EXECUTIVE SUMMARY

Purpose and Scope of Document. This document first provides a historical basis for existing cancer risk assessments for formaldehyde (HCHO). Summaries of the epidemiological studies, laboratory animal studies, and supporting data are then provided. These are used to develop a comprehensive hazard characterization for the carcinogenicity of formaldehyde and to establish the mode of action by which formaldehyde causes tumor development. A biologically-based, dose-response model is described that incorporates current mechanistic understandings of the ways in which formaldehyde induces cancer in rats. Both the hazard characterization and dose-response model presented in this document have been developed using the U.S. Environmental Protection Agency (U.S. EPA) latest draft guidelines for carcinogenic risk assessment. The use of mechanistic data (rather than standard default assumptions) offers the potential to decrease the uncertainties inherent in the extrapolation of data, both across species (e.g., rat to human) and from high concentrations to low concentrations (e.g., from the experimental bioassay concentrations to those relevant to human exposures). Finally, the biologically-based dose-response modeling approach is contrasted with a benchmark dose approach.

Synopsis of Existing Assessments

IARC – The first qualitative assessment of formaldehyde by the International Agency for Research on Cancer (IARC) was in 1981 (IARC, 1982); this assessment was followed by updates in 1987 (IARC, 1987) and 1995 (IARC, 1995). The latest review of IARC reconfirmed their earlier classification of formaldehyde in Group 2A (probably carcinogenic to humans). This classification was based on limited evidence of carcinogenicity in humans (based primarily on nasopharyngeal cancer) and sufficient evidence in laboratory animals (based on nasal tumors in rats).

Health & Welfare Canada – In 1988, Health & Welfare Canada completed an assessment in which carcinogenic risks were estimated based on nasal tumors in rats, but incorporating DNA-protein cross-links (DPX) as the measure of dose (Report to the Federal-Provincial Working Group on Indoor Air Quality - Formaldehyde). Several limitations of this approach were recognized including the lack of chronic data and the unknown relationship between DPX formation and tumor development.
OSHA – In 1992, the Occupational Safety and Health Administration (OSHA) issued final amendments to its formaldehyde standard, including a reduction in the permissible exposure limit (PEL). The amendments to the OSHA standard for formaldehyde lowered the PEL from the existing level of 1 ppm in air as an 8-hour time-weighted average (TWA) to an 8-hour TWA of 0.75 ppm. A 15-min short-term exposure limit (STEL) of 2 ppm and an action level of 0.5 ppm in the prior standard continued to be in effect.

ACGIH – The American Conference of Governmental Industrial Hygienists (ACGIH) has determined threshold limit values (TLV) for formaldehyde for the protection of workers who might be exposed occupationally. The TLVs published by ACGIH for formaldehyde have decreased 30-fold over the last 50 years. In 1995, the ACGIH set a ceiling (i.e., as opposed to a TWA) for a TLV for formaldehyde of 0.3 ppm. This was based on evidence of irritation from reports of occupational and other exposures to formaldehyde. ACGIH (1991) designated formaldehyde as being in Group A2 (suspected human carcinogen) but maintains that protection against irritant effects is the most appropriate basis for a TLV. While the ACGIH (1999) publication of TLVs retains 0.3 ppm as the TLV for formaldehyde, their notice of intended changes include the addition of sensitizer and dropping the qualifier “nasal” when listing cancer as a critical effect considered as part of the basis for the TLV.

rat and the monkey, the nasal anatomy of which is much more similar to humans. The unit risk calculated by U.S. EPA (1991) was $2.8 \times 10^{-3}$ per ppm based on rat data and $3.3 \times 10^{-4}$ per ppm based on monkey data. The Agency never finalized the 1991 draft risk assessment.

**Exposure**

Exposure to formaldehyde is ubiquitous since it is found in the environment as the result of both natural and anthropogenic mechanisms. The uses for formaldehyde are quite extensive and include many consumer products as well as industrial uses.

**Occupational Exposure.** Occupational exposure to formaldehyde occurs in many lines of work, mostly by inhalation of formaldehyde gas. There are also solid (e.g., paraformaldehyde) and liquid (e.g., formalin) forms that can lead to exposures by other routes or can contribute to inhalation of formaldehyde as a result of off-gassing. The Occupational Safety and Health Administration (OSHA, 1985) estimated that over 22,000 workers had average formaldehyde exposures over 1 ppm, and about 500,000 were potentially exposed to 0.5 to 1 ppm. With the lowering of the OSHA PEL in 1992 from 1 ppm (8-h TWA) to 0.75 ppm (8-h TWA), the levels to which workers are likely to be exposed have recently decreased.

**Environmental Exposure.** Outdoor air in nonurban environments generally contains levels of formaldehyde that are less than 1 ppb. Preuss et al. (1985) reported the mean and maximum concentrations of formaldehyde in a nonpopulated region to be 0.4 ppb and 0.8 ppb, respectively. In the Los Angeles area, levels of 10 to 30 ppb were reported, with even higher levels (48 ppb) found during severe air inversions. Other cities in North America have been found to have somewhat lower levels, averaging 3.8 to 6.6 ppb (Grosjean, 1982). In the indoor environment, concentrations of formaldehyde tend to be higher. As with other indoor air pollutants, levels of formaldehyde can be increased by the use of measures that decrease air exchange with the outside. In conventional homes, mean indoor air concentrations have been reported to be 0.03 ppm; in mobile homes, levels as high as 0.1 to 0.5 ppm have been reported (summarized in ACGIH, 1991).
Toxicokinetics

Absorption. Inhaled formaldehyde is rapidly and almost entirely absorbed in the respiratory tract. In obligate nasal breathers such as rats, formaldehyde is almost completely absorbed in the nasal passages (Heck et al., 1983); in monkeys, absorption occurs primarily in the nasal passages but also to a lesser extent in the trachea and proximal regions of the major bronchi (Monticello et al., 1989). The species-specific differences in the actual sites of formaldehyde uptake, and hence sites of nasal lesions, are determined by complex interactions among nasal anatomy, ventilation, and breathing patterns (e.g., nasal versus naso-oral) (Morgan et al., 1991). Formaldehyde reacts rapidly at the site of contact and is quickly metabolized by enzymes in the target tissue. It can also be metabolized by enzymes in erythrocytes, so that exposure to even high concentrations of atmospheric formaldehyde does not result in an increase in blood concentrations. Heck et al. (1985) exposed human volunteers to 1.9 ppm formaldehyde for 40 min. and reported that the level of formaldehyde in blood was not increased above preexposure levels. Bogdanoffy et al. (1987) suggested that a portion of inhaled formaldehyde is retained in the mucous blanket of the respiratory tract. This was based on the demonstration that incubation of human nasal mucus with formaldehyde in vitro led to the formation of protein adducts.

Metabolism. Formaldehyde is efficiently metabolized to formate by all tissues of the body as a normal metabolic process. Endogenous and exogenous formaldehyde are handled metabolically by the same processes. Following inhalation, formaldehyde is rapidly metabolized in the respiratory tissues with which it first comes into contact. Formaldehyde dehydrogenase is the primary enzyme involved in the metabolism of formaldehyde and is widely distributed in all tissues. The specific pathway for the metabolism of absorbed formaldehyde involves reaction with glutathione to form S-hydroxymethyl-glutathione. This hemithioacetal is then oxidized by formaldehyde dehydrogenase to form S-formylglutathione, which is subsequently hydrolyzed to release glutathione and formate.

Hazard Assessment for Carcinogenicity

Human Studies. Possible associations between formaldehyde and cancers of various organs have been examined extensively in epidemiological studies in occupationally exposed populations. There have been over 30 cohort and case-control studies of
professionals, including pathologists and embalmers, and of industrial workers. In addition, several meta analyses of the available data have been conducted. This document provides only a brief overview of the available data with emphasis on information relevant to bounding of estimated risks derived from studies in animals.

Case-Control and Cohort Studies. Though most epidemiological studies on the potential association between exposure to formaldehyde and cancer have focused on the respiratory tract, in some case-control and cohort studies occasionally increased risks of various non-respiratory tract cancers (e.g., multiple myeloma, non-Hodgkin's lymphoma, ocular melanoma, brain, connective tissue, pancreatic, leukemic, lymphoid and haematopoietic, colon) have been observed (Boffetta et al., 1989; Heineman et al., 1992; Pottem et al., 1992; Blair et al., 1993; Holly et al., 1996; Stroup et al., 1986; Stayner et al., 1988; Matanoski, 1991; Hayes et al., 1990). Risk measures for these excesses have been reported only sporadically, and there does not appear to be any consistent pattern. Thus, the primary focus was on the well studied associations between exposure to formaldehyde and cancers of the respiratory tract (i.e., nasal, nasopharyngeal and lung).

Nasal and Nasopharyngeal Cancer. In case control studies, there have been increases in cancers of the nasal or nasopharyngeal cavities that fulfill, at least in part, traditional criteria of causality, with tumors having been observed in workers with the highest levels or duration of exposure. Of note though is that measures of exposure in these population-based investigations were considerably less extensive than those in the cohort studies of occupationally exposed populations. Excesses of cancers of the nasal or nasopharyngeal cavities have not been observed consistently in cohort studies. Where there have been excesses, there has been little evidence of exposure-response; however, the total number of observed tumors in these investigations was small.

While sometimes no increase was observed overall (Vaughan et al., 1986a), significantly increased risks of nasopharyngeal cancer (up to 5.5-fold) were observed among workers with 10 to 25 years duration of exposure or in the highest exposure category in 3 out of 4 investigations (Vaughan et al., 1986a; Roush et al., 1987; West et al., 1993), though there were limitations to most of these studies. In the only investigation in which the association between exposure to formaldehyde and adenocarcinoma of the nasal cavity was examined, there was a non-significant
increase that was exacerbated in the presence of wood dust (Luce et al., 1993), though possible residual confounding by wood dust exposure could not be excluded.

There is little convincing evidence of increased risks of nasopharyngeal cancer in cohort studies of populations of professionals or industrial workers occupationally exposed to formaldehyde, though it should be noted that the total number of cases of this rare cancer in all of the studies was small. The results of the largest industrial cohort mortality study of 26,561 workers first employed before 1966 at ten plants in the United States (4% of cohort exposed to > 2ppm) indicated an approximately three-fold excess of deaths due to nasopharyngeal cancer associated with occupational exposure to formaldehyde (Blair et al., 1986). However, subsequent analyses revealed that five of the seven observed deaths were among individuals also having been exposed to particulates; four of the seven observed deaths occurred at one specific industrial plant (Blair et al., 1987; Collins et al., 1988; Marsh et al., 1996). Three of the seven observed deaths due to nasopharyngeal cancer occurred in individuals with less than one year of employment (Collins et al., 1988), and the four deaths at one specific plant occurred equally in both short-term and long-term workers (Marsh et al., 1996).

**Lung Cancer.** There is little evidence for a causal association between exposure to formaldehyde and lung cancer in case control and cohort studies conducted to date. Increases in mortality or incidence have not been observed consistently, and where examined, there has been consistently no evidence of exposure response. In most case-control studies, there have been no increases in lung cancer (Bond et al., 1986; Brownson et al., 1993; Andjelkovich et al., 1994; Gerin et al., 1989). In the single study where it was examined, there was no significant increase in adenocarcinoma of the lung for those with “long-high” occupational exposure; though the odds ratio was greater than for lung cancer, the number of cases on which this observation was based was small (Gerin et al., 1989).

In smaller cohort studies of professional and industrial workers (Table 4-2), there have been no significant excesses of cancers of the trachea, bronchus, and lung (Andjelkovich et al., 1995; Hayes et al., 1990), the buccal cavity or pharynx (Matanoski, 1991; Hayes et al., 1990; Andjelkovich et al., 1995), the lung (Stroup et al., 1986; Bertazzi et al., 1989; Hansen and Olsen, 1995) or respiratory system (Matanoski, 1991). In the largest industrial cohort mortality study of 26,561 workers first employed before 1966 at ten plants in the United States (4% of cohort exposed to > 2ppm), Blair et al. (1986) observed a slight but significant (1.3-fold) excess of deaths due to lung
cancer among the subcohort of white male industrial workers with $\geq 20$ years since first exposure. However, results of a number of follow-up studies within this industrial group have provided little additional evidence of exposure-response (i.e., cumulative, average, peak, duration, intensity) except in the presence of other substances (Blair et al., 1986, 1990a; Blair and Stewart, 1994b; Marsh et al., 1992a, 1996; Callas et al., 1996).

**Meta Analyses.** Meta analyses of data from epidemiological studies published between 1975 and 1991 were conducted by Blair et al. (1990b) and Partanen (1993). These analyses revealed no increased risk of cancer of the oral cavity associated with exposure to formaldehyde (Blair et al., 1990b; Partanen, 1993). In both meta analyses, there was a significantly increased cumulative relative risk (ranging from 2.1 to 2.74) of nasopharyngeal cancer among those in the highest category of exposure to formaldehyde, while in the lower or low-medium exposure categories, the cumulative relative risks for nasopharyngeal cancer ranged from 1.10 to 1.59 (Blair et al., 1990b; Partanen, 1993). More recently, Collins et al. (1997) determined the cumulative relative risks of death due to nasal, nasopharyngeal, and lung cancer associated with potential exposure to formaldehyde, based upon a meta analysis of data from case-control and cohort investigations published between 1975 and 1995. In contrast to the findings of Blair et al. (1990b) and Partanen (1993), Collins et al. (1997) concluded there was no evidence of increased risk of nasopharyngeal cancer associated with exposure to formaldehyde. The differing results were attributed to inclusion of more recent studies for which results were negative (particularly Gardner et al., 1993) and correction for underreporting of expected numbers.

**Exposure-Response.** There is little evidence of a causal association between exposure to formaldehyde and lung cancer. Results of studies in a rather extensive database do not fulfill traditional criteria of causality in this regard such as consistency, strength and exposure-response. The data for nasal and nasopharyngeal cancer are less clear. In case control studies, there have been increases in cancers of the nasal or nasopharyngeal cavities that fulfill, at least in part, traditional criteria of causality, with tumors having been observed in workers with highest levels or duration of exposure. It should be noted, though, that measures of exposure in these population-based investigations are rather less reliable than those in the larger, most extensive cohort studies of occupationally exposed populations; moreover, methodological limitations complicate interpretation of several of the case-control studies. Excesses of
cancers of the nasal or nasopharyngeal cavities have not been observed consistently in cohort studies. Where there have been excesses, there has been little evidence of exposure-response, though the total number of observed tumors was small.

Laboratory Animal Studies. Cancer bioassays for formaldehyde have been conducted in several species and strains of laboratory animals, and by several routes of administration. For the purpose of subsequent dose-response analysis, emphasis is placed here on the inhalation bioassays. Studies by other routes of exposure are described only to the extent that they contribute to the weight-of-evidence for carcinogenicity of formaldehyde.

Several inhalation bioassays conducted in the rat have shown that high concentrations of formaldehyde result in the formation of nasal squamous cell carcinomas (SCC). Stratified squamous epithelium lines the most anterior portion of the rat nasal cavity, where most of the nasal tumors induced by formaldehyde are found to originate. The first evidence for the carcinogenicity of formaldehyde came from an inhalation bioassay in F344 rats (Kerns et al., 1983). Rats (119-120/sex/group) were exposed to 0, 2.0, 5.6, or 14.3 ppm formaldehyde [0, 2.5, 6.9 or 17.6 mg/m³] for 6 h/day, 5 days/week for up to 24 months, followed by an additional 6-month observation period. Necropsies revealed no gross or histopathological lesions in any tissue other than the nasal cavity, where dose-related increases in the incidence and severity of lesions were seen.

The nasal tumors found in the Kerns et al. (1983) bioassay were reported to originate in the anterior portion of the nasal cavity, but their precise location had not been determined. Morgan et al. (1986) reexamined the histologic sections from the nasal passages of these rats and recorded the location of each SCC and polypoid adenoma. In rats exposed to the highest concentration of formaldehyde (14.3 ppm), 57% of nasal SCCs were found in the anterior portion of the lateral aspect of the nasoturbinate and adjacent lateral wall (level 1), 26% in the midventral nasal septum (level 2), 10% in level 3, and 3% in level 4. The location of the remaining 4% could not be determined. In a subsequent bioassay conducted at CIIT, Monticello et al. (1996) exposed male F344 rats to 0, 0.7, 2, 6, 10, or 15 ppm formaldehyde [0, 0.86, 2.46, 7.38, 12.3, or 18.45 mg/m³] for 6 h/day, 5 days/week for up to 24 months. The results of this study were similar to those reported previously by Kerns et al. (1983).

Two inhalation bioassays of formaldehyde have been conducted in mice. The first was a partial lifetime study in C3H mice (42–60/group) exposed to 0, 0.05, 0.1, or 0.20 mg/L formaldehyde [0, 50, 100, or 200 mg/m³] for 1 h/day, 3 days/week for 35
weeks (Horton et al., 1963). The high-concentration group exhibited significant mortality, resulting in discontinuation of this treatment group in the fourth week. At the time of this study, the nasal epithelium had not been identified as a target organ for formaldehyde, and no examination of these tissues was conducted. The trachea and bronchi of most exposed mice exhibited basal cell hyperplasia, squamous cell metaplasia, and atypical metaplasia. No pulmonary tumors were seen in any group.

The second inhalation bioassay in mice involved a 30-month study of B6C3F1 mice (119-121/sex/group) exposed to 0, 2, 5.6, or 14.3 ppm formaldehyde [0, 2.5, 6.9, or 17.6 mg/m³] for 6 h/day, 5 days/week for up to 24 months (Kerns et al., 1983). Among mice killed at 24 months, only two squamous cell carcinomas were seen, with both of these occurring in male mice exposed to the highest concentration of formaldehyde (14.3 ppm). No nasal tumors were seen in controls, any group of female mice, or in male mice exposed to the low or mid concentrations of formaldehyde.

In comparison with the level of tumor induction observed in the rat, the mouse appears to be less susceptible to the development of SCCs following exposure to a given concentration of formaldehyde. The mouse is well known, however, for decreasing its minute volume in response to inhalation of noxious chemicals (Barrow et al., 1986). When these breathing differences are taken into account, the cancer risk is comparable between rats and mice (Barrow et al., 1986).

Weight of Evidence of Laboratory Animal Studies. The overall weight-of-evidence in laboratory animals is that formaldehyde is carcinogenic by the route of inhalation only at high concentrations. There is no evidence for a carcinogenic response at concentrations that do not also cause a variety of nonneoplastic effects. Therefore, at concentrations to which humans are typically exposed, formaldehyde is not likely to be carcinogenic. Also, because of the irritant properties of formaldehyde, exposure is self-limiting: e.g., humans are not likely to be exposed to concentrations above 1–2 ppm formaldehyde for other than short periods of time because of local irritation to the eyes, nose, and mucus membranes.

Genotoxicity Studies. Several human cytogenetic population monitoring studies have been conducted on groups of individuals who had formaldehyde exposure, frequently at undocumented levels. The results of these are somewhat equivocal as noted in the IARC review (1995). An increased incidence of micronucleated buccal or nasal mucosal cells has been reported in some surveys of individuals occupationally-exposed to formaldehyde. Evidence of genetic effects (i.e., chromosomal aberrations,
sister chromatid exchanges) in peripheral lymphocytes from individuals exposed to formaldehyde vapor has also been reported in some studies but not others. The interpretation of these studies is frequently confounded by their small size, lack of appropriate matching of exposed and controls and the contribution of co-exposures. Thus, at best, a very weak positive response is indicated, usually in nontarget cells for tumor formation.

In studies involving the exposure of laboratory animals to formaldehyde, there have been genotoxic effects primarily at the site of first contact (i.e., within the respiratory or gastrointestinal tracts) following inhalation or ingestion, though distal effects cannot be precluded, on the basis of available data. These in vivo laboratory animal studies are similar to the human studies reported above in that there is an inconsistency on the positive mutagenicity of formaldehyde. It can be described as being weakly mutagenic in some assays and not detectable as mutagenic in others.

The in vitro genotoxicity of formaldehyde has been assessed based on a wide variety of endpoints (IARC, 1995). Generally, the results of these studies have indicated that formaldehyde is genotoxic in bacterial and mammalian cells in vitro. Formaldehyde induces mutations in S. typhimurium and in E. coli, with positive results obtained in the presence or absence of metabolic activation systems. Formaldehyde increases the frequency of chromatid/chromosome aberrations, sister chromatid exchange, as well as gene mutations in a variety of rodent and human cell types. Exposure to formaldehyde increases DNA damage (strand breaks) in human fibroblasts and rat tracheal epithelial cells and increases unscheduled DNA synthesis in rat nasoturbinate and maxilloturbinate cells. On the basis of in vitro assays in which a variety of endpoints were examined in bacterial and mammalian cells, formaldehyde is described as being mutagenic, although weakly so.

**DPX Formation Following Inhalation of Formaldehyde.** The formation of DPX following inhalation of formaldehyde has been shown to be highly nonlinear above concentrations of about 6 ppm. Glutathione-mediated detoxification of formaldehyde becomes saturated in rats at exposures above 4 ppm (Casanova and Heck, 1987), so that a nonlinear increase in DPX formation would be expected from exposures to higher concentrations.

Casanova et al. (1991) exposed rhesus monkeys to 14C-formaldehyde at concentrations of 0.7, 2, or 6 ppm [0.9, 2.4, or 7.3 mg/m³] for 6 h and measured the formation of DPX. Per milligram of DNA, the highest concentrations of DPX were found in the middle turbinates, followed by the anterior lateral wall-septum and nasopharynx.
Very low concentrations were found in the larynx-trachea-carina and in the proximal portions of the major bronchi in the animals exposed to 6 ppm. The location of highest DPX concentrations is consistent with the site and severity of lesions seen in monkeys following inhalation of formaldehyde. In comparison to DPX formation in rats exposed to the same atmospheric concentration of formaldehyde, the yield of cross-links in the nose of monkeys was about 10-fold lower. Experiments were conducted to determine whether the formation of DPX is cumulative with continued exposure. It was found that the cumulative yield of DPX was similar in both acutely and subchronically exposed rats, suggesting that rat nasal DPX are rapidly repaired (Casanova et al., 1994).

A model has been proposed in which the concentration of DPX formed in specific anatomical regions of different species can be predicted based on a pharmacokinetic parameter that takes into account species-specific minute volumes and the quantity of mucosal DNA in that particular region. The model has currently been developed for the rat and monkey (Heck and Casanova, 1995; Casanova et al., 1991). It has been suggested that the concentrations of DPX that would result in the human nose following inhalation of formaldehyde would be even lower than those seen in the monkey. CIIT has developed models of site-specific airflow in the nose of rats (Kimbell et al., 1997a), monkeys (Kepler et al., 1998), and humans (Subramaniam et al., 1998) to enable state-of-the-art cross-species extrapolation.

Cell Proliferation. Cell proliferation has been postulated to play a key role in the carcinogenic process (Ames and Gold, 1990; Cohen and Ellwein, 1990). It may contribute by fixing chemically-induced DNA alterations that are not repaired prior to cell division or by increasing the number of cells that are available to undergo spontaneous mutations. In either case, an increase in cell proliferation results in a shortening of the cell cycle such that less time is available for the repair of promutagenic lesions. Additionally, cell proliferation plays a role in the progression stage of carcinogenesis. Exposure to formaldehyde at high concentrations has been found to result in cellular toxicity followed by a compensatory increase in the rate of epithelial cell proliferation in the rat (Chang et al., 1983), monkey (Monticello et al., 1989), and xenotransplanted human nasal respiratory epithelium (Klein-Szanto et al., 1989).

Monticello et al. (1991a) measured regional increases in rat nasal epithelial cell proliferation following acute and subchronic inhalation of formaldehyde. F344 rats were exposed to concentrations of 0, 0.7, 2, 6, 10, or 15 ppm formaldehyde for 6 h/day, 5 days/week for up to 6 weeks. A direct correlation was not apparent between sites of
increased cell proliferation and sites susceptible to formaldehyde-induced nasal cancer (that is, the lateral meatus and nasal septum of the anterior nasal passages (Morgan et al., 1986a)). Monticello (1990) also conducted a parallel experiment in rhesus monkeys involving a 6-week exposure. Results were similar to those seen in the rat, with exposure to 6 ppm resulting in increased cell proliferation and morphologically similar lesions to those seen in rats. However, the distribution of lesions was species-specific, with lesions in the rat being confined to the anterior nasal passages and lesions in the monkey being more widespread and extending into the lower airways.

Monticello et al. (1996) reported that there were no treatment-related increases in cell proliferation indices in the 0.7, 2, or 6 ppm groups but statistically significant increases in the 10 and 15 ppm groups. This suggests that sustained cell proliferation is an important component in the carcinogenic process. The nonlinearity and site specificity of formaldehyde-induced nasal SCC in rats can probably be accounted for by taking into account both the target cell population size and sustained increases in cell proliferation in these populations, with this latter being determined by differences in regional doses of formaldehyde resulting from species-specific airflow patterns.

**Mucociliary Studies.** A protective mucous layer, which is secreted by the nasal submucosal glands and goblet cells, covers the epithelium of the nasal passages of several species including rats, monkeys, and humans. The mucous layer provides an extra line of defense against inhaled microbes and toxicants. Formaldehyde has been shown to react with the proteins and polysaccharides in the mucous layer so that the concentration actually reaching the target epithelial cells is reduced (Morgan et al., 1984). However, high concentrations of formaldehyde have been shown to inhibit mucociliary action. This inhibition of mucociliary function by high concentrations of formaldehyde may also contribute to the nonlinearity of the dose-response relationship for formaldehyde-induced toxicity and carcinogenicity.

**Mode and Mechanism of Action for Formaldehyde-Induced Nasal Tumors**

For considerations of dose-response characterization, distinguishing between mode of action and mechanism of action is appropriate. Establishing a mode of action is much more feasible since this only requires the identification of the necessary but not sufficient steps whereby a particular agent causes tumor development. Since cancer can be considered as a genetic disease, a mode of action should describe the
processes by which mutations can be induced directly or indirectly by chemical exposure. Thus, the mode of action for the production of nasal tumors by formaldehyde has to include data pertinent to mutation induction. The question that has to be addressed by mode of action is how mutations needed for tumor development arise after formaldehyde exposure.

*Mode of Action.* Formaldehyde induces DPX in nasal mucosal cellular DNA of rats with a concentration response that is highly nonlinear. For a DNA adduct or a cross-link (DNA-DNA or DNA-protein) to be converted into a mutation (gene or chromosomal), DNA replication has to occur. The genetic alteration arises as an error of DNA replication on a damaged template. The normal cell turnover rate is very low in the nasal mucosa, and so the probability of a mutation being produced by a replication error, especially at low levels of DPX, is also very low (close to zero). Regenerative cell proliferation following formaldehyde-induced cytotoxicity increases the number of DNA replications and, thus, increases the probability of a DPX initiating a DNA replication error resulting in a mutation. The concentration-response curve for formaldehyde-induced cytotoxicity and regenerative proliferation is highly nonlinear. This nonlinearity is a consequence of the fact that saturable protective mechanisms (e.g., mucous layer, oxidative metabolism) reduce the amount of formaldehyde reaching the squamous epithelium at low exposure concentrations. Not until these protective mechanisms are overwhelmed will a cytotoxic (or carcinogenic) effect be observed. The two factors that therefore appear to be necessary, but not sufficient, for tumor formation following formaldehyde exposure, namely DPX and regenerative cell proliferation, both have highly nonlinear responses as a function of concentration or of flux of formaldehyde into tissue.

Using a mode of action approach, it is reasonable to argue that the shape of the tumor response curve below the level of observation is nonlinear. Either DPX (i.e., genotoxicity) or cell proliferation values (or both) could be used as a cancer surrogate for describing the shape of the dose response curve. Given that formaldehyde is weakly mutagenic, one might presume that DPX, a direct DNA response, is an appropriate surrogate for tumor response. However, we currently do not know what the probability is of a DPX producing a mutation. In addition, we do not know how many mutations will be in genes critical for the formation of squamous cell carcinomas following formaldehyde exposure. Conservatively, we can assume: (1) that a DPX has a probability of 1 of producing a mutation by a replication error, and (2) that there are 10 genes essential for SCC development, mutations in any one of which can lead to
tumor initiation or promotion via a two-stage clonal growth model. It follows that a tumor response predicted from DPX would be $1 \times 10^{-4}$ of the induced DPX (10 tumor genes/100,000 total genes), which is clearly an overestimate because of the conservative assumptions and because it does not make allowance for the fact that much less than 50% of the human genome is transcribed. An additional overestimate that is of undetermined magnitude is the result of DPX being repaired or removed prior to DNA replication and mutation formation.

Cell proliferation appears to be essential for mutations in the development of tumors and since there appears to be a more direct relationship between regenerative cell proliferation and tumor formation than for DPX, the models described here more appropriately use regenerative cell proliferation as a required step in the development of benchmark dose or biologically-based dose response models. In addition, there is a contribution from mutagenicity (not defined specifically by DPX) that is of particular impact at low exposures.

Proposed Mechanisms of Action. Additional confidence in the proposed mode of action can be obtained if data are available to support a mechanism of formaldehyde as a carcinogen that is consistent with this mode of action. Literature sources and CIIT data on the mutagenicity of formaldehyde, its reactivity with DNA, its effects on the tumor suppressor gene p53, and its cytolethality at high concentrations can be used to develop a general mechanistic hypothesis to add confidence to the proposed mode of action.

Formaldehyde is utilized for the biosynthesis of thymidine, deoxyadenosine, and deoxyguanosine, which are incorporated into DNA during DNA replication. When rats or monkeys are exposed to $[^{14}C]$formaldehyde, the radiolabel is rapidly incorporated into these nucleosides, which are utilized for the biosynthesis of new DNA in every cell that is undergoing DNA synthesis. Incorporated formaldehyde can be separated from covalently bound formaldehyde by hydrolysis of the DNA and HPLC (Casanova et al., 1989). The amount of $^{14}C$ incorporated into the DNA is reflective of the number of cells undergoing DNA replication.

Although the amount of $^{14}C$ incorporated into DNA is a measure of DNA synthesis and increases with increasing dose, the rate of increase declines with increasing concentrations of formaldehyde (Casanova et al., 1994). At low formaldehyde concentrations (< 2 ppm), this can be ascribed to saturation of metabolic pathways, i.e., the concentration of $[^{14}C]$formaldehyde in the nasal epithelium becomes high enough locally to saturate the pathways leading to the incorporation of
14C into DNA. However, a toxicologically more important factor, particularly at high concentrations, is the inhibition of DNA replication by DPX, as discussed below. This inhibition is likely to be a major cause of formaldehyde-induced mutations (Heck and Casanova, 1999).

Measurements of the incorporation of 14C into the nasal mucosal DNA of rats and monkeys have revealed an important inhibitory effect of formaldehyde on the replication of DNA. The inhibition of DNA replication by DPX could be explained by several mechanisms. In one possible mechanism, formaldehyde could form a cross-link between DNA and an associated histone molecule (a reaction that has been studied extensively (Ohba et al., 1979; O'Connor and Fox, 1989)), which would prevent the histone from dissociating from the DNA. Since histone dissociation is necessary for DNA replication to proceed (Alberts et al., 1983), replication would be blocked, thereby decreasing the amount of 14C incorporated into DNA in portions of the DNA that contain DPX.

As noted by Permana and Snapka (1994), blockage of DNA replication by a DNA-protein cross-link would not necessarily result in cell death. Rather, such damage could be repaired completely or with accompanying errors leading to mutation or deletion. Lethality would occur only if an essential gene was inactivated by the mutation or deletion. Carcinogenesis could result from damage to a protooncogene or tumor suppressor gene. Blockage of DNA replication would be expected to be followed by the removal of that portion of the DNA that contains the cross-link. This removal could result in a deletion if the parental strand were not replicated exactly. The induction of large and small deletions in DNA is one of the primary mutational effects of formaldehyde in eukaryotic cells (Benyajati et al., 1983; Crosby et al., 1988).

Point mutations in the p53 tumor suppressor gene were identified in 5 of 11 tumors taken from the noses of rats exposed to formaldehyde (Recio et al., 1992). Although the point mutations occurred at different sites in the p53 gene, all of the mutations were apparently homozygous at the cDNA level. This is suggestive of a deletion of one copy of p53 and a point mutation in the other. The deletion-point mutation hypothesis is clearly not restricted to p53 but could occur with any critical gene. In addition, other mechanisms of mutation that do not involve DPX at all are also possible. For example, mutations might be induced through cytolethality and sustained cell proliferation at high formaldehyde concentrations. However, these mechanisms are unlikely to play an important role at low concentrations of formaldehyde (≤ 2 ppm) that are not cytolethal and do not induce cell proliferation.
Characteristic genotoxic effects of formaldehyde are sister chromatid exchanges, chromosomal aberrations and micronuclei (IARC, 1995). These characteristic genotoxic effects of formaldehyde suggest that DNA-protein cross-links could play an important mechanistic role in the development of formaldehyde-induced tumors.

**Integrated Assessment of Mode of Action**

Formaldehyde is likely to be carcinogenic to humans by the route of inhalation only at excessively high and prolonged exposures. It is not likely to be carcinogenic to humans under low exposure conditions, specifically, those exposures that do not cause cytotoxic effects. The weight of evidence of human carcinogenicity following inhalation exposure is based on (a) limited evidence of an association between exposure to formaldehyde and nasopharyngeal cancers, (b) indisputable evidence in rats for the induction of nasal cancer by high concentrations of formaldehyde; and (c) the weak genotoxic potential of formaldehyde.

The mode of action of formaldehyde is not fully understood. Although formaldehyde exhibits weak genotoxic activity, whether this contributes to an increased risk at low exposure levels is not clear. Nonetheless, clearly the dose-response relationship for effects mediated by formaldehyde exposure is highly nonlinear, including that for the induction of cancer. Therefore, the dose-response assessment should assume nonlinearity. A biologically-based model that reflects this nonlinearity and incorporates the extensive mechanistic data and state-of-the-art analyses for species-specific dosimetry will provide the best basis for a quantitative dose-response characterization.

**Regional Respiratory Tract Dosimetry Modeling of Formaldehyde**

Site-specificity of HCHO-induced lesions in rats and primates implicates regional tissue susceptibility and dose as major factors contributing to tissue damage. The observation that lesions occur only in areas lined by epithelial types other than squamous suggests that these regions may be more susceptible to damage than areas lined by squamous epithelium. Species-specific patterns of lesions in rats and primates suggest that regional dose is a factor. Regional dose is a function of the amount delivered by inhaled air and the absorption characteristics of the nasal lining. The amount delivered by inhaled air depends on major airflow patterns, air-phase...
diffusion, and absorption at the air-lining interface. Dose to cells depends on the amount absorbed at the air-lining interface, mucus/tissue-phase diffusion, chemical interactions such as reactions and solubility, and clearance rates. Species differences in these factors determine species-specific lesion distributions. For the current risk assessment effort, site-specific estimates of formaldehyde dose to the upper respiratory tract (URT) (nares to larynx) are needed for rats. For humans, estimates of formaldehyde dose to the tissue are needed throughout the entire respiratory tract.

Upper Respiratory Tract Dosimetry for Formaldehyde: The F344 rat and rhesus monkey nasal surface for one side of the nose and the nasal surface for both sides of the human nose were mapped at high resolution as part of an effort to develop three-dimensional, anatomically-accurate computational fluid dynamics (CFD) models of rat, primate, and human nasal airflow and inhaled gas uptake (Kimbell et al., 1997; Kepler et al., 1998; Subramaniam et al., 1998). The approximate locations of squamous epithelium and the portion of squamous epithelium that is mucus-coated have been mapped onto the reconstructed nasal geometry of the CFD models. These CFD models provide a means for estimating the amount of inhaled gas reaching any site along the nasal passage walls and allow direct extrapolation of exposures associated with tissue damage in animals to human exposures via regional nasal uptake.

The CFD models allow the entire interior surface of the nose to be partitioned or segregated by the flux, or rate of transport, of chemical passing from the inhaled air into the airway wall. The amount of nasal surface area receiving a specific range of flux values (called a flux bin) can be compared across species and used in mathematical models that predict tumor response as a function of the number of cells at risk. An arbitrary number of flux bins can be defined up to the limit imposed by the spatial resolution of the CFD model; sensitivity analyses showed stable results with 15 or more bins. We used 20 flux bins for all nasal dosimetry simulations.

Rat, primate, and human nasal epithelial types were assumed to be similar in all characteristics except thickness, extent, and location in nasal passages. Species-specific thickness, extent, and location were estimated from the literature or by direct measurements. The nasal passages of all three species were assumed to have a continuous mucus coating over all surfaces except specific areas in the nasal vestibule. The location and extent of nonmucus-coated regions were estimated in each species from descriptions given by Morgan and colleagues (Morgan et al., 1984), Harkema and colleagues (Harkema et al., 1987; Harkema, 1992), and Mygind and colleagues (Mygind et al., 1982), respectively.
The major patterns of inhaled airflow during resting breathing were considered to be similar to those seen at steady-state (Subramaniam et al., 1998). We conservatively assumed that steady-state inspiratory airflow patterns at flow rates equal to twice the resting minute volumes of rats, monkeys, and humans provided reasonable estimates for inspiratory airflow patterns during resting cyclic breathing. Simulations of formaldehyde uptake in the entire respiratory tract over a single breathing cycle showed that formaldehyde exhaled from the lower respiratory tract (LRT) does not significantly affect dose to nasal tissues. Thus, we assumed that HCHO was absorbed only during inspiration so that for each breath, flux into nasal passages walls was assumed to be zero during exhalation.

The air-phase diffusivity of HCHO was 0.15 cm²/s and was assumed to be constant throughout the nasal passages of all three species. We assumed that the rate of HCHO transport into tissue far exceeds mucus velocity rates, so that mucus transport was not included in the CFD models. The rate of HCHO absorption at air-lining interfaces was considered to be proportional to the air phase HCHO concentration adjacent to the nasal lining layer, where the constant of proportionality, called a mass transfer coefficient, depended on whether the nasal lining was mucus-coated or not. A mass transfer coefficient for nonmucus-coated squamous epithelium was estimated from measurements of HCHO absorption made for human epidermal tissue. A mass transfer coefficient for mucus-coated regions in the primate and human was assumed to be the same as the value obtained for the rat by fitting overall HCHO uptake predictions in the rat to HCHO uptake data measured in the rat (Patterson et al., 1986).

Steady-state airflow simulations were carried out at flow rates equivalent to twice the estimated minute volume in all three species for comparison purposes (0.576 L/min in the rat, 4.8 L/min in the monkey, and 15.0 L/min in the human) by using a fluid dynamics analysis package (FIDAP) to solve the full Navier-Stokes equations of motion for incompressible Newtonian fluid flow in the three nasal geometries. Estimates of HCHO uptake were also needed at additional flow rates in the human corresponding to an ICRP66 (1994) activity pattern for a heavy working adult male (cyclic breathing flow rates of 7.5 L/min, 9 L/min, 25 L/min). Airflow simulations compared well with descriptions and measurements of flow in nasal molds for each species (Kimbell et al., 1997; Kepler et al., 1998; Subramaniam et al., 1998).

Regional uptake of inhaled HCHO was simulated in each species with the mass transfer coefficient for the nonmucus-coated region, \( k_{nm} \), set to 0.41 cm/s, and the mass transfer coefficient for the mucus-coated remainder of the nose, \( k_m \), set to 4.7 cm/s.
Overall uptake of inhaled HCHO by the rat nose at a steady-state flow rate of 0.576 L/min was 90% (rat uptake was fitted to 97% at 0.288 L/min). With $k_m = 4.7$ cm/s in the monkey and human, HCHO uptake by the nose was estimated to be 67% in the primate and 76% in the human at steady-state airflow rates of 4.8 L/min in the primate and 15.0 L/min in the human. The maximum HCHO wall mass flux value averaged over the breathing cycle obtained in the nonsquamous epithelium of each species at twice the minute volume flow rate was 2,620 pmol/mm$^2$/hr/ppm in the rat, 4,492 pmol/mm$^2$/hr/ppm in the monkey, and 2,082 pmol/mm$^2$/hr/ppm in the human. Comparison of average flux estimates in the entire human nose for several flow rates with nasal uptake predicted by the LRT model showed good agreement, indicating that calibration of the LRT model to the CFD nasal model was appropriate. The flux values determined for the upper respiratory tracts of rats and humans were used in the two-stage clonal growth model to determine potential human risk for nasal cancer.

*Human Respiratory Tract Dosimetry For Formaldehyde:* For the human, regional flux in the entire respiratory tract is needed to characterize dose for risk assessment purposes. In addition to the human nasal CFD model, a typical-path, one-dimensional model of formaldehyde uptake was developed for the LRT. The LRT model consisted of the tracheobronchial and pulmonary regions in which uptake was simulated for four ventilatory states based on an ICARP66 (1994) activity pattern for a heavy working adult male. Nasal uptake in the LRT model was calibrated to match overall nasal uptake predicted by the human CFD model.

For examining lung cancer risk in humans, some model adjustments had to be made in order to estimate formaldehyde flux to tissues in the LRT. While rodents are obligate nasal breathers, humans switch to oronasal breathing when the level of activity requires a minute ventilation of about 35 L min$^{-1}$. Thus, two anatomical models for the URT, one for oral breathing and one for nasal breathing were needed, each of which was basically a tubular geometry. For the mouth cavity, the choice of tubular geometry is consistent with Fredberg et al. (1980). The rationale for using the simple tubular geometry for the nasal airway is due mainly to the need to remove HCHO from the inhaled air at the same rate as does a corresponding three-dimensional CFD simulation. This is because, in the risk assessment calculations, the nasal airway fluxes predicted by the CFD simulations, not those predicted by the single path model, were used for the URT fluxes.

In order to account for oronasal breathing, two simulations were used. In one simulation, the nasal airway model was used for the proximal URT and in the other, the
mouth cavity model was used for this region. In both simulations, the fractional airflow rate that occurred in the mouth cavity or in the nasal airway was taken into account. The airflow rate in all segments distal to the proximal URT was not reduced, but corresponded to that expected for the full minute volume. The same airflow rate split was assumed for both inhalation and exhalation.

For each segment distal to the proximal URT, the dose (fluxes) of HCHO from both simulations were added to obtain the estimated dose for oronasal breathing. Testing this method using the present oral and nasal airway models would have been possible if dosimetry models existed that simultaneously accounted for the two paths in one simulation. Since this was not feasible, special anatomical models of the regions were devised for testing. Test results indicate that the approach should give reasonable respiratory tract dose estimates for oronasal breathing.

As expected, the highest fluxes are predicted for the most proximal airways, the nasal airways and mouth cavity. As minute volume increases, HCHO penetrates further into the respiratory tract. For nasal breathing, the average nasal airway flux is at least 3 times greater than predicted for any other model segment or generation. During oronasal breathing (50 L/min minute volume), 46% of the inhaled air flows through the nasal airways (ICRP66, 1994), which corresponds to a rate that is less than for the 25 L/min nasal breathing state. This results in the nasal flux during oronasal breathing being less than that for the 25 L/min minute volume nasal breathing state.

Simulations were carried out for four ventilatory states based on an ICRP66 (1994) activity pattern for a heavy-working adult male and were used to account for various activities throughout a day. Defining the representative human as a heavy-working adult male should result in conservative risk estimates. The formaldehyde flux values generated by the dosimetry model were used in the two-stage clonal growth model to predict the risk of cancer in humans.

**Biologically-Based Approach to Dose-Response Assessment: Case Specific Model for Formaldehyde**

The goal of this dose-response assessment for formaldehyde carcinogenicity was to minimize risk assessment uncertainties by maximizing the use of scientific data. Anatomically realistic models were used to describe the regional dosimetry of formaldehyde in the nose of the F344 rat and in the entire respiratory tract of humans. Sufficient anatomical detail was captured in these models to accurately describe the effects of interspecies differences in anatomy on respiratory tract dosimetry of
formaldehyde. In addition, the human dosimetry model describes how the regional dosimetry of formaldehyde is affected by variations in breathing rate on an hourly basis as a function of activity level.

Data on precursor and neoplastic effects of formaldehyde were used in the context of a two-stage model of carcinogenesis involving clonal expansion. Formaldehyde dose to target cells is linked to specific parameters of the clonal growth model. Separate clonal growth models have been developed for rats and for humans. The human model predicts additional risk of formaldehyde-induced cancer in the respiratory tract for various scenarios. The final clonal growth model includes a directly mutagenic effect of formaldehyde and provides a good description of the high dose rodent tumor data. The model describes a low dose linear response for both rats and humans at exposure levels where cytotoxicity and regenerative proliferation do not play a role.

Two modes of carcinogenic action are described. First, formaldehyde is assumed to act as a direct mutagen. The intensity of the direct mutagenic effect is described in the model as being proportional to the tissue concentration of DPX. The dose-response curve for DPX formation is linear in the low dose region and increases in a greater-than-linear manner at the high doses that were used in the rodent bioassays. Thus, the overall dose-response model for formaldehyde has a linear low dose component related to DPX formation. The second mode of action is based on formaldehyde being a potent cytotoxicant, with cell killing being followed by regenerative cellular proliferation. A hockey stick dose response curve (i.e., dose threshold curve; Cornfield, 1977) for regenerative cellular proliferation is used. These modes of carcinogenic action are embedded in the two-stage clonal growth cancer model.

Various sensitivity analyses were conducted that examined the validity of assumptions or of choices made between alternative approaches to model development. For example, we tested the assumption that tumors are rapidly fatal by optimizing the rat clonal growth model using the likelihood for incidental (i.e., nonfatal) tumors. The resulting optimal fit was visually much poorer than the fit obtained assuming that the rat nasal tumors are rapidly lethal.

When the cell proliferation data measured in various sites in the nose of the rat were expressed as unit length labeling indices (ULLI, i.e., cell labeling data expressed as number of labeled cells per millimeter of basement membrane examined) and were plotted against formaldehyde concentration, a J-shaped curve was obtained. Since tumor risk predicted by clonal growth models is sensitive to cell kinetics, we used both
the J-shaped data and a hockey stick transformation of the data for risk predictions to evaluate the sensitivity of predicted risks to the shape of the ULLI data curve. Use of a hockey stick model is conservative for risk prediction compared to a J-shaped model. The inflection point in the hockey stick-shaped ULLI data is set at 2 ppm. An optimal fit of a hockey stick to the ULLI data has its inflection closer to 5 ppm. Our use of the lower inflection point is conservative as it means that formaldehyde exposure increases cell division rates at lower concentrations than would be the case if we were using the optimal hockey stick. This is a minor point with respect to predictions of human risk as the only situation addressed in this document where human exposure is greater than 2 ppm is in the analysis of the epidemiological data. A relatively small number of individuals in the highest exposure quintile in the Wallingford, CT cohort were exposed to 2.2 ppm (NCI exposure estimates).

Two of the parameter values of the human model (the probability of mutation per cell division and the growth advantage for preneoplastic cells) are estimated statistically by fitting the model to human 5-year age group lung cancer incidence data. Incidence data for smokers, nonsmokers, and a mixed cohort of smokers and nonsmokers are available. The model was adjusted to fit these different data sets by varying the values of the two parameters. In the clonal growth model, as currently defined, the values of these parameters are also affected by formaldehyde. Since smoking and formaldehyde both affect the same parameters, predicted risks associated with formaldehyde exposure are affected by smoking status. In other words, the model describes interactions between smoking behavior and formaldehyde exposure. The difference between smokers and nonsmokers in additional cancer risk due to formaldehyde exposure is predicted to be about a factor of 10.

The human clonal growth model using hockey stick-shaped ULLI predicted nonzero additional risk throughout the exposure ranges examined. The dose-response curves for environmental exposures (0.001 to 0.1 ppm continuous for 80 yr) are linear, reflecting the linear behavior of the DPX model over this concentration range. Formaldehyde-related effects on cell proliferation do not play a role in the risk predictions for exposures between 0.001 and 0.1 ppm. The predicted dose-response curves for occupational exposure are nonlinear between 0.1 and 1.0 ppm. We have not fully investigated the relative contributions of cell division rates and DPX to this nonlinearity, but the nonlinear formation of DPX is likely the driving force. A summary of predicted additional risk of respiratory tract cancer for various environmental and occupational exposures to formaldehyde is given in the following table.
Predicted human additional risk of respiratory tract cancer due to environmental and occupational exposures to formaldehyde.

<table>
<thead>
<tr>
<th>Formaldehyde Exposure Concentration (ppm)</th>
<th>Environmental</th>
<th>Occupational</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-smoking</td>
<td>Mixed</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>---------------</td>
<td>-------</td>
</tr>
<tr>
<td>0.001</td>
<td>2.3X10⁻¹⁰</td>
<td>3.9X10⁻⁹</td>
</tr>
<tr>
<td>0.02</td>
<td>4.8X10⁻⁹</td>
<td>1.0X10⁻⁷</td>
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<tr>
<td>0.04</td>
<td>1.0X10⁻⁸</td>
<td>2.1X10⁻⁷</td>
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<td>0.06</td>
<td>1.5X10⁻⁸</td>
<td>3.3X10⁻⁷</td>
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<td>0.08</td>
<td>2.1X10⁻⁸</td>
<td>4.5X10⁻⁷</td>
</tr>
<tr>
<td>0.10</td>
<td>2.7X10⁻⁸</td>
<td>5.8X10⁻⁷</td>
</tr>
<tr>
<td>0.30</td>
<td>---³</td>
<td>---</td>
</tr>
<tr>
<td>0.50</td>
<td>---³</td>
<td>---</td>
</tr>
<tr>
<td>0.70</td>
<td>---³</td>
<td>---</td>
</tr>
<tr>
<td>1.00</td>
<td>---³</td>
<td>---</td>
</tr>
</tbody>
</table>

³80 year lifetime continuous exposure at indicated ppm.

³³80 year lifetime continuous exposure at 0.004 ppm with 40 years occupational exposure (8hr/day, 5 days/week) at indicated ppm beginning at age 18 years. ICRP66 (1994) "light working" breathing pattern.

³³³Simulations not done.

The 2-stage clonal growth model is a parameter- and data-rich model in the context of cancer risk assessment. Even with the richness of the formaldehyde data, calculating all of the parameters of the clonal growth model directly from data is not possible. Some parameter values were estimated by calculating the maximum likelihood of the data. Further, there were points in the model development process where choices between alternative possible approaches were required. If insufficient
information was available to make the choice on a scientific basis, we attempted to identify the alternative that would lead to a prediction of higher risk in the final model. This means that, overall, the final clonal growth model should be conservative in the sense that it tends to overpredict risk in the face of scientific uncertainty. The predicted risk levels for formaldehyde are considerably lower than in previous cancer risk assessments. This is in general the expected behavior when assessments based largely on default assumptions are replaced by more mechanistically based assessments.

Overall Conclusions

Mode of Action

Formaldehyde induces DNA-protein cross-links (DPX) and cytotoxicity that results in regenerative cell proliferation. All of these changes are highly nonlinear with flux. Cancer is a genetic disease that requires a cell to accumulate mutations in several genes (frequently unknown) critical for tumor development. DPX are proposed to be able to cause mutations as a result of errors of DNA replication on the damaged template. At low concentrations (fluxes) of formaldehyde, the DNA replication rate will be the normal rate of cell turnover that, with the low induced frequency of DPX, will lead to a very low to negligible mutation frequency. At higher concentrations of formaldehyde when cytotoxicity is induced, the probability of a DPX giving rise to a mutation via DNA replication is much higher. The flux-response curve for mutations will be highly nonlinear. Thus, the mode of action for tumor induction at higher concentrations is different from that at low concentrations because of the involvement of regenerative cell proliferation. The present model incorporates both directly mutagenic and cytotoxic modes of action. Direct mutation is described as being proportional to DPX while cytotoxicity is based directly on cell replication data.

Clonal Growth Model

The goal of the dose-response assessment used for formaldehyde carcinogenicity was to minimize risk assessment uncertainties by maximizing the use of scientific data. Anatomically realistic models were used to describe the regional dosimetry of formaldehyde to specific regions of the respiratory tracts of rats and humans. These models also incorporated information on human breathing rates and patterns as a function of activity level. Predictions of the regional dosimetry of
formaldehyde in the respiratory tract thus reflect a daily activity pattern described on an hourly basis.

Data on precursor and neoplastic effects of formaldehyde were used in the context of a two-stage clonal growth cancer model. Formaldehyde dose to target cells is linked to specific parameters of the clonal growth model. Separate clonal growth models were developed for rats and for humans. The human model predicts additional risk of formaldehyde-induced cancer in the respiratory tract for various scenarios. Two modes of carcinogenic action are described. First, formaldehyde is assumed to act as a direct mutagen. The second mode of action is based on formaldehyde being a potent cytotoxicant, with cell killing being followed by regenerative cellular proliferation. These modes of carcinogenic action are embedded in the clonal growth model. The dose-response behaviors defined by the airflow, DPX, and regenerative cellular proliferation models directly affect the parameters of the clonal growth model.

Dose-response assessments using the human clonal growth model were developed for environmental exposures (continuous exposure throughout an 80-year lifetime) and for occupational exposures (8 hr/day, 5 day/week exposures for 40 years beginning at age 18). The occupational exposures were described as occurring simultaneously with a 0.004 ppm environmental background level of formaldehyde. The human clonal growth model predicted nonzero additional risk throughout the exposure ranges examined. The difference in predicted risk between smokers and nonsmokers is large, being somewhat more than a factor of 10 across the formaldehyde concentration ranges examined. The dose-response curves for environmental exposures are linear, reflecting the linear behavior of the DPX model over this concentration range. Formaldehyde-related effects on cell proliferation do not play a role in the risk predictions between 0.001 and 0.1 ppm. Overall, the predicted risk levels for formaldehyde are considerably lower than in previous cancer risk assessments. For example, in the 1991 assessment by EPA, additional risks from continuous exposure to 0.1 ppm formaldehyde varied between $3.3 \times 10^{-5}$ and $2.8 \times 10^{-4}$, depending upon which model was used. Here, the assessment of additional risks for such an exposure scenario is $2.7 \times 10^{-8}$ for nonsmokers and $6.7 \times 10^{-7}$ for smokers.

Efforts were made to determine if the clonal growth model provides cancer risk estimates for occupational exposure to formaldehyde that are consistent with the results of epidemiological studies. For the analyses undertaken, the clonal growth modeling results indicate that none of the lung cancer or total respiratory tract cancer deaths can be attributed to exposure to formaldehyde.
Benchmark Dose Model

Benchmark dose (BMD) analysis is a statistical curve-fitting approach that uses a mathematical model structure fit to experimental data. Two approaches to interspecies extrapolation were taken in this analysis. In both approaches a benchmark dose (exposure level) is first identified in rats. The first approach then used computational fluid dynamics (CFD) modeling techniques to extrapolate directly from the benchmark dose based on rat data to an equivalent dose in humans. The second approach estimated the concentration of DNA-protein crosslinks (DPX) in rats at the end of a 6-hr exposure to the benchmark dose using a HCHO flux-DPX pharmacokinetic model. A human flux-DPX model was then used to estimate the human HCHO dose that generated an equal level of DPX (after a 6-hr exposure) as noted for the rat.

A benchmark dose (BMD) was calculated for either the combined nasal squamous cell carcinomas (SCC) incidence data of Kerns et al. (1983) and Monticello et al. (1996) or for the time-weighted, site-averaged unit-length labeling index (ULLIs) data of Monticello et al. (1996). Among the mathematical structures used, the Weibull model with a variable x-intercept produced the best fits to the tumor data. Based on the rodent tumor incidence data, the upper bound on the estimates of 1% extra risk for humans exposed to 0.1 ppm formaldehyde is $4.2 \times 10^{-4}$ when extrapolating using the flux-DPX model. In contrast, the airflow only extrapolation approach to calculating the BMD produced an estimate of $2.5 \times 10^{-4}$ as the upper bound on risk for exposure to 0.1 ppm formaldehyde. As one would expect, BMD modeling of a precursor endpoint such as the unit-length labeling index of cells in the nose of rats results in upper bound risk estimates for such events that are somewhat greater than the risk estimates for tumors (e.g. upper bounds on 1% extra risks of $7.9 \times 10^{-4}$ and $3.9 \times 10^{-4}$ for BMDs using flux-DPX modeling and CFD extrapolation, respectively).

Advantages of a Biologically-Based Approach to Risk Assessment

The clear advantage of using a biologically-based model is that it can provide more accurate prediction of the tumor response as a function of flux (or concentration) at low levels. The model takes into account information on the cancer process itself, namely that it involves multiple stages together with the proposed mode-of-action whereby formaldehyde can produce the genetic alterations required to obtain the various steps in this multistep cancer process. In this same vein, it allows for more accurate predictions of effects in humans based on data obtained in laboratory animals. These are predictions of effect (tumors) as well as dose to the target cells...
The use of a biologically-based model clearly demonstrates the nonlinearity of tumor response. This argues against the use of a linear extrapolation from the lowest point of observation for tumors.

Interspecies extrapolation of the clonal growth model involves using human data directly or indirectly for identification of several model parameters. The biologically-based structure of the clonal growth model facilitates the use of these data. In benchmark dose modeling, data on tissue dosimetry can be used for interspecies extrapolation, thereby gaining some of the advantages that biologically-based models offer. However, in general, the lack of a specific description of mode of action within the benchmark dose model makes incorporation of relevant mechanistic data more difficult.

The clonal growth model describes interactions between smoking behavior and formaldehyde exposure with respect to predictions of human respiratory tract cancer risk. These predictions are based on mechanistic considerations and not on cancer incidence data for humans exposed to mixtures of formaldehyde and cigarette smoke. Separate benchmark dose models could adequately describe the individual cancer incidence data sets for smokers and nonsmokers, but a benchmark dose model could not, as a consequence of its structure, predict an interaction between smoking and formaldehyde exposure. The biologically-based approach we have taken creates new possibilities for predicting cancer risks for important real world scenarios such as combined cigarette smoking and formaldehyde exposure.

Sources of Uncertainty

A major byproduct of the current risk assessment is a reduction in uncertainties associated with estimating potential cancer risks to humans from exposure to formaldehyde gas. Worth noting is that specific sources of uncertainty can be identified in the framework of biologically-based modeling to a far greater extent than can usually be done using default or benchmark dose methodologies. The increased ability to identify uncertainty sources using biologically-based modeling does not mean that uncertainty is increased. The informed use of biologically-based models in risk assessment represents an inherent reduction in uncertainty because uncertainty sources can be identified and quantified.

For formaldehyde dosimetry, parameter estimates that have low uncertainty are the values of numerical simulation parameters, the air-phase diffusivity of formaldehyde, and activity pattern and ventilation parameters. Anatomical issues that do not contribute significantly to uncertainty are the assumption that the distributions of
epithelial types and of mucus- and nonmucus-coated nasal regions remain constant in
time and the use of a typical-path human lung structure to represent healthy children
and adults.

Sources of uncertainty in dosimetry for which sensitivity analyses are
appropriate include the use of individual rat, primate, and human nasal anatomies as
representative of the general population, the use of a typical-path human lung
structure to represent people with compromised lungs, the sizes of specific lung
airways, the use of a symmetric Weibel model for lung structure, the estimation of the
location and extent of squamous and olfactory epithelium and of mucus- and
nonmucus-coated nasal regions in the human, and the values of mass transfer and
dispersion coefficients.

A major finding of the analyses conducted to determine the sensitivity of risk
estimates to the number of bins used to characterize the dose (flux) of formaldehyde to
specific sites in the rat nose is particularly worth of comment here. Site-specific nasal
lesions observed in species-specific patterns (rats and primates) suggest that there
might be significant regional and species-specific variation in formaldehyde uptake.
Our sensitivity analyses showed that the use of one flux bin (as used previously by the
U.S. EPA), which has been the default approach in risk assessment, substantially
underestimates the additional risk in rats. Further, the use of more than 15 bins does
not significantly change risk estimates from the values obtained using 15 bins. Thus,
our ability to incorporate regional variation in nasal formaldehyde flux into risk
estimates significantly reduced uncertainty for both uneven formaldehyde distributions
within the nose and species-specific differences in nasal distributions.

The relative merits of the BMD and clonal growth modeling approaches can be
compared with respect to interspecies and low dose extrapolations. In BMD modeling,
a point of departure is identified that is either within, or at worst not far from, the data to
which the model is fit. With formaldehyde, the extrapolation from the point of departure
should be consistent with its two modes of action—cytotoxicity/regenerative cellular
proliferation and direct mutation.

Unlike the BMD model, integration of the 2-stage clonal growth model with the
dose-response submodels for the two modes of action provides a single exposure-
response model that predicts tumor risk across the full exposure range of interest.
Comparison of the uncertainty of the BMD and clonal growth approaches hinges on
how one evaluates their relative abilities to accurately describe the combined dose-
response behavior of the two modes of action in the region of low dose extrapolation.
With respect to interspecies extrapolation, the biologically motivated structure of the clonal growth model lends itself naturally to being scaled up from rodents to humans. Anatomically realistic CFD models are available for both the rat and human noses, and a single path model is available for the human lung. The availability of data on the incidence of human lung cancer allows the formaldehyde-independent parameters of the human clonal growth model to be calibrated directly against human data. The lack of human data on formaldehyde related changes in the values of key parameters of the clonal growth model is a limitation that accounts for much of its uncertainty. In the longer run, this limitation may be viewed as an advantage as it leads to identification of specific data gaps that may be filled by targeted experimentation. Interspecies scale-up of BMD models is inherently more problematical as there usually is no underlying biological structure to manipulate.

The main qualitative difference between the biologically-based clonal growth and BMD models is in the flexibility that the biologically-based approach provides in bringing scientific data to bear to address key issues in extrapolation and interactions with host-specific factors such as cigarette smoking. When the data needed to support development of a biologically-based model are not available, BMD modeling may be the preferred alternative. The limitations of BMD modeling relative to biologically motivated clonal growth modeling should be kept in mind though, as they suggest that considerably more uncertainty is generally associated with the benchmark approach.

The biologically-based clonal growth model is deemed to be the best approach for providing realistic risk estimates for formaldehyde exposure to humans. The risk estimates developed maximize the use of mechanistic data in a quantitative model. The two-stage biological structure on which the model is based is an over simplification of the actual mechanism by which formaldehyde-induced tumors arise, but the model incorporates vastly more scientific data than any previous cancer risk assessment. The two-stage clonal growth model is a much more realistic representation of the carcinogenic process than can be represented in a benchmark dose model. The incorporation within the clonal growth model structure of both mutagenic and cytotoxic modes of action is a significant advance over previous assessments. Overall, the predicted risk levels for formaldehyde are considerably lower than in previous cancer risk assessments. This is in general the expected behavior when assessments based largely on default assumptions are replaced by more mechanistically-based assessments.
**Additional Research for Reducing Uncertainty**

Reducing the uncertainties in any risk assessment model requires knowledge of the mechanisms underlying the adverse health outcome, in the present case, formaldehyde-induced tumors. In addition, a quantitative assessment of the parameters incorporated into the model is necessary.

In order to better define mode-of-action, establishing a direct relationship between DPX and mutation induction is desirable. In addition, the probability of converting a DPX into a mutation would need to be obtained. The mode by which regenerative cell proliferation is involved in the production of mutations required for tumor development has yet to be determined. Also, data are not available on the time course of DPX loss in rats or monkeys. Uncertainties in the use of allometric scaling to derive human metabolic parameters associated with DPX formation are amenable to experimental studies. To better use these quantitative mode-of-action data in a mechanism-based model, knowing which genes are mutated in formaldehyde-induced tumors would be desirable.

Perhaps the most significant area where additional research would lead to a reduction in uncertainty relates to the development of data that would allow one to judge whether the hockey stick-shaped or J-shaped curve is the most appropriate to relate ULLI to formaldehyde exposure level. Difference in risk predictions between the hockey stick-shaped and J-shaped versions of the human model were dramatic. The J-shaped model predicted no environmental or occupational additional risk over the concentration ranges examined (i.e., the risk predictions of the J-shaped model were below control). This behavior of the model reflects the powerful influence of cell kinetics on risk predictions in clonal growth models. Uncertainties in the overall dose-response assessment could be reduced by additional experimental work to determine if the J-shaped ULLI curve is reproducible. In addition, data on how the ULLI curve changes as a function of exposure scenario would be extremely useful. These data would allow the risk assessment to incorporate more fully the competitive processes of injury and repair.

There is a clear need for additional basic biological data in a number of areas. The lack of data on nasal epithelial density of cells in the rat and human required the current assessment to use data on tracheal cell density and assume that the data are applicable to the nose. Also, quantitative data on the specific stages and on the total cell cycle time in rats and humans for respiratory tract epithelial cells are needed. The lack of such data required a compilation and synthesis of information across various species and an assumption concerning the reasonableness of using information in the
two-stage clonal growth model. Inherent in the analyses conducted here is the assumption that human nasal cells will respond like rat nasal cells if exposed to the same dose of formaldehyde. Experimental data are needed to establish the appropriateness of this assumption.

The computational fluid dynamics (CFD) models for the rat and human that were used to determine airflow patterns and formaldehyde flux values were developed from measurements on a single rat as well as human. Quantification of anatomical sources of uncertainty involves determining the extent to which normal variation in these model inputs affects risk estimates.

Summary

As our understanding increased on how different modes of action can lead to carcinogenic outcomes, the suitability of the LMS model for use in carcinogenic risk assessments became controversial. In 1996, the U.S. EPA issued new draft guidelines for carcinogenic risk assessments. These latest guidelines are oriented around the objective of minimizing risk assessment uncertainty by maximizing the use of scientific data. Benchmark dose modeling was introduced as a default methodology for use when sufficient data are not available to develop a biologically-based dose-response model. In addition, for chemicals for which a rich database is available, the U.S. EPA guidelines provided for the development of case-specific models.

While we have included several BMD models as default alternatives, our primary emphasis was on the development of a case-specific model for formaldehyde carcinogenicity using a two-stage clonal growth model. The model incorporates data on normal growth curves for rats and humans, cell cycle times, and cells at risk in the different regions of the respiratory tract. Additional animals from one of CIIT's cancer bioassays were examined for nasal tumors so that statistical power could be increased in the use of formaldehyde-induced tumor incidence data. Formaldehyde-induced cell proliferation and DNA-protein cross-link data were integral to our analyses. Computational fluid dynamics (CFD) models of the rat and human noses were used to predict regional formaldehyde flux. Lower respiratory tract flux was predicted in humans using a single-path model of the nasal, oral, and lung airways. The rat CFD model was used to estimate a relationship between regional formaldehyde dose and enhanced cell proliferation rates measured regionally in rats exposed to 0, 0.7, 2, 6, 10, and 15 ppm. This result was used with formaldehyde flux predictions throughout the rat nose and human respiratory tract to infer regional cellular proliferation rates.
Maximum likelihood estimate methods were implemented to fit the clonal growth model to cancer incidence data. A number of sensitivity analyses were run to determine the significance of specific modeling assumptions. Age-adjusted data on the incidence of lung cancer in humans was used to calibrate the human model for background tumor incidence.

The clonal growth model was used to make human cancer risk estimates for workplace and environmental exposures with incorporation of appropriate activity breathing patterns. As can be seen from the following table, risk estimates from the clonal growth model are substantially lower than those from EPA’s previous assessments or from our BMD analyses. However, the reader should keep in mind that the risk estimates in the table are not all totally comparable because of differences in assumptions and methodologies. While the clonal growth model is doubtless an oversimplification of the actual mechanisms of formaldehyde carcinogenesis, it is nevertheless a parameter-rich model in the context of cancer risk assessment. There were points in the model development process where choices between alternative approaches were required. If insufficient information was available to make the choice on a scientific basis, we attempted to identify the alternative that would lead to a prediction of higher risk in the final model. This means that, overall, the final clonal growth model should be conservative in the sense that it tends to overpredict risk in the face of scientific uncertainty.
Evolution of risk assessments for formaldehyde.

Human risk at 0.1 ppm inhaled formaldehyde, 6 h/d, 5 d/wk

<table>
<thead>
<tr>
<th>Risk Assessment</th>
<th>Risk Estimates Upper bound (MLE)</th>
<th>MOEs vs. LEDo1 (MLEo1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal growth modeling(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Workplace scenario(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>1.0x10(^{-7})</td>
<td>—</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>4.1x10(^{-9})</td>
<td>—</td>
</tr>
<tr>
<td>Environmental scenario(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>6.7x10(^{-7})</td>
<td>—</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>2.7x10(^{-8})</td>
<td>—</td>
</tr>
<tr>
<td>CIIT, BMD (1% risk)(^d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flux-DPX modeling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Based on tumors</td>
<td>4.2x10(^{-4}) (3.9x10(^{-4}))</td>
<td>23.6 (25.8)</td>
</tr>
<tr>
<td>Based on labeling index</td>
<td>7.9x10(^{-4}) (5.3x10(^{-4}))</td>
<td>12.6 (18.8)</td>
</tr>
<tr>
<td>Airflow extrapolation only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Based on tumors</td>
<td>2.5x10(^{-4}) (2.4x10(^{-4}))</td>
<td>39.9 (42.3)</td>
</tr>
<tr>
<td>Based on labeling index</td>
<td>3.9x10(^{-4}) (2.9x10(^{-4}))</td>
<td>25.5 (34.2)</td>
</tr>
<tr>
<td>(rat-based, from q1)(^d)</td>
<td>2.8x10(^{-4})</td>
<td></td>
</tr>
<tr>
<td>(rat-based, using full model)</td>
<td>3.1x10(^{-4}) (3.1x10(^{-5}))</td>
<td></td>
</tr>
<tr>
<td>(monkey-based, full model)</td>
<td>3.3x10(^{-5}) (4.2x10(^{-7}))</td>
<td></td>
</tr>
<tr>
<td>EPA, 1987 (U.S. EPA, 1987)</td>
<td>1.6x10(^{-3}) (5x10(^{-7}))</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Clonal growth risk estimates derived from a biologically-based model that incorporated various toxicological, mechanistic, and dosimetric data, that estimated parameters and optimized the likelihood from the data, and that provided an integrated approach to dose-response characterization.

\(^b\) Workplace exposure scenario involved 40 years of exposure to 0.1 ppm for 8 hr per day 5 days per wk beginning at age 18. All nonwork hours from birth to age 80 involved exposure to 0.004 ppm.

\(^c\) Environmental scenario involved 80 years of continuous exposure to 0.1 ppm.

\(^d\) Weibull model calculation with y-intercept.