A Survey on Health Effects in a Human Population Exposed to Permanent-Waving Solution Containing Thioglycolic Acid

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Abstract: A Survey on Health Effects in a Human Population Exposed to Permanent-Waving Solution Containing Thioglycolic Acid: Hui-Fang Gan, et al. Department of Environmental Toxicology, School of Public Health, Harbin Medical University, China—Thioglycolic acid (TGA) is the active ingredient of permanent-waving solution (PWS). TGA has been shown to be a chemical of high toxicity, which can be absorbed through intact skin and cause damage to organs or systems in animals. This study evaluated the effect of TGA-containing PWS on the health of a human population in 3 substudies. Firstly, 57 female hairdressers exposed to TGA-containing PWS (cases) and 64 female schoolteachers (controls) were studied. Their menstruation state was evaluated with information obtained from interviews. The results revealed that the menoxenia rate in the cases was significantly higher than that in the controls (22.81% vs 9.38%, p<0.05). Secondly, 8 female hairdressers selected from those that participated in the above survey underwent a fluctuation test for the mutagenic activity of urine. Eight female medical students were chosen as controls. Difference in the mutagenic activity of urine on TA100 between the two groups was highly significant (110.30 ± 45.95 vs 28.43 ± 19.33, p<0.01). Finally, a micronucleus assay was carried out on scalp hair follicle cells in healthy volunteers. Scalp hair with the follicle cell mass was sampled from 8 male and 8 female volunteers before permanent waving and at 24, 48 and 72 h after waving. One thousand hair follicle cells were examined by light microscopy. The number of cells containing a micronucleus and the number of micronuclei in each cell was determined. The permillages of micronuclei in hair follicle cells before and after permanent waving were compared. Micronuclei presence reached its peak value (12.44‰) 24 h after permanent waving, which was significantly higher than that before waving (3.13‰, p<0.001). The rate decreased progressively after 24 h. Our results suggest that the reproductive function of hairdressers may be affected by long-term exposure to PWS, probably due to the presence of TGA, and more attention should be paid to its potential carcinogenic effects.

Key words: Thioglycolic acid, Mutagenic activity, Micronucleus, Hairdressing

Hairdressers are exposed to a variety of chemicals on a daily basis, which may be absorbed, inhaled or possibly even ingested\(^1\). Chemical waving is the most common means of hair styling and permanent-waving solution (PWS) is usually used for this purpose. The active ingredient of PWS is thioglycolic acid (TGA), which may potentially damage the health of both the hairdressers and their customers.

Few data regarding the health effects of TGA are available in the literature. Early toxicological data on TGA were found in a Russian periodical in the 1960s. Rotenberg et al. reported parameters of its acute oral toxicity in rat and mouse, acute inhalation toxicity in mouse, acute dermal toxicity in rabbit, and chronic inhalation toxicity in rat\(^2\). Thereafter a few more toxicological studies on TGA have been published. According to the US EPA document, LC\(_{50}\) for TGA as determined by an acute inhalation toxicity study on rat (4-h exposure) was 0.21 mg/l (standard error 0.040 mg/l)\(^3\). RW Tyl reported the results of studies which evaluated the developmental toxicity of sodium thioglycolate administered topically to CD rats and New Zealand white rabbits from days 6 to 19 or 29 of gestation\(^4,5\). As to the mutagenicity of TGA, the results were negative in the Ames test system on Salmonella typhimurium strains TA100, TA1535, TA97 and TA98, with and without metabolic activation\(^6\). Although epidemiological studies conducted by Kersemaekers and Blatter indicated that exposure to chemicals increased the risk of reproductive
disorders in hairdressers\textsuperscript{7–10}, little information regarding the potential adverse effect of TGA exposure on health exists. Data on the mutagenic and reproductive effects of TGA in humans have not been found so far.

Our early studies demonstrated that TGA could be absorbed through intact skin and cause damage to organs or systems in animals. Percutaneous administration of TGA disturbed the oestrous cycle in rats, and increased the rate of micronucleus occurrence in polychromatic erythrocytes in the bone marrow of mice\textsuperscript{11,12}. In view of its adverse effect on animals, greater attention should be paid to the health of hairdressers exposed to TGA in PWS.

This study was to evaluate the health effects of TGA-containing PWS in a human population by assessing the menstruation state and evaluating the mutagenic activities of TGA in hairdressers, their customers and healthy controls.

**Methods**

*Survey of the menstruation state*

Study cases and controls for the survey were selected from hairdressing shops and primary schools respectively, in Harbin, China. Both cases and controls were interviewed by one of the investigators. The following key points were included in the questionnaire: employment history, permanent waving procedure, work load, protective measures, personal habits, obstetrical history, anamnesis, medical history, and details regarding the state of menstruation before and after employment. A total of 200 female hairdressers were interviewed and those who met the selection criteria were further investigated. The selection criteria for the survey were: age between 20 and 40 yr, involvement of permanent-waving procedure >1 yr, no exposure to hair dyes, no smoking and alcohol abuse, not on contraceptive or hormone therapies, and in good health. After further sorting of the collected data, subjects who had delivered within one year, had undergone abortion or a sterilising operation within 3 months prior to the survey, or had menstruation disorder due to other causes were excluded from the study.

The mean age of the selected hairdressers (n=65) was 30.1 yr. The average length with exposure to PWS was 6.7 (1–10) yr. Of the 65 hairdressers, 47 worked for over 8 h, the others for 6–8 h. Our study hairdressers served 2 to 10 (average 6.2) customers for permanent waving per day, spending approximately 2.5 h applying PWS. The overwhelming majority of them did not use protective gloves or respirators. The predominant means of exposure to PWS was direct dermal contact in the study subjects. The chemical composition of the PWS used was: TGA (99% pure) 10%–12%, ammonia solution 5%–8%, sodium carbonate <2g (adjusted pH 8.5–9), deionised water 80%–85%, essence.

Of the 65 hairdressers investigated, 8 were excluded from the final analysis because of incomplete data or an unsure history of menstruation. Finally, data from 57 cases were analysed.

Sixty-four healthy female teachers (age 20–40 yr, mean 30.5) were selected from primary schools to act as controls. They were matched with the study hairdressers for working body posture, working hours, working load, income and/or living standard. No controls had any occupational exposure to PWS or exposure to TGA from any source. Individuals who smoke, abuse alcohol, use contraceptive and hair dyes were excluded from the study.

Menstruation abnormalities were defined as the following. *Disorder of menstrual cycle*: menstrual cycle shorter than 21 d, longer than 35 d or cycle fluctuation exceeding 7 d. *Disorder of menstrual period*: menstruation period exceeds 7 d or is less than 3 d. *Menorrhagia*: analgesic must be taken during the period of menstruation. *Disorder of menstruation* (menoxenia): having any one of the above three disorders.

*Evaluation of the mutagenicity*

Testing mutagenic activity of urine

Study subjects—Study cases consisted of 8 female hairdressers (age 20–30 yr) selected from those who participated in the menstruation state survey. None of the study cases had previous exposure to carcinogens or suspected carcinogens, no family history of malignant tumours, no virus infection within three months and were not on any medication. Eight female medical students without any exposure to PWS were studied as controls, and were matched with the cases for age.

Urine sampling and pretreatment—Mutagenic activity of urine was examined with the fluctuation test. Urine was collected from each study subject between 6 am and 7 am (morning urine). Morning urine was strictly defined as the first urination after getting up. First, the urine creatinine level was determined. Second, the collected urine was filtered to remove crystalline salts, and the pH was adjusted to neutral. Afterwards, 200 ml of filtered urine was passed through a column of GDX-102 resin to adsorb organic substances, which were then eluted with ethyl ether and volatilised in a dessicator. The concentrate obtained was dissolved in DMSO.

Fluctuation tests—The characteristics of *S. typhimurium* strains TA98 and TA100 had been determined and satisfied the requirements of the test before the present study. The procedure employed by Falck et al. was used\textsuperscript{13}. The test was divided into 5 sets: 3 sets of concentrate equivalent to 20 ml, 10 ml and 5 ml urine samples, 1 set of solvent (DMSO) for controls; 1 set of dexon for a positive control. Each set consisted of 50 test tubes. Concentrate, enrichment and indicator solutions were added to each tube. After 96-h incubation, the number of positive tubes in each set was determined. The highest value among the 3 sets was taken as the result of the assay. The mutagenic activity value for a 10 ml
urine sample was calculated with the following formula:

\[
\text{Mutagenic activity} = \frac{\text{Number of positive tubes in test sample} - \text{Number of positive tubes in solvent control}}{\text{Urine creatinine (mmol/l)}}
\]

Micronucleus assay on scalp hair follicle cells
This test was carried out on hair follicle cells obtained from 8 male and 8 female volunteers aged between 20 and 40 yr. All volunteers were in good health. None of them used hair dyes or was exposed to PWS within 6 months prior to the test. Each study subject had his/her hair perm by the same hairdresser with the same PWS. Two or three samples of scalp hair with the follicle cell mass were removed before waving and at 24, 48 and 72 h after waving. The hair follicle cells were treated and stained with Wright’s solution in a procedure developed at our laboratory. One thousand hair follicle cells were examined by light microscopy. The number of micronucleus-containing cells and the number of micronuclei in each cell were determined. The micronucleus was identified based on Heddle’s criteria. For each subject, the micronucleus occurrence rate in hair follicle cells at each time point was calculated. The micronucleus-occurrence permillage in hair follicle cells before and after waving were compared.

## Results
**Findings of the survey**
Most study cases (hairdressers) complained of headache, dizziness, dryness in the eyes or throat, dryness and rhagades of skin on the hands. A few reported “allergic reaction”. The results for the menstruation state are shown in Table 1. Differences between cases and controls in the occurrence of menstrual cycle disorder, menstrual period disorder and menorrhagia were not statistically significant, but the frequency of menoxenia was significantly higher in cases than in controls (22.81 % vs 9.38 %, \( p < 0.05 \)).

**Mutagenic activity of urine**
Tables 2 and 3 list the results of the fluctuation test for urine mutagenic activity. No significant difference in mutagenic activity on strain TA98 was found between cases and controls (53.08 ± 20.71 vs 43.85 ± 15.64, \( p > 0.05 \)), whereas the difference in mutagenic activity on TA100 between the two groups was highly significant (110.30 ± 45.95 vs 28.43 ± 19.33, \( p < 0.01 \)).

### Table 1. Menstruation state of hairdressers exposed to PWS containing TGA

<table>
<thead>
<tr>
<th>Menstruation</th>
<th>Observed group (n=57)</th>
<th>Control group (n=64)</th>
<th>( \chi^2 )</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disorder of menstrual cycle</td>
<td>7 (12.28 %)</td>
<td>2 (3.13 %)</td>
<td>3.671</td>
<td>0.055</td>
</tr>
<tr>
<td>Disorder of menstrual period</td>
<td>2 (3.51 %)</td>
<td>0</td>
<td>2.283</td>
<td>0.131</td>
</tr>
<tr>
<td>Menorrhagia</td>
<td>6 (10.53 %)</td>
<td>4 (6.25 %)</td>
<td>0.727</td>
<td>0.394</td>
</tr>
<tr>
<td>Menoxenia</td>
<td>13 (22.81 %)</td>
<td>6 (9.38 %)</td>
<td>4.109</td>
<td>0.043</td>
</tr>
</tbody>
</table>

### Table 2. Mutagenic activity of urine in fluctuation test (strain TA98)

<table>
<thead>
<tr>
<th>Code</th>
<th>Urine volume (ml)</th>
<th>Urine creatinine (mmol/l)</th>
<th>Positive tube* (number)</th>
<th>Mutagenic activity</th>
<th>Urine volume (ml)</th>
<th>Urine creatinine (mmol/l)</th>
<th>Positive tube* (number)</th>
<th>Mutagenic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>20.8</td>
<td>6</td>
<td>28.8</td>
<td>5</td>
<td>16.8</td>
<td>3</td>
<td>35.7</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>29.2</td>
<td>9</td>
<td>61.6</td>
<td>10</td>
<td>18.6</td>
<td>8</td>
<td>43.0</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>32.0</td>
<td>11</td>
<td>68.8</td>
<td>10</td>
<td>15.8</td>
<td>8</td>
<td>50.6</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>19.5</td>
<td>8</td>
<td>41.0</td>
<td>5</td>
<td>7.5</td>
<td>2</td>
<td>53.3</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>17.7</td>
<td>2</td>
<td>22.6</td>
<td>5</td>
<td>15.0</td>
<td>3</td>
<td>40.0</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>7.7</td>
<td>8</td>
<td>51.9</td>
<td>5</td>
<td>14.2</td>
<td>4</td>
<td>56.3</td>
</tr>
<tr>
<td>7</td>
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<td>14.4</td>
<td>10</td>
<td>69.4</td>
<td>20</td>
<td>13.3</td>
<td>3</td>
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<tr>
<td>8</td>
<td>5</td>
<td>17.4</td>
<td>7</td>
<td>80.5</td>
<td>5</td>
<td>16.5</td>
<td>5</td>
<td>60.6</td>
</tr>
</tbody>
</table>

*The number of positive tubes of urine minus that of solvent
The rates of micronucleus occurrence in hair follicle cells before and after waving in each study subject are shown in Table 4. The micronucleus rate reached its peak value (12.44‰) after 24 h of waving, and was significantly higher than that before waving (p < 0.001). The rate decreased progressively after 24 h.

**Discussion**

Although a series of observations on the health status of hairdressers have been reported, few of the study subjects were involved in waving with PWS containing TGA. There have been few toxicological studies on TGA reported, particularly in a human population. Based on the findings in animal experiments, we conducted these studies to evaluate the adverse effects of TGA-containing PWS on the health of a human population. The results of toxicity tests on animals suggested that TGA may induce menstruation disorder, chromosome damage and other mutagenic effects in humans. This study revealed an increased rate of menstruation disorder in hairdressers exposed to PWS. Although the evidence to prove a causal relation between TGA exposure and increased menstruation disorder may not be sufficient, this finding suggests that the effect of PWS on menstruation function cannot be ignored. Since rats...
exposed to TGA percutaneously displayed apparent histological changes in the ovary and disorder of the oestrous cycle\(^{11}\), there is a concordance in the organ affected in animals and humans.

Determination of toxicants or its metabolites in urine is usually used as a measure in biological monitoring. Mutagenic activity of urine reflects individual exposure to potential mutagens. Fluctuation test of urine is regarded as a sensitive biological monitoring tool for occupational and environmental exposure to mutagens or carcinogens\(^{13}\). Increased mutagenic activity of urine on strain TA100 was observed in hairdressers but not in controls. This finding suggests that excessive uptake of mutagens had taken place in the observed group and that the reverse mutation had resulted from base-pair substitution.

TGA is the active ingredient of PWS and is very easily absorbed through intact skin in animals. Besides TGA, PWS also contained ammonia solution, sodium carbonate and essence. Ammonia is an irritant gas but sodium carbonate is generally regarded as practically non-toxic. It is therefore plausible to speculate that the increased mutagenic activity and the adverse health effects in humans may be attributed to TGA.

Micronucleus assay is a rapid approach for assessing chromosomal damage\(^{10}\). It is well known that epithelial cells are the common targets for carcinogens. Hair follicle cells originate in the epidermis and is easy to have them separated effectively. Hair follicle cells are therefore suitable for biological assay. Kuroki \textit{et al} reported that human epidermal and dermal cells both metabolised benzo(a)pyrene (BP), producing almost all the proximate metabolites of BP\(^{15}\). Vermorken \textit{et al} showed that the hair follicle enzyme is very similar to the liver monooxygenase system\(^{10}\). So that, hair follicle cells could be proper testing material for screening carcinogens. In the present study, the micronucleate rate in hair follicle cells showed a satisfying time-effect relationship, and it fully met the general requirements of the micronucleus test.

In conclusion, our findings suggest that reproductive function in hairdressers can be affected by long-term exposure to PWS, possibly due to the effects of TGA. These data are consistent with the findings of previous toxicological studies on animals and show PWS is mutagenic to the human population. More attention should be paid to the potential risk of carcinogenesis in the occupational population.

References

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