ISSN-0976-0245 (Print) • ISSN-0976-5506 (Electronic)

Volume 9 | Number 1 | January-March 2018

Indian Journal of Public Health Research & Development

An International Journal

SCOPUS IJPHRD CITATION SCORE

Indian Journal of Public Health Research and Development
Scopus coverage years: from 2010 to 2016 Publisher: R.K. Sharma, Institute of Medico-Legal Publications
ISSN:0976-0245E-ISSN: 0976-5506 Subject area: Medicine: Public Health, Environmental and Occupational Health
CiteScore 2015- 0.02
SJR 2015- 0.105
SNIP 2015- 0.034

Covered by Scopus
EMBASE
Approved by MCI

Website:
www.ijphrd.com

Safe Limits Concentration of Ammonia at Work Environments through CD8 Expression in Rats

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ABSTRACT

It has been widely reported incidence caused by acute and chronic effects of exposure to ammonia in the working environment in Indonesia, but ammonia concentration was found to be below the threshold value. The purpose of this study was to determine the safety limit concentration of ammonia in the working environment through the expression of CD8 as a reference for determining the threshold value of ammonia in the working environment.

This research was a laboratory experimental with post test only controlgoup design using experimental animals as subjects experiment.

From homogeneity test results indicated that the weight of white rats exposed and control groups had a homogeneous variant with a significant level of p (0.701) > α (0,05). Description of the average breathing rate is 0.0013 m3 / h. Average weight rats based group listed exposure is 0.1405 kg. From the calculation IRS IRS CD8, CD8 highest score in the doses contained 0.0154, with the location of the highest dose of ammonia without any effect on the lungs of rats is 0.0154 mg/ kg body weight of mice. Safe Human Dose (SHD) ammonia is 0.002 mg/ kg body weight workers. The safety limit concentration of ammonia gas in the working environment of 0,025 ppm.

Keywords: *SafeLimitsConcentration,CD8, immunity, exposure, ammonia, chemicals, toxicity, rats, working environment*

INTRODUCTION

Ammonia is one of the hazardous chemicals that are often used in Indonesia. Many case reports caused by acute and chronic effects of ammonia exposure.According to Hutabarat study (2010)[11] about The Analysis of Ammonia and Chlorine Gas Impact to Lung Physiology of Factory Workers In Rubber Gloves "X" in Medan obtained as follows : that the exposure of ammonia causes complaints of dry throat (80%), dry respiratory roads (73.3%), sore eyes (66.67%), nasal irritation and cough (53.3%), and fainting (6.67%) with the safe limits level of ammonia gas in the air according to Regulation of Ministry of Manpower and Transmigration No.13-2011. Test results showed that levels of air in the working environment is below the threshold,grade 0,05 ppm.

Another study results reported by Daud (2012)[4] about Risk Analysis of $NH_3$and H_2S Exposure on Scavengingat Land fill Tamangapa Makassar. The results showed that theconcentrations ofNH_3in the Land fill is 0.637mg/m^3 and the safe duration of The Scavenger to work at the Landfill is about 2.6 years.

Ammoniahas an impact onthe body's immune system. The results Goto (2003)[7] about Helicobacterpylori and gastric diseases pylorishowed that the bacteriacanmetabolizestarchandproteininto ammoniathat causeinflammation of the stomach. The result of this studyalsofound that the systemic IgA immune response is the large stand most important agent for the invasion of Helicobacterpylori.

Based on several cases and the results of studies on the effects of ammonia on human and workers, it is needed to measure back the threshold limits value (TLV) of ammonia, especially in the workplace. As is known,

the TLV of NH_3 in Indonesia by Minister of Manpower and Transmigration No: 1/1997; ISO 2005 is 25 ppm or 17 mg/m^3. This number is the same as those issued by the ACGIH and lower than issued by OSHA 50 ppm.

Conceptually, the TLV of a toxin in the workplace is directly proportional to the weight of the human body (Williams, 1985)[17]. The average weight of workers in Western countries is greater than the average weight of workers in Indonesia. Therefore, the TLV of ammonia in Indonesia should be lower than the TLV of ammonia in Western countries, including those issued by the ACGH and OSHA from America.

The objectiveof research was to analyze anatomical differences in CD8 expression ofrats'slymphocytescell lungin exposedgroup and controlgroup; to determinethe highest dose of ammonia without any effect onrat; to determine thesafe dose of ammoniaon workersor Safe Human Dose (SHD) are exposed to ammonia;to determineSLC of ammonia gas in the workplace.

MATERIAL AND METHOD

The research was conducted in three stages: the first stage is experimental study, stage II is observational study, and stage III is determination of the safe limit of ammonia gas concentration in workplace.

The research used the speciesof Rattusnorvegicusor the rats that free from ammonia exposure as research object. The rats came from Laboratoryof Animals Faculty of Pharmacy, University of Airlangga. Rats were selected by its sex, the weight of the rats is between 138-142 g and aged 2-3 months (rat breeding). Maturity is expected to be relatively non- rodents will have a different weight.

Results of preliminary research : The lowest dose that had no effecton theratis 0,004552 mg/kg (with concentration of **0,058159** mg/m^3),being the highest dose is the dose that has an effectin rats but not lethal rat is0,045523 mg/kg (with concentration of **0,58159** mg/m^3). Between the 2 groups was made 5 variations of ammonia exposure with each multiple of 5, so that the variation of the concentration of ammoniain there search phase I is: control group (0,0000 mg/m^3, exposed group (I:0,087239; II:0,130858; III: 0,196287; IV:0,294439; V:,441645 (mg/m^3). Each group contained 4 rattail. Required a total of 24 rattails.

FINDINGS

a) The results of observations of rats age in the early stages of this research has been selected samples of rat aged 1.5 to 2 months. Because this study runs for 1.5 months then aged rats during the study ranged from 3 months - 3.5 months. There are no influence of the rats immunity by the age and sex, except there is a treatment do for the rats. Data age ratindicated that the age homogenity test on the exposed group and control group had homogeneous variance with significance level p (0.983) > α (0,05). The average age of rat was 105.2 days.

b) Results of weight measurements of rat

In animal studies using rats, in this study measured that the weight factor will be known to its development, because it is done by using animal testing to observe the immune response. Weight factors can affect the results of the research (30). For this purpose the weight of rats performed statistical tests to determine homogeneity.From the results of homogeneity test indicated that the weight of rats exposed and control groups have homogeneous variance with significance level p (0.701) > α (0,05). Average weight 140.50 g rats.

Measurement of independent variables

a) Concentration of ammonia gas

Accordance with ammonium hydroxide evaporation equation into ammonia gas, by knowing the concentration of NH_4OH in the work environment can be known concentrations of ammonia gas in the workplace after taking into account the temperature and pressure of the air at the site of exposure

Table 1. Ammonia gas concentrations per group

Groups	N	Concentration (mg/m^3)
Control	4	0,0000
Exposed: Concentration I	4	0,0872
Concentration II	4	0,1309
Concentration III	4	0,1962
Concentration IV	4	0,2944
Concentration V	4	0,4416

Sources: Primary Data (2013)

Table 1 above is the smallest concentration of ammonia gas in the control group without exposure to ammonia, the first group with the highest exposure to 0.0872 mg/m^3 and 0.4416 mg/m^{3}konentrasi exposure. The ammonia concentration obtained from NH_4OH solution. By using the formula NH_4OH conversion to ammonia concentrations of ammonia gas generated is in units of mg/L. Ammonia gas concentration in mg/L is then converted to ammonia gas concentration with units of mg/m^3 using the Ideal Gas Theorem after adjusting the temperature and pressure conditions at study sites in the Laboratory Animals Faculty of Pharmacy, University of Airlangga. For temperature, the results of temperature measurement by Airlangga University Lab of PH Department OHS UA obtained 29.6°C. For air pressure, measured by PP BTKL Surabaya is 759 mmHg.

b) Measurement of respiratory rate of rat

Respiratory rate measurement results are listed in the rat following table.

Table 2.Rat Respiratory Rate

Groups	Number	Average respiration rate (L/hr)
Control	4	1,3750 ± 0,0000816
Exposed 1	4	1,3755 ± 0,0001633
Exposed 2	4	1,3809 ± 0,0001633
Exposed 3	4	1,3809 ± 0,0000816
Exposed 4	4	1,3657 ± 0,0000816
Exposed 5	4	1,3754 ± 0,0001633

Sources: Primary Data (2013)

Table 2 in the rat average breathing rate is the lowest in the group of rats exposed to 4 is1 .3657L/ hr while the tallest is a group of rats exposed to 2 and 3 is 1.3809L/ h

c) Determination of ammonia gas concentration limit in safe work environment

The method used to determine the concentration of ammonia gas safety ofworkers in the workplace is a formulation method with the following steps (30):

a. Determine the highest dose ofammonia NOAEL or no effect on rat by using the formula:

$$Dose = \frac{(\alpha)(BR)(C)(t)}{(W)} (mg/kg)$$

α =% absorbed substances lungs, = 100% if not known.

BR = animal respiration rate (m^3/hr)

t = longer working time (hours)

C =concentration of toxins in the air (mg/m^3).

b. SHD determine safe human dose (mg/kg) with the formula:

$$SHD = NOAEL \frac{(Animal\ Km)}{(Human\ Km)}$$

Note: NOAEL = No Observed Adverse Effect Level

1. Animal Km = W/ BSA

BSA=0.09W0.67

BSA= Body Surface Area

W = weight of rats (kg)

2. HumanKm=W/BSA

w = weight of human

h = height of human

c. Determine the safe limits of ammonia gas concentration in the working environment by using the following formula:

Safe Limits [NH3] = $\frac{(SHD)(W)}{(\alpha)(BR)(t)}$ (mg/m^3)

Safe Limits [NH_3] = $\frac{\#(mg/m^3)x(R.T)}{(P)(Mr.NH_3)}$ ppm

α =% NH3 is absorbed through the lungs If not known α= 100%)

BR= Breathing Rate= rate ofrespiration in workers (m^3/hr)

BR = [(Vrespd.td.VT) + (Vrespb.tb.VT) + (Vrespj.tj.VT)]:8

V resp= Speed respiration

td = number of hours of time to sit

tb = number of hours of time to stand

tj = the number of hours of time to run

t = td + tb + tj = 8 hours of work (hours)

VT= tidal volume lung

SHD = safe human dose (mg /kg)

R = Rydberg constant (0.082 L.atm/mol.°K.)

T = (273 + T°C)

P = air pressure (atm)

Mr = molecule relative

Measurement of the dependent variable

Based on data from the ammonia gas concentration in units of mg/m^3 above, can be determined dose of ammonia in the body of rats by using a conversion formula known concentration to dose after respiratory rate of rats, rats body weight and duration of exposure to ammonia in the rat. Long exposure to ammonia gas in the rat is 8 hours every day.

- Description of the average breathing rate of rat

Description of the average respiratory rate in the first phase of the study was 0.0013 m^3/hr.

- Description of the average weight of rats

Description of the average weight of rats in the study phase I.

Dose of ammonia in the body of rats in each group of by ammonia gas concentration, the percentage of ammonia is absorbed, the average breathing rate per group, the average weight per group and a long exposure on a daily basis are listed in.

Table 3. Calculated Dose ammonia in the body of the Old rat Exposure of 8 hours/day

NH_3 concentration (mg/m^3)	Percentage of ammonia is absorbed (α)	Average respiration rate (BR)(L/ hr)	W (average weight) (kg)	Dose NH_3(mg/ kg)
0,0000	100%	1,3750	0,1405	0,0000
0,0872	100%	1,3755	0.1405	0,0068
0,1309	100%	1,3809	0,1410	0.0103
0,1963	100%	1,3809	0, 1410	0,0154
0,2944	100%	1,3657	0.1395	0,0231
0,4416	100%	1,3754	0.1405	0,0346

Sources: Primary Data (2013)

Table 3 above is based on concentration data, the percentage of ammonia is absorbed, the average rate of respiration, prolonged exposure to ammonia every day and the weight of rats in each dose group it is known that ammonia is absorbed by each group of rats. Dose of ammonia in the control group was 0.0000 mg/kg, in group I was exposed to 0.0068 mg/ kg, in the exposed group II 0.0103 mg/ kg, in the exposed group III 0.0154 mg/ kg, in the exposed group IV 0.0231 mg/ kg and in group V 0.0346 mg/ kg body weight of rats.

Group

Based on the results of the calculation of the dose of ammonia in rat exposed group and control over, the lowest dose of ammonia in the body of a rat control group is 0,000 mg/kg body weight while the rat was on the highest dose group V rats exposed to ammonia is 0.0346 mg/ kg of body weight of rat.

Observations on the expression of CD8 lymphocytes

K represents the control group without exposure to ammonia, and 1, 2, 3, 4 and 5 respectively represent the group exposed to ammonia in a row with a dose of ammonia 0.0068; 0.0103; 0.0154; 0.0231; 0.0346 (mg/kg), it seems the number of cells immunoreaktif (arrows) increased both the number and intensity of the color began to control group exposed group 2 and 3 decreases ranging exposed group and lowest in the exposed group 5.

Table 4. Observations CD8 Expression on Rat Lung Cells Lymphocytes

Groups of	Dose NH_3 (mg/ kg)	Ekspresi CD8
Control	0,0000	2,00
Group I	0,0068	4,00
Group II	0.0103	5,50
Group III	0,0154	6,75
Group IV	0,0231	4,00
Group V	0,0346	2,75

p = 0,042 ; α = 0,05

Sources: Primary Data (2013)

IRS calculation of CD8 as shown in Table 4 above the highest scores are those of CD8 IRS III (6.75) or at a dose of 0.0154. Of that number then decreased to 3.00 IRS. From these data it can be concluded that the location of the highest dose of ammonia with no effect on the lungs of rats were in group III, the dose of 0.0154 mg/ kg body weight of rat.

From the results of statistical analysis using the Kruskal Wallis test a significant difference between the number of CD8 IRS with a control group exposed group I, II exposure, exposure III, IV and exposed exposed group V with significance p (0.042) <α (0,05).

From the above description it can be stated that the highest dose of ammonia without effect (NOAEL) in the rat lies in the exposed group III at a dose of 0.0154 mg/ kg body weight of rat.

Based on the findings ofthe value ofthe highest dose of ammonia with no effecton rat (0.0154mg/kg), Animal Km rats on average (with an average weight140.5g) is 5.81and the Human Km workers in the poultry industry average the average (mean weight 61.46 kg and mean height 158.7cm) is 37.34, using an extrapolation formula (30) safe dose of ammonia to workers or safe Human dose (SHD) is 0.0154x5.81/37.34=0.0024 mg/kg body weight of workers

CONCLUSION

The safe limit of ammonia gas concentrations findings from this study is differrent significantly with ammonia NAB issued by ACGIH and NIOSH (USA), which is often the branchmark of Indonesian government in determining NAB. This is not the same difference in the determination of the NOAEL, physical condition (weight and height), temperature and air pressure in Indonesia and in Western countries.

There was significant differences in CD8 with p = 0.042 <α (0.05) in the lungs of rats axposed to ammonia and control groups.Highest dose of ammonia without effect in rats, is mentioned as NOAEL was 0.0154 mg/kg rat body.Based on this research, Animal Km rat factor average (with an average weight 140.5 g) is 5.81, Km Human factors of poultry industry workers in the average (mean weight 61.46 kg and average height 158.7 cm) is 37.34. Extrapolation factor is Animal Km factordevided with Human Km factor. The results obtained NOAEL was a safe dose of ammonia to the worker/safe human dose (SHD) exposed to ammonia is 0.0024 mg/kg body weight workers.

Conflicts of Interest: All authors have no conflicts of interest to declare.

Source of Funding: This is an article "Safe Limits Concentration of Ammonia at Work Environments through CD8 Expression in Rats" of Occupational Health and Safety Department that was supported by Activity Budget Plans 2013, Faculty of Public Health, Airlangga University.

Ethical Clearance: The study was approved by the institutional Ethical Board of the Public Health, Airlangga University.

All subjects were fully informed about the procedures and objectives of this study and each subject prior to the study signed an informed consent form.

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