

**8EHQ-0303-14711**  
**THE AN GROUP**

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March 25, 2003

US Environmental Protection Agency  
Office of Pollution Prevention and Toxics  
CBI Center  
EPA East Building, Room 6428  
1201 Constitution Avenue, NW  
Washington, DC 20004  
Attention: 8(e)



**Contain NO CBI**

Re: File [8EHQ-400-14711]

Dear 8(e) Coordinator:

In April of 2000 and again in November of 2000, the Acrylonitrile Group Inc., (The AN Group), a trade association representing producers and users of acrylonitrile, submitted to the TSCA 8(e) Coordinator translations of several Chinese articles (some published, some unpublished) relating to studies of acrylonitrile. The AN Group was formed to facilitate the protection of human health and the environment through all stages of the acrylonitrile product lifecycle. Members of the AN Group include: Bayer Corporation; BP Chemicals, Inc.; Cytec Industries Inc.; The Dow Chemical Company; DuPont Company; GE Plastics; Solutia, Inc.; and Sterling Chemicals, Inc.

We are hereby today submitting the following three additional Chinese articles and English translations:

- Effects of Acrylonitrile Exposure on Male Sexual Hormone;
- Effects of Acrylonitrile on Reproductive and Endocrine Systems of Female Rats; and,
- Lipid Peroxidation Effects of Acrylonitrile in Male Rats.

The English versions were prepared by a translator at the National Institute of Health Library. The AN Group is not involved with these studies and has not assessed the significance of the findings. We are, nonetheless, providing these articles to EPA for its information and suggest that these materials be added to the previously established docket # [8EHQ-0400-14711].

Please contact me at the address above if you have any questions.

Sincerely,

  
Robert J. Fensterheim  
Executive Director



2003 APR - 8 AM 8:59

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# 丙烯腈对男工性激素水平影响研究

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**摘要** 为探讨丙烯腈(AN)对作业男工性激素水平的影响,选择71名长期接触AN的男工为接触组,选与其生活环境、经济条件、年龄相近的不接触任何毒物的男工50人为对照组,测定其血清中T、LH、FSH和E<sub>2</sub>水平,结果发现接触组血清中T水平明显下降而E<sub>2</sub>明显升高,与对照组差异均有高度显著性(P<0.01),作者认为其原因是长期接触较低浓度AN,由于AN及其代谢产物CEO对睾丸组织的细胞发生了脂质过氧化作用致使间质细胞受损所致,确切机制尚需深入研究。

**关键词** 丙烯腈(AN); 睾酮(T); 黄体生成素(LH); 卵泡刺激素(FSH); 雌二醇(E<sub>2</sub>)

## Effect of Acrylonitrile Exposure on Male Sexual Hormone

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**Abstract:** To evaluate the effects of acrylonitrile(AN) exposure on male sexual hormone, blood samples were collected from 71 male workers exposed to AN occupationally and 50 male workers with no exposure history. The levels of serum testosterone (T), luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol (E<sub>2</sub>) were determined by radioimmunoassay (RIA). The results showed that the level of T in exposed workers was lower than that of non-exposed workers, while the level of E<sub>2</sub> was elevated in workers exposed to AN significantly (P<0.01). It was considered that AN exposure could induce lipid peroxidation and produce Leydig's cell injury in testis through AN and CEO, but the mechanism still need study deeply.

**Key Words:** Acrylonitrile(AN); testosterone(T); luteinizing hormone (LH); follicle stimulating hormone (FSH); estradiol (E<sub>2</sub>)

丙烯腈(Acrylonitrile, AN)是有机合成工业应用广泛的单体。近年对其一般毒性、致突变、致癌和致畸作用的研究报道较多<sup>[1-3]</sup>, 但对其生殖毒性研究较少。国内张玉敏等报道, 对雄性大鼠皮下注射染毒 AN 25 mg kg<sup>-1</sup> 体重 77 天可致血中睾酮(T)水平下降, 黄体生成素(LH)升高, 睾丸组织 T 下降, LH、卵泡刺激素(FSH)和雌二醇(E<sub>2</sub>)含量升高, 并对间质细胞及生精上皮产生不同的损伤作用<sup>[4]</sup>。为进一步探讨长期接触 AN 是否对男工的性激素水平产生影响, 为全面评价 AN 的毒作用, 保护工人及子代健康提供科学依据, 进行本研究。

### 材料与方方法

1. 研究对象 以某腈纶化工厂化工、丙烯腈和仓储车间接触 AN 的男工 71 人为接触组, 平均年龄 33.9±6.3 岁(22~55 岁), 平均工龄 14.3±6.4 年(4~35 年), 选同一地区, 生活环境、经济状况相近的其他工厂不接触任何毒物和有害作业男工及男性行政人员 50 人为对照组, 平均年龄 34.9±7.2 岁(24~55 岁) 平均工龄 14.1±6.7 年(4~34 年)

2. 作业场所空气中 AN 的监测 1990 年前车间空气 AN 浓度为每两个月监测一次, 1990 年起改为每月监测一次。按《劳动卫生工作规范》分别在化工、丙烯腈、仓储车间设定监测点, 以气相色谱法定量分析测定。

3. 性激素水平测定 早餐禁食,于上午8~9点抽取上肢静脉血,分离血清备检。测定血中T、LH、FSH和E<sub>2</sub>水平。各指标测定均采用放射免疫法(RIA)试剂盒由天津放射研究所提供,按试剂盒方法操作<sup>[5]</sup>。对照组和接触组为同批试剂盒同一天完成测定。

4. 统计方法 用Foxpro建立数据库,采用SAS统计软件进行相应的统计分析。

### 结 果

1. 车间空气中AN监测结果 该厂始建于70年代,1990年前污染严重,车间空气中AN浓度超过国家标准约10~20倍。1990年该工厂进行彻底改造,引进了全套美国设备,生产过程全部管道化、密封化、自动化操作,露天框架,通风良好。新设备使用后,1990年~1997年车间年平均AN浓度波动在1.54~6.09mg/m<sup>3</sup>,平均超过国家标准1~2倍。1996年1月~2000年6月车间年平均AN浓度明显下降,测定结果见表1。

表1 1997年~2000年各车间空气中丙烯腈平均浓度测定结果

车 间	监测 点数	年度平均浓度 (mg/m <sup>3</sup> )				
		1996年	1997年	1998年	1999年	2000年
化工	17	1.95	3.52	1.40	0.74	1.64
丙烯腈	8	1.90	2.20	0.84	0.43	1.76
仓储	3	2.15	3.05	0.43	0.14	0.41

2. 自觉症状 接触组和对照组肝肾功能均正常。接触组工人主诉有头昏、失眠、记忆力减退、胸闷等非特异症状,有少数工人主诉有性功能减退症状,因被检人数仅71人,主诉症状分散,而对照组又多无上述症状,故未进行统计学处理。

3. 血清中性激素水平测定结果 见表2。接触组T水平明显下降,但在本方法正常值范围(12.4~35.6nmol/L<sup>-1</sup>)内,靠近正常下限值。

表2 丙烯腈作业男工血清性激素水平测定结果(x±s)

组 别	人数	年龄(岁)	T(nmol/L)	LH(IU/L)	FSH(IU/L)	E <sub>2</sub> (pmol/L)
对照组	50	34.9±7.2	23.64±11.54	10.62±4.32	8.86±6.55	157.8±142.9
接触组	71	33.9±6.3	15.99±3.63**	10.22±4.42	9.75±6.06	421.4±177.3**

\*\* 与对照组比较差异有高度显著性 P<0.01

4. 不同工龄组AN作业男工血清中性激素水平测定结果 见表3。从表3可见各工龄组间各性激素水平差异均无显著性(P>0.05)。

表3 不同工龄丙烯腈作业男工血清性激素水平测定结果(x±s)

工龄	人数	工龄(年)	T(nmol/L)	LH(IU/L)	FSH(IU/L)	E <sub>2</sub> (pmol/L)
≤15年	44	10.3±3.2	16.14±3.47	9.83±3.67	9.69±6.85	427.5±185.2
>15年	27	20.8±4.7	15.77±3.92	10.87±5.45	9.83±6.16	411.5±166.7

5. 不同车间AN作业男工性激素水平测定结果 见表4。从表4可见各车间男工各性激素水平差异均无显著性(P>0.05)。

表4 不同车间丙烯腈作业男工血清性激素水平测定结果(  $\bar{x} \pm s$  )

组别	人数	T( nmol/L )	LH( IU/L )	FSH( IU/L )	E <sub>2</sub> ( pmol/L )
化工	27	16.26±3.54	9.94±3.68	10.88±7.10	389.6±173.1
丙烯腈	22	15.16±4.07	10.98±5.38	8.56±5.78	419.7±202.9
仓储	22	16.36±3.38	9.83±3.68	8.46±7.10	461.7±159.8

#### 6. 90年后参加工作男工性激素水平测定结果

见表5。从表5可见,接触较低浓度AN的男工与对照组比较血清T下降,E<sub>2</sub>升高,差异均有高度显著性(P<0.01)。

表5 90年后接触低浓度丙烯腈男工血清性激素水平测定结果(  $\bar{x} \pm s$  )

组别	人数	年龄(岁)	T( nmol/L )	LH( IU/L )	FSH( IU/L )	E <sub>2</sub> ( pmol/L )
对照组	50	34.9±7.2	23.64±11.54	10.62±4.32	8.86±6.55	157.8±142.9
接触组	13	26.7±2.8	16.51±4.61**	9.05±3.61	7.79±6.05	451.5±189.3**

\*\* 与对照组比较差异有高度显著性 P<0.01

### 讨 论

本研究在较严格控制影响性激素水平的因素,例如年龄等混杂影响的条件下,发现在较长期接触高浓度AN后并在近10年内接触较低浓度AN的作业男工,血清中T下降,E<sub>2</sub>升高而LH、FSH均正常,T与E<sub>2</sub>的改变与对照组差异均有高度显著性(P<0.01)。说明上述改变确为该浓度下AN所致。

本研究接触组平均工龄为14.3±6.4年,71人中90年及其后参加工作的仅13人,平均工龄为6.2±2.0年。1990~1997年车间空气中AN浓度波动在1.54~6.09 mg/m<sup>3</sup>,平均超过国家标准1~2倍,1998年起车间空气中AN浓度均在国家标准以下,但该13名工人血清中T明显下降,E<sub>2</sub>明显升高,均与对照组比较差异有高度显著性(P<0.01),LH、FSH与对照组比较差异不显著(P>0.05),说明在该浓度下工作4年以上仍可对性激素的分泌产生损害作用。陈玉清等报道丙烯腈作业工人在低于国家浓度标准的情况下工作,仍可使工人血中γ-谷氨酰转肽酶(γ-GT)和尿中硫氰酸盐(USCN)明显高于对照组(P<0.05),外周血淋巴细胞微核率明显高于对照组(P<0.05),说明在低于国家标准下AN仍可对作业工人产生损害作用,因此本研究产生上述损害是完全可能的<sup>[7]</sup>。但为更确切地证实上述发现尚需进行一段时间的动态观察。

在该浓度下作业男工血中T下降,E<sub>2</sub>升高而LH未发生明显改变,其机理可能如下:AN及其代谢产物2-氯环氧乙烷(CEO),是一种强亲电化合物,CEO有比AN更强的亲电性,二者均可使生物膜发生脂质过氧化作用,与各种生物大分子如类脂、蛋白质结合,改变了生物膜上各种酶的活性,同时AN、CEO更易与DNA、RNA结合,使遗传物质发生改变,细胞突变后使机体某些酶的合成、活化发生改变<sup>[8]</sup>。马明月研究发现雄性大鼠染毒AN 25 mg/kg<sup>-1</sup>77天可致血中GSH含量和GSH-Px活性降低,可对睾丸组织产生脂质过氧化作用<sup>[9]</sup>。张玉敏报道给雄性大鼠染毒AN 25 mg/kg<sup>-1</sup>77天可使血中T明显下降,E<sub>2</sub>明显升高,光镜下可见睾丸间质细胞水肿,轻度变性,电镜下可见间质细胞线粒体肿胀,嵴消失,有的线粒体破碎,粗面内质网脱颗粒<sup>[4]</sup>。由此说明AN及其代谢产物CEO可使合成T的线粒体受损,线粒体上合成T的酶活性下降,T合成减少致使血中T下降是主要原因之一。另一原因可能是由于睾丸外组织使T降解加快,同时T转化为E<sub>2</sub>的量增加致使外周血中T下降E<sub>2</sub>升高。男性血中E<sub>2</sub>除肾上腺少量分泌外,主要由睾丸外脂肪组织、神经组织、结缔组织中的芳香化酶催化下由T转化<sup>[10]</sup>。本研究发现E<sub>2</sub>升高推测可能是AN和/或CEO诱导了芳香化酶的活性,因而使T转化为E<sub>2</sub>的量增加。

至于LH未发生明显改变可作如下解释:一般说来由于T水平下降通过负反馈作用使LH升高,促进T合成以维持血中T浓度稳定。本研究中LH未发生改变可能是由于男工血中E<sub>2</sub>升高,E<sub>2</sub>对LH的释放可产生一些抑制作用,由于E<sub>2</sub>主要来源于外周T的芳香化,这种抑制可被认为是一种间

接的雄激素效应，因而使 LH 未升高。

为明确损伤的机理有必要进一步测定血中与 T 和 E<sub>2</sub> 合成相关酶的活性，例如 C<sub>17-20</sub> 裂解酶、芳香化酶等。如有可能可作男工精液质量分析、睾丸组织活检和尿中代谢产物 USCN 的测定及 AN、CEO 与红细胞形成的加合物水平的测定，并对接触低剂量的男工血中性激素水平进行动态观察以进一步明确毒作用机理，确定产生生殖系统损伤作用的阈剂量，为修订现行卫生标准提供毒理学和人群流行病学的依据。

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## EFFECTS OF ACRYLONITRILE EXPOSURE ON MALE SEXUAL HORMONE

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Acrylonitrile (AN) is an important monomer in the organic synthetic chemical industry. In recent years, there have been many studies published on its general toxicity, mutagenicity, carcinogenicity, and deformation activity [1-3]. In China, Zhang Yumin et al. have reported that when male rats are treated with 25 mg of AN per 1 kg of body weight administered subcutaneously for 77 days, it can lead to a decrease in testosterone (T) levels in the blood, a rise in luteinizing hormone (LH), a decrease in T in testicular tissue, an increase in LH, follicle-stimulating hormone (FSH) and estradiol (E<sub>2</sub>) content, as well as different harmful effects on interstitial cells and sperm production [4]. In order to study more deeply whether or not long-term exposure to AN affects the level of sexual hormones production in male workers, and in order to evaluate the overall toxic effects of AN, this study was conducted on a scientific basis with the aim of protecting workers and the health of their progeny.

### MATERIALS AND METHODS

**1. Subjects:** A group of 71 male workers in a certain nitrile fiber plant exposed to acrylonitrile in the manufacturing process and to acrylonitrile in warehouse work areas, with a mean age of  $33.9 \pm 6.3$  (22-55 years of age), a mean working time of  $14.3 \pm 6.4$  years (4-35 years), and a control group of 50 male administrative workers and male workers who perform hazardous work but who have not been exposed to toxic substances, and work in another factory in the same area, with the same living environment, and the same economic conditions. The mean age was  $34.9 \pm 7.2$  (24-55 years of age), and the mean working time was  $14.1 \pm 6.7$  years (4-34 years).

**2. Monitoring of AN in the Air at the Work Place:** AN concentration in air in warehouse work areas was monitored every two months before 1990, and starting in 1990, it was monitored once a month. According to the "Labor Health and Working Standards," in each factory, monitoring sites for acrylonitrile are to be established in the warehouse work areas, and measurements are to be performed using gas chromatography.

**3. Measurement of Sexual Hormone Levels:** Subjects were forbidden to eat breakfast, venous blood was drawn from an upper extremity between 8:00 am and 9:00 am, and serum was isolated and prepared for testing. Blood levels of T, LH, FSH, and E<sub>2</sub> were determined using radioimmunoassay (RIA). The sample containers were provided by Tianjin Radiological Laboratory, and used in accordance with their sample container methodology [5]. Measurements for both the control group and the exposure group were made on the same day using the same batch sample box.

**4. Statistical Method:** The relevant statistical analyses were conducted using a Foxpro-constructed database and SAS statistical software.

## RESULTS

**1. Results of Monitoring of AN in Work Area Air:** The factory in question was first built in the 1970's, and prior to 1990, it was seriously polluted, with AN concentration in work area air exceeding national standards 10 to 20-fold. In 1990, the factory underwent thorough renovation, with a complete set of facilities introduced into the factory from the United States. The manufacturing processes were all channelized, sealed, and automated, with open-air frames and good ventilation. After using the new facilities, the annual mean AN concentration fluctuated between 1.54 and 6.09 mg/m<sup>3</sup>, which exceeded the national standards 1 to 2-fold. From January 1996 to June 2000, there was a significant drop in annual mean AN concentration. The results are given in Table 1.

**Table 1. Results of Measurement of the Mean Concentration of Acrylonitrile in Work Area Air from 1997 to 2000**

Work area	Monitoring Sites	Annual Mean Concentration (mg/m <sup>3</sup> )				
		1996	1997	1998	1999	2000
Chemical plant	17	1.95	3.52	1.40	0.74	1.64
Acrylonitrile	8	1.90	2.20	0.84	0.43	1.76
Warehouse	3	2.15	3.05	0.43	0.14	0.41

**2. Symptoms:** The exposed group and the control group both exhibited normal liver and kidney function. The principal complaints of the exposed workers were dizziness, loss of sleep, loss of memory, tightness in the chest, and other non-idiosyncratic symptoms. A small number of workers complained of sexual dysfunction. Since the number of tested individuals was only 71, the distribution of the principal symptoms, as well as a large number of symptoms in the control group not given above, were not subjected to statistical treatment.

### 3. Results of Measurement of Serum Levels of Sexual Hormones

See Table 2. The exposed group exhibited a significant decrease in testosterone levels, but they were within a normal range (12.4-35.6 nmol/L<sup>-1</sup>), or close to the normal lower limit.

**Table 2. Results of Measurement of Serum Levels of Sexual Hormones In Male Workers Involved in Work with Acrylonitrile ( $\bar{x} \pm s$ )**

Group	Number	Age	T (nmol/L)	LH (IU/L)	FSH (IU/L)	E <sub>2</sub> (pmol/L)
Control group	50	34.9±7.2	23.64±11.54	10.62±4.32	8.86±6.55	157.8±142.9
Exposed group	71	33.9±6.3	15.99±3.63**	10.22±4.42	9.75±6.06	421.4±177.3**

\*\* High significance in comparison to the control group ( $P < 0.01$ )

#### 4. Results of Measurement of Serum Levels of Sexual Hormones in Male Workers Involved in Work with AN in Groups with Different Length of Working Time

See Table 3. Table 3 shows that the rise in sexual hormone levels in workers who had worked for different lengths of time was not significant ( $P > 0.05$ )

**Table 3. Results of Measurement of Serum Levels of Sexual Hormones In Male Workers Involved with Acrylonitrile with Different Length of Working Time ( $\bar{x} \pm s$ )**

Group	Number	Years Worked	T (nmol/L)	LH (IU/L)	FSH (IU/L)	E <sub>2</sub> (pmol/L)
≤15 Years	44	10.3±3.2	16.14±3.47	9.83±3.67	9.69±6.85	427.5±185.2
> 15 Years	27	20.8±4.7	15.77±3.92	10.87±5.45	9.83±6.16	411.5±166.7

#### 5. Results of Measurement of Serum Levels of Sexual Hormones in Male Workers Involved with AN in Different Work Areas

See Table 4. Table 4 shows that the rise in sexual hormone levels in male workers in different work areas was not significant ( $P > 0.05$ )

**Table 4. Results of Measurement of Serum Levels of Sexual Hormones in Male Workers Involved with Acrylonitrile in Different Work Areas ( $\bar{x} \pm s$ )**

Group	Number	T (nmol/L)	LH (IU/L)	FSH (IU/L)	E <sub>2</sub> (pmol/L)
Chemical plant	27	16.26±3.54	9.94±3.68	10.88±7.10	389.6±173.1
Acrylonitrile	22	15.16±4.07	10.98±5.38	8.56±5.78	419.7±202.9
Warehouse	22	16.36±3.38	9.83±3.68	8.46±7.10	461.7±159.8

## 6. Results of Measurement of Serum Levels of Sexual Hormone in Male Workers Involved After 1990

See Table 5. Table 5 shows that in comparison with the control group, male workers exposed to lower concentrations of AN show lower serum testosterone and higher E<sub>2</sub>, with a high degree of significance (P < 0.01).

**Table 5. Results of Measurement of Serum Levels of Sexual Hormones in Male Workers Exposed to Low Concentrations of Acrylonitrile After 1990 (x ± s)**

Group	Number	Age	T (nmol/L)	LH (IU/L)	FSH (IU/L)	E <sub>2</sub> (pmol/L)
Control group	50	34.9 ± 7.2	23.64 ± 11.54	10.62 ± 4.32	8.86 ± 6.55	157.8 ± 142.9
Exposed group	13	26.7 ± 2.8	16.51 ± 4.61**	9.05 ± 3.61	7.79 ± 6.05	451.5 ± 189.3**

\*\* High significance in comparison to the control group (P < 0.01)

## DISCUSSION

This study strictly controlled the factors affecting sexual hormone levels, such as age and other mixed influences. The study found that after a relatively long period of exposure to high concentrations of AN, and less than nearly 10 years of exposure to relatively low concentrations of AN, male workers exhibited decreases in serum testosterone levels, increases in serum E<sub>2</sub> levels, normal levels of serum FSH, with fluctuations in serum T and E<sub>2</sub> levels significantly higher than in the control group (P < 0.01). These fluctuations are explained by the concentrations of AN.

The mean number of working years of the exposed group was 14.3 ± 6.4 years, and among the 71 workers, there were only 13 who had worked there since 1990, with a mean length of time working at the plant of 6.2 ± 2.0 years. Between 1990 and 1997, the AN concentration in air in work areas fluctuated between 1.54 and 6.09 mg/m<sup>3</sup>, with the mean value exceeding the national standards from 1 to 2-fold. Starting in 1998, the AN concentration in air in work areas was below the national standards, but there was a marked drop in testosterone levels and a marked rise in E<sub>2</sub> levels in the aforementioned 13 workers, with a significant increase over the control group (P < 0.01). LH and FSH did not show significant increases over the control group (P > 0.05), indicating that working 4 years or more under said concentrations could still have a harmful affect on secretion and production of sexual hormones. Chen Yuqing et al. reported that acrylonitrile workers who work in conditions where the concentration is lower than national standards can still have blood concentrations of γ-GT and urine concentrations of USCN that are higher than the control group (P < 0.05), and peripheral blood lymphocyte micronucleus percentage was markedly higher than in the control group (P < 0.05). This

indicates that even when AN is lower than the national standards, it can produce harmful effects on workers. Because of this, the aforementioned damage found in this research is entirely possible [7]. However, in order to obtain more certain prove of this, the dynamics need to be observed over longer periods of time.

Regarding possible mechanisms for the decrease in blood levels of testosterone, elevated  $E_2$ , and lack of significant change in LH levels: AN and its metabolite CEO are strongly electrophilic compounds, and CEO is more strongly electrophilic than AN. Both induce lipid peroxidation in biomembranes, and bind to biological macromolecules such as lipids and proteins, modifying various types of enzymatic activity in biological membranes, and at the same time, AN and CEO readily bind to DNA and RNA, inducing changes in the genetic material and cell mutations, after which there are changes in enzymatic synthesis and activity in the organism [8]. Ma Mingyue studied decreases in blood GSH and GSH-Px activity in male rats treated with  $25 \text{ mg/kg}^{-1}$  acrylonitrile for 77 days and found lipid peroxidation in testicular tissue [9]. Zhang Yumin reported that when male rats were treated with  $25 \text{ mg/kg}^{-1}$  acrylonitrile for 77 days, blood testosterone levels decreased significantly and  $E_2$  levels increased significantly. Examination with an optical microscope found edema in Leydig's cells, with mild deformation, and examination with an electron microscope revealed swelling of the mitochondria in the interstitial cells, disappearances of mitochondrial ridges, break-up of mitochondria, and degranulation of the endoplasmic reticulum [4]. Thus, AN and its metabolite CEO can damage the mitochondria involved in the synthesis of testosterone, reducing the enzyme activity in the mitochondria involved in the synthesis of testosterone, so that the decrease in testosterone synthesis is one of the principle causes of the decrease in blood testosterone levels. Another possible cause is accelerated testosterone degradation in extratesticular tissues, and at the same time, testosterone conversion results in increased levels of  $E_2$ , leading to a decrease in peripheral blood levels of testosterone and increased levels of  $E_2$ . Except for the secretion by the adrenal gland of small quantities of  $E_2$  into the blood of males, testosterone conversion occurs due to aromatase catalysis principally in extratesticular fatty tissues, nerve tissues, and connective tissues [10]. This study found that with increased levels of  $E_2$ , there is the possibility that AN and/or CEO induced the aromatase activity, with the result that testosterone conversion leads to increased  $E_2$ .

Possible explanations of LH not changing significantly: In general, as a result of the decrease in testosterone level leading to negative feedback and causing a rise in LH, it promotes testosterone synthesis, so as to stabilize the concentration of testosterone in the blood. The fact that LH does not undergo change in this study could be due to elevated blood levels of  $E_2$ , and the release of  $E_2$  to LH could give rise to a kind of inhibitory effect, with the result that  $E_2$  is principally derived from the aromatization of peripheral testosterone, and this type of inhibition can be known through a kind of indirect male hormone effect, so that the LH level is not raised.

In order to elucidate the mechanism of damage, it is necessary to conduct more measurements of the interactive enzyme activity contributing to testosterone and estradiol synthesis, such as that of C<sub>17-20</sub> desmolase and aromatase. If it is possible to perform a qualitative analysis of the seminal fluid of the male workers, to perform biopsies on testicular tissue, to measure the metabolite USCN in urine, to measure the levels of AN, CEO, and erythrocyte forming adducts, as well as to observe the dynamics of sexual hormone levels in the blood of male exposed to low levels of acrylonitrile, this will be a step toward elucidating the mechanism of toxic action, and determining the threshold doses with harmful effects on the reproductive system. Revisions in the current public health standards will be made on the basis of toxicology and epidemiology.

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# 丙烯腈对雌性大鼠生殖内分泌影响的研究

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**摘要** 目的 研究丙烯腈对雌性大鼠血清中雌二醇( $E_2$ )、卵泡刺激素(FSH)及黄体生成素(LH)水平及卵巢、子宫、肾上腺病理改变的影响。方法 采用放射免疫法测定皮下注射染毒 30 天雌性大鼠血清中上述各激素的水平,光镜观察卵巢、子宫和肾上腺的病理改变。结果 各染毒组与对照组比较三种激素水平差异均无显著性( $P>0.05$ )但卵巢及肾上腺有不同程度的病理改变。结论 在此条件下,丙烯腈对雌性大鼠的内分泌激素无影响,但对生殖系统有直接损害作用。

**关键词** 丙烯腈; 大鼠; 雌二醇( $E_2$ ) 卵泡刺激素(FSH) 黄体生成素(LH)

## Effects of Acrylonitrile on Reproductive and Endocrine System of Female Rats

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**Abstract Objective** To study the effects of acrylonitrile(AN) on the levels of estradiol( $E_2$ ), follicle stimulating hormone(FSH) and luteinizing hormone(LH) in serum of female rats, as well as histopathological changes of uterus, ovaries and adrenal gland. **Methods** Female rats were administered by subcutaneous injection for 30 days, and their levels of above hormones were determined by RIA, as well as histopathological examinations were performed for above organs with light microscope. **Results** There was no significant difference between the exposed groups and the control group in the levels of the three hormones ( $P>0.05$ ), while there were histopathological changes of different degree in ovaries and adrenal gland. **Conclusion** Under this condition, AN has no effect on hormones of female rats, but it has direct damage effect on reproductive system.

**Key words** Acrylonitrile; Rats; Estradiol( $E_2$ ); Follicle stimulating hormone(FSH); Luteinizing hormone(LH)

丙烯腈(Acrylonitrile, AN)是有机合成工业中的重要单体,广泛应用于合成腈纶纤维,ABS工程塑料及生产食品包装材料等[1]。动物实验及流行病学调查结果表明,丙烯腈对动物及人类生殖结局有不同程度的损害,能造成一定的出生缺陷,但机制未明。本次试验试图通过用不同剂量丙烯腈对雌性大鼠皮下注射染毒 30 天,测定血中  $E_2$ , LH, FSH 水平,研究丙烯腈对雌性大鼠激素的影响,观察生殖系统主要脏器的病理改变,以期找到早期损害指标。

### 材料与方 法

1. 实验动物分组及处置 由沈阳医学院实验动物中心提供的 Wistar 雌性大鼠 40 只,体重 180—220g 随机分为四组:一个对照组、三个染毒组。对照组给予生理盐水,染毒组分别给予丙烯腈 5mg/kg、15mg/kg、25mg/kg,按 0.2ml/100g 容积皮下注射,每日染毒一次,连续染毒 30 天。

2. 受试物 丙烯腈由上海试剂三厂出品,批号 941210。丙烯腈含量大于 99%,用生理盐水配制成相应浓度,临用现配。

3. 实验方法 大鼠染毒 30 天后,乙醚麻醉,腹主动脉采血,然后摘取脏器用天平称重。血液凝固后离心分离血清,放射免疫法测定血清中 E<sub>2</sub>、LH、FSH 的水平,光镜下观察卵巢、子宫、肾上腺的病理改变。

4. 统计分析 建立 Foxbase 数据库,应用 SAS 软件进行统计分析和显著性检验。

## 结 果

1. 中毒症状:染毒一周后,各染毒组呈现不同程度的烦躁不安、躁动等症状。二周后出现兴奋,呼吸急促,易怒等症,约经 1 小时后恢复正常。三周后,高剂量组出现精神萎靡,嗜睡等症状。各组动物体重增长速度随染毒剂量增大而程下降趋势,均低于对照组,但无显著性差异( $P>0.05$ )

2. 丙烯腈对大鼠内分泌激素的影响

由表 1 可知各激素水平与对照组相比,均无显著性差异( $p>0.05$ )

表 1 丙烯腈对大鼠内分泌激素的影响( $\bar{x}\pm s$ )

组别	n	FSH(MIU/ml)	LH(mIU/ml)	E <sub>2</sub> (pg/ml)
对照组	10	15.37±5.99	5.48±1.37	37.61±21.24
5mg/kg	10	16.64±6.77	4.95±2.30	46.08±26.34
15mg/kg	10	21.07±8.96	3.97±2.25	34.71±22.81
25mg/kg	10	15.70±7.59	4.57±0.79	54.64±18.74

3. 丙烯腈对大鼠生殖系统损害的病理检测

卵巢:各组卵巢外表光滑,附有致密的结缔组织被膜,仍可分清被膜、皮质和髓质三部分,皮质层内含有不同发育阶段的卵泡。不同剂量染毒丙烯腈大鼠卵巢组织中,初级卵泡和次级卵泡的发生数与对照组比较无明显增加或减少。与对照组相比,各染毒组次级卵泡内可见颗粒细胞增多,并有不同程度的脂肪变性、水样变性。高剂量组卵泡腔内有点状、固缩性坏死的细胞及炎细胞浸润,同时血管扩张充血。成熟卵泡不多见,但可见许多黄体 and 间质腺形成,黄体细胞有变性,其间质无明显纤维性增生等改变。

子宫:各组子宫内膜局部均有大量嗜中性粒细胞及嗜酸性粒细胞浸润,血管扩张充血,腺体增生活跃。各染毒组间无差异。

肾上腺:肾上腺各层组织结构清晰可辨,高剂量组略有部分细胞胞浆疏松或呈颗粒状,血窦扩张充血,其余正常。

## 讨 论

雌性生殖周期的变化主要通过丘脑下部、垂体前叶和卵巢分泌的激素进行调节的,即通过下丘脑—垂体—卵巢轴来调节。下丘脑和垂体分泌的激素调节和影响卵巢的周期性变化,卵巢产生的激素又调节子宫内膜的周期性变化。当卵泡接近成熟时,雌激素水平最高,此时垂体分泌的 FSH 和 LH 也处于最高峰,血液内高浓度的雌激素又可反馈影响垂体和下丘脑的分泌活动,使 LH 分泌增强。在

LH 与 FSH 的协同作用下, 卵巢排卵和黄体形成[2]。外来化合物对雌性生殖损伤的作用点特别多, 内分泌紊乱是最常见的损伤之一。如果外来化合物干扰内分泌激素的合成与释放, 则会使这些激素在血液中的浓度发生变化, 从而影响正常的生殖功能。丙烯腈是一种蓄积作用不明显的化学物[3]。它在动物体内的吸收与染毒途径有关, 经口或呼吸道可有 95%吸收, 经皮只有 1%吸收。丙烯腈有两种主要代谢途径, 一是直接与谷胱甘肽(GSH)结合解毒, 二是在细胞色素氧化酶作用下形成氧环乙烷(CEO)然后与 GSH 结合后由尿液排出体外[4]。邵静等给予雌性大鼠腹腔注射染毒丙烯腈(8, 16mg/kg)16 周, 测定雌性大鼠血清 LH、FSH、E2 含量, 发现剂量达到 16mg/kg 时雌性大鼠血清中 LH 含量显著下降。病理学检查光镜下可见卵巢的生长卵泡中卵母细胞核消失, 次级卵泡腔内充满污染的无定形物。电镜下可见卵泡细胞的线粒体数日减少, 少数肿胀早空泡变性[5]。说明丙烯腈可通过直接损伤生殖器官及影响生殖内分泌功能而产生生殖毒作用。本次实验结果表明, 丙烯腈对雌性大鼠的 E2、FSH、LH 无显著影响, 但光镜病理检查可见, 丙烯腈对雌性大鼠生殖系统有较明显的影响, 且随剂量的增加卵巢内变性坏死稍有加重。说明丙烯腈对雌性生殖系统有直接损害作用, 进一步证明, 如果达到一定剂量, 丙烯腈可直接损伤雌性大鼠生殖系统, 对卵泡的生长过程造成影响。至于内分泌激素无显著性改变, 原因大致如下几点:

1. 丙烯腈的半衰期较短, 给药时间间隔较长且染毒期限短, 使丙烯腈不能在体内蓄积, 因而不会发挥太大的毒作用。张玉敏等[6]曾报道以 25mg/kg 体重剂量给雄性大鼠皮下注射染毒, 染毒 38 天时各观察指标未见有意义变化, 而当染毒 77 天时, 大鼠血中睾酮(T)下降, LH 升高, 睾丸中 T 下降, LH、FSH、E2 均升高, 因此, 当累积染毒达到一定剂量时, 内分泌系统应受到一定的损害。

2. 缺少动态分析。本次实验大鼠染毒共 30 天, 如果染毒后 1~2 周就测定大鼠血清中 FSH、LH、E2 的水平或许结果与 30 天后实验结果不一致。因为经过 1~2 周后, 大鼠体内经过代偿对丙烯腈产生了一定的适应性, 机体内分泌系统趋于平衡状态, 消除了外来物对自身的影响。对丙烯腈毒性研究尚需增加染毒时间和提高染毒剂量, 进一步探讨其对雌性激素的影响。

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## EFFECTS OF ACRYLONITRILE ON REPRODUCTIVE AND ENDOCRINE SYSTEMS OF FEMALE RATS

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Acrylonitrile (AN) is an important monomer in the organic synthetic chemical industry. It is widely used in the synthesis of nitrile synthetic fibers, ABS processed plastics, packaging materials for processed foods, and other applications [1]. Animal studies and epidemiological surveys show that acrylonitrile brings about different degrees of damage to animal and human reproduction, and can cause certain birth defects, but the mechanism has yet to be elucidated. Our experiments used varying levels of acrylonitrile in subcutaneous injections in female rats for 30 days, to study the effects of acrylonitrile on blood levels of E<sub>2</sub>, LH, and FSH, as well as the effects on hormone levels in female rats. We observed histopathological changes in the reproductive system, and found evidence of damage in the early stages.

### MATERIALS AND METHODS

**1. Subjects and Treatment:** We used 40 female Wistar rats weighing 180-220 g provided by the Shenyang Medical College Experimental Animal Center. The animals were divided into 4 groups: 1 control group and 3 treatment groups. Using subcutaneous injection, saline was administered to the control group, and 5 mg/kg, 15 mg/kg, 25 mg/kg were administered to the treatment groups, on the basis of 0.2 mL/100g. The animals were treated once a day for 30 consecutive days.

**2. Test Sample:** The acrylonitrile was from Shanghai Reagent Third Factory, lot number 941210. Acrylonitrile content was 99%, and prepared in the appropriate concentrations using saline, and distributed for clinical use.

**3. Experimental Method:** 30 days after treating the rats, they were anesthetized with ether, blood was drawn from the ventral aorta, and then the organs were removed and weighed. After the blood clotted, the serum was removed by centrifugation, and RIA was used to determine serum levels of E<sub>2</sub>, LH and FSH, and histopathological changes in the ovaries, uterus, and adrenal gland were observed under an optical microscope.

**4. Statistical Analysis:** A Foxbase database was set up, and SAS software was used to perform statistical analyses and significance studies.

## RESULTS

**1. Toxic Symptoms:** One week after treatment, each treatment group exhibited symptoms such as differing degrees of agitation, unease, restlessness, and the like. Two weeks after treatment, they exhibited symptoms such as excitement, shortness of breath, and irritability, and after about one week, they returned to normal. Three weeks after treatment, the high-dose group exhibited symptoms such as listlessness and lethargy. Body weight in each group of animals increased rapidly along with increases in dose amount, and there was a tendency to decrease in the control group, but the difference was not significant ( $P > 0.05$ ).

### 2. Effect of Acrylonitrile on the Endocrine System of Rats

Table 1 shows a comparison of endocrine levels ( $P > 0.05$ )

Table 1. Effect of Acrylonitrile on the Endocrine System of Rats ( $\bar{x} \pm s$ )

Group	n	FSH(MiU/ml)	LH(mIU/ml)	E <sub>2</sub> (pg/ml)
Control	10	15.37±5.99	5.48±1.37	37.61±21.24
5mg/kg	10	16.64±6.77	4.95±2.30	46.08±26.34
15mg/kg	10	21.07±8.96	3.97±2.25	34.71±22.81
25mg/kg	10	15.70±7.59	4.57±0.79	54.64±18.74

### 3. Histopathological Studies of the Effect of Acrylonitrile on the Rat Reproductive System

**Ovary:** The outer surface of the ovaries in each group appeared smooth, with a membrane of fine tissue, and it was still possible to distinguish the three parts, namely, the membrane, the cortex, and the medulla, and within the cortex layer there were follicles in different stages of development. Among the rat ovarian tissues treated with differing doses of acrylonitrile, the number of primary follicles and growing follicles that developed did not exhibit a clear increase or decrease in comparison with the control group. A greater number of granulocytes could be observed in the growing follicles of each treated group, in comparison to the control group, as well as differing degrees of fatty degeneration and aqueous degeneration. Inside the cavities of the follicles of high-dose group were found punctate and pyknotic necrotic cells, and inflamed cells which had infiltrated, and at the same time, the blood vessels had expanded and were gorged with blood. Mature follicles were few in number, but a large number of corpora lutea and interstitial glands could be observed to have formed. The lutein cells were degenerate, and their interstices did not exhibit evidence of changes such as fibrous hyperplasia.

**Uterus:** The endometrium in each group exhibited local infiltration of a large number of neutrophilic granulocytes and acidophilic granulocytes, as well as expanded blood vessels gorged with blood. Glandular hyperplasia was dramatic, with no significant difference among the treatment groups.

**Adrenal Gland:** The structure of the histological layers could be clearly distinguished, and the high-dose group had some cells that were fibrous or granular, with the hemal sinus expanded and gorged with blood.

## DISCUSSION

Changes in the female reproductive cycle are primarily regulated by hormones secreted by the hypothalamus, anterior lobe of the pituitary, and ovaries, via the hypothalamus-pituitary-ovary regulatory axis. Hormones secreted by the hypothalamus and pituitary regulate and influence periodic changes in the ovaries, and the ovaries produce hormones and regulate periodic changes in the endometrium. When the follicles approach maturity, the level of female hormones is high, and the FSH and LH secreted by the pituitary reach their highest peaks. The elevated blood levels of female hormones can generate feedback that influence the secretory activity of the pituitary and hypothalamus, increasing the secretion of LH. Under the joint action of LH and FSH, the ovaries release ova corpora lutea are formed [2]. External compounds have many harmful effects on the female reproductive system, and disruption of the endocrine system is one of the harmful effects most commonly seen. If an external compound interferes with the synthesis and release of hormones, then the concentration of this hormone in the blood will give rise to changes, and affect normal reproductive function. Acrylonitrile is a chemical substance with unclear cumulative effects [3]. Its absorption in animals is related to the means by which it is administered. Absorption is 95% in the case of oral or respiratory intake, but only 1% in the case of cutaneous administration. Acrylonitrile has two principal metabolic pathways, one of which is detoxification through direct conjugation with glutathione (GSH), and the second of which is the formation of 2-cyanoethylene oxide (CEO) under the effects cytochrome oxidase activity, after which it is conjugated with GSH and then eliminated from the body in the urine [4]. Shao Jing et al. treated female rats with acrylonitrile by peritoneal injection (8, 16 mg/kg) for 16 weeks, and measured serum levels of LH, FSH, and E<sub>2</sub>, and when the dosage reached 16 mg/kg, the serum level of LH fell significantly. Histopathological examinations performed with an optical microscope revealed nuclear dissolution, and the cavities of growing follicles were filled with amorphous matter. There was a decrease in the number of mitochondria of follicle cells visible under the microscope, and a small number of them were swollen, giving rise to bubble degeneration [5]. The explanation is that acrylonitrile is able to directly damage the reproductive organs and affect reproductive endocrine function, and produce a toxic effect on reproduction. These experimental results show that acrylonitrile does not significantly affect E<sub>2</sub>, FSH, and LH in female rats, but optical microscope examination can show that acrylonitrile has a relatively clear effect on the reproductive system of female rats, and as the dose increases, degenerative necrosis increases within

the ovaries. The results explain the direct harmful effects of acrylonitrile on the reproductive system of female rats, and furthermore, they explain that if a certain dose level is reached, acrylonitrile can directly harm the reproductive system of female rats, affecting follicle growth throughout the process of formation. The hormones do not undergo significant changes, and the main reasons are as follows:

1. The half-life of acrylonitrile is short, the administration time intervals are relatively long and the treatment period is short, and acrylonitrile cannot be stored in the body, so it cannot exhibit a large toxic effect. Zhang Yumin et al. [6] reported on the subcutaneous administration of 25 mg/kg in male rats for 38 days, and each observation showed no significant change, and when treated for 77 days, there was a drop in blood testosterone (T) levels in rats, a rise in LH, a drop in T, and increases in LH, FSH, and E<sub>2</sub> in the testes. Because of this, when the cumulative dosage reached a certain level, the endocrine system sustained a certain level of damage.

2. Analysis of Dynamics: In these experiments, the rats were all treated for 30 days, and if the serum levels of FSH, LH, and E<sub>2</sub> in rats are measured 1-2 weeks after treatment, the results are not in agreement with the test results after 30 days. The reason is that after the passage of 1-2 weeks, a certain compensatory adaptation to acrylonitrile occurs in the bodies of the rats, and the endocrine system within the organism rapidly reaches a state of equilibrium, canceling out the effect of the external substance. Toxicity studies on acrylonitrile need to increase the treatment time and raise the treatment dose, so as to further explore the effects on female hormones.

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## 丙烯腈对雄性大鼠脂质过氧化作用研究

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**摘要** 为了研究丙烯腈的脂质过氧化作用,给雄性大鼠皮下注射丙烯腈,测定染毒38天和77天血及睾丸组织脂质过氧化指标;并对睾丸、附睾进行病理学检查。结果染毒38天15mg·kg<sup>-1</sup>、25mg·kg<sup>-1</sup>组大鼠血中谷胱甘肽(GSH)含量显著升高,5mg·kg<sup>-1</sup>、15mg·kg<sup>-1</sup>、25mg·kg<sup>-1</sup>组大鼠睾丸中谷胱甘肽过氧化物酶(GSH-Px)显著下降且与染毒剂量呈高度负相关( $r=-0.464, p<0.01$ );染毒77天25mg·kg<sup>-1</sup>组大鼠血中GSH-Px显著下降,5mg·kg<sup>-1</sup>、15mg·kg<sup>-1</sup>、25mg·kg<sup>-1</sup>组GSH显著下降且与染毒剂量呈高度负相关( $r=-0.789, p<0.01$ ),5mg·kg<sup>-1</sup>、15mg·kg<sup>-1</sup>、25mg·kg<sup>-1</sup>组大鼠睾丸中GSH-Px显著下降;病理所见曲细精管、精原细胞发生纤维变性,精子生成减少,附睾中精子数量减少。我们可以得出丙烯腈能使大鼠睾丸组织产生脂质过氧化作用。

**关键词** 丙烯腈 大鼠 脂质过氧化作用 睾丸

丙烯腈是职业环境中重要的有害空气污染物之一[1],为腈纶纤维,丁腈橡胶、ABS工业塑料及某些合成树脂等的有机合成单体。近年来对丙烯腈的毒理学研究进一步深入,重点是关于丙烯腈的特殊毒性[2],丙烯腈对生殖系统的毒作用有一定的报道[3,4]。丙烯腈的代谢研究热点集中在活性氧上[5,6],但脂质过氧化是否为丙烯腈对雄性生殖细胞损伤的机理之一,作者尚未见报道。本文通过丙烯腈亚慢性染毒检测雄性大鼠血中及睾丸组织中脂质过氧化物及抗氧化物含量和抗氧化物酶活力,研究丙烯腈生殖毒作用的可能机理。

### 1 材料与方 法

#### 1.1 受试物

丙烯腈为化学纯试剂,纯度>99%,含氰酸0.05%。

#### 1.2 动物与分组

选择体重180~220克健康雄性Wistar大鼠80只,随机区组分为阴性对照组、5mg·kg<sup>-1</sup>、15mg·kg<sup>-1</sup>、25mg·kg<sup>-1</sup>丙烯腈染毒组,每组20只。Wistar大鼠由沈阳医学院实验动物中心提供。

#### 1.3 实验动物处 置

将丙烯腈按设计剂量用生理盐水现配成相应浓度的水溶液,按0.2ml·100g<sup>-1</sup>皮下注射,阴性对照组给予等容积的生理盐水,一天一次,染毒77天,于染毒第38天和第77天每组随机处死10只,经腹主动脉采血,并取同侧睾丸用生理盐水制成1:10组织匀浆备检。

#### 1.4 检测指标及方 法

测定血和睾丸匀浆中的SOD、CAT、GSH-Px的活力及MDA、GSH的含量。上述指标测定所用试剂盒由南京建成生物工程研究所提供。测定方法按试剂盒中相应指标检测说明书操作[7]。

#### 1.5 统计方 法

应用Foxbase软件建立数据库,应用SAS软件进行统计分析。

## 2 结 果

### 2.1 染毒丙烯腈38天雄性大鼠血中SOD、CAT、GSH-Px、MDA、GSH测定结果

见表1,从表1可见血中GSH含量各组间经F检验有显著性( $p<0.05$ ),GSH含量显著升高,其它指标

差异均无显著性(  $P>0.05$  )。

表 1 染毒丙烯腈38天雄性大鼠血中MDA, SOD, CAT, GSH-Px, GSH水平测定结果(  $\bar{x}\pm s$  )

组别	动物数 (只)	MDA ( $\text{nmol}\cdot\text{ml}^{-1}$ )	SOD ( $\text{NU}\cdot\text{ml}^{-1}$ )	CAT ( $\text{U}\cdot\text{glb}^{-1}$ )	GSH-Px ( $\text{U}$ )	GSH ( $\text{mg}\cdot\text{glb}^{-1}$ )
阴性对照组	10	14.91 $\pm$ 3.78	386.75 $\pm$ 50.85	448.30 $\pm$ 136.31	30.91 $\pm$ 9.05	2.39 $\pm$ 0.26
5mg. kg <sup>-1</sup>	10	13.85 $\pm$ 2.32	330.50 $\pm$ 95.05	454.40 $\pm$ 132.21	31.87 $\pm$ 7.85	2.93 $\pm$ 0.63
15mg. kg <sup>-1</sup>	10	15.89 $\pm$ 0.87	350.08 $\pm$ 98.79	441.25 $\pm$ 90.86	32.03 $\pm$ 12.40	3.10 $\pm$ 0.55*
25mg. kg <sup>-1</sup>	10	16.09 $\pm$ 2.25	335.98 $\pm$ 37.41	476.44 $\pm$ 90.28	27.30 $\pm$ 15.41	2.92 $\pm$ 0.39*

\* 与阴性对照相比差异有显著性  $P<0.05$

## 2.2 染毒丙烯腈38天大鼠睾丸中SOD、CAT、GSH-Px、MDA、GSH测定结果

见表2, 由表2可见, 38天大鼠睾丸匀浆中GSH-Px各组间经F检验差异有显著性(  $p<0.05$  ), 且与染毒剂量呈高度负相关(  $r=-0.464, P<0.01$  ), 其它指标的差异均无显著性(  $p>0.05$  )。

表 2 染毒丙烯腈38天大鼠睾丸中MDA, SOD, CAT, GSH-Px, GSH水平测定结果(  $\bar{x}\pm s$  )

组别	动物数 (只)	MDA ( $\text{nmol}\cdot\text{mgPr}^{-1}$ )	SOD ( $\text{NU}\cdot\text{mgPr}^{-1}$ )	CAT ( $\text{U}\cdot\text{gPr}^{-1}$ )	GSH-Px ( $\text{U}\cdot\text{mgPr}^{-1}$ )	GSH ( $\text{mg}\cdot\text{gPr}^{-1}$ )
阴性对照组	10	0.69 $\pm$ 0.12	39.91 $\pm$ 4.19	32.71 $\pm$ 10.77	48.79 $\pm$ 8.81	4.52 $\pm$ 0.40
5mg. kg <sup>-1</sup>	10	0.62 $\pm$ 0.11	48.10 $\pm$ 11.11	25.61 $\pm$ 7.01	39.57 $\pm$ 7.42*	4.31 $\pm$ 0.58
15mg. kg <sup>-1</sup>	10	0.63 $\pm$ 0.13	52.06 $\pm$ 9.69*	25.55 $\pm$ 8.13	39.96 $\pm$ 7.29*	4.43 $\pm$ 0.85
25mg. kg <sup>-1</sup>	10	0.56 $\pm$ 0.14	49.03 $\pm$ 4.73	26.05 $\pm$ 5.32	36.15 $\pm$ 5.89*	4.19 $\pm$ 0.62

\* 与阴性对照相比差异有显著性  $P<0.05$

## 2.3 染毒丙烯腈77天雄性大鼠血中SOD、CAT、GSH-Px、MDA、GSH测定结果

见表3, 从表3可见血中GSH-Px活力25mg. kg<sup>-1</sup>组与阴性对照组比较差异有显著性(  $p<0.05$  ), 其它指标差异均无显著性(  $P>0.05$  )。

表 3 染毒丙烯腈77天雄性大鼠血中MDA, SOD, CAT, GSH-Px, GSH水平测定结果(  $\bar{x}\pm s$  )

组别	动物数 (只)	MDA ( $\text{nmol}\cdot\text{ml}^{-1}$ )	SOD ( $\text{NU}\cdot\text{ml}^{-1}$ )	CAT ( $\text{U}\cdot\text{glb}^{-1}$ )	GSH-Px ( $\text{U}$ )	GSH ( $\text{mg}\cdot\text{glb}^{-1}$ )
阴性对照组	10	11.60 $\pm$ 1.64	331.88 $\pm$ 41.64	528.87 $\pm$ 94.20	38.54 $\pm$ 10.57	2.64 $\pm$ 0.91
5mg. kg <sup>-1</sup>	10	8.47 $\pm$ 1.91	325.87 $\pm$ 73.06	463.52 $\pm$ 138.37	43.52 $\pm$ 10.55	2.40 $\pm$ 0.65
15mg. kg <sup>-1</sup>	10	9.54 $\pm$ 0.87	307.69 $\pm$ 41.14	526.63 $\pm$ 180.88	51.62 $\pm$ 14.67*	3.17 $\pm$ 0.98
25mg. kg <sup>-1</sup>	10	10.17 $\pm$ 1.41	296.29 $\pm$ 26.21	535.46 $\pm$ 107.59	26.76 $\pm$ 8.15*	2.45 $\pm$ 0.45

\* 与阴性对照相比差异有显著性  $P<0.05$

## 2.4 染毒丙烯腈77天大鼠睾丸中SOD、CAT、GSH-Px、MDA、GSH测定结果

见表4, 由表4可见, 77天大鼠睾丸匀浆中GSH-Px各组间经F检验差异有显著性(  $p<0.05$  ), 各组GSH显著下降, 且与染毒剂量呈高度负相关(  $r=-0.789, P<0.01$  ), 其它指标的差异均无显著性(  $p>0.05$  )。

表 4 染毒丙烯腈77天大鼠睾丸中MDA, SOD, CAT, GSH-Px, GSH水平测定结果(  $\bar{x}\pm s$  )

组别	动物数 (只)	MDA ( $\text{nmol}\cdot\text{mgPr}^{-1}$ )	SOD ( $\text{NU}\cdot\text{mgPr}^{-1}$ )	CAT ( $\text{U}\cdot\text{gPr}^{-1}$ )	GSH-Px ( $\text{U}\cdot\text{mgPr}^{-1}$ )	GSH ( $\text{mg}\cdot\text{gPr}^{-1}$ )
阴性对照组	10	0.70 $\pm$ 0.17	38.16 $\pm$ 5.74	26.36 $\pm$ 6.00	37.79 $\pm$ 11.63	3.64 $\pm$ 0.91
5mg. kg <sup>-1</sup>	10	0.61 $\pm$ 0.15	37.73 $\pm$ 8.87	20.54 $\pm$ 4.26	24.00 $\pm$ 9.24*	2.70 $\pm$ 0.86* 1
5mg. kg <sup>-1</sup>	10	0.70 $\pm$ 0.15	42.07 $\pm$ 7.48	25.49 $\pm$ 5.08	25.49 $\pm$ 6.39*	1.88 $\pm$ 0.68**
25mg. kg <sup>-1</sup>	10	0.56 $\pm$ 0.09	33.28 $\pm$ 12.17	21.40 $\pm$ 7.70	33.87 $\pm$ 8.62	1.12 $\pm$ 0.34**

\* 与阴性对照相比差异有显著性  $P<0.05$

## 3 讨论

目前已知丙烯腈在体内有两种主要代谢途径[8], 一是与GSH结合而解毒, 二是在细胞色素氧化酶的作用下形成氰环氧乙烷( Cyanoethylene oxide CEO)。丙烯腈及其代谢产物 CEO具有较强的亲电性和氧化性[2], 其大部分都将与GSH结合由尿液排出体外。GSH与体内亲电物质和氧化物物质结合的过程中, 需要谷胱甘肽-S-转移酶( GST)和谷胱甘肽过氧化物酶( GSH-Px)的作用。

本研究结果发现染毒丙烯腈38天时, 15mg. kg<sup>-1</sup>、25mg. kg<sup>-1</sup>组血中GSH显著升高, 而各染毒组睾丸组织中GSH-Px活力显著下降, 且存在剂量一效应关系; 染毒丙烯腈77天, 25mg. kg<sup>-1</sup>组血中GSH-Px活力显著下降, 各染毒组血中GSH显著下降, 且存在剂量一效应关系。这是由于丙烯腈及其代谢产物CEO大量进入体内, 机体为了抵抗其损害作用, 首先造成GSH应激性合成增加致使血中GSH升高, 使之与丙烯腈及CEO结合保护相关组织特别使睾丸组织免受损伤, 但随染毒时间延长, 体内丙烯腈和CEO亦增加, 机体合成GSH的速度低于结合速度致使血中GSH逐渐消耗而减少, 且随染毒剂量升高, GSH消耗不断增加, 因而血中GSH含量明显下降; 对睾丸组织来说, 通过GSH-Px活力降低, 即在GSH与丙烯腈及CEO的结合过程中GSH-Px活力下降, 说明体内抗氧化系统占优势, 故睾丸组织受到一定损伤, 病理所见亦证实了丙烯腈使生精细胞发生脂质过氧化, 细胞损伤而致纤维化, 使生精能力受破坏故而精子生成减少、数量下降。

由上述结果, 我们可以初步认为, 丙烯腈在体内代谢活化中, 对机体产生一定的脂质过氧化作用, 这可能是丙烯腈致雄性生殖系统损伤的机理之一。但本次研究睾丸组织中脂质过氧化其它指标变化不明显, 主要是由于组织中的GSH保护作用相对稳定, 使组织免受脂质过氧化作用的损伤。因此GSH对丙烯腈造成的氧化性损伤有很好的拮抗作用。丙烯腈对机体产生的脂质过氧化作用其特征表现为血和睾丸组织中GSH水平下降, 因此推测丙烯腈对人体损伤的早期变化也可能为血中GSH水平下降。如经人体推测证实此结果, 建议可将检测血中GSH含量变化作为丙烯腈产生损伤作用的早期效应指标。

流行病学资料显示[9, 10]在丙烯腈污染严重的工厂, 男工、女工的生殖系统均受到一定损害, 本研究亦证实丙烯腈可对雄性大鼠生殖系统产生脂质过氧化作用而损伤雄性生殖功能, 因此仍需进一步对丙烯腈的生殖损伤机理进行人群流行病学机理研究, 以确保作业工人及其下一代的健康。

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作者简介

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## LIPID PEROXIDATION EFFECTS OF ACRYLONITRILE IN MALE RATS

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**Abstract:** In order to study the lipid peroxidation effects of acrylonitrile, subcutaneous injections of acrylonitrile were administered to male rats, measurements were taken over 38 days and 77 days, to obtain a lipid peroxidation index for blood and testicular tissues, and pathological examinations were done on the testes and epididymis. The results showed that in groups treated for 38 days with  $15 \text{ mg/kg}^{-1}$  and  $25 \text{ mg/kg}^{-1}$  there was a significant increase in blood levels of GSH, and in groups treated with  $5 \text{ mg/kg}^{-1}$ ,  $15 \text{ mg/kg}^{-1}$ , and  $25 \text{ mg/kg}^{-1}$  there was a significant decrease in GSH-Px levels in the testes, indicating a negative correlation with dosage ( $r = 0,464, P < 0.01$ ). In groups treated for 77 days with  $25 \text{ mg/kg}^{-1}$ , there was a significant decrease in blood levels of GSH-Px, and in groups treated with  $5 \text{ mg/kg}^{-1}$ ,  $15 \text{ mg/kg}^{-1}$ , and  $25 \text{ mg/kg}^{-1}$ , there was a significant decrease in GSH, indicating a negative correlation with dosage ( $r = 0,789, P < 0.01$ ). In groups treated with  $5 \text{ mg/kg}^{-1}$ ,  $15 \text{ mg/kg}^{-1}$ , and  $25 \text{ mg/kg}^{-1}$ , there was a significant decrease in GSH-Px levels in the testes. Pathological observations indicated deformation of the seminiferous tubules, fibrous deformation of the spermatogonium, reduction in sperm production, and reduced sperm count in the epididymis. We were able to conclude that acrylonitrile can induce lipid peroxidation effects in the testicular tissues of rats.

**Key Words:** Acrylonitrile, rat, lipid peroxidation effects, testes

Acrylonitrile is one of the important hazardous air pollutants in the work environment [1], and it serves as an organic monomer in the production of nitrile fibers, acrylonitrile-butadiene rubber, ABS industrial plastics, and certain synthetic resins. In recent years, there has been greater interest in toxicological studies on acrylonitrile, with an emphasis on the specific toxicity of acrylonitrile [2], and there have been some reports on toxic effects of acrylonitrile on the reproductive system [3,4]. Metabolic research on acrylonitrile has focused on reactive oxygen [5,6], but the authors have yet to see any reports on whether or not lipid oxidation due to acrylonitrile is one of the mechanisms of damage to male reproductive cells. This paper reports on a possible mechanism of toxic effects of acrylonitrile on the reproductive system, arrived at by monitoring subacute exposure to acrylonitrile, and lipid oxidation products and antioxidation products in the blood and in testicular tissues, as well as antioxidase activity.

### 1. Materials and Methods

#### 1.1 Test Material

Chemically pure specimen of acrylonitrile, with a purity of 99%, and cyanic acid content of 0.05%.

## 1.2 Test Animals and Groups

80 healthy male Wistar rats were selected, with body weight 180-220 g, and divided randomly into a negative control group, acrylonitrile treatment groups of 5 mg/kg<sup>-1</sup>, 15 mg/kg<sup>-1</sup>, and 25 mg/kg<sup>-1</sup>, each group containing 20 rats. The Wistar rats were provided by the Shenyang Medical College Experimental Animal Center.

## 1.3 Treatment of Experimental Animals

Aqueous solutions in the relevant concentrations were prepared with acrylonitrile and saline, with subcutaneous injections based on 0.2 mL/100 g<sup>-1</sup>, with the negative control groups receiving equivalent volumes of saline, with treatment once a day for 77 days, and 10 animals were randomly sacrificed on the 38<sup>th</sup> day and on the 77<sup>th</sup> day, and blood was drawn from the ventral aorta, and testes were removed from the same side, and saline was used in a ratio of 1:10 to prepare homogenized test specimens.

## 1.4 Determination of Index and Methodology

Measurements were made of SOD, CAT, and GSH-Px activity in blood and homogenized testicular specimens, as well as MDA and GSH content. Determination of the above-mentioned index used specimen containers provided by the Nanjing Jiancheng Living Organism Processing Laboratory. The measurement method followed the manual for index determination utilizing the specimen containers.

## 1.5 Statistical Method

Using a database created with Foxbase software, SAS software was applied to statistical analysis.

## 2. Results

### 2.1 Measurement Results for Blood SOD, CAT, GSH-Px, MDA, and GSH in Male Rats Treated with Acrylonitrile for 38 Days

See Table 1. From Table 1 we can see that blood GSH has significant levels among the groups, in accordance with F analysis ( $P < 0.05$ ), and that GSH levels increased significantly, with the index not increasing significantly ( $P > 0.05$ ).

Table 1. Results of Measurement of Blood Levels of SOD, CAT, GSH-Px, and GSH In Male Rats Treated with Acrylonitrile for 38 Days ( $\bar{x} \pm s$ )

Group	No. of Animals	MDA (nmol. ml <sup>-1</sup> )	SOD (NU. ml <sup>-1</sup> )	CAT (U. gHb <sup>-1</sup> )	GSH-Px (U)	GSH (mg. gHb <sup>-1</sup> )
Negative control	10	14.91±3.78	386.75±50.85	448.30±136.31	30.91± 9.05	2.39±0.26
5mg. kg <sup>-1</sup>	10	13.85±2.32	330.50±95.05	454.40±132.21	31.87± 7.85	2.93±0.63
15mg. kg <sup>-1</sup>	10	15.89±0.87	350.08±98.79	441.25± 90.86	32.03±12.40	3.10±0.55*
25mg. kg <sup>-1</sup>	10	16.09±2.25	335.98±37.41	476.44± 90.28	27.30±15.41	2.92±0.39*

\*  $P < 0.05$  when compared with respective negative control

## 2.2 Measurement Results for Testicular SOD, CAT, GSH-Px, MDA, and GSH in Male Rats Treated with Acrylonitrile for 38 Days

See Table 2. From Table 2 we see that GSH-Px levels in rat testicular homogenate in groups treated for 38 days rise significantly ( $P < 0.05$ ) in accordance with F analysis, and that there was a negative correlation to the dose level ( $r = -0.464$ ,  $P < 0.01$ ), with the index not increasing significantly ( $P > 0.05$ ).

Table 2. Results of Measurement of Testicular Tissue Levels of SOD, CAT, GSH-Px, and GSH in Male Rats Treated with Acrylonitrile for 38 Days ( $\bar{x} \pm s$ )

Group	No. of Animals	MDA (nmol. mgPr <sup>-1</sup> )	SOD (NU. mgPr <sup>-1</sup> )	CAT (U. gPr <sup>-1</sup> )	GSH-Px (U. mgPr <sup>-1</sup> )	GSH (mg. gPr <sup>-1</sup> )
Negative control	10	0.69±0.12	39.91± 4.19	32.71±10.77	48.79±8.81	4.52±0.40
5mg. kg <sup>-1</sup>	10	0.62±0.11	48.10±11.11	25.61± 7.01	39.57±7.42*	4.31±0.58
15mg. kg <sup>-1</sup>	10	0.63±0.13	52.06± 9.69*	25.55± 8.13	39.96±7.29*	4.43±0.85
25mg. kg <sup>-1</sup>	10	0.56±0.14	49.03± 4.73	26.05± 5.32	36.15±5.89*	4.19±0.62

\*  $P < 0.05$  when compared with respective negative control

## 2.3 Measurement Results for Blood SOD, CAT, GSH-Px, MDA, and GSH in Male Rats Treated with Acrylonitrile for 77 Days

See Table 3. From Table 3 we see that rat blood levels of GSH-Px activity in groups treated with 25 mg/kg<sup>-1</sup> rise significantly ( $P < 0.05$ ), with the index not increasing significantly ( $P > 0.05$ ).

Table 3. Results of Measurement of Blood Levels of SOD, CAT, GSH-Px, and GSH in Male Rats Treated with Acrylonitrile for 77 Days ( $\bar{x} \pm s$ )

Group	No. of Animals	MDA (nmol. ml <sup>-1</sup> )	SOD (NU. ml <sup>-1</sup> )	CAT (U. gHb <sup>-1</sup> )	GSH-Px (U)	GSH (mg. gHb <sup>-1</sup> )
Negative control	10	11.60±1.64	331.88±41.64	528.87± 94.20	38.54±10.57	2.64±0.91
5mg. kg <sup>-1</sup>	10	8.47±1.91	325.87±73.06	463.52±138.37	43.52±10.55	2.40±0.65
15mg. kg <sup>-1</sup>	10	9.54±0.87	307.69±41.14	526.63±180.88	51.62±14.67*	3.17±0.98
25mg. kg <sup>-1</sup>	10	10.17±1.41	296.29±26.21	535.46±107.59	26.76± 8.15*	2.45±0.45

\*  $P < 0.05$  when compared with respective negative control

## 2.4 Measurement Results for Testicular SOD, CAT, GSH-Px, MDA, and GSH in Male Rats Treated for 77 Days with Acrylonitrile

See Table 4. From Table 3 we see that GSH-Px levels in rat testicular homogenate in groups treated for 77 days rise significantly ( $P < 0.05$ ) in accordance with

F analysis, that GSH decreased significantly, and that there was a negative correlation to the dose level ( $r = -0.789$ ,  $P < 0.01$ ), with the index not increasing significantly ( $P > 0.05$ ).

Table 4. Results of Measurement of Testicular Levels of SOD, CAT, GSH-Px, and GSH in Male Rats Treated with Acrylonitrile for 77 Days

Group	No. of Animals	MDA ( $\mu\text{mol. mgPr}^{-1}$ )	SOD ( $\text{NU. mgPr}^{-1}$ )	CAT ( $\text{U. gPr}^{-1}$ )	GSH-Px ( $\text{U. mgPr}^{-1}$ )	GSH ( $\text{mg. gPr}^{-1}$ )
Negative control	10	0.70 $\pm$ 0.17	38.16 $\pm$ 5.74	26.36 $\pm$ 6.00	37.79 $\pm$ 11.63	3.64 $\pm$ 0.91
5mg. kg <sup>-1</sup>	10	0.61 $\pm$ 0.15	37.73 $\pm$ 8.87	20.54 $\pm$ 4.26	24.00 $\pm$ 9.24*	2.70 $\pm$ 0.86* 1
5mg. kg <sup>-1</sup>	10	0.70 $\pm$ 0.15	42.07 $\pm$ 7.48	25.49 $\pm$ 5.08	25.49 $\pm$ 6.39*	1.88 $\pm$ 0.68**
25mg. kg <sup>-1</sup>	10	0.56 $\pm$ 0.09	33.28 $\pm$ 12.17	21.40 $\pm$ 7.70	33.87 $\pm$ 8.62	1.12 $\pm$ 0.34**

\*  $P < 0.05$  when compared with respective negative control

### 3. Discussion

It was previously known that acrylonitrile possess two principal metabolic pathways in biological organisms [8]. One of them is conjugation with GSH and detoxification, and the other is the formation of cyanoethylene oxide (CEO) under the effects of cytochrome oxidase within the cell. Acrylonitrile and its metabolite CEO possess relatively strong electrophilic and oxidative properties [2], and they are conjugated with GSH and eliminated from the body in the urine almost completely. In the process of binding with electrophilic and oxidative compounds, there is required the activity of GSH - S - GST and GSH-Px.

The results of our study shows that when treated with acrylonitrile for 38 days, blood levels of GSH rose in groups receiving 15 mg/kg<sup>-1</sup> and 25 mg/kg<sup>-1</sup>, GSH-Px activity decreased in testicular tissues, indicating a relationship between dosage and effect. When treated with acrylonitrile for 77 days, GSH-Px activity in blood decreased significantly in groups receiving 25 mg/kg<sup>-1</sup>, and there was a significant decrease in blood levels of GSH in all treated groups, indicating a relationship between dosage and effect. This is due to the introduction into the body of large quantities of acrylonitrile and its metabolite CEO, and since the body resists the harmful effects thereof, it first stimulates increased production of GSH, leading to higher blood levels of GSH, and the conjugation of acrylonitrile and CEO protects the relevant tissues from damage, in particular, the testicular tissues. However, when exposure occurs over an extended period, acrylonitrile and CEO both increase within the organism, prompting accelerated synthesis of GSH, and the rate of conjugation decreases, resulting in a gradual depletion of GSH in the blood, and when the treatment dose increases, GSH depletion progresses continuously, leading to a significant decrease in GSH in the blood. With regard to the effect on testicular tissues it can be said that due to a decrease in GSH-Px activity, there is a decrease in GSH-Px activity in the participation of GSH in the process of conjugation of acrylonitrile and CEO, thereby assisting the oxidative system within the organism,

causing some damage to testicular tissues. From a pathological standpoint, this demonstrates that acrylonitrile brings about lipid peroxidation in sperm producing cells, as well as cellular damage, fibrous formations, damaging sperm production, and reducing sperm count and volume.

Based on the above results, we can realize for the first time that when acrylonitrile undergoes metabolic activity, it has a certain lipid peroxidation effect on the organism, and this can constitute one of the mechanisms of harm to the male reproductive system. However, in our study, the lipid peroxidation and changes in the lipid peroxidation index in testicular tissues are not clear, and the protective effect of GSH in the tissues is relatively stable, so the tissues avoid being damaged by lipid peroxidation. Accordingly, GSH exhibits very good effects against the oxidative damage caused by acrylonitrile. There is a decrease in GSH in blood and tissues in which there are characteristic lipid peroxidation effects produced by acrylonitrile in organisms, leading to the conjecture that acrylonitrile causes damage to the human body, bringing about changes in early stages of exposure, and lowers blood levels of GSH. If this conjecture is proven to be true, then this would suggest that changes in blood levels of GSH would indicate that acrylonitrile produces damage at early stages of exposure.

Epidemiological data [9,10] indicate that in factories where there is serious acrylonitrile contamination, a certain amount of harm is done to the reproductive systems of both male and female workers. Our research demonstrates that acrylonitrile can produce lipid peroxidation effects in the reproductive systems of rats, as well as harm to sexual function. Further research is needed regarding the mechanism of reproductive damage effected by acrylonitrile, so as to promote research into epidemiological mechanisms, and to ensure the health of workers and their descendants.

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