

Evaluation of the oral toxicity of formaldehyde in rats

David^{*a}, and Hanslavina Arkeman*

ABSTRACT

*Department of Histology,
Medical Faculty,
Trisakti University

Correspondence

^adr. David
Department of Histology,
Medical Faculty,
Trisakti University
Jl. Kyai Tapa 260 - Grogol
Jakarta 11440
Telp 021-5672731 ext.2702
Email : davesaboch@yahoo.com

Univ Med 2008; 27: 106-112

Formaldehyde is used in the production of plywood, particleboard, a wide variety of molded or extruded plastic items, and for preserving cadavers. Experimental posttest only control group design was used to determine the histopathological changes of rat stomach tissue exposed to formaldehyde for 12 weeks in thirty adult female Sprague-Dawley rats weighing 150–200 g. The animals were randomized into three groups, namely the control group, the treatment group given 4 mg/L of formaldehyde, and the group treated with 6 mg/L of formaldehyde. The results showed that there was a significant difference in the thickness of the mucous layer of the gastric fundus between the control group and the group treated with 6 mg/L ($p = 0.011$), and also a significant difference in the thickness of the mucous layer of the gastric fundus between both treatment groups (4 mg/L vs. 6 mg/L) ($p = 0.003$), indicating that there was necrosis of the superficial layers of the gastric mucosa due to high concentrations of formaldehyde. In histopathology sections from all groups, similar changes were observed in both treatment groups, in the form of vacuolar (hydropic) degeneration of gastric fundus glands. In conclusion, administration of formaldehyde in drinking-water for 12 weeks caused histopathologic effects on the gastric mucosa in rats.

Keywords : Formaldehyde, oral, drinking water, toxicity

INTRODUCTION

Formaldehyde is used in many industries, in the production of fertilizer, paper, plywood, and polyacetal, phenolic, urea, and melamine resins. The latter three kinds of resins are widely used as adhesives and binders for wood products, pulp and paper, synthetic fibers, and in the finishing of textiles. Furthermore, formaldehyde may be converted into a number of chemicals, such as 1,4-butanediol, 4,4'-

diphenylmethane diisocyanate, pentaerythritol and hexamethylene-tetramine. Formaldehyde is also used in many hospitals and laboratories to preserve tissue specimens,⁽¹⁻³⁾ and in mortuaries as an embalming fluid. Formaldehyde in drinking water commonly originates from the oxidation of natural organic matter during the ozonization and chlorination process. Formaldehyde in drinking water may also come from deterioration of the protective finish on polyacetal plastics.^(4,5)

Formaldehyde is a gas widely encountered in natural and man-made environments and is readily soluble in water to form a 40% solution (Formalin®). The hazards of exposure to this gas by inhalation have been the subject of many studies, leading to the reclassification by the International Agency for Research on Cancer (IARC) of formaldehyde from group 2A to group 1 (carcinogenic to humans), which expresses the current concern about the carcinogenic effects of inhalation of formaldehyde.^(2,6) However, in Indonesia the most recent issue was about the indiscriminate use of formaldehyde as a food preservative, especially for sources of protein, such as beef, poultry, fish, as well as tofu and tempeh.

Formaldehyde is a colorless, flammable gas at room temperature. It has a pungent, distinct odor and at high concentrations may cause a burning sensation to the eyes, nose, and lungs. Formaldehyde is also known as methanal, methylene oxide, oxymethylene, methylaldehyde, and oxomethane. Formaldehyde can react with many other chemicals, and at very high temperatures it will break down into methanol (wood alcohol) and carbon monoxide. A number of experimental studies on the effects of formaldehyde by ingestion have been conducted in rodents, particularly in rats.

Owing to formaldehyde's high reactivity, effects in the tissue on first contact following ingestion are more likely to be related to the concentration of the formaldehyde consumed than to its total intake.^(7,8) The aim of the present study was to assess the effects of orally administered formaldehyde on the thickness and histopathology of the gastric fundal mucosa in rats.

METHODS

Design of the study

A posttest only control group design was

used to attain the objectives of the study.

Sample

Thirty adult female Sprague Dawley rats weighing 150–200 g. The sample size was calculated using the formula of Federer⁽⁹⁾: $(n-1)(t-1) > 15$. When using three groups ($t = 3$), the optimal sample size required will be 10 adult female rats per group.

Intervention

The experimental animals were randomly assigned to three groups, namely group I comprising the control group which was not given formaldehyde-containing drinking water, group II as the treatment group that was given drinking water containing formaldehyde in a concentration of 4 mg/L daily for 12 weeks, and group III as the treatment group that was given drinking water containing formaldehyde in a concentration of 6 mg/L daily for 12 weeks.

Methods

All three groups of experimental animals were given food pellets and plain drinking water, and it was only in the intervention groups (groups II and III) that formaldehyde was added to the drinking water. The formaldehyde used was Formalin® diluted with distilled water to obtain the required dose. After 12 weeks the experimental animals were sacrificed and the organs taken for histological sections stained with Hematoxylin-Eosin (HE).

Data collection

The weight of the rats in each group was measured at the beginning and at the end of the experiment to monitor increases in the weights of the rats in each group. Measurements were also taken of the thickness of the fundal mucosa in the rats of each group using a micrometer in a double-blind manner.

Data analysis

Continuous data were expressed as mean \pm SD. One-way analysis of variance (ANOVA) was used for comparing the difference in the body weights of the rats before and after exposure to Formalin® between the three groups, at a significant

level of 0.05. The same method was used for comparing the thickness of the fundal mucosa of the rats in each group at a significant level of 0.05. When significant differences were found between the three groups, the analysis was continued with the Tukey multiple comparison method.

Table 1. Mean difference in body weight and between thickness of the fundal mucosa the three groups

Mean	Control (n=10)	Formaldehyde 4 mg/L (n=10)	Formaldehyde 6 mg/L (n=10)	P
Difference in body weight (g)	32.9 \pm 12.5	33.0 \pm 15.6	31.7 \pm 7.6	0.953
<i>Tunica mucosa</i> of gastric fundus (μ m)	1051.24 \pm 132.07	1075.00 \pm 65.04	919.00 \pm 138.91*	0.004

* Significantly different from control group and group on 4 mg formaldehyde

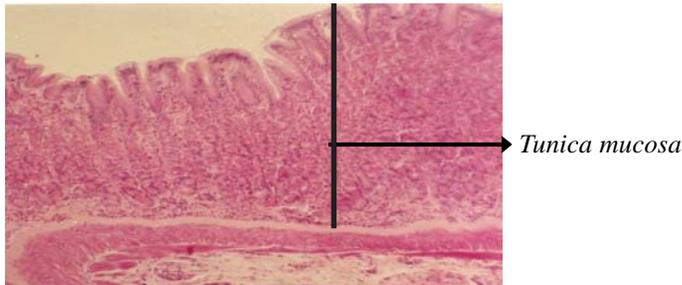


Figure 1a. *Tunica mucosa* of rat gastric fundus of control group stained with HE, at 100X magnification

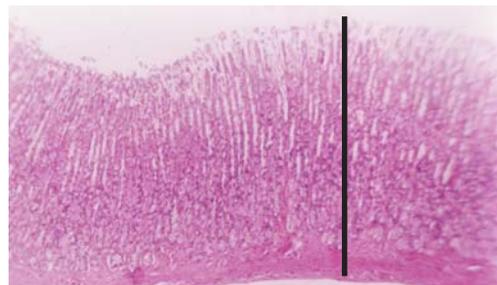


Figure 1b. *Tunica mucosa* of rat gastric fundus of 4 mg formaldehyde group stained with HE, at 100X magnification

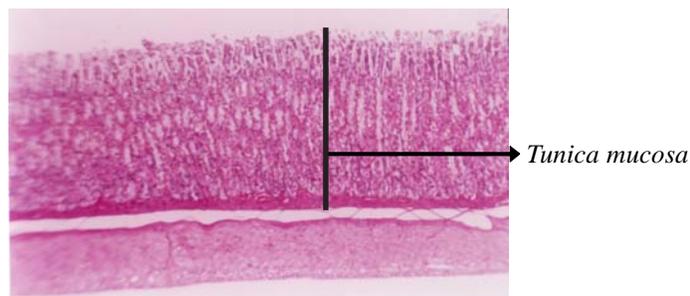


Figure 1c. *Tunica mucosa* of rat gastric fundus of 6 mg formaldehyde group stained with HE, at 100X magnification

RESULTS

After 12 weeks' exposure to formaldehyde, the mean difference (\pm SD) in body weight before and after exposure was 32.9 ± 12.5 g in the control group and 33.0 ± 15.6 g in the group treated with a formaldehyde concentration of 4 mg/L, whereas in the group treated with 6 mg/L formaldehyde a mean difference in body weight of 31.7 ± 7.6 was found (Table 1). The results of the ANOVA revealed that there was no significant difference in the body weights of the rats before and after exposure to formaldehyde between the three groups ($p = 0.953$). The ANOVA results for the mean thickness of the *tunica mucosa* of the rat gastric fundus showed a significant difference between the three groups ($p = 0.004$). Multiple comparison analysis according to Tukey showed

that the mean thickness of the *tunica mucosa* of the gastric fundus in the group on 6 mg/L formaldehyde was significantly different from that of the control group ($p = 0.011$) and that of the group on 4 mg/L formaldehyde ($p = 0.003$).

In the histological sections structural changes may be seen in the cells of the fundal mucosa of rats exposed to formaldehyde in concentrations of 4 mg/L and 6 mg/L, respectively. Necrosis of fundal mucosal cells occurred especially in the cylindrical epithelium, affecting a higher percentage of cells in the group exposed to 6 mg/L formaldehyde (Figures 1a, 1b, and 1c).

This group also showed hydropic degeneration of the lamina propria and associated glands, which percentage was more severe than that in the group on 4 mg/L of formaldehyde (Figures 2a, 2b, and 2c).

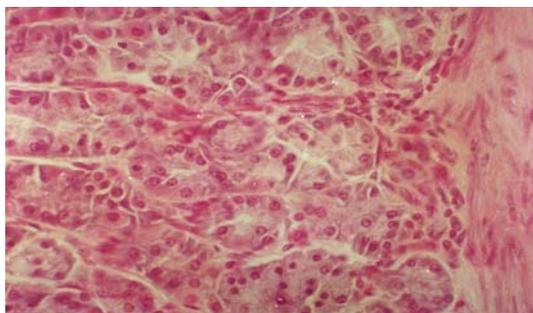


Figure 2a. Cells of the glands in the fundal mucosa stained with HE, at 400X magnification

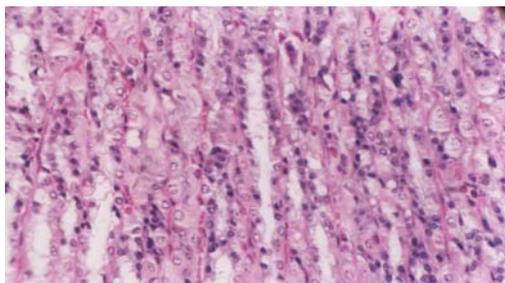


Figure 2b. Hydropic degeneration in the fundal mucosa stained with HE, at 400X magnification

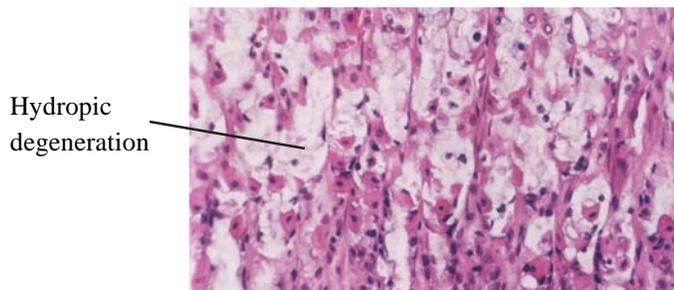


Figure 2c. Hydropic degeneration in the fundal mucosa stained with HE, at 400X magnification

DISCUSSION

This study shows that administration of drinking water containing formaldehyde in a concentration of 6 mg/L to experimental rats for 12 weeks, resulted in a mean thickness of the fundal mucosa of $919.00 \pm 138.91 \mu\text{m}$. The fundal mucosa in the group given drinking water containing formaldehyde in a concentration of 6 mg/L was thinner, in comparison with that in the control group and in the group given drinking water containing formaldehyde in a concentration of 4 mg/L. This indicates the occurrence of necrosis in the superficial cells that were directly exposed to irritation by formaldehyde. For purposes of comparison, a number of previous studies by various investigators may be mentioned.

In one study, Wistar rats (10 per sex per dose) were given formaldehyde in drinking-water at 0, 10, 50, or 300 mg/kg of body weight per day. At the end of 12 months, all rats of both sexes in the high-dose group were observed to have gastric erosions, ulcers, squamous cell hyperplasia, hyperkeratosis, and basal cell hyperplasia. However, only one male and one female from the mid-dose group showed hyperkeratosis.⁽⁶⁾

In a carcinogenicity study, a group of 10 rats was given drinking-water containing 0.5% Formalin® (0.2% formaldehyde) for 32 weeks. Histopathological changes were observed in the stomach, as well neoplastic changes in the forestomach and papillomas.

Takahashi et al. reported that formaldehyde enhanced gastric two-stage carcinogenesis in rat stomach induced by N-methyl-N'-nitro-N-guanidine.⁽¹⁰⁾ Subsequently, Furihata et al. administered formaldehyde at doses of 11 to 110 mg/kg body weight by gastric intubation to male F344 rats, using ornithine decarboxylase activity and DNA synthesis as markers of tumor-promoting activity. Their study showed

an increase in ornithine decarboxylase activity (up to 100-fold) and in DNA synthesis (up to 49-fold) in the pyloric mucosa of the glandular stomach, both with a maximum after 16 hr. These results suggest that formaldehyde has tumor-promoting activity in carcinogenesis in the glandular stomach.⁽¹¹⁾

A number of other long-term studies by the oral route have been conducted, and these have been reviewed in detail by the International Programme on Chemical Safety.⁽⁶⁾ The conclusion of these reviews was that formaldehyde is a normal mammalian metabolite and is not carcinogenic at low levels of exposure. Similarly, Bosetti et al reviewed cohort studies on formaldehyde risk of nasopharyngeal and selected other cancers, and found a pooled RR of 0.63 for nasopharyngeal cancer. They concluded that occupational exposure to formaldehyde shows no appreciable excess risk for oral and pharyngeal, sinonasal or lung cancers, and that the non-significantly increased RR for nasopharyngeal cancer among industry workers was attributable to a cluster of deaths in a single plant.⁽¹²⁾ These views have still to be reconciled with the reclassification of formaldehyde by the IARC.

The histological changes observed in the current study showed that the group given drinking water with a high concentration of formaldehyde experienced hydropic degeneration in a larger percentage of the cells of the fundal glands, compared with the group given drinking water with a low concentration of formaldehyde. This indicates that the extent of the histopathological changes was proportional to the formaldehyde concentration to which the rats had been exposed. This is in accord with the view that, owing to the high reactivity of formaldehyde, its effects in the tissue of first contact following ingestion are more likely to be related to the concentration of the formaldehyde consumed than to its total intake.^(7,8)

The current study also found that even short exposure times were sufficient for inducing histological changes in the rat gastric fundus. The observed histopathological changes confirm the study conducted by M Azim Khan,⁽¹¹⁾ which clearly demonstrated that one of the effects of formaldehyde is induction of cell vacuolization. However, the study by Til et al. revealed that long-term administration of formaldehyde in drinking water (2 years) may induce the development of gastric papillomas.⁽⁶⁾ The hydropic degeneration induced after 12 weeks in our study could represent the initial stage of such a development.

One study in male rats showed an increased incidence of forestomach papillomas, while another study in male and female rats found that the incidence of gastrointestinal leiomyosarcomas was increased in females and in males and females combined. In a third study, also using male and female rats, the number of males bearing malignant tumors and the incidences of hemolymphoreticular tumors (lymphomas and leukemias) and testicular interstitial-cell adenomas in males were increased. However, a fourth study gave negative results.⁽¹³⁾

A health-based value can be derived on the basis of a tolerable concentration, since irritation by formaldehyde is a concentration-dependent outcome and is not directly related to the total intake. The International Programme on Chemical Safety has established a tolerable concentration of 2.6 mg/L for ingested formaldehyde based on the no-observed-effect level (NOEL) of 260 mg/L for histopathological effects in the oral and gastric mucosa of rats.⁽¹⁴⁾

The Environmental Protection Agency (EPA) recommends that an adult should not drink water containing more than 1 milligram of formaldehyde per liter of water (1 mg/L) for a lifetime exposure, and a child should not drink water containing more than 10 mg/L for 1 day

or 5 mg/L for 10 days. The Occupational Safety and Health Administration (OSHA) has set a permissible exposure limit for formaldehyde of 0.75 parts per million (ppm) for an 8-hour workday, 40-hour workweek. The National Institute for Occupational Safety and Health (NIOSH) recommends an exposure limit of 0.016 ppm.⁽¹⁵⁾

The weight of evidence indicates that ingested formaldehyde is not carcinogenic in humans. There are good grounds for confidence that the present understanding of the two step nature of formaldehyde carcinogenesis requiring a high level of exposure to formaldehyde in the first instance remains valid. The World Health Organisation (WHO) in its Concise International Chemical Assessment Document outlined that "the weight of evidence indicates that formaldehyde is carcinogenic only at concentrations that induce the obligatory precursor lesion of proliferative regenerative response associated with cytotoxicity, although interaction with the DNA must be taken into account".⁽⁶⁾

CONCLUSIONS

Short-term administration of formaldehyde in drinking water was capable of effecting histopathological changes in the cells of the gastric fundal glands in rats. The severity and extent of the histopathological changes were proportional to the formaldehyde concentration to which the rats had been exposed.

REFERENCES

1. Dutch expert committee on occupational standards (DECOS). Formaldehyde health-based recommended occupational exposure limit. Health Council of the Netherlands (Gezondheidsraad) 2003; 02 OSH: 124.
2. International Agency for Research on Cancer (IARC) Monographs on the evaluation of the

- carcinogenic risk of chemicals to humans Vol: 88; 2004.
3. Thomsen GK. Health effects of selected chemicals. Nord 1995; 28: 211–37.
 4. Organization for Economic Co-operation and Development (OCED). SIDS initial assessment report for SIAM 14. Formaldehyde. Paris: Organization for Economic Co-operation and Development; 2002.
 5. World Health Organization. Guidelines for drinking water quality. Formaldehyde in drinking water. Geneva: World Health Organization; 2005.
 6. World Health Organization. Concise International Chemical Assessment Document: formaldehyde. Geneva: World Health Organization; 2002.
 7. Cogliano VJ, Yann G, Baan RS, Kurt S, Secretan MB, El Ghissassi F, et al. Advice on formaldehyde and glycol ethers. *Lancet Oncol* 2004; 5: 528.
 8. Pitcher M. LCA treatment of human health exemplified by formaldehyde within the furniture industry. Presented at the 4th Australian LCA Conference, Sydney. February 2005.
 9. Federer W. Statistics and society: data collection and interpretation. 2nd ed. New York: Marcel Dekker, 1991.
 10. Takahashi M, Hasegawa R, Furukawa F, Toyoda K, Sato H, Hayashi Y. Effects of ethanol, potassium metabisulfite, formaldehyde and hydrogen peroxide on gastric carcinogenesis in rats after initiation with N-methyl-N'-nitro-N-guanidine. *Jpn J Cancer Res* 1986; 77: 118-24.
 11. Furihata C, Yamakoshi A, Matsushima T. Inductions of ornithine decarboxylase and DNA synthesis in rat stomach mucosa by formaldehyde. *Jpn J Cancer Res* 1988; 79: 917-20.
 12. Bosetti C, McLaughlin JK, Tarone RE, Pira E, La Vecchia C. Formaldehyde and cancer risk: a quantitative review of cohort studies through 2006. *Ann Oncol* 2008; 19: 29-43.
 13. International Agency for Research on Cancer. Formaldehyde. Available at: <http://www.monographs.iarc.fr/ENG/Meetings/88-formaldehyde.pdf>. Accessed June 2, 2008.
 14. International Programme on Chemical Safety (IPCS). Formaldehyde. Geneva: World Health Organization (Concise International Chemical Assessment Document 40); 2002.
 15. Agency for toxic substances and disease registry (ATSDR). 1999. Toxicological profile for formaldehyde. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services. Available at: www.atsdr.cdc.gov/toxprofiles/tp111-c1.pdf. Accessed March 3, 2007.