RESULTS OF A 28-MONTH CHRONIC INHALATION TOXICITY STUDY OF FORMALDEHYDE IN MALE FISHER-344 RATS

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ABSTRACT — Male F-344 rats were exposed by inhalation to gaseous formaldehyde at 0.3, 2, and 15 ppm 6 h/day, 5 days/week for 28 months. Nasal tumors were macroscopically evident in the 15 ppm group from the 14th month and 8 of 32 rats bore such tumors at the 24th month. Histopathological examination revealed both squamous cell papillomas and carcinomas. No nasal tumors were observed in the lower exposure groups (0.3 and 2 ppm groups).

In the high exposure group (15 ppm group), frequent face washing, coughing and/or crouching position, lacrimation, nasal discharge, and yellow discoloration of the haircoat were observed. Significant decrease in food consumption and body weight was noted, and 20 (88.3%) rats died by the 24th month. Reduced triglyceride levels and liver weights, presumed to be related to the drop in food intake, were also seen in the 15 ppm group. Epithelial cell hyperplasia, hyperkeratosis, and the squamous metaplasia were apparent in all exposure groups. Inflammatory cell infiltration, erosion or edema were evident in all groups, including the 0 ppm and room (RC group) controls. In this study, a no effect level of formaldehyde vapor could not be obtained because toxicological signs were obvious even with the low exposure group. The benchmark doses for squamous metaplasia and epithelial hyperplasia were 0.25 and 0.24 ppm, respectively.

KEY WORDS: Formaldehyde, Inhalation toxicity study, Fisher-344 rat, Benchmark dose

INTRODUCTION

Formaldehyde is one of the most important and widely used chemicals. The most common commercially available form is a 30-50% aqueous solution known as formalin. Methanol is usually added as a stabilizer to prevent polymerization. Most formaldehyde is consumed in the manufacture of urea-formaldehyde, phenolic, polyacetal, and melamine resins. These resins are primarily applied as adhesives when making particle boards, fiberboards, and plywood. Urea-formaldehyde concentrates are also used in various coating processes, in paper products, and in thermal insulating materials.

In Japan, the concentration of formaldehyde in fabrics has been regulated since 1974, when it became clear that its presence in textiles...
could cause allergies (Oba et al., 1975). Formaldehyde released from plywood and furniture similarly presented a problem, and therefore the Japanese Agricultural Standard (JAS) was revised in 1980 to provide a standard for maximum allowable levels (Kojima et al., 1982).

In the USA, the residents of mobile homes have been reported to suffer from headaches as well as eye and nose ailments, which are believed to be caused by gaseous formaldehyde from the urea resins present in the heat-insulating materials used in such homes (ECETOC, 1981).

The first symptoms related to exposure to formaldehyde at concentrations ranging from 0.1-0.5 ppm are burning of the eyes, lacrimation, and general irritation to the upper respiratory tract. High concentrations (10 to 20 ppm) may cause coughing, tightening in the chest, a sense of pressure in the head, and palpitations (NIOSH/OSHA, 1980).

Watanabe et al. (1954) documented the first evidence indicating a possible carcinogenic action of formaldehyde. Two of 10 rats treated with aqueous formaldehyde subcutaneously developed sarcomas at the injection site. Because of lack of control, the significance of the results was questioned, but a subsequent inhalation study using F-344 rats and B6C3F1 mice at the Chemical Industry Institute of Toxicology (CIIT) unequivocally demonstrated that formaldehyde induces carcinomas in the nasal cavity. Groups of rats and mice were exposed to 2, 6, and 15 ppm formaldehyde vapors 6 h/day, 5 days/week for 24 months. Squamous cell carcinomas in the nasal epithelium were observed in rats of the 6 and 15 ppm exposure groups and in mice of the 15 ppm group (Swenberg et al., 1980). Albert et al. (1982) confirmed the induction of squamous carcinomas of the nasal cavity in SD rats exposed to 14 ppm gaseous formaldehyde 6 h/day, 5 days/week, over their life span.

Tobe et al. (1989) earlier reported no significant increase in the incidence of tumors in Wistar rats given formaldehyde in their drinking water at concentrations of 0.5, 0.1, 0.02% for 24 months compared to the control group, and Til et al. (1989) demonstrated that administration of 1.2 to 109 mg/kg/day in drinking water for 18 months was not associated with any gastric tumors.

From a comprehensive IARC evaluation it was concluded that there is limited evidence in humans of formaldehyde carcinogenicity but sufficient evidence in experimental animals (Group 2A; probable human carcinogen) (WHO, 1996).

A number of WHO working groups concerned with the public health impact of indoor air pollutants, and other review bodies such as the National Academy of Science's Committee on Indoor Air Pollutants, have cited volatile organic compounds (VOCs) as an important category of indoor air pollutants. In 1983 WHO defined the sick-building syndrome as characterized by a high frequency of irritative symptoms of the eyes, throat and lower airways, skin reactions, nonspecific hypersensitivity, mental fatigue, headache, nausea, and dizziness among individuals residing in a particular building (Fishbein et al., 1991).

Formaldehyde is often cited as a likely indoor odorant/irritant responsible for health complaints in building illness outbreaks. It is sometimes present at nearly TLV levels in homes as well as offices, resulting in a higher biological plausibility for toxic or irritant effects than other odorants present at orders of magnitude below the TLV in such environments (Cone et al., 1991).

Rusch et al., (1983) and Woutersen et al., (1989) previously reported effects of exposure to low concentrations of formaldehyde vapor for long periods. F-344 rats exposed for 22 hours per day, seven days per week for 26 weeks to 0.98 ppm or 0.19 ppm formaldehyde demonstrated no formaldehyde-related lesions (Rusch et al., 1983). When Wistar rats were exposed to 0, 0.1, 1.0 or 10 ppm of formaldehyde vapor for 6 hours per day, five days per week for 28 months, compound-related nasal changes were only found in animals given the highest dose (Woutersen et al., 1989).

The Japan Society for Occupational Health (JSOH) has recommended 0.5 ppm of formaldehyde for the Occupational Exposure Limits (OELs) in Japan (JSOH, 1996).

A guideline for indoor air levels of formaldehyde in non-industrial buildings was set by WHO with a recommended 30-minute average concentration of formaldehyde below 0.1 mg/m$^3$. 
(0.083 ppm) (WHO, 1987). However, there is no regulation of the indoor air levels of formaldehyde in Japan.

The purpose of the present study was to cast further light on formaldehyde effects by evaluating the long-term response to a low concentration which can be perceived by human olfaction.

MATERIALS AND METHODS

The exposure concentrations of formaldehyde were set at 15.0 ppm, for which carcinogenesis was reported in rats by Swenberg et al., (1980), 2.0 ppm, at which no nasal cavity malignancies were recognized (Swenberg et al., 1980) and 0.3 ppm, which can be perceived by human olfaction and may be encountered as a rather high concentration in room air (Matsumura et al., 1983).

The inhalation system employed is illustrated in Fig. 1. A modified Kamei method (1981) was applied to produce formaldehyde vapor. The concentration of formaldehyde in formalin (37.0 % formaldehyde aqueous solution containing 10 % methanol as anti-polymerization, Wako Pure Chemicals, Osaka, Japan) was confirmed by an iodometric titration method (Yamate et al., 1968). Flow of the aqueous formalin solution was controlled at 4 ml/h with a

![Diagram of formaldehyde exposure system](image1)

Fig. 1. Schematic illustration of the formaldehyde exposure system.

![Processing levels for histology](image2)

Fig. 2. Processing levels for histology in a ventral view of the hard palate of the rat.
Perista pump [A] (SJ-1211L, ATO, Tokyo, Japan), introduced into a sprayer in a glass bottle [B] heated to 70°C. The formaldehyde vapor was then diluted with room air adjusted to 600 l/h after passage through a HEPA filter before introduction into an exposure chamber [C] (height 1.90 m, width and depth 0.81 m, volume 560 l, stainless steel pyramidal type, NATESUME, Tokyo, Japan). The number of air changes was 13 times per hour. The actual concentration of formaldehyde in the chamber was measured by the acetyl acetone method (Yamate et al., 1968), twice a day.

Male Fisher-344 rats (F-344/DuCrj, 4 weeks old) were purchased from Charles River Japan Inc. (Atsugi, Japan) and acclimated for one week in an animal room controlled at 24±1°C, with a humidity of 55±5%, and a 12 h dark and 12 h light cycle. The 160 rats were divided into five groups (15, 2, 0.3, 0 ppm and a room control, no exposure group [RC group], and exposed for 6 h per day (10am-4pm), 5 days per week for 28 months. Rats in the 0 ppm group inhaled methanol at the same concentration as the 15 ppm group (4.2 ppm methanol). During the exposure periods, food and water were withdrawn, and 4 animals in each group, except for the RC group, were housed in stainless steel mesh cages (180×380×190 mm) in the chamber. After exposure, all animals were transferred to polycarbonate cages (400×240×160 mm including white flake chips) with food (F-2, Funabashi Farm, Chiba, Japan) and water available ad libitum.

All animals were observed and recorded for clinical signs once a day during the study. Body weights and food consumption were recorded weekly. Animals found dead or moribund were also subjected to a necropsy. Five animals per group were randomly selected at the end of the 12th, 18th, and 24th months, and surviving animals at 28 months were sacrificed for hemato-

![Graph](image-url)  

**Fig. 3.** Mortality of male F-344 rats inhaling formaldehyde for 28 months.  
* : Significant at 5% level as compared with 0 ppm group.  
** : Significant at 1% level as compared with 0 ppm group.  
○, 0 ppm group; ▲, 0.3 ppm group; ■, 2 ppm group; ●, 15 ppm group; X, room control group.
logical, biochemical, and pathological examinations. Blood samples were collected via the jugular vein under anesthesia.

The red blood cell count (RBC), hemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and white blood cell count (WBC) for each animal were determined with a Coulter counter SP (Coulter Electronics Inc., California, USA). Differential leukocyte counts were made with Giemsa-stained preparations. Serum samples were analyzed for total protein (T-PRO), albumin (ALB), the albumin/globulin ratio (A/G), blood urea nitrogen (BUN), glucose (GLU), phospholipids (PL), triglyceride (T-GLY), total cholesterol (T-CHO), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) using a Gemsaec auto analyzer type IV (Electro Nucleonics Inc., New Jersey, USA) and sodium (Na), potassium (K), and chloride (Cl) with a Hitachi ion-selective electrode 702 (Hitachi Ltd., Tokyo, Japan).

Autopsies were performed and the wet weights of the brain, heart, lungs, liver, kidneys, spleen, testis, and adrenal gland of each rat were measured. Histopathological examinations were performed on the pituitary, thyroid, nasal region, trachea, esophagus, stomach, small and large intestine, prostate gland, urinary bladder, muscle, femur, sciatic nerve, spinal cord, mesenteric lymph nodes, and any other gross lesions. After fixation in 10% buffered formalin and decalcification, histopathological sections of the nasal tubinates were obtained from five anatomical levels (Fig. 2). A constant amount (3 ml) of 10% neutral formalin solution was injected into the left lung in order to examine alveolar changes in detail. Sections were routinely processed and stained with hematoxylin and eosin, with special stains such as azocalmine.

![Graph](image_url)

**Fig. 4.** Body weights of male F-344 rats inhaling formaldehyde for 28 months. **** Significant at 1% level as compared with 0 ppm group. ● 0 ppm group; ▲ 0.3 ppm group; ■ 2 ppm group; ◆ 0.15 ppm group; X, room control group.
Ganilone blue, silver or mucicarmin being performed when necessary. Soft X-ray photographs of skulls with obvious nasal tumors were taken with a Softex apparatus (CMB-2, SOFTEX Co., Tokyo, Japan).

The mortality rate was calculated by the life table technique (Cox, 1972). Mortality and histopathological incidences were statistically evaluated by the Fisher's exact test (Fisher, 1955). The hematology, clinical chemistry and organ weight data were statistically evaluated using Bartlett's test for heterogeneity of variance. If the variance was not heterogenous, standard one-way ANOVA was used. If there were significant differences among the means, Dunnet's or Scheffé's tests were applied to deter-

<p>| Table 1. Significant Changes in Biochemical examination and Organ Weights for male F-344 rats inhaling formaldehyde for 28 months. |
|---|---|---|---|---|---|
| Item | Unit | Months |   | Groups |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th>0 ppm</th>
<th>0.3 ppm</th>
<th>2 ppm</th>
<th>15 ppm</th>
<th>RC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Animals</td>
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<tr>
<td></td>
<td>24</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>9</td>
<td>11</td>
<td>7</td>
<td>0</td>
<td>6</td>
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<tr>
<td>T-GLY mg/dl</td>
<td>12</td>
<td>181±23</td>
<td>158±17</td>
<td>149±21</td>
<td>112±20**</td>
<td>169±19</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>212±62</td>
<td>209±30</td>
<td>253±15</td>
<td>129±28</td>
<td>313±53*</td>
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<td>24</td>
<td>170±23</td>
<td>196±58</td>
<td>221±135</td>
<td>71±13</td>
<td>218±36</td>
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<td></td>
<td>28</td>
<td>207±78</td>
<td>250±81</td>
<td>229±66</td>
<td>140±75</td>
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<tr>
<td>Body weight g</td>
<td>12</td>
<td>434.3±16</td>
<td>439.0±19.3</td>
<td>444.0±25.7</td>
<td>347.1±30.2**</td>
<td>425.9±29.3</td>
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<tr>
<td></td>
<td>18</td>
<td>464.6±33.1</td>
<td>472.6±27.7</td>
<td>473.6±18.4</td>
<td>345.6±20.9**</td>
<td>474.6±33.0</td>
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<td></td>
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<td>431.8±27.2</td>
<td>438.8±50</td>
<td>457.9±20.8</td>
<td>300.4±15.8**</td>
<td>469.0±14.4</td>
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<td></td>
<td>28</td>
<td>402.4±47.9</td>
<td>452.1±72.5</td>
<td>433.9±32.5</td>
<td>404.5±62.4</td>
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</tr>
<tr>
<td>Absolute Organ Weights</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Liver g</td>
<td>12</td>
<td>9.61±0.45</td>
<td>9.47±0.42</td>
<td>10.00±0.87</td>
<td>7.57±0.56**</td>
<td>9.67±0.72</td>
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<td>18</td>
<td>11.57±1.05</td>
<td>11.29±0.84</td>
<td>11.73±0.76</td>
<td>7.87±0.67**</td>
<td>12.14±0.65</td>
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<td>11.34±1.88</td>
<td>12.28±2.72</td>
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<td>7.03±1.25*</td>
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<td>11.90±3.07</td>
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<td>10.71±2.34</td>
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<tr>
<td>Adrenals mg</td>
<td>12</td>
<td>40±9</td>
<td>40±2</td>
<td>38±5</td>
<td>43±3</td>
<td>38±6</td>
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<td></td>
<td>18</td>
<td>83±88</td>
<td>41±3</td>
<td>42±7</td>
<td>43±3</td>
<td>43±4</td>
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<td></td>
<td>24</td>
<td>49±14</td>
<td>79±73</td>
<td>63±14</td>
<td>54±2</td>
<td>49±1</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>414±708</td>
<td>181±400</td>
<td>88±65</td>
<td>79±51</td>
<td></td>
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<tr>
<td>Relative Organ Weights</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver g%</td>
<td>12</td>
<td>2.22±0.13</td>
<td>2.16±0.11</td>
<td>2.25±0.09</td>
<td>2.18±0.07</td>
<td>2.27±0.04</td>
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<td></td>
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<td>2.49±0.13</td>
<td>2.39±0.04</td>
<td>2.48±0.10</td>
<td>2.28±0.14*</td>
<td>2.56±0.13</td>
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<tr>
<td></td>
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<td>2.56±0.57</td>
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<td>2.48±0.27</td>
<td>2.33±0.29</td>
<td>2.51±0.16</td>
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<td></td>
<td>28</td>
<td>3.00±0.92</td>
<td>2.82±1.30</td>
<td>2.50±0.28</td>
<td>2.64±0.40</td>
<td></td>
</tr>
<tr>
<td>Adrenals mg%</td>
<td>12</td>
<td>9.1±2.2</td>
<td>9.1±0.6</td>
<td>8.5±1.0</td>
<td>12.5±1.3**</td>
<td>8.7±1.6</td>
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<tr>
<td></td>
<td>18</td>
<td>17.1±16.4</td>
<td>8.6±0.5</td>
<td>8.8±1.1</td>
<td>12.4±1.0</td>
<td>9.0±1.3</td>
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<td></td>
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<td>11.2±2.5</td>
<td>19.6±21.3</td>
<td>13.9±3.7</td>
<td>18.1±0.2</td>
<td>10.5±0.3</td>
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<tr>
<td></td>
<td>28</td>
<td>98.4±161.4</td>
<td>44.4±102.8</td>
<td>21.3±18.3</td>
<td>18.8±11.7</td>
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</tr>
</tbody>
</table>

Values are Mean ± S.D.
RC : Room Control group.
* : Significant at the 5% level as compared with the 0 ppm group.
** : Significant at the 1% level as compared with the 0 ppm group.
mine which group was significantly different from the controls. If the variance was heterogeneous, the Kruskal-Wallis test and nonparametric Dunnet’s or Scheffé’s tests were applied (Yamazaki et al., 1981). The benchmark dose (Crum, 1984) was calculated by the THRESH computer program (ICF Kaiser Engineers, INC. Louisiana, USA).

RESULTS

Actual concentrations of formaldehyde in the low (0.3 ppm), medium (2 ppm), and high (15 ppm) concentration groups were 0.30 ± 0.07 (mean ± S.D.), 2.17 ± 0.32, and 14.85 ± 2.22, respectively.

In the 15 ppm group, at an early time in the study, frequent face washing, coughing and/or crouching position, lacrimation and nasal discharge were observed. These symptoms decreased gradually after one month of exposure. A yellow discoloration of the haircoat was noted approximately one month after the start of the exposure in the 15 ppm and to a slight extent in the 2 ppm group.

Rats started to die from the 19th month in the 0 ppm group, with a total of eight animals dying spontaneously by the end of the study. In the 0.3 ppm, 2 ppm and RC groups, mortalities were evident from the 13th, 23rd, and 8th months, respectively. Six rats died in the 0.3 ppm group, 10 in the 2 ppm group, and 11 in the

<table>
<thead>
<tr>
<th>Table 2. Incidences of non-proliferative lesions in the nasal cavities of male F-344 rats inhaling formaldehyde for 28 months.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups (ppm)</td>
</tr>
<tr>
<td>Months</td>
</tr>
<tr>
<td>No. of animals examined</td>
</tr>
<tr>
<td>Cell infiltration</td>
</tr>
<tr>
<td>Erosion</td>
</tr>
<tr>
<td>Edema</td>
</tr>
<tr>
<td>Squamous cell metaplasia</td>
</tr>
</tbody>
</table>

Values are no. of animals.
RC : Room Control group.
D : Dead animals.
* : Significant at the 5% level as compared with the 0 ppm group.

<table>
<thead>
<tr>
<th>Table 3. Incidences of proliferative lesions in the nasal cavities of male F-344 rats inhaling formaldehyde for 28 months.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups (ppm)</td>
</tr>
<tr>
<td>Months</td>
</tr>
<tr>
<td>No. of animals examined</td>
</tr>
<tr>
<td>Epithelial cell hyperplasia</td>
</tr>
<tr>
<td>with squamous cell metaplasia</td>
</tr>
<tr>
<td>Epithelial cell hyperkeratosis</td>
</tr>
<tr>
<td>Papillary hyperplasia</td>
</tr>
<tr>
<td>Squamous cell papilloma</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>Unclassified sarcoma</td>
</tr>
<tr>
<td>Sarcoma</td>
</tr>
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</table>

Values are No. of animals.
RC : Room Control group.
D : Dead animals.
** : Significant at the 1% level as compared with the 0 ppm group.
RC group. In the 15 ppm group, the first death occurred in the 6th month, and a total of 20 rats died by the 24th month. The mortality rates at the 28th month were 59.6% in the RC group, 45.5% in the 0 ppm group, 31.8% in the 2 ppm group, 55.9% in the 2 ppm group, and 88.3% in the 15 ppm group (Fig. 3).

Significant decreases in body weights and food consumption were observed in the high concentration group throughout the exposure period (Fig. 4). No significant differences between the controls and the other groups were seen.

No abnormal hematological findings related to the inhalation were observed. On biochemical examination of serum, the RC group exhibited a significant increase in T-GLY as compared to the 0 ppm group at the 18th month, and in the 15 ppm group a significant decrease was observed at the 12th month and a tendency for decrease at the 18th and 24th months (Table 1).

In the 15 ppm group, significant decreases in the absolute and relative liver weights were observed at the 18th month and a tendency for increased adrenal weights at the 12th month (Table 1).

Macroscopic observation did not reveal any lesions other than nasal tumors, recognized from the 13th month in the 15 ppm group. Similarly, no microscopic lesions attributable to formaldehyde exposure were detected in the organs other than the nasal cavity. Data for non-proliferative lesions in the latter for the 0, 0.3, 2 ppm and RC groups are summarized in Table 2. The results for the 15 ppm group were complicated by the many neoplastic lesions observed in this group. Inflammatory cell infiltration, erosion and edema were observed in all exposure groups without any dose-relation. Squamous cell metaplasia without epithelial hyperplasia (photo 2) was apparent in all exposure groups, and a significantly increased incidence was recognized in the 2 ppm group as compared to the 0 ppm group. The first such lesions were recognized in the 0.3 and 2 ppm group from the 18th month (Fig. 5). Most non-proliferative lesions were found in the respiratory epithelium of the nasal turbinates and maxillotubinates at cross levels B and C.

![Graph](image-url)  
**Fig. 5.** Incidences of squamous metaplasia in F-344 male rats inhaling formaldehyde for 28 months.  
- ○, 0 ppm group; ▲, 0.3 ppm group; ■, 2 ppm group; ◆, 15 ppm group;  
- X, room control group.
They were very rare in the nasal septum and in the olfactory epithelium of the ethmoturbinates at cross levels D and E.

Data for the proliferative lesions in the nasal cavity are summarized in Table 3. Epithelial cell hyperplasia with squamous cell metaplasia was present in all exposure groups and at significantly increased incidences in the 2 ppm and 15 ppm groups (Photo 3) as compared to the 0 ppm group. This lesion was first recognized in the 21st, 18th and 6th months in the 0.3, 2 and 15 ppm groups, respectively (Fig. 6). Epithelial cell hyperkeratosis was observed in one animal of the 2 ppm group and in 26 of the 15 ppm group. Papillary hyperplasia, squamous cell papillomas and squamous cell carcinomas (Photo 4) were only observed in the 15 ppm group. Most tumors were located in levels B and C. Large tumors invaded the subcutis through the nasal bones (Photo 5).

There were various neoplastic changes in other organs in each group, but there were no significant inter-group differences in their incidences.

DISCUSSION

The lack of any differences in physiological or behavioral parameters, mortality, body weight gain and food consumption, and clinical signs was noted between the 0 ppm group and the RC group. It was indicated that 4.2 ppm of methyl alcohol vapor alone is without significant effect. White et al. [1983] similarly reported no significant changes in the lungs of SD rats after inhalation of methanol vapor for up to 6 weeks, even at 10,000 ppm.

Hematological examination did not reveal any adverse signs related to the formaldehyde exposure. The yellow hair coat discoloration noted in the 15 ppm and 2 ppm groups was also reported by Woutersen et al. (1987) and Appelman et al. (1988). The same symptom appeared in F-344 rats inhaling 2, 6, or 15 ppm for 24 months (Kerns et al., 1983), and in Wistar rats administered formaldehyde in drinking water (Til et al., 1983 and Til et al., 1989). Brendel (1964) had earlier described a yellow coat in albino rats administered hexamethyl-
Photo 1. Histopathological findings for the nasal septum.
Normal epithelium of a 0 ppm group animal exposed for 28 months. H.E. stain, ×200.

Photo 2. Histopathological findings for the nasal septum.
Squamous cell metaplasia without epithelial hyperplasia in a 2 ppm group animal inhaling formaldehyde for 28 months. H.E. stain, ×200.
Chronic Inhalation Toxicity Study of Formaldehyde.

**Photo 3.** Histopathological findings for the nasal septum.
Epithelial cell hyperplasia with squamous cell metaplasia in a 15 ppm group animal inhaling formaldehyde for 18 months. H.E. stain, ×200.

**Photo 4.** Histopathological findings for the nasal septum.
Squamous cell carcinoma in a 15 ppm group animal inhaling formaldehyde for 18 months. H.E. stain, ×200.
enetetramine, for which formaldehyde is a decomposition product. One gram of rat hair contains about 3.3 mg of kynurenine (Rebell, 1966), with which formaldehyde can react to produce a yellow dyestuff (Kewitz et al., 1966).

The decrease in body weight gain and food consumption, increase in mortality and decrease in T-GLY and liver weight observed for the 15 ppm group were in line with the CIIT report (Kerns et al., 1983). Rusch et al. (1983) found that when F-344 rats were exposed to formaldehyde at 0.0, 0.19, 0.98, and 2.95 ppm, for 22 hours per day, 7 days per week for 26 weeks, decrease in body weight and liver weight was only significant in the highest dose group. A study of food restriction for four weeks reported in male Wistar rats showed depression of body weight, decrease of T-GLY, and liver weight loss (5, 10, and 15 g food/kg B.W./day) as compared to the unrestricted diet group (Oishi et al., 1979). They suggested that the change of liver weight was more evident under food restriction than in the normal condition. In the present study, the decrease of body weight, the decrease of T-GLY and the liver weight in the high-concentration group were accompanied by decreased food consumption.

Beall et al., (1984) suggested that exposure to formaldehyde might be associated with hepatotoxicity in many species, and Strubelt et al., (1989) reported that formaldehyde inhibits aerobic energy supply by destruction of mitochondria in isolated perfused livers of rats. However, no hepatic damage was observed with the present hematological, biochemical or histopathological examinations.

When the Wistar rats were exposed to 0, 0.1, 1.0 or 10 ppm of formaldehyde vapor for 6 hours per day over five days per week for 28 months, an increased incidence of squamous metaplasia of the respiratory epithelium was encountered with the 10 ppm dose (Woutersen et al., 1989). Our results are thus in line with the literature. Cynomolgus monkeys and F-344 rats similarly demonstrated this lesion in response to formaldehyde but not Syrian golden hamsters after exposure to doses up to 2.95 ppm for 22 hours per day, seven days per week for 26 weeks.

Photo 5. Histopathological findings for the nasal bone. A subcutaneous tumor has invaded the nasal bone in a 15 ppm group animal inhaling formaldehyde for 15 months. Soft X-ray.
(Rusch et al., 1983). Since squamous metaplasia may be an early step in progression to neoplasia (Burger et al., 1989), long-term exposure to 0.3 ppm formaldehyde vapor may induce nasal cancer. However, squamous metaplasia in the respiratory tract is often associated with such conditions as viral infection (Baskerville et al., 1974), vitamin A deficiency (Shields et al., 1987), tobacco smoke (Dalbey et al., 1980), or exposure to urban pollutants, and Burger et al. (1989) also reported that vitamin C exposure itself produced mild to moderate squamous metaplasia in the larynx of rats receiving 0.76 mg/liter for 6 hours per day for 2 weeks and, in this case, the irritant properties of an acidic pH were presumably responsible.

The dose response and time of first observation in the present study also provide evidence that squamous cell metaplasia and epithelial hyperplasia may be due to irritation by formaldehyde vapor. However, squamous cell metaplasia in the 0.3 ppm group did not progress to squamous cell carcinoma.

The relatively high incidences of squamous cell papillomas and carcinomas observed in the high concentration group are in agreement with earlier studies. Kerns et al. (1983) reported that F-344 rats exposed to 14.3 ppm formaldehyde vapor for 24 months developed 103 squamous cell carcinomas in 232 rats. Polypoid but no squamous cell papillomas were also described. Twenty-five squamous cell carcinomas were found in 99 SD rats after exposure to 14 ppm formaldehyde vapor over their life span (Albert et al., 1982), and 38 squamous cell carcinomas and 10 polyps or papillomas were noted in 100 SD rats (Sellakumar et al., 1985). The toxicological reason why squamous cell papillomas developed in our and Sellakumar's studies, but only polypoid adenomas in the Kerns study is not clear, because we and Kerns used the same strain (F-344) whereas Sellakumar used SD rats.

The ACGIH's current threshold limit value (TLV) for formaldehyde (0.3 ppm) is based primarily on its irritant rather than its carcinogenic effects (Conolly et al., 1995). WHO reported that formaldehyde is an odorant with an odor detection threshold of 0.06 mg/m³, at which level there is little or no concern. The 30-minute average concentration of formaldehyde is recommended to be below 0.1 mg/m³ (0.083 ppm) in indoor air in non-industrial buildings as an air-quality guideline (WHO, 1987). The Japan Society for Occupational Health (JSOH) has recommended 0.5 ppm of formaldehyde for Occupational Exposure Limits (OELs) in Japan (JSOH, 1996).

Safe levels or Acceptable Daily Intake (ADI) values are derived from experimental no-observed effect level (NOEL) or no-observed-adverse-effect level (NOAEL) by applying appropriate safety factors (WHO, 1995). For the risk assessment of some chemicals, the US EPA has typically calculated a reference dose (RfDs) or reference concentrations (RfCs) using the no-observed-adverse-effect level (NOAEL) approach (Barnes et al., 1988). Alternatively, a benchmark dose may be developed by mathematical modeling of the dose-response data as an alternative to the uncertainty factor in extrapolating for NOAEL (WHO, 1994). In the present study, the NOAEL of formaldehyde vapor could not be obtained, because squamous metaplasia and epithelial hyperplasia were recognized in the nasal cavities of the rats given the lowest concentration (0.3 ppm) but not in the 0 ppm or RC groups. Benchmark doses for squamous metaplasia and epithelial hyperplasia were found to be 0.25 and 0.24 ppm, respectively, in our present investigation.

Hilton et al. (1996) reported that formaldehyde is a potent contact allergen but it lacks any significant potential to cause sensitization in the respiratory tract, while Yoshida et al. (1997) reported that formaldehyde exposure may accelerate type I allergy and moderate type IV allergy in female B6C3F1 mice exposed to 500 ppb daily for 8 hr, 6 days per week for 6 weeks.

The need for assessing the noncancer risk of formaldehyde to which humans are routinely exposed indoors arises from the large amount of time spent indoors. Therefore, even if species differences are ignored, recommendations regarding the maximum concentrations of formaldehyde should be reconsidered carefully.

REFERENCES

Albert, R.E., Sellakumar, A.R., Laskin, S., Kuschneret, M., Norton, N. and Snyder,


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