[CANCER RESEARCH 43, 4382–4392, September 1983]

Carcinogenicity of Formaldehyde in Rats and Mice after Long-Term Inhalation Exposure[1]

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ABSTRACT

Groups of approximately 120 male and 120 female Fischer 344 rats and C57BL/6 × C3H F_1 mice were exposed by inhalation to 0, 2.0, 5.6, and 14.3 ppm of formaldehyde gas 6 hr/day, 5 days/week, for 24 months. This exposure period was followed by up to 6 months of nonexposure. Interim sacrifices were conducted at 6, 12, 18, 24, 27, and 30 months. Significant formaldehye-induced lesions were restricted to the nasal cavity and proximal trachea. The distribution and severity of these lesions were concentration dependent. Rhinitis, epithelial dysplasia, and squamous metaplasia occurred in all exposure groups of rats and in the intermediate and high exposure groups of mice. There was regression of rhinitis, dysplasia, and metaplasia at 27 months (3 months postexposure) in the 14.3- and 5.6-ppm groups of mice and in the 2.0- and 5.6-ppm groups of rats. Squamous cell carcinomas were observed in the nasal cavities of 103 rats (52 females and 51 males) and 2 male mice exposed to 14.3 ppm and in 2 rats (one male and one female) exposed to 5.6 ppm of formaldehyde gas. Formaldehyde inhalation was also weakly associated with an increase in the frequency of polypoid adenomas in the nasal cavity of male rats.

INTRODUCTION

Formaldehyde is the most important commercially produced aldehyde in the United States with over 9 million pounds produced annually (12). Because of the extensive uses of formaldehyde in building materials, textiles, insulation, and other industries, there is potential for occupational and environmental exposure (42). Considerable human exposure to formaldehyde gas occurs at concentrations up to 1 ppm (9). Formaldehyde is known to cause eye, nose, and throat irritation as well as dermal irritation (2) and allergic contact dermatitis (26, 28).

Formaldehyde is mutagenic in some bacteria, fungi, and *Drosophila* (3), and it induces unscheduled DNA synthesis in HeLa cells (24). Formaldehyde induces DNA-protein cross-linkage in bacterial and mammalian cells (33, 34, 43). It is reported to be nonmutagenic in the Chinese hamster ovary assay (19), but it does induce sister chromatid exchange in cultured Chinese hamster ovary cells and human lymphocytes (27). Furthermore, formaldehyde can act as a weak initiating agent (32) and as a weak promoter in the C3H/10T½ mouse embryo fibroblast transformation assay (7).

Preliminary results of this bioassay demonstrated extensive toxicity and carcinogenicity in rats exposed to 14.3 ppm of formaldehyde for 18 months (38). More recently, the induction of squamous cell carcinomas in the rat nasal cavity by formaldehyde has been reported in another laboratory (1). This paper reports the final results of our 30-month bioassay on the effects of formaldehyde exposure in rats and mice.

MATERIALS AND METHODS

Animals and Exposure

Seven-week-old Fischer 344 rats (Charles River Breeding Laboratories, Inc., Portage, Mich.) and 6-week-old C57BL/6 × C3H F_1 (hereafter called B6C3F_1) mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were assigned randomly on the basis of body weights to 3 exposure groups and one control group. There were 119 to 121 animals of each sex in each of the exposure and control groups.

All animals were exposed for 6 hr/ day, 5 days/week, for a period of up to 24 months. This exposure period was followed by a 6-month period of nonexposure. Intended concentrations of formaldehyde for rats and mice were 15, 6, 2, or 0 ppm. The mean exposure concentrations of formaldehyde over the 2-year exposure period were 14.3 ± 0.04 (S.E.), 5.6 ± 0.02, and 2.0 ± 0.01 ppm. The mean value for chamber temperature was 22.7° (95% confidence interval; range, 22.6 to 22.8°) and for relative humidity was 51.5% (95% confidence interval; range, 51.2 to 51.9%).

The exposures took place in 5-cu m Hinners-type chambers that were operated at approximately 1 inch of water subatmospheric pressure with 12 air changes per hr. The concentration of formaldehyde gas, generated by heating paraformaldehyde (Aldrich Chemical Co., Milwaukee, Wis.), was monitored with a Miran 1-A IR spectrophotometric gas analyzer (38). The internal environment of each chamber was maintained at 20–22° and 51 ± 5% humidity. The cage positions within the chambers were rotated one position from top to bottom and left to right each day throughout the exposure period.

Rats were housed individually in stainless steel wire mesh cages, and mice were housed similarly with 4 animals of the same sex per cage. During the 18-hr nonexposure period, the rats and mice were housed in environmentally controlled holding rooms and were fed Purina Rodent Chow 5001 and allowed free access to water. All test animals were housed separately from control animals and were maintained in holding rooms on a 12-hr light-dark cycle throughout the experiment.

All animals were observed twice daily throughout the study. Weekly body weight determinations were made for the first 6 months and biweekly determinations thereafter. The rats were weighed individually, and the mice were weighed by cage groups.

Hematology, serum chemistry, and urinalysis determinations were made from animals selected randomly (10/sex/group) at each scheduled sacrifice. Neurofunction and ophthalmoscopic examinations were also done at selected intervals in the study. Detailed methods for these examinations have been described (29).

Pathology

Gross pathological examinations were performed on all animals that died or were sacrificed at the 6-, 12-, 18-, 24-, 27-, and 30-month scheduled intervals during the course of the study (22). All major tissues

[1] This investigation was supported by the Chemical Industry Institute of Toxicology, Research Triangle Park, N. C. 27709.

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Received January 20, 1983; accepted June 8, 1983.

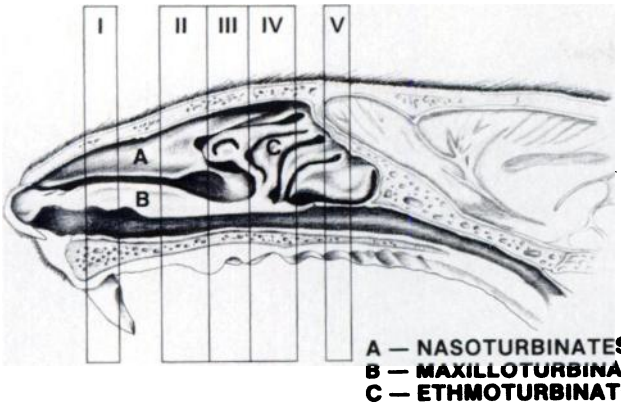

Chart 1. Midsagittal section of a rat head that demonstrates the turbinates that are included in each level (*I* to *V*) for microscopic evaluation.

from each organ system (approximately 50 tissues/animal) in the control and high exposure groups were evaluated histologically. The tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned to a thickness of 5 μm, and stained with hematoxylin and eosin. Tissue masses observed at necropsy were evaluated microscopically in all animals for confirmation.

Multiple sections of nasal turbinates were evaluated as target tissues in all rats and mice. The nasal turbinates from both rats and mice were processed in a similar manner. Histological sections were evaluated from 5 anatomical levels in the rat and from Levels II, III, and V in the mouse (Chart 1). After fixation in 10% buffered formalin and decalcification, the nasal cavity was trimmed at the appropriate levels (22, 44). The tissues were maintained in correct anatomical orientation through the embedding process. Level I was oriented in the cassette so the posterior surface was sectioned, and the remaining levels were positioned so their anterior surfaces were cut.

Statistics

Weights and Clinical Pathology. Data were tested for homogeneity of variances using Bartlett's test (4), and, when not statistically different ($p > 0.05$), ANOVA[3] to test for equality of exposure group means was done. When significant differences in means were observed (ANOVA), exposure level *versus* control comparisons were made by Dunnett's test (14)). When Barlett's test was significant, the Kruskal-Wallis test (23) replaced the ANOVA. Specific exposure levels *versus* control comparisons were made using Dunn's nonparametric equivalent (13) to the Dunnett test.

Clinical Observations. χ^2 tests for homogeneity were done on clinical, ophthalmological, and neurobehavioral data.

Histomorphological Observations and Survival. Histomorphological lesions were analyzed using the actuarial life table method (6) and the National Cancer Institute's bioassay analysis program (10, 16, 40, 41). The life table method treats the survival times of lost animals, those that died of trauma, or those terminated at scheduled sacrifices as censored observations. Cumulative tumor rates and survival curves were calculated from these data by the method of Kaplan and Meier (21).

Recognizing that differential mortality patterns among the exposure groups could create an associated bias in the analysis of tumor incidence, both unadjusted and adjusted data were analyzed. Unadjusted data, which ignore survivorship information, were analyzed for overall comparisons between the exposure groups by a generalization of the Fisher-Irwin exact test for linear trend. Adjusted data, which consider time to lesion observation and survivorship, were analyzed for both overall and pairwise comparisons by the methods of Cox (10) and Tarone (40). These tests evaluate the comparison of each tumor or event with the total number of animals in all groups surviving to or beyond a specific time point.

The presentation of adjusted data (which reflect time dependency of the event) as well as unadjusted data (which reflect final ratios of the event) minimizes the basis of early mortality that could preclude a sufficient number of animals being present for meaningful comparisons at the later time intervals. Similarly, the presentation of both group and pairwise analysis allows for examination of events among groups as well as linear trends between groups. Both observations are necessary for evaluating the presence or absence of a trend.

The level of significance used in all test procedures with unadjusted data was $p < 0.05$, and for adjusted pairwise analyses, Bonferroni's correction was used with a significance level of $p < 0.0167$ (16).

[3] The abbreviation used is: ANOVA, one-way analysis of variance.

RESULTS

Body Weights

From Exposure Week 3 to Exposure Week 103, mildly (15 to 35 g) decreased body weights ($p < 0.05$) in male and female rats (5.6 and 14.3 ppm) were observed when compared with control values (Charts 2 and 3). Animals in the 2.0-ppm exposure group had sporadically reduced body weights ($p > 0.05$) throughout the exposure period. At the 12-month interim sacrifice, decreases in body weight in all groups of rats were thought to be associated with typical histomorphological lesions of sialodacryoadenitis virus infection (20). Mean body weights returned to previous values by Study Week 53 (Charts 2 and 3). At 27 months (3 months postexposure), the body weights of the male rats in the 14.3-ppm exposure group and males and females in the 5.6- and 2.0-ppm groups were not statistically different from the body weights of the control animals.

In male mice, significant body weight differences were sporadic and inconsistent with a test agent effect during the course of the study. In female mice (14.3 ppm), there was a trend toward lower body weights beginning after 72 weeks on study. The body weights in this group also returned to normal after the exposures were discontinued (Chart 4).

Mortality

Male and female rats in the 14.3-ppm exposure group exhibited significantly increased mortality ($p < 0.001$) when compared with control animals from the 12th month of exposure to the end of the study (Charts 5 and 6). Male rats in the intermediate- and low-exposure groups showed an apparent concentration-dependent decrease in cumulative survival from 17 months onward. The increased mortality was, however, only significant ($p < 0.05$) in the intermediate-exposure group. After the exposures were discontinued, the survival rates in the groups continued to be below the control group values.

In male mice, there were no differences in survival between exposure groups, although the exposed mice appeared to have slightly poorer survival (not concentration dependent) from 6 to 24 months (Chart 7). Generally poor survival in all groups of males was attributed to fighting and infections of the genitourinary tract associated with group housing (22). Of the mice that died, 21% had macroscopic evidence of inflammatory lesions of the penis, kidneys, and/or urinary bladder. Gross lesions in the genitourinary system were associated histomorphologically with purulent balanoposthitis, prostatitis, seminal vesiculitis, pyelonephritis, and cystitis in animals from all groups. In spite of the

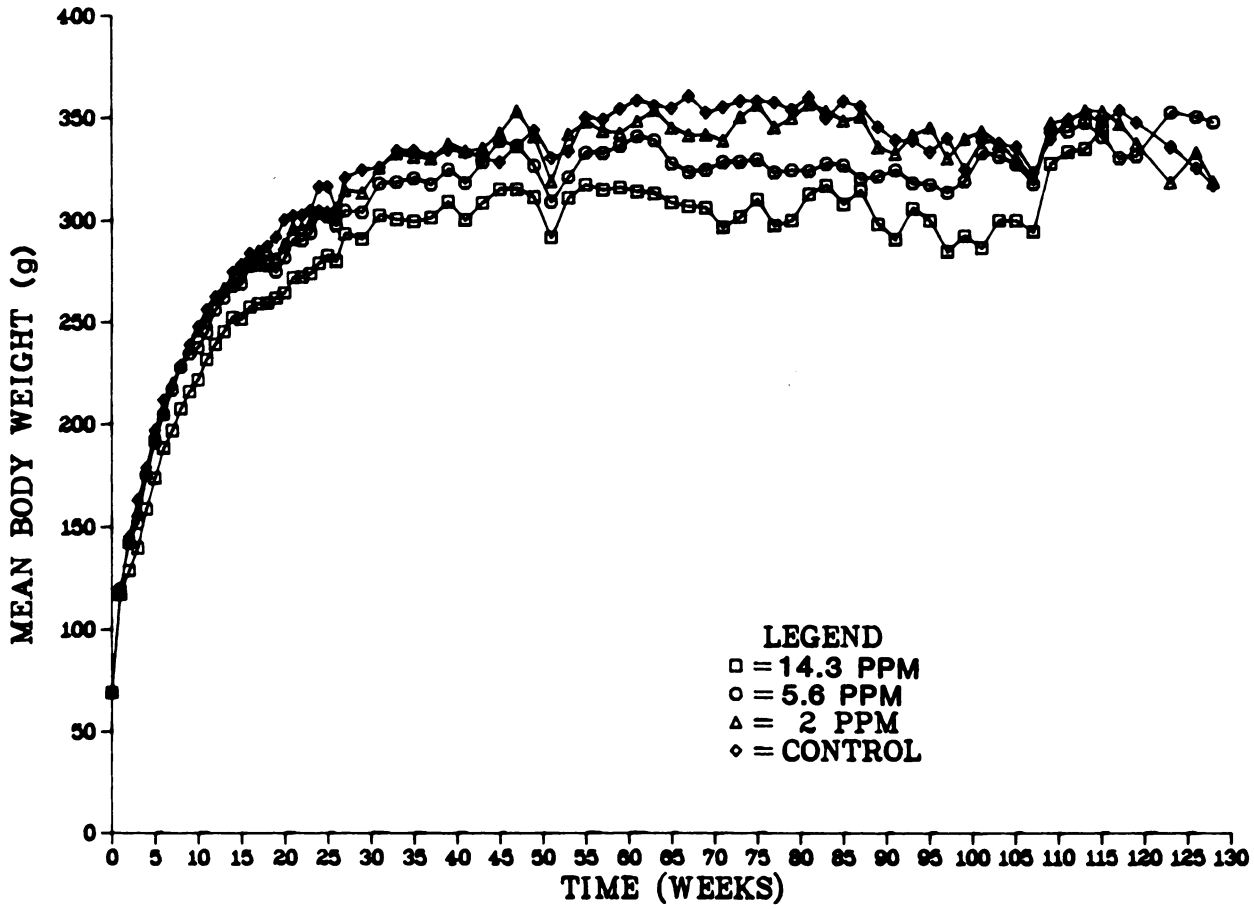

Chart 2. Mean body weight for male rats exposed to formaldehyde gas.

increased mortality, the numbers of male mice surviving a minimum of 18 months were 41, 33, 32, and 25 for the 0-, 2-, 5.6-, and 14.3-ppm exposure groups, respectively. There were no differences in cumulative survival among the groups of female mice, with 89 (0 ppm), 83 (2 ppm), 92 (5.6 ppm), and 88 (14.3 ppm) surviving a minimum of 18 months.

Hematology, Urinalysis, Clinical Chemistry, and Ophthalmological and Neurofunction Examinations

There were no alterations in the clinicial pathology or ophthalmological or neurofunctional data that were considered to be related to formaldehyde exposure.

Clinical Observations and Pathology

Rats. Exposure to formaldehyde produced a concentration-dependent increase in yellow discoloration of the hair coat. Three months after the exposures were discontinued, hair coat coloration was essentially normal. Other significant clinical and macroscopic observations were limited to the 14.3-ppm exposure group. Clinically, rats in this group were dyspneic ($p < 0.01$) and emaciated ($p < 0.05$), and many had large s.c. facial swellings that, on closer inspection, were interpreted to be proliferative lesions (carcinomas) protruding from the nasal cavity (Fig. 1). Neoplastic lesions were first observed clinically at Day 358 in females and Day 432 in males (Chart 8). Macroscopically, these lesions originated in the anterior portion of the nasal cavity, and in a few instances, they extended into the ethmoturbinates.

Formaldehyde-induced microscopic lesions were confined to the nasal cavity and the proximal trachea. In the nose, lesions were first noted in anterior sections (Levels I, II, and III) from animals that were terminated at 6 months in the 14.3-ppm exposure group. Alterations of the epithelium were initially restricted to the ventral portion of the nasal septum and the distal tips of the nasoturbinates and maxilloturbinates. As the study progressed, the distribution and severity of lesions within the nasal cavity increased in all exposure groups.

In the 2.0-ppm exposure group, purulent rhinitis, epithelial dysplasia, and squamous metaplasia were present in Level I turbinates at 12 months. The mucosa at this location was characterized by a transition from normal nonciliated simple cuboidal epithelium to an epithelial lining that was several cells thick and squamoid in appearance. The organization and the polarity of the individual epithelial cells had changed from vertical to horizontal with respect to the basement membrane. These alterations were termed zones of epithelial dysplasia. Similar histomorphological alterations have also been called basal cell hyperplasia and epidermoid metaplasia (1, 4). The morphological diagnosis, squamous metaplasia, was used to designate zones of altered epithelium that were characterized by the presence of a well-differentiated germinal cell layer (stratum germinativum) and superficial layers of epithelium (stratum spinosum and stratum corneum). Keratin was produced only in areas of squamous metaplasia. In all exposure groups, epithelial dysplasia was

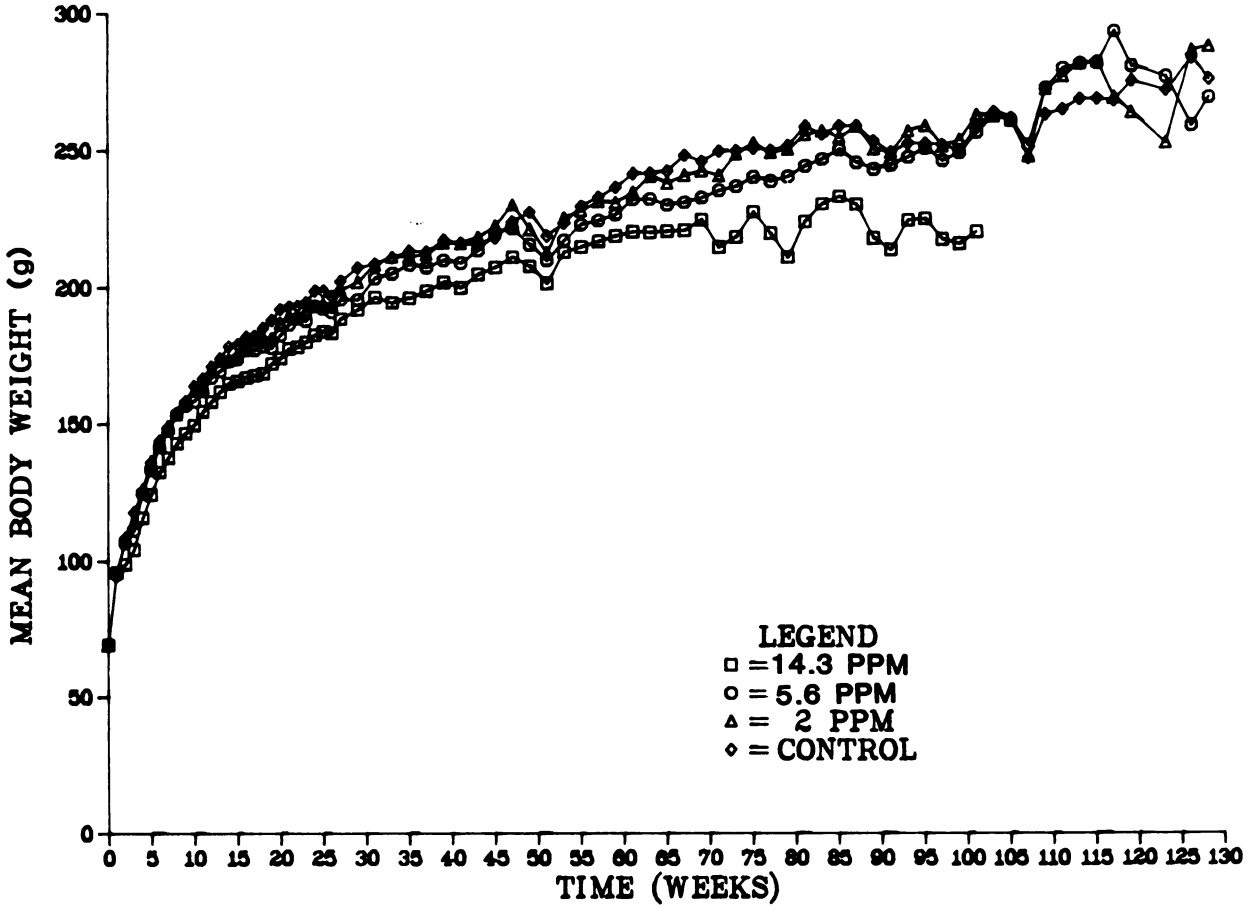

Chart 3. Mean body weight for female rats exposed to formaldehyde gas.

detected earlier than was squamous metaplasia. At 24 months, in the 2.0-ppm exposure group, the frequency of metaplasia exceeded that of prior sacrifice intervals (Chart 9); however, dysplasia and metaplasia were observed in Level I only. At 27 months (3 months postexposure), there was a significant decrease ($p < 0.05$) in the frequency of metaplasia in the 2.0-ppm exposure group.

In the 5.6-ppm exposure group, purulent rhinitis, epithelial dysplasia, and squamous metaplasia were observed in Levels I, II, and III. At 27 months, there was regression ($p < 0.05$) of squamous metaplasia in all affected levels of the 5.6-ppm exposure group (Chart 9) and Levels IV and V in the 14.3-ppm group (Chart 9). As in the 2.0-ppm exposure group, the severity of the lesions in the 5.6- and 14.3-ppm exposure groups was most intense in Level I; however, in these groups, there were also exposure-related compound effects in Levels II, III, IV, and V (Chart 9). These data are contrasted with a lesion (dysplasia or metaplasia) frequency of less than 15% of the 0-ppm exposure group where lesions were present in Level I only.

Eight rats (4 males and 4 females) from the low-exposure group, 6 male rats from the intermediate-exposure group, and 5 rats (4 males and one female) from the high-exposure group had benign proliferative lesions (polypoid adenomas) of the nasal mucosa in Level I, II, or III. One control male rat had a similar lesion (Table 1). In a few animals, the polypoid adenomas were visible grossly in the nasal cavity after decalcification and sectioning (Fig. 2). The tumors grew into the lumen of the nasal cavity where, in some cases, they caused obstructive lesions and were associated with focal purulent rhinitis. The cells comprising these neoplasms were cuboidal and rarely ciliated, and they often formed acinar-like structures that were filled with detritic cellular and noncellular debris (Fig. 3). The exact origin of the polypoid adenomas (respiratory or glandular epithelium) could not be determined with light microscopy. The adenomas in the control rat and rats exposed to 2.0 or 5.6 ppm of formaldehyde gas were not associated with zones of epithelial dysplasia or squamous metaplasia. When adjusted and unadjusted data were analyzed, no significant differences were observed in pairwise analyses; however, a significant adjusted trend ($p < 0.05$) was present for male rats (10, 16, 40).

Squamous cell carcinomas (Table 1) were observed in 2 rats (one male and one female) exposed to 5.6 ppm of formaldehyde ($p > 0.05$) and in 103 rats (51 males and 52 females) from the 14.3-ppm exposure group ($p < 0.001$). The adjusted cumulative incidence rate of squamous cell carcinomas in male and female rats from the 14.3-ppm exposure groups at 24 months was 67 and 87%, respectively (Chart 8). In the 14.3-ppm exposure group, squamous metaplasia with zones of squamous epithelial hyperplasia and increased keratin production appeared to precede areas of squamous papillary hyperplasia with foci of cellular atypia. More advanced lesions included carcinomas *in situ* and invasive squamous cell carcinomas of the nasal turbinates. The neoplasms were extremely osteolytic and were associated with excessive keratin production and mild to severe purulent rhinitis

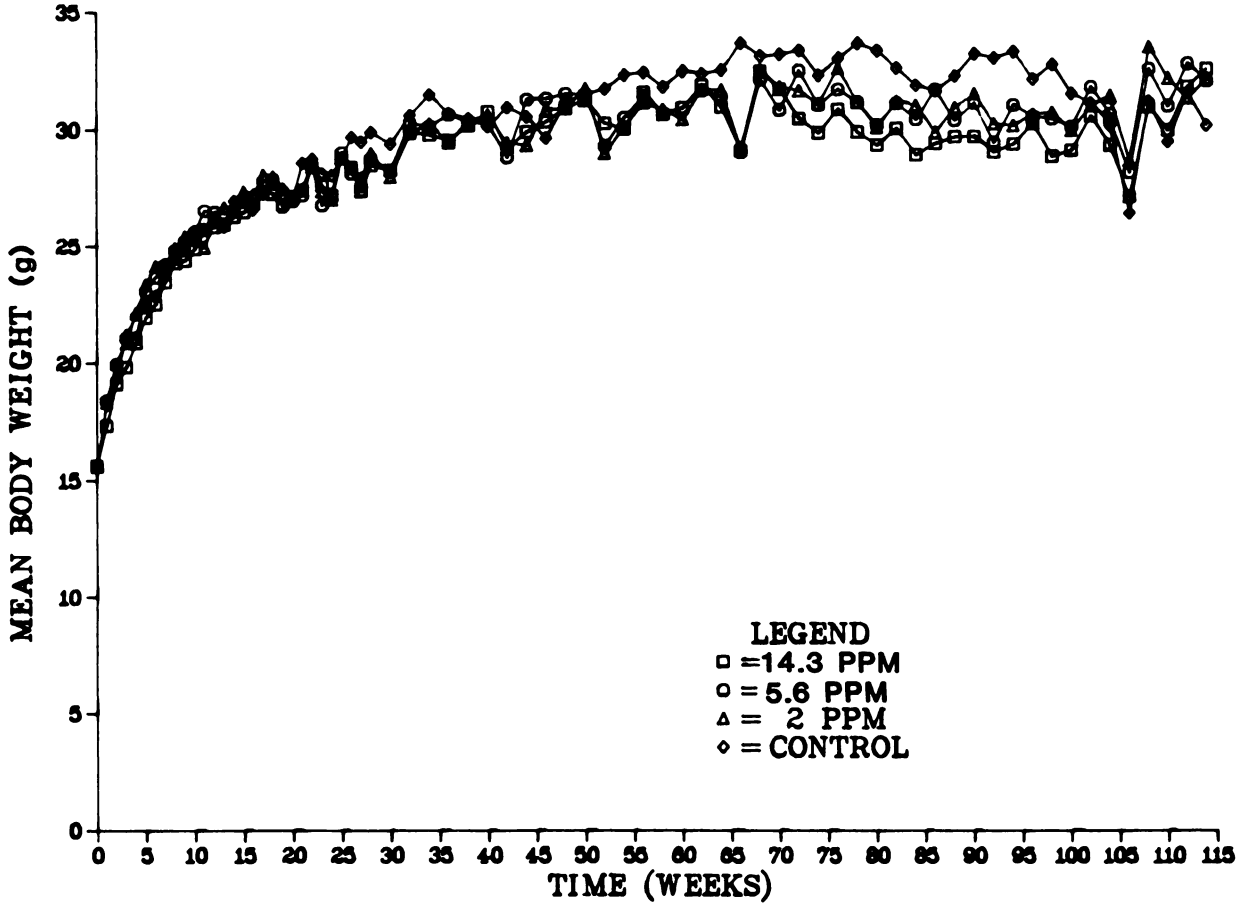

Chart 4. Mean body weight for female mice exposed to formaldehyde gas.

(Fig. 4). In a few animals, the carcinomas had grown through the ethmoid plate and invaded the rhinencephalon. Others grew in an anterioventral direction and invaded the vomeronasal organ, but they never protruded through the hard palate into the oral cavity. In one rat, there was detectable metastasis of malignant squamous epithelium to the mandibular lymph nodes. One carcinosarcoma, one undifferentiated carcinoma, and an undifferentiated sarcoma were also observed in the 14.3-ppm exposure group. There were 2 additional animals with carcinomas of the respiratory epithelium (nasal carcinoma).

In many animals from the high-exposure group, with or without carcinoma, the excessive accumulation of keratin and inflammatory exudate within the lumen of the nasal cavity caused severe dyspnea and death. This is not unexpected, since rats are obligatory nose breathers (17, 31).

In rats exposed to 14.3 ppm of formaldehyde that were killed at 18 months, there were a few animals that had multifocal areas of minimal to mild epithelial hyperplasia, epithelial dysplasia, or squamous metaplasia of the proximal tracheal mucosa. Similar lesions were also observed with an increased frequency ($p < 0.05$) in rats from the unscheduled death group and in rats from 24-month sacrifice. There were no significant tracheal lesions present in the 0-, 2.0-, or 5.6-ppm exposure groups, and tracheal lesions were not observed during the postexposure period in the 14.3-ppm exposure group. Neoplasia of the tracheal epithelium was not observed.

Mice. Significant formaldehyde-induced lesions were observed in the upper respiratory tract of mice. These included inflammatory, dysplastic, and squamous metaplastic alterations of the respiratory epithelium. Lesions in the nasal cavity of mice were first detected at 12 months, when animals in the 14.3-ppm exposure group exhibited serous rhinitis in Levels III and V. By 18 months, most animals in the 14.3-ppm exposure group had dysplastic and metaplastic alterations of the nasal mucosa in Level II (Chart 10), and these changes were associated with a shift in the nasal exudate from serous to purulent. In the 5.6-ppm exposure group at the 18-month sacrifice, a few mice had dysplastic changes that were associated with serous rhinitis in Level II, and no alterations were detected in the nasal cavity from animals in the 2.0-ppm exposure group. By 24 months, a majority (>90%) of mice in the 14.3-ppm exposure group had dysplastic and metaplastic alterations that were associated with seropurulent rhinitis. At that time period, there were only a few mice in the 5.6-ppm exposure group with dysplasia, metaplasia, or serous rhinitis in Level II. At 24 months, the mice exposed to 2.0 ppm of formaldehyde were free of significant nasal lesions; however, a few animals had serous rhinitis in Level II.

At 24 months, there were mice in all exposure groups with minimal to moderate hyperplasia of the squamous epithelium lining the nasolacrimal duct. This lesion was most extensive, both in frequency and distribution, in mice from the 14.3-ppm exposure group. Animals from the high-exposure group also had focal areas of atrophy of the olfactory epithelium lining the ethmoturbinates. This lesion also occurred in the 5.6-ppm group,

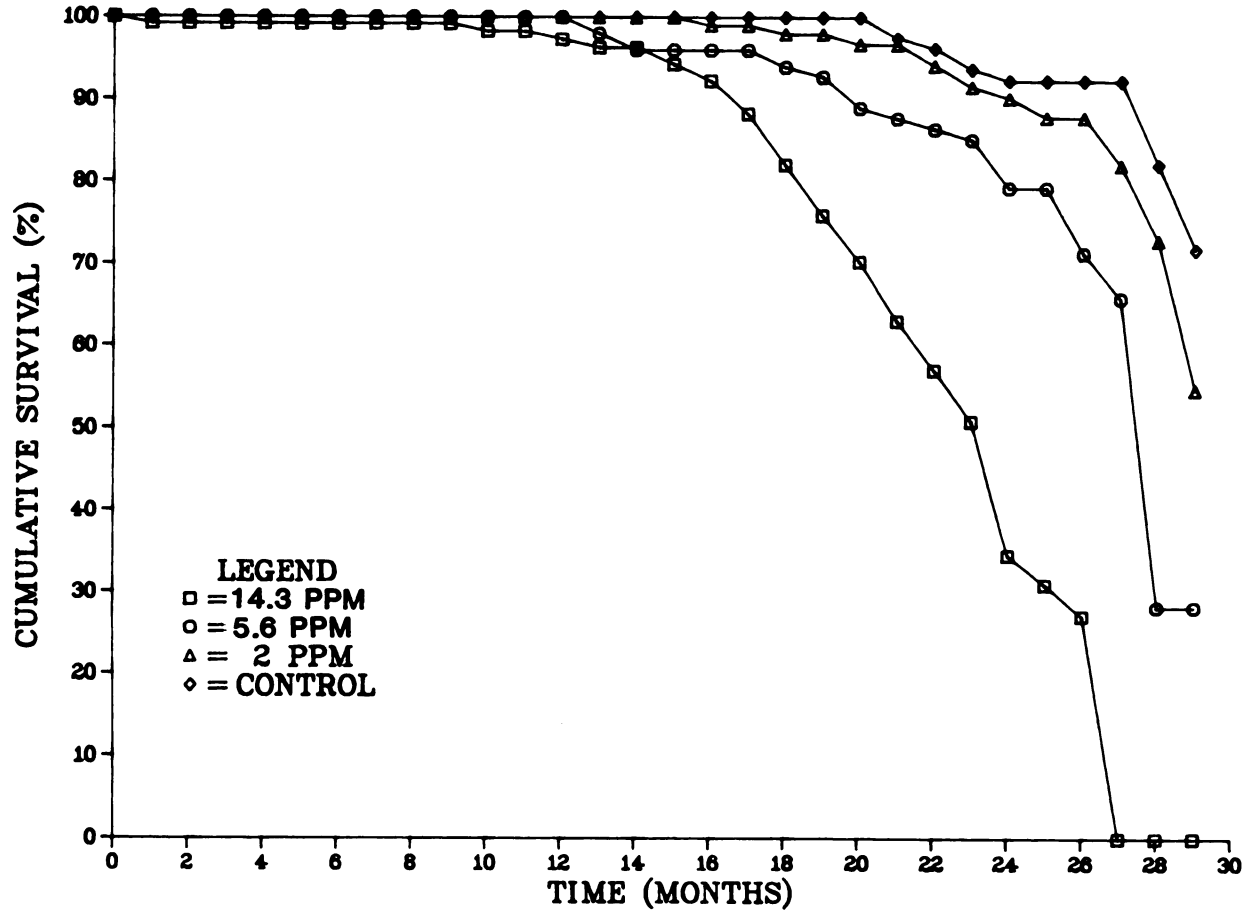

Chart 5. Cumulative survival of male rats exposed to formaldehyde gas.

but the frequency was greatly reduced. Tracheal lesions were not observed in mice.

Two male mice (14.3 ppm) from the 24-month sacrifice had squamous cell carcinomas in the nasal cavity ($p > 0.05$). Both carcinomas originated unilaterally on the nasoturbinates, and the location and the histomorphology of these tumors were simiar to those observed in rats; however, they were not as invasive and did not cause death.

At 27 months, dysplastic epithelial lesions were present only in the 14.3-ppm exposure group, and the exudate associated with these lesions was more serous than purulent. Squamous metaplasia was not present at this time interval ($p < 0.05$) (Chart 10), and the low- and intermediate-exposure groups were free of significant compound-related lesions.

DISCUSSION

Exposure to 14.3 ppm of formaldehyde for 24 months produced a high incidence of nasal cancer in male and female rats. The tumors exhibited a sharp concentration-response relationship, with the 2 carcinomas in the intermediate-exposure group being identical to the 103 squamous cell carcinomas found in rats exposed to 14.3 ppm of formaldehyde, while none occurred in the 2.0-ppm group or control rats. The spontaneous incidence of nasal neoplasia in age-matched rats is extremely low (30). Although the incidence of polypoid adenomas in the nasal cavity was not statistically significant (adjusted pairwise analysis), there was a positive concentration response for the occurrence of these benign neoplasms in male rats, suggesting that they represent a formaldehyde-enhanced lesion. There was no evidence of progression from polypoid adenoma to squamous cell carcinoma. This is similar to observations of Takano *et al.* (39) who found that 1,4-dinitrosopiperazine-induced papillomas did not progress to adenocarcinomas of the nasal cavity. Rather, a strong correlation existed for progression from nodular hyperplasia to adenocarcinoma. In the present investigation, polypoid adenomas were similar to the papillomas of Takano *et al.*, while squamous hyperplasia and squamous papillary hyperplasia with foci of cellular atypica were the squamoid counterparts of nodular hyperplasia described by Takano *et al.* (39).

The spontaneous incidence of nasal tumors in mice is also extremely low, with one neuroepithelioma and one angiosarcoma being described (35). Two male mice exposed to 14.3 ppm of formaldehyde in our study developed squamous cell carcinomas in the nasal cavity that were similar to neoplasms oserved in rats. This strongly suggests that these tumors in mice resulted from formaldehyde exposure. While the sensitivity of the bioassay in male mice for identifying a carcinogenic end point was lower than in rats due to increased nontumor mortality, the number of male mice surviving at least 18 months does meet the standards recently proposed for evaluation of bioassay data, *i.e.*, 25 mice/group for 18 months (15).

In rats, formaldehyde inhalation was associated with an exposure-dependent increase in the frequency, severity, and dis-

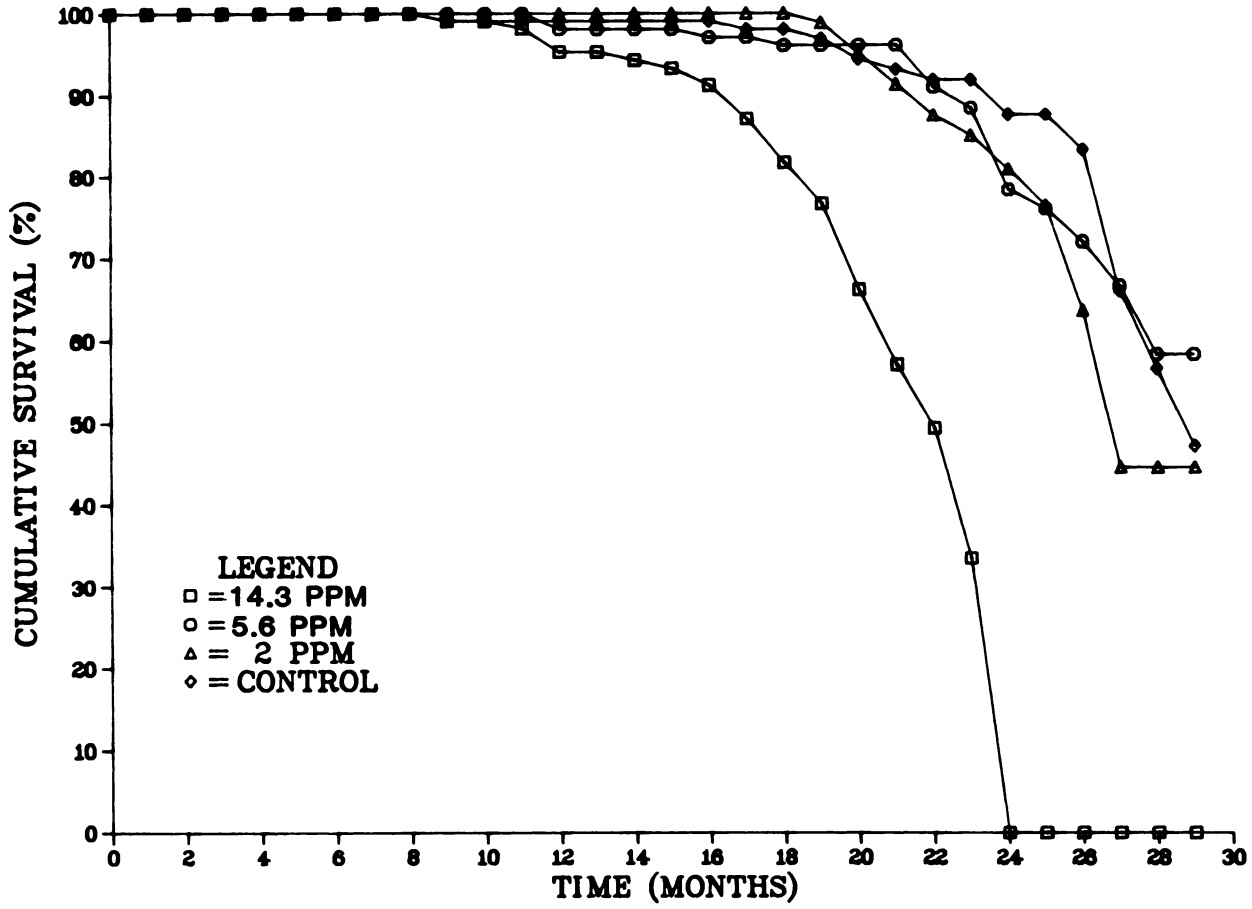

Chart 6. Cumulative survival of female rats exposed to formaldehyde gas.

tribution of rhinitis, dysplasia, and squamous metaplasia of the respiratory epithelium lining the anterior nasal cavity. In contrast to rats, mice exhibited marked irritant-induced effects (rhinitis, dysplasia, and squamous metaplasia) only at the highest exposure level.

Three months after formaldehyde exposure was discontinued in rats, there was regression (frequency and severity) of squamous metaplasia and rhinitis in all affected levels of the nasal cavity in both the low and intermediate exposure groups as well as in Levels IV and V in the high-exposure group. In mice, regression of squamous metaplasia and rhinitis was evident in all affected levels of the nasal cavity in both the intermediate- and the high-exposure groups.

Formaldehyde-induced lesions (squamous metaplasia and inflammation) in mice were much less severe than similar lesions in rats from the same exposure group. Likewise, dramatic differences were apparent in the incidence of squamous cell carcinomas between rats and mice exposed to 14.3 ppm of formaldehyde. It is of interest to note that the incidence of squamous cell carcinoma was similar in mice exposed to 14.3 ppm and rats exposed to 5.6 ppm of formaldehyde. These differences in response between the 2 species may be related to differences in their physiological responses to formaldehyde inhalation. Exposure of mice to 15 ppm of formaldehyde results in a 50% reduction in minute volume, whereas rats exhibit a 20% decrease (8). If the minute volumes for rats and mice are used to calculate the amount of formaldehyde inspired, and this amount is normalized to the surface area of the nasal cavity in accordance with its patterns of deposition, then the "dose" of formaldehyde available for absorption and local toxicity is greater in rats than mice exposed to 14.3 ppm of formaldehyde. For mice, the dose is approximately one-half the amount that rats are exposed to at 14.3 ppm (8, 36).

Additional support for this dose concept is provided by data on the effect of formaldehyde exposure on cell turnover in the nasal cavities of rats and mice (36). A 10- to 20-fold increase in the labeling index of the respiratory epithelium occurs following 3 days of exposure (6 hr/day) to 6 or 15 ppm of formaldehyde in rats, but in mice, this occurred only when they were exposed to 15 ppm. No increase in cell turnover was demonstrable at exposures of 0.5 or 2 ppm in rats or 0.5, 2, or 6 ppm in mice. The increase in cell proliferation represents a compensatory response to formaldehyde toxicity. Microscopically, the response is characterized primarily by replacement of dead cells in rats exposed to 15 ppm and a combination of replacement and epithelial hyperplasia in mice exposed to 15 ppm and rats exposed to 6 ppm. Since formaldehyde is mutagenic and genotoxic (3, 24), increased cell turnover may result in fixation of formaldehyde-DNA damage, resulting in mutations and initiation of neoplastic transformation. Subsequent exposure with increased cell turnover may serve as a promotional event, leading to a high incidence of carcinoma in the nasal cavity. At lower exposure concentrations, host defense mechanisms, such as mucociliary clearance or metabolic detoxification, may reduce the likelihood

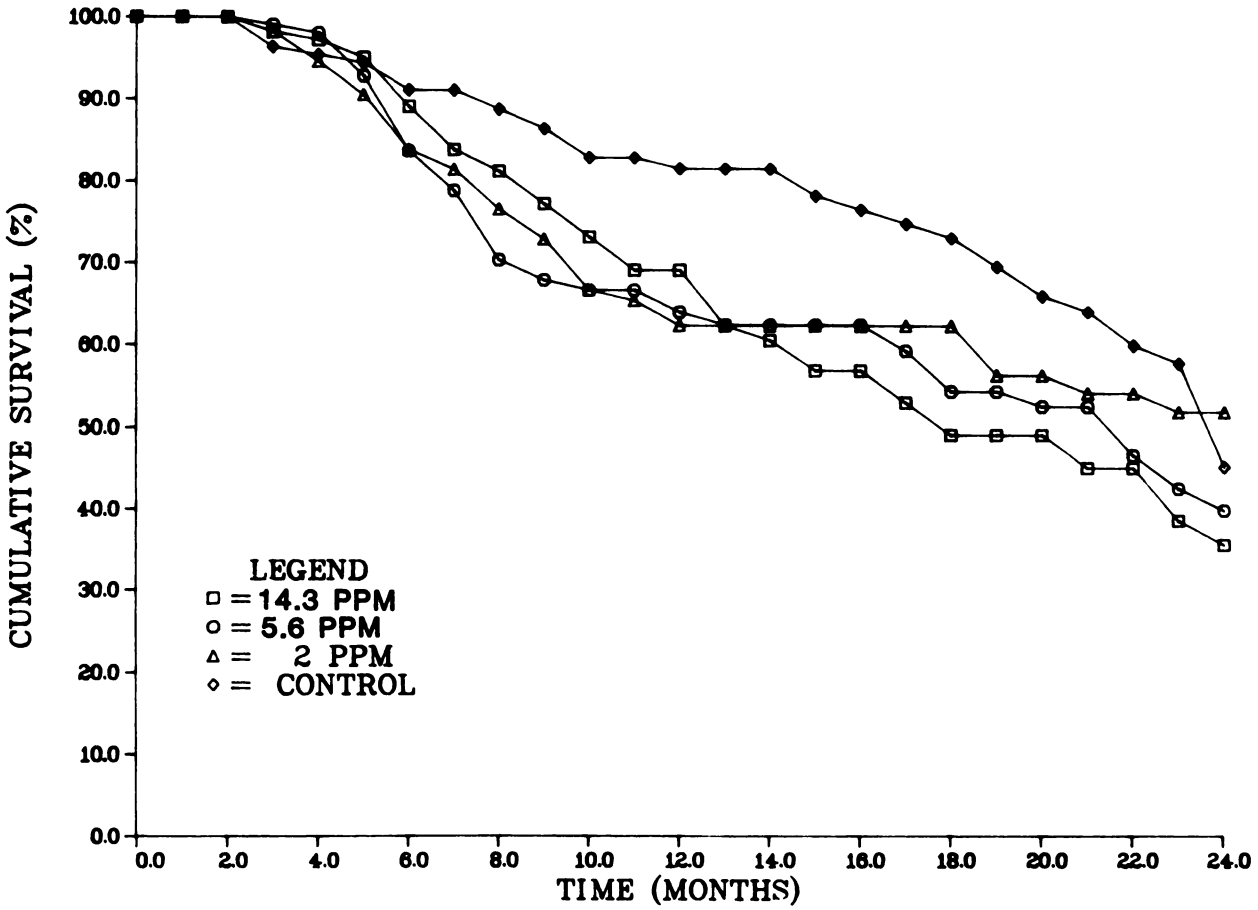

Chart 7. Cumulative survival of male mice exposed to formaldehyde gas.

of such events in a nonlinear fashion. The nasal respiratory epithelium is normally covered by a dynamic protective layer of mucus (31). The moving superficial gel phase contains glycoproteins which may react with formaldehyde and provides a saturable diffusion barrier to the gas. If this reaction occurs with no appreciable decrease in mucus flow rate, the mucus coat could provide an effective barrier for prolonged low-level exposure. Formaldehyde is, however, known to be ciliastatic (1). Preliminary studies utilizing an *in vitro* frog palate preparation have shown that exposures to 15 ppm of formaldehyde result in an initial stimulation of mucus flow, followed by rapid cessation of flow (25). Reduced ciliary beat frequency and ciliastasis occurred subsequent to the complete cessation of mucus flow. Exposures to 2 ppm of formaldehyde caused a slight increase in mucus flow but no cessation or ciliastasis. No effect was demonstrable during exposures to 0.5 ppm of formaldehyde. Thus, exposures to high concentrations for a few hr are likely to cause greater insult than do longer exposures at lower concentrations. Data from recent studies in our laboratory strongly support this concept. Rats exposed to 12 ppm of formaldehyde for 3 hr/day have much more extensive nasal lesions and cell replication than do rats exposed to 3 ppm for 12 hr/day (37).

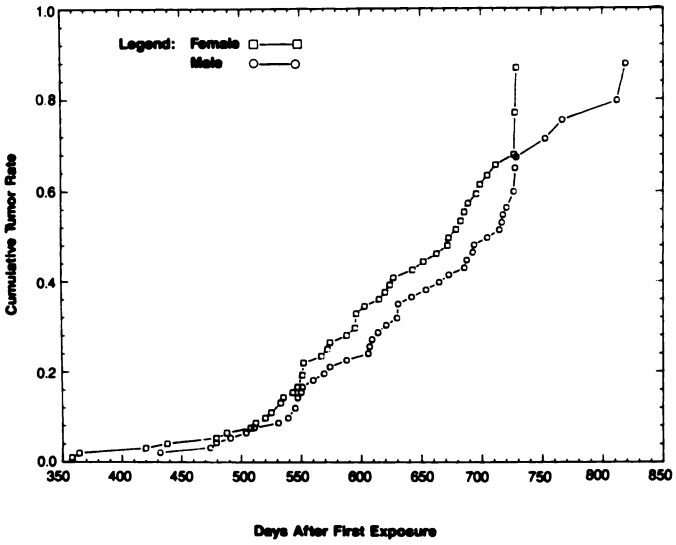

Chart 8. Cumulative incidence rate of squamous cell carcinomas in rats exposed to 14.3 ppm of formaldehyde gas (the Kaplan-Meier life table analysis). Exposure was terminated at 24 months.

The bioassay data reported above, as well as data on cytotoxicity and cell replication (36, 37), represent nonlinear responses to formaldehyde concentration. Such nonlinear responses are believed to result from overloading of host protective mechanisms such as mucociliary clearance, metabolic detoxification, and DNA repair. Hoel *et al.* (18) have suggested recently that, when nonlinear dose-response data are present, risk estimates shoud be based on dose to target site, rather than ambient air concentration. Unfortunately, quantitative data on the *in vitro*

Table 1

Summary of neoplastic lesions in the nasal cavity of Fischer 344 rats exposed to formaldehyde gas

Formaldehyde (ppm)	Sex	No. of nasal cavities evaluated	Squamous cell carcinoma	Nasal carcinoma	Undifferentiated carcinoma or sarcoma	Carcinosarcoma	Polypoid adenoma	Osteochondroma
0	M	118	0	0	0	0	1	1
	F	114	0	0	0	0	0	0
2.0	M	118	0	0	0	0	4	0
	F	118	0	0	0	0	4	0
5.6	M	119	1	0	0	0	6	0
	F	116	1	0	0	0	0	0
14.3	M	117	51	1[a]	2[a]	1	4	0
	F	115	52	1	0	0	1	0

[a] A rat in this group also had a squamous cell carcinoma.

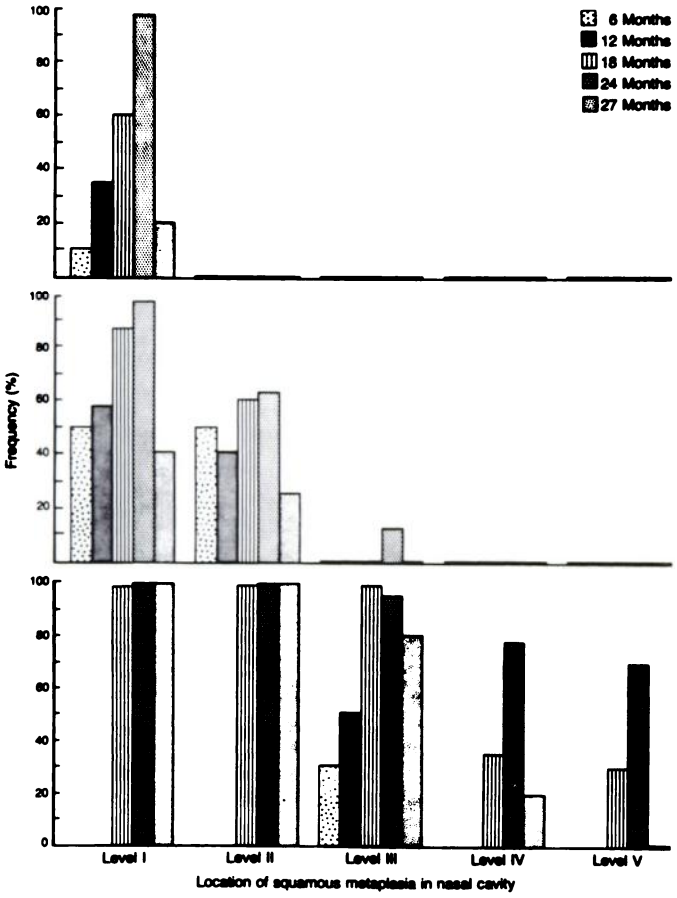

Chart 9. Frequency of squamous metaplasia in the nasal cavity of Fischer 344 rats exposed to 2.0 ppm (*top*), 5.6 ppm (*middle*), or 14.3 ppm (*bottom*) of formaldehyde gas for 24 months. Nasal cavity Levels I, II, IV, and V were not evaluated at the 6- and 12-month interim sacrifices in the 14.3-ppm exposure group.

molecular dosimetry of formaldehyde-induced DNA adducts are not yet available. In humans, where respiration is both oral and nasal, the site and degree of formaldehyde toxicity may be different than in rodents. An assessment of human risk should incorporate a collective evaluation of animal toxicity studies, epidemiology studies, as well as a thorough understanding of the mechanisms involved in the expression of toxicity with formaldehyde.

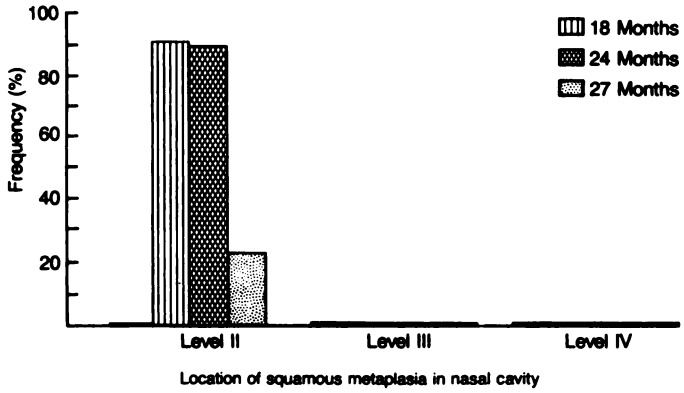

Chart 10. Frequency of squamous metaplasia in the nasal cavity of B6C3F$_1$ mice exposed to 14.3 ppm of formaldehyde gas.

ACKNOWLEDGMENTS

The authors wish to acknowledge the following persons who participated in one or more phases of this study: Daryl C. Thake, Melanie M. Connell, Ralph I. Mitchell, Gerald L. Fisher, Ronald Joiner, Anna D. Barker, Grace M. Henry, Susan L. Icely, Joni K. Holley, Barbara K. Wood, and Jane T. Daniels.

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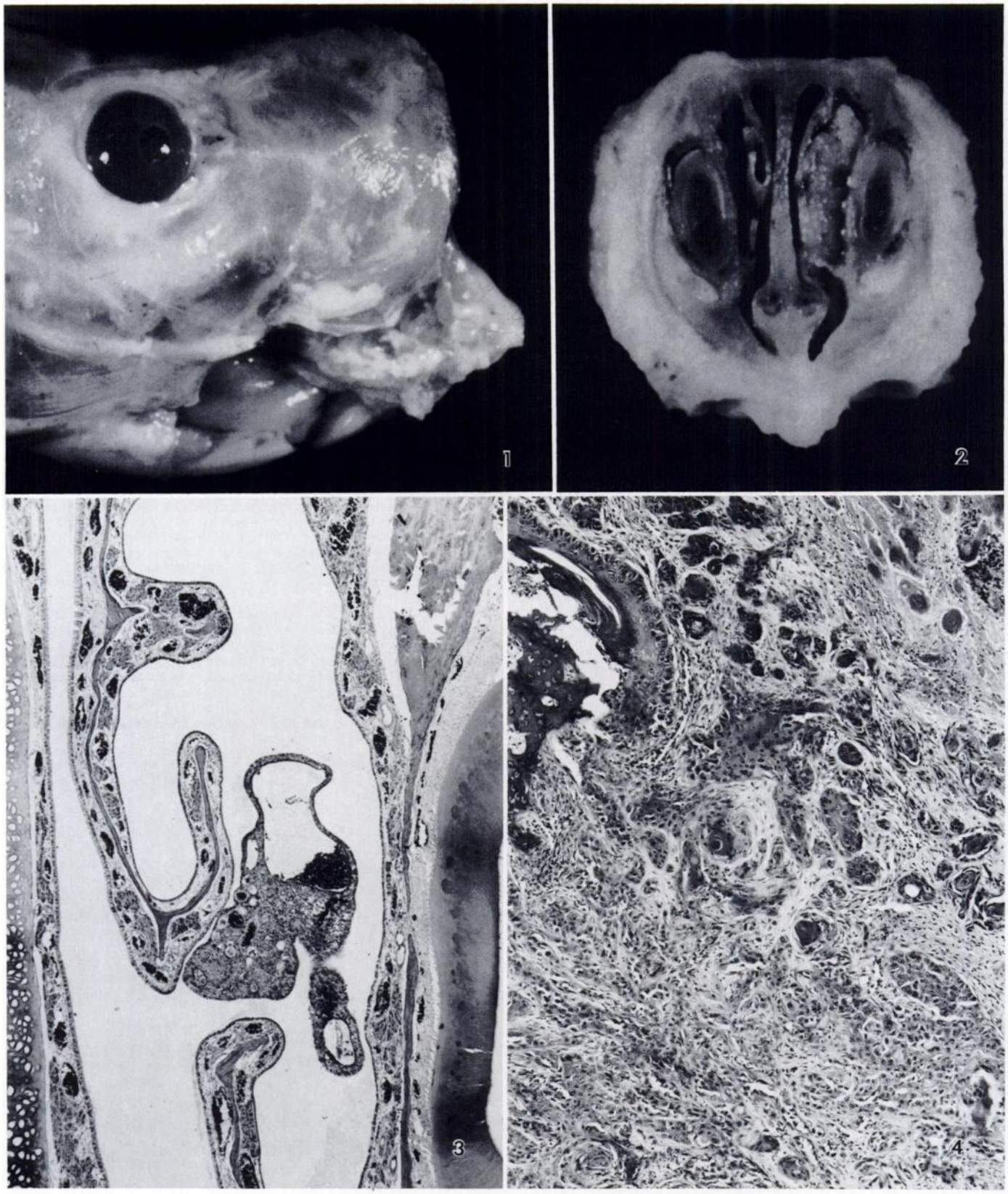

Fig. 1. Gross photograph of a 14.3-ppm formaldehyde-exposed rat bearing a large invasive squamous cell carcinoma of the nasal cavity.
Fig. 2. Cross-section of a decalcified 5.6-ppm formaldehyde-exposed rat with a polypoid adenoma obstructing the right nasal passage.
Fig. 3. Level II. Polypoid adenoma arising in the nasoturbinate of a rat exposed to 5.6-ppm formaldehyde vapor for 24 months. H & E, × 45.
Fig. 4. Level II. Advanced squamous cell carcinoma that has invaded the maxilla of a 14.3-ppm formaldehyde-exposed rat. H & E, × 96.