## TABLE OF CONTENTS

### EDITORIAL

### RESEARCH ARTICLE

01 Detection Study Toxicity of Toluene Exposure in Male Wistar Rat Kidney  
Setriane Bernadi Kristanto, Muchtaruddin Mamyur, Sutjipto Endardjo

09 Detection of Acute Toluene Exposure to Increase Value Malondialdehyde (MDA) and Increase in Number of Lipid Vacuoles Male Wistar Rat Myocardium  
Heriemesan Tendunan, Dewi S Soemarko, Muchtaruddin Mamyur, Sutjipto Endardjo

17 Early Neurotoxicity Detection of Toluene Acute Exposure on Male Wistar Rat  
Luth Nuruliyah, Fadil Effendi, Ahmad Aulia Yusef

23 Early Hepatotoxicity Detection of Toluene Acute Exposure on Male Wistar Rat  
Yustita Permana Sari, Muchtaruddin Mamyur, Ernie Kristoefd

31 Toluene Toxicity Male Wistar Rats in the Blood Examination and Blood Bank Plasma Malondialdehyde  
Margenta Dewi, I. Fary Effendi, Muchtaruddin Mamyur, Diana Aulia

39 Detection of Acute Exposure to Toluene Against Reduction Male Wistar Rat Sertoli cells  
Dihan P. Raksy, Dewi S Soemarko, Sutjipto Endardjo, Muchtaruddin Mamyur

45 The Suitability Test Between Vision Tester and Tno Stereoscopic Vision Test In Stereoscopic Vision Screening  
Wiluwes, Sutjipto, Fadillah, Muchtaruddin Mamyur

49 Impaired Color Vision Screening Using the Ishihara Test and Multifunction Vision Tester to Workers  
Makrywah Kosuma, Arif W., Sulistono, Fary Effendi, M. Rith, Muchtaruddin Mamyur

### EVIDENCE-BASED CASE REPORT

53 Chromate in Cement as a Cause of Contact Dermatitis in Cement Worker : Evidence-based Case Report  
Endriana S Lubis, Tresno Fokas

59 Association Between Heat Exposure and Urolithiasis in Workers Evidence-based Case Report  
Remy Mulyani, Indah Suci Widyaningsih, Dewi S Soemarko

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ARTIKEL PENELITIAN

Detection of Acute Exposure to Toluene Against Reduction Male Wistar Rat Sertoli cells

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Abstrak
Toluena sudah diketahui sebagai toksikan yang dapat menimbulkan toksisitas pada manusia sehingga ditetapkan nilai ambang batas pada pekerja sebesar 50 ppm. Hingga saat ini data mengenai efek pajanan toluena dibawah nilai ambang batas terhadap gangguan pada tingkat molekuler masih terbatas. Penelitian mengenai dosis toluena dibawah nilai ambang batas masih diperlukan sebagai upaya perlindungan yang lebih baik terhadap pekerja. Penelitian toksisitas pada dosis toluena yang lebih rendah dari nilai ambang batas dapat dilakukan pada hewan coba. Rancangan penelitian eksperimental murni terhadap 30 ekor tikus Wistar jantan dengan tingkat pajanan 1.6 ml; 3.2 ml; 6.4 ml; 12.8 ml dan kontrol. Pajanan dilakukan selama 14 har berturut-turut dengan durasi 4 jam per hari, dengan mengalirkan toluena cair ke dalam chamber yang dipertahankan pada jumlah yang tetap. Analisis data dilakukan untuk memperoleh perbandingan jumlah sel Sertoli dan kadar malondialdehid (MDA) tes antar kelompok penelitian dengan uji ANOVA, untuk mengendalikan faktor suhu dan kelambahan lingkungan digunakan uji MANOVA. Jumlah sel Sertoli pada kelompok 1.6 ml; 3.2 ml; 6.4 ml; 12.8 ml; dan kelompok kontrol masing-masing nilai memiliki nilai rerata dengan simpang baku 2.35±1.99 per 10 lagap pandang; 4.47±1.43 per 10 lagap pandang; 4.9±1.43 per 10 lagap pandang; 5.00±1.56 per 10 lagap pandang; dan 4.83±1.75 per 10 lagap pandang yang secara statistik tidak berbedanya pada p<0.067. Kadar MDA tests pada kelompok 1.6 ml; 3.2 ml; 6.4 ml; 12.8 ml; dan kelompok kontrol untuk masing-masing kelompok memiliki nilai rerata 0,10 (0.00-0.17) mmol/mg; 0.09 (0.02-0.22) mmol/mg; 0.12 (0.04-0.27) mmol/mg; 0.06 (0.04-0.15) mmol/mg; dan 0.07 (0.05-0.10) mmol/mg, yang secara statistik tidak berbeda pada nilai p<0.856. Disimpulkan dosis pajanan kurang dari sama dengan 12.8 ml tidak menyebabkan perubahan kadar MDA tests dan penurunan jumlah sel sertoli.

Kata Kunci : toluena; toksisitas; MDA jaringan tesis; sel sertoli.
Detection of Acute Exposure to Toluene Against Reduction Male Wistar Rat Sertoli cells

Dyah P Rahayu, Dewi S Soemarko, Sutjahjo Endardjo, Muchtaruddin Manuyur

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Abstract
Toluene has known as toxicant that can cause human toxicity which the threshold is 50 ppm. Nowadays, we have lacked data of effects of toluene exposure below the threshold that can lead molecular disturbances. The experiment of toluene exposure below the threshold is necessary to prevent the worker. The experiment of toluene toxicity by toluene exposure below the threshold can do in animal. The true experimental study of 30 male Wistar rats, administered by 1.6 ml; 3.2 ml; 6.4 ml; and 12.8 ml toluene liquid and control. Exposure given by flows the liquid toluene on the chamber with the duration of 4 hours per day, for 14 consecutive days. Statistical analysis to comparison the Sertoli cell and malondialdehyde (MDA) level in each group do by ANOVA test and MANOVA test do to control the environment. Sertoli cell on each group 1.6 ml; 3.2 ml; 6.4 ml; and 12.8 ml; and control has each mean 2.35 per 10 field of view; 4.47 per 10 field of view; 4.08 per 10 field of view; 5.00 per 10 field of view; and 4.83 per 10 field of view, which is not significant in p=0.067. The testicular MDA level on each group 1.6 ml; 3.2 ml; 6.4 ml; 12.8 ml; and control has each median 0.10 mmol/mg; 0.09 mmol/mg; 0.12 mmol/mg; 0.06 mmol/mg; and 0.07 mmol/mg, which is not significant in p=0.856. Conclusions the exposure dose less then equal to 12.8 ml cannot lead the testicular MDA level and decreasing of Sertoli cells.

Keywords: toluene; toxicity; testicular tissue; MDA; Sertoli cells
INTRODUCTION
Toluene is an organic solvent that is still widely used in various industries, such as paint solvents, thinners, inks, adhesives, pharmaceutical products, cosmetic additives, pesticide industry, crude petroleum, industrial plastics, disinfectants, adhesives and synthetic fibers although the number of limited.\(^1\) Toluene is known as a toxicant that can cause toxicity in humans that set a threshold value in accordance with Circular 1997 of the Minister of Labour of Indonesia by 50 ppm.\(^2\) Until now, data on the effects of toluene exposure below the threshold value of the disturbance at the molecular level is still limited. Studies in humans showed that toluene has toxic effects on the male reproductive organs, one case of testicular atrophy and decreased spermatogenesis due to degeneration of spermatogonia and Sertoli cells as well as the suppression of spermatogenesis. Based on previous studies in mice gained weight reduction and epididymal sperm counts were significantly rats due to exposure to toluene 2000 ppm (7500 mg/m\(^3\)). 6 hours per day for 90 days.\(^1\) Research on toluene doses below the threshold value is necessary in order better protection to workers. Toluene toxicity studies at doses lower than the threshold value can be done in experimental animals.

This study uses a lower exposure dose below the threshold, ie 0 ml, 1.6 ml, 3.2 ml, 6.4 ml, and 12.8 ml with a shorter exposure time, 14 days, from previous studies aimed at obtain the lowest dose below the threshold value which can cause changes in testicular tissue MDA levels of male Wistar rats and the decrease in the number of Sertoli cells in male Wistar rats, as the basic stage dose response relationship for the development of science.

METHOD
This study uses a true experimental design with 30 male Wistar rats with exposure levels of 1.6 ml, 3.2 ml, 6.4 ml, 12.8 ml, and control. Calculation of the sample size is determined by the formula Freeder for experimental research using experimental animals with the addition of 20% in anticipation of the too small number of samples due to death.\(^1\) The entire rats are placed inside the chamber measures 80 x 40 x 40 cm\(^3\) = 128,000 cm\(^3\) = 128 liters, which aims to provide a wider space for the animals try and maintain concentration during exposure to toluene. Exposure was for 14 consecutive days with a duration of 4 hours per day, with toluene liquid flow into a chamber maintained at a fixed amount, by adding additional doses of toluene per hour with respect to the speed of air flow out of the neighborhood in chamber. Conditions in the chamber is maintained at a temperature of 27 to 30.5 °C and humidity 60-90% in order to maintain the physical condition of the animal and facilitate dispersion of toluene.\(^1\)

Termination is done immediately after the treatment to -14 or immediately after the animal died while receiving treatment by decapitation. MDA measurements performed at the Laboratory of Biochemistry Faculty of Medicine, University of Indonesia by using the thiobarbituric acid-reactive test substance (TBARS) method of Wills (1987), which is calculated in units of pg/mg.\(^1\)

Histopathological examination of the tests conducted in the Department of Anatomical Pathology Laboratory of the Department of Pathology Faculty of Medicine, University of Indonesia.\(^1\) The reading results are centered on all Sertoli cells, with as many as 10 field of view of the evaluation of each preparation with a magnification of a light microscope equipped with a photography Nikon Eclipse E 600 W 400s, calculations are performed on each of Sertoli cells that have a nucleus with the help of software MBF_Imago J.

Quality assurance was conducted in order to avoid bias caused by environmental factors or due to an error condition in both the maintenance procedures, treatment, making preparations, and reading the results. Data collection was conducted in two phases, namely pre-experimental stage and experimental stage. The preliminary test aims to get the default values will be used as a reference at the time of data collection. Data analysis was carried out to obtain the ratio of the number of Sertoli cells, and levels of testicular tissue
malondialdehyde (MDA) between research groups with ANOVA test, and to control the temperature and humidity environment factors used MANOVA test.13,14

RESULTS

Based on ANOVA, all study subjects have equal weight in each study group, where the average standard deviations for the group of 1.6 ml, 3.2 ml, 6.4 ml, 12.8 ml, and each control was 237.33 ± 6.35 g, 237.33 ± 4.18 g, 239.00 ± 5.62 g, 244.00 ± 3.52 g, and 239.67 ± 8.07 g on the value of \( p = 0.281 \).

Ambient temperature is not equal for each study group 1.6 ml, 3.2 ml, 6.4 ml, 12.8 ml, and controls with a mean for each group was 30.32 (30.13 to 30.50 \( p = 0.138 \)), 30.14 (30.09 to 30.18 \( p = 0.138 \)), 29.23 (29.01 to 29.44 \( p = 0.003 \)), 28.83 (28.83 to 28.83 \( p = 0.002 \)), and 29.68 (29.66 to 29.70 \( p = 0.003 \)).

Ambient humidity is not equivalent to each research group 1.6 ml, 3.2 ml, 6.4 ml, 12.8 ml, and controls with a mean for each group was 51.19 (43.70 to 55.07, \( p = 1.000 \)), 51.87 (51.86 to 51.87 \( p = 1.000 \)), 61.90 (61.50 to 62.30 \( p = 0.003 \)), 57.13 (57.13 to 57.13 \( p = 0.002 \)), and 65.04 (64.97 to 65.10 \( p = 0.003 \)).

Comparison of the number of Sertoli cells in 1.6 ml: 3.2 ml, 6.4 ml, 12.8 ml, and the control group each value has a mean value with standard deviations of 2.35 ± 1.99 per 10 field of view, 4.47 ± 1.43 per 10 field of view, 4.08 ± 1.43 per 10 field of view, 5.00 ± 1.56 per 10 field of view, and 4.83 ± 1.75 per 10 field of view not statistically significant at \( p = 0.067 \).

Comparison of testicular tissue MDA levels in the group of 1.6 ml, 3.2 ml, 6.4 ml, 12.8 ml for the control group and each group has an average value of 0.10 (0.00 to 0.17) nmol / mg; 0.09 (0.02 to 0.22) nmol / mg; 0.12 (0.04 to 0.27) nmol / mg; 0.06 (0.04 to 0.15) nmol / mg; and 0.07 (0.05 to 0.10) nmol / mg, which was not statistically significant at \( p = 0.856 \).

Table 1: Comparison of the number of Sertoli cells and testicular tissue MDA in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>0</th>
<th>1.6</th>
<th>3.2</th>
<th>6.4</th>
<th>12.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sertoli cells</td>
<td>Mean</td>
<td>4.83</td>
<td>2.35</td>
<td>4.47</td>
<td>4.08</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.75</td>
<td>1.99</td>
<td>1.43</td>
<td>1.43</td>
</tr>
<tr>
<td></td>
<td>( p^* )</td>
<td>0.067</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testicular tissue MDA</td>
<td>Median</td>
<td>0.09</td>
<td>0.00</td>
<td>0.09</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>0.05</td>
<td>0.00</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>0.10</td>
<td>0.17</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>( p^{**} )</td>
<td>0.856</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* : ANOVA test  
**: Kruskal Wallis test

Independent association between exposure dose, ambient temperature, and ambient humidity with Sertoli cell number and testicular tissue MDA levels are known to perform MANOVA test, it was found that the ambient temperature (\( p = 0.003 \)) and ambient humidity (\( p = 0.012 \)) has significant effect on testicular tissue MDA, but the ambient temperature (\( p = 0.915 \)) and ambient humidity (\( p = 0.431 \)) did not have significant effect to the number of Sertoli cells.

Table 2: Independent association between dose exposure, ambient temperature, and ambient humidity with the number of Sertoli cells and testicular tissue MDA

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sum of square*</th>
<th>( p^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sertoli cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>0.033</td>
<td>0.915</td>
</tr>
<tr>
<td>Humidity</td>
<td>1.802</td>
<td>0.431</td>
</tr>
<tr>
<td>Dosis</td>
<td>20.825</td>
<td>0.153</td>
</tr>
<tr>
<td>Testicular tissue MDA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>0.031</td>
<td>0.003</td>
</tr>
<tr>
<td>Humidity</td>
<td>0.021</td>
<td>0.012</td>
</tr>
<tr>
<td>Dosis</td>
<td>0.011</td>
<td>0.016</td>
</tr>
</tbody>
</table>

\* : MANOVA test

DISCUSSION

This study is expected to at least the early stages may contribute to the determination of dose effects in humans, so to achieve that it needs to be proven by research to a higher level, such as in primates is considered closer to humans.
RESULTS
Based on ANOVA, all study subjects have equal weight in each study group, where the average standard deviations for the group of 1.6 ml, 3.2 ml, 6.4 ml, 12.8 ml, and each control was 237.23 ± 6.35 g, 237.33 ± 4.18 g, 239.00 ± 5.62 g, 244.00 ± 3.52 g, and 239.67 ± 8.07 g on the value of p = 0.281.

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Ambient humidity is not equivalent to each research group 1.6 ml, 3.2 ml, 6.4 ml, 12.8 ml, and controls with a mean for each group was 51.19 (50.70 to 51.60 p = 0.005), 51.87 (51.87 to 51.87 p = 1.000), 61.50 (61.50 to 62.30 p = 0.003), 57.13 (57.13 to 57.13 p = 0.002), and 65.04 (64.97 to 65.10 p = 0.003).

Comparison of the number of Sertoli cells in 1.6 ml, 3.2 ml, 6.4 ml, 12.8 ml, and the control group each value has a mean value with standard deviations of 2.35 ± 1.99 per 10 field of view; 4.47 ± 1.43 per 10 field of view; 4.08 ± 1.43 per 10 field of view; 5.00 ± 1.56 per 10 field of view; and 4.83 ± 1.75 per 10 field of view not statistically significant at p = 0.067.

Comparison of testicular tissue MDA levels in the group of 1.6 ml, 3.2 ml, 6.4 ml, 12.8 ml for the control group and each group has an average value of 0.10 (0.00 to 0.17) nmol / mg; 0.09 (0.02 to 0.22) nmol / mg; 0.12 (0.04 to 0.25) nmol / mg; 0.06 (0.04 to 0.15) nmol / mg; and 0.07 (0.05 to 0.10) nmol / mg, which was not statistically significant at p = 0.856.

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<th>6.4</th>
<th>12.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sertoli cells</td>
<td>4.03</td>
<td>2.35</td>
<td>4.47</td>
<td>4.08</td>
<td>5.00</td>
</tr>
<tr>
<td>Mean</td>
<td>1.75</td>
<td>1.99</td>
<td>1.43</td>
<td>1.43</td>
<td>1.56</td>
</tr>
<tr>
<td>SD</td>
<td>0.067</td>
<td>0.067</td>
<td>0.067</td>
<td>0.067</td>
<td>0.067</td>
</tr>
<tr>
<td>Median Testicular tissue MDA</td>
<td>0.006</td>
<td>0.005</td>
<td>0.007</td>
<td>0.008</td>
<td>0.009</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.00</td>
<td>0.00</td>
<td>0.02</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.10</td>
<td>0.17</td>
<td>0.22</td>
<td>0.27</td>
<td>0.15</td>
</tr>
<tr>
<td>p**</td>
<td>0.006</td>
<td>0.005</td>
<td>0.007</td>
<td>0.008</td>
<td>0.009</td>
</tr>
</tbody>
</table>

* : ANOVA test  ** : Kruskal-Wallis test

Independent association between exposure dose, ambient temperature, and ambient humidity with Sertoli cell number and testicular tissue MDA levels are known to perform MANOVA test, it was found that the ambient temperature (p = 0.003) and ambient humidity (p = 0.002) have significant effect on testicular tissue MDA, but the ambient temperature (p = 0.012) and ambient humidity (p = 0.031) did not have significant effect to the number of Sertoli cells.

Table 2: Independent association between dose exposure, ambient temperature, and ambient humidity with the number of Sertoli cells and testicular tissue MDA

<table>
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<tr>
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<th>Sum of square*</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sertoli cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>0.03</td>
<td>0.915</td>
</tr>
<tr>
<td>Humidity</td>
<td>1.802</td>
<td>0.451</td>
</tr>
<tr>
<td>Dosis</td>
<td>20.825</td>
<td>0.153</td>
</tr>
<tr>
<td>Testicular tissue MDA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>0.03</td>
<td>0.903</td>
</tr>
<tr>
<td>Humidity</td>
<td>0.021</td>
<td>0.012</td>
</tr>
<tr>
<td>Dosis</td>
<td>0.011</td>
<td>0.015</td>
</tr>
</tbody>
</table>

* : MANOVA test

DISCUSSION
This study is expected to at least the early stages may contribute to the determination of dose effects in humans, so to achieve that it needs to be proven by research to a higher level, such as in primates is considered close to humans.


