the situation like abortion, illness, drug, drinking, X-ray exposed during the pregnancy were all excluded.

**STUDY ON THE TOXIC EFFECTS OF  
ACRYLONITRILE ON LIVER**

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## STUDY ON THE TOXIC EFFECTS OF ACRYLONITRILE ON LIVER

By Xiao Wei

**Abstract:** The toxic effects of acrylonitrile were investigated by occupational epidemiological and experimental studies. The results show: the incidence of abnormal SGPT and the mean activities of SGPT were increased in exposed workers, which were higher in male workers than female workers ( $p < 0.01$ ). The major damage of experimental animals were the structural and functional changes of mitochondria, endoplasmic reticulum and cytomembrane in liver. Alcohol could speed up and make the hepatic injury worse. It is considered that the toxicity of acrylonitrile on liver is not an acute reaction.

**Keywords:** Acrylonitrile; Liver; SGPT; Ultra-structure; Enzyme histochemistry

Acrylonitrile is an important synthetic organic monomer and chemical intermediate. It is used widely and professional exposed to large amount of people. This article did a survey on the occupational AN exposed workers and researched on the experimental rats about their subacute liver toxicity effects. It provides scientific evidence to completely and systematically explore non-acute liver toxicity effects of AN, helps to understand toxic effect mechanism and protect the health of occupational workers.

### 1. Occupational Epidemiological Survey

#### 1.1 Survey Target

Exposed group had 372 workers in a chemical industry company. Among them, there were 221 male workers whose ages were 18-56, and average age was 37. Exposed time of AN was 1-31 years, average exposure time was 16 years. The control group had 186 administrators and researchers who had no exposure to AN in a research institute. All the people surveyed had no history of liver problems and no exposure or usage of the drug that affected their liver.

#### 1.2 Test Methods

Regular clinical tests were done to those people surveyed. First collect blood from the vein with empty stomach. Do the test in the lab right away. Anybody whose SGPT was  $19\mu\text{mol}/(\text{min}\cdot\text{L})$ , they were positive.

### 2. Experiment on Animals

#### 2.1 Animals and their disposal

AN group: 48 healthy adult Wistar rats that were 10 weeks old. Their weight was 180-200g. The ratio of female and male was 2:1. They were divided into 1 comparison group and 3 contaminated groups. In the 3 contaminated groups, use 20, 40 and  $60\mu\text{g}/(\text{g}\cdot\text{d})$  of AN to pour into their empty stomach in the morning, once a day and 6 days

a week, total was 12 weeks; AN ethanol group: 24 healthy and adult Wister rats that were 10 weeks old. Their weight was 180-200g. The ratio of female and male was 2:1. They were divided into 1 comparison group and 3 contaminated groups. In the 3 contaminated groups, use 40 of AN, 1000 of ethanol and AN ethanol mixed liquid (40+1000) $\mu$ g/(g.d), to pour into their empty stomach in the morning, once a day and 6 days a week, total was 8 weeks. Weight the animals in each group every week at the same time. Calculate the amount of toxic liquid given according to their weight. After contamination, release blood from their vein and let them die. Collect each specimen and observe them. AN was provided by Lanzhou Chemical Company. The analysis of color method showed the purity was 99.78%.

## 2.2 Data and Methods

### 2.2.1 Serum Enzymes:

Each enzyme and test methods used are shown in Table 1.

Table 1. Test Methods of Serum Enzymes

Enzymes	Test method
ChE	Color development process of acetyl-chlorine
LDH	Oxidation of lactic acid
$\gamma$ -GT	Spec. method
ZnTT	Turbidimetry
TTT	Turbidimetry
GPT	Lai's method
GOT	Lai's method
AKP	Jin's method

### 2.2.2 Light Microscope Observation:

After animals were put to death, get left side and center of liver tissue and use 10% formaldehyde to firm them. Make specimen at a conventional way. Use HE to color it. Observe under light microscope.

### 2.2.3 Enzyme Observation:

After animals were put to death, get left side and center of liver tissue and use liquid nitrogen to freeze them. Do culture under the temperature of  $-20^{\circ}\text{C}$ . Use conventional enzyme and chromatic method. Observe them under light microscope. Items and methods are shown in Table 2.

**Table 2. Liver Enzyme Tissue and Chromatic Methods**

Name	Chromatic Methods
SDH	Standard dehydrogenate method
ICDH	Standard dehydrogenate method
GDH	Standard dehydrogenate method
CCO	DAB
β-OHBD	Standard dehydrogenate method
NADPHD	Standard dehydrogenate method
G-6-P	Pb-method
LDH	Standard dehydrogenate method
G-6-PD	Standard dehydrogenate method
GPDH	Standard dehydrogenate method
ACP	Azo coupling method
5'-N	Pb-method
ATPase	Mg-method

**2.2.4 Electron Microscope Observation:**

Pick 3 rats, two male and 1 female. After putting them to death, quickly open their stomach, take center liver tissue, use to firm them. Make conventional electron microscope specimen. Use LKB-II Super Microtome to cut sections. Double chromate with acetic acid uranium and ICDH. Use JEW100-CX electron microscope to observe.

**3 The Result**

**3.1 Occupational Epidemiological Survey**

Comparison was done to the positive people with SGPT activity increase in the exposed group and control group. The result is shown in Table 3 and 4.

**Table 3.  
The positive rate of activity increase of SGPT among exposure and control**

	Number of positive reaction	Number of negative reaction	Total	Rate of positive (%)
Exposure	153	219	372	41.13(1)
Control	9	177	186	4.80
Sub-total	162	396	558	29.03

(1) P<0.01

(2) SGPT -- serum glutamicpyruvic transaminase

**Table 4.**  
**The positive rate of activity increase of SGPT of male and female in exposure group**

	Number of positive reaction	Number of negative reaction	Total	Rate of positive (%)
Male	111	110	221	50.23(1)
Female	42	109	151	27.82
Sub-total	153	219	372	41.13

(1) P<0.01

Activity of SGPT of different sexes in exposed and control groups are shown in Table 5,6,7.

**Table 5. Activity of SGPT of male and female in control  $\mu\text{mol}/(\text{min.L})$**

	n	$\bar{x}$	s	P
Male	125	7.65	4.94	>0.05
Female	61	6.89	5.42	
Sub-total	186	7.39	5.1	

**Table 6.**  
**Comparison of SGPT activity of male and female among control and exposure  $\text{mol}/(\text{min.L})$**

	n	$\bar{x}$	s	F	P
Control	186	7.39	5.1	71.99	<0.01
Exposure (male)	221	25.29	21.12		
Exposure (female)	151	15.29	12.39		

**Table 7.**  
**Comparison of SGPT activity between exposure and control, and between male and female**

Control Group	Xa-Xb	a	q	Bound of q (1)		P
				0.05	0.01	
Control vs. Exposure of male	17.89	3	16.07	3.31	4.13	<0.01
Male vs. Female in exposure	9.99	2	8.97	2.77	3.64	<0.01
Control vs. Exposure of female	7.9	2	7.1	2.77	3.64	<0.01

(1) Newman - Kculs method

Comparison of SGPT level of workers with different service length in the exposed group is shown in Table 8, 9 and 10.

**Table 8: The average SGPT level of male with different service length in exposure**

Service Length	n	$\bar{x}$	s	F	P
(A) 1-	36	18.95	10.57	3.63	<0.01
(B) 5-	52	31.15	20.97		
(C) 10-	27	20.86	15.73		
(D) 15-	55	26.99	18.72		
(E) 20-	51	21.84	17.03		

**Table 9. The average SGPT level of female with different service length in exposure**

Service Length (a)	n	$\bar{x}$	s	F	P
1-	30	16.57	12.62	1.28	>0.05
5-	28	13.96	10.4		
10-	24	20.71	20.59		
15-	29	14.98	13.52		
20-	40	13.15	9.06		

**Table 10. Comparison of SGPT Level of male among different exposed years**

	$X_A - X_B$	a	q	bound of q		P
				0.05	0.01	
A - E	12.2	5	4.18	3.86	4.6	<0.05
A - D	10.29	4	3.23	3.63	4.4	
A - C	9.31	3	3.5	3.31	4.12	<0.05
A - B	4.16	2	1.6	2.77	3.64	
B - E	8.04	4	2.78			
B - D	6.13	3	1.94			
B - C	5.15	2	1.97			
C - E	2.89	3	0.99			
C - D	0.98	2	0.31			
D - E	1.91	2	0.56			

### 3.2 Experiment Results on Animals

#### 3.2.1 Regular Condition and Serum Enzyme Data:

Thirty (30) minutes after pouring the toxicant, all animals appeared to be excited, restrainable and light anesthetic, losing appetite and weak. The level and time sequence of their expression were related to the amount of toxicant. Each serum enzyme in the animals tested had changes but no statistical difference.

### 3.2.2 Light Microscope Observation: Control group:

No liver cell and tissue were seen for special changes. Exposed group: one sample in the low dosage group, three samples in the medium dosage group and four samples in the high dosage group. In the high dosage group, liver had extravasated blood, cell color was not well distributed, cell and cell nucleus were swollen, cell was loosely distributed and sizes of cell nucleus were different. The problems were more obvious in the group of high dosage and the group with AN ethanol mixed.

### 3.2.3 Electron Microscope Observation: Control Group:

Liver tissue bound was clear. No shape change in cell membrane and cell nucleus. Exposed group mainly showed tissue loose under low multiple electron microscope in the medium and high dosage groups, and oedema of cell nucleus, imbalance of density, obscurity of cell membrane structure under high multiple electron microscope. Linear particle is swollen and its structure was damaged; inner net of rough surface was enlarged, depolymerized and threshing; cell was swollen. The change in the high dosage group and AN ethanol mixed contamination group was very clear.

### 3.2.4 Enzyme Tissue Chemical Changes:

Activity changes of each enzyme to the comparison group are shown in Table 11. The characteristics of enzyme tissue chemical changes are: the activity of SDH, ICDH,  $\beta$ -OHBD in each contaminated group was all reduced, the high dosage group had the most changes; CCO, ATPase, LDH were reduced in the medium and high dosage groups; G-6-Pase, GDH, GPDH were reduced in the high dosage group; all three groups had increase of NADPH and decrease of G-6-PDH. The difference between the groups were not big. Beside CCO, GPDH had no obvious change, other enzyme activity change was the same as that of in the high dosage group, and AN ethanol mixed contaminated group.

**Table 11. The chemical changes of enzyme tissue in the contaminated group (1)**

Enzymes	Low dosage group	Medium dosage group	High dosage group	Mix contaminated group
SDH	↓	↓	↓	↓
ICDH	↓	↓	↓	↓
GDH	(-)	(-)	↓	↓
CCO	(-)	↓	↓	(-)
β-OHBD	↓	↓	↓	↓
NADPHD	↑	↑	↑	↑
G-6-P	(-)	(-)	↓	↓
LDH	(-)	↓	↓	↓
G-6-PD	↓	↓	↓	↓
GPDH	(-)	(-)	↓	(-)
ACP	(-)	(-)	(-)	(-)
5'-N	(-)	(-)	(-)	(-)
ATPase	(-)	↓	↓	↓

#### 4 Discussion

In recent years, occupational health protection reports point out AN causes nausea, vomiting, loss of appetite, liver pain and liver enlargement. It has a potential liver toxic effect. In this occupational health survey, the above symptoms were found. SGPT was used as a fixed test quota and had a good objectivity. The other elements of increasing SGPT activity was eliminated. In the exposed group, positive rate was still higher than the control group ( $P < 0.05$ ), SGPT level in male workers was clearly higher than that of in female workers ( $P < 0.05$ ). Reports also point out occupational workers repeatedly exposed to AN for a long time will increase their abnormal positive rate of SGPT and average level. Although there is no statistical meaning for SGPT change of the experimental animals, multiple quotas show that AN has subacute liver contamination effects.

SDH is scattered in the inner membrane of linear particle and ICDH is scattered in the linear particles. GDH is in the linear particles and relates to the GD oxidation; β-OHBD relates to the fat acid oxidation(3). The reduced activity of the above enzyme means linear particle function goes down as well. Electron microscope shows the shape of the linear particle is abnormal. Therefore, AN can obstruct the function of linear particle, damage the structure, and block cell energy metabolism and reduce production of ATP. Because the energy that the cell system needs is not enough, damage therefore done to the cell and tissue.

NADPH and G-6-Pase are the symbols of enzyme in the inner net. The former is involved in antidotal activity and the latter is involved in the process of sugar production. When NADPH of the animals in the contaminated group increases, the antidotal effects in the inner net increase; When G-6-Pase in the high dosage group and AN-ethanol mixed contaminated group reduces, the inner net is damaged. The ultramicroscopic structure observation discovers that some of the specimen of the contaminated group have damages of their inner net structure on the rough surface. This is the same result done by other authors(4). Since the structure function of the inner net on the rough surface is abnormal, it will affect protein compound in the cell (5).

ATPase exists in the cell membrane, and it is an important energy metabolite enzyme. It resolves ATP and releases energy. AN can reduce ATPase, in addition, it can reduce organic membrane enzyme, such as SDH and CCO. which means organic membrane is affected. Even though the electron microscope does not find direct and obvious damages of the organic membrane, cell is dropsy and cell nucleus and some cell are swollen in each contaminated group. And it also relates to the amount of dosage. In addition, energy metabolite and protein compound blockage caused by AN also affect the normal metabolism and structure maintenance of the organic membrane.

Alcohol does not damage animal liver. But under the same dosage and same time of contamination, AN and ethanol mixed contaminated group has more animal liver damages than other groups. Changes are the same as in the high dosage and long time AN exposed group. This points out that ethanol can speed up and make the hepatic damages worse. Occupational epidemiological survey result shows that among workers exposed to AN, there are more abnormal SGPT and a higher average level in male workers than that of in female workers. Whether this relates to the alcohol drinking or not needs to be explored further.

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**EFFECTS OF ACRYLONITRILE ON ACTIVITY OF  
BLOOD CHOLINESTERASE**

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# EFFECTS OF ACRYLONITRILE ON ACTIVITY OF BLOOD CHOLINESTERASE

By XIAO Wei

**Abstract:**

**Objective:** To recognize the toxic effects of acrylonitrile by studying of blood cholinesterase of the workers and rats exposed to acrylonitrile

**Methods:** Collected the blood of the exposed workers and rats and assessed the activity of the blood cholinesterase.

**Results:** The activity of blood cholinesterase is much lower with the exposed workers ( $p < 0.01$ ), and it has the dose response relationship.

**Conclusion:** Acrylonitrile reduces the activity of blood cholinesterase

{Key word}: Acrylonitrile; Blood; Cholinesterase

Acrylonitrile is an important material for organic synthesis chemistry. With more and more broad-spectrum study on the toxic effects of acrylonitrile, we have gained better understanding on its toxicology. However, there has no reports on acrylonitrile effects on the activity of cholinesterase. In order to understand the chronic toxic effects of acrylonitrile, and to research for meaningful diagnosis and/or health monitoring indicators for chronic acrylonitrile poisoning, this study conducted whole-blood cholinesterase activity tests on 273 workers working on acrylonitrile from a chemical industry company. We also conducted corresponding animal studies.

## 1 Subjects and Methods

### 1.1 Epidemiological Study

#### 1.1.1 Epidemiological Subjects

The exposed group was composed of 237 workers exposed to acrylonitrile in three separate departments of a chemical industry company. Among which were 183 males, aged 18-56, averaging 37 years old. They had been occupationally exposed to acrylonitrile for 1-31 years, averaging 16 years. The control group was composed of 184 workers from a local research institute who were not exposed to acrylonitrile. Among which 125 were males, aged 19-59, averaging 39 years old. All the subjects had no history of organo-phosphate exposure.

### 1.1.2 Analysis Methods

The acrylonitrile concentration present in the air of the individual departments was measured by SP-2305 gas chromatography. Measurement of whole-blood cholinesterase activity: blood samples were collected from elbow veins of the subjects through venipuncture, and analyzed on-site by ferric trichloride colometric method <sup>(1)</sup>. Control standards were simultaneously measured in the process of all sample analyses using 751 spectrophotometer.

## 1.2 Animal Study

### 1.2.1 Animals and Methods.

Animals used were 24 healthy, adult Wistar rats, 10 weeks old, weighing 180-200g, with male to female ratio of 1:1. Animals were randomly grouped into 1 control group and 3 exposed groups. Two of the exposed groups were administered 20, 40, 60  $\mu\text{g}/\text{g}^{-1}\cdot\text{d}$  of acrylonitrile daily in the morning by gavage tube feeding on empty stomach, once per day, 6 days per week for 8 weeks. Animals from each group were weighed every Monday morning, and the dosages were adjusted by weight. After the intoxication was completed, blood samples were collected from muscular artery and cholinesterase activity was analyzed as described above. Acrylonitrile used in the study, with purity of 99.78%, was provided by the chemical industry company where the subjects of the exposed group in 1.1.1 worked.

## 2 Results

### 2.1 Epidemiological Results

Constant air monitoring of processing acrylonitrile departments showed that values above standard were detected in all three departments studied. Concentration of acrylonitrile varied extensively depending on location, time, and production operation. The results were summarized in Table I.

Table 1. The concentration of acrylonitrile in workshop

Workshop	Times of Measure	Range of Concentration mg/m <sup>3</sup>	Average of concentration mg/m <sup>3</sup>	Times of over standard (Average - 2)/2
A	38	0 - 101.6	15.27	6.89
B	35	0 - 35.08	7.10	2.55
C	25	0.5 - 40.02	6.62	2.31
Total	98		8.92	3.46

Maximum allowed concentration of acrylonitrile in workshop is 2 mg/m<sup>3</sup>

We also categorized subjects in the control group and the exposed group by gender and length of service. No statistically significant difference was found on the activities of whole-blood cholinesterase, as summarized in Tables 2 and 3.

**Table 2. Comparison of activity of blood cholinesterase between male and female**  
( $\mu\text{Kat/L}$ )

Group	Sex	n	$\bar{x}$	s	P Comparison in the group
Control Group	Male	125	82.61	4.80	> 0.05
	Female	59	82.47	3.78	
	Total	184	82.53	4.57	
Exposure Group	Male	183	45.75	7.44	>0.05
	Female	90	44.71	7.65	
	Total	273	44.93	6.96	

**Table 3. Comparison of activity of blood cholinesterase among worked years**  
( $\mu\text{Kat/L}$ )

Group	Worked Years	n	$\bar{x}$	s	P Comparison in the group
Control Group	0 -	57	82.74	4.22	> 0.05
	10 -	38	82.48	3.35	
	20 -	52	82.39	4.07	
	30	37	81.72	4.37	
Exposure Group	0 -	118	40.22	8.54	>0.05
	10 -	93	40.72	9.61	
	20 -	24	38.89	5.06	
	30 -	38	38.1	4.56	

The activities of whole-blood cholinesterase were compared between subjects in the control group and the 3 exposed groups from different departments. The results were shown in Tables 4 and 5.

**Table 4. The activity of blood cholinesterase of control group and each workshops**  
( $\mu\text{Kat/L}$ )

Group	n	$\bar{x}$	s	P
Control group	184	82.53	4.57	<0.01
Workshop A	138	38.44	5.21	
Workshop B	107	43.14	11.30	
Workshop C	28	53.67	10.97	

**Table 5 Comparison between control group and exposure group, and in exposure group**

	$\bar{x}_A - \bar{x}_B$	a	q value	Bound of q value	P
				0.01	
Control and workshop A	43.89	4	62.7	4.4	<0.01
Control and workshop B	39.19	3	55.9	4.12	<0.01
Control and workshop C	28.66	2	40.9	3.64	<0.01
Workshop C and workshop A	15.23	3	21.7	4.12	<0.01
Workshop C and workshop B	10.53	2	15	3.64	<0.01
Workshop B and workshop A	4.7	2	6.71	3.64	<0.01

Health examinations were conducted on subjects from the control group and the exposed groups. Symptoms related to blood cholinesterase activity were summarized in Table 6.

**Table 6. Comparison of symptom between control and exposure group**

Items	Group	Number of positive reaction	Rate of positive reaction %	$\chi^2$	P
General symptom of neurasthenia	Exposure	139	50.92	106.33	<0.01
	Control	9	4.89		
Depress of breast	Exposure	63	23.08	35.97	<0.01
	Control	5	2.71		
Sweater	Exposure	53	19.41	34.87	<0.01
	Control	2	1.09		
Tremble	Exposure	104	38.10	67.66	<0.01
	Control	8	4.35		

### 1.1 Animal Study Results

Comparisons of whole-blood cholinesterase activity in the animal study were summarized in Tables 7 and 8.

**Table 7. Comparison of activity of blood cholinesterase between control and exposed animals**

	n	$\bar{x}$	s	(μkat/L)	
				F	P
(C) Control group	6	62.17	16.05	3.56*	<0.05
(H) High dosage	5	31.2	2.68		
(M) Middle dosage	6	50.5	15.22		
(L) Low dosage	6	45.17	21.54		

\*  $F(3.19) = 3.13$

Table 8. Comparison of animals between control group and exposure group

	$\bar{x}_A - \bar{x}_B$	a	q value	Bound of q value 0.01	P
C - H	30.97	4	4.77	3.96	<0.05
C - L	16.93	3	2.62	3.58	>0.05
C - M	11.67	2	1.80	2.95	>0.05
M - H	19.30	3	2.97	3.58	>0.05
M - L	5.33	2	0.82	2.95	>0.05
L - H	13.97	2	2.15	2.95	>0.05

### Discussion

Health monitoring and protection procedures conducted on chemical industry workers in recent years have found that workers exposed to acrylonitrile complain about dizziness, headache, weakness, blurry vision, excessive sweating and irritability. Health examinations found varying degree of symptoms, such as pupil constriction, excessive sweating, low blood pressure and tremor. In our study, the whole-blood cholinesterase activities for workers in the 3 exposed groups were significantly lower than the control group. The whole-blood cholinesterase activity also showed statistically important differences among workers from different departments, and the reduction of cholinesterase activity became more apparent with increased acrylonitrile concentration detected in the air of the department. Health examination results showed that exposed workers were prone to symptoms related to lowered cholinesterase activity. Four indicators of lowered cholinesterase activity, i.e., neurological disorder, chest discomfort, excessive sweating, and tremor were all apparently higher for the exposed groups, suggesting that acrylonitrile reduces the blood cholinesterase activity. In the animal study, whole-blood cholinesterase activities of the exposed groups were apparently lower than the control group. Although the difference in cholinesterase activity was statistically significant only between the high dose group and the control group, the medium and low dose groups both showed apparent reduction tendency. Epidemiological study and animal study results both showed the toxic effect of acrylonitrile on reduction of cholinesterase activity.

Organo-phosphate insecticides are strong inhibitors of cholinesterase activity. During Organo-phosphate insecticides poisoning, whole-blood cholinesterase activity reduces drastically, mainly because Organo-phosphate insecticides bind to cholinesterase, blocking access to its activity center and causing it to lose its function of breaking down acetylcholine. Acrylonitrile is a highly toxic organic nitrile. The mechanism of its toxic effect is inhibition of cytochrome oxidase, causing tissue hypoxia.

The mechanism of acrylonitrile on reduction of cholinesterase activity, however, has not been reported. The molecular formula of acrylonitrile is  $\text{CH}_2 = \text{CH} - \text{CN}$ , which is analogous to Organo-phosphate molecules in their molecular charges. Whether this is related to the reduction of cholinesterase activity remains to be determined. Moreover, several possibilities exist with regards to acrylonitrile-induced reduction of cholinesterase activity.

Does acrylonitrile itself or its metabolites cause the effect? Does it direct or indirect effect? Is the reduction of activity caused by blocked metabolism or blocked activity? All of these issues await for further studies <sup>(5,6)</sup>.

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**Study on Acrylonitrile to Teratogenesis of  
Rats Sperm**

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## Study on Acrylonitrile to Teratogenesis of Rats Sperm

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Acrylonitrile (AN) is a volatile, highly toxic organic acrylic substance. As the modern industry develops quickly, the numbers of industrial workers exposed to AN are increasing. In recent years, effects on liver, blood and immunity are studied widely and deeply. There are reports on teratogenesis, carcinogenesis and embryonic of AN toxicity to experimental animals<sup>[1-4]</sup>. But there are no reports on reproductive cell toxicity to female animals. This article will talk about using inhalation of contamination through the respirator tract to observe the toxicity of sperm in rats.

### 1. Material and method

Acrylonitrile was made at Lanzhou Chemical Industrial Corporation. The purity was over 99.5%. Animals were provided by the Animal Department of Lanzhou Medical College. 96 seven-weeks old male rats of Kunming type were selected. Their weight was  $24.47 \pm 3.46g$ . They were divided into 8 groups. Normal control group, positive control group and three groups with different amounts of contamination ( $60mg/m^3$ ,  $90mg/m^3$ ,  $120mg/m^3$ ), were contaminated for 28 days; three groups with different days of contamination (7 days, 14 days and 28 days) were contaminated with  $120mg/m^3$  of AN. Rats in the positive control group were injected 50mg/kg of cyclophosphamide to their stomach once a day for two consecutive days. According to experiment design, rats in all other contamination groups inhaled AN vapor quietly for two hours every day. Contamination was done for 6 consecutive days a week, and one day there was no contamination. During those 6 days, no food or water was fed to the animals. Normal control group had same condition as the contamination group, except no inhalation of AN vapor.

After contamination, normal execution method of cervical vertebrae dislocation of all animals in each group was used to make slices. Cecity method was used for observation. Under the microscope, the count outline of each animal was very clear. There were 500 sperm with good dispersion. Judgment of sperm aberration shape was made in accordance with classification counting requirements written in the "Experimental Methods of Mutagenesis, Teratogenesis and Carcinogenesis of Environmental Chemical Substances" by Huang Xingshu. Rates of sperm aberration and formation of each type of sperm aberration were calculated.

## 2. Results:

2.1 Effects to sperm aberration rates in rats with similar AN contamination time, but different contamination concentration (See Table 1)

**Table 1. Effect of exposed acrylonitrile on the distortion of mouse's sperms**

	Doses (mg/m <sup>3</sup> )	No. of alive mice	Number of observed sperms	Number of Distorted sperms	Rate of distortion (%)
Normal Control		12	6000	73	1.22
Positive control		10	5000	204	4.08
Exposure of acrylonitrile	60	10	5000	129	2.58*
Exposure of acrylonitrile	90	12	6000	166	2.77*
Exposure of acrylonitrile	120	12	6000	187	3.12*

\* P<0.005

Table 1 shows after the group had 60mg/m<sup>3</sup>, 90mg/m<sup>3</sup>, 120mg/m<sup>3</sup> of AN contamination for 28 days, their sperm aberration rates were 2.58%, 2.77% and 3.12% which were higher than that of control group that was only 1.22%. After statistics was calculated, it showed a significant difference. Also as the contamination concentration increased, sperm aberration rate increased as well. This indicated a positive relationship. (related coefficient =0.9712; P<0.05).

2.2 Effects of formation rates of sperm aberration in rats with similar AN contamination days, but different concentration (See Table 2)

**Table 2. Comparison of distortion of mouse's sperms by different exposed doses**

	Doses (mg/m <sup>3</sup> )	Type of distortion of sperms (%)							Total
		No fixed Shape	No hook	Two heads	Two tails	Banana	Folding of tail	Big head	
Normal Control		81.7	11.5	4.1	2.7	0	0	0	100
Positive control		52.9	18.7	5.7	3.4	10.4	3.3	5.6	100
Exposure of acrylonitrile	60	83.0	8.5	3.1	3.1	0	0	2.3	100
Exposure of acrylonitrile	90	84.4	7.8	3.6	3.0	0	0.6	0.6	100
Exposure of acrylonitrile	120	87.5	7.5	3.2	1.6	0	0	0.5	100

**Table 2. Comparison of results of reproductive organs of female between couple and female workers exposed to AN**

	couple exposure			Female exposure			R/Ra	R/Rb	Ra/Rb
	Number of cases	positive	rate of positive	Number of cases	positive	rate of positive			
Sterility %	104	3	2.88	275	7	2.55*	1.13	3.69	3.27
Gestation Complications %	133	28	21.05**	343	71	20.70**	1.02	2.95	2.9
Spontaneous abortion %	133	13	9.77	343	24	7	1.4	1.51	1.08
Immature delivery %	120	19	15.83**	319	32	10.03**	1.58	3.35	2.13
Later born %	120	7	5.83**	319	10	3.13	1.86	3.22	1.73
Stillborn fetus %	120	4	3.33*	319	2	0.63	5.29	4.01	0.76
Congenital defects ‰	116	4	34.5**	317	7	22.1**	1.56	8.21	5.26
Mortality of newborn ‰	116	1	8.62	317	4	12.6	0.68	3.08	4.5
Mortality of pregnancy ‰	120	5	41.7**	319	6	18.8	2.22	3.76	1.69

R--rate of couple exposure group; Ra--rate of female exposure group; Rb--rate of control group

When comparing the rate of stillborn fetus (3.33%) in the couple exposure group and 0.63% in the female exposure group, their difference was obvious ( $P < 0.05$ ). The rates of complication, premature delivery, overdue delivery and deficiency were higher in the couple exposure group than that of in the control group ( $P < 0.01$ ). Besides, stillborn fetus, mortality of pregnancy in the couple exposure group was also higher than that of in the control group. The rates of complication, premature delivery and deficiency in the female exposure group were also higher than that of in the control group. Plus sterility was higher than that of in the control group.

### 3. Discussion and Analysis

It is reported that AN has embryo toxicity and deformative effect to animals. We found in our survey that the rates of sterility, complication of pregnancy, premature delivery, overdue delivery and deficiency were all higher than those of in the control group ( $P < 0.05$ ); Especially when the couple both exposed to AN, the rates of sterility, complication of pregnancy, natural miscarriage, premature delivery, overdue delivery, stillborn fetus, deficiency and mortality of pregnancy were all higher than those of in the female exposure group. So we think AN damages female reproductive organs. We need to further study AN toxicity to male reproductive organs. The reason of causing unusual child-bearing danger should be explored further.

Since shop air has a higher density of AN than the standards and workers work under that condition, the rate of unusual child-bearing danger of the female workers increases. In order to insure the health of the workers and their children, it is recommended to reduce the density of shop air.

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## 2. Survey Results

### 2.1 AN Density in the Shop Air

Monthly test data of 1989 and 1990 provided by the factory were used. Among the 155 test data, average AN density was 16.35mg/m<sup>3</sup> (0-152.88mg/m<sup>3</sup>). 92.90% exceeded the state health standards. (2mg/m<sup>3</sup>)

### 2.2 Result of Reproductive Organs of Female Workers Exposed

Based on statistic analysis, in the exposed group, there were complications (20.80%) and premature delivery (11.62). Deficiency rate was much higher than the control group (25.4%) (P<0.01). In the exposed group, sterility was 2.64% and overdue delivery was 3.87% which were higher than the control group (p<0.05). Although the difference of mortality of new-born and pregnancy had no statistic meaning, their relative data was 4.11 and 2.26 and had a tendency to increase. See Table 1.

Table 1. Comparison of results of reproductive organs of female between exposed group and control

	Exposed			Control			X <sup>2</sup>	RR
	Number of cases	positive	rate of positive	Number of cases	positive	rate of positive		
Sterility %	379	10	2.64*	511	4	0.78	4.84	3.38
Gestation Complications %	476	99	20.80**	770	55	7.14	50.64	2.91
Spontaneous abortion %	476	37	7.77	770	50	6.49	0.74	1.2
Premature delivery %	439	51	11.62**	720	34	4.72	19.08	2.46
Overdue delivery %	439	17	3.87*	720	13	1.81	4.62	2.14
Stillborn fetus %	439	6	1.37	720	6	0.83	0.76	1.65
Congenital defects %	433	11	25.4**	714	3	4.2	10.05	6.05
Mortality of newborn %	433	5	11.5	714	2	2.8	3.4	4.11
Mortality of pregnancy %	439	11	25.1	720	8	11.1	3.29	2.26

\* P<0.05; \*\*  
P<0.01.

In order to discuss the results of reproductive organs of both male and female exposed to AN and female only exposed to AN, we further divided the exposed group into 2 groups, one was for couple exposure, and the other was for female exposure. We found that except mortality of new-born in the couple exposure group, all other data was higher than the female group. For details, see Table 2.

Table 2 shows after contamination, the main sperm aberration shape was not finalized. Compared to natural aberration, they were 83.0%, 84.4% and 87.2%. Examination found no obvious difference.

2.3 Effects to sperm aberration rates in rats with different AN contamination time (See Table 3).

Table 3. Effect of exposed acrylonitrile by different days on the distortion of mouse's sperms

	Exposed Days	No. of alive mice	Number of observed sperms	Number of Distorted sperms	Rate of distortion (%)
Normal Control		12	6000	73	1.22
Positive control		10	5000	204	4.08
Exposure of acrylonitrile	7	10	5000	67	1.12
Exposure of acrylonitrile	14	11	5500	84	1.63
Exposure of acrylonitrile	28	12	6000	187	3.12*

\* P<0.005

Table 3 indicates that using AN concentration of 120mg/m<sup>3</sup> for 7 days, 14 days and 28 days to contaminate rats, their sperm aberration rates were 1.11%, 1.53% and 3.12%. Compared to that of control group, only after 28 days of contamination, sperm aberration rate was higher than that of control group. Therefore, this does not show any significant difference.

### 3. Discussion

Reproductive toxicity<sup>[5]</sup> of any substances can be judged from the sperm aberration experiment. Numbers, shapes and moving abilities of the sperm are the three indexes in the experiment. Among those, observation of sperm shape is more objective and easy; plus, its sensitivity is very close to the other two indexes. Therefore, in this experimental research, sperm shape was selected as observing index. Sperm aberration rate in the normal control group was 1.22% which is close to 1.30% reported by Tophan<sup>[6]</sup> about animal sperm natural aberration rate. This means result is believable.

AN causes the increase of rats aberration rate; besides, it is related to dosage and time. This proves that AN is somewhat toxic to the male rate reproductive cells. Its toxicity mechanism might be because of the following reasons. (1) AN inhaled is oxidized

in the animal body to epoxide, and together with nucleic acid they show conjugate binding <sup>[1,7]</sup> causing DNA damages which are not recoverable and removable <sup>[1]</sup>. (2) AN might disturb endocrine function <sup>[1]</sup>, and cause changes of sex hormone level. (3) The binding between AN and the organism of thiokinase might inhibit lactate dehydrogenase activity of the sperm and cause energy metabolism disorder of the sperm process.

#### 4. Summary

This experiment research has discovered that in a short period of time inhalation of low dosage AN by rats can cause sperm aberration rate to increase, while most of other animals showed no obvious intoxication. This proves AN is harmful to reproductive cells and is potentially mutagenic. Further study needs to be done. Enhancement of protection to the AN workers must be made.

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**STUDY ON THE JOINT EFFECTS OF ACRYLONITRILE  
AND ALCOHOL IN RATS**

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## STUDY ON THE JOINT EFFECTS OF ACRYLONITRILE AND ALCOHOL IN RATS

XIAO Wei

### Abstract:

In order to learn the joint effects of acrylonitrile and alcohol, acrylonitrile (40  $\mu\text{g/g}$ ), alcohol (1000  $\mu\text{g/g}$ ) and the mixed liquid of both were given to rats by gavage for 8 weeks. The results revealed the joint effects of AN and alcohol. Hypo-function and structural lesion in hepatocyte were found in rats. The numbers of white blood cells decreased remarkably in these two groups.

{Key words}: Acrylonitrile, Alcohol, Combined effects, Liver, Blood.

The purpose of this study is to understand the influence of daily alcohol consumption on the toxicity of acrylonitrile, and to explore the relation between daily life behavior and acrylonitrile occupational hazards. Based on this, the study wishes to improve protecting measures against occupational injuries and the health of acrylonitrile workers, and to enhance the overall efficiencies of industrial enterprises.

### 1. Materials and Methods

#### 1.1 Animals

Animals used were 32 healthy adult Wistar rats, 10-week old and weight of  $190 \pm 10\text{g}$ , of which half were male and half were female. Animals were randomly grouped into 1 control group (A) and 3 intoxicated groups (B, C, D). The latter groups were intoxicated as follows: (C) acrylonitrile 40  $\mu\text{g/g}$  (B) ethanol 1000  $\mu\text{g/g}$  (equivalent to daily alcohol consumption of 50g for human) and (D) mixture of acrylonitrile (40  $\mu\text{g/g}$ ) + ethanol (1000  $\mu\text{g/g}$ ). Intoxication was performed daily morning by gavage feeding on empty stomach, once per day, 6 days per week for 8 weeks. Animals from each group were weighed every Monday, and doses were adjusted by weight. After the course of intoxication, animals were sacrificed by femoral artery bleeding. Various samples were then collected for observations and analyses. Acrylonitrile used in the study was chemical reagent used for industrial synthesis, and was analyzed by gas chromatography to have a purity of 99.68%.

## **1.2 Observation Indexes and Analytical Methods**

### **1. Light Microscopic Pathology**

Hepatic tissues from the left middle lobe of the liver were removed immediately after sacrifice and fixed with 10% formaldehyde. A week later the tissue samples were dehydrated, embedded and sectioned according to regular pathological sample preparative methods. The samples were then stained with H & E, and observed under light microscopy.

### **2. Electron Microscopic Observations**

One male and one female rats were randomly selected from each group and sacrificed. Hepatic tissues from the left middle lobe of the liver were immediately removed (2 mm × 3 mm × 5 mm), and fixed with para-formaldehyde in numbered vials. Ten days later the samples were washed, dehydrated, embedded, and sectioned with LKB-I ultramicrotome. The sections were double-stained with unary acetate and lead citrate, and observed with JEW-100-CX electron microscope.

### **3. Enzyme Histochemistry Observations**

Hepatic tissues from the left middle lobe of the liver (2 mm × 5 mm × 5 mm) were removed immediately after sacrifice, wrapped in aluminum foils and snap frozen in liquid nitrogen. The frozen tissues were then sectioned in cryostat (-20 °C). The sections were then stained by enzyme histochemistry and observed with light microscopy.

### **4. Blood Indexes**

Indexes measured included serum enzymes, erythrocyte hemoglobin, serum proteins and complements.

## **I. Results**

### **A. General Conditions**

After gavage feeding, all animals in the acrylonitrile - and mix-intoxicated groups showed signs of repression, paralysis, loss of appetite and lethargy. This was more so for the mix-intoxicated group, which had an earlier onset and lasted longer and was more severe.

## 2.2 Liver Changes

### 1. Hepatic Pathology

For animals in the control and ethanol-intoxicated groups, there were no changes in the size, the morphology or the structure of hepatic cells or its organelles. For the acrylonitrile - and mix-intoxicated groups, there were varying degrees of cell size and nuclear size differences. The changes identified under the microscope included: (1) blurred and incomplete structures of mitochondrial inner and outer membranes and broadened spaces between cristae; damaged or vanished cristae structures; (2) broken, expanded or dissociated structure of the rough endoplasmic reticulum; (3) obvious nuclear swelling with loose nucleoplasm, uneven density, blurred nuclear membrane structure with rough and broken edges; and (4) apparent swelling of cytoplasm with large patches of low electron density regions. The above observations were more obvious in the mix-intoxicated group.

### 2. Changes in Enzyme Histochemistry

A total of 13 enzymes were studied. Activity changes of each enzyme relative to the control group are shown in Table 1.

**Table 1. Comparison of enzyme in liver between control and exposure group**

Enzyme	Exposure of acrylonitrile	Exposure of mixed poison
SDH	↓	↓
ICDH	↓	↓
GDH	(-)	↓
CCO	(-)	(-)
β OHBD	(-)	↓
NADPH	↑	↑
G-6-Pase	(-)	↓
LDH	↓	↓
G-6-PDH	↓	↓
GPDH	(-)	(-)
ACP	(-)	(-)
5-N	(-)	(-)
ATPase	↓	↓

The results showed that there were obvious enzyme histochemistry changes for the acrylonitrile - and mix-intoxicated groups. (1) The activities of the mitochondria marker enzymes SDH and ICDH were both reduced in the acrylonitrile - and mix-intoxicated groups in comparison to the control group; while the activity reductions of GDH and β-OHBD were seen only in the mix-intoxicated group. (2) The activity of endoplasmic

enzyme NADPH increased in both the acrylonitrile - and mix-intoxicated groups; while the activity reduction of G-6-Pase was found only in the mix-intoxicated group. (3) The activity of cytoplasm enzyme LDH, G-6-PDH were similarly reduced in the acrylonitrile - and mix-intoxicated groups. (4) The activity of biological membrane enzyme ATPase was reduced in the acrylonitrile - and mix-intoxicated groups, and there was no appreciable difference between the two groups <sup>(2)</sup>.

### 2.3 Results of Blood Indexes Analyses

There were no statistically important differences for various serum enzymes, serum proteins, and complements measured in the study. But total leukocyte counts, neutrophil counts, lymphocyte counts, and granulocyte counts showed statistical differences. Results of paired-comparisons are shown in Table 2.

Table 2. Comparison of cell counts of animal

	Leukocyte counts	Neutrophils counts	Lymphocyte counts	Granulocyte counts
A - D	< 0.01	< 0.01	< 0.01	< 0.01
A - C	< 0.01	< 0.05	< 0.01	< 0.01
A - B	< 0.01	< 0.05	< 0.05	< 0.01
B - D	< 0.05	< 0.05	< 0.05	< 0.01
B - C	< 0.05	< 0.05	< 0.05	< 0.01
C - D	< 0.05	< 0.05	< 0.05	< 0.05

## I. Discussion

### A. Influences on the Liver

The damage to the liver by combined intoxication mainly manifested as microstructural changes and corresponding activity changes in enzyme histochemistry. (1) SDH, an enzyme distributed throughout the inner membrane of mitochondria, ICDH, present within the matrix of mitochondria, are both components of the tricarboxylic acid cycle enzyme system and involved in aerobic metabolism of glucose. GDH is present in the matrix of mitochondria, associated with alanine metabolism.  $\beta$ -OHBD is mainly involved in fatty acid metabolism.

The activity reductions of the above mentioned mitochondria enzymes indicate reduction of mitochondria functions. This is consistent with the structural damages of mitochondria observed microscopically. (2) G-6-Pase is present in the endoplasmic reticulum and involved in gluconeogenesis. The reduction of its activity suggests endoplasmic reticulum damages. Electron microscopic observations of rough ER expansion, dissociation, de-granulation and reduction in number further substantiate the

suspected damages. (3) ATPase is present on cell membrane responsible for ATP hydrolysis and energy release. The activity of this enzyme was also reduced by the mix-intoxication. Moreover, the activity reductions of other biological-membrane enzymes SDH and G-6-Pase, cytoplasm enzyme LDH, G-6-PD and GPDH along with the electron microscopic observations of swelled cells and nucleus all indicate cell membrane damages suffered by liver cells. The electron microscopic observations that the structures of the cell membrane and nuclear membrane were blurred and broken further substantiate this conclusion<sup>(3, 4)</sup>.

#### **B. Influences on the peripheral blood system**

Mixed-intoxication reduced the WBC counts of the experimental animals. This result is consistent with health monitoring results conducted on acrylonitrile workers over the years. The total WBC counts of human populations exposed to acrylonitrile were obviously lower in comparison to the control population. Furthermore, the abnormality rate of total WBC counts being lower than  $4.0 \times 10^9/L$  was apparently higher than the control population. This proves that mixed-intoxication has the effect of reducing total peripheral WBC counts in both rats and human population. Among differential leukocyte counts, the neutrophil counts, lymphocyte counts, and granulocyte counts of the acrylonitrile - and mixed-intoxicated groups were obviously lower in comparison to the control group. Lymphocyte count of the mix-intoxicated group was only 17.38% that of the control group. It is apparent that reducing the total numbers of immune cells is one of the mechanisms by which acrylonitrile affects the immune system. The conclusion is drawn that mixed-intoxication has apparent toxic effects on leukocytes.

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**Study on Combined Effects of Acrylonitrile and Ethanol on  
Peripheral Blood In Rats**

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## Study on Combined Effects of Acrylonitrile and Ethanol on Peripheral Blood In Rats

### Abstract:

The purpose of this study is to examine the combined effects of acrylonitrile and ethanol on peripheral blood by way of gavage intoxication of experimental animals. Results: (1) Total leukocyte counts of the intoxicated groups are all lower than those of the control group ( $p < 0.01$ ); (2) Among differential leukocyte counts, the absolute values of neutrophils, lymphocytes, and granulocytes of the acrylonitrile-intoxicated and the mix-intoxicated groups are all lower than those of the control group ( $p < 0.01$ ); and (3) Hemoglobin level, average erythrocyte hemoglobin content, and average erythrocyte hemoglobin concentration of the acrylonitrile-intoxicated and the mix-intoxicated groups are all lower than those of the control group. Based upon this, we conclude that acrylonitrile has apparent toxic effects on the blood, and ethanol aggravates the toxic effects of acrylonitrile on cells of the leukocytic lineage.

**Key words:** Acrylonitrile, ethanol, combined effects, blood

With extensive productive and everyday utilization of acrylonitrile, population in direct and indirect exposure to acrylonitrile has increased. The purpose of this study is to further understand the chronicle toxic effects of acrylonitrile, to examine its toxic effects on the immune system and its carcinogenic mechanism, and to study the influences of everyday alcohol drinking on the toxicity of acrylonitrile. We intoxicated Wistar rats by gavage feeding, and under combined effects of acrylonitrile and ethanol, we examined the toxic influences of the latter on the peripheral blood of the Wistar rats.

### 1. Materials and Methods

#### 1.1 Reagents

Acrylonitrile used in the study was provided by Lanzhou Chemical Industry Company. Gas chromatography showed a purity of 99.78%.

#### 1.2 Animals

Animals used were 32 healthy adult Wistar rats, 10 weeks old, weighing 180-200g, with male to female ratio of 1:1. Animals were randomly grouped into 1 control group and 3 intoxicated groups. The intoxicating doses were based on pilot screening studies and on values calculated from an average worker's daily alcohol consumption. The three

intoxicated groups were group A (40  $\mu\text{g/g}$  acrylonitrile), group B (1000  $\mu\text{g/g}$  ethanol), and group C (40  $\mu\text{g/g}$  acrylonitrile + 1000  $\mu\text{g/g}$  ethanol). Doses were administered daily in the morning by gavage feeding on an empty stomach, once per day, 6 days per week for 8 weeks. Animals from each group were weighed on fixed weekly schedule, and doses were adjusted by weights. After the course of the intoxication, blood samples were collected from muscular artery and blood components were analyzed.

### 1.3 Indices and Methods

Indices, including total and differential leukocyte counts, erythrocyte counts, hemoglobin level, average erythrocyte hemoglobin content, and average erythrocyte hemoglobin concentration, were all measured by automated blood cell counter; Serum proteins (A,  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$ ,  $\gamma$ ) were examined using regular electrophoresis devices; Complement 4 ( $C_4$ ) was measured by using automated immunobiochemical analyzer.

## 2 Results

### 2.1 General Conditions

Thirty minutes after feeding, all animals in the intoxicated groups showed signs of excitement, repression, loss of appetite, and subsequent lethargy. The occurrence of abscess rate was high in the intoxicated groups during the intoxicating period.

### 2.2 Statistical Results

- (1) Several animal groups showed substantial differences in their leukocyte counts ( $P < 0.01$ ). Results of paired-comparison are shown in Table 1.

Table 1. Comparison of leukocyte counts of animal

Compared group	Differences $\bar{x}_A - \bar{x}_B$	No. of group a	q value	Bound of q value		P
				0.05	0.01	
A - D	15.00	4	7.46	4.05	5.19	< 0.01
A - C	12.10	3	6.02	3.65	4.79	< 0.01
A - B	8.48	2	4.22	3.00	4.13	< 0.01
B - D	6.52	3	3.24	3.65	4.79	> 0.05
B - C	3.62	2	1.80	3.00	4.13	> 0.05
C - D	2.90	2	1.44	3.00	4.13	> 0.05

- (2) Several animal groups showed substantial differences in their neutrophils counts ( $P < 0.01$ ). Results of paired-comparison are shown in Table 2.

Table 2. Comparison of neutrophils counts of animal

Compared group	Differences $\bar{x}_A - \bar{x}_B$	No. of group a	q value	Bound of q value		P
				0.05	0.01	
A - D	1.78	4	7.41	4.05	5.19	< 0.01
A - C	1.07	3	4.46	3.65	4.79	<0.05
A - B	0.64	2	2.67	3.00	4.13	>0.05
B - D	1.14	3	4.75	3.65	4.79	<0.05
B - C	0.43	2	1.79	3.00	4.13	>0.05
C - D	0.71	2	2.96	3.00	4.13	>0.05

(3) Several animal groups showed substantial differences in their lymphocyte counts (P<0.01). Results of paired-comparison are shown in Table 3.

Table 3. Comparison of lymphocyte counts of animal

Compared group	Differences $\bar{x}_A - \bar{x}_B$	No. of group a	q value	Bound of q value		P
				0.05	0.01	
A - D	13.07	4	7.18	4.05	5.19	< 0.01
A - C	10.64	3	5.85	3.65	4.79	<0.01
A - B	7.38	2	4.06	3.00	4.13	<0.01
B - D	5.69	3	3.13	3.65	4.79	>0.05
B - C	3.26	2	1.79	3.00	4.13	>0.05
C - D	2.43	2	1.34	3.00	4.13	>0.05

(4) Several animal groups showed substantial differences in their granulocytes counts (P<0.01). Results of paired-comparisons are shown in Table 4.

Table 3. Comparison of lymphocyte counts of animal

Compared group	Differences $\bar{x}_A - \bar{x}_B$	No. of group a	q value	Bound of q value		P
				0.05	0.01	
A - D	13.07	4	7.18	4.05	5.19	< 0.01
A - C	10.64	3	5.85	3.65	4.79	<0.01
A - B	7.38	2	4.06	3.00	4.13	<0.01
B - D	5.69	3	3.13	3.65	4.79	>0.05
B - C	3.26	2	1.79	3.00	4.13	>0.05
C - D	2.43	2	1.34	3.00	4.13	>0.05

- (5) There were apparent differences among groups in their hemoglobin values ( $P < 0.05$ ). Results of paired-comparison are shown in Table 5.

Table 5. Comparison of hemoglobin of animal

Compared group	Differences $\bar{x}_A - \bar{x}_B$	No. of group a	q value	Bound of q value 0.05	P
B - D	22.20	4	5.00	4.05	<0.05
B - C	19.60	3	4.41	3.65	<0.05
B - A	11.40	2	2.58	3.00	>0.05
A - D	10.80	3	2.43	3.65	>0.05
A - C	8.20	2	1.85	3.00	>0.05
C - D	2.60	2	0.59	3.00	>0.05

- (6) Erythrocyte counts and average erythrocyte sizes are shown in Table 6. All three indices did not show statistically significant differences among different groups.

Table 6. Testing results of hemoglobin counts, specific volume and average volume of hemoglobin of animals

	hemoglobin counts (X 10 <sup>12</sup> /L)		Specific volume of hemoglobin		Average volume of hemoglobin (fl)	
	$\bar{x}$	S	$\bar{x}$	S	$\bar{x}$	S
Control group	6.80	0.49	0.44	0.03	63.80	1.64
Exposure of ethanol	6.82	0.81	0.44	0.04	64.60	2.51
Exposure of acrylonitrile	7.03	0.48	0.45	0.03	64.20	1.30
Exposure of ethanol and acrylonitrile	7.09	0.72	0.46	0.05	64.60	1.34

- (7) There were apparent differences among groups in their average erythrocyte hemoglobin content ( $P < 0.05$ ). Results of paired-comparison are shown in Table 7.

Table 7. Comparison of average erythrocyte hemoglobin content of animal

Compared group	Differences $\bar{x}_A - \bar{x}_B$	No. of group a	q value	Bound of q value 0.05	P
B - D	3.94	4	4.92	4.05	<0.05
B - C	3.60	3	4.50	3.65	<0.05
B - A	1.70	2	2.13	3.00	>0.05
A - D	2.24	3	2.80	3.65	>0.05
A - C	1.90	2	2.38	3.00	>0.05
C - D	0.34	2	0.43	3.00	>0.05

- (8) There were apparent differences among groups in their average erythrocyte hemoglobin concentration ( $P < 0.01$ ). Results of paired-comparison are shown in Table 8.

**Table 8. Comparison of average erythrocyte hemoglobin concentration of animal**

Compared group	Differences $\bar{x}_A - \bar{x}_B$	No. of group a	q value	Bound of q value		P
				0.05	0.01	
B - D	61.50	4	5.65	4.05	5.19	<0.01
B - C	53.30	3	4.90	3.65	4.79	<0.01
B - A	21.70	2	1.99	3.00	4.13	>0.05
A - D	29.80	3	3.66	3.65	4.79	<0.05
A - C	31.60	2	2.90	3.00	4.13	>0.05
C - D	8.20	2	0.75	3.00	4.13	>0.05

- (9) Electrophoretic analyses of various serum proteins and instrumental measurement of complement 4 ( $C_4$ ) did not show any statistically significant difference among animal groups. No regular pattern was observed for their variability. Mononucleated cell counts of all the intoxicated groups were lower than those of the control group. This was especially obvious for the mix-intoxicated groups. However, there were no statistically significant differences among the groups.

### 3 Discussion

The results of the study showed that leukocyte counts for group C was 60% of group A, 40% of group B, and 22% of the control group. This is consistent with the health monitoring results conducted over the years on population exposed to acrylonitrile. Among differential leukocyte counts, the neutrophils counts, lymphocyte counts, and granulocytes counts of all the intoxicated groups were obviously lower than those of the control group. Among these lowered counts, the reduction of lymphocyte counts was the most apparent. Lymphocyte count of the mix-intoxicated group was only 17.38% of the control group. Accordingly, acrylonitrile shows leukocyte toxicity on both experimental animals and exposed human population. Ethanol aggravates acrylonitrile-induced toxic effects on leukocytes, which is associated with its toxic effects on the immune system<sup>(1-3)</sup>.

The three major indices of hemoglobin level, average erythrocyte hemoglobin content, and average erythrocyte hemoglobin concentration of both acrylonitrile-intoxicated group and mix-intoxicated group were lower than those of the control group, while those of the ethanol-intoxicated group were higher than those of the control group.

Also, the average erythrocyte hemoglobin concentration of group C was substantially lower than that of the control group. Therefore, we conclude that mixed acrylonitrile and ethanol intoxication reduces erythrocyte hemoglobin content. The mechanism of the intoxication may include (1) acrylonitrile inhibits the activities of multiple enzymes including cytochrome oxidase, leading to impeded energy metabolism and syntheses, which further influences hemoglobin synthesis; and (2) acrylonitrile releases cyanide, which binds to  $\text{Fe}^{+3}$ , depriving iron supply required for the process of hemoglobin synthesis, and leads to inadequate hemoglobin synthesis<sup>(4-5)</sup>.

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**Tests of Four Biochemical Indexes of AN Workers**

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## **Tests of Four Biochemical Indexes of AN Workers**

**By XIAO Wei**

In order to discuss AN chronic intoxication diagnosis and occupational health prevention indexes, this article will report the test results of 273 AN workers about their whole blood cholinesterase activity, reduction glutathione (GSH), sulfocyanate and serum sulfur level.

### **1 Target and method**

#### **1.1 Survey target**

##### **1.1.1 Exposed Group:**

There were 273 AN workers. Among them, 183 were males whose ages were between 18-56 years old with an average age of 37 years old. Their years of AN exposure were between 1 to 31 years with an average of 16 years.

##### **1.1.2 Comparative Group:**

Local scientific and administrative workers that were not exposed to AN: Among them 184 people were scientific and supporting people, male - 125; age ranged from 19 through 59 with an average of 39 years old.

#### **1.2.2 Test method**

##### **1.2.1 AN vapor concentration in the AN Plant:**

Instrument of SP-2305 type of gas chromatography was used for testing.

##### **1.2.2 Blood biochemical indexes:**

- (1) Iron trichloride calorimetric method was used to test whole blood cholinesterase activity;
- (2) Sodium nitroferricyanide color developing method was used to test GSH;
- (3) Acetocaustin - high iron nitric acid method was used to test sulfocyanate;
- (4) Modified heavy nitrogen calorimetric method was used to test serum sulfur.

All the instruments used were 721 type of spectrophotometer made in Shanghai.

## 1 Results and discussion

### 1.2 AN concentration in the plant

AN vapor concentration in the AN plant was tested in all year round. All shops had records of exceeding the standards. AN concentration changed greatly in different stations, time and production situation. But they did not have patterns. To sum up all situations in the 98 tests, it was found that 74.47% of the shops exceeded the maximum allowable AN concentration target regulated by the State. The average concentration was  $8.92\text{mg}/\text{m}^3$ .

### 1.3 Physical exam results

Analysis of physical exam results of survey target showed positive rates in the exposed group was obviously higher than those of control group. The difference was highly significant. The orders of positive rates from high to low in the exposed group were neurasthenic syndrome (50.9%), tremor (38.1%), chest distress (23.1%) and sweating (19.4%). The percentages in the control group were 4.9%, 4.4%, 2.7% and 1.1%.

### 1.4 Biochemical indexes

Comparative test results of four biochemical indexes to the control group is shown in the following table.

Table 1. Comparison of biochemical test between exposure and control group

	n	Cholinesterase (mg/ml) ± SD	Reductive glutathion (mg%) ± SD	Sulfocyanide (mg/dl) ± SD	Thiohydroxy (mg%) ± SD
Exposure group	273	47.95 ± 9.85*	27.20 ± 4.91*	1.63 ± 0.54*	1.62 ± 0.72*
Control group	284	99.43 ± 5.51	35.29 ± 5.05	1.32 ± 0.52	1.96 ± 0.81

\* P<0.01

Test results showed in the exposed group the three indexes, which were whole blood cholinesterase activity, reduction GSH and serum sulfur, were apparently lower than those in the control group, but blood-sulfocyanate amount was higher than that of control group. The difference was highly significant. Since the decrease of whole blood cholinesterase activity was more apparent than the changes of the other three indexes, this meant its was highly related to the AN vapor concentration in the AN plant. Also because there were no obvious differences in terms of sex, length of service and age, it was believed that the whole blood cholinesterase activity in AN workers might be a sensitive biochemical test index.

**Health Effects of Acrylonitrile to AN Workers**

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**1999**

## Health Effects of Acrylonitrile to AN Workers

By LI Feng, XIAO Wei and LI Zhilan, ZHANG Zhengying  
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Acrylonitrile (AN), also known as vinyl cyanide, is an important monomer in the organic synthetic industry. It makes activities to vinyl and nitrile grouping and is highly toxic<sup>[1]</sup>. As a main product of petrochemical industry, AN is widely used to synthesize butadiene-acrylonitrile rubber, acrylic fiber, ABS plastics, etc. Since organic chemical industry has developed rapidly, there are more and more AN workers now. Therefore, study of AN toxicity effect is more important. In order to discuss early effects of AN to worker's health, meaningful biochemical indexes were selected. Also in order to provide scientific basis for diagnosing AN chronic toxicity, we did an occupational health examination to 538 AN workers at Lanzhou Chemical Corporation in March, 1990.

### Target and Method

#### A Target:

ABS plant, #11 plant, 101 plant and another plant of 533 were selected as exposed group. Among them, 358 were males, 179 were females. Ages were 18-54 years old. Exposure length was 1-35 years. Control group included 195 people from the same area, with similar ages, genders and length of services, but no exposure to any toxicity. Among them, 132 were males and 63 were females. Ages were 21-57 years old. Length of service was 1-32 years.

#### A. Method:

(1) Test of AN concentration in the plant air: gas chromatography was used.

(2) Physical exams: included normal exams and lab indexes. Normal exams included work history, past medical history, internal medicine, neurology, five sense organ, etc. Lab test indexes included white cell counts, determination of hemoglobin blood protein and contents of whole blood thiocyanate, as well as whole blood cholinesterase activity and GPT. Acetocaustin - high ferric nitrate method was used to test the contents of whole blood thiocyanate. Iron trichloride calorimetric method was used to test cholinesterase activity and modified Lan's method was used to test GPT.

## Results

A. See Table 1 for AN concentration in the plant air

Table 1 shows the highest value and average value of the concentration in the exposed group were all higher than the national standards. It was 1-6.64 times over the limit.

**Table 1 Concentration of acrylonitrile in workshop**

Workshop	mg/M <sup>3</sup>		
	Maximum Concentration	Average Concentration	Times over Standard
11#	101.6	15.27	6.64
ABS	17.78	7.1	2.55
Raw materials	31	4	1

A. Physical exam results: 538 people were analyzed from the following aspects:  
 a. Neology: Results are shown in Table 2 and 3.

**Table 2. Comparison of main symptom between exposure and control**

Main symptom	Exposure ( 538 )		Control (195)	
	NO.	%	NO.	%
Headache	152	28.3**	6	3.1
Dizzy	233	41.5**	3	1.5
Insomnia	167	31.0**	6	3.1
hobby of sleepy	68	12.6*	3	1.5
Too much dream	218	40.5**	6	3.1
Tinnitus	74	13.8*	6	3.1
Failing memory	145	27.0**	1	0.5
Lack of physical strength	115	21.4*	6	3.1
Palpitation	84	15.6*	10	5.1
Breast checking	87	16.2*	8	4.1
Nauseating	60	11.2*	1	0.5
Liver-ache	26	4.8	7	3.6
Skin-itch	116	21.6*	1	0.5
Sweater	86	16.0**	0	0

\* P < 0.05    \*\* P < 0.01

**Table 3. The relationship between worked years and symptom for control group**

Worked years		>5	>10	>15	>20
Total number		129	81	77	89
Headache	NO.	17	26	38	24
	%	13.2	32.1	49.4	26.9
Dizzy	NO.	25	36	43	34
	%	19.4	44.4	55.8	38.2
Insomnia	NO.	15	21	34	27
	%	11.6	25.9	44.2	30.3
Failing memory	NO.	14	21	27	26
	%	10.8	25.9	35.1	29.2
Lack of physical strength	NO.	11	20	24	22
	%	3.5	24.7	31.2	24.7
Palpitation	NO.	3	12	20	17
	%	2.3	14.8	25.9	19.1
Depress of breast	NO.	8	15	16	15
	%	6.2	18.5	20.8	16.9

Table 2 shows the comparison results between exposed and control group, except liver pain which had no obvious difference ( $P>0.05$ ), all others showed significant differences, especially headache, dizziness, memory falling, nightmare, and sweating were more obvious. Neurasthenia and vegetative nervous function disorder were the main symptoms, although they had nothing to do with ages and genders, they did have close relationship with the exposure length. See Table 3.

Tables 3 shows self-awareness symptoms of AN workers increased as their exposure length increased, especially 5-15 years of service was more obvious ( $P<0.05$ ).

B See Table 4 for content changes of whole blood thiocyanate

Table 4 results show in comparison between the exposed group and control group, there is a significant difference in the contents of whole blood thiocyanate ( $P<0.01$ ). That is to say occupational exposure and the contents of whole blood thiocyanate were closely related.

**Table 4. Comparison of concentration of sulfocyanate in blood between exposure and control**

	Number of head	Concentration (mg/de) ± S
Exposure group	446	1.5945± 0.5263**
Control group	158	1.3100 ± 0.5441

\*\* P < 0.01

C See Table 5 for cholinesterase activity changes

Table 5. Comparison of the activity of cholinesterase between exposure and control group

	Number of people	Concentration ( mg/de) ± S
Exposure group	358	47.8851± 10.4954**
Control group	188	99.4920 ± 5.4970

\*\* P < 0.01

Table 5 shows occupational exposure and cholinesterase activity are closely related. Cholinesterase activity in the exposure group was decreased sharply.

D See Table 6 for blood picture and white cell counts

Table 6. Comparison of leukocyte counts between exposure and control group

	Number of people	Number of Positive	%
Exposure group	538	54	10.1**
Control group	195	3	1.5

Note: Positive: Counting of leucocyte < 4000/mm<sup>3</sup>

\*\* P<0.01

Table 6 shows the high and low counts of white cells are obviously related to occupational exposure (P<0.01).

E See Table 7 for SGPT change

Table 7. Comparison of positive rate of SGpT between exposure and control group

	Number of people	Number of Positive	%	Relative risk (RR)
Exposure group	471	212	45.0*	
Control group	187	10	6.3	3.5

Note: Positive: SGpT > 40 unit

\*\* P<0.05

SGPT positive rate in the exposed group is obviously higher than that of control group (P<0.05). It is 8.5 times higher.

#### F Effects on women's menstruation mechanism and pregnancy

37 female workers were tested in the petrochemical inspection shop of the 303 plant. Among them, 19 people had menstruation disorder, which was 51.4% of the total. 2 workers had premature delivery, which was 5.4%. 8 workers had spontaneous abortion which was 8.1% of the total.

## Discussion

1. This survey and analysis show that there are significant differences between the exposed group and control group when comparing their neurasthenia and vegetative nervous function disorder. Among them, headache, dizziness, sleep disorder, memory falling, fatigue are the main symptoms in neurasthenia. Palpitation, chest distress, sweating on palm of hand are the main symptoms in vegetative nervous function disorder. This also is related to the exposure length. If length of service is under 20 years, the symptoms are more obvious as the service years increase. For over 20 years of service, symptoms show decreasing trend which has the same result as the domestic report [2].

Acrylonitrile is a highly toxic substance. It is easy to get into human bodies through their respiratory system, skin and digestive system. In the AN intoxication, precipitation dicyan radical [8] mainly causes inhibition of cell biochemical oxidation process and therefore brings oxygen deficit and cell suffocation. Central nervous system is very sensitive to oxygen deficit and is easy to be damaged. Neurasthenia group are shown in clinic.

2. Thiocyanate is a test index of cyanide in biochemistry. Comparison between AN exposed group and control group showed significant differences. It proves that this is closely related to occupational exposure of acrylonitrile. On a certain degree, it reflects metabolism after AN's entrance to the body. Therefore, it is considered a reference index for diagnosis.

3 Whole blood cholinesterase activity of people exposed to AN has shown obvious reduction, besides, it decreases when AN concentration increases. It is therefore considered a sensitive index to reflect the condition of long-time AN exposure.

4 These results indicate decrease rate of total white cells of AN workers is 10.1%. This shows a significant difference ( $P < 0.01$ ) which is the same as some related reports [5,6]. It is considered to be connected with immune function by AN toxicity effects.

In regards of AN effects to liver, positive test result of GSPT in the exposed group is obviously higher than that of control group ( $P < 0.05$ ). It is 8.5 times of that of control group. Long-time exposure to AN causes liver injuries.

5 This survey also has found that AN does harm to menstruation mechanism and pregnancy of female workers, this is especially shown in the original liquid workshop of the 303 Plant. Occurrence rate of premature delivery, spontaneous abortion, menstruation disorder were a lot higher. This is similar to the female survey reports in the synthetic rubber industry<sup>[8]</sup>. But this type of effect is only a trend and phenomenon, its cause and mechanism need to be discussed further.

## Summary

1. This article summarizes physical examination results of 538 AN workers, discusses and analyzes clinical symptoms and physical appearances of long-time AN workers exposed. It proves that acrylonitrile is harmful to the health of human beings, mainly neurasthenia and vegetative nervous function disorder, and it is closely related to the length of service.

2. This research has done a preliminary exploration to biochemical indexes which help diagnosis. It proves that long-time exposure to AN is harmful to counts of white cells and whole blood thiocyanate, whole blood cholinesterase activity and GPT.

3 Preliminary discussion is done for menstruation mechanism and pregnancy of AN female workers.

4 Further observation on AN chronic toxicity to human bodies needs to be done.

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**JUNE AND DECEMBER 1989  
ACRYLONITRILE AIR MONITOR**

**AT**

**LANZHOU INDUSTRIAL CHEMICAL CORPORATION**

**BY**

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Lanzhou Chemicals Industrial Corp.: ACRYLONITRILE AIR MONITOR AT ACRYLONITRILE PLANT

DATE	AN AVERAGE CONCENTRATION (MG/CUBIC METERS)			NOTES
	Cent Control	Reactor	AN Backend	
	Room	Room	Section	
	ITEM 1	ITEM 2	ITEM 3	
Jan-98	0.410	0.410	0.410	1. AN PLANT PRODUCTION: 1998: 25,000 TONS 1999: 30,000 TONS
Feb-98	0.310	0.310	0.310	
Mar-98	0.310	0.310	0.420	2. EXPOSED PEOPLE: ITEM 1: 72 PEOPLE ITEM 2: 8 PEOPLE ITEM 3: 16 PEOPLE
Apr-98	0.310	0.420	0.310	
May-98	0.310	0.310	0.310	
Jun-98	0.310	0.310	0.310	
Jul-98	0.310	0.310	0.310	
Aug-98	0.310	0.310	0.310	
Sep-98	0.320	0.310	0.310	
Oct-98	0.310	0.310	0.310	3. COMPANY TOTAL EMPLOYEES IS
Nov-98	0.310	0.320	0.310	8,000 PEOPLE
Dec-98	0.310	0.310	0.310	4. COMPANY PRODUCTS: A. Fertilizer (ammonia and urea) B. Plastic (ABS, PE and PP) C. SAR (styrene-acrylonitrile-rubber) D. Benzene E. Organic-CN chemicals F. None-organic chemicals G. Toxic chemicals
<b>AVERAGE</b>	<b>0.319</b>	<b>0.328</b>	<b>0.328</b>	
Jan-99	0.310	0.310	0.460	5. Original exposed people - 560 people Of this number, 380 were women  <i>Y. Ray Hing's comments</i> It is not a surprise to see AN concentration a. Lowest in central control room b. Highest in Purification section; i.e. AN-backend section
Feb-99	0.310	0.310	0.310	
Mar-99	0.310	0.300	0.470	
Apr-99	0.410	0.720	0.590	
May-99	N/A	N/A	N/A	
Jun-99	0.310	0.300	0.310	
Jul-99	0.710	0.720	0.470	
Aug-99	0.307	0.307	0.310	
Sep-99	0.307	0.306	0.307	
Oct-99	0.234	0.234	0.234	
Nov-99	0.310	0.310	0.310	
Dec-99	0.310	0.310	0.310	
<b>AVERAGE</b>	<b>0.348</b>	<b>0.375</b>	<b>0.371</b>	
Jan-00	0.960	1.600	1.390	
Feb-00	0.430	0.530	0.530	
Mar-00	0.850	0.640	0.960	
Apr-00	0.320	0.320	0.320	
May-00	0.640	0.750	0.530	
<b>AVERAGE</b>	<b>0.640</b>	<b>0.768</b>	<b>0.746</b>	

From Dr. Xiao Wei's Presentation 07-17-00 in Beijing

DECEMBER 1989 COMPLEX AUDIT OF ALL GASEOUS HAZARDOUS CHEMICALS ANALYSIS AND RESULTS

1	2	3	4	5	6	7	8	9	10	11	12	13
PROCESS	LOCATION	CHEMICAL	ALLOWAB	TEST No. 1	TEST No. 2	TEST No. 3	TEST No. 4	MONTH	SAMPLING	EXCEEDED	MONTHLY	HIGHEST
UNIT		MONITORED	LE	MG/M3	MG/M3	MG/M3	MG/M3	Ave.	FREQUENCY	4	OVER	Conc.
		MG/M3	MG/M3	MG/M3	MG/M3	MG/M3	MG/M3	MG/M3			Y	MG/M3
Butylonitrile	Walking path	AN	2	aborted	3.35	0	aborted	1.83	2	1		3.35
	Polymerization	Butadiene	100	3.8	3.1	1.3	20	4.2	4	0		20
Purification 3rd deck		AN	2	13.4	24.79	0	29.48	9.95	4	3		29.48
		Butadiene	100	2.4	6.3	30.4	62.4	13	4	0		62.4
Purification ground		AN	2	11.73	13.4	52.26	830.8	51.11	4	4		830.8
		Butadiene	100	1.5	12.9	0.3	27.6	3.6	4	0		27.6
Reaction Section		AN	2	7.71	3.35	0	415.4	10.18	4	3		415.4
		Butadiene	100	aborted	aborted	aborted	aborted	aborted	aborted	aborted		
Pumps houses		AN	2	aborted	aborted	aborted	aborted	aborted	aborted	aborted		
		Butadiene	100	4.3	20.3	aborted	aborted	9.3	2	0		20.3
ABS	Polymerization	AN	2	14.74	36.18	aborted	aborted	23.09	2	2		36.18
		Butadiene	100	2.1	1.9	0	0.7	1.3	4	0		2.1
Latex Section	Centrifugal Dust Collection Section	Styrene	40	0.3	0.8	1.5	18.76	6.8	4	4		18.76
		Butadiene	100	0.5	8	0	1.6	1.3	4	0		8
		AN	2	1.34	0	0	0	1.08	4	0		1.34
		Dust	10	10	2.1	6	12.2	6.3	4	1		12.2
Catalytical	Catalysis transfer area	Dust	2	aborted	aborted	aborted	aborted	aborted	aborted	aborted		
Phenol	Building 3-3 first floor	Benzene	40	aborted	aborted	aborted	aborted	aborted	aborted	aborted		
		Cumin	100	aborted	aborted	aborted	aborted	aborted	aborted	aborted		
Water	#1 Chemical Waste	Benzene	40	5.2	3.5	3.1	3.8	4.7	4	0		8.8

From Dr. Xiao Wei's Presentation 07-17-00 in Beijing

DECEMBER 1989 COMPLEX AUDIT OF ALL GASEOUS HAZARDOUS CHEMICALS ANALYSIS AND RESULTS

1	2	3	4	5	6	7	8	9	10	11	12	13
PROCESS	LOCATION	CHEMICAL	ALLOWAB	TEST No. 1	TEST No. 2	TEST No. 3	TEST No. 4	MONTH	SAMPLING	EXCEEDED	MONTHLY	HIGHEST
UNIT		MINITORED	IE	MG/M3	MG/M3	MG/M3	MG/M3	MG/M3	Ave. FREQUENCY	4	OVER THE	Conc.
												MG/M3
WATER	No. 1 Chemical Waste	Toluene	100	1	0.6	0.5	1.2	0.8	4	4	0	1.2
		Ethyl Ben	100	10.2	5.1	3.9	8.6	6.5	4	4	0	10.2
		Cumlin	100	1.4	1	0	0	1.1	4	4	0	1.4
		Styrene	40	5.1	2.3	1.5	3.4	2.8	4	4	0	5.1
		Benzene	40	3	4.5	22.4	125.8	14	4	4	1	125.8
		Toluene	100	5	4.7	12	8.3	7	4	4	0	12
		Ethyl Ben	100	1.8	1.7	2.6	10.3	3	4	4	0	10.3
		Styrene	40	2.6	1.9	1.1	10.1	2.7	4	4	0	10.1
		NH3	30	10	7.4	7.6	2.4	6.1	4	4	0	10
		Equipment										
Raw Mat'l	AN Pump House	AN	2	45.56	522.6	34.17	NA	93.35	4	4	3	45.68
	2 P Pump House	Benzene	4	49.8	13.8	1.5	14.4	11	4	4	1	49.8
Poly-styrene	Detached Extrusion Packaging	Styrene	40	1.1	6.2	6.8	15	10.5	4	4	0	19.1
		Dust	10	18.4	8.5	53.1	23	20.9	4	4	3	53.1
Selected area	No. 7 Line 3 (2)	Styrene	40	14.3	NA	NA	NA	14.3	1	1	0	14.3
		Styrene	40	116.3	NA	NA	NA	116.3	1	1	1	116.3
Polymerization	No. 6 1st Fl Butadiene Recovery Sec	Butadiene	100	0.4	NA	NA	NA	0.4	1	1	0	0.4
		Ethyl Benz	100	4.6	NA	NA	NA	4.6	1	1	0	4.6
Carbon 4	No. 74 First Floor	Butadiene	100	0.4	NA	NA	NA	0.4	1	1	0	0.4
		Ethyl Benz	100	4.6	NA	NA	NA	4.6	1	1	0	4.6
Raw Mat'l	Styrene	Butadiene	100	0.4	NA	NA	NA	0.4	1	1	0	0.4
		Ethyl Benz	100	4.6	NA	NA	NA	4.6	1	1	0	4.6

From Dr. Xiao Wei's Presentation 07-17-00 in Beijing

**JUNE 1989 COMPLEX AUDIT OF ALL GASEOUS HAZARDOUS CHEMICALS ANALYSIS AND RESULTS**

1	2	3	4	5	6	7	8	9	10	11	12	13
PROCESS	LOCATION	CHEMICAL	ALLOWAB	TEST No. 1	TEST No. 2	TEST No. 3	TEST No. 4	MONTH	SAMPLING	EXCEEDED	OVER THE	HIGHEST
UNIT		MONITORED	LE	MG/M3	MG/M3	MG/M3	MG/M3	MG/M3	FREQUENCY	4	MONTHLY	CONC.
								Ave.			THE	MG/M3
								MG/M3			HIGHEST	
Styrene	Pump House # 15A	Benzene	40	1.1	1.6	4.5	3.5	2.3	4	0		4.5
		Toluene	100	0.4	0.6	0.2	1.5	0.5	4	0		1.5
		Ethyl Benz	100	1.6	14.3	2.6	2	3.3	4	0		14.3
		Styrene	40	3	7.3	0.8	0.8	1.9	4	0		7.3
		Di-ethyl-B	100	0	0	0.5	0	0.8	4	0		0.5
		Benzene	40	135.8	133	151.3	116.4	133.5	4	4	2.34	151.3
		Toluene	100	1.2	2.6	2.6	1	1.9	4	0		2.6
		Ethyl Benz	100	11.7	17.4	18.9	11.1	14.4	4	0		18.9
		Di-ethyl-B	100	1.7	0.7	1.4	1.3	1.2	4	0		1.7
		Benzene	40	66.4	6.4	185.2	2.1	20.2	4	2		185.2
ABS	Building 13 2nd floor	Toluene	100	0.7	1	3.2	0	1.2	4	0		3.2
		Ethyl Benz	100	7.7	6.7	23.3	1.1	6	4	0		23.3
		Di-ethyl-B	100	2.2	2	1.4	1.3	1.7	4	0		2.2
		Butadiene	100	3.4	0.5	0	16.2	2.3	4	0		16.2
		AN	2	0	17.25	16.5	540	19.8	4	3	8.9	540
		Styrene	40	1	0.8	1.3	8.3	1.7	4	0		8.3
		Butadiene	100	13	1	0.9	23	4.1	4	0		23
		AN	2	0	1.5	13.5	948	11.77	4	2	4.89	948
		Dust	10	6.4	2.1	2.2	8.7	4	4	0		8.7
		Dust	2	6.4	2.1	2.2	8.7	4	4	0		8.7
Catalytical Section	Catalysis transfer area	Dust	2	6.4	2.1	2.2	8.7	4	4	0		8.7
		Dust	2	6.4	2.1	2.2	8.7	4	4	0		8.7

From Dr. Xiao Wei's Presentation 07-17-00 in Beijing

**JUNE 1989 COMPLEX AUDIT OF ALL GASEOUS HAZARDOUS CHEMICALS ANALYSIS AND RESULTS**

1	2	3	4	5	6	7	8	9	10	11	12	13	
PROCESS	LOCATION	CHEMICAL	ALLOWABLE	TEST No. 1	TEST No. 2	TEST No. 3	TEST No. 4	MONTH	SAMPLING	EXCEEDS	MONTHLY	HIGHEST	
UNIT		MONITORED	MG/M3	MG/M3	MG/M3	MG/M3	MG/M3	Ave. MG/M3	FREQUENCY	D	OVER THE	Conc. MG/M3	
Butylnitrile	Walking path	Butadiene	100	aborted	aborted	81.6	aborted	81.6	1	0		81.6	
		AN	2	aborted	aborted	78.75	aborted	78.75	1	1	38.38	78.75	
	Polymerization	Butadiene	100	0	0	1.5	aborted	1.9	1.4	3	0		1.9
		AN	2	0	0	4.5	aborted	0	1.65	3	1		4.5
	Purification 3rd deck	Butadiene	100	7.8	16.2	195	aborted	25.2	14.7	3	0		25.2
		AN	2	48.75	8.2	aborted	9	44.06	4.3	3	3	21.03	195
	Purification ground	Butadiene	100	2.4	82.5	aborted	4.1	2.25	5.7	3	0	1.85	8.2
		AN	2	0	9.4	aborted	4.1	6.21	2.12	3	2		82.5
	Reaction Section	Butadiene	100	aborted	4.5	aborted	0	4.1	6.21	2	0		9.4
		AN	2	aborted	18.4	aborted	261	57	29.24	2	1	0.06	4.5
	Pumps houses	Butadiene	100	aborted	15	aborted	261	57	69.3	2	1		261
		AN	2	aborted	14.5	38.8	17.7	14.3	19.4	2	2	13.62	57
Phenol	Building 33 Ground Fl (4)	Benzene	40	13.4	17	5.9	13	11.5	4	0		38.8	
		Cummin	100	26	26	20.8	11.4	20	4	0		17	
Storage	Liquid Chemicals Pump house	Butylene	100	92.6	61.4	405.6	77	115.4	4	1		405.6	
		Butylene	100	92.6	61.4	405.6	77	115.4	4	1		405.6	
Raw Mat'l	AN Pump House	AN	2	53.25	60.75	337.5	62.25	90.8	4	4	44.4	337.5	
		Benzene	40	1124.8	387.9	58.2	28.1	164.1	4	3	3.1	1124.8	
Latex	Polymerization	Butadiene	100	aborted	7.7	aborted	aborted	7.7	1	0		7.7	
		Butadiene	100	aborted	7.7	aborted	aborted	7.7	1	0		7.7	
PE	Packaging	Dust	10	49	6.4	2.2	6.5	8.2	4	1		49	
		Benzene	10	49	6.4	2.2	6.5	8.2	4	1		49	

From Dr. Xiao Wei's Presentation 07-17-00 in Beijing

**Two (2) Examples of Chemically Burn of Eyes by AN**

**By ZENG Suhua**

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**Maoming, Guangdong Worker Occupational Hospital**

**Two (2) Examples of Chemically Burn of Eyes by AN****By ZENG Suhua****Article submitted on October 19, 1998**

Acrylonitrile (AN) is a colorless, inflammable and volatile liquid with a special cherry smell. Relative molecular weight is 53.06 and the boiling point is 77.3°C. It is easily resolved in the organic solvent. It is highly toxic and very sensitive to human beings. Inhalation of a large quantity of vapor within a short period of time can cause intoxication and death. But there are only few reports about eye burn. Two examples of chemically burn of eyes by AN are reported in the following.

**Clinical information****A. General information**

Both patients were male instrument makers. Their ages were 30 and 28 year old. The two workers opened the valve to exam the trouble of the pipeline without wearing gas masks. AN liquid in the pipeline was ejected by high pressure to their eyes and faces. Three minutes later, water was used to rinse their eyes, faces and hair. Plant clinic gave them isoamyl nitrile to inhale. Sodium hyposulfite was used to wash their eyes, faces and skin. Clean clothes were put on. Two hours later, they were sent to the hospital.

**B. Clinical manifestation and lab tests**

Both workers had symptoms of needling pain, burning and lacrimation of the eyes. Ophthalmologic exam results: edema and congestion of both eyelids, congestion of conjunctive and eye ball conjunctive of both cheeks. Fluorescent staining slit-lamp observation showed there was diffuse punctate scaling, cornea parenchymatous superficial layer edema opacity on the cornea. No abnormal situation was found after the exam of the whole body. Urine sulfocyanate amounts were in the normal range.

### C. Treatment and changes

After the two workers got to the hospital, 0.64g of sodium hyposulfite and 500ml of physiological saline were mixed together to wash their eyes. Eye drops, aureomycin eye ointment were put into their eyes. 1.28g of sodium hyposulfite was injected. Cephalosporin and Vitamin C were taken orally to prevent infection and to protect cornea from adhesion. Treatment lasted for 8 days and they were discharged after fully recovered.

### Discussion

Based on the workers' AN exposure history, clinical manifestation and lab test results, the conclusion of mechanism of eye burning were (1) high pressure impact force when AN was ejected; (2) It was related to the cornea stimulation from AN. Since AN has a strong osmotic force and toxicity, fast cornea tissue inhalation, if rinse and antidote are not done right away, it easily causes intoxication of the whole body. Two patients mentioned in this article were rescued at the scene. Water and antidotes, sodium hyposulfite were used to rinse and clean; antidote, such as iscamyl nitrile, was inhaled. Other proper antidotes were used after they got into the hospital, therefore, they avoided two times of contamination and AN intoxication (Only eye burn happened). At the same time cure rate was improved, and the osmotic force was reduced.

#### *Note from Y. Ray Hing, BP Chemicals Consultant:*

**Maoming Guangdong built an Acrylonitrile plant with a capacity of 8,000 tons per year, using Chinese own developed technology. The plant was started up in early 1990s. Due to heavy financial losses and violations of the Chinese Environmental Regulations, this plant was shutdown two years after start-up.**

**The plant accidentally discharged waste acrylonitrile to the near-by river, killing all the inhabitant in the river, including all fish. This accident was the second largest spilled of AN that I've known, the first being in Lanzhou in 1970s.**

**Progress on Clinical Study of Acute  
Acrylonitrile Intoxication**

by

**FONG Sanwui, ZHANG Ruopeng, CHEN Yuqing**

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## Progress on Clinical Study of Acute Acrylonitrile Intoxication

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Acrylonitrile (AN) is an important monomer of organic synthesis widely used in making acrylic fibers, acrylonitrile-butadiene rubber, ABS engineering plastics, certain types of resins, and tobacco pesticide fumigants and insecticide. Total U.S. production of AN in 1990 was 3 billion pounds <sup>(1)</sup>. In 1995, our country had an annual AN production capacity of 399,000 tons. With increased AN production, the population exposed to it has increased drastically. AN-caused intoxication incidents and patients have also been increased continuously. As a result, the past few years have seen extensive clinical study and experience about acute AN intoxication. And toxicology researches have brought about new insights. This article will review the progress of clinical studies for acute AN intoxication in recent years.

### I. Physical and Chemical Properties

AN is a colorless, flammable, and volatile liquid, with a bitter almond odor. Its chemical formula is  $\text{CH}_2 = \text{CH} - \text{CN}$ , with relative molecular weight of 53.06, specific weight of 0.798 (25/4°C), boiling point of 77.3°C, and vapor pressure of 110-115 mmHg (25°C). Its solubility in water is 7.3% (20°C), and is miscible with most organic solvents. Its vapor forms an explosive mixture with air, with explosion limits of 3.05-17.00% (volume). AN hydrolyzes to become acrylic acid and reduces to produce propane nitrile.

### II. Effects of Acute Toxicity <sup>(1-5)</sup>

AN is highly toxic. Its routes of entry to human body are through respiratory tract, skin and gastrointestinal tract. Its toxic effect is similar to that of hydrocyanic acid. Its oral LD<sub>50</sub> in rats is 20-102 mg/kg, and ID<sub>50</sub> is 571 ng/m<sup>3</sup> (2h).

Human is very sensitive to AN. The olfactory threshold of Chinese population is 46.6 mg/m<sup>3</sup>. The maximum allowable concentration in working environment is 45 mg/m<sup>3</sup>. Human exposure to AN at 1000 mg/m<sup>3</sup> for 1- 2 hours leads to death, and at 300-500 mg/m<sup>3</sup> for 5-10 minutes produces poisoning symptoms. Children are more sensitive than adults. The more condense the inhaled AN, the faster the onset of disease. The greater the dose, the more severe the disease. Otherwise, the onset is slow, and the symptoms are mild, with an obvious dose-response relationship. The latent period for AN intoxication varies, ranging from 0.5 to 24 hours. A few cases showed instantaneous onset after

exposure to large doses. A great amount of clinical cases have proven that AN acute toxicity centers on the central nervous system, with complications of cardiac, hepatic, and pulmonary injuries.

Acute AN toxicity has become one of the important issues for debates among researchers. The focus of the debate, which closely relates to its treatment of clinical patients, is whether its metabolite or its molecule, causes systemic poisoning. Most researchers believe that cyanide (CN<sup>-</sup>), as an important metabolite of AN, is at least partially responsible for its acute toxicity. Studies proved that in AN intoxicated animals, CN<sup>-</sup> concentrations in blood and tissues are the highest, and over 40 enzymes are affected by CN<sup>-</sup>. Among them, cytochrome oxidase is the most sensitive enzyme, resulting in inhibited cell respiration/oxidation, and manifested as a serious of poisoning symptoms. Moreover, conventional cyanide antidotes are capable of protecting humans and animals against AN toxic effects. Nonetheless, CN<sup>-</sup> release alone can't explain all the clinical manifestations of acute AN toxicity. Therefore, AN toxicity is effected not only by its CN<sup>-</sup> release, but also by its intrinsic toxicity, for example, in paralyzing the CNS, especially direct paralyzing the respiratory system. Indeed, clinical findings of acute AN intoxication differ greatly from CN<sup>-</sup> intoxication. Not only is the onset slower, acute AN intoxication also produces complications of pulmonary edema and causes abnormal electro-cardiogram or cardiomuscular enzyme patterns and increased serum amino-transferase besides the major CNS signs and symptoms.

### 1. Toxic Effects on the Nervous System <sup>(8-18)</sup>

During acute AN intoxication, one of the earliest and most prominent manifestations is the intoxication of the central nervous system. Clinical cases have proven that the early phase of intoxication sees cholinergic nerve excitation, followed by central nervous system malfunctions.

A summary of 334 cases occurred globally over the last 10 years finds that the early phase produces clinical manifestations of cholinergic nerve excitation — dizziness, headache, nausea, vomiting, chest distress, unstable blood pressure and heart beat, and upper abdominal discomfort. Most cases show tendon hypereflexia, intensified muscular tension, and somnolence or confusion.

Severe cases show unconsciousness and pathologic signs and symptoms of CNS excitation/depression, and all kinds of motor neuron excitatory manifestations such as muscular tremor and convulsion of extremities. Clinical observations by Ding Yunpeng of acute toxicity patients found symptoms of pupil constriction, ocular muscle tremor, extraocular muscle paralysis, followed by manifestations of CNS obstruction, such as convulsion of the extremities, tremor, and coma.

Xu Lili reported 3 cases of acute intoxication with vomiting, convulsion, confusion, hypereflexia, loss of abdominal and bulbocavernosus reflexes, and electro-

encephalogram abnormality. AN releases cyanide (CN<sup>-</sup>), which inhibits cell respiratory oxidation and results in tissue hypoxia. Since the central nervous system is the most sensitive system for hypoxia, it becomes the major target organ during acute AN intoxication. Study by Ghanayem et al. proved that acute AN intoxication in rats showed an early phase of cholinomimetic neurotoxicity, which is dose-dependent. The higher the dose, the more rapid the onset. A late phase showed depression, convulsions, and respiratory failure followed by death. In human bodies, the rate of AN metabolism is slow, the level of CN<sup>-</sup> from such metabolism is low (about 15%) and the clinical manifestations from its toxic effect also appear slower. Therefore, cyanide-induced central nervous system malfunction appears after the manifestations of cholinergic nerve excitation.

AN also incur damages to the peripheral nerves, causing numbness and weakness in the extremities. Physical examinations of patients find signs and symptoms of peripheral nerve injuries, such as sensory deprivation, reduced muscular tension and reduced tendon reflex. However, these symptoms are often ignored during emergency treatments of acute AN intoxication. Since there are inadequate electromyography data to support the finding, its establishment requires further observations and studies. Nonetheless, clinical cases and animal studies have both proven that AN is a confirmed neurotoxin. Although there are many reports on acute AN intoxication, there are few studies and reports about its sequelae, probably because diagnostically it is hard to distinguish between chronic intoxication and sequelae from acute intoxication. Domestic reports over the recent years have described that most clinical symptoms of severely intoxicated patients subside after treatments. But a few clinical symptoms and signs of its sequelae persist — mainly from injuries to the nervous system — such as neurasthenic syndrome, muscular atrophy, sensory type multiple neuritis, and disturbed vegetative nerve functions.

A few cases of acute and severe intoxication reported persistent headache, vertigo, weakness, numbness of extremities, and electromyography abnormalities still exist after 3-month treatment. Zhang Yifeng once diagnosed 4 cases of patients of acute intoxication, all with sequelae. Zhang Zhengyin reported 49 cases of acute AN intoxication. Among them, there were no sequelae for the mildly intoxicated, and there was sequelae for the severely intoxicated. As a summary, sequelae of the nervous system are closely related to the degree of intoxication. This fits the rules in a typical intoxication— the higher the dose of exposure, the faster the onset; the more severe the intoxication, the more severe the injuries of the nervous system, and hence the higher rate of the sequelae.

## 2. Toxic Effects on the Respiratory System <sup>(6, 11)</sup>

AN is a respiratory stimulant and depressant and has a strong paralyzing effect on the respiratory system. The symptoms of upper respiratory tract stimulation is most prominent, including pharyngalgia, paroxysmal cough, chest distress, dyspnea, nasopharynx congestion and edema. In 1980, O'Berg first reported 2 cases of death from pulmonary edema after high concentrations of AN was inhaled. After that there have been

7 cases of domestic reports of pulmonary edema complication from severe AN intoxication.

The characteristics of this complication included, besides the upper respiratory tract symptoms described above, rapid onset, paroxysmal cough, cough with frothy sputum and dyspnea. Pulmonary auscultation heard dry and moist rale. X-ray finds increased shadow in the hilus of the lung with blurred margin and lowered translucency, accompanied by scattered dotty or reticular shadow. WBC counts were slightly higher. Blood gas analysis found varying degrees of metabolic acidosis with reduced arterial oxygen saturation and disturbed electrolyte balance.

The electrolyte and acid-base imbalance were corrected by administering of glucocorticoid. The reversal of the X-ray signs varied greatly, ranging from several hours to 2-3 days. There was a case of emergency treatment of acute AN intoxication in China with complications of alveolar pulmonary edema, cerebral edema, and upper digestive tract hemorrhage. After treatments, the patient recuperated in 7 days. AN intoxication-induced acute pulmonary edema has attracted the attention of international medical organizations.

In 1984, CEC (Commission of the European Communities) first raised the finding that AN inhalation might cause pulmonary edema. In 1987, WHO-IARC (International Agency for Research on Cancer) called for attention to the etiology of AN intoxication-induced pulmonary edema. In November of 1993, the WHO-IPCS (International Program on National Safety) discussed the etiology of AN intoxication-induced pulmonary edema. Therefore, sufficient clinical attention should be paid to such pulmonary edema complication.

### 3 **Cardiotoxicity** (6, 9, 12, 13)

AN causes cardiovascular injuries. But there has yet unified conclusion about its mechanism of toxicity. One hour after onset of the disease, patients of acute AN intoxication show symptoms of cardiovascular injuries, such as downshifted ST interval and lowered, flattened, or reversal T wave.

Some patients show sinus tachycardia, sinus bradycardia, bundle branch blockage, and abnormal cardiovascular enzyme patterns. All patients are able to recover after treatment. Some researchers believe that cardiovascular injury is caused by tissue hypoxia during AN intoxication. But clinically all severe intoxications do not manifest cardiovascular injury, while some mild intoxication shows electro-cardiogram or cardiovascular enzyme abnormalities. Therefore, hypoxia alone can not fully explain the injury, and further studies are required to understand whether cardiovascular toxicity of AN is caused by hypoxia, AN itself, or AN metabolites.

Animal studies have found that AN can cause opacity of cardiac muscle, swelling and fiber fracture and lightly fatty degeneration. Therefore, both clinical case and animal study have proven that AN causes cardiac muscular injuries.

#### 4 **Hepatotoxicity** (8, 10, 12, 13, 16-18)

Varying degree of hepatic injury appears in patient 24-42 hours after acute AN intoxication. Hepatic enzymes increase, and a few patients develop hepatomegaly. But severe hepatic illnesses are rare, and hepatic injury is not limited to patients suffering from acute intoxication. In 1991, Xiao Wei proved in animal studies that AN causes increased activity of coenzyme nicotinamide adenine dinucleotide phosphate (NADPH), suggesting increased endoplasmic detoxification. High dose group showed reduced glucose-6-phosphatase, indicating damaged endoplasmic network. Electron microscopy found reduced and structurally damaged rough endoplasmic reticulum. Research institutions in the United States including Louisville University conducted a series of studies and concluded that AN binds to glutathione, forming cyanoethyl thioluria as the major metabolite after intoxication. In hepatic cells, 80-90% of AN is converted into thioluria. Hepatic injury might have been caused by the toxic effect of the metabolites produced in the liver during AN metabolism.

#### 5 **Toxic Effects on the Circulatory System** (8, 10, 18, 19)

There are only a few reports about toxic effects on the circulatory system during acute AN intoxication. In 1992, US reported 2 cases of acute AN intoxication with mild complications of anemia and jaundice. Domestically, there has been no such report. Study by Farooqui et al. draws 2 conclusions: (1) high concentration of AN inhibits erythrocyte membrane  $\text{Na}^+ - \text{K}^+$ -ATP activity; and (2) low concentration of AN depletes erythrocyte glutathione, degrades hemoglobin, induces lipid peroxidation, and changes erythrocyte structure. These conclusions can well explain the clinical manifestations of anemia and jaundice during acute AN intoxication.

A summary of 334 cases over the last 10 years found that cyanosis appears in only 9.9% of acute AN intoxication. But all the data did not indicate at what stage symptom appears. In the 3 cases of acute AN intoxication reported by Xu Lili, no cyanosis was found. In the 144 cases of acute AN intoxication reported by Chen Yuqing, of which 42 were severe and 102 were mild cases, no cyanosis was found. Blood gas analysis of some of the cases found normal  $\text{PaO}_2$ , normal  $\text{PaCO}_2$ , but increased  $\text{PvO}_2$ , which is not sufficient to explain cyanosis caused by acute AN intoxication. This suggests that cyanosis might be caused by complications from AN intoxication.

## 6 Toxic Effects on the Skin <sup>(7, 20)</sup>

ACN has a strong stimulatory effect on the skin. Local skin contamination can cause dermatitis. YU Zhang once reported a case of acute AN dermal intoxication with complication of dermal ulcerative necrosis. The patient was recovered after treatment.

### III. Treatments

Currently, nitrite and sodium thiosulfate are still being used globally as the first choice of antidotes for acute AN intoxication. According to 278 published cases and 56 unpublished cases with descriptions of treatments on acute AN intoxication, reliable effects can be achieved in mild intoxication by intravenous injection of sodium thiosulfate, without the use of methaemoglobin. For cases of severe intoxication, small doses of methaemoglobin combined with sodium thiosulfate can be administered. Repeated doses of sodium thiosulfate can be applied if the symptoms do not subside 30 minutes after first administration. AC intoxication causes tissue cells to lose their oxygen utilization. Since brain and heart are very sensitive to this deprivation, oxygen treatment is essential. Regular oxygen supply should be administered to patients with mild or severe acute intoxication. If condition is allowed, hyperbaric oxygen treatment can be used. For patients of severe acute intoxication, adequate amount of glucocorticoid should be simultaneously administered with the antidotes described above in the early phase of the treatment to clear free radicals in the neurons, and to prevent cerebral edema.

### IV. Issues of Carcinogenicity, Malformation and Mutagenicity <sup>(4, 18, 21)</sup>

In mammals, AN can be oxidized to become unstable acrylonitrile epoxide through the action of microsomal mixed-function oxidase. Acrylonitrile epoxide is a potential carcinogenic and mutagenic compound. Long-term carcinogenicity experiments conducted on rats proved that AN induces tumor regardless of the routes of administration (drinking water, gavage feeding, and inhalation). The position and characteristics of induced tumors are fairly consistent in all studies, with a dose-response relationship. In rats, AN can induce central nervous system glioma, Zymbal adenoma, squamous papilloma of the esophagus, mammary gland tumor, mammary gland fibroma, small intestine cancer, and adenoma of the auditory canal. But data on AN carcinogenicity in humans are still insufficient. International Agency for Research on Cancer (IARC) classified AN as a 2A chemical substance, meaning "probable human carcinogen, with nearly sufficient evidence." Therefore, health monitoring should be enforced on occupationally exposed AN workers.

## V. Existing Problems and Suggestions

Over the last 10 years, considerable amount of data has been generated on AN biological monitoring indices, on its neurotic, hepatic, blood, pulmonary and reproductive toxicology studies, and on epidemiological studies. But systematic clinical data are largely deficient. For most of the acute AN intoxication patients, data on the onset and course of the disease are incomplete. For some cases, classifications of intoxication level are not well defined. Measurements of AN concentration are inadequate. Moreover, there is usually no early-phase monitoring of cardiac, pulmonary, and hepatic injuries. Usually, no attention on these aspects is paid until there appear obvious electro-cardiogram and liver function abnormalities, leading to fatal delay in treatments. Since emergency management and repair facilities are not properly maintained, and safety education has not been sufficiently enforced, cases of acute AN intoxication occur continuously.

Therefore, we suggest studying and employing monitoring measures suitable for use by basic organizations, and conducting systematic and careful observations on patients of acute AN intoxication, with special attention paid to monitoring and protection of vital organs, such as heart, lung, and liver. Finally, safety education should be adequately enforced and protection facilities properly maintained in order to prevent further occurrence of intoxication accidents.

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