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THE MECHANISTIC TOXICOLOGY OF FORMALDEHYDE AND ITS IMPLICATIONS FOR QUANTITATIVE RISK ESTIMATION

Thomas B. Starr and James E. Gibson

Chemical Industry Institute of Toxicology, P. O. Box 12137, Research Triangle Park, North Carolina 27709

INTRODUCTION

In 1980 the Chemical Industry Institute of Toxicology (CIIT) issued preliminary results from a chronic toxicity and carcinogenicity study of inhaled formaldehyde in rats and mice. These results, which pertained to 18 months of formaldehyde exposure, indicated that formaldehyde is carcinogenic in rats (1). A final report published in 1983 (2) confirmed the preliminary findings. Specifically, inhalation exposure to formaldehyde concentrations of 5.6 or 14.3 ppm, six hours per day, five days per week, for 24 months caused squamous cell carcinomas in the nasal cavities of approximately 50% of the rats in the high-exposure group and 1% of the rats in the low-exposure group (5.6 ppm). Mice also proved susceptible to formaldehyde's carcinogenicity, but tumors were observed only at the highest exposure concentration (14.3 ppm) and with an incidence of 1%.

Because formaldehyde is a major building-block chemical used in a multitude of industrial processes and consumer products, these findings have provoked widespread concern about the possible effects of formaldehyde exposure on human health (3, 4). In 1983, for example, the estimated US production of

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formaldehyde as a 37% solution was 5.7 billion pounds (5). Formaldehyde's major end uses include adhesives (60%) and plastics (15%), with the main derivatives being urea-formaldehyde resins, phenol-formaldehyde resins, polyacetal, and butanediol. Formaldehyde-derived resins are used primarily in manufacturing particleboard, plywood, insulation, appliances, and automobiles, and residual formaldehyde vapors are known to off-gas from some of these products. The potential for both occupational and environmental exposures to this chemical is thus considerable.

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In order to assess adequately the cancer risk from low-level formaldehyde exposures, critical issues of mechanism must be considered in addition to the basic finding that formaldehyde is carcinogenic in rats and mice. Two important mechanistic factors are the physiologic responses to sensory irritation produced by formaldehyde exposure and the effects of such exposure on the mucociliary clearance apparatus of the nasal cavity. These factors control the local rate of delivery of airborne formaldehyde to underlying target tissues. Also critical are the cellular proliferation response to formaldehyde-induced cytotoxicity and the disposition of delivered formaldehyde within target cells via metabolism and macromolecular binding. All four of these mechanistic aspects of formaldehyde toxicology are directly relevant to both the low-dose and interspecies risk extrapolation problems because they determine the form of the functional relationship between the concentration of formaldehyde in ambient air and the amount of formaldehyde that ultimately reaches and interacts with the genetic material of target cells. When such mechanistic information is incorporated properly into quantitative dose-response models, more accurate and scientifically defensible estimates of low-dose cancer risk should result.

Following a brief summary of the major findings of the CIIT chronic inhalation bioassay, this review focuses on results from selected mechanistic studies that appear to be directly applicable to the problem of assessing carcinogenic risk from exposure to gaseous formaldehyde. The concluding section illustrates how the data regarding biological defense mechanisms can be incorporated into the quantitative risk assessment process.

A SUMMARY OF THE CIIT CHRONIC FORMALDEHYDE INHALATION BIOASSAY FINDINGS

The Chemical Industry Institute of Toxicology commissioned the Battelle Memorial Institute, Columbus Laboratory, in Columbus, Ohio, to undertake a 24-month toxicity and carcinogenicity study of inhaled formaldehyde in male and female B6C3F1 mice and Fischer-344 rats. One hundred-twenty animals of each sex and species began inhalation exposure to formaldehyde at target concentrations of 0, 2, 6, and 15 ppm six hours per day, five days per week.

The mean formaldehyde concentrations in the test chambers over the 24-month exposure period were: 2.0 ± 0.6, 5.6 ± 1.2, and 14.3 ± 2.8 ppm. Interim necropsies of randomly selected animals were completed at 6, 12, 18, and 24 months after exposure commenced. Some female mice and male and female rats were followed for an additional three-to-six months after completion of the planned 24-month exposures.

The major toxicological finding from this study was the induction of squamous cell carcinomas in the nasal cavities of 103 rats in the 14.3-ppm group, two rats in the 5.6-ppm group, and two male mice in the 14.3-ppm group (Table 1). Two nasal carcinomas, one carcinosarcoma, one undifferentiated carcinoma, and one undifferentiated sarcoma were also observed in rats in the 14.3-ppm exposure group. In addition, an exposure-related induction of squamous metaplasia was found in the respiratory epithelium of the anterior nasal passages of rats in all formaldehyde-exposed groups (2). In mice, however, the irritant-induced effects were essentially limited to the group exposed to 15 ppm and no effects were observed at lower concentrations. The animals from the low and intermediate exposure groups that were allowed to recover after 24 months of formaldehyde exposure showed an apparent regression of metaplasia in all affected sites of the nasal cavity (2).

A number of polypoid adenomas were also observed in the nasal cavities of exposed and control rats (Table 1). Although the incidence of these benign lesions in the exposed animals was not significantly elevated over that in controls in an adjusted pairwise analysis, an adjusted trend test indicated that formaldehyde exposure increases the frequency of this lesion (2). However, the observed incidence of adenomas did not increase as a function of airborne formaldehyde concentration. Adenomas were found in 3.4, 2.6, and 2.2% of the animals exposed to 2.0, 5.6, and 14.3 ppm formaldehyde respectively. This compares to an incidence of 0.4% (one case) in control animals. An independent review of these findings (Pathology Working Group, unpublished observations) yielded a consensus among participating pathologists regarding the benign nature of the observed polypoid adenomas. Moreover, these reviewers concluded that "there was no morphological evidence that these lesions progressed to squamous cell carcinomas." The squamous cell carcinomas and polypoid adenomas were thought to be readily separable lesions that should not be combined solely for statistical and risk estimation purposes.

PHYSIOLOGIC RESPONSES TO SENSORY IRRITATION

The respiratory tract membranes contain a wide variety of sensory nerve endings that can respond to chemical and/or physical stimuli. One group of these nerve endings, located in the nasal mucosa, is associated with the maxillary and ophthalmic divisions of the trigeminal nerve. Stimulation of

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Table 1 Summary of neoplastic lesions in the nasal cavity of Fischer-344 rats exposed to formaldehyde gas[a]

Exposure group	0 ppm		2.0 ppm		5.6 ppm		14.3 ppm	
Sex	M	F	M	F	M	F	M	F
Diagnosis / Number of nasal cavities evaluated	118	114	118	118	119	116	117	115
Squamous cell carcinoma	0	0	0	0	[redacted]	1	51	52
Nasal carcinoma	0	0	0	0	0	0	1[b]	1
Undifferentiated carcinoma/sarcoma	0	0	0	0	0	0	2[b]	0
Carcinosarcoma	0	0	0	0	0	0	1	0
Polypoid adenoma	[redacted]	0	4	4	6	0	4	1
Osteochondroma	[redacted]	0	0	0	0	0	0	0

[a] Adapted from (16).
[b] One rat in this group also had a squamous cell carcinoma.

these nerve endings by airborne chemical irritants such as formaldehyde evokes a painful burning sensation, a desire to withdraw from the contaminated atmosphere, and a decrease in the rate of respiration (6). Collectively, these responses to sensory irritation have been termed the common chemical sense, separating them from more specialized chemical senses such as olfaction and gustation (7). All substances that excite the common chemical sense are potentially noxious and lung damaging, and the reflex responses to sensory irritation comprise an important respiratory tract defense mechanism that serves to minimize the inhalation of noxious agents and warn of their presence through the perception of pain (6, 8, 9).

Decreases in respiratory rate during inhalation exposure can be used to quantitate the sensory irritation potential of chemicals (6). For formaldehyde, this property is well established in laboratory animals (10–12). Experimental results indicate that mice are far more sensitive to formaldehyde exposure than rats. For example, the formaldehyde concentration required to elicit a 50% decrease in respiratory rate (RD_{50}) in naive animals is 3.13 ppm for Swiss-Webster mice (10) and 4.9 ppm for B6C3F1 mice (12) but is 31.7 ppm for Fischer-344 rats (12). Chang and colleagues have shown that associated changes in tidal volume do not compensate entirely for the decreased respiratory rate in both rats and mice (12). As a result, minute volumes (the product of respiratory rate and tidal volume) for naive animals of these species decrease during exposure to sufficiently high formaldehyde concentrations.

Significant differences between rats and mice in the magnitude of their response to sensory irritation have also been observed following pretreatment with four six-hour-per-day exposures to 2, 6, and 15 ppm formaldehyde (12). While respiration was depressed in a concentration-dependent manner in both species, the amplitude of the response was always greater in mice. This can be interpreted as a shift in the concentration-response curves for mice relative to those for rats to lower concentration ranges, with RD_{50} values ranging from 2–6 ppm in the mice compared to 23–32 ppm in rats. Minute volumes at the RD_{50} values are also decreased by approximately 50%. However, it is notable that rats pretreated to 15 ppm did have some degree of tidal volume compensation, as evidenced by a decrease of only 37.5% in minute volume following pretreatment to 15 ppm formaldehyde (12). These results suggest that the B6C3F1 mouse respiratory tract may be better protected against chronic exposure to high airborne formaldehyde concentrations than is the respiratory tract of the Fischer-344 rat.

Testing this hypothesis of differential protective capability in these two species has been possible because the formaldehyde toxicity induced by chronic inhalation exposure appears to be limited to the nasal cavity (1, 2). The localization of toxicity is due in large part to the high-water solubility of formaldehyde and the fact that rodents are obligatory nose breathers. Assuming

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that all inhaled formaldehyde is deposited in the nasal cavity, a theoretical deposition rate on the mucosal surface can be obtained by dividing the amount of formaldehyde inhaled per unit time by the nasal cavity surface area. For a ten-minute exposure to 15 ppm formaldehyde, this deposition rate for rats is twofold larger than that for mice (13, 14). Although the disparity between these species in the calculated deposition rate is consistent with the parallel disparity in nasal tumor incidence following chronic exposure (2), additional studies were required to determine whether or not this difference in delivered dose is maintained during chronic exposure conditions.

The persistence of this effect has been evaluated in rats and mice pretreated to 6 and 15 ppm formaldehyde six hours per day for four days (15). On the fifth day, minute volume was measured during a six-hour exposure to 6 and 15 ppm. During exposure to 15 ppm formaldehyde, the time-weighted average deposition rate for mice continued to be approximately half that for rats, while at 6 ppm both species appeared to receive similar delivered doses.

The species difference predicted at 15 ppm was also assessed by comparative autoradiography, histopathology, and cell turnover studies (15). Whole-body autoradiographic studies of rats and mice exposed to [^{14}C]formaldehyde revealed that radioactivity was heavily deposited in the anterior nasal cavity, with much less deposition in olfactory regions. This anterior-posterior gradient is consistent with the high-water solubility and chemical reactivity of formaldehyde. In addition, whole-body autoradiography qualitatively confirmed the difference in delivered dose in rats compared with mice identically exposed to 15 ppm formaldehyde (15). Histopathologic examination of the nasal cavities of rats and mice after one and five days of exposure to 15 ppm formaldehyde demonstrated that rats had more severe lesions than mice. Cell turnover studies of nasal respiratory epithelium have also revealed much higher cell proliferation in rats compared with that observed in mice (15). When comparing the responses of different species to the same airborne concentration of formaldehyde, it is therefore essential to adjust for the interspecies differences in pulmonary ventilation and nasal cavity surface area.

The effects of exposure on pulmonary ventilation must also be considered when comparing the responses of a single species to different airborne concentrations of a sensory irritant. This is particularly important when estimating the shape of the dose-response curve for risk assessment purposes. For example, rats exposed to 15 ppm formaldehyde for six hours inhale only twice the amount of formaldehyde per unit time as do rats similarly exposed to 6 ppm (16). This mildly nonlinear relationship between the inhalation rate for rats and the concentration of formaldehyde in ambient air is due to the larger depression of minute volume induced in rats by exposure to 15 ppm formaldehyde relative to that induced by exposure to 6 ppm (16). Thus, the precipitously steep rise in squamous cell carcinoma incidence from 1% among rats chronically exposed to

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5.6 ppm formaldehyde to nearly 50% among rats similarly exposed to 14.3 ppm (Table 1) actually appears even steeper, i.e. more severely nonlinear, when the amount of formaldehyde inhaled per unit time, rather than ambient air concentration, is used as the measure of exposure.

THE EFFECTS OF EXPOSURE ON MUCOCILIARY FUNCTION

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Many factors are expected to influence the distribution of lesions induced by irritant materials in the nasal passages (17). These include species-specific anatomy and physiology, nasal cavity airflow dynamics, mucociliary flow rate and direction, as well as exposure level and tissue-specific susceptibility. The amount of airborne formaldehyde that reaches the surface of the nasal epithelium is clearly dependent on its ambient air concentration and the rate at which inspired air passes through the nose. Furthermore, airflow patterns within the nose modulate the amount of gas reaching specific areas, while the nature and movement of the surface secretions are likely to affect absorption of formaldehyde and its subsequent fate.

As noted previously, chronic exposure to high concentrations of formaldehyde induced squamous cell carcinomas in the nasal passages of rats, and exposure was also weakly associated with an increased incidence of polypoid adenomas (2). Because detailed mapping of the locations of these neoplasms within the nasal passages was not attempted during the original chronic inhalation study (1, 2), the histologic sections of this bioassay were reexamined to determine the locations and apparent site of origin of the observed tumors (17a).

The majority of the squamous cell carcinomas occurred in two main locations. The first region is lateral to the nasoturbinate, extending from the ventral margin of this turbinate to the lateral wall just dorsal to the maxilloturbinate, at a level measured along the long axis of the nose just posterior to the incisor tooth. The second region is comprised of the ventral and middle nasal septum approximately at the level of the incisive papilla. In contrast, polypoid adenomas occurred just posterior to the vestibule, on the naso- and maxilloturbinates and the adjacent lateral wall. These three regions of the nose are lined with respiratory epithelium, which is protected by the nasal mucociliary apparatus (18). Because of the potential importance of the mucociliary apparatus in modulating the delivery of formaldehyde to target cells in the nasal passages, studies of the effects of formaldehyde on the mucociliary apparatus have also been undertaken. The current state of knowledge about this system and the results of some initial studies are summarized below.

The nasal mucociliary apparatus of the rat provides a continuous layer of watery mucus that covers the respiratory epithelium (19, 20). Formaldehyde

would be expected to dissolve readily in this layer and thus be removed from the inspired airstream. It has been demonstrated that almost 100% of inspired gaseous formaldehyde is removed in the upper airways in dogs (21), but the retention efficiency of the rat nose for formaldehyde has yet to be established directly. The approximate thickness of the mucus (20), its flow patterns (20, 22), and its flow rate (20) have been determined in the rat nose. The layer of nasal mucus flows continuously over the surface of the nasal mucosa and is cleared eventually toward the nasopharynx (20, 22), where it is swallowed with any entrapped or dissolved materials. Recent studies have demonstrated that the nasal and tracheal mucociliary clearance mechanisms in the guinea pig can respond to formaldehyde exposure with an increased rate of clearance (23). If mucus clearance does result in the removal of formaldehyde from the nose, then increased clearance rates in response to exposure would be expected to increase the efficiency of this potentially protective mechanism.

However, exposure to sufficiently high formaldehyde concentrations is also known to have inhibitory effects upon mucociliary function. The mucociliary apparatus consists of several main components that have been reviewed in detail elsewhere (18). Each component may be influenced adversely by exposure to formaldehyde. Cilia, which are microscopic, hair-like processes of the epithelial cells, drive the mucus over the surface by their coordinated beating. Formaldehyde has been found to be ciliastatic in several species (19), and it causes slowing of mucus flow in the anterior nasal passages of humans during inhalation exposure (24). Studies with a frog palate preparation indicate that formaldehyde induces slowing of mucus flow before it inhibits ciliary activity, probably as a result of biochemical reactions with constituents of the mucus blanket (25). Nasal mucus consists principally of water (~95%), mucus glycoproteins (0.5–1%), free proteins and salts, and other materials in much smaller amounts (25a). Formaldehyde reacts readily with proteins (25b) and with polysaccharides at room temperature (25c). Similar reactions may be responsible for the slowing of mucus flow in humans.

At high concentrations, formaldehyde has been found to induce both mucostasis and ciliastasis in rats following in vivo inhalation exposure (19). Studies with rats using a rapid postmortem assessment of nasal mucociliary function following inhalation exposures to formaldehyde revealed a clear concentration-response relationship for the inhibition of nasal mucociliary function, with 0.5 ppm being a no-observed-effect concentration (26). This concentration-response relationship closely parallels that for formaldehyde-induced lesions observed in the chronic inhalation study (2). It therefore has been postulated that localized disruption of mucociliary function accounts in part for the subsequent appearance of epithelial lesions in the affected locations (16).

The areas of inhibition of mucociliary function and acute cytotoxicity in the nasal epithelium (20) include the regions in which squamous cell carcinomas

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occurred. However, acute changes also appeared consistently on the medial aspect of the maxilloturbinate at 6 and 15 ppm (20, 26). The latter region was rarely a site of squamous cell carcinoma development, indicating possible regional differences in the susceptibility of the rat nasal epithelium to the carcinogenic effects of formaldehyde.

In the chronic inhalation study (2), the polypoid adenomas occurred in the anterior nasal passages, a region lined by sparsely ciliated respiratory epithelium with generally very slow mucus flow rates (20). Mucus in this region is derived from mucus streams that originate in more dorsal or more posterior regions of the nose. Mucus flow results in the translocation of materials deposited on the surface and may thus influence the final site at which the nasal epithelium is exposed to inspired materials. Formaldehyde absorbed more posteriorly might be carried forward toward the point at which the polypoid adenomas occurred. Macklin has proposed that mucus flow patterns in the lower respiratory tract account for the distribution of air-pollutant-induced cancer in the trachea and bronchi of humans (27), and similar reasoning may be applicable to the distribution of formaldehyde-induced lesions in the rat nasal cavity.

Mucus flow may thus play an important role in determining the distribution and frequency of neoplasia in the nasal passages of rats exposed to formaldehyde by modulating delivery of this chemical to the nasal epithelium in specific regions of the nose. However, anatomic or physiologic characteristics of the rat nose may also render this species either hyper- or hyposensitive to formaldehyde-induced nasal cancer. Thus, comparative studies in rats and humans of nasal air flow, mucus flow, other physiologic characteristics, and their effects on the delivered dose will provide additional information valuable for risk-assessment purposes.

THE CELL PROLIFERATION RESPONSE TO TISSUE INJURY

Cell proliferation is a critical factor in chemical carcinogenesis. Numerous studies with a broad range of chemicals have demonstrated that cell replication is required for the initiation and promotion of chemical carcinogenesis. When promutagenic DNA adducts are present during de novo DNA synthesis, the likelihood of inserting a wrong nucleotide greatly increases, and such events, if unrepaired before replication, result in permanent mutations. Cell proliferation is also responsible for expanding the clonal population of initiated cells to a cancerous mass. Furthermore, in the case of formaldehyde-induced neoplasia, cell replication is thought to be important in the initial binding of the chemical to DNA, since formaldehyde is known to bind only to single-stranded DNA (28, 29). The number of single-stranded sites is much greater in replicating

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DNA than in non-replicating DNA. Thus, the likelihood of formaldehyde binding to DNA, errant DNA synthesis, and expansion of initiated cell populations to neoplasia are all related to cell proliferation.

Morphologic changes in the nasal respiratory mucosa of Fischer-344 rats have been observed following a single six-hour exposure to 15 ppm formaldehyde gas. These changes consisted of acute degeneration and swelling, with the formation of dense bodies and vacuoles within epithelial cells (15, 30). Three to five similar exposures (one per day) produced ulceration of the respiratory epithelium in a high proportion of the animals. After nine days of exposure, restorative hyperplasia and metaplasia were observed. Rats exposed to 6 ppm exhibited milder degenerative changes but prominent hyperplasia of the respiratory epithelium. In contrast, no morphologic changes were evident by light microscopy in rats exposed to 0.5 or 2 ppm formaldehyde.

In order to better understand these cytotoxic and restorative responses to different concentrations and durations of formaldehyde exposure, a series of investigations was undertaken to identify and elucidate the effects of formaldehyde exposure on cell replication in the respiratory epithelium of rodent nasal passages. Initial studies (31) have demonstrated that marked increases in cell proliferation were present in the second level of the nasal passages, the same region that had the acute pathology and that developed most of the squamous cell carcinomas in the CIIT bioassay (2). Rats exposed to 6 or 15 ppm formaldehyde for three six-hour-per-day exposures had ten to twenty fold increases in [^{3}H]thymidine labeling. No increase over controls was detected in rats exposed to 0.5 or 2 ppm nor in mice exposed to 0.5, 2, or 6 ppm formaldehyde. Mice exposed to 15 ppm formaldehyde showed a tenfold increase in de novo DNA synthesis. Subsequent studies have shown that administration of [^{3}H]thymidine at 18 rather than two hours after the last exposure is a more sensitive method for evaluating the effects of formaldehyde on cell proliferation (31). Rats exposed to 15 ppm formaldehyde for a single six-hour period already showed a greater than tenfold increase in cell proliferation relative to controls. By five days of exposure, this increase exceeded twentyfold. Similar increases have been demonstrated in mice.

It is important to know how much of this response is due to the duration of exposure and how much is due to formaldehyde concentration, since markedly different results were evident in the histopathology of rats exposed for six months to 15 ppm formaldehyde, six hours per day, five days per week, (450 ppm-hours per week) (1, 2, 32) compared to that of rats exposed to 3 ppm, 22 hours per day, seven days per week (462 ppm-hours per week) (33). Animals on the latter exposure regimen exhibited much less toxicity. To evaluate this discrepancy, rats and mice were exposed to 12 ppm formaldehyde for three hours per day, 6 ppm for six hours per day, or 3 ppm for twelve hours per day (31). Exposures were conducted for three and ten days, with [^{3}H]thymidine

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Table 2 Effects of formaldehyde concentration versus cumulative exposure on cell turnover in Fischer-344 rats[a]

	Percent labeled cells[b]		
	Level 1	Level 2	
Exposure	3 days	3 days	10 days
Control	3.00 ± 1.56	0.54 ± 0.03	0.26 ± 0.02
3 ppm × 12 hours	16.99 ± 1.50	1.73 ± 0.63	0.49 ± 0.19
6 ppm × 6 hours	15.46 ± 10.01	3.07 ± 1.09	0.53 ± 0.20
12 ppm × 3 hours	16.49 ± 2.02	9.00 ± 0.88	1.73 ± 0.65

[a] Adapted from (31).
[b] Mean ± standard error.

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administered 18 hours after the last exposure. Sections from the most anterior and the second level of respiratory mucosa were prepared for autoradiography and the labeling index was determined. Table 2 shows that the most anterior level of the respiratory epithelium (level 1) had a similar fivefold increase in cell proliferation in all three concentration-time groups. This is in marked contrast to the adjacent, more posterior section (level 2), where a distinct relationship between concentration and cell turnover is evident. In this section, the marked increase in proliferation is also a somewhat transient event, since its magnitude decreased with time.

These concentration-time data are consistent with the recent data on the effects of formaldehyde on the mucociliary clearance apparatus (20, 26). The lack of a concentration effect in the anterior section reflects the fact that this region has minimal mucociliary clearance. In contrast, the adjacent more posterior section normally has a continuous flow of mucus over its surface. As the concentration of formaldehyde increases, larger areas of the mucus blanket become immobilized, effectively removing this protective mechanism (26). The efficacy of mucociliary clearance is thus likely to be greatest at low concentrations of formaldehyde.

In ongoing experiments following a protocol similar to that employed by Swenberg et al (31) except that the pulse of [^{3}H]thymidine was administered 18 hours after the last exposure, slight increases in cell proliferation were evident in rats exposed to 0.5 and 2 ppm formaldehyde in one six-hour exposure, but not after three or nine such exposures (33a). In contrast, much higher labeling indices were observed in 6-ppm exposed rats after one and three days. These data show a distinct nonlinear dependence of the labeling indices on formaldehyde concentration. A threefold increase in formaldehyde concentration from 2 to 6 ppm resulted in an eightfold increase in cell proliferation after one day of exposure and nearly a 25-fold increase after three days of exposure.

These data are consistent with nonlinear data on the covalent binding of formaldehyde to respiratory mucosal DNA (34) and carcinogenesis (16).

THE BIOCHEMICAL DISPOSITION OF INHALED FORMALDEHYDE

Formaldehyde is known to react with DNA in cultured mammalian cells in vitro, forming DNA-protein cross-links (35, 36), and this reaction may be a critical factor in the transformational (37), mutagenic (38), and carcinogenic (2) actions of formaldehyde. It is therefore important to determine whether inhaled formaldehyde can react with DNA in vivo and, if this reaction occurs, to quantify the amount of formaldehyde that reacts with DNA in target tissues as a function of its airborne concentration.

Evidence that inhaled formaldehyde reacts with respiratory mucosal DNA has been obtained by Casanova-Schmitz & Heck (39). These researchers observed that exposure of Fischer-344 rats to formaldehyde concentrations equal to or greater than 6 ppm resulted in a statistically significant decrease in the amount of DNA that could be extracted from proteins in homogenates of the respiratory mucosa. The extraction of the solubilized tissue homogenates was carried out using a strongly denaturing aqueous-immiscible organic solvent mixture. When the tissue was extracted in this manner, the DNA separated into two fractions (39). The aqueous (AQ) phase contained DNA that by all spectrophotometric and chromatographic criteria appeared to be pure, double-stranded DNA. An interfacial (IF) layer also contained DNA, but this DNA appeared to be cross-linked to proteins, since the DNA could not be released without digestion of the interface using proteinase K. Importantly, the quantity of IF DNA was dependent on the airborne formaldehyde concentration to which the rats had been exposed, increasing as the concentration increased. The investigators therefore concluded that formaldehyde does react with respiratory mucosal DNA following in vivo inhalation exposures, and that this reaction might well play a critical role in the development of nasal cancer during chronic formaldehyde inhalation exposures.

The inability to extract DNA from proteins does not, however, constitute proof of the formation of DNA-protein cross-links. Additional evidence is required to ensure the validity of this conclusion. To obtain such evidence, and to determine, if possible, the amount of formaldehyde that becomes covalently bound, additional experiments have been performed to investigate the mechanisms of labeling of respiratory mucosal DNA following inhalation exposure of rats to [^{14}C]- and [^{3}H]formaldehyde (34). The labeling of macromolecules (DNA, RNA, and protein) in the respiratory mucosa has been studied in rats that were pre-exposed for six hours to unlabeled formaldehyde on the day preceding exposure to the labeled compound. Pre-exposure was undertaken to

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stimulate cell turnover in the respiratory mucosa, a physiological response to toxic injury that appears to play an important role in the induction of nasal cancer by formaldehyde (30). The pre-exposure to unlabeled formaldehyde and the exposure to [^{14}C]- and [^{3}H]formaldehyde were both carried out at the same airborne concentrations. The specific activities of IF and AQ DNA obtained from the respiratory mucosa following in vivo exposure to 0.3, 2, 6, 10, and 15 ppm of [^{14}C]- or [^{3}H]formaldehyde were determined. The [^{14}C]-specific activity of the total DNA rose to a peak at 6 ppm and then decreased at higher concentrations. In addition, the ^{14}C-specific activity of the AQ DNA fraction was found to be significantly greater than that of the IF DNA fraction at 6 ppm, but no significant difference between the specific activities of IF and AQ DNA was found at other concentrations.

Based on several arguments advanced by Casanova-Schmitz et al (34), it was concluded that the major route of DNA labeling in the respiratory mucosa is metabolic incorporation. The maximum in the specific activity of the DNA at 6 ppm therefore implies that the metabolic incorporation of [^{14}C]formaldehyde into respiratory mucosal DNA is maximal at this concentration. This result is consistent with the observation that the incorporation of [^{3}H]thymidine into respiratory mucosal DNA after exposure to formaldehyde at 6 ppm is higher than after exposure to 15 ppm (31). The smaller amount of metabolic incorporation of ^{14}C into DNA that occurs at 10 and 15 ppm relative to that at 6 ppm is probably due to the cytotoxic effects of formaldehyde at these high concentrations.

The finding that the ^{14}C-specific activity of respiratory mucosal AQ DNA is significantly higher than that of IF DNA at 6 ppm is an extremely important one. This result implies that the AQ DNA incorporates a significantly larger amount of ^{14}C than the IF DNA at 6 ppm. In addition, this result implies that the two DNA fractions must differ structurally, for otherwise they could not have been separated by solvent extraction into portions with differing specific activities. However, no difference between the specific activities of AQ and IF DNA has been found at either 0.3 or 2 ppm, demonstrating that the structural difference noted above is not an inherent property of respiratory mucosal DNA but must have been induced in the DNA by exposure to formaldehyde at 6 ppm. A plausible explanation for this structural difference is that formaldehyde exposure at 6 ppm results in the formation of DNA-protein cross-links in the IF DNA fraction.

It may at first seem contradictory that the specific activity of IF DNA is lower than that of AQ DNA at 6 ppm since the IF DNA is presumed to contain covalently bound formaldehyde. However, this result is consistent with DNA-protein cross-linking because the major route of DNA labeling is metabolic incorporation. The formation of cross-links decreases the rate of incorporation of [^{14}C]formaldehyde metabolites into DNA by preventing the dissociation of

proteins from DNA necessary for de novo DNA synthesis to occur. An inhibition of DNA synthesis by formaldehyde has been shown to occur in yeast under conditions in which DNA-protein cross-links were induced (40).

The most direct evidence for the formation of covalently bound formaldehyde in DNA and proteins in the respiratory mucosa has been provided by determinations of the $^3H/^{14}C$ ratios in respiratory mucosal macromolecules following exposure of rats to [^{14}C]- and [^3H]formaldehyde (34). The $^3H/^{14}C$ ratios in the IF DNA and proteins increased with increasing formaldehyde concentrations, but no increases in the $^3H/^{14}C$ ratios of the AQ DNA and RNA have been observed. As discussed in detail by Casanova-Schmitz et al (34), increased $^3H/^{14}C$ ratios in macromolecules with increasing formaldehyde concentrations provide evidence of the covalent binding of formaldehyde. Thus, only the IF DNA and proteins contain measurable quantities of covalently bound formaldehyde.

The differences between the $^3H/^{14}C$ ratios in IF and AQ DNA are statistically significant at formaldehyde concentrations equal to or greater than 2 ppm. These differences therefore indicate that at these concentrations the mechanisms of labeling of IF and AQ DNA are significantly different: AQ DNA is labeled primarily or exclusively by metabolic incorporation, whereas IF DNA is labeled by both metabolic incorporation and covalent binding. Strong support for the conclusion that the difference between the $^3H/^{14}C$ ratios in the two DNA fractions is due to DNA-protein cross-linking is provided by the strong correlation between the difference in the $^3H/^{14}C$ ratios in IF and AQ DNA fractions and the percent interfacial DNA that was obtained in each experiment (34). It should be noted that, although a significant difference between the $^3H/^{14}C$ ratios of IF and AQ DNA has been detected at 2 ppm and above, the percentage of interfacial DNA is significantly increased relative to the level in control rats only at concentrations equal to or greater than 6 ppm (39). This indicates that the isotope ratio method is a more sensitive technique for the detection of DNA-protein cross-links than is the measurement of DNA extractability.

Casanova-Schmitz et al (34) also have shown that the $^3H/^{14}C$ ratio of a macromolecule following exposure to formaldehyde labeled with both isotopes is quantitatively related to the fraction of the total ^{14}C that is due to covalent binding, f_b, as follows:

$$f_b = \frac{(^3H/^{14}C)_o - (^3H/^{14}C)_m}{(^3H/^{14}C)_b - (^3H/^{14}C)_m}.$$

The terms in this equation include: $(^3H/^{14}C)_o$ = the observed $^3H/^{14}C$ ratio of macromolecule; $(^3H/^{14}C)_m$ = $^3H/^{14}C$ ratio characteristic of metabolic incorporation of ^3H and ^{14}C (derived from [^3H]- and [^{14}C]formaldehyde) into the macromolecule; $(^3H/^{14}C)_b$ = $^3H/^{14}C$ ratio characteristic of covalent binding of

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Table 3 Absolute and relative concentrations of covalently bound [^{14}C]formaldehyde in respiratory mucosal DNA of Fischer-344 rats[a]

Airborne formaldehyde concentration (ppm)	Absolute concentration of covalently bound [^{14}C]formaldehyde (nmol/mg DNA)	Relative concentration of covalently bound [^{14}C]formaldehyde[b] (nmol/mg DNA/ppm)
0.3	0.002 ± 0.003[c]	0.007
2.0	0.022 ± 0.006	0.011
6.0	0.233 ± 0.023	0.039
10.0	0.406 ± 0.099	0.041
15.0	0.631 ± 0.064	0.042

[a] Adapted from (41).
[b] Absolute amount of covalent binding in nmol/mg DNA divided by airborne formaldehyde concentration.
[c] Mean ± standard error as determined by Casanova-Schmitz et al (34).

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[^{3}H]- and [^{14}C]formaldehyde to the macromolecule under the reaction conditions. The values of these isotope ratios can be determined by methods described in Casanova-Schmitz et al (34). Hence, the fraction of covalently bound [^{14}C]formaldehyde can be calculated. Knowing the total ^{14}C concentration in the macromolecule permits determination of the concentration of covalently bound formaldehyde. The results of calculating the concentrations of covalently bound formaldehyde in respiratory mucosal DNA at 0.3, 2, 6, 10, and 15 ppm of inhaled formaldehyde are summarized in Table 3.

These data show that the concentration-response profile for covalent binding of [^{14}C]formaldehyde to DNA is sigmoidal, increasing gradually between 0.3 and 2 ppm, steeply between 2 and 6 ppm, and less steeply at the higher concentrations. The observed concentration of covalently bound formaldehyde at 2 ppm is significantly lower than the value predicted by linear extrapolation from the concentration measured at 6 ppm to the origin. In contrast to the results obtained with respiratory mucosal DNA, covalent binding of [^{14}C]formaldehyde to respiratory mucosal proteins depends in an apparently linear manner on the formaldehyde concentration throughout the concentration range (34).

The explanation for nonlinearity in the binding of formaldehyde to respiratory mucosal DNA is presently unknown. However, at least two mechanisms may account for the nonlinear behavior. First, the physiological and biochemical defense mechanisms such as mucociliary clearance, metabolism, and DNA repair could be inactivated or become less efficient with increasing formaldehyde concentrations, resulting in a disproportionate increase in the concentration of DNA-protein cross-links. Second, the marked increase in cell turnover caused by formaldehyde exposure at 6 ppm relative to that at 2 ppm

(31) could increase the availability of sites in DNA for reaction with formaldehyde. As noted above, we know that formaldehyde binds to single-stranded regions of DNA but not to double-stranded regions (28, 29).

Also important is the fact that the relative disposition of formaldehyde in respiratory mucosal tissues is concentration dependent. Casanova-Schmitz et al (34) have shown that the percentage of total ^{14}C in respiratory mucosal DNA and proteins due to covalent binding increases with concentration. If the disposition of formaldehyde in the respiratory mucosa were governed by purely linear kinetics, then the percentage of the total ^{14}C due to covalent binding would be constant, i.e. independent of the airborne concentration. Nonlinear kinetics must therefore be involved.

THE IMPLICATIONS FOR QUANTITATIVE RISK ESTIMATION

Nearly four years have elapsed since the first report that the chronic inhalation of gaseous formaldehyde induces nasal cancer in Fischer-344 rats (1). During that time, research into the mechanisms of formaldehyde toxicity has yielded a great deal of additional information directly relevant to concerns about the potential adverse effects of formaldehyde exposure on human health. As reviewed above, this research has focused on the biological defenses that protect organisms from toxicity at low-level formaldehyde exposures. It has also elucidated the mechanisms by which high-level formaldehyde exposures impair these defenses and thereby enhance nonlinearly the probability of irreversible toxic effects.

Nevertheless, low-dose risk estimates that result from the typical approach to quantitative risk assessment, namely, a linearized multistage model analysis of bioassay tumor incidence versus administered dose, do not utilize this additional mechanistic information. Indeed, such risk estimates would be no different had none of the research described above been undertaken. The process of quantitative risk estimation can be improved in this regard by utilizing the available mechanistic data to construct a measure of exposure that is more realistic and biologically meaningful than administered dose, as is summarized below.

The key issue in the use of mechanistic data in quantitative risk estimation concerns the form of the relationship between two distinct measures of exposure denoted by the terms *administered dose* and *delivered dose* (41). The administered dose is an external measure of exposure directly controlled in laboratory studies of toxicity. For inhalation studies, it refers to the concentration of a test chemical in the inhalation chamber air. In contrast, the delivered dose is an internal measure of exposure referring to the quantity or concentration of the biologially active form of a test chemical present in specific target

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tissues. This latter measure is presumed to be the direct causative variable in mechanistic descriptions of the carcinogenic process at the cellular and molecular levels (42).

The relationship between administered and delivered doses reflects the entire spectrum of biological responses to exposure, ranging from physiologic responses of the whole organism to intracellular biochemical responses in target tissues. Thus, the administered dose actually provides no more than an indirect, surrogate measure of the delivered dose, and the relationship between these two measures of exposure need not be a simple linear one. This is especially important because low-dose risk extrapolations based on the assumption of linearity are known to yield risk estimates that are either excessively conservative (too high) or anticonservative (too low) when the true administered-delivered dose relationship is nonlinear (42).

In the case of formaldehyde, the extensive mechanistically oriented studies reviewed above have identified four biological responses that appear to be important determinants of the formaldehyde dose delivered to target tissues in the rodent nasal cavity. The first of these is the minute volume depression in response to sensory irritation. In Fischer-344 rats and B6C3F1 mice it is an important factor only at formaldehyde concentrations above 6 ppm. Still, the fact that it is induced at these concentrations has three important consequences. First, the amount of formaldehyde entering the rat or mouse nasal cavity is not linearly proportional to formaldehyde concentrations in inspired air greater than 6 ppm. Second, the precipitously steep rise in squamous cell carcinoma incidence from about 1% among rats chronically exposed to 5.6 ppm formaldehyde to nearly 50% among rats similarly exposed to 14.3 ppm (Table 1) is actually steeper, i.e. more severely nonlinear, when the amount of formaldehyde inhaled per unit time is used as the measure of exposure rather than the ambient air formaldehyde concentration. Third, the marked disparity in tumor response between rats and mice identically exposed to 14.3 ppm formaldehyde (Table 1) can be reconciled by measuring exposure in terms of the rate, adjusted for the interspecies difference in nasal cavity surface area, at which formaldehyde is actually deposited in the nasal cavity.

Two other factors that must be considered are the inhibition of mucociliary clearance and the stimulation of cell proliferation that are both induced by exposure to high but not low formaldehyde concentrations. Both tend to increase disproportionately the dose delivered to target tissues at high formaldehyde concentrations, thus counterbalancing and probably overriding any reduction in delivered dose associated with minute volume depression. The inhibition of mucociliary clearance contributes to this effect by eliminating a pathway for the removal of formaldehyde from the nasal cavity before it penetrates to underlying epithelial cells. Increased cell proliferation enhances the likelihood of irreversible genotoxic events once formaldehyde reaches the

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target cells by increasing the number of single-stranded DNA sites at which formaldehyde may covalently bind and by decreasing the amount of time available for the repair of such lesions before they become fixed during cell replication.

Finally, studies of the disposition of formaldehyde in nasal cavity tissues have provided the first direct quantitative measurements of the amount of formaldehyde delivered to target-tissue DNA. These studies are of critical importance for several reasons. First, they have demonstrated that the delivered dose/administered dose relationship is distinctly nonlinear, as would be expected from considering the observed spectrum of effects of inhaled formaldehyde on minute volume, mucociliary clearance, and cell proliferation. Second, the studies also provide evidence that in target tissues, metabolic incorporation, a process by which delivered formaldehyde is detoxified, is less efficient at high airborne formaldehyde concentrations than it is at low concentrations. Thus, another removal pathway that protects against formaldehyde toxicity at low airborne concentrations appears to be compromised at formaldehyde concentrations greater than 2 ppm. Third, the data for the covalent binding of formaldehyde to target-tissue DNA are in a form that makes it possible to reanalyze the nasal tumor results from the chronic bioassay with the delivered formaldehyde dose rather than the airborne formaldehyde concentration as the measure of exposure. Such a reanalysis has been completed recently (41), and a brief summary of the principal results is provided below.

Tumor incidence rates nearly identical to those used by Cohn (43) were employed since his analysis of the chronic bioassay results figured prominently in the US Consumer Product Safety Commission's decision to ban the sale of urea-formaldehyde foam insulation in the United States (3). Concentrations of formaldehyde covalently bound to respiratory mucosal DNA corresponding to the airborne formaldehyde concentrations employed in the chronic bioassay were derived from those reported by Casanova-Schmitz et al (34). Four commonly used quantal response models, namely, the multistage, Weibull, logit, and probit, were used for low-dose extrapolation. Model parameters were estimated using standard maximum likelihood (ML) techniques. Both ML estimates of risk and their upper 95% confidence bounds were calculated for three airborne formaldehyde concentrations, 0.1, 0.5, and 1.0 ppm. For these concentrations, it was assumed that the delivered dose/administered dose relationship was linear and given by a straight line passing from the origin through the concentration of covalently bound formaldehyde observed at 2 ppm (34). As noted by Starr & Buck (41), this low-dose linearity assumption probably overestimates the amount of covalent binding that actually occurs at these airborne concentrations.

The maximum likelihood (ML) estimates of risk and their upper 95% confidence bounds are shown in Tables 4 and 5 respectively. It is readily

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Table 4 Maximum likelihood estimates of risk based on administered dose (A) and delivered dose (D) at selected ambient air formaldehyde concentrations[a]

Airborne concentration (ppm)	Dose measure	Maximum likelihood risk estimates			
		Probit	Logit	Weibull	Multistage
0.1	A	< 1.00(−26)[b]	3.92(−11)	2.20(−10)	2.51(−7)
	D	< 1.00(−26)	7.40(−13)	6.20(−12)	4.70(−9)
0.5	A	5.16(−17)	9.85(− 8)	2.75(− 7)	3.14(−5)
	D	< 1.00(−26)	9.76(−10)	4.27(− 9)	5.88(−7)
1.0	A	2.65(−11)	2.87(− 6)	5.94(− 6)	2.51(−4)
	D	4.00(−20)	2.15(− 8)	7.13(− 8)	4.70(−6)

[a] Reprinted with permission from (41).
[b] Values in parentheses are powers of ten.

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Table 5 Upper 95% confidence bounds on risk based on administered dose (A) and delivered dose (D) at selected ambient air formaldehyde concentrations[a]

Airborne concentration (ppm)	Dose measure	Upper 95% confidence bounds on risk			
		Probit	Logit	Weibull	Multistage
0.1	A	< 1.00(−26)[b]	2.84(−10)	1.57(− 9)	1.56(−4)
	D	< 1.00(−26)	6.19(−12)	5.12(−11)	6.19(−5)
0.5	A	7.69(−16)	5.13(− 7)	1.41(− 6)	8.09(−4)
	D	< 1.00(−26)	6.31(− 9)	2.73(− 8)	3.10(−4)
1.0	A	2.58(−10)	1.24(− 5)	2.54(− 5)	1.80(−3)
	D	7.09(−19)	1.22(− 7)	3.98(− 7)	6.24(−4)

[a] Reprinted with permission from (41).
[b] Values in parentheses are powers of ten.

apparent that the estimates obtained with delivered dose are unilaterally lower than the corresponding estimates obtained with administered dose. For ML estimates, the ratios of risk based on administered dose to risk based on delivered dose range from 35 (Weibull: 0.1 ppm) to more than nine orders of magnitude (probit: 0.5 ppm). The multistage ML risk estimates based on delivered dose are uniformly lower by a factor of 53. For upper 95% confidence bounds, corresponding risk reduction factors range from 2.5 (multistage: 0.1 ppm) to more than ten orders of magnitude (probit: 0.5 ppm).

These results demonstrate that the incorporation of delivered dose into low-dose extrapolation procedures leads to a unilateral reduction in estimates of cancer risk associated with exposure to low airborne formaldehyde concentrations. Because the use of this exposure measure allows much of the information already obtained from mechanistic studies of formaldehyde toxicity to enter the risk assessment process in a meaningful and relevant manner, the resulting risk estimates reflect what is known of the underlying biological reality more faithfully than do previous estimates based solely on findings from the chronic bioassay. Additional research is of course required to further refine and elaborate the delivered dose concept, especially for humans. Comparative studies of the physiologic and biochemical responses to formaldehyde exposure are especially important. In addition, predictive mechanistic models that explicitly incorporate the influences of these phenomena on the covalent binding of formaldehyde to target tissue DNA and on the promotional stages of the carcinogenic process must be constructed and validated.

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