

Induction of Squamous Cell Carcinomas of the Rat Nasal Cavity by Inhalation Exposure to Formaldehyde Vapor

James A. Swenberg,¹ William D. Kerns, Ralph I. Mitchell, Edward J. Gralla, and Kenneth L. Pavkov

The Chemical Industry Institute of Toxicology [J. A. S., E. J. G.], Research Triangle Park, North Carolina 27709, and Battelle Columbus Laboratories, [W. D. K., R. I. M., K. L. P.], Columbus, Ohio 43201

ABSTRACT

Groups of 120 male and 120 female rats were exposed by inhalation to 0, 2, 6, or 15 ppm formaldehyde vapor 6 hr/day, 5 days/week, for 18 months of a 24-month study. The present communication describes interim findings based on data available after 18 months of exposure. Squamous cell carcinomas occurred in the nasal cavities of 36 rats exposed to 15 ppm formaldehyde. The tumors ranged from small early carcinomas of the nasal turbinate to large invasive osteolytic neoplasms which extended into the subcutis of the premaxilla. Similar tumors were not detected in rats exposed for 18 months to 2 or 6 ppm or in mice exposed to 2, 6, or 15 ppm formaldehyde. Rhinitis, epithelial dysplasia, and squamous metaplasia occurred in rats from all exposure levels of formaldehyde; however, the severity and extent of the lesions were dose related. In contrast, papillary hyperplasia and squamous atypia occurred only in animals exposed to 15 ppm formaldehyde.

INTRODUCTION

Formaldehyde is a commodity chemical with an annual production of over 9 billion pounds in the United States (2). Despite its widespread use, known chemical reactivity, and potential for human exposure, little data are available on its chronic toxicity and potential carcinogenicity. Formaldehyde is known to cause local irritation and skin sensitization following acute and subacute exposure (3). Adequate chronic toxicology and carcinogenicity studies have not been reported. When Sprague-Dawley rats were exposed concurrently to formaldehyde (14.6 ppm) and hydrochloric acid (10.7 ppm), nasal carcinomas developed in 25% of the animals (8). Speculation was presented that the tumors were due to the formation of bis(chloromethyl)ether, an agent shown previously to induce nasal tumors in rats (6). Shooter has suggested, however, that the carcinogenicity of bis(chloromethyl)ether may be due to hydrolysis to formaldehyde (9).

Formaldehyde is mutagenic in some bacteria, fungi, yeast, and *Drosophila* (1) and induces unscheduled DNA synthesis in HeLa cells (7). In addition, formaldehyde causes protein-DNA and DNA-DNA cross-links in *Escherichia coli* (11) and mammalian cells treated *in vitro*.²

In order to assess more thoroughly the chronic toxicity and carcinogenicity of formaldehyde, long-term toxicity and carci-

nogenicity studies utilizing inhalation exposure of rats and mice were initiated. The present communication describes the interim results of the rat study based on data available after 18 months of exposure. A high incidence of squamous cell carcinomas has been diagnosed in the nasal turbinates of rats exposed to 15 ppm formaldehyde for 6 hr/day, 5 days/week. Such tumors have not occurred at 6 or 2 ppm or in mice exposed to 2, 6, or 15 ppm formaldehyde vapor. Exposure-related neoplasia has not been detected in other tissues; however, histopathological examination of all tissues has not been completed. These data and the histopathology of the mouse study will be reported following termination of the experiments.

MATERIALS AND METHODS

Animals. Seven-week-old Fischer 344 rats (Charles River Laboratories, Portage, Mich.) were randomly assigned to 3 exposure groups and one control group, each group contained 120 males and 120 females. The animals were housed individually in stainless steel wire mesh cages, which were transferred to the inhalation chambers during exposure. During nonexposure periods, the animals were housed in animal rooms with food (Purina Lab Chow 5001) and water available *ad libitum*.

Exposure. Rats were exposed to 0, 2, 6, or 15 ppm formaldehyde vapor in 5-cu m Hanners-type test chambers constructed of stainless steel and glass. The environment was maintained at 20–22° and 45 ± 5% humidity, with 12 air changes/hr. Cage positions were rotated one position from top to bottom and left to right each day throughout the exposure period. The test atmosphere was generated by heating solid paraformaldehyde (Aldrich Chemical Co., Inc., Milwaukee, Wis.) to a temperature of 54–82° in a depolymerization chamber and metering this vapor into filtered incoming air. Chamber concentrations were monitored at least every 30 min using an automatic sampling system and a MIRAN-1A portable gas analyzer. Peak absorption was read with this IR spectrophotometer at 3.58- μ m wavelength and slit setting of 1.0 mm. The response time was set at 10 sec, and the sampling flow rate was 10 liters/min. Concurrent analyses of the same samples were examined by the chromatographic acid analytical procedure at least once every 7 to 10 days, to provide a reference standard and to confirm the IR spectrophotometer procedure.

Observations. All animals received caged-side inspections at least twice daily throughout the study for abnormal behavior, signs of toxicity, general physical condition, and mortality. The rats were examined more closely and weighed weekly for the first 6 months and biweekly from 6 to 18 months. Ten animals per sex per exposure level were randomly selected for interim necropsies at 6 and 12 months. This number was raised to 20

¹ To whom requests for reprints should be addressed.

² G. L. Petzold and J. A. Swenberg. The induction of DNA crosslinks in V-79 cells exposed to hexamethylphosphoramide and formaldehyde, manuscript in preparation.

Received April 8, 1980; accepted June 16, 1980.

animals per sex per exposure level for the 18 month necropsy. Rats which died or were found moribund were also subjected to a complete necropsy. Complete gross examinations were performed on all animals, and a standard set of 43 tissues plus all gross lesions were fixed in 10% neutral-buffered formalin. Tissues from the control and 15-ppm animals were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. This included 4 cross-sections of nasal turbinate from animals of all exposure levels that died or were killed through 18 months of exposure.

RESULTS

Measured formaldehyde concentrations during the first 18 months of exposure averaged 14.1, 5.6, 2.1, and 0 ppm on a daily basis as compared to the respective target concentrations of 15, 6, 2, and 0 ppm. Formaldehyde exposure caused a dose-related decrease in body weight in both males and females (Charts 1 and 2). Exposure to formaldehyde also produced a dose-related yellow discoloration of the haircoat. Typical lesions of enzootic sialodacryoadenitis infection were found in animals from all exposure groups at the 12-month necropsy. A transient decrease in body weight was also associated with the infection (Charts 1 and 2). Early mortality was minimal during the first year of formaldehyde exposure. However, a sharp increase in the number of deaths occurred in rats exposed to 15 ppm during the subsequent 6 months (Chart 3). This was primarily related to the development of squamous cell carcinomas in the nasal turbinates (Chart 4). One of the earliest clinical signs of tumor formation was the development of a unilateral ocular discharge. This was frequently followed by a localized swelling over the nasal bones, which progressed to large osteolytic tumors (Fig. 1). In other clinically normal animals, the neoplasms were not detected until the turbinates were grossly sectioned following decalcification. The earliest stages of tumor formation were evident only histologically. Examination of the nasal cavities of rats found dead or killed

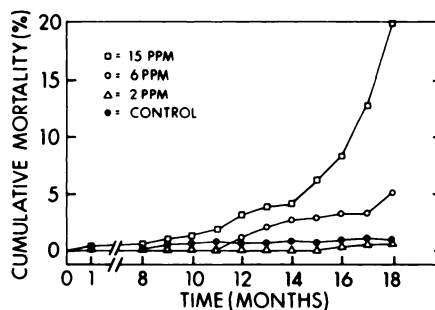


Chart 3. Cumulative mortality of rats exposed to formaldehyde vapor.

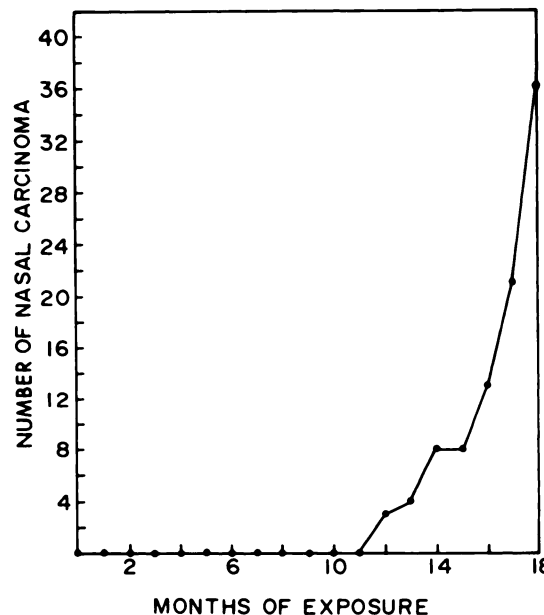


Chart 4. Cumulative number of nasal carcinomas in 200 Fischer 344 rats exposed to 15 ppm formaldehyde vapor.

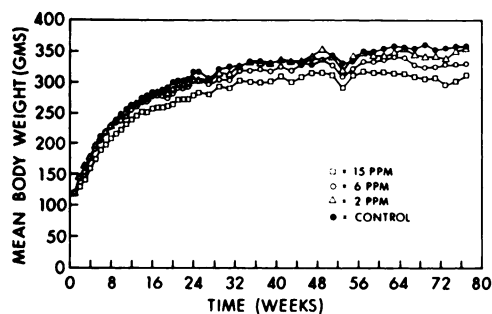


Chart 1. Mean body weights of male rats exposed to formaldehyde vapor.

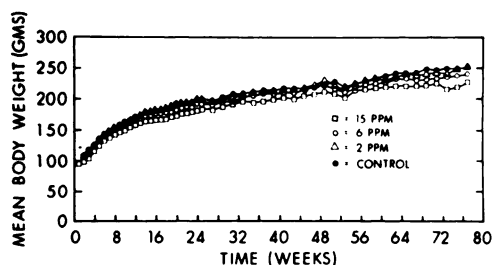


Chart 2. Mean body weights of female rats exposed to formaldehyde vapor.

when moribund revealed a high incidence of neoplasia in animals from the 15-ppm exposure group (Table 1). The neoplasms were usually located on or lateral to the nasal turbinate and the maxilloturbinate (Fig. 2), whereas the ethmoid turbinates were relatively unaffected. Large tumors eroded through the nasal bones and invaded the subcutis. The tumors were characterized by the formation of epithelial pearls, invasive epithelial pegs, a moderate mitotic index, and accumulations of keratin and exudate (Fig. 3). Five of the neoplasms contained areas of basal cell carcinoma, and one was associated with a spindle cell sarcoma. Squamous papillomas developed in 4 rats exposed to 15 ppm formaldehyde. No gross or microscopic squamous cell carcinomas of the nasal turbinates have been detected in rats exposed to 0, 2, or 6 ppm formaldehyde. One rat exposed to 6 ppm developed a squamous cell carcinoma of the facial skin; however, it did not extend into the turbinates.

Histopathology of all tissues from control and from 15 ppm rats sacrificed after 6 and 12 months of exposure revealed that compound-related lesions were restricted to the nasal cavity. These changes included mild to severe mucopurulent rhinitis, epithelial dysplasia, and squamous metaplasia (Fig. 4). Lesions were dose related with respect to both severity and extent of involvement. Similar nonneoplastic lesions were evident in the nasal cavities of rats which died (Table 1) or were sacrificed at

Table 1
Summary of histopathology in the nasal turbinates of rats dying during the first 18 months of exposure to formaldehyde

Exposure level (ppm)	No. of rats examined	Diagnosis									
		Rhinitis, acute suppurative or seropurulent	Osteomalacia	Epithelial dysplasia	Squamous metaplasia	Squamous or epithelial hyperplasia	Squamous papillary hyperplasia	Squamous metaplasia with cellular atypia	Squamous papilloma	Squamous cell carcinoma	Spindle cell sarcoma
0	2	0	0	0	0	0	0	0	0	0	0
2	2	0	0	1	0	0	0	0	0	0	0
6	8	2	0	8	6	0	0	0	0	0	0
15	44	38	1	1	38	11	6	3	4	28	1

Table 2
Summary of histopathology in the nasal turbinates of rats sacrificed after being exposed to formaldehyde for 18 months

Exposure level (ppm)	No. of rats examined	Diagnosis									
		Rhinitis, acute suppurative or seropurulent	Focal turbinate atrophy	Epithelial dysplasia	Squamous metaplasia	Squamous or epithelial hyperplasia	Squamous papillary hyperplasia	Squamous metaplasia with cellular atypia	Adenomatous polyp	Squamous cell carcinoma	
0	40	0	0	0	0	0	0	0	0	0	
2	40	2	0	35	24	0	0	0	1	0	
6	40	7	0	37	35	0	0	0	1	0	
15	40	39	2	13	39	15	4	7	1	8	

18 months (Table 2). The disease had progressed, however, in rats exposed to 15 ppm formaldehyde to include areas of squamous papillary hyperplasia (Fig. 5), cellular atypia (Fig. 6), and squamous cell carcinomas. No papillomas were present in animals killed at the scheduled 6-, 12-, or 18-month sacrifices. One adenomatous polyp was detected in one rat from each of the 2-, 6-, and 15-ppm exposure groups.

By combining the results of Tables 1 and 2, a total of 36 squamous cell carcinomas were detected in the 200 rats exposed to 15 ppm formaldehyde vapor for 18 months, providing an interim incidence of 18%. This number will probably increase during the final 6 months of exposure. The number of rats at risk during this interim period was adjusted to exclude the 40 animals sacrificed at 6 and 12 months. Since 8 carcinomas were detected in the 40 rats sacrificed at 18 months, these animals have been included in the incidence. As stated above, no carcinomas have been detected in 0-, 2-, or 6-ppm exposure groups. Likewise, no other gross or microscopic lesions attributable to formaldehyde exposures have been detected in other tissues. However, histopathological examination of all tissues from 18- and 24-month animals has not been completed. These findings will be reported as they become available.

DISCUSSION

While a full evaluation of the carcinogenicity of formaldehyde vapor must await the completion of this and related studies, the evidence presented demonstrates that exposure of rats to 15 ppm formaldehyde, 5 days/week for 18 months, results in a high incidence of nasal tumors. Presently, the data exhibit a sharp dose response, since similar tumors have not been diagnosed in animals exposed to 2 or 6 ppm formaldehyde

vapor. Distinguishing whether the hyperplastic and metaplastic changes found in all exposure groups represent adaptive responses to a potent irritant, or the first signs of a progressive spectrum leading to overt neoplasia, requires completion of the entire study. Papillary hyperplasia and squamous atypia have occurred only in rats exposed to 15 ppm formaldehyde. The overall incidence of squamous cell carcinomas continues to increase during the final months of exposure. Because of this, final calculations of data such as mean latency time will not be available until the study is complete.

Formaldehyde is known to cause a variety of low-level mutagenic and genotoxic events (1, 7).² Formaldehyde-DNA adducts are thought to involve the formation of labile methylol products, which can be easily removed or further react to form a stable methylene bridge (4). The latter frequently involve amino groups of proteins and DNA. With V-79 cells and alkaline elution, we have shown that these DNA-protein cross-links are repaired within 24 hr and that they are susceptible to proteinase K treatment.² Similar lesions are induced in DNA by hexamethylphosphoramide; however, metabolic activation is required which liberates formaldehyde. Hexamethylphosphoramide also causes squamous cell carcinomas of the rat nasal cavity following inhalation exposure (12).

Current studies underway in our laboratory demonstrate that there is very little cell turnover in the nasal epithelium of control rats and that a marked increase occurs following exposure to 15 ppm formaldehyde vapor. Such increased cell turnover may result in fixation of the rather labile formaldehyde-DNA damage, resulting in mutations and initiation of neoplastic transformation. Subsequent exposure may serve as a promoter, leading to the high incidence of nasal tumors. Similarly, initiating and/or promoting activities of sialodacryoadenitis virus cannot be ruled out, since mice exposed to the same concentrations of

formaldehyde did not develop tumors of the nasal cavity.

In contrast to humans, rats and mice are obligatory nose breathers (5). Thus, the site and degree of formaldehyde toxicity may be modified in humans. For instance, while bis(chloromethyl)ether induces nasal tumors in rats (6), it causes lung tumors in humans (10). Currently, no adequate epidemiological studies exist which address this problem, despite the fact that occupational and environmental exposure to formaldehyde is prevalent. Cogent human risk assessment for formaldehyde exposure can be achieved only by combining extensive and thorough epidemiology, quantitative toxicology, and a better understanding of the mechanisms involved.

REFERENCES

1. Auerbach, C., Moutschen-Dahmen, M., and Moutschen, M. Genetic and cytogenetical effects of formaldehyde and related compounds. *Mutat. Res.*, **39**: 317-362, 1977.
2. *Directory of Chemical Producers: United States of America* (1979). pp. 637-638. SRI International, Menlo Park, 1979.
3. Fassett, D. W. Aldehydes and acetals. *In*: F. A. Patty, *Industrial Hygiene and Toxicology*. II. 2nd Rev. Ed., (ed), pp. 1959-1984. New York: John Wiley & Sons, Inc., 1963.
4. Feldman, M. Y. Reactions of formaldehyde with bases, nucleosides and nucleotides. *Prog. Nucleic Acid Res. Mol. Biol.*, **13**: 1-48, 1973.
5. Hatch, T. F., and Gross, P. Experimental studies on deposition of inhaled aerosols. *In*: *Pulmonary Deposition and Retention of Inhaled Aerosols*, pp. 45-68. New York: Academic Press, Inc., 1964.
6. Kuschner, M., Laskin, S., Drew, R. T., Capiello, V., and Nelson, N. The inhalation carcinogenicity of alpha halo ethers. III. Lifetime and limited period inhalation studies with bis(chloromethyl)ether at 0.1 ppm. *Arch. Environ. Health*, **30**: 73-77, 1975.
7. Martin, C. N., McDermid, A. C., and Garner, R. C. Testing of known carcinogens and noncarcinogens for their ability to induce unscheduled DNA synthesis in HeLa cells. *Cancer Res.*, **38**: 2621-2627, 1978.
8. Sellakumar, A. R., Albert, R. E., Rusch, G. M., Katz, G. V., Nelson, N., and Kuschner, M. Inhalation carcinogenicity of formaldehyde and hydrogen chloride in rats. *Proc. Am. Assoc. Cancer Res.*, **21**: 106, 1980.
9. Shooter, K. V. Assays for phosphotriester formation in the reaction of bacteriophage R17 with a group of alkylating agents. *Chem.-Biol. Interact.*, **11**: 575-588, 1975.
10. Weiss, W., Moser, R. L., and Auerbach, O. Lung cancer in chloromethyl ether workers. *Am. Rev. Respir. Dis.*, **120**: 1031-1037, 1979.
11. Wilkins, R. J., and MacLeod, H. D. Formaldehyde induced DNA-protein crosslinks in *Escherichia coli*. *Mutat. Res.*, **36**: 11-16, 1976.
12. Zapp, J. A. HMPA: a possible carcinogen. *Science (Wash. D. C.)*, **190**: 422, 1975.

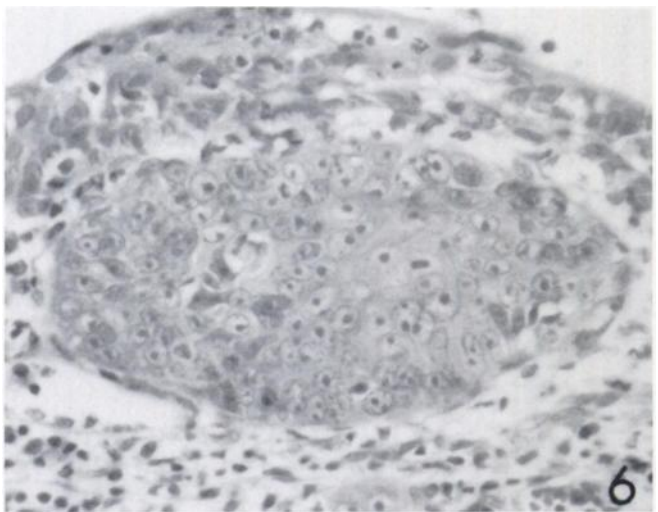
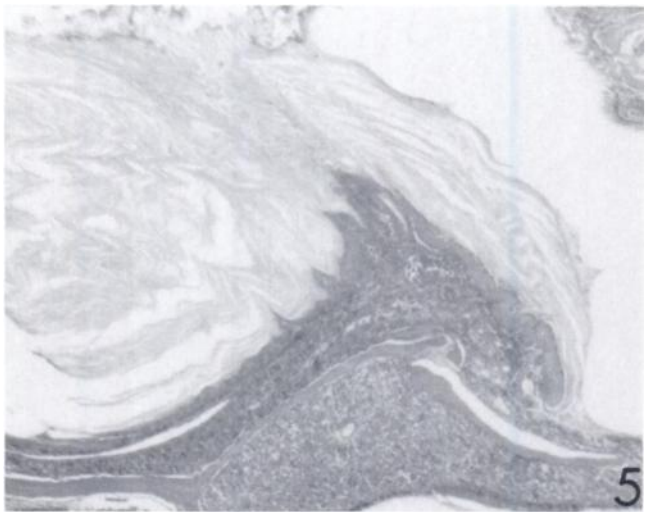
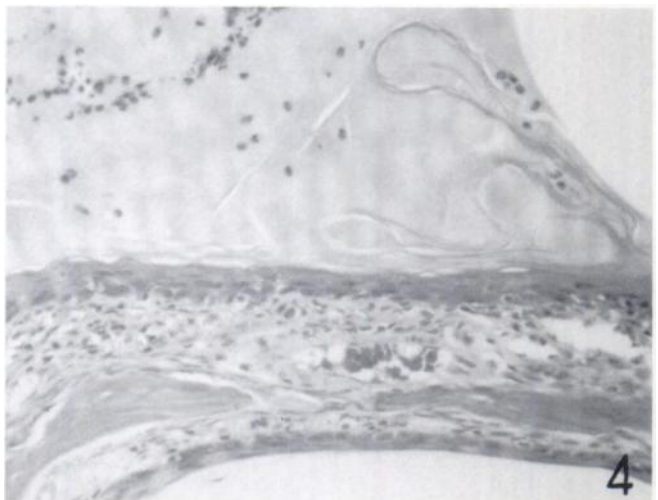
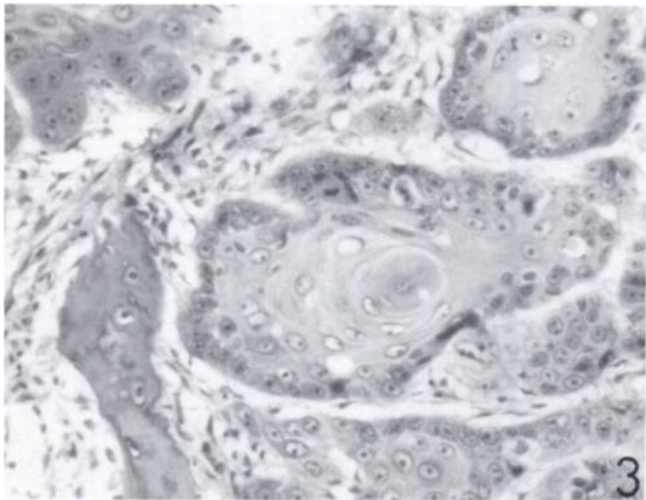
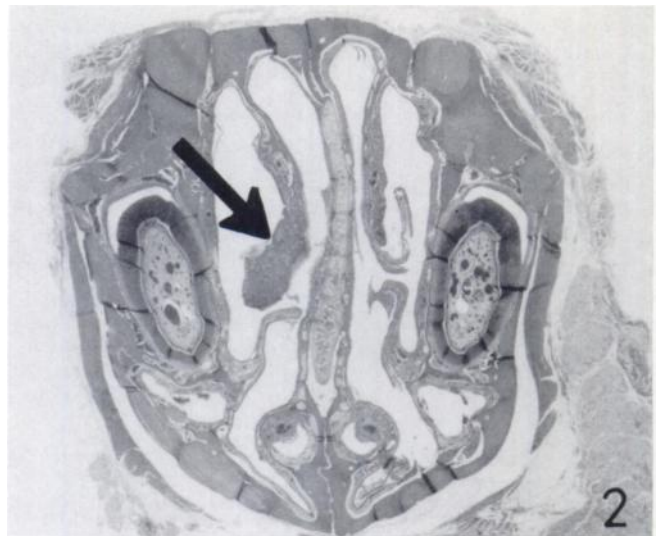


Fig. 1. Gross photograph of a 15-ppm formaldehyde-exposed rat bearing a large squamous cell carcinoma of the nasal cavity which extends into the subcutis over the premaxilla.
 Fig. 2. Macrophotograph of an early squamous cell carcinoma arising in the nasoturbinates (arrow) of a rat exposed to 15 ppm formaldehyde vapor. H & E, $\times 9$.
 Fig. 3. Invasive squamous cell carcinoma exhibiting early epithelial pearls and occasional mitoses. H & E, $\times 440$.
 Fig. 4. Squamous metaplasia of the nasoturbinates with accumulation of keratin and exudate in a rat exposed to 6 ppm formaldehyde for 18 months. H & E, $\times 160$.
 Fig. 5. Squamous papillary hyperplasia on the maxilloturbinate of a rat that was exposed to 15 ppm formaldehyde vapor. H & E, $\times 43$.
 Fig. 6. Nest of cellular atypia in the submucosa of the median septum of a rat exposed to 15 ppm formaldehyde. H & E, $\times 440$.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Induction of Squamous Cell Carcinomas of the Rat Nasal Cavity by Inhalation Exposure to Formaldehyde Vapor

James A. Swenberg, William D. Kerns, Ralph I. Mitchell, et al.

Cancer Res 1980;40:3398-3402.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/40/9/3398>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link <http://cancerres.aacrjournals.org/content/40/9/3398>. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.