FINAL DRAFT

FORMALDEHYDE RISK ASSESSMENT UPDATE

June 11, 1991

Office of Toxic Substances
U.S. Environmental Protection Agency
Washington, D.C.

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PREFACE

This document was prepared by the U.S. EPA Office of Toxic Substances with the assistance of the Office of Research and Development, and it provides an updated assessment with data that have become available since publication of "Assessment of Health Risks to Garment Workers and Certain Home Residents from Exposure to Formaldehyde" by EPA in 1987. Various approaches for estimating cancer risk from formaldehyde exposure are discussed. Thus, this document is not intended to replace the 1987 assessment, but its purpose is to provide an evaluation of recent data and risk assessment approaches.

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1.0 EXECUTIVE SUMMARY

The EPA has conducted a review of epidemiologic and toxicological data developed since the release of EPA's "Assessment of Health Risks to Garment Workers and Certain Home Residents from Exposure to Formaldehyde" (EPA, 1987), and has done an evaluation of the potential human health risks due to inhalation of formaldehyde vapor released from pressed wood products in new mobile and conventional homes.

Sensory irritation of the mucus membranes of the eyes and the respiratory tract, and cellular changes in the nasal cavity are the principal noncancer effects of exposure to low airborne concentration of formaldehyde. The latter effect may lead to the secondary complication of respiratory disease. Although there is inadequate information to quantitate the noncancer risks to the general population from inhaled formaldehyde, fewer and less severe responses are expected to be associated with less frequent and less intense exposures. Further, little risk from noncancer effects after exposure to formaldehyde is expected in situations where exposures are ten-fold less than a no-observed effect level, or one hundred-fold less than a lowest-observed effect level in humans.

Precise thresholds for the irritant effects of formaldehyde for the eyes and upper airway have not been firmly established. However, a large number of observations from clinical and nonclinical settings supports a conclusion that the range of airborne formaldehyde concentrations over which most people respond is 0.1 to 3.0 ppm. Recent studies in humans indicate that symptoms of lower airway irritant effects may occur at about 1 ppm or higher, which are slightly lower than levels reported previously (EPA, 1987). Limited data in humans and laboratory animals indicate that low level exposure to formaldehyde may impair nasal mucociliary flow. The inhibition of nasal mucus flow occurred at 0.4 ppm in humans and in laboratory animals at 2 ppm of formaldehyde. Repeated and long-term exposures to formaldehyde vapor may also induce histologic changes of the nasal epithelium (e.g. cell degeneration, inflammation, hyperplasia, squamous metaplasia, and rarely dysplasia). In laboratory animals, the no observed effect level for these cellular changes is about 1 ppm.

EPA has classified formaldehyde as a "Probable Human Carcinogen" (Group B1) in accordance to its Guidelines for Carcinogenic Risk Assessment (1986). Available epidemiologic studies support the conclusion made in 1987 that there is "limited" evidence to indicate that formaldehyde may be a carcinogen in humans. The human evidence for potential carcinogenicity is based mainly on the association of formaldehyde exposure with modest increases of cancers of the nasopharynx, nasal cavity, and sinus. The evidence relied in a

small degree on observations of increases in lung cancer. However, no consistent patterns between formaldehyde exposure and lung cancer are apparent across studies. Further, there is a suggestion from recent analyses that combined exposures to formaldehyde and other substances in resins and molding material processes may be involved in the development of lung cancer.

The epidemiologic evidence is corroborated by experimental findings showing that formaldehyde is genotoxic and carcinogenic to rats and mice. Data developed since 1987 confirm the previous findings that formaldehyde is a nasal carcinogen in rats at high exposure concentrations and provide a better understanding of its mechanism of carcinogenic action, though it is still not completely understood. Evidence from recent inhalation studies in rats indicates that formaldehyde concentration may be more important than the accumulated dose (i.e., concentration x time) in the induction of cellular effects, and suggests that sustained tissue damage and cell proliferation associated with exposure to high concentrations of formaldehyde are important in the induction of nasal tumors. Major advances have also been made in determining the delivered dose in respiratory tract tissues in experimental animals.

In the absence of quantifiable human data, dose-response data in rats at high concentrations were used to estimate low-dose human cancer risk for formaldehyde. The cancer quantitative risk assessment has been modified by using the rate of formation of DNA-protein cross links as surrogates for delivered concentrations in the target cells. EPA has calculated an upper bound inhalation incremental unit risk for lifetime exposure based on rat (2.8×10^{-3} per ppm) and monkey (3.3×10^{-4} per ppm) dosimetry by using the linearized multistage procedure. Of the risk estimate options, EPA favors that based on monkey DPX because the monkey is considered a closer surrogate to humans than the rat. The resultant risk estimates are about 50-fold less than estimates based on airborne exposure concentrations performed in the previous EPA assessment (EPA, 1987). The largest contribution to the reduction in the cancer risk estimates is the use of DNA-protein cross-links data as delivered doses. Limitations and associated uncertainties of the cancer risk estimates are discussed.

2.0 BACKGROUND

A previous review, "Assessment of Health Risks to Garment Workers and Certain Home Residents from Exposure to Formaldehyde" (EPA, 1987), examined the noncancer and cancer effects associated with formaldehyde exposure. EPA conclusions on the noncancer effects associated with exposure to formaldehyde (EPA, 1987) were based mainly upon already-existing reviews by the National Research Council (1981), Consensus Workshop on Formaldehyde (1984), and Interagency Risk Management Council (IRMC, 1984).

The major noncancer human health effects posed by inhalation exposure to formaldehyde were due to the irritating nature of the chemical (EPA, 1987). These effects were sensory irritation and cellular changes. The evidence of cellular damage in humans, although limited, was considered important. A number of lower airway and pulmonary effects, including asthma, may occur with formaldehyde exposure. Formaldehyde-induced cellular changes in the nasal passages of rats, mice, hamsters, and monkeys were reported from a number of studies (EPA, 1987). These changes ranged from rhinitis and epithelial hyperplasia and squamous metaplasia to dysplasia, depending on the duration of exposure, the concentration, and the species tested.

There is also considerable evidence, with inhalation exposure, for the carcinogenicity of formaldehyde in animals. This was based on the increased incidence of a rare malignant tumor, nasal squamous cell carcinoma in two species (rats and mice), and in both sexes of two rat strains (Fischer 344 and Sprague-Dawley), in multiple inhalation experiments at high concentrations.

EPA (1987) detailed the ability of formaldehyde to induce gene mutations in several test systems including viruses, bacteria, yeast, fungi, Drosophila, grasshoppers, and mammalian cells in culture. The document further described the ability of formaldehyde to induce cellular transformation in several systems; its co-mutagenic ability when tested with agents such as x-rays, ultraviolet light, and hydrogen peroxide; and its increased activity in test systems which were deficient in excision repair mechanisms. Formaldehyde was further characterized as inducing both chromosome aberrations (CA) and sister chromatid exchanges (SCE) <u>in vitro</u> as well as causing single-strand breaks in DNA, inhibiting the resealing of single-strand breaks produced by ionizing radiation and inducing cross-links between DNA and protein, in both epithelial and fibroblastic cells. It was proposed that formaldehyde may act both by damaging DNA and by inhibiting DNA repair in treated cells. A review of studies on the metabolic incorporation of formaldehyde versus covalent binding to DNA in treated cells were also included in the document. Human studies which were concerned with the occurrences of CA and SCE in the blood, and mutagenic products in the urine of exposed workers, were also detailed.

In 1987, EPA also reviewed 28 epidemiologic studies and concluded based upon EPA's Guidelines for Cancer Risk Assessment (EPA, 1986) that "limited" evidence existed for an association between formaldehyde and human cancers. The "limited" classification recognizes that a credible argument for a causal association can be made, but that bias, chance, and confounding factors cannot be ruled out. The evidence weighted most heavily for an association with cancers of the upper respiratory tract

(nasopharynx, nasal cavity and sinus, and buccal cavity). A lesser portion of evidence suggested that excesses in lung and brain cancers, and leukemia, may have been associated with formaldehyde exposure in some studies. However, the biological explanation for cancers beyond the site of contact (e.g. brain cancer and leukemia) remained unsupported.

Based on limited epidemiologic evidence for an association of upper respiratory tract cancer with formaldehyde exposure and sufficient animal evidence for an induction of nasal tumors in formaldehyde-exposed animals along with supporting genotoxicity evidence, the EPA classified formaldehyde as a probable human carcinogen (Group B1).

The epidemiologic studies were considered inadequate for quantitative risk assessment. Therefore, the quantitative risk assessment of formaldehyde reported in 1987 was based on the Kerns et al. (1983) F344 rat bioassay, in which nasal squamous cell carcinoma incidences were increased with increasing formaldehyde levels in both males and females (see Table 4.1). The accounting of the tumor incidence and of the number of animals at risk was in accord with the recommendations of the Interagency Risk Management Council (IRMC, 1984). A five stage model was found to fit the data adequately. This process resulted in an upper limit inhalation incremental unit risk estimate of 1.3×10^{-5} per ug/m^3, or 1.6×10^{-2} per ppm.

Casanova-Schmitz et al. (1984) proposed to use the formation of DNA-protein cross-links (DPX) as a surrogate dose for risk estimates based on animal inhalation studies. These authors developed methodology that would allow differentiation between metabolically incorporated and covalently bound formaldehyde (DPX). Metabolic reactions of formaldehyde via the one-carbon pathway <u>in vivo</u> involve the loss of hydrogen from formaldehyde whereas chemical reactions do not. Casanova-Schmitz et al. (1984) had used this fact to sort out the relative amounts of ^{3}H- and ^{14}C-labeled formaldehyde which were metabolized or reacted chemically when administered to rats by inhalation. They concluded that formaldehyde produced DNA-protein cross-linking (DPX) in the rat nasal mucosa at doses of 2 ppm and greater, and that the extent of cross-linking increased with dose in a nonlinear manner from 2 to 6 ppm. They believed these data suggested a potential overestimate of risk at low levels of exposure.

In 1985, the Environmental Protection Agency (EPA), Consumer Product Safety Commission (CPSC) and National Toxicology Program (NTP) published their reservations to the Chemical Industry Institute of Toxicology (CIIT) interpretations of the experimental data, and these were upheld by a panel of expert scientists. Additionally, it was found that whereas ^{2}H had little effect on the rate of formaldehyde metabolism, ^{3}H had a major slowing effect. This isotope effect reduced the accuracy

of the[^{3}H]/[^{14}C] ratio as a quantitative means of distinguishing bound formaldehyde from metabolically-incorporated formaldehyde; this observation along with other critiques led CIIT to develop new methodology (Casanova et al., 1989) in order to support the existence and importance of DPX in the nasal mucosa of rats dosed with formaldehyde. The new information was responsive to the reservations and it has been utilized in this revised assessment.

3.0 METABOLISM AND PHARMACOKINETICS

Formaldehyde may undergo several reactions in vivo. These reactions are characterized as either metabolic or chemical. Metabolic reactions - those which require enzyme catalysis - may be subdivided into the processes of degradation and incorporation. The biotransformations involved in these processes are oxidation to formic acid and carbon dioxide; and incorporation into biosynthetic pathways leading to proteins and nucleic acids. The latter biotransformations utilize either formaldehyde or its metabolite, formic acid. The oxidative step is mediated by formaldehyde dehydrogenase, a glutathione (GSH)-dependent enzyme (Votila and Koivusalo, 1983). The initial step in the oxidative process is formation of S-hydroxymethyl GSH ($GS-CH_2OH$). Hydride abstraction from this intermediate produces S-formyl GSH (GS-CHO). Enzymatic hydrolysis of the thioester, mediated by S-formylglutathione hydrolase, produces formic acid with concomitant regeneration of GSH.

Chemical reactions do not require enzyme participation. They involve interactions of nucleophilic amine, sulfur or hydroxyl groups with formaldehyde. The initial product of reaction is the addition of nucleophile to the carbonyl group of formaldehyde leading to formation of hydroxymethyl derivatives. Amine, sulfur and oxygen nucleophiles form aminals (R_2-N-CH_2OH), thioacetals ($RS-CH_2OH$), and hemiacetals ($R-O-CH_2OH$), respectively. These reactions are reversible, the equilibrium is dependent on pH and the nature of the nucleophile. Hydroxymethyl derivatives of primary amines or amides can react further with either formaldehyde to form N-bis-hydroxymethyl derivatives, or with a second molecule of nucleophile. The latter reaction is particularly favorable with basic amines and produces structures of the type R_2N-CH_2-N in which two molecules of amine are linked by a methylene bridge. This latter transformation is also reversible although more drastic conditions (pH and temperature) are required to hydrolyze the methylene-linked products relative to the corresponding hydroxymethyl derivatives.

Formaldehyde is known to react with amino acids, proteins, nucleotides, RNA and DNA with predominant formation of N-hydroxymethyl derivatives which react further to form methylene-linked products. Cross-links are formed (intermolecularly) between proteins, protein and DNA, and between DNA molecules. DNA-DNA cross-links have been identified in pure DNA preparations incubated with formaldehyde (Chaw et al., 1980), but such direct

cross-links have yet to be identified following treatment of intact cells. Some evidence suggests that indirect DNA-DNA cross-links may occur by the linkage of two DNA strands to the same chromatin to form protein bridges between DNA strands (Wilkins and McLeod, 1976). The reactions of formaldehyde with DNA are of mechanistic interest because of the potential association of these transformations with genotoxic effects.

Cross-linking of DNA and protein by formaldehyde has been observed in a variety of mammalian cells in culture following treatment with formaldehyde (Bedford and Fox, 1981; Ross et al., 1981; Craft et al., 1987). Inhalation exposure of rats to formaldehyde resulted in decreased extractability of DNA from proteins in the respiratory mucosa. This result was interpreted as evidence that formaldehyde forms DNA-protein cross-links (DPX) under in vivo conditions (Casanova-Schmitz and Heck, 1983).

CIIT recognized the potential implications of DPX for quantitative risk assessment and sought to develop methodology that would allow quantitation of protein-DNA cross-links. Results of this effort were published in Casanova-Schmitz et al. (1984). The proposed method was based on the inhalation exposure of rats to a mixture of [^{14}C]- and [^{3}H]-formaldehyde as a way to differentiate between metabolic incorporation and covalent binding. Two observations were key to this approach: first, cross-linking of DNA to proteins decreases its extractability - a conventional extraction procedure separates DPX material from non-cross-linked DNA; and second, oxidative metabolism of formaldehyde involves hydride abstraction which would result in loss of [^{3}H] from formaldehyde; material covalently bound would retain its original [^{3}H] specific activity. Macromolecules radiolabelled by metabolic incorporation of formaldehyde would show a lower value for the ratio [^{3}H]/[^{14}C] than those labelled by covalent binding since [^{14}C] is constant in either situation. Thus, by measuring the ratio of [^{3}H]/[^{14}C] in radiolabeled macromolecules it would be possible to establish the relative amount of covalently bound formaldehyde and hence DPX. CIIT used this fact to sort out the relative amounts of [^{3}H]- and [^{14}C]-labeled formaldehyde which were metabolized or reacted chemically when administered to rats by inhalation. They concluded that formaldehyde produced DPX in the rat respiratory mucosa at doses of 2 ppm and greater, and that the extent of cross-linking increased with dose between 2 and 6 ppm.

CPSC, EPA and NTP published their reservations to the interpretation of the experimental data by CIIT (Cohn et al., 1985). A panel of experts, convened with the express purpose of evaluating the CIIT study, concurred with the Agency's conclusion that several shortcomings precluded the use of DPX as a measure of intracellular dose in the derivation of risk estimates. The comments and recommendations given by the expert group were summarized in the Agency's risk assessment document (EPA, 1987) as follows: First, the methodologies must be validated to assure

that the experimental assumptions were scientifically sound and that the formaldehyde-DNA-protein complexes were identified properly; second, the single intracellular target used in the study may be inadequate; and third, the use of an acute exposure model in the CIIT study may not be appropriate because chronic, not acute exposure, is most relevant to risk assessment. Specific issues raised were: the lack of validation of the extraction procedure (chloroform/iso-amyl alcohol/phenol) in terms of efficiency and identity of the materials separated by this procedure; the incursion of kinetic isotope effects in the disposition of [^{3}H]-formaldehyde which confound the use of the proposed approach; the use of other covalent adducts such as protein cross-links, and hydroxmethyl adducts with proteins, DNA, or RNA, as dosimetric markers; and insufficient evidence to state unequivocally that all the DPX occurs in the interfacial DNA fraction.

3.1 Review of New Studies

The relevance attached to the use of DPX as a measure of intracellular concentration of formaldehyde is emphasized in recent publications by CIIT. An important finding, that verified a concern expressed by the expert panel, was the demonstration of a [^{3}H] isotope effect in the formaldehyde dehydrogenase oxidation of formaldehyde to formic acid (Heck and Casanova, 1987). Oxidation of [^{3}H]- and [^{14}C]-formaldehyde in rat nasal mucosal homogenates occurred with an isotope effect equal to 1.8$\pm$ 0.11 (ratio of V_{max}/K_m values for corresponding dehydrogenase reactions with [H^{14}CHO] and [^{3}HCHO]). A small isotope effect in the formation of cross-links was determined using rat hepatic nuclei; binding of [^{3}HCHO] to DNA was slightly favored (3.4%) over reaction with [H^{14}CHO]. The net result of an isotope effect during the oxidation step is [^{3}H] enrichment of intracellular formaldehyde relative to extracellular or administered formaldehyde. This event reduces the accuracy of the [^{3}H/[^{14}C] ratio as a quantitative means of distinguishing bound from metabolically incorporated formaldehyde. Casanova and Heck (1987) proposed a pharmacokinetic model for formaldehyde disposition in the nasal mucosa. As shown in Figure 3-1, the model consists of three components: 1) influx of ^{3}HCHO and H^{14}CHO into the cells of the nasal mucosa with rate constants characteristic for each isotope; 2) oxidative metabolism of intracellular formaldehyde with rate constants for each isotope; and 3) elimination of formaldehyde by routes other than oxidation including covalent binding to macromolecules, diffusion out of the cells and any metabolic transformations not involving cleavage of the ^{3}H-carbon bond. Based on this model, the discrepancy between intracellular and extracellular isotope ratios can be attributed to the isotope effect during oxidation. The authors further assumed that the isotope effect *in vivo* is equivalent to that determined *in vitro* and that the isotope effect on covalent binding, because of its small magnitude, can be neglected. The result of these assumptions was to establish an upper limit, equal to the measured isotope effect, for the increase in the intracellular

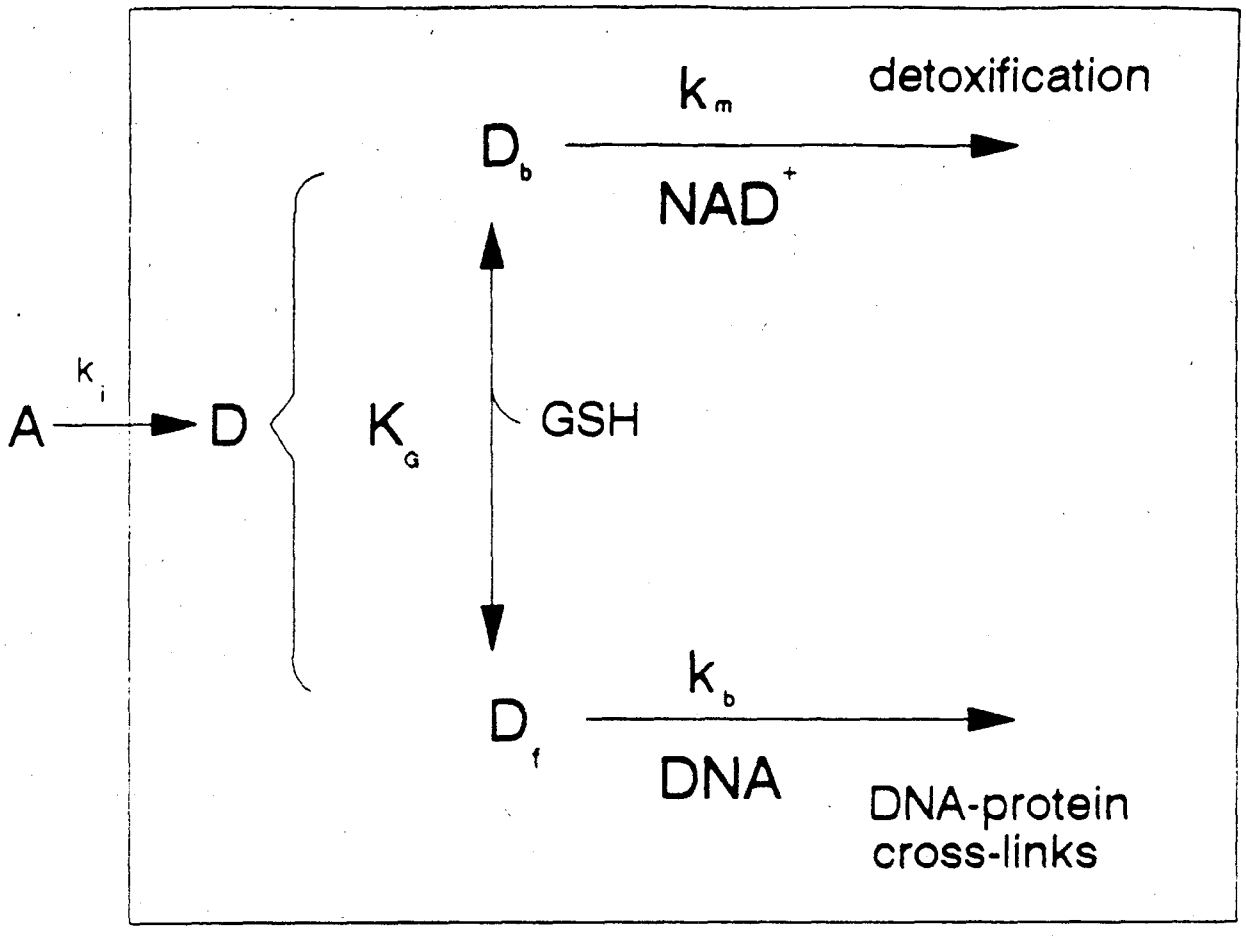


Figure 3-1. Pharmacokinetic model for disposition of inhaled HCHO in the rat nasal mucosa to metabolites (detoxification) or to DNA-protein cross-links. Abbreviations are as follows: A is extracellular (administered) HCHO and D is intracellular (delivered) HCHO consisting of free (D_f) and GSH-bound (D_b) forms. Rate and equilibrium constants are defined in the text. (from Casanova and Heck, 1987)

isotope ratio relative to the isotope ratio of administered formaldehyde. The concentration response curve for DPX following 6 hr exposure to $^{3}HCHO$ and $H^{14}CHO$ was normalized using this factor (1.8±1.1) to account for ^{3}H enrichment. The resulting curve showed that ignoring the isotopic effect on oxidation would lead to overestimates of the extent of DPX formation, particularly at low levels of formaldehyde. These findings prompted CIIT to develop new methodology in order to support the existence and potential importance of DPX in the nasal mucosa of rats dosed with formaldehyde.

Earlier research at CIIT was interpreted as indicating that formaldehyde cross-linked DNA with protein in the rat nasal mucosa and that the product (DPX) was present only in the interfacial (IF) DNA fraction. Additional studies were performed in an effort to establish these points.

In one study (Casanova et al., 1989), triplicate groups of four male Fischer 344 rats were exposed in nose-only exposure chambers to a mixture of ^{14}C- and ^{3}H-labeled formaldehyde (6 ppm; 6 hr). Animals were not pre-exposed to formaldehyde as in previous experiments. Immediately after exposure, the rats were decapitated and their respiratory mucosal tissue was removed. The tissue specimens were homogenized, treated with sodium borohydride to reduce reversibly bound Schiff base adducts to methylamines, dissolved in buffer, and sheared by passage through a 20-gauge needle. The sheared solution was separated into aqueous (AQ) DNA and interfacial (IF) DNA fractions with a mixture of chloroform, isoamyl alcohol and phenol. The two DNA fractions were purified and assayed separately. The reaction products of formaldehyde and DNA in rat nasal mucosa were studied by HPLC (high pressure liquid chromatography) analyses of hydrolysates of AQ and IF fractions. Chromatography of hydrolyzed AQ DNA samples showed that in the AQ DNA, essentially all of the radioactivity was eluted with the normal deoxyribonucleosides, dG, dT, and dA, indicating that the radioactivity in the AQ DNA fraction was almost entirely due to metabolic incorporation. In addition, [^{3}H] (but not [^{14}C]) was eluted early in the chromatography profile; this early-eluting [^{3}H] was considered by the authors to be caused by isotopic exchange of [^{3}H]-H_2O with the DNA.

In the IF DNA, radioactivity was also eluted with the normal deoxyribonucleosides, dG, dT, and dA, implying that nucleosides in the IF DNA were also labeled by metabolic incorporation. However, unlike the AQ DNA, a substantial quantity of both [^{3}H] and [^{14}C] was eluted before the deoxyribonucleosides. This early eluting radioactivity was identified as formaldehyde by chemical derivatization with dimedone. The observations that [^{3}H]- and [^{14}C]-formaldehyde were present only in the IF DNA and that the [^{3}H] activity of the early-eluting peak was higher than the [^{14}C] activity suggested to the authors (Casanova et al., 1989) that formaldehyde was bound as DPX.

The difference between the hydrolysates of the two DNA fractions was that only IF DNA hydrolysate contained formaldehyde, meaning that only IF DNA was cross-linked. No other radioactive organic compounds were detected in the DNA hydrolysates. Specifically sought and not found were N^2-hydroxy-methyl-deoxyadenosine (hm6dA), N^2-hydroxy-methyl-deoxyguanosine (hm2dG), N^2-hydroxy-methyl-deoxycytidine (hm4dC) and N^2-hydroxy-methyl-thymidine (hm3dt) which had been synthesized and characterized by their UV spectra and migration during reversed-phase HPLC.

A second experiment by Casanova et al. (1989) was designed to determine the extent of DNA-formaldehyde covalent binding in the rat respiratory mucosa at each of five exposure levels. Triplicate groups of four rats each were exposed for six hours in nose-only chambers to approximately 0.3, 0.7, 2, 6 and 10 ppm of ^{14}C-formaldehyde. The mucosal tissue was collected and treated as in the previously described double-label study to prepare solutions of sheared DNA. Then followed incubation with proteinase K, solvent extraction, etc. leading to the release and assay of ^{14}C-formaldehyde by HPLC and dimedone derivatization. It should be noted that this experimental procedure did not separate IF DNA and AQ DNA, but collected total DNA.

No evidence of formation of N-hydroxymethyl derivatives was obtained at any exposure level. The study did show that formaldehyde-DNA binding resulted from all exposure levels, including 0.3 ppm (1.4 $\pm$ 0.6 pmol/mg of DNA). The latter airborne concentration is in the range of measured ambient concentrations of formaldehyde in urban or indoor environments. The results obtained by this procedure were about two-fold lower than those obtained by the [^{3}H]/[^{14}C] ratio approach (Casanova-Schmitz et al., 1984). The difference was explained by the authors as a result of exposure conditions (naive vs. pre-exposed animals) and the isotope effect in the oxidation of ^{3}HCHO which leads to overestimation of the extent of covalent binding to DNA. The shape of the concentration-response for DPX appeared to remain nonlinear with respect to formaldehyde exposure concentration.

Similar findings were reported for rhesus monkeys exposed in a head-only inhalation chamber to H^{14}CHO (0.7, 2, or 6 ppm; 6 hr). Analysis of DPX was carried out by HPLC as described for the rat study (Heck et al., 1989). The mean concentration of cross-links in the turbinates and anterior nose was calculated, with weighting according to the amount of DNA in each tissue, to allow comparison with the corresponding rat data. Other tissues were examined for formation of DPX, and results are summarized in Table 3-1.

The results in Table 3-1 show predominant formation of DPX in the turbinates and anterior nose areas with lower levels detected in the nasopharynx and trachea. No cross links were detected in the sinus, proximal lung or bone marrow. Formation

Table 3-1

Concentration of DNA-protein Cross-links in the Respiratory Tract and Bone Marrow (femur) of Rhesus Monkeys Exposed to $H^{14}CHO$.

Tissue	Concentration of DNA-cross-links (pmol/mg DNA).		
	0.7 ppm	2.0 ppm	6.0 ppm
Turbinates, anterior nose	0.36 ± 0.10*	2.56 ± 0.31	18.2 ± 3.4
Sinus	ND	ND	ND
Nasopharynx	(0.09± 0.09)	(0.47 ± 0.09)	5.8 ± 2.4
Larynx/ trachea/carina	ND	(0.4)	9.4 ± 5.4
Airways**	ND	(0.70 ± 0.15)	5.7 ± 5.7
Proximal lung	ND	ND	ND
Bone marrow	ND	ND	ND

* Mean ± S.E.; ND = not detectable; values in parentheses are uncertain, as dpm for these samples were less than twice the background.
** Major intrapulmonary airways greater than 2 mm in diameter.

of DPX in turbinates and anterior nasal mucosa in the monkey was established at all exposure levels with concentrations of cross-links being lower than in rats, by as much as an order of magnitude (Casanova et al., 1989). Both studies (Casanova et al., 1989; Heck et al., 1989) reported that DPX was stable in unhydrolyzed DNA but was dissociated by enzymatic hydrolysis of the DNA which resulted in release of radiolabelled formaldehyde. Structural characterization of specific DPX components was made difficult by the hydrolytic lability of such adducts.

Data for each species were fitted to a pharmacokinetic model (Casanova and Heck, 1987), and the results are shown graphically in Figure 3-2.

The results described above indicate that (a) the formation of DNA-protein cross-links likely is a nonlinear function of the exposure dose in both species and (b) monkeys had lower levels of cross-links in corresponding tissues than did rats.

Heck et al. (1989) interpreted the preceding studies as strongly suggesting that the intracellular concentration of formaldehyde is modulated by two saturable defense mechanisms, namely, covalent binding to protein in nasal mucosa and metabolism by formaldehyde dehydrogenase. They added that the repair of DPX cross-links may also be saturable.

3.2 Summary and Conclusions

The recent CIIT work has clarified most of the concerns expressed in the expert panel report outlined earlier in this section. The major experimental design issues concerned the validation of the extraction procedure for separation of AQ DNA, IF DNA and proteins; possible existence of an isotope effect in the reactions of labelled formaldehyde; and substantiation of the claim that all the formaldehyde bound as DPX was present in the IF DNA. These issues were addressed by development of a method to determine the extent of formaldehyde binding to DNA that utilizes only $H^{14}CHO$ and thus is not subject to isotope effects as in the previous $^{3}H/^{14}C$ approach. This method is based on the release of labelled formaldehyde from DNA by enzymatic digestion. HPLC is used to separate deoxynucleosides from formaldehyde released by hydrolysis. The latter provides the basis for the quantitation of DPX, and it was also used to demonstrate that only IF DNA contained DPX since no formaldehyde was released by hydrolysis of AQ DNA. This method also proved to be more sensitive than the isotope ratio procedure allowing detection of DPX at the lowest exposure concentration used (0.3 ppm), significantly lower than the previously reported detection threshold of 2 ppm (Casanova-Schmitz et al., 1984). Levels of DPX were determined in the turbinates and anterior nose of the monkey at all exposure concentrations tested (0.7, 2, and 6 ppm).

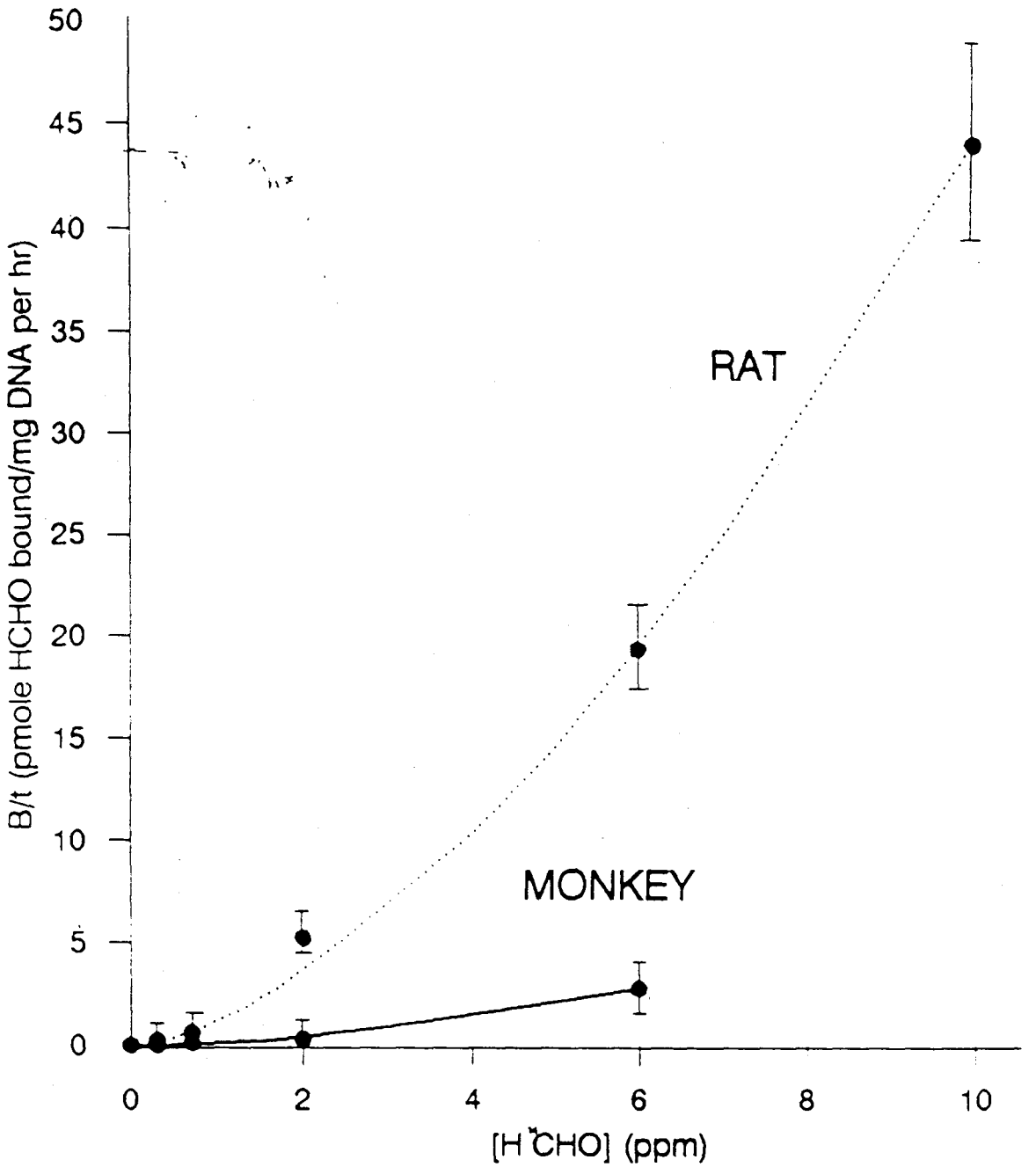


Figure 3-2. Formation of DNA-protein cross-links in the turbinates and anterior nose of F-344 rats and rhesus monkeys exposed for 6 hr to H^{13}CHO. Values shown are mean ± S.E., D = 3 exposure groups or animals per concentration. (Data in the rat and monkey are from Casanova et al., 1989 and Heck et al., 1989, respectively).

The study by Casanova et al., 1989 also addressed the question posed by the expert panel with regard to the use of other covalent adducts as indicators of intracellular formaldehyde. No evidence was obtained for formation of hydroxymethyl derivatives of DNA, although it may be still be argued that formation of such derivatives at levels below the detection limit of the current methodology cannot be ruled out. An important finding was the demonstration that hydroxymethyl derivatives of deoxynucleosides may be formed during hydrolysis of DPX; hydroxymethyl adducts are stable to HPLC conditions and detection by this analytical procedure is possible. The choice of buffer is critical in the formation of hydroxymethyl compounds from deoxynucleosides. The use of Tris buffer effectively eliminates formation of these intermediates because the primary amine group in Tris interacts with the released, "free" formaldehyde. Buffers not "interactive" with formaldehyde allow formation of hydroxymethyl derivatives. It is possible that a previous report (Beland et al., 1984) on the isolation of hm6dA in DNA hydrolyzates, isolated from Chinese hamster ovary cells incubated with ^{3}HCHO (1 mM) may have dealt with an artifact produced during the isolation procedure.

An important issue that is not addressed by the current work is the suitability of using an acute exposure model to predict effects under chronic exposure. A source of uncertainty is the replacement of normal respiratory epithelial cells by squamous cells that takes place within a week following the first exposure. Whether the pharmacokinetic parameters for formaldehyde in these two different cell types are comparable remains to be established.

An important aspect of the <u>in vivo</u> disposition of formaldehyde, and not discussed in the current studies, is the mechanism by which formaldehyde penetrates into the cell and eventually the nucleus. It is improbable to presume that formaldehyde could penetrate cellular membranes as free material. It is more likely that a "latent" form of formaldehyde is carried into the cell possibly bound to protein(s) as an N-hydroxymethyl derivative. This "latent" formaldehyde carrier could either deliver formaldehyde to a critical target (DNA), or react directly with DNA. A certain selectivity governs this type of reaction. It has been reported that only proteins that have binding affinity for DNA form cross-links in the presence of formaldehyde (Solomon and Varsharsky, 1985), an observation that may suggest that perhaps N-hydroxymethyl derivatives of proteins may constitute an "active" form of formaldehyde. Mechanistically, reactivity considerations would favor reaction of formaldehyde with nucleophilic groups in proteins rather than DNA. DNA affinity as a requisite for protein cross-linking reactions is not unreasonable, or unexpected, if one considers that the length of the cross-link established is rather short, thus requiring close proximity of reactants. Other observations

from chemical reactions of formaldehyde with weak nucleophiles such as amides indicate that reaction of N-hydroxymethyl derivatives takes place more readily than the corresponding reaction with formaldehyde (Walker, 1975). The latter may be simply a matter of reversibility.

These observations could eventually be incorporated in the design of a pharmacokinetic model of the disposition of formaldehyde, i.e., the model could incorporate the potential role of protein N-hydroxymethyl adducts in the formation of DPX. The current model relates extracellular formaldehyde to the intracellular concentration of formaldehyde not bound to GSH. A possible scheme for the reaction of formaldehyde leading to formation of DPX that incorporates the above observations would involve initial formation of a protein N-hydroxymethyl derivative -probably more than one hydroxymethyl group per protein - which transports formaldehyde into the cell. Since N-hydroxymethyl reactions are reversible, the same protein may not be the one that transports formaldehyde into the nucleus, binds and eventually reacts with DNA. The slow step in this process could be formation of the first cross-link; reaction of other N-hydroxymethyl groups present in the same protein should take place at a faster rate, i.e., a contributing factor to the nonlinear kinetics observed may also be the presence of multiple intermediates (protein N-hydroxymethyls) reacting with DNA at different rates.

The conclusions based on currently available evidence are that: formaldehyde reacts <u>in vivo</u> with proteins and DNA to form cross-linked adducts (DPX); formation of DPX is nonlinear with respect to exposure concentration, the source of nonlinearity is attributed to saturation of detoxication mechanisms that include binding to extracellular (mucosal) proteins and oxidation to formic acid - an alternative is to consider the role of protein N-hydroxymethyl derivatives as an "active" form of formaldehyde which would also be expected to exhibit nonlinear kinetics; identification of specific adducts found in DPX remains an issue; and, the role of N-hydroxymethyl derivatives of proteins as described above remains unexplored.

The current pharmacokinetic analysis of DPX formation have adequately addressed most of the reservations expressed in the expert panel's report. The use of DPX as the surrogate dose for risk estimates appears appropriate although some uncertainties remain which may limit the application of these data. These uncertainties, which have a strong pharmacodynamic component, are associated with the use of an acute model to predict chronic effects and the biological significance of DPX as it relates to formaldehyde-induced carcinogenesis. These issues are discussed in more detail in Section 5 of this document.

4.0 CARCINOGENIC HAZARD

4.1 Animal Carcinogenicity Studies

4.1.1 Long-Term Bioassays

The principal evidence indicating that formaldehyde is carcinogenic in laboratory animals derives from the inhalation studies in rats and mice conducted by CIIT. In the first study (Kerns et al., 1983), groups of 120 male and 120 female Fischer 344 strain rats and C57BL/6 X C3HF1 strain mice were exposed by inhalation to 0, 2.0, 5.6 and 14.3 ppm of formaldehyde gas for 6 hr/day, 5 days/week, for 24 months. Squamous cell carcinomas of the nasal cavity were observed in 103 of 232 rats (44%; 51/117 males and 52/115 females) and 2 of 215 mice (0.9%) exposed to 14.3 ppm of formaldehyde and in 2 of 235 rats (0.9%; 1/119 male and 1/116 female) exposed to 5.6 ppm formaldehyde gas. Research recently completed at CIIT (Monticello, 1990) supports the results of the 1983 study in rats in that nasal tumors were found with formaldehyde exposures to 10 (4/17, 23% tumor incidence) and 15 ppm (27/45, 60% tumor incidence). At 6 ppm, no tumors were observed in Monticello (1990). The reasons for the difference in these results at 6 ppm compared to those in Kerns et al. (1983) may be due to fewer animals allotted to dose groups in Monticello (1990).

The spontaneous incidences of nasal neoplasia in these strains of rats and mice are extremely low. Although not statistically significantly elevated in incidence in comparison with matched control mice, the nasal carcinomas observed in 2 (out of a total of 215) mice exposed to a concentration of 14.3 ppm were, therefore, regarded as related to formaldehyde exposure. The difference in susceptibility of rats and mice has been postulated to be due, at least in part, to a greater reduction in respiratory rate and minute volume, and thus the "dose" available for deposition in the nasal cavity, in mice than in rats during exposure to this irritating vapor (see Section 4.3). A lifetime inhalation study was also conducted in hamsters exposed to 10 ppm formaldehyde for 5 hours/day, 5 days/week Dalbey, 1982). There was no tumorigenic response in the formaldehyde-exposed hamsters, but survival time was reduced compared to unexposed controls.

Kerns et al. (1983), also reported a small number of polypoid adenomas and other neoplasms (adenocarcinoma, poorly differentiated carcinoma/sarcoma) of the nasal mucosa in the rats. However, according to a Pathology Workgroup (Consensus Workshop, 1984), these polypoid adenomas are not likely to represent the benign counterpart of squamous cell carcinomas. Therefore, the polypoid adenomas were not combined with squamous cell carcinomas for risk estimation.

Knowledge of the anatomic sites of origin of formaldehyde-induced neoplasms is important for a better understanding of the nasal carcinogenicity of formaldehyde. In this chronic inhalation study (Kerns et al., 1983), the tumors were reported to originate in the anterior portion of the nasal cavity but their precise location in the nose was not determined. Morgan et al. (1986) have defined more precisely the localization of nasal tumors associated with chronic exposure of F334 rats to formaldehyde vapor by reexamining histologic sections from the nasal passages of 98 of the 103 tumor-bearing rats (slides of 5 rat noses were missing) in the inhalation study (Kerns et al., 1983). In their analysis (Morgan et al., 1986), the location of 121 squamous cell carcinomas in 98 rat noses (some had multiple neoplasms) were recorded on diagrams of cross sections of the nose. It was found that the majority of squamous cell carcinomas occurred on the lateral side of the anterior portion of the nasoturbinate and adjacent lateral wall (57%) or the midventral nasal septum (26%); about 10% were on the dorsal septum and the roof of the dorsal meatus. Only one squamous cell carcinoma (out of 121 reexamined) was found on the medial aspect of the maxilloturbinate, a region which presumably also has a high "delivered dose" due to airflow patterns influencing regional exposure to formaldehyde. It had also been shown that inflammation and sustained cell proliferation but not tumors occurred in this region following chronic exposure of formaldehyde (see Section 4.3). These findings suggested to the authors (Morgan et al., 1986) that not only regional exposure but local tissues susceptibility may be important for the distribution of formaldehyde-induced neoplasms in the nose of rats.

Additional studies by other investigators have confirmed the carcinogenicity of formaldehyde in Fischer 344 and other strains of rats by inhalation exposure. In a study by Tobe et al. (1985), groups of 32 male Fischer 344 rats were exposed to 0, 0.3, 2.0, and 15 ppm formaldehyde vapor 6 hours/day, 5 days/week, for 28 months. Squamous cell carcinomas of the nasal cavity were seen in 14/27 high-dose (15 ppm) rats surviving past 12 months; no such neoplasms were observed in the control or the low-dose groups (0.3 and 2.0 ppm). In addition, nasal papillomas, considered to represent the benign counterpart of the squamous cell carcinomas, were observed in 5 high-dose rats in this study.

In two reports (Albert et al., 1982; Sellakumar et al., 1985), groups of 99-100 male Sprague-Dawley rats were exposed 6 hours/day, 5 days/week for life by inhalation to: hydrochloric acid (HCl) alone, mixtures of HCl and formaldehyde, or formaldehyde alone. The results show that significant incidences (27-38%) of squamous cell carcinomas of the nasal cavity were induced in all rats exposed to formaldehyde (14-15 ppm) regardless of the concurrent exposure to HCl. No carcinogenic response was observed in the rats exposed to air (control) or to HCl alone.

Feron et al. (1988) exposed male Wistar rats to 0, 10, or 20 ppm formaldehyde vapor 6 hr/day, 5 days/week, for 13 weeks and then were held up to 117 weeks. A low incidence (6/132, 4.5%) of nasal tumors (3 squamous cell carcinomas, 1 carcinoma in situ and 2 polypoid adenomas) was observed in the rats exposed to 20 ppm formaldehyde vapor. Squamous cell carcinoma was also noted in 1/44 rats at the dose level of 10 ppm. No such neoplasms were found in 45 control rats. These data indicate a carcinogenic potential of formaldehyde after short-term exposure to very high concentrations.

Formaldehyde-induced nasal tumors were found mainly at exposure concentrations which also induced severe degenerative, hyperplastic and metaplastic changes in nasal epithelium. Therefore, it has been suggested that increased cytotoxicity and cell replication may play an essential role in the induction of nasal cancer by formaldehyde. To study the significance of cytotoxicity to the nasal mucosa for the induction of nasal tumors by formaldehyde in rats, a long-term inhalation study was conducted in which groups of 60 male Wistar rats with either severely damaged or undamaged nose were exposed to 0.1, 1.0, or 10 ppm formaldehyde, 6 hr/day, 5 days/week for 28 months (Woutersen et al., 1989). The damage to the nasal mucosa was induced by bilateral intranasal electrocoagulation. The results of these experiments showed that compound-related hyperplasia, rhinitis (inflammation of the nasal cells with squamous cells) in the respiratory mucosa developed in rats with either a damaged or an undamaged nose at 10 ppm, although a higher incidence of these lesions occurred in the rats with a damaged nose. Exposure to 10 ppm formaldehyde for 28 months produced significant incidence (15/58) of nasal squamous cell carcinomas in rats with damaged nose but not in rats with intact nose. No compound-related nasal neoplasms or cytotoxic effects were observed in rats (with either a damaged or undamaged nose) exposed to 0.1 or 1.0 ppm formaldehyde for 28 months. The findings of these studies, therefore, suggest that the nasal mucosa damaged by electrocoagulation was more susceptible to the induction of nasal tumors by formaldehyde. The roles of cytotoxicity and cell proliferation in formaldehyde-induced carcinogenesis are discussed in Section 4.3.2.

As shown in Table 4-1, the combined results of four long-term inhalation studies indicate that formaldehyde induces high incidences of nasal tumors (38-50%) in two strains of rats at approximately 15 ppm (Albert et al., 1982; Kerns et al., 1983; Tobe et al., 1985; Sellakumar et al., 1985; Monticello, 1990). There is also weak evidence of carcinogenicity at 10 ppm or lower concentrations. The observed tumor incidence in these studies produces a very steep dose-response curve for the carcinogenic effect of formaldehyde. An exception to the above is the observation that rats with noses damaged by electrocoagulation had a high incidence of nasal tumors following inhalation

Table 4-1

Incidences of Nasal Squamous Cell Carcinomas in Rats After Inhalation Exposure to Formaldehyde

Rat Strain	Dose Level (ppm)	Tumor Incidence Male	Female	Reference
Fischer 344	0	0/118	0/114	Kerns et al., 1983
	2.0	0/118	0/118	
	5.6	1/119	1/116	
	14.3	51/117	52/115	
Fischer 344	0	0/17		Monticello, 1990
	0.7	0/17		
	2.0	0/16		
	6.0	0/18		
	10.0	4/17		
	15.0	27/45		
Fischer 344	0	0/32		Tobe et al., 1985
	0.3	0/32		
	2.0	0/32		
	15.0	14/27		
Sprague-Dawley*	0	0/99		Albert et al., 1982; Sellakumar et al., 1985
	14.0-15.0	27/100-38/100		
Wistar**	0	0/45		Feron et al., 1988
	10.0	1/44		
	20.0	3/132		
Wistar***	0	1/54		Woutersen et al., 1989
	0.1	1/58		
	1.0	0/56		
	10.0	15/58		

* With or without concurrent exposure to hydrochloric acid; hydrochloric acid alone did not induce any tumors.

** Exposed for 13 weeks, then observed up to 117 weeks.

*** Rat noses were damaged by bilateral intranasal electrocoagulation. No tumors were found in exposed rats with undamaged noses.

exposure to formaldehyde at 10 ppm. These data strongly suggest that increased cytotoxicity and cell proliferation play a role in the induction of nasal cancer by formaldehyde.

Several investigators (Takahashi et al., 1986; Til et al., 1989; Tobe et al., 1989; Soffritti et al., 1989) have also studied the carcinogenic activity of formaldehyde by administration to the rat in the drinking water.

In one study (Takahashi et al., 1986), squamous cell papillomas of the forestomach were observed in 8 of 10 Wistar rats given 0.5% formalin (about 0.2% formaldehyde) in the drinking water for 32 weeks.

In another study by Til et al. (1989), groups of 70 male and 70 female Wistar rats were administered 0.19% formaldehyde in their drinking water for up to 104 weeks. Significant incidences of hyperplastic and inflammatory changes in the fore- and glandular stomach were reported, but there was no evidence of tumor formation. Til et al. (1989) proposed that the differences in findings between these two studies might be ascribed to the use of different criteria for the classification of a lesion as a papilloma or as papillary epithelial hyperplasia.

Similarly, Tobe et al. (1989) observed high incidences of forestomach and glandular hyperplasia in the rats given formaldehyde solution in drinking water for 12 months at a concentration of 0.5%. In this study, group of 20 male and 20 female Wistar rats were given formaldehyde at concentrations of 0, 0.02, 0.10 and 0.5% in their drinking water ad libitum. Six randomly chosen rats from each group were sacrificed after 12 and 18 months. All 12 rats (6 males and 6 females) in the 0.5% group sacrificed at 12 months developed squamous cell hyperplasia in the forestomach. Hyperplasia of the glandular stomach was observed in 10 of the 12 high-dose rats (6 males and 4 females) sacrificed at 12 months. The experiment was terminated at 24 months and all surviving rats were killed for necropsy. Various types of tumors were observed in organs such as pituitary, thyroid, testis, adrenals, mammary gland and skin; however, there were no significant difference in the incidences of any tumors among the treated and control groups. This drinking water study may not be adequate to evaluate the carcinogenic potential of formaldehyde because of the high early mortality of the high-dose group and the use of a small number of animals. At 12 months, the mortality rates in the high-dose (0.5%) group were 45% and 55% in males and females, respectively. At 18 months, the respective mortality rates reached 67% and 70%, and by 24 months, all animals in the 0.5% group had died.

In a study conducted by Soffritti et al. (1989), groups of 50 male and 50 female Sprague-Dawley rats of different ages at the start of the study (12 day embryos -- transplacental

carcinogenesis, 7- and 25-week old rats) were administered formaldehyde in drinking water at different concentrations (0, 0.001, 0.005, 0.01, 0.1, 0.15, and 0.25%) for 104 weeks. A low incidence (2-21%) of gastrointestinal neoplasms was observed in some treated animals. These gastrointestinal neoplasms, which were not seen in the matched controls and were reported to be extremely rare in the historical controls (1.39% incidence) of this rat strain, included benign tumors (papillomas, acanthomas, adenomas and leiomyomas) and malignant tumors (squamous cell carcinomas, adenocarcinomas and leiomyosarcomas) of the forestomach and the small intestine (duodenum, jejunum, and ileum). The lack of clear reporting of the test results limits the usefulness of this study.

Collectively, the findings of these drinking water studies with formaldehyde (Takahashi et al., 1986; Til et al., 1989; Tobe et al., 1989; Soffritti et al., 1989) provide only suggestive evidence for the carcinogenicity formaldehyde by the oral route. in the rat by the oral route.

In the study of Soffritti et al. (1989), a slightly increased incidence of leukemia was also noted in some formaldehyde-treated rats (11-18%) as compared with the controls treated with drinking water alone (3.5-5.5%). However, no statistical analysis and historical control data were reported; the biological significance of these low incidences of neoplasms is questionable.

The carcinogenicity of formaldehyde has also been studied by a variety of other routes of administration and/or in other animal species including application to buccal mucosa in rabbits (Meuller et al., 1978), subcutaneous injection in rats (Watanabe et al., 1954; Watanabe and Sugimoto, 1955), skin painting in mice (Iversen, 1986), and inhalation in hamsters and monkeys (Rusch et al., 1983). Although some of the data suggest that formaldehyde was carcinogenic, none of these studies is conclusive regarding formaldehyde carcinogenicity because of various limitations in the experimental protocols used (e.g., small number of animals, short duration).

4.1.2 Tumor Promotion and Co-Carcinogenicity Studies

The tumorigenesis-promoting potential of formaldehyde has been investigated in classical skin painting experiments in mice (Iversen, 1986). Two groups of 32 mice (hr/hr Oslo strain) were painted once with 51.2 ug dimethylbenz(a)anthracene (DMBA) in 100 ul acetone; one group was followed 9 days later with applications of 200 ul 10% formaldehyde in water twice a week, and the other was given no further treatment. The animals were treated and/or observed for 60 weeks. The incidence of skin tumors after DMBA initiation followed by formaldehyde (11/32, 37.5%) was not significantly different from that after DMBA alone (85/225,

37.7%). However, the time of appearance of the first tumor and the mean latency time were significantly reduced.

As benzo[a]pyrene (B[a]P) is a well-known respiratory carcinogen, the interaction of B[a]P and formaldehyde in respiratory tract carcinogenesis was studied in rat tracheal explants (Cosma and Marchok, 1987). The induction of carcinogenesis was quantitated as the number of growth-altered cell populations (designated as "tumor-initiation sites", TIS) isolated in cell cultures generated from explants cut from the preexposed tracheas. While exposure twice-weekly for 4.5 months to 0.2% formaldehyde solution gave only a weak response (0.25 TIS/trachea), 2.37 TIS per trachea were detected after exposure to 20 ug B[a]P in the same regimen. The combination of B[a]P followed by formaldehyde had a greater response than either compound alone (7.83 TIS/trachea), while the reverse exposure gave 1.49 TIS per trachea, which was less than B[a]P alone. Thus, the induction of TIS by combined exposure to B[a]P and formaldehyde was dependent on the order of exposure. The suppression of the cellular response to B[a]P when it was preceded by exposure of formaldehyde was believed to be due to the inhibition of enzymes involved in the metabolism of B[a]P.

The effects of formaldehyde on gastric carcinogenesis in rats after initiation with N-methyl-N-nitro-N-nitrosoguanidine (MNNG) were studied by Takahashi et al. (1986). Male outbred Wistar rats were given MNNG in the drinking water (100 mg/liter) and a diet supplemented with 10% sodium chloride for 8 weeks. Thereafter, they were maintained on drinking water containing 0.5% formalin (about 0.2% formaldehyde) for 32 weeks and then sacrificed for necropsy and histological examination. The results showed that formaldehyde significantly increased the MNNG-induced incidences of neoplasms in the forestomach and glandular stomach of the rats. Papillomas in the forestomach were observed in 88% (15/17) of animals treated with MNNG and formaldehyde; no such neoplasm was seen in 30 rats treated only with MNNG. The incidences of glandular stomach adenocarcinomas in rats treated with MNNG alone and in rats treated with both MNNG and formaldehyde were 13% and 29%, respectively.

No evidence was found for any co-carcinogenic effects of formaldehyde when C3H mice were exposed simultaneously to coal tar aerosol and formaldehyde (Horton et al., 1963). There was suggestive evidence of a co-carcinogenic effect of formaldehyde on diethylnitrosamine-induced carcinogenesis in hamsters (Dalbey, 1982), but high mortality and other factors discussed in EPA (1987) limit the usefulness of this study.

4.1.3 Summary and Conclusions of Animal Studies

An earlier review (EPA, 1987) has indicated that there is sufficient evidence for the carcinogenicity of formaldehyde in

experimental animals exposed by inhalation at high concentrations. Recent animal studies confirm previous findings of an increased incidence of squamous cell carcinomas of the nasal cavity in rats exposed by chronic inhalation or less than lifetime exposure to high concentration of formaldehyde. In addition, the localization of nasal tumors in rats has been better defined; the findings suggest that not only regional exposure but also local tissue susceptibility may be important for the distribution of formaldehyde-induced neoplasms. The nasal mucosa damaged by electrocoagulation has been found to be more susceptible to the induction of nasal tumors by formaldehyde.

Results obtained from four studies in which formaldehyde was administered to rats in the drinking water provide only suggestive evidence by the oral route for the carcinogenicity of formaldehyde.

In recent tumor promotion studies, formaldehyde enhanced the tumor response in mouse skin, rat trachea, and rat stomach, indicating that formaldehyde has tumor promotion potential at least in some tissues but not in hamster respiratory tract. No co-carcinogenic effects were seen in any available studies.

4.2 Mutagenicity

The EPA (1987) document detailed the ability of formaldehyde to induce mutation in several test systems, including viruses, bacteria, yeast, fungi, *Drosophila*, grasshoppers, and mammalian cells in culture. The document further described the ability of formaldehyde to induce cellular transformation in several systems, to act as a co-mutagen when tested with agents such as x-rays, ultraviolet light, and hydrogen peroxide, and to have enhanced activity in test systems deficient in excision-repair mechanisms. Formaldehyde was further characterized as inducing both chromosome aberrations and sister chromatid exchanges *in vitro* as well as causing single-strand breaks in DNA, inhibiting the resealing of single-strand breaks produced by ionizing radiation and inducing cross-links between DNA and protein in both epithelial and fibroblastic cells. Formaldehyde was proposed to act by damaging DNA and by inhibiting DNA repair in treated cells. Studies on the metabolic incorporation of formaldehyde versus covalent binding to DNA in treated cells were also included in the document.

Formaldehyde was reported neither to induce micronuclei *in vivo* nor to cause sperm head abnormalities in mice (EPA, 1987). There was question about the ability of formaldehyde to induce mutation in the dominant lethal assay in mice and in an *in vivo* assay for sister chromatid exchanges. Limitations in these studies were stated in the document.

Human studies concerned with the occurrences of chromosomal aberrations and sister chromatid exchanges in the blood and mutagenic products in urine of exposed workers were also detailed.

New literature in the area of mutagenicity/genotoxicity was reviewed and included studies on covalent binding and the induction of DNA strand breaks; studies on induction of chromosomal aberrations in vitro; co-mutagenesis studies of x-rays and formaldehyde in Drosophila; cytotoxicity and mutation in human lymphocytes and Salmonella and a study on sister chromatid exchange formation in anatomy students exposed to formaldehyde embalming solution (Bogdanffy et al., 1987; Casanova et al., 1989; Craft et al., 1987; Crosby, 1988; Dresp and Bauchinger, 1988; Dowd et al., 1986; Ecken and Sobels, 1986; Liber et al., 1989; Heck et al., 1989, in press; Schmid et al., 1986; Snyder and Van Houten, 1986; Yager et al., 1986).

The new studies corroborate previous findings on the genotoxicity of formaldehyde (EPA, 1987).

4.3 Possible Mechanisms of Formaldehyde-Induced Carcinogenicity

Formaldehyde has been shown to be mutagenic in a variety of assay systems, and there is convincing evidence that formaldehyde is genotoxic in mammalian cells, including human lymphoblasts (see Section on Mutagenicity; also EPA, 1987; Ma and Harris, 1988). The mechanisms of genotoxicity of formaldehyde, however, are not yet completely understood. Formaldehyde is chemically reactive and has been shown to interact with various biological macromolecules including amino acids, proteins, nucleic acid bases, nucleosides, RNA and DNA (EPA, 1987). The reactions of formaldehyde with DNA and its components are of particular interest since such reactions are believed to be involved in the genotoxic effects of chemicals (see Section 3.0). DNA-DNA cross-links have been identified in pure DNA preparations incubated with formaldehyde (Chaw et al., 1980), but such direct cross-links have yet to be identified following treatment of intact cells. Some evidence suggests that indirect DNA-DNA cross-linking may occur by the linkage of two DNA strands to the same chromatin to form protein bridges between DNA strands (Wilkins and MacLeod, 1976). Snyder and Van Houten (1986) have shown that the reaction of formaldehyde with synthetic DNA polymers in protein-free mixtures resulted in an increased frequency of misincorporation by polymerase, suggesting the production of potentially mutagenic adducts.

The understanding of the mechanisms of the carcinogenic effects is important for the risk assessment of formaldehyde. The findings that formaldehyde is a nasal carcinogen in rats and that the dose-response for the nasal squamous cell carcinomas is

nonlinear (0% incidence at 0 and 2 ppm, about 1% incidence at 5.6 ppm and 44% incidence at 14.3 ppm; see Section 4.1.1) have prompted considerable research into its mechanism(s) of carcinogenic action. As a result, a number of factors and hypotheses have been proposed to understand this type of carcinogenic response. Recent mechanistic studies on formaldehyde have focused on the roles of DNA-protein cross-linking (see Section 3.0) and cell proliferation. Examinations of the species differences in carcinogenic response and the role of the nasal mucociliary apparatus has led to the conclusion that the "dose" available for deposition in the nasal cavity and epithelium may be critical for the carcinogenic effects of formaldehyde.

4.3.1 Role of DNA-Protein Cross-linking

DNA-protein cross-links (DPX) are removable by excision repair, which may increase the frequency of single-strand breaks in DNA and represent a possible mutagenic response of cells exposed to formaldehyde. Cross-linking of DNA and protein via a methylene bridge N-CH_2-N, has been observed in a variety of mammalian cells in culture following treatment with formaldehyde (Bedford and Fox, 1981; Ross et al., 1981; Craft et al., 1987). Evidence that formaldehyde may form DPX in vivo was provided by studies from CIIT (as discussed in Section 3.0). Crosby et al. (1988) have shown that formaldehyde mutagenesis in human lymphoblasts is characterized by large deletions of DNA (which may result from DPX formation) and that in E. coli, different types of mutations (large deletions, base-pair transversions and transitions) are induced at different concentrations of formaldehyde. It seems possible that a shift in the type of mutations induced by formaldehyde as a function of concentration may be related to the highly nonlinear nature of formaldehyde carcinogenesis in rat nasal cavity.

However, the relevance of DPX to formaldehyde carcinogenesis needs to be further explored. For instance, the types of mutations and the relationships between DNA-protein cross-links and formaldehyde mutagenesis have not yet been examined in the rat nasal mucosa; the repair of formaldehyde-induced DNA-protein cross-links has not been investigated to define the kinetics or mechanism of this process; and this lack of knowledge constitutes a major gap in our understanding of this important mechanism postulated for formaldehyde carcinogenesis.

4.3.2. Role of Cytotoxicity and Cell Proliferation

It is generally accepted that the multi-stage process of carcinogenesis involves DNA damage (mutation) and clonal expansion of the initiated or altered cells. Theoretically, there are several roles that cell replication may play in the process of carcinogenesis. The chance for an initiating event is

enhanced during cell replication when the DNA is single stranded and experiences greater exposure and susceptibility to chemically-induced DNA damage. Furthermore, enhanced cell replication may increase the probability of converting DNA adducts into mutations before they can be repaired. However, cell proliferation is an ongoing process in normal living tissue. Enhanced cell proliferation is generally believed to be an important but not a sufficient step to induce tumors. Not all chemicals that induced cell proliferation or hyperplasia are equivalent in their ability to promote/induce tumor formation. Chemically-induced cell proliferation may just represent regenerative cell growth ("adaptation"), which does not necessarily lead to tumorigenesis. It appears that clarification between "adaptive" versus "preneoplastic" responses is needed. Measurement of the extent and duration of site-specific cell proliferation, in conjunction with elucidation of the exact biochemical and molecular alterations underlying the effects is important in understanding the relationship of chemically-induced cell proliferation to carcinogenesis.

Formaldehyde-induced nasal tumors were found mainly at exposure concentrations which also induced severe degenerative, hyperplastic and metaplastic changes in nasal epithelium. Therefore, it has been proposed that increased cytotoxicity and cell replication may play an essential role in the induction of nasal cancer by formaldehyde.

Studies by Woutersen et al. (1989) in Wistar rats have demonstrated a higher susceptibility of the nasal mucosa damaged by electrocoagulation than the undamaged nasal mucosa to the induction of nasal tumors by formaldehyde). Bilateral intranasal electrocoagulation was reported to cause damage in all structures in the anterior of the nasal cavity, with perforation of the nasal septum and loss of turbinates being a common occurrence. The higher susceptibility of the damaged than the undamaged nasal mucosa to the carcinogenic action of formaldehyde may be due to irreparable damage to nasal defense mechanisms (e.g., the mucociliary apparatus) caused by the electrocoagulation, thus rendering the metaplastic and hyperplastic nasal respiratory mucosa more susceptible to formaldehyde. It is plausible that the cytotoxic effects caused by electrocoagulation may enhance the carcinogenic response of formaldehyde by providing an increased opportunity for formaldehyde to interact with single-strand DNA during cell proliferation or by promoting the formaldehyde-initiated cells.

Studies investigating formaldehyde-induced toxicity have shown that increased cell proliferation occurs following acute exposure and is an early and sensitive indicator for cytotoxic effects in the nasal passages of rats (Swenberg et al., 1983; Zwart et al., 1988). Using a labeling technique with ^{3}H-thymidine, Swenberg et al. (1983) found a 10- to 20-fold increase

in the labeling index (defined as the proportion of cells in a given population that are in the process of DNA replication) in the nasal epithelium of rats 2 or 18 hours after exposed to 6 or 15 ppm 6 hr/day, for 3-5 days; only slight increases were detected in rats exposed to 2 or 0.5 ppm formaldehyde as compared with the controls. In mice, increased cell proliferation was evident only at 15 ppm and was much lower than that in rats (Table 4-2).

It should be noted, however, that increases in cell proliferation following acute exposure to rats occurred in the respiratory epithelium of both the central portion (the primary site of squamous cell carcinomas) and the most anterior portion of the nasal passages. Furthermore, the observed cell proliferation was substantially decreased after 10 days of exposure (Swenberg et al., 1983).

To study in detail possible effects of low concentrations of formaldehyde on the nasal epithelium, Zwart et al. (1988) exposed Wistar rats to 0, 0.3, 1 and 3 ppm formaldehyde vapor for 6 hr/day, 5 days/week during the 3-day or 13-week cell proliferation studies. A statistically significant increase in cell proliferation in the respiratory epithelium of the central portion (the primary site of squamous cell carcinomas; Level III in a 6 standard cross levels of the nose) of the nose was observed in rats exposed to 1 and 3 ppm formaldehyde for 3 days; the dose-response showed an approximate log-linear positive relationship with about a 10-fold increase in the 3 ppm group. After 13 weeks of exposure, the cell turnover rate at this nose region, however, tended to be slightly lower than in the controls. At the anterior part of the nose (Cross level II), on the other hand, an increase in cell proliferation was observed in the rats after both 3 days and 13 weeks of exposure to 3 ppm formaldehyde only; cell turnover in rats exposed to 0.3 and 1 ppm formaldehyde was not different from controls.

The findings of these studies (Swenberg et al., 1983; Zwart et al., 1988), therefore, suggest that restorative cell proliferation occurs in nasal passages of rats in response to formaldehyde exposure and that: (1) a significant increase in cell turnover occurred above 1 ppm, (2) the response lacks site specificity with respect to tumor induction and, (3) the response seems to be transient.

Recent acute and subacute studies have also demonstrated that there is a correlation between cytotoxicity and cell proliferation induced by formaldehyde in the rat nasal epithelium and that the cell proliferation rate is concentration-dependent. In these studies (Monticello, 1990), groups of Fischer 344 rats were exposed to 0, 0.7, 2, 6, 10, or 15 ppm formaldehyde gas, 6 hours/day, for 1, 4, or 9 days, or 6 weeks. There were no treatment-induced lesions or responses in cell proliferation in

Table 4-2

Effect of Formaldehyde Exposure on Cell Proliferation in the Central Portion Of the Nasal Passages*

	% of Labelled Respiratory Epithelial Cells	
Exposure**	Rat	Mouse
Control	0.22±0.03***	0.12±0.02
0.5 ppm	0.38±0.05	0.09±0.04
2 ppm	0.33±0.06	0.08±0.04
6 ppm	5.40±0.82	0.15±0.06
15 ppm	2.83±0.81	0.97±0.04

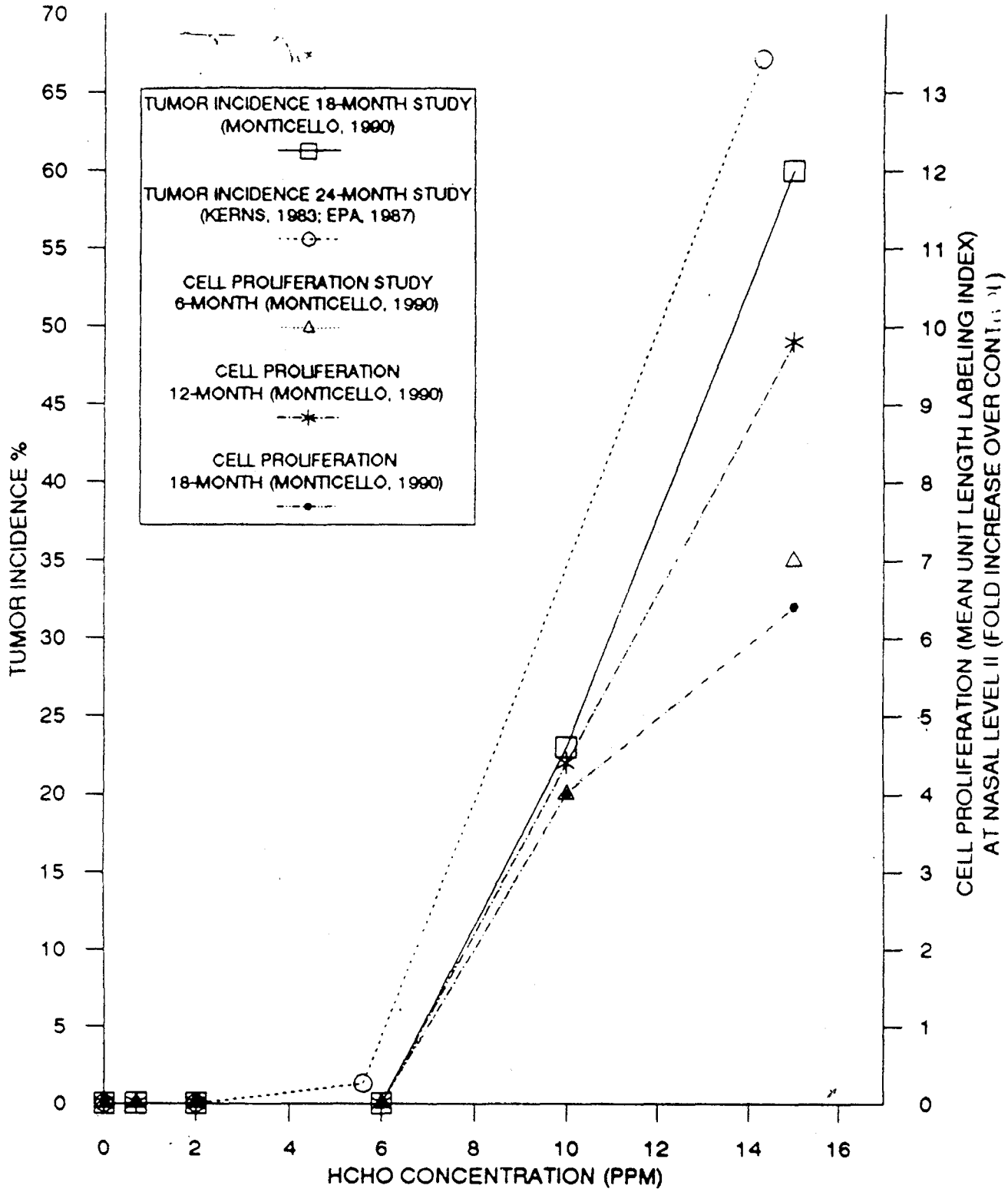
*Swenberg et al. (1983).
**All animals exposed for 6 hrs/day for 3 days.
***Mean ± standard error.

the two lowest concentration groups, 0.7 and 2.0 ppm. However, formaldehyde-induced lesions and increased cell proliferation were detected in the nasal passages of the 6, 10, and 15 ppm groups. Elevations in cell proliferation were detected following 1 day at concentrations of 6 ppm or higher and remained elevated after 6 weeks of exposure for some locations (the lateral wall and the maxilloturbinate) of the anterior nasal cavity.

Further insights into the relationship between both sustained cell proliferation and inflammation and formaldehyde-induced nasal carcinogenesis have recently been provided by the results of a chronic study in Fischer 344 rats. In these studies (Monticello, 1990; Monticello and Morgan, 1990), regional nasal respiratory epithelial lesions and cell proliferation (labeled cells/mm basement membrane) were examined following 6, 12, or 18 months exposure (6 hr/day, 5 day/week), to 0, 0.7, 2, 6, 10, or 15 ppm formaldehyde. Tumor incidence was assessed for all animals (scheduled and unscheduled sacrifices) through 18 months exposure of this 2-year study. The data showed that statistically significant elevations in cell proliferation were confined to the high-dose (10 and 15 ppm) groups following chronic exposures (6, 12 and 18 month values expressed as fold-increase over control; 10 ppm group: 4.0-, 4.3-, and 4.0-fold, respectively; 15 ppm group: 7.0-, 9.8-, and 6.6-fold, respectively). Nasal epithelial cell necrosis, inflammation, hyperplasia, metaplasia and squamous cell carcinomas also were present only in the 10 (4/17, 23% tumor incidence) and 15 ppm (27/45, 60% tumor incidence) groups. These results lend some support to the hypothesis that cell proliferation plays an important role in formaldehyde-induced nasal carcinogenesis since there appears to be a correlation between sustained cell proliferation and nasal tumor induction in rats exposed to formaldehyde at high doses (see Figure 4-1). These results also point to cell proliferation as a contributor to the observed nonlinear dose-response for formaldehyde-induced nasal tumors in rats; the cell proliferation data appear important for the development of biologically-based models for quantitative cancer risk assessment of formaldehyde.

It should be noted, however, as in the acute studies (Swenberg et al., 1983; Zwart et al., 1988), that the cell proliferation responses observed in these chronic studies (Monticello and Morgan, 1990) lack site-specificity with respect to tumor induction; similar increases in cell proliferation and inflammation scores occurred also at the medial aspect of the maxilloturbinate, a region where no nasal tumors were seen. These results, therefore, suggest that sustained increases in cell proliferation may not be solely responsible for the increase in the predisposition of the nasal cavity to formaldehyde carcinogenesis.

Figure 4-1.
Tumor incidence and cell proliferation in rats exposed to formaldehyde



As in the rat, studies in monkeys showed that formaldehyde-induced lesions were correlated with increases in cell proliferation (Monticello and Morgan, 1989). Groups of three rhesus monkeys were exposed either to room air or 6 ppm formaldehyde for 5 days per week for 1 or 6 weeks. Formaldehyde-induced lesions in the respiratory tract were associated with increases in cell proliferation rates up to 18-fold over controls. Whereas the greatest response in cell proliferation occurred in the anterior nasal cavity, increases in cell proliferation in the monkey were also detected in the larynx, trachea, and carina; this is in contrast to the rat where lesions were confined to the anterior nasal passages. Furthermore, after 6 weeks of exposure to 6 ppm formaldehyde, the labeling index in the monkey nasal mucosa remained elevated. Based on the extent of lesions and cell proliferation data, it was concluded that the monkey may be more sensitive than the rat to the acute and subacute effects of formaldehyde at 6 ppm. This is further supported by the finding that monkeys had considerably less DPX than rats exposed to the same formaldehyde airborne concentration (6 ppm) (Casanova et al., 1989). Under the conditions of this study, the maxillary sinuses of the monkey did not exhibit any evidence of histologic responses or changes in cell proliferation rate.

Formaldehyde-induced lesions and cell proliferation in human tracheobronchial epithelium that has been transplanted into rat tracheas have been studied (Klein-Szanto et al., 1989; Ura et al., 1989). In these experiments, de-epithelialized rat tracheas repopulated with normal human tracheobronchial epithelium were exposed in vivo to slow-releasing devices containing 0, 0.5, 1, and 2 mg paraformaldehyde. These studies showed that all doses elicited a proliferative response in the human respiratory epithelium similar to that observed in the rat epithelium when exposed to formaldehyde; the labeling index of the human respiratory epithelium showed dose dependence between 2 and 4 weeks after initiation of exposure. Based on these findings, the authors (Klein-Szanto et al., 1989) concluded that the similarity of response of the rats and human respiratory epithelia exposed to formaldehyde further supports the cancer risk concern of humans exposed to formaldehyde.

4.3.3 Role of the Protective Effect of the Mucociliary Apparatus

The mucociliary apparatus presents a continuous layer of mucus which flows over the surface of the nasal epithelium. In the rats, the mucociliary apparatus has been suggested to play an important role in protecting the nasal epithelium against the cytotoxicity of formaldehyde (Zwart et al., 1988). Both in vitro and in vivo studies (Morgan et al., 1983, 1986) have shown that there is a clear dose-dependent effect of formaldehyde on the mucociliary apparatus of the rats. At 15 ppm, there was

significant inhibition of mucociliary activity whereas at 2 or 6 ppm, only slight effects were noted. At 0.5 ppm, no effects were observed. This range appears to correspond to the range where the dose-responses in nasal mucosa cytotoxicity (Wilmer et al., 1989) and in carcinogenicity (Kerns et al., 1983) of formaldehyde were seen. It has, therefore, been postulated that at low formaldehyde concentrations, the mucus layer of the rat nasal cavity may trap and remove much inhaled formaldehyde, thus preventing it from reaching underlying cells. If the mucus layer is saturated (at high concentrations), the mucociliary clearance system could be seriously compromised and may allow a greater amount of formaldehyde to reach the underlying respiratory epithelium.

Although there is a dose-dependent effect of formaldehyde on the mucociliary apparatus (and on nasal cytotoxicity) of the rats, it is not known to what degree the mucus layer protects against the cytotoxic effects of formaldehyde.

In humans, nasal mucociliary function was inhibited by exposure to 0.3 ppm formaldehyde for 1-5 hours (Anderson and Molhave, 1983). It is also known that formaldehyde at levels below 1 ppm can be detected in the olfactory region of the human nose, indicating that formaldehyde is not completely removed by the mucus layer, even at low concentrations.

4.3.4 Importance of Dose Available for Deposition

The sensory irritant effects of formaldehyde are well documented. It has been shown that mice are more sensitive and reduce their respiratory rate and minute volume to a greater extent than rats in response to the irritancy of formaldehyde. When both species were exposed to 14.3 ppm formaldehyde, the mice inhaled only half as much formaldehyde per unit time as did rats (Chang et al., 1983). Therefore, it has been postulated that it may be this difference in the "dose" available for deposition to the nasal cavity and epithelium that accounts for the different carcinogenic response in rats and mice observed in the studies of Kerns et al. (1983). However, this factor alone may not be totally responsible for the difference in response between rats and mice. It is not known whether similar carcinogenic response would be observed if mice could have been exposed to levels of formaldehyde comparable to the amount effectively inhaled by rats.

4.3.5 Air Concentration vs. Total Daily Dose

An inhalation toxicity study was conducted by Wilmer et al. (1989) in order to investigate the importance of formaldehyde concentration vs. total dose. One hundred and fifty male Wistar rats (albino SPF) were allocated to 5 groups of 25 animals for histopathology, with 5 additional groups of 5 animals assigned to

a 3-day cell-proliferation study. Five rats from each group of 25 were further examined at the end of the 13-week period for histopathology and cell-proliferation. Two groups were exposed continuously to 1 or 2 ppm for a total duration of 8 hours, with concentration-time products (Ct) of 8 or 16 ppm-hr/day. Two groups were exposed intermittently at 30 minute intervals, total duration of 4 hours, to 2 or 4 ppm with Ct equal to 8 or 16 ppm-hr/day. A fifth group was exposed to air.

The cell-proliferation study showed no statistically significant difference in the number of [^{3}H]thymidine-labeled nasal epithelial cells between exposed and control groups after 3 days and 13 weeks formaldehyde exposure. After 13 weeks, the rats from each of the histopathology groups were sacrificed, and the posterior surface of the nasal cavity was sectioned and examined. The animals in the 4 ppm group intermittently exposed group exhibited significantly higher incidences than controls of treatment-related histopathological changes (diffuse disarrangement and squamous metaplasia). An increased incidence of these lesions were not found in the group receiving the same total dose of formaldehyde (2 ppm continuously exposed for 8 hours) or in any of the other groups. Thus the rate (the authors call this "concentration") at which a dose is applied is more important than the total dose in producing lesions commonly associated in rats with formaldehyde exposure. As a general observation, this study points to the possibly greater importance of concentration rather than total dose in determining the response of rats to formaldehyde.

In another study of dose rate by Feron et al. (1988), 465 male Wistar rats (albino SPF) were assigned to 9 different groups. Exposure regimens and observation periods are as follows.

Formaldehyde Concentration	Exposure Duration (6 hours/day, 5 days/week)		
0 ppm	4 weeks	8 weeks	13 weeks
10 ppm	4 weeks	8 weeks	13 weeks
20 ppm	4 weeks	8 weeks	13 weeks
Follow-up	126 weeks	122 weeks	117 weeks

Slightly lower body weights were observed in rats exposed to 10 ppm for 8 and 13 weeks; however, those animals exposed for 4 weeks at that concentration were not significantly affected. In all instances, rats exposed at 20 ppm had lower body weights compared to controls. Regardless of exposure duration, rats exposed to 10 ppm showed incidence of hyperplasia and stratified squamous metaplasia higher than in the controls. The incidence was higher in those animals exposed for 13 weeks. Rats exposed

to 20 ppm showed an increased incidence of rhinitis and replacement of the olfactory epithelium by respiratory or regenerating olfactory epithelium (in addition to hyperplasia and stratified squamous metaplasia) when compared with the controls.

Two squamous cell carcinomas were found in the control group exposed for 8 weeks, 1 each in the 10 ppm group exposed for 8 weeks and 13 weeks, and 5 in the 20 ppm group (1 each in the 4- and 8-week exposed groups and 3 in the group exposed for 13 weeks). The 20 ppm group also exhibited 2 polypoid adenomas (1 each in the 4- and 8-week exposed groups), 1 carcinoma in situ (13-week exposed group), 1 cystic squamous cell carcinoma (13 week-exposed group), and 1 ameloblastoma (13 week-exposed group). (It was not possible to determine from the study report if any of the four different types of lesions at the 20 ppm group occurred in the same animals).

The highest incidence occurred in the groups exposed for the longest period of time (13 weeks) and at the highest concentration (20 ppm). There is also a slight suggestion from the data that the total dose of formaldehyde may be a predictor for incidence of squamous cell carcinomas; however, the actual tumor counts are small and the periods for which the animals were exposed are too short for definitive determinations of the predictive potential of total dose versus concentration to be properly assessed.

4.3.6 Summary and Conclusions

Recent mechanistic studies have focused on the roles of DNA-protein cross-linking, cell proliferation, mucociliary apparatus, and concentration versus dose in formaldehyde-induced carcinogenesis.

A study by CIIT demonstrated that formaldehyde produced DNA-protein cross-linking in the rat nasal mucosa in a nonlinear manner. In view of the chemical reactivity of the compound, a plausible hypothesis is that formaldehyde may act by DNA-protein cross-linking and/or other direct genome-damaging reactions. Formaldehyde may also act in ways other than DNA-protein cross-linking or directly damaging the genome; however, additional issues that remain unresolved include determination of the types of mutations and the relationship between DNA-protein cross-links and mutagenesis in the rat nasal mucosa; and the possible role of other effects of formaldehyde on the genome in the carcinogenesis process.

Acute exposure studies show that cell proliferation in the rat was enhanced significantly at formaldehyde concentrations of 1 ppm or greater. The dose response for cell proliferation shows a steep increase from 3 to 10 ppm. An important observation is that the cell proliferation in the rat is sustained only at high

levels of exposure (10-15 ppm). The results of chronic studies show an apparent correlation between sustained, increased levels of cell proliferation and nasal tumor induction. These results lend support to the hypothesis that cell proliferation plays an important role in formaldehyde-induced nasal carcinogenesis. These results also point to cell proliferation as a contributor to the observed nonlinear dose-response for formaldehyde-induced nasal tumors in rats. However, as in the acute studies, cell proliferation responses observed in the chronic studies lack site-specificity with respect to tumor induction; similar increases in cell proliferation and cytotoxicity occurred also at the medial aspect of the maxilloturbinate, a region where no nasal tumors were seen. These results, therefore, indicate that sustained increases in cell proliferation may not be solely responsible for the increase in the predisposition of the nasal cavity to formaldehyde carcinogenesis.

Recent studies of monkeys and of human tracheal tissues indicate that the proliferative response in these species is qualitatively similar to that in rats.

Although a dose-dependent effect of formaldehyde on the mucociliary apparatus of the rats has been established, it is not known to what degree the mucus layer protects against the cytotoxic effects of formaldehyde, especially in humans.

Recent studies have also examined the relative importance of air concentration and total dose in formaldehyde cytotoxicity and cell proliferation. There is evidence to suggest that concentration is of greater importance than total dose in eliciting toxic responses.

4.4 Epidemiologic Studies

The EPA (1987) review of epidemiologic evidence based on 28 studies concluded that "limited" evidence existed for an association between formaldehyde and human cancer using criteria as defined in EPA's Cancer Risk Assessment Guidelines (EPA, 1987). The evidence is based mainly on well-conducted cohort and case-control studies of populations occupationally exposed to formaldehyde during chemical production, in textiles, in plastics production, and in various wood industries. One study examined residency in mobile homes.

Three studies were designed specifically to detect moderate elevations in underlying cancer risk among populations with formaldehyde exposure. These studies (Blair et al., 1986, 1987; Stayner et al., 1988; and Vaughan et al., 1986a, 1986b) observed statistically significant elevations in risk of site-specific upper respiratory cancers (nasopharynx or buccal cavity) with measures of formaldehyde exposure. In all three studies, the

site-specific cancer risks appeared to increase with increasing exposure level or with increasing duration of exposure.

Other investigations (Olsen et al., 1984; Hayes et al, 1986) showed possible increases in sinonasal cancer, while others (Partanen et al., 1985; Bertazzi et al., 1986; Blair et al., 1986, 1987; and Acheson et al., 1984) indicated that lung cancer also may be associated with occupational exposure to formaldehyde. The risk associated with sinonasal cancer appeared to be specific for the histologic type, squamous cell carcinoma. The relative risks observed for upper respiratory tract cancers in all the reviewed studies ranged from just above 1.0 (a risk of 1.0 implies no association between exposure and disease) to 3.0, depending on the site.

Several of the epidemiologic studies (Blair et al., 1987; Hayes et al., 1987; Olsen et al., 1984), also, suggested that nasopharyngeal and sinonasal cavity cancer risks may be enhanced with simultaneous exposure to particulates or wood dust. There is limited evidence for an association between wood dust exposure itself and sinonasal cavity cancer (IARC, 1987). Both Hayes et al. (1987) and Olsen et al. (1984) observed that the risk (statistically significantly elevated) associated with combined formaldehyde and wood dust exposure appeared higher than the individual risks associated with wood dust exposure, adjusted for formaldehyde exposure, or for formaldehyde exposure, adjusted for wood dust exposure. Risks in analyses which attempted to control for wood dust exposure, however, were generally not statistically significant.

As discussed in EPA (1987), elevations in risks from cancers of the nasopharynx, nasal cavity and sinuses, and lung were not detected in every reviewed study. One reason the epidemiologic evidence on formaldehyde can appear inconsistent is that many of the previously reviewed studies had limited ability to detect small to moderate elevations in site-specific cancer risks. This is particularly true for those sites with lower incidence rates such as cancer of the nasal cavity and sinuses. Contributing factors that reduce a study's detection ability include small study populations, shortened follow-up periods which did not consider sufficient disease latency, and a low proportion of highly exposed individuals. In addition, errors in the exposure measurements introduce a bias which limits the detection ability.

The EPA (1987) review also discussed studies of populations occupationally exposed to formalin, a solution containing formaldehyde. These investigations reported excesses of leukemia, and cancer of the brain and prostate were reported in embalmers, morticians, anatomists, and pathologists (Harrington and Shannon, 1975; Harrington and Oakes, 1984; Levine et al., 1984; Matanoski, 1982; Stroup, 1984; Walrath and Fraumeni 1983; Walrath and Fraumeni 1984). The biological support for an

association with cancer at these sites was weak. In addition, the contribution of other agents such as viruses and certain solvent exposures was considered more likely with populations exposed to formalin than with the industrial populations.

Since publication of EPA (1987), eleven new reports have become available. Four of these studies contain new data (Roush et al., 1987; Stern et al., 1987; Malker et al., 1990; Hayes et al., 1990). The remaining are either reanalyses of the Blair et al. (1986, 1987) study (Collins et al., 1988; Sterling and Weinkam, 1988; Robins et al., 1988; Blair et al., 1990a), or further investigations of existing data sets (Gerin et al., 1989; Partanen et al., 1990). Data presented by Stayner et al. (1988) were already reviewed in EPA (1987) and will stand as discussed there. In addition to the above reports, three reviews have been published (IARC, 1987; UAREP, 1988; Blair et al., 1990b).

This section provides an evaluation of the new reports and reviews, along with a discussion of exposure information and the total epidemiologic evidence on formaldehyde. More detailed information regarding the new reports and selected existing studies (as reviewed in EPA (1987)) are presented in Appendix A. The tables in Appendix A contain a description of the study design and population, the risks associated with site-specific cancers, and an estimate of exposure levels as the study authors described them.

4.4.1 Case-Control Studies

Gerin et al. (1989) conducted a case-control analysis to further investigate possible associations of occupational formaldehyde exposure and cancer at specific sites. Cases and controls came from a study which was originally designed to generate hypotheses on many occupational exposures and 14 site-specific cancers (Siemiatycki et al., 1987a). Cancers of the nasopharynx and sinonasal cavity were not included in either the case or control series. For the present analysis, Gerin et al. (1989) assessed the job histories of all cases and controls for potential formaldehyde exposure.

No odds ratios (OR) were significantly elevated in this study. These investigators, however, observed an apparent increased odds ratio between adenocarcinoma of the lung and long duration-high occupational exposure to formaldehyde (OR=2.3, 95% confidence interval: (0.9, 6.0)). The long duration-high exposure classification was believed most likely a time-weighted-average exposure below 1.0 ppm. This apparent increase did not meet statistical significance at the 0.05 level in an analysis which took into account age, ethnic group, socio-economic status, cigarette smoking, and white-collar/blue-collar status. Although no trend analysis was presented by Gerin et al. (1989), for those with long durations of exposure alone, the risks for

adenocarcinoma of the lung appeared to increase with increasing exposure level (long-low, OR = 0.8, 95% C.I.: (0.3, 1.3); long-medium, OR = 0.8, 95% C.I.: (0.4, 1.6); long-high, O.R. = 2.3, 95% C.I.: (0.9, 6.0)). The authors concluded that the apparent increase in adenocarcinoma of the lung may be indicative of increased risk with formaldehyde exposure. They noted, however, that this finding may be due to chance.

Roush et al. (1987) conducted a case-control study specifically designed to examine associations between sinonasal and nasopharyngeal cancer cases and possible occupational formaldehyde exposure. They selected cases covering the last 41 years from the Connecticut Tumor Registry. Controls were drawn from men dying in Connecticut in the same period. Occupational exposure information was indirectly ascertained for each of the cases and controls from death certificate and city directories. These investigators did not observe statistically significant increases in risk between sinonasal cavity cancer and probable occupational formaldehyde exposure, although an apparently elevated risk remained after taking into account a 20+ year latency and probable high formaldehyde exposure.

An apparently elevated odds ratio was observed for nasopharyngeal cancer when there had been probable formaldehyde exposure at high levels 20 or more years prior to death (OR = 2.3, 95% C.I.: (0.9, 6.0)). This odds ratio appeared to increase with age (68+ years vs. <68 years) and was statistically significantly elevated for the older age group (OR = 4.0, 95% C.I.: (1.3, 12.0)).

These authors concluded, for the older age and higher exposure category, that the elevated odds ratio suggested an association between cancer at this site and formaldehyde. They attributed the increased risk partially to lower background nasopharyngeal cancer risks for males in this age group. The detection ability of this study may have been lowered by several factors, including the availability of only indirect information on occupational exposure. In addition, formaldehyde exposures from other-than-occupational sources, for instance, from residential dwellings, could not be ascertained in this study, nor could possible confounding exposures such as smoking.

Partanen et al. (1990) examined possible associations between formaldehyde and respiratory cancer in a nested case-control study of 136 respiratory cancers among 7307 male Finnish woodworkers. These men were employed in jobs in particleboard, plywood, construction carpentry, furniture manufacturing, and glue manufacturing plants, and in sawmills. In a previous study (Partanen et al., 1985), these investigators found an apparently increased odds ratio (OR=1.4, $p>0.10$) between respiratory cancer and formaldehyde in a nested case-control study based upon a cohort of 3805 of these workers. For the current study, Partanen

et al. augment their original cohort to include other wood working facilities and they update mortality an additional year, until 1982. As in their earlier study, three controls were matched to each case according to year of birth.

After accounting for a minimum latency period of 10 years, smoking, and vital status at the time of data collection, Partanen et al. observed an apparently elevated odds ratio for respiratory cancer (OR=1.4, 90% C.I.: 0.4, 4.1) with exposure to cumulative formaldehyde (either dustborne or as a gas) (≥3 ppm-months). It appeared from other analyses examining upper respiratory and lung cancer separately, that the excess risk associated with cumulative formaldehyde exposure concentrated into the category of upper respiratory cancer (OR=2.4, 90% C.I.: 0.4, 13.2; adjusted for vital status and a 10 year latent period) rather than to lung cancer (OR=0.9, 90% C.I.: 0.3, 3.0; adjusted from vital status, a 10 year latent period, and smoking). Analyses examining exposure to only dustborne formaldehyde were similar to analyses examining total formaldehyde. In addition, no exposure-response relationships were observed between respiratory cancer and either level, duration, or cumulative formaldehyde exposure. In all these analyses, Partanen et al. did not control for wood dust exposure.

Partanen et al. (1990) believed these results are compatible either with chance or with a weak elevated risk mainly due to cancers of the upper respiratory organs. Identified limitations in this study include low power, inconsistencies between the data from the original cohort and the augmented cohort, possible misclassification of early exposure, pooling of different "respiratory" cancers, respondent bias regarding information on smoking, and possible confounding by phenol. Phenol exposure was highly correlated with formaldehyde since glue formulations in the manufacturing of plywood contained both formaldehyde and phenol.

4.4.2. Proportional Mortality Studies

Hayes et al. (1990) conducted a proportional mortality ratio (PMR) study of embalmers and funeral directors in the U.S. In all, 3649 white and 397 nonwhite male deaths between 1975 and 1985 were identified from licensing boards and state funeral directors' associations, from occupational information on death certificates, and from a listing of deaths among members of the National Funeral Directors' Association. Proportions of deaths for 1975-1985 in the U.S. population were used as referents. The investigators provided exposure information from recently conducted exposure monitoring of 24 embalmings in a mortuary college. Formaldehyde levels averaged over the entire embalming procedure ranged from 0.98 ppm (associated with high ventilation) to 3.99 ppm (for low ventilation); total dust levels averaged 0.42 mg/m^3 (range 0.07 to 0.78 mg/m^3). Wipe samples in the

embalming room contained blood contamination, suggesting that aerosolization of blood and other biologic tissue may occur. The authors use these exposure measures as qualitative indicators of exposure for deaths in this study.

Statistically significantly elevated proportions of deaths were found from the following causes: all cancers (whites, PMR=107, 900 deaths, 95% C.I.: 101-115), ischemic heart disease (whites, PMR=113, 1418 deaths, 95% C.I.: 107-119; nonwhites, PMR=145, 135 deaths, 95% C.I.: 122-172), and suicide (whites, PMR=130, 74 deaths, 94% C.I.: 102-164). Significant decreases in the proportion of deaths for both races were noted for diseases of the respiratory system (whites, PMR=85, 233 deaths, 95% C.I.: 74-97; nonwhites, PMR=53, 13 deaths, 95% C.I.: 28-91). Among neoplastic deaths, statistically significant increases were observed for cancers of the colon (nonwhites, PMR=231, 16 deaths, 95% C.I. 132-376) and lymphatic and hematopoietic system (whites, PMR=131, 100 deaths, 95% C.I.: 106-159; nonwhites, PMR=241, 15 deaths, 95% C.I.: 135-397). These investigators also noted that the risk associated with nasopharyngeal cancer appeared elevated for whites (PMR=189, 3 deaths, 95% C.I.: 39-548), but the elevation was not statistically significant. Only one death due to nasopharyngeal cancer was observed among nonwhites, whereas, only 0.25 would have been expected.

The authors believed the apparent elevated proportions of death due to nasopharyngeal cancer and leukemia were consistent with previous observations in formaldehyde-exposed industrial cohorts and other studies of professionals (embalmers, morticians, pathologists, and anatomists). This study is limited by its study design. Since the comparison is based on a proportion, if the number of deaths other than the cause of interest is low, then the PMR for the cause of interest may be artificially inflated. In addition, selection bias related to leukemia diagnosis could not be discounted and lack of specific exposure information limits these conclusions.

4.4.3 Cohort Studies

Stern et al. (1987) conducted a cohort study of 9,365 leather tannery workers. The exposures of concern were chrome and other numerous chemicals, including formaldehyde. Formaldehyde was detected in measurable levels in the tannery's finishing department. The investigators observed one death (0.4 expected) due to squamous cell carcinoma of the nasal cavity. This person had worked more than 18 years in the finishing department. Although formaldehyde could not be specifically implicated, these investigators concluded that the death was most likely attributable to an occupational origin since it occurred sufficient years after entry to the department to be consistent with an induction period for occupational nasal carcinoma.

Malker et al. (1990) examined the relationship between nasopharyngeal cancer incidence and several occupational groupings with potential formaldehyde exposure. Incident male cases of nasopharyngeal cancer were identified from the Swedish Cancer-Environment Registry for the period 1961-1979. Expected numbers of cases were derived using age- and sex-specific cancer incidence rates applied to the distributions of the various occupational and industrial categories. Employment was defined for 1960 from information given to the cancer registry. The authors observed statistically significantly elevated ($p<0.05$) standardized incidence ratios (SIR) for men employed in fiberboard plants (SIR=3.9, 95% C.I.: 1.3, 9.3) and shoe repair (SIR=4.0, 95% C.I.: 1.0, 9.9). No statistically significantly elevated risks were seen among textile workers, furniture makers, or chemical workers.

These investigators consider the association between nasopharyngeal cancer and fiberboard employment as noteworthy, given previous reports of elevated risks between cancer at this site and formaldehyde exposure. The authors further believe that the lack of an association between other job categories with exposure to formaldehyde and nasopharyngeal cancer may reflect limitations in the job coding scheme used by the cancer registry.

Four groups of investigators have reexamined the Blair et al. (1986, 1987) data, using either different exposure measures or statistical methodology (Collins et al., 1988; Sterling and Weinkam, 1989, 1990, personal communication; Robins et al., 1988; Blair et al, 1990a). Blair et al. (1986, 1987) studied 26,561 workers from 10 U.S. plants with occupational formaldehyde exposure through manufacture of formaldehyde, resins, laminate, molding compounds, photographic films, or particleboard. Blair et al. (1986) examined the relationship between mortality and formaldehyde exposure as categorized three ways: 1) time-weighted average (TWA), 2) peak exposure, and 3) cumulative exposure in ppm-years.

Collins et al. (1988) reanalyzed the cohort data of Blair et al. (1986, 1987) using two approaches. The first partitioned the nasopharyngeal mortality experience into that of the Cyanamid cohort and that of the remaining nine plants. The second examined nasopharyngeal cancer mortality and simultaneous formaldehyde and particulate exposure utilizing a revised exposure classification scheme. Formaldehyde exposure was considered important only when it simultaneously occurred in the presence of particulates. Blair et al. (1986, 1987), on the other hand, classified an individual as particulate exposed if he was ever exposed to particulates at any time in his working history. For a particulate-exposed study subject in Blair et al. (1986, 1987), all formaldehyde exposure, whether incurred in the presence or absence of particulates, was summed in the cumulative exposure measure.

Collins et al. concluded from the first approach that the excess in nasopharyngeal cancer mortality observed by Blair et al. (1986, 1987) was limited in the Cyanamid plant. By using their exposure classification (the second approach), Collins et al. (1988) concluded that the dose-response relationship between increasing cumulative formaldehyde exposure and increasing nasopharyngeal cancer risk apparent in Blair et al. (1986, 1987), disappeared. Collins et al. (1988) drew support for their conclusion from the lack of a statistically significant trend in risks with increasing formaldehyde-particulate exposure.

The Collins et al. (1988) reanalysis appears well-conducted, however, strong conclusions cannot be drawn. Their reanalysis is based upon few deaths and as these deaths are distributed across four exposure categories, the risks for each category are subject to wide variation, and become very unstable. For this reason, any strong interpretation of the Collins et al. (1988) reanalysis is difficult.

Sterling and Weinkam (1988; 1989; personal communication with Dr. James Weinkam, April 12, 1990) performed several analyses examining deaths due to all cancers, all respiratory cancers, and all lung cancer observed in Blair et al. (1986, 1987) by reclassifying exposure using a time-integrated exposure score, and by comparing mortality among the higher-exposed to that of lower-exposed (an internal comparison group). Blair and Stewart (1989) and Blair et al. (1990a) questioned the Sterling and Weinkam (1988; 1989) conclusions with respect to the correct number of person-years and the correct number of deaths. For example, Blair et al. (1986) observed 280 deaths, yet the Sterling and Weinkam (1989) reanalysis was based upon 299 lung cancer deaths. The 299 deaths included all respiratory system cancer deaths, which includes cancers of the sinonasal cavity, larynx, and lung (Weinkam, personal communication).

A reanalysis has been performed based only on the 280 lung cancer deaths (personal communication with Dr. James Weinkam, April 12, 1990). Cumulative exposure to formaldehyde was ranked into four hierarchical categories and odds ratios for cumulative exposure to formaldehyde and lung cancer were as follows: <0.1 ppm-yr, OR = 1.0; 0.1-0.5 ppm-yr, OR = 1.12, 95% C.I.: (0.76, 1.84), 0.5-2.0 ppm-yr, OR = 1.12, 95% C.I.: (0.76, 1.84), and 2+ ppm-yr, O.R. = 1.46, 95% C.I.: (0.87, 2.49). Exposure-response relationships between lung cancer and cumulative formaldehyde were also examined using logistic regression analysis. This analysis suggested an increase in lung cancer risk with increasing cumulative exposure, however, the estimated odds ratio for the highest cumulative exposure was not statistically significant. Weinkam believes that this analysis along with the observations in the 1988 and 1989 articles supported the conclusion that lung cancer mortality is associated with formaldehyde exposure. This conclusion is limited since the 1988

and 1989 analyses appeared to be based upon a incorrect number of site-specific cases, and the most recent results did not achieve statistical significance.

Robins et al. (1988) reanalyzed the lung cancer and nonmalignant respiratory disease mortality data of Blair et al. (1986) using analytical methods newly developed by the authors that accounted for the survivor effect. These analyses were carried out to address comments that the lack of a dose-response trend for lung cancer observed in Blair et al. (1986) may be due to bias from the survivor effect. The survivor effect is the tendency of workers at increased risk of death to preferentially terminate employment earlier than those not at increased risk. These workers, thus, tend to have less cumulative exposure. If a survivor effect is operating, an artifactual inverse relationship will be seen between cumulative exposure and the site-specific death.

By examining mortality rates among workers who terminated employment and those that did not, Robins et al. (1988) observed that nonmalignant respiratory disease mortality was higher among workers who terminated employment than among those workers who continued to worker over a 15 year period. This finding supports the hypothesis that survivor effect bias was present in the Blair et al. (1986) analyses examining nonmalignant respiratory disease. In their examinations of lung cancer, Robins et al. did not find the effects of survivor bias when formaldehyde exposure was assessed using cumulative exposure, peak exposure, and combined exposure to formaldehyde and particulates. They believed these findings confirmed the Blair et al. (1986) original conclusions that formaldehyde was not associated with lung cancer mortality.

One limitation of the Robins et al.'s reanalysis of lung cancer is that it is based upon the entire cohort. This includes both salaried and wage workers. Blair et al. (1986) did not observe an increased lung cancer risk in the entire cohort, only among workers classified as wage workers and among wage workers with 20 or more years since first exposure. Any small lung cancer effect limited to a selected subgroup such as wage workers may be diluted in analyses which examine the lung cancer experience of the entire cohort.

Blair et al. (1990a) examined in greater detail than in their 1986 article exposure-response relationships for lung cancer mortality and formaldehyde, and for lung cancer and exposure to other substances found in conjunction with formaldehyde. These analyses were limited to wage workers. The authors did not observe any consistent pattern of increasing trends between lung cancer and formaldehyde exposure, as assessed by peak, average, intensity, duration, and cumulative exposure. They reported positive exposure-response associations between

lung cancer and cumulative formaldehyde level for persons hired before the study's start date, and for those workers with exposure to formaldehyde and particulates from resin and molding compound operations. Neither of these associations was statistically significant.

In analyses that examine lung cancer mortality by specific manufactured products, the SMR for plywood was 180; molding compounds or resins, SMR=120; and plastic products, SMR=170 (confidence intervals could not be calculated due to missing elements of observed/expected ratios in the paper). Among specific plants (work sites), statistically significant elevations were noted for three plants (Nos. 1, 6, and 9) for those workers with any formaldehyde exposure. Particulate exposure would have been expected for plants 1 and 6. Exposure-response associations were suggested for three individual plants (Nos. 1, 3, and 4) with cumulative exposure. The trend was statistically significant for one plant (No. 3); however, the trends for the other two plants were not.

In analyses which examine lung cancer mortality and exposure to formaldehyde alone, and to substances found in conjunction with formaldehyde, statistically significantly elevated SMRs were noted among wage workers with a 20-year latency for exposure to antioxidants, asbestos, hexamethylenetetramine, melamine, plasticizers, urea, and wood dust. These SMRs were greater than or equal to 150. In addition, lung cancer mortality was statistically significantly elevated among formaldehyde-exposed who also had exposure to other substances (SMR=140, 95% C.I.: 116, 167). Persons exposed only to formaldehyde had an SMR of 103 (95% C.I.: 83, 127). Statistically significant positive exposure-response trends were noted for melamine and urea. Positive trends were also suggested for phenol and wood dust, but they were not statistically significant.

Blair et al. (1990a) concluded that the lack of clear exposure-response trends for lung cancer in this study was consistent with results of their previous study (Blair et al., 1986). They feel that the small, but not statistically significant positive association for persons with exposure to formaldehyde and particulates may reflect the risks associated with substances in resin and molding compound operations. This conclusion was further supported by positive exposure-response trends for several compounds (e.g., melamine and urea) found in the resin and molding compound operations. They also believed that interpretation of the apparent exposure-response gradient for exposure to formaldehyde and particulates from resin and molding compound operations is difficult because of small increases in risk, lack of statistical significance, and inconsistency among individual plants.

4.4.4 Evaluation of New Studies

The reports received since the EPA (1987) assessment focused on cancer of the respiratory tract following inhalation exposure. Dermal exposure, in addition, was probable in one study (Hayes et al., 1990). Four new studies were added (Stern et al., 1987; Malker et al., 1990; Roush et al., 1987; and Hayes et al., 1990). The study of Roush et al. (1987) was specifically designed to assess the role of formaldehyde exposure, thus, the conclusions of this study carry greater weight than the other new studies. The remainder were reanalyses of previously published studies (Collins et al., 1988; Sterling and Weinkam, 1988, 1989; Robins et al., 1988; and Blair et al., 1990), or further investigations of existing data sets (Gerin et al., 1989; Partanen et al., 1990).

The new studies provide small additional support to the conclusions made in EPA (1987) that suggested an association between formaldehyde and respiratory tract cancer. This new evidence, however, does not provide causal evidence of human cancer. These new studies are limited because of lack of power to detect small to moderate excess cancer risks associated with exposure and possible exposure misclassification.

Two of the new studies investigated sinonasal cancer (Roush et al., 1987; Stern et al., 1987). Neither showed any indication of a formaldehyde-associated effect. Nasopharyngeal cancer was evaluated in several of the analyses. Roush et al. (1987) found the highest risk among those who were older, who had the longest latency, and with the highest occupational exposure. This finding suggests that an occupational agent, most likely formaldehyde, is responsible for the observed increased cancer risk. Malker et al. (1990) observed a statistically significant elevation in nasopharyngeal cancer risk with employment in fiberboard plants where formaldehyde and particulate (wood dust and resin) exposures are expected. No such observations were noted for other industries with potential formaldehyde exposure. Exposure is not as well characterized in this study as in those where information exists on individual jobs held by each worker, and limits the Malker et al. (1990) observations. Hayes et al. (1990) noted an elevation in the proportion of nasopharyngeal deaths among embalmers. This elevation was not statistically significant ($p>0.05$).

The reanalysis of the Blair et al. (1986, 1987) data using different exposure measures or statistical methodology, including those done by Collins et al. (1988), Sterling and Weinkam (1988; 1989), Robins et al. (1988) and Blair et al. (1990a), enables an examination of the stability of prior conclusions and allows the generation of alternative interpretations and limitations around these alternatives. Different conclusions than those of Blair et al. (1986, 1987) have been drawn by two investigators (Collins et

al., 1988; Sterling and Weinkam, 1988, 1989, personnel communication). Conclusions from two other reanalyses (Robins et al., 1988; Blair et al., 1990) were similar to the original investigation. With respect to lung cancer, Sterling and Weinkam (1988, 1989, personal communication) believed their analyses suggested an exposure-response relationship between increasing lung cancer risk and increasing cumulative formaldehyde. On the other hand, both Blair et al. (1990) and Robins et al. (1988) believed their analyses did not demonstrate that an exposure-response relationship existed with formaldehyde. With respect to nasopharyngeal cancer, Collins et al. (1988) did not find a statistically significant relationship between increasing nasopharyngeal cancer risks and increasing cumulative formaldehyde using an exposure classification scheme which considered important only formaldehyde exposure when it simultaneously occurred in the presence of particulates.

The EPA has carried out other analyses on data presented by Collins et al. (1988) (see Appendix A) using a Poisson trend statistic (Armitage, 1955; Tarone, 1982; as cited in Chapter 3 of Breslow and Day, 1980). Analyses which are based upon exposure as defined by interval midpoints (0 ppm/yr, 0.25 ppm/yr, 3.0 ppm/yr, and 12.5 ppm/yr) and by rank score (1, 2, 3, 4) show statistically significant trends between increasing nasopharyngeal cancer risk and increasing formaldehyde exposure. These results support the Blair et al. (1987) previous observations of an association between formaldehyde and nasopharyngeal cancer.

New investigations on lung cancer do not provide much support that formaldehyde, by itself, is responsible for excesses in lung cancer. No statistically significant increases in risk were noted in Gerin et al. (1989) and Partanen et al. (1990) nor in reanalyses (Blair et al., 1990a; Robins et al., 1988; Sterling and Weinkam, personal communication) of Blair et al. (1986). One study (Blair et al., 1990), however, suggests that combined exposures, such as those in operations involving resins and molding materials, may be associated with excesses in lung cancer. This analysis observed positive trends with exposure to two resins (urea and melamine), where exposure to both gaseous formaldehyde and particulates is expected.

4.4.5 Non-EPA Reviews of the Formaldehyde Literature

The International Agency for Research on Cancer (IARC) (1987) reviewed essentially the same literature as EPA (1987) and concluded that there was "limited" evidence of carcinogenicity to humans according to the IARC's Classification of the Evidence for Carcinogenicity. IARC defines "limited evidence of carcinogenicity" as "a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or

confounding could not be ruled out with reasonable confidence" (IARC, 1987).

IARC (1987) believed the evidence was strongest for cancers of the nose and nasopharynx since exposure-response relationships had been observed in several studies. This group also noted an excess in lung cancer in several studies, but that exposure-response gradients were not consistently demonstrated across all studies. The excesses in mortality from leukemia and brain cancer were primarily limited to occupations with formalin exposure, but IARC believed that factors other than formaldehyde might have increased the risk for these cancers.

A panel under the auspices of the University Associated for Research and Education (UAREP) (1988) reviewed the same body of literature as IARC (1987) and EPA (1987) using a metanalysis approach. The UAREP panel commented only on the determination of causality. Unlike the IARC and EPA, the UAREP panel did not attempt to categorize the epidemiologic evidence other than whether causality could be established. The panel concluded that a causal relationship has not been established for cancer at any site. In addition, the panel noted that if such a causal relationship exists, the excess risk must be small. The panel noted elevated risks in nasopharyngeal cancer with formaldehyde exposure in several studies, and concluded that the evidence for causality was weak. With respect to observed excesses in nasal cavity and sinus cancers and any formaldehyde exposure, several studies suggest an approximate doubling of the risk, while other studies could not exclude an elevation of this size. Overall, the panel concluded that the presence or absence of an association could not be firmly established. With respect to lung cancer, the panel thought the evidence was not consistent and did not indicate a causal association with formaldehyde exposure.

For sites which are not directly in contact with formaldehyde (denoted non-topical sites in the UAREP report), the panel stated that the rapid metabolism of formaldehyde makes it unlikely that formaldehyde is the agent responsible for increased brain tumors observed in the group that uses formalin. For the excesses in leukemia observed in several studies of anatomists, embalmers, and pathologists, the panel concluded that socioeconomic factors influencing diagnosis may explain the elevations observed in these groups.

Blair et al. (1990b) performed a metanalysis on essentially the same body of literature as reviewed by IARC (1987), UAREP (1988), and EPA (1987) with the addition of more recent findings, either published or in press. From the metanalysis, Blair et al. found excesses in deaths due to cancers of the nasal cavities, nasopharynx, lung, and brain, and due to leukemia. The investigators believed that a causal role for formaldehyde was

most probable for cancers of the nasopharynx and, to a lesser extent, the nasal cavities.

Blair et al. (1990b) derived their support for the conclusion regarding nasal cavity cancer from statistically significant elevations in nasal cavity cancer risk, from the apparent specificity of the association with squamous cell carcinoma, and from histological changes in the nasal mucosa of woodworkers and chemical workers which corresponded to lesions observed in rodents. Potential confounding from other exposures (i.e., wood dust) in these studies still remained a problem, however. With regard to nasopharyngeal cancer, their conclusions were based on statistically significant excesses observed in three studies and the observation of a dose-response trend between increasing risk and increasing formaldehyde exposure observed in two of these studies. In addition, they believed that the occurrence of cancer at a site with possible direct contact with formaldehyde made sense in terms of the biological knowledge. Small numbers, inconsistency among studies, and a possible independent role for particulates precluded definitively labeling formaldehyde as a nasopharyngeal carcinogen.

Blair et al. (1990b) further concluded that the excesses in lung cancer were difficult to interpret due to inconsistencies among studies and lack of trends with either level or duration of exposure. In addition, the excesses of leukemia and brain and colon cancer observed among professionals (i.e., those with formalin exposure) were most likely not related to formaldehyde since similar excesses were not observed among the industrial workers.

4.4.6 Discussion and Conclusions

The accumulated epidemiologic evidence is consistent with the conclusion reached in EPA (1987) that there is "limited" evidence of human carcinogenicity as defined in EPA's Cancer Risk Assessment Guidelines (EPA, 1986). Because collectively the data do not conclusively demonstrate a causal relationship, the evidence is not considered "sufficient".

Increases in respiratory tract cancer are seen in several well-designed and well-conducted studies. Observed relative risks have not been large, risks ranged from just above 1.0 to 3.0, depending on the site. There also appears to be a gradient of support, with greater support for cancers near the portal of entry (cancers of the nasal cavity and sinus, and the nasopharynx) than further down the respiratory tract (lung cancer). Table 4-3 presents selected results from reviewed studies cited in Appendix A.

Table 4-3

Selected findings from epidemiologic studies examining inhaled formaldehyde

Authors (year)	Cancer Site	Risk Measure	Type of Exposure	Risk Ratio (95% Confidence Interval)[a]
Hayes et al. (1986)	Nose and Nasal Sinuses	OR[b]	Formaldehyde; No to low wood dust	
			Classification A[c]	2.5* (1.2[d], 5.0[e])
			Classification B[c]	1.6 (0.9[d], 2.8[e])
	(Squamous cell carcinoma)		Formaldehyde; Low wood dust	
			Classification A	3.0* (1.3[d], 6.4[e])
			Classification B	1.9 (1.0[d], 3.6[e])
Olsen et al. (1984)	Nose and Nasal Sinuses	OR	Formaldehyde and wood dust	2.8* (1.3[d], 6.4[e])
			Formaldehyde; adjusted for wood dust	1.6
Olsen and Asnaes (1986)	Nose and Nasal Sinuses (Squamous cell carcinoma)	OR	Formaldehyde; adjusted for wood dust	2.3 (0.9[d], 5.8[e])
Vaughan et al. (1986b)	Nasopharynx	OR	Mobile home residency	
			1-9 years	2.1 (0.7, 6.6)
			10+ years	5.5* (1.6, 19.4)
Roush et al. (1987)	Nasopharynx	OR	Formaldehyde	
			High level exposure (≥ 1 ppm at 20+ yrs. prior to death)	2.3 (0.9, 6.0)
			High level exposure (≥ 1 ppm at 20+ yrs. prior to death); 68+ yrs. old at death	4.0* (1.3, 12.0)
Malker et al. (1990)	Nasopharynx	SIR[f]	Fiberboard manufacturing	3.9* (1.1, 10.2)

Table [redacted] (continued)

Authors (year)	Cancer Site	Risk Measure	Type of Exposure	Risk Ratio (95% Confidence Interval)[a]
Blair et al. (1986, 1987)	Nasopharynx	SMR[g]	Ever exposed to formaldehyde (ppm-year)	300* (110, 653)
			Ever exposed to particulates (ppm-year)	
			< 0.5	192 (5, 1114)
			0.5 -5.5	403 (48, 1445)
			> 5.5	746 (81, 2408)
Collins et al. (1988) (reanalysis of Blair et al., 1986)	Nasopharynx	SMR	Simultaneous formaldehyde and particulates (ppm-year)	
			0	215 (25, 802)
			< 0.5	343 (37, 1204)
			0.5 -5.5	216 (3, 1113)
			> 5.5	826* (112, 3611)
Hayes et al. (1990)[h]	Nasopharynx	PMR[i]	Embalmers and funeral directors	189 (39, 548)
Blair et al. (1986)	Lung	SMR	Ever exposed to formaldehyde (ppm-year)	110 (97, 125)
			Wage workers, >20 years of latency	122* (106, 139)
Blair et al. (1990a) (reanalysis of Blair et al., 1986)	Lung	SMR	Formaldehyde alone (ppm-year)	
			< 0.5	110 (80, 149)
			0.5 -5.5	110 (78, 151)
			> 5.5	74 (41, 124)
			Formaldehyde and other agents (ppm-year)	
			< 0.5	154* (110, 212)
			0.5 - 5.5	118 (87, 158)
			> 5.5	154* (113, 207)

Table 4-3 (continued)

Authors (year)	Cancer Site	Risk Measure	Type of Exposure	Risk Ratio (95% Confidence Interval)[a]
Sterling and Weinkam (personal communication-reanalysis of Blair et al., 1986)	Lung	OR	Formaldehyde (ppm-year)	
			< 0.1	1.0
			0.1 - 0.5	1.1 (0.8, 1.8)
			0.5 - 2.0	1.1 (0.7, 1.8)
			> 2.0	1.5 (0.9, 2.5)
Acheson et al. (1984)	Lung	SMR	All 6 plants	105 (91, 120)
			BIP plant	118* (100, 137)
Bertazzi et al. (1986)	Lung	SMR	Resin workers (total cohort)	236* (140, 373)
			Formaldehyde (a subset)	136 (44, 315)
Gerin et al. (1989)	Lung	OR	Formaldehyde - Long duration	
			Low level	0.5 (0.2, 1.3)
			Medium level	1.0 (0.4, 2.5)
			High level	2.2 (0.7, 7.6)
Partanen et al. (1990)	Lung	OR	Formaldehyde - ≥3 ppm-months	0.9 (0.3[d], 3.0[e])

Statistically significant.

[a] Confidence interval as presented by the authors or calculated by EPA, with the exception of Olsen et al., 1984.

[b] Odds ratio.

[c] Work history was assessed by using two independent classifications.

[d] Lower 90% confidence limit.

[e] Upper 90% confidence limit.

[f] Standard incidence ratio.

[g] Standard mortality ratio.

[h] Inhalation and dermal exposure.

[i] Proportional mortality ratio.

4.4.6.1. Exposure Consideration

Exposure to formaldehyde in the epidemiologic studies has been primarily occupational to formalin or in the manufacture or production of formaldehyde or formaldehyde products. Only one study examined population exposure to formaldehyde, and this was indirectly assessed by residency in a mobile or manufactured home.

Exposure to formaldehyde for the industrial populations occurs in the manufacture of formaldehyde or formaldehyde-containing products. Exposure can result while manufacturing and handling formaldehyde, paraformaldehyde or other formaldehyde derivatives; from gaseous formaldehyde released from resins which are being subjected to heat or pressure processes (e.g., making plywood and particleboard, or plastic molding compounds); or from off-gassing from formaldehyde-containing materials (e.g., manufacturing apparel from fabrics treated with formaldehyde resins which are used to impart wrinkle and crease resistance). For these studies, formaldehyde exposure was examined directly by quantitating formaldehyde levels or indirectly through particular occupations where formaldehyde exposure has been know to occur. In the settings where formaldehyde-based resins are used to make another product such as a molded plastic compound, plywood, or particleboard, exposure to particulates is a potential. Particles can either be composed of the resin itself, or in the case of plywood or particleboard manufacturing, the particles are wood dusts with the potential for formaldehyde to become attached to them.

Formalin is a solution containing 5% formaldehyde. Occupational titles with potential formalin exposure can include morticians, embalmers, anatomists, and pathologists. Exposure to formaldehyde for these professional is by inhalation and by dermal absorption. In addition to formaldehyde, this group has diverse chemical exposures, for example, to solvents and viruses. The levels to which the studied populations were exposed to formaldehyde are absent in all these studies. In some cases, the investigator inferred formaldehyde exposure to the studied population based upon data from industrial hygiene surveys conducted in the 1970's and 1980's of similar occupational settings, from selected funeral homes or anatomy laboratories. It is not known whether the individuals under study did or did not have formaldehyde exposure at the levels identified in the industrial hygiene surveys. In other cases, the investigators do not even attempt to infer level of exposure. In this situation, no information on exposure level can be obtained.

Only one study (Vaughan et al., 1986) examined general population exposure to formaldehyde. Actual exposure levels experienced by the individual under study were not known, but the potential for formaldehyde exposure was assessed indirectly by

examining length of residence in a manufactured or mobile home. Longer exposure durations inferred higher cumulative formaldehyde exposure than shorter durations of exposure. Potential exposure to formaldehyde in manufactured or mobile homes comes from off-gassing from formaldehyde-containing materials such as particleboard and plywood. These materials are used as underlayment for flooring and in wall paneling, shelves, and cabinets.

It is difficult to infer how exposure patterns experienced by the individuals under study influence risk. One reason is that information on past exposure is absent from many studies. All that can be assumed is that past exposure was most likely higher than indicated by present monitoring data. When past exposures are absent, it is not known whether process changes may have altered exposure patterns.

Another reason is the difficult in knowing the most appropriate exposure measure. Exposure in these studies has been defined several ways, primarily as time-weighted averages or cumulative exposure. Several investigators present some information on peak exposure. In the Blair et al. (1986) cohort study of workers manufacturing formaldehyde or formaldehyde-based products, for example, peaks were examined as the highest time-weighted average (TWA) or peak excursion level of exposure experienced by a study participant. Peak patterns of exposure would also be expected for occupations using formalin where short-term high-level formaldehyde exposure would be expected during the embalming or autopsy processes.

4.4.6.2. Evaluation of the Body of Human Evidence

The evidence for potential human carcinogenicity associated with formaldehyde exposure rests heavily on associations with cancers of the nasal cavity and sinus and of the nasopharynx. The EPA (1987) concluded that cancer of the nasal cavity and sinus may be related to formaldehyde exposure. This conclusion was largely supported by the finding of statistically significant elevated risks between sinonasal cavity cancer and formaldehyde/wood dust exposure in two well-conducted case-control studies (Olsen et al., 1984; Hayes et al., 1986). Both investigators attempted to adjust for wood dust exposure. The risk observed by Olsen et al. (1984) appeared elevated but was not statistically significant. In analyses which examined only those with no to low wood dust exposure, Hayes et al. (1986) observed that risk associated with formaldehyde was statistically significantly elevated under one exposure classification but not in another exposure classification.

Additional support came from two studies (Olsen and Asnaes, 1986; Hayes et al., 1986) which noted squamous cell carcinoma as the histological type most specifically associated with

formaldehyde exposure. Both studies adjusted for wood dust exposure. In these analyses, Hayes et al. (1986) showed the highest risks were statistically significant associated with squamous cell carcinoma, whereas, no such association was observed between formaldehyde exposure and adenocarcinoma. The risks associated with squamous cell carcinoma in Olsen and Asnaes (1986) appeared elevated, but were not statistically significant.

Findings from four studies (Vaughan et al., 1986b; Blair et al., 1986, 1987; Roush et al., 1987; and Malker et al., 1990) carried out under differing study designs and exposure settings support the finding of a possible association between nasopharyngeal cancer and formaldehyde exposure. Nasopharyngeal cancer risk appeared to increase with either increasing exposure level (Blair et al., 1986, 1987; Roush et al., 1987) or with increasing duration of exposure (Vaughan et al., 1986b).

The observation in the human studies of elevated risks due to cancers of the nasopharynx, and the nasal cavity and sinuses are supported by results from toxicological studies. Rats exposed for two years to formaldehyde developed squamous cell carcinoma of the nasal turbinates (Kerns et al., 1983). Likewise, DNA-protein crosslinks and nonneoplastic histopathological changes have been found in the nasopharynx and nasal cavity of rats and monkeys (Heck et al., 1989; Casanova et al, 1989). These observations indicate that formaldehyde reacts with biological material at the point of contact. Human exposure to formaldehyde is predominately by inhalation, thus, sites along the respiratory tract are most likely to be "at risk". DPX-protein crosslinks and histopathological changes have not been noted in the maxillary sinuses of monkeys (Monticello and Morgan, 1989). This finding may indicate that pooling cancers of the nasal cavity with those of the nasal sinuses in the epidemiologic studies may not best reflect disease in humans.

Further biological support for a possible association between formaldehyde and cancer of the nasal cavity and sinuses comes from three studies (Edling et al., 1988; Holmstrom et al., 1989; Boysen et al., 1990) examining cellular changes among formaldehyde-exposed workers. These studies observed dysplasia and squamous cell metaplasia in nasal biopsies of woodworkers and chemical workers exposed to formaldehyde.

Firm conclusions regarding possible associations between nasal cavity and sinuses cancers and nasopharyngeal cancer and formaldehyde can not be drawn. Support for nasal cavity and sinus cancers is based on only two studies and on four studies for nasopharyngeal cancer. With respect to nasal cavity and sinus cancer, elevated risks were not detected in any of the cohort studies. For each study, the expected number of deaths was less than three, thus, the detection levels in these studies were greater than the risks for sinonasal cavity cancer observed

in Olsen and Asnaes (1984), Olsen et al. (1986), and Hayes et al. (1986). Other cohort studies did not observe any elevations in nasopharyngeal cancer risk. The number of participants in these studies tended be smaller than in those of Vaughan et al. (1986b), Blair et al. (1986, 1987), and Roush et al. (1987) and possible limitations in these remaining studies tended to conservatively bias the results.

In addition, the role of wood dust and particulate exposure remains unclear. Even though the studies of Olsen et al. (1984), Olsen and Asnaes (1986), and Hayes et al. (1986) tried to adjust for wood dust exposure, its influence may not have been entirely removed. This is important since Vaughan and Davis (1990) have recently reported an association between wood dust exposure and increased risk of squamous cell cancer of the sinonasal cavity. Simultaneous particulate exposure also may have a role in the development of nasopharyngeal cancer. Two studies (Blair et al., 1987; Malker et al., 1990) report statistically significant elevations in nasopharyngeal cancer risk with formaldehyde and particulate exposure. It is more difficult to assess the contribution of particulate exposure for the case-control studies of Roush et al. (1987) and Vaughan et al. (1986a,b) since information on its presence is lacking.

The classification of the epidemiologic evidence relies in small degree on observations that lung cancer may be associated with formaldehyde exposure. Statistically significant elevations in lung cancer relative risks have been noted in several studies: Blair et al. (1986) (for a subgroup of white men with greater than 20 years of latency), Acheson et al. (1984) (for a subgroup of workers from one plant), and Bertazzi et al. (1986). Exposure in these studies were to formaldehyde and to particulates. Because the elevation in lung cancer risk has not been consistently observed across all studies, these observations carry lesser weight. In addition, further analyses of Blair et al. (1990a) suggest that exposure to formaldehyde and other substances might be important in the development of lung cancer. The role of these exposures, however, needs to be further elucidated.

Excesses in leukemia and brain cancer are observed in studies of populations occupationally exposed to formalin; these are most likely related to exposures other than formaldehyde. These populations have exposures to viruses and solvents in addition to the formalin exposure; none of the studies adjusted for these confounding factors. Second, the absence of excesses of leukemia and brain cancer among the industrial cohorts argues against a role of formaldehyde. Third, the biological support for an association with cancers at these sites was weak. Formaldehyde is rapidly metabolized upon contact with biological materials. No measurable increase in formaldehyde, as indicated by DNA-protein cross-links, have been found in the blood, and

there is an absence of induced pathology at sites distant to the portal of entry (Heck et al., 1989; Casanova et al, 1989; Monticello and Morgan, 1989).

4.5 Weight of Cancer Evidence

4.5.1. Human Evidence

The associations observed in the newer studies between lung, nasal cavity and sinus cancer, and nasopharyngeal cancer and formaldehyde have not been large. Point estimates for relative risks are of the order of 3.0 or less for high exposure scenarios. Studies reviewed in 1987 also suggested that relative risks associated with exposure are of this magnitude.

Increased nasopharyngeal cancer risks have been observed in four studies (Vaughan, et al.,1986; Blair et al., 1986, 1987; and Roush et al., 1987; Malker et al., 1990) carried out under differing circumstances. The remaining studies did not observe any elevations in nasopharyngeal cancer risk. Three studies (Vaughan et al., 1986; Blair et al., 1986, 1987; and Roush et al., 1984) were designed to detect moderate increases in formaldehyde risks. Limitations in these remaining studies tend to conservatively bias the results. EPA (1987) also concluded that cancer of the nasal cavity and sinus may be related to formaldehyde exposure based on two well-conducted studies (Olsen et al., 1984; Olsen and Asneas, 1986; Hayes et al., 1986).

Elevated risks for nasopharyngeal, and nasal cavity and sinus cancers were observed among various subgroups: those who were older, those with the greatest latency since first exposure, those with the highest duration or level of exposure, and those with occupational particulate exposure. These observations add support for a possible causal association between cancer at these sites and formaldehyde exposure. In addition, the suggested increase in metaplasia and dysplasia in workers in formaldehyde-related industries provides biological support for a possible association between formaldehyde and nasal cavity and sinus cancers.

Elevations in lung cancer relative risks have been noted in several studies (Blair et al., 1986; Acheson et al., 1984, Bertazzi et al., 1986). However, no consistent patterns with increasing level or duration of exposure to formaldehyde have been observed. In addition, one study (Blair et al., 1990a) suggests that formaldehyde, by itself, is not associated with lung cancer, but, in conjunction with exposure to compounds found in resin and molding operations (i.e., melamine and urea), may be responsible for elevated lung cancer risks. The studies of Acheson et al. (1984) and of Bertazzi et al. (1986) were of populations with combined exposures to formaldehyde and resins.

The excesses in leukemia and brain cancer observed in those studies of populations occupationally exposed to formalin are most likely related to exposures other than formaldehyde.

When examined in the context of previously reviewed studies, the studies released since 1987 support the conclusions drawn in EPA (1987). That is, limited evidence exists for an association between formaldehyde and human cancer. Collectively, however, the evidence does not demonstrate conclusively a causal relationship.

4.5.2. Animal Evidence

The principal evidence indicating that formaldehyde is able to elicit a carcinogenic response in laboratory animals comes from three chronic inhalation studies (Kerns et al., 1983; Albert et al., 1982 and Sellakumar et al., 1985; Tobe et al., 1985). All three studies showed statistically significant increases in squamous cell carcinomas of the nasal cavity of Fischer 344 rats or Sprague-Dawley rats at comparable incidences (38-50%), following exposure to high concentrations of formaldehyde (about 15 ppm). Tumor induction was also seen at lower formaldehyde concentrations but the carcinogenic dose-response curve was very steep (Kerns et al., 1983; Monticello, 1990). A tumor incidence of 1% was seen at 6 ppm (Kerns et al., 1983) and no tumorigenic response was observed at 2 ppm or less (Kerns et al., 1983; Albert et al., 1982 and Sellakumar et al., 1985). Results of a recent inhalation study by Feron et al. (1988) confirm the carcinogenic potential of formaldehyde at high concentrations (20 ppm) following shorter term exposure (13 weeks), with a lifetime follow-up.

As previously discussed (EPA, 1987), there are species differences in the response to inhalation of formaldehyde. In mice, there was a marginal tumorigenic response. Only a small number of nasal squamous cell carcinomas were seen in male mice exposed to high concentrations of formaldehyde (Kerns et al., 1983). No tumorigenic response was found in hamsters (Dalbey, 1982). No cancer studies have been conducted in nonhuman primates. However, two studies demonstrated that at least for nonneoplastic lesions (hyperplasia and squamous metaplasia), rats and monkeys respond similarly to inhalation of formaldehyde (Rusch et al., 1983; Monticello et al, 1989).

Recent studies also provide suggestive evidence for the carcinogenicity of formaldehyde based on oral exposure data. Marginal increases in incidences of forestomach and intestinal tumors were reported in an extensive drinking water study in rats (Soffritti et al., 1989), and similar increases in forestomach neoplasms were reported in another drinking water study (Takahashi et al., 1986). In two other drinking water studies in rats (Til et al., 1989; Tobe et al., 1989), there was no

carcinogenic evidence but hyperplastic and inflammatory changes in the fore- and glandular stomach were reported. These studies further support the suggestion that formaldehyde is likely to cause toxicity at the initial site of contact but not at distant sites.

The combined data support the conclusion in the EPA (1987) review that there is sufficient evidence of carcinogenicity of formaldehyde in animals based on inhalation data. This is based on the induction by formaldehyde of an increased incidence of a rare type of malignant tumor (i.e., nasal squamous cell carcinomas) in both sexes of rats, in multiple inhalation experiments, and in more than one species (i.e., rats and mice).

4.5.3. Supporting Evidence

Formaldehyde induces a variety of toxic effects other than neoplasia. It is a potent upper respiratory tract irritant in humans and animals. Short-term exposure to formaldehyde causes cytotoxicity, increases rates of cell turnover and cell proliferation in the nasal passages, and induces hyperplasia and squamous metaplasia in the noses of rats and mice, and the upper respiratory tract of monkeys. Formaldehyde also induces a number of genotoxic effects in a variety of cell culture systems (e.g. bacteria, viruses, fungi, mammalian cells) and in vivo studies in insects. The observed effects include gene mutations, single strand breaks, chromosomal aberrations, sister chromatid exchanges, and cell transformation. In addition, formaldehyde has been found to form adducts with DNA in both in vivo and in vitro tests. Its ability to interfere with DNA repair mechanisms has also been demonstrated (EPA, 1987).

Recent studies indicate that formaldehyde has tumor promotion potential, at least in some tissues. Formaldehyde enhances the tumorigenic response in mouse skin induced by DMBA (Iversen, 1986), rat respiratory trachea induced by benzo[a]pyrene (Cosma and Marchok, 1987), and rat stomach induced by MNNG (Takahashi et al., 1986).

Formaldehyde is not the only aldehyde which is carcinogenic in animals. Acetaldehyde, the closest aldehyde to formaldehyde in structure, is carcinogenic in rats causing nasal cancers via inhalation exposure. It also induces cancers of the nose and trachea in hamsters. Other aldehydes such as glycidaldehyde and malondialdehyde have also been shown to be carcinogenic.

4.5.4. Categorization of Overall Cancer Evidence

The lines of evidence presented above, i.e. human, animal, and supporting evidence, can be integrated into a weight of evidence assessment of human carcinogenicity for formaldehyde. These consist of (1) a sufficient animal evidence, mainly based

on multiple inhalation studies demonstrating nasal cancers in rats, and some nasal tumor response in mice; (2) limited human evidence for an association of formaldehyde exposure with cancers of the nose and nasopharynx, and possibly lung cancers seen in epidemiologic studies; and (3) considerable other key evidence. This latter information includes (1) structure-activity considerations that associate aldehyde exposure with carcinogenic activity; (2) the known genotoxic activity of formaldehyde including the formation of DNA adducts; and (3) the ability of formaldehyde to injure cells and affect cell division. EPA classifies all this information on formaldehyde as indicating that it is a probable human carcinogen (Group B1) according to EPA's Guidelines for Carcinogen Risk Assessment (1986).

5.0 ESTIMATES OF CANCER RISK

Data from studies of humans are always preferred for making numerical risk estimates. As was the case in 1987, however, the available epidemiologic data on formaldehyde are still not suitable for low dose quantitative cancer risk estimation, mainly because of a lack of adequate exposure information in the studies. Accordingly, results from animal studies are relied upon to estimate low-dose human cancer risk.

Since the 1987 EPA review, updated data on nasal DNA-protein cross-link (DPX) data have been reported for rats, as well as DNA-binding data for monkeys. Risk estimates based on these data also have been published by Starr (1990). This section reviews the 1987 approach and provides risk estimates based on the new information, along with an evaluation of the assumptions and considerations in the estimation of risks.

The accuracy of risk assessment depends on the mechanistic basis of the model used to calculate risks. Biologically based models, of the type developed by Moolgavkar and Knudson (1981), have potential for a more comprehensive approach to risk estimation. Although certain aspects of the mechanism of formaldehyde induced carcinogenesis have been established, it is still not completely understood. A biologically based approach has more prospects to be constructively applied when more definitive data become available concerning cell proliferation, dose rate effects, actual carcinogenic moieties and their mechanisms of action, and the interactions among these processes (See discussion in Section 8.3.2.2). In the absence of these data, the linearized multistage procedure is used as in 1987 to yield a plausible upper limit to the cancer risk based on the cancer incidence observed in rats at high concentrations.

Discussions of the choice of animal study for modelling, and tumor response are found in the 1987 document.

5.1 Review of Quantitative Assessment by EPA (1987)

The quantitative risk assessment of formaldehyde reported in 1987 (outlined in Figure 5-1) was based on the Kerns et al. (1983) F344 rat bioassay. This study provides a dose-response relationship between increased nasal squamous cell carcinoma incidences in both males and females with increasing formaldehyde concentrations. The accounting of the tumor incidence and of the number of animals at risk was in accord with the recommendations of the Interagency Risk Management Council (IRMC, 1984); serial sacrifice and mortality were taken into account, and squamous cell carcinoma incidences in males and females were combined. The administered exposures were assumed to pose equivalent carcinogenic risk across species. A continuous lifetime average exposure correction factor of 5.6 was used (by multiplying each exposure rate in a 6 hours/day, 5 days/week inhalation bioassay by a factor of (5 days/7 days) x (6 hr/24 hr) = 1/5.6). A "five-stage" model (i.e., one incorporating a fifth degree polynomial) was found to fit the data adequately, using GLOBAL83 (ICF Clement Associates, Ruston, LA). This process resulted in an upper limit inhalation incremental unit risk estimate of 1.3×10^{-5} per $\mu g/m^3$, or 1.6×10^{-2} per ppm, for exposure levels below 0.6 ppm.

Since 1987, EPA has changed its policy for interpreting the linearized multistage procedure (LMP). EPA currently uses GLOBAL86 rather than GLOBAL83 for fitting the LMP. Rather than fitting a polynomial of fixed degree, this new implementation fits models of all degrees 1 through 6, and, among those that fit the data, chooses the one with the minimum q_1^*. This results in the choice of a model of different order for the 1987 data set with administered exposures, a "three-stage" model giving the lowest unit risk (6.1×10^{-3} per ppm).

5.2 Issues in the Use of DPX as Delivered Dose

Insight into the exposure of target tissues to reactive formaldehyde can be helpful. Formaldehyde has been shown to react with macromolecules in the target cells to form detectible DPX. The DPX levels following certain levels of inhalation exposure, therefore, may serve as a useful dosimeter. This is true whether or not the cross-links are involved (directly or indirectly) in the actual mechanisms of formaldehyde-induced carcinogenesis. That is, under certain assumptions, the DPX can serve as an index of the presence of the reactive compound in the target cells. The considerations for using DPX levels in this way may be outlined as follows.

First, one must assume that, from moment to moment, the concentration of formaldehyde at the targets for DPX formation (i.e., DNA and certain proteins) is equal or at least proportional to the concentration at the target sites, whatever they may be, for formaldehyde's carcinogenic effects. This seems

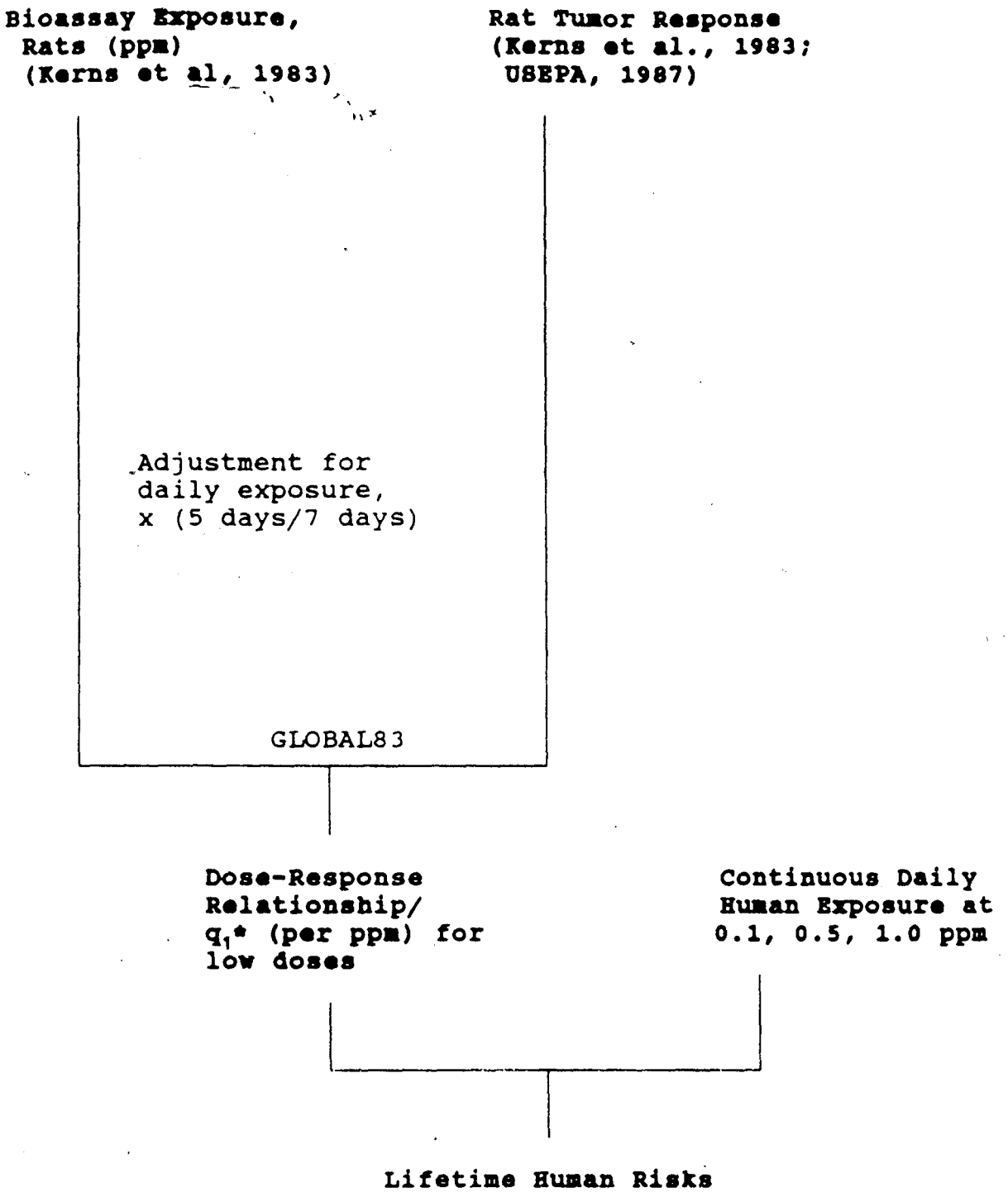


Figure 5-1: Steps in the derivation of lifetime human cancer risks based on rat carcinogenicity and administered dose, as described in 1987.

reasonable in the present case; if the targets of carcinogenic action and DPX formation are not one and the same, they are at least side-by-side within the same cells. Second, the rate of DPX formation at any one moment should be proportional to the formaldehyde concentration at that moment; i.e., the rate of cross-linking should be first order and not saturable over the range of conditions encountered. This also seems reasonable; the cross-linking reactions are spontaneous and not enzyme-mediated. There would seem to be a large excess of linkable nucleophilic sites on DNA and protein, so their concentrations remain essentially constant.

Under these conditions, at any given moment the rate of new DPX-formation in target cells is proportional to intracellular formaldehyde concentration. As a consequence, the total number of cross-links accumulated during the course of the tissue's exposure – that is, the integral of the instantaneous rate of formation from the onset of inhalation until the disposal of all inhaled and absorbed formaldehyde – is proportional to the integral of tissue formaldehyde concentration over that time span. That is, the total DPX is an index of the target tissue "area under the exposure-time curve" (AUC). The AUC is often used as a measure of target tissue exposure, since it encompasses both the amount of compound present and the duration of its presence.

In practice, what is measured is not the *total* amount of cross-linking during a bout of exposure, but the *net* amount, reflecting some degree of repair during the exposure. When DPX is used as a dosimeter of the presence of formaldehyde in the target cells, the total rather than the net cross-link formation is desired. Repair tends to obscure the desired datum. One must assume that such repair is negligible, or at least that it constitutes a constant proportion of the total cross-linking. (In other contexts, however, the net cross-linking may be the appropriate thing to examine – e.g., when exploring the mechanisms whereby cross-links may affect the target cells or the carcinogenic process.)

5.2.1 Use of DPX for High-to-Low-Dose Extrapolation

Having discussed the use of DPX levels as an indicator of target-tissue AUC, it remains to explore how this "delivered-dose" measure is proportional (or not proportional) to inhalation exposure on the one hand, and to carcinogenic effect on the other. Turning first to the question of high-to-low-dose extrapolation, the DPX determinations following inhalation of different air concentrations (Casanova et al., 1989; Heck et al., 1989) indicate that higher exposures have more-than-proportionally higher levels of DPX. Since the dose-response curve for formaldehyde-induced nasal carcinomas in rats is markedly convex, the question is raised as to whether this

nonlinearity can be explained as a consequence of the pharmacokinetics of nasal tissue exposure.

A number of potential causes for pharmacokinetic nonlinearity were discussed earlier, in Sections 3 and 4. Most prominent is the presumed saturability of oxidative detoxification mediated by formaldehyde dehydrogenase. In addition, the role of the mucociliary apparatus in trapping and removing formaldehyde may be compromised at higher exposures. Heck et al. (1989) also mention possible saturation of protein binding and repair of DNA-protein cross-links; if these two factors do indeed figure prominently, the rationale for linking DPX to intracellular formaldehyde exposures is weakened, since basic assumptions, discussed above, would be violated.

The empirical data on DPX levels in rats (Casanova et al., 1989) show that, whatever saturation of protective effects are responsible, there is indeed a higher-than-proportional exposure of nasal epithelial cells to formaldehyde at high air concentrations. Use of DPX levels as a surrogate measure of target-cell dose will, therefore, tend to account for its nonproportionality to air concentration. That is, it will empirically adjust for observed nonlinearities in formaldehyde pharmacokinetics, removing this source of nonlinearity from the expression of the dose-response relationship.

Examination of the rat DPX data (Casanova et al., 1989) and the nasal carcinoma data of Kerns et al. (1983) suggests that the convexity of tumor response is much sharper and extends to much lower doses than does the cross-linking nonlinearity. The convexity in DPX levels is noticeable but rather shallow, and the data indicate substantial cross-linking at lower exposure levels – levels at which tumor response was no longer seen. Moreover, DPX formation tends to become linear with air concentration at lower levels. Thus, although pharmacokinetics may explain an important part of the convexity in the tumor dose-response relationship, there are other sources of nonlinearity as well. This raises the question of the proportionality of "delivered dose" to carcinogenic effect.

Once it is taken that DPX levels provide an index of area under the target-tissue formaldehyde concentration curve (AUC), it should be clear that a number of factors can cause tumorigenic response to be convex with respect to this "delivered dose." Being an integrated, summary measure of the tissue's exposure, the AUC contains no information about the shape of the concentration curve over time, including its peak levels. Peak levels and average concentration will be higher immediately after breathing higher air concentrations.

For a mechanism of carcinogenic action in which the impact on critical cellular targets accumulates from moment to moment at

a rate proportional to concentration, the AUC will give a good prediction of the cumulative effect of a given tissue exposure. The argument is the same as that tying cross-links to the AUC for dosimetric purposes, since cross-links accumulate in this way. Indeed, it is literally the same if the cross-links are the sole agent of carcinogenicity, say, by causing transforming mutations. If, however, the mechanism of carcinogenic action is such that doubling the target-tissue concentration more than doubles the rate at which damage is done, integrating the concentration (the AUC) will no longer be proportional to summing the damage. For example, a receptor-mediated mechanism may have receptor occupancy at a given time in proportion to tissue concentration of ligand, but the carcinogenic effect of an exposure that propels occupancy above the critical level for triggering signal transduction will be out of proportion to the consequences of a slightly lower concentration that just falls short of such triggering levels. Carcinogenesis mediated by cytotoxicity would also be expected to show such effects. Identifying and accounting for such effects requires rather detailed specification of how the carcinogenic process proceeds, including how the rates of the key processes vary moment by moment as a function of carcinogen concentration, and how the engendered effects accumulate over time.

Judging the impact of such factors for formaldehyde is difficult, owing to lack of comprehensive knowledge about the mechanism of carcinogenic action. As detailed in Section 4, there are considerable data showing cytotoxicity and at least transitory cell proliferation at high, but not low, inhaled formaldehyde concentrations. At the same time, the documented genotoxic effects of formaldehyde would be expected to operate in proportion to the AUC. Perhaps the two mechanisms operate synergistically at higher exposure levels (resulting in the sharp convexity), while the genotoxic effect may (or may not) operate alone at lower levels. Just as the dosimetric nonlinearities may be analyzed and compensated for by pharmacokinetic analysis, these "pharmacodynamic" nonlinearities may be approached using a biologically based approach to dose-response modeling (e.g., Moolgavkar and Knudson, 1981). The understanding of formaldehyde's carcinogenic mechanisms, however, is insufficiently developed to apply this approach at present.

It should be evident from the foregoing that the use of measured DNA-protein cross-links as a surrogate for target-tissue exposures to reactive formaldehyde allows one to take into account certain pharmacokinetic nonlinearities, but does not address what may be called pharmacodynamic nonlinearities in the dose-response relationship. These must be approached empirically by fitting a dose-response curve of rat nasal carcinoma incidences (as seen in the Kerns et al. [1983] study) versus target tissue exposure (i.e., versus DPX level). The resulting curve will be less convex than one of tumors versus air

concentration, by virtue of its ability to eliminate the effect of lack of proportionality of inhaled air concentration to the resulting exposure of cells of the nasal epithelium to reactive formaldehyde. It will, however, retain curvature ascribable to the variation in carcinogenic effects of high and low delivered doses.

Some potential pitfalls in this analysis should be noted. First, as discussed above, the validity of DPX levels as a dosimeter is dependent on some assumptions, which, although reasonable, are unproven. Second, the DPX data of Casanova et al. (1989) are for a single 6-hour exposure to naive rats, while rats in the Kerns et al. (1983) bioassay received such exposures daily for a lifetime. One must assume that the dosimetric adjustment identified for the first day's exposure obtains throughout life. Skepticism about this is warranted, since the long-term bioassay rats lost their normal nasal epithelial tissue in favor of a proliferation of squamous cells, which may have very different metabolic abilities, formaldehyde uptake, and detoxifying mechanisms than the epithelial cells examined in the DPX experiments.

5.2.2 Use of DPX Data for Interspecies Extrapolation

The result of the approach outlined above is a dose-response curve of rat nasal carcinoma risk as a function of nasal epithelial exposure, as indexed by its surrogate, DPX. The question then arises how such a curve should be applied to the calculation of potential human risks from formaldehyde inhalation. This question can be decomposed into two main components: how to determine the relationship of inhalation exposure to target tissue AUC in humans (i.e., the question of human pharmacokinetics), and how to determine the expected lifetime cancer risk for a human with a certain daily target tissue exposure, using the rat-based dose-response curve (i.e., the question of human pharmacodynamics vis-à-vis the bioassay rats). These will be considered in turn.

Since the carcinogenicity of formaldehyde is being expressed in terms of DPX, for its application to humans one first needs a determination of the relation between inhaled air concentration and such target tissue exposure. There are no data on formaldehyde-induced DNA-protein cross-links in humans, nor is the simple pharmacokinetic model of Casanova et al. (1987) readily scaled up to provide human DPX estimates. Two empirical approaches may be entertained to estimate this relationship by analogy with another species: using the observations on DPX levels following inhalation of various formaldehyde concentrations in rats (Casanova et al., 1989) or in rhesus monkeys (Heck et al., 1989).

If the inhalation-DPX relationship in rats is used to characterize dose delivery in both rats and humans, it is clear that differences between these two species cannot be addressed. Such an analysis can, however, illuminate high-to-low dose differences. The convexity of the dose-response relationship is a key issue, and, as noted above, accounting for the lack of proportionality between air concentration and resultant DPX can account for much, but not all, of this convexity. The shortcomings of this approach are several. The obvious one is that any potential differences between humans and rats in the DPX levels resulting from given inhalation exposures cannot be identified or accounted for. There may be such differences not only in the general levels of DPX, but also in the degree of nonlinearity between high and low air concentrations.

There is also the question of pharmacokinetic dose-rate effects. The observations of rat DPX formation are for constant 6-hour exposures followed by exposure to pure air, matching the daily exposures in the Kerns et al. (1983) cancer bioassay. In contrast, human exposures may arise in a variety of patterns, from continuous exposure to episodic peaks. Since the degree of saturation of capacity-limited processes and other sources of pharmacokinetic nonlinearity depends on the moment-by-moment tissue concentration profile, the DPX resulting from exposures of different pattern yet equal ppm-hours may not be equivalent. Fortunately, we are usually interested in rather low-level human exposures, and the importance of dose-rate effects to pharmacokinetics tends to be minimal under such circumstances (Hattis, 1990).

An alternative to using the rat inhalation-DPX relationship for characterizing the one in humans is to rely on the observations in rhesus monkeys (Heck et al., 1989). The respiratory tract of monkeys is more similar in anatomy and geometry to humans than is that of the rat (see Appendix C). Moreover, monkeys may share facultative oral-breathing with humans, while rodents are obligate nose-breathers. These factors would tend to suggest that observations of the degree of DPX formation in monkeys may be more likely to be representative of the case in humans. There are remaining differences in the respiratory tract of humans and monkeys, however. In addition, there are differences in size scale and respiratory rhythms that have unknown effects on the ability of formaldehyde to be absorbed and react. Finally, the enzymatic rates of detoxification, binding, and cross-linking reactions must be assumed similar in monkeys and humans, although such rates are often considered to scale allometrically across species of different body size. These questions bear on whether the proportionality of DPX levels to the AUC of formaldehyde is similar across species; i.e., whether the use of DPX as an index of tissue exposure needs to be recalibrated. In sum, any claim that use of the monkey DPX data introduces a correction for

species differences in pharmacokinetics (as well as the high-to-low dose correction) needs to be tempered by the uncertainty about how well the monkeys actually represent the situation in humans.

If it is granted that a useful estimate of DPX levels in humans following a defined exposure can be derived from either the rat or monkey DPX data, and that such an estimate provides an adequate index of area under the curve of formaldehyde concentration in the target nasal epithelial cells, it remains to determine how the dose-response relationship of nasal carcinoma incidence as a function of DPX--determined in rats--is to be applied to the calculation of human risk.

EPA commonly applies so-called surface area scaling to applied doses—i.e., administered amounts are equal in terms of $mg/kg^{2/3}/day$—to determine doses of expected equal carcinogenicity across species. Such scaling is intended to adjust for the fact that physiological processes operate more slowly in larger species, even when the basic anatomical and biochemical machinery is the same. Rates of such processes as uptake, cardiac output, metabolic activation and detoxification, and excretion of a material from the body tend, in general, to maintain proportionality to body weight to the 2/3 power. A consequence of such allometric scaling of the rate of processing an administered dose is that, again as a default expectation, the area under the curve of the daily target tissue concentration of the proximate carcinogen (be it the administered compound or a metabolite) is expected to be equalized across species when the doses are scaled in proportion to this processing rate, that is, proportional to the 2/3 power of body weight. Since the volume of air breathed per unit time stays roughly in proportion to body weight to the 2/3 power, this method is essentially equivalent to equating exposure on a ppm-basis, as was done in EPA (1987).

As discussed above, the AUC is an index of the carcinogen's opportunity to interact with the tissues, and measures both the amount of compound present at the target and the duration of its presence. When dosing that is adjusted to equalize daily AUCs across species is continued for a lifetime, it results in equal lifetime average concentrations of the toxicant in the target tissue. Such lifetime average concentrations are taken to be equivalent in lifetime cancer risk, despite the fact that a human is exposed for a longer total amount of time. Just as pharmacokinetic processes tend to proceed at a slower pace in humans, so does the pace of carcinogenesis, as shown by the fact that age-specific incidence curves of cancer and cancer latency tend to be congruent when time is compared on a fraction-of-lifetime basis.

In the present case, the DPX measurements have been taken as an index of the area under the curve of formaldehyde in the

target cells. Thus, an equal amount of newly created cross-links per day per unit of tissue would be expected to indicate equal daily AUCs, and hence, by assumption, equal lifetime cancer risk. In other words, no further "cross-species scaling factor" need be applied, since AUCs are being compared directly. This is in contrast to a situation in which the pharmacokinetic analysis produces estimates of *amount* of a compound metabolically activated to its carcinogenic form. The expectation from allometric variation in pharmacokinetics is that doses scaled by surface area will result in an equal fraction of the lower human administered dose being metabolized. That is, amounts metabolically activated are less per unit of tissue in humans, but equal in proportion to $kg^{2/3}$. Owing to the expectation of slower processing of the activated material, this lesser amount in humans may nonetheless be expected to lead to the same AUC as in the experimental animals. In sum, empirical pharmacokinetic data on "delivered doses," should be compared against an expectation based on pharmacokinetic allometry - i.e., against the pattern that one assumes for delivered doses when such case-specific data are not available. The same target tissue exposure, when characterized by different units of measurement, can have different cross-species ratios. Definitions of toxicological equivalency of tissue doses across species must account for which measure is being used.

The question of cross-species extrapolation of carcinogenic effects is a difficult one, subject to ongoing debate. The issue of how to incorporate pharmacokinetic information, and of the need for additional cross-species scaling corrections on "internal doses," is particularly problematic. The scheme outlined above for formaldehyde is intended to be compatible with EPA's default methodology, modified to take special account of the nature of the DPX data. A document presenting a fuller discussion of EPA's cross-species scaling methods, their scientific rationale, and their modification when using pharmacokinetic data, is in preparation.

5.2.3 Summary

To summarize the rationale for using DPX in the quantitative cancer risk assessment of formaldehyde, it is argued that DPX should provide an index of the area under the curve of reactive formaldehyde in the target nasal epithelial cells (AUC), both in rats and in other species. This is so whether or not the DPX are mechanistically involved in the carcinogenic process or simply passive markers of the presence of formaldehyde in the target cells.

General allometric arguments lead to the *a priori* expectation that relative AUCs across species from a given inhalation exposure might tend to be equal, but the observed facts contradict this expectation. In fact, there is a markedly

lower DPX level in monkeys than in rats, indicating a lower target tissue exposure to formaldehyde, which may be attributed to differences in respiratory anatomy and airflow patterns (see Appendix C). Moreover, there is a lack of proportionality of DPX levels to air concentration, indicating that saturable pharmacokinetic processes are encountered at higher air concentrations, leading to higher-than-proportional tissue doses. By figuring the dose-response curve in terms of DPX, rather than externally encountered air concentration, this high-to-low dose nonlinearity can be identified and taken into account. In addition, if the difference between rats and humans is similar to that between rats and monkeys, then humans are expected to have a lower nasal epithelium exposure than rats. The curve of carcinoma risk versus DPX is taken to apply across species, on the assumption that equal tissue AUCs (indexed by DPX) experienced for a lifetime may be expected to yield equal lifetime cancer risks. A human low-dose unit risk can then be developed by estimating the DPX level from a low unit of exposure, and determining the projected risk for such a DPX level. The nonlinearity of the curve of carcinoma risk versus DPX is an empirical estimate of the nonproportionality of risk to the various levels of target tissue exposure. Further knowledge of the underlying biological processes of carcinogenesis as they operate at different tissue exposure levels could illuminate the estimation of this relationship, but such biologically based dose-response modeling has not been attempted in the present analysis.

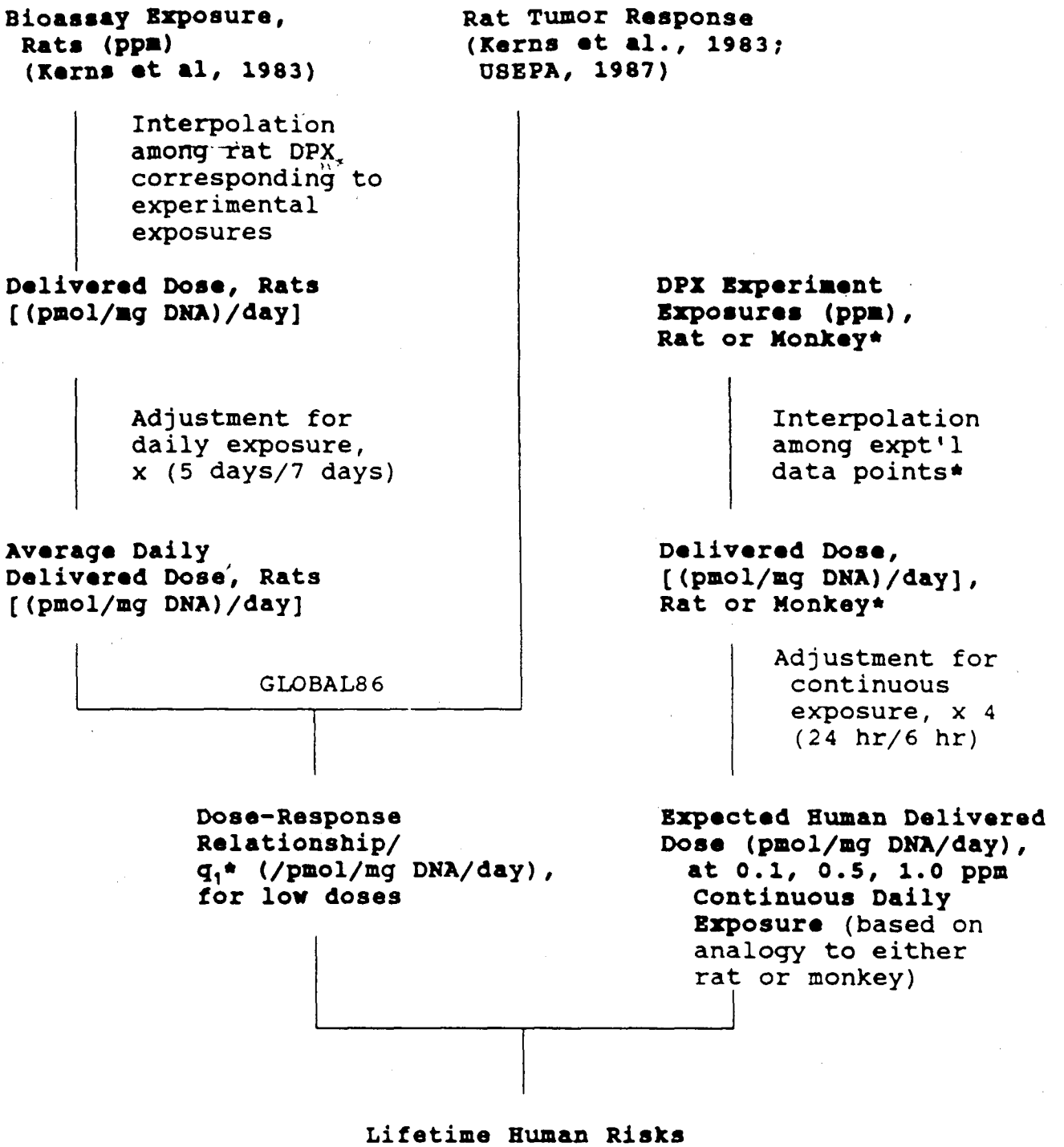
5.3 Calculation of Risk Estimates Using DPX

As discussed in Section 5.2, DPX may be considered as a measure of formaldehyde delivered to target cells. The following sections detail the development of risk estimates using DPX data of rats and monkeys. The risk assessment procedures are schematically described in Figure 5-2.

5.3.1 Estimation of Delivered Dose

The first step in modelling cancer risk is to convert the bioassay exposure rates to delivered dose equivalents, using the data reported by Casanova et al. (1989) and Heck et al. (1989). Table 5-1 reports the total amount of formaldehyde equivalents covalently bound to DNA and protein in nasal mucosa, resulting from a 6-hour exposure to formaldehyde at approximately 0.3, 0.7, 2, 6, or 10 ppm for F344 rats, and 0.7, 2, or 6 ppm for Rhesus monkeys.

A variety of curve-fitting techniques are available for relating the administered exposures to the observed DPX levels. One approach is to use the pharmacokinetic model developed by Casanova and coworkers (Casanova and Heck, 1987; Casanova et al., 1989; see Figure 5-3). The model, however, has several



* Rat dosimetry data from Casanova et al. (1989); monkey dosimetry data from Heck et al. (1989).

Figure 5-2: Steps involved in the derivation of lifetime cancer human risks based on rat carcinogenicity data, and the use of DNA-protein cross-linking (DPX) as dosimeter.

Table 5-1

Measured Delivered-Dose Levels in Nasal Mucosa at Experimental Administered Dose Levels in Rats and Monkeys, and Interpolation Equations

Administered Formaldehyde Concentration (ppm)	Formaldehyde Equivalents (pmol/mg DNA/6 hours)	Linear Interpolation Relationship[c]
Rats[a]:		
0.0	0.0 (assumed)	
0.32	1.4 ± 0.6	Y = 4.4X
0.71	3.9 ± 0.4	Y = -0.65 + 6.41X
1.93	19.9 ± 3.7	Y = -5.41 + 13.1X
5.92	106 ± 6	Y = -21.8 + 21.6X
9.87	266 ± 30	Y = -134 + 41.5X
Monkeys[b]:		
0.0	0.0 (assumed)	
0.7	0.36 ± 0.10	Y = 0.51X
2.0	2.56 ± 0.31	Y = -0.82 + 1.69X
6.0	18.2 ± 3.4	Y = -5.26 + 3.91X

[a] Casanova et al., 1989: Analytical chamber concentrations were reported as mean ± s.e. (n = 3).

[b] Heck et al., 1989: Analytical concentrations were not reported for the monkey DNA-binding experiment.

[c] Equations listed apply only to the associated range of bracketed doses: Y = DPX in pmol/mg DNA; X = exposure in ppm.

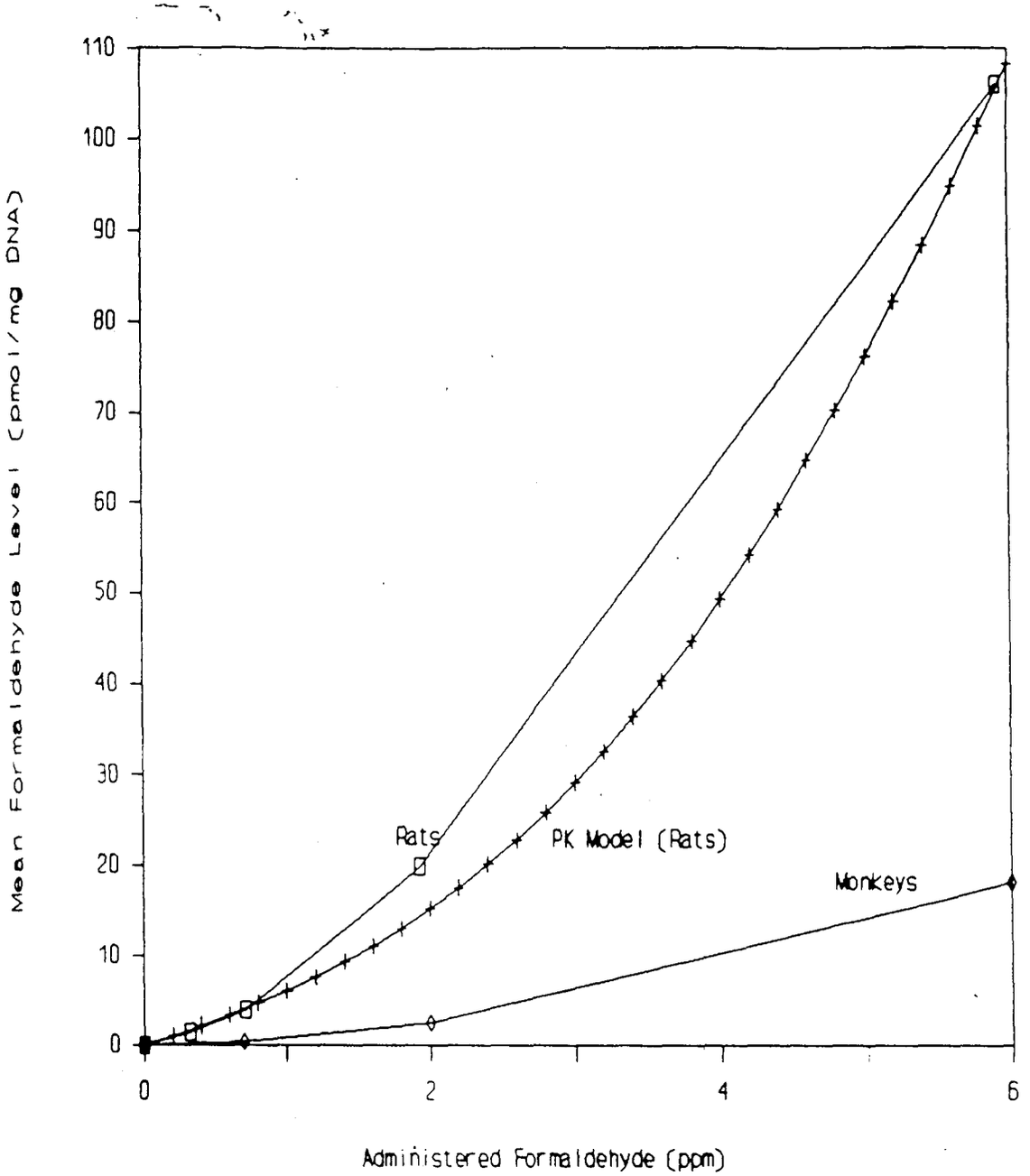


Figure 5-3: Observed and model-predicted concentrations of DPX in nasal mucosa of F-344 rats (Casanova et al., 1989) and Rhesus monkeys (Heck et al., 1989).

shortcomings, as acknowledged by the authors

> "it ignores other elimination routes (mucociliary clearance, diffusion out of the cells, metabolism by enzymes other than FDH, and covalent binding to macromolecules other than DNA), and it does not incorporate the possibility of DNA repair or possible changes in numbers or types of respiratory mucosal cells."

While each of these processes would generally be expected to decrease DPX levels, their relative rates and interactions are not well characterized. These omissions in the pharmacokinetic model, therefore, may account for the reported model's underestimation of the mean observed DPX concentration in rats at a nominal concentration of 2.0 ppm (analytical concentration 1.93 ppm) by approximately 25%. The model predicts about 14.8 pmol/mg DNA, while the mean observed level was 19.9 pmol/mg DNA after 6 hours exposure. Accurate estimation of the magnitude of the lowest dose can be critical, since the estimate of low-dose risk can be strongly influenced by the lowest dose even when, as in this case, there is no tumor response in the low-dose animals. Use of delivered dose estimates based on the pharmacokinetic model would lead to a higher unit risk than using the observed DPX concentration, about 25% higher in this case, all other factors remaining equal.

Alternatively, linear and quadratic regression methods both fit the data adequately from an overall perspective (high correlation coefficients) but neither method accurately estimates the observations in the low-dose region, where there is the greatest need for accuracy.

In order to better reflect the experimental data, therefore, a linear-interpolation approach was applied to the experimental data presented in Table 5-1. Cross-linking corresponding to a concentration falling between experimental concentrations has been estimated by linearly interpolating between the two experimental concentrations closest to that concentration. This approach was chosen because it will not under- or overestimate the observed points. A concentration of 0 ppm was assumed to correspond to 0 pmol of formaldehyde bound to DNA and protein. For concentrations greater than 10 ppm, the linear extrapolation was based on the DPX observed at 6 and 10 ppm. The equations of the lines connecting each consecutive pair of points are also listed in Table 5-1.

5.3.2 Lifetime Average Daily Exposure: Dose-Rate Considerations

In the 1987 risk assessment, an adjustment of the bioassay doses to continuous daily exposures was made before modelling risk estimates. This may be inappropriate for the DPX-based

procedure; there is some qualitative evidence that dose rate, rather than average exposure concentration, can be more critical in determining the severity of endpoints such as cell proliferation and nonmalignant histological changes (see Section 4.3.5).

It is evident from examination of the Casanova et al. (1987) and Heck et al. (1989) data on DPX (Figure 5-2) that inhalation of different levels of formaldehyde lead to DPX levels that are not proportional to air concentration. Since the curves are convex, the predicted DPX corresponding to a 24-hour averaged exposure will tend to be less than for a 6-hour exposure to a 4-times higher air concentration. Since the aim is to express response in rats in terms of their DPX levels, the DPX corresponding to the unaveraged, 6-hour exposures should be used. Since such DPX levels were experienced by bioassay rats for only 5 days per week, they should be corrected by a factor of 5/7 to produce the average daily DPX experienced for a lifetime.

The above procedure tends to account for dose-rate effects in the formation of DPX (and hence AUC of formaldehyde in the target tissue) by different inhalation regimens. At low dose levels, as evidenced by the lower dose linearity of the curves in Figure 5-2, such effects become minimal, and the delivered dose for a 24-hour exposure is equivalent to that of a 6-hour exposure to a 4-times higher formaldehyde air concentration that produces the same cumulative dose. As noted previously, however, non-proportionality of DPX to carcinogenic effect and dose-rate effects in this relationship, are not addressed by such an analysis.

5.3.3 Quantitative Estimation of Risk

In order to characterize the empirical relationship of rat nasal tumors to DPX, the linearized multistage procedure, as programmed in GLOBAL86 (ICF Clement Associates, Ruston, LA), was applied to the interpolated rat DPX concentrations adjusted to daily exposure levels, and the IRMC (1984) tumor incidences used in the 1987 assessment. The modelling procedure yielded a "two-stage" model (goodness-of-fit p = 0.07). The input data and the resulting model parameters are summarized in Table 5-2. The low-dose slope of the linearized upper bound curve (q_1^*) represents an upper bound for the incremental risk per unit of tissue dose - that is, 1.6×10^{-4} per pmol of bound formaldehyde per mg of DNA per day. As discussed in section 5.2.2, this incremental risk per unit of nasal DPX is presumed to apply to humans as well.

In general, risks corresponding to lifetime daily 6-hour exposures at rates below 0.7 ppm can be calculated using an upper limit inhalation incremental unit risk estimate of 1.6×10^{-4} per (pmol/mg DNA)/day, after adjusting the exposure rate to reflect the relevant DPX formation rate. The full model as given in

Table 5-2

Calculation of Cancer Risk Based on Incidence of Squamous Cell Carcinomas and on Estimates of Delivered Dose in F344 Rats

Bioassay Exposure (ppm)	Delivered Dose (pmol/mg DNA/day)[a] Unadjusted	Adjusted[b]	Tumor-bearing animals/ Number at risk[c]
0	0	0	0/156
2.0	21.4	15.3	0/159
5.6	99.1	70.8	2/153
14.3	445.2	318	94/140

[a] Interpolated from the rat DPX data (see Table 5-1).
[b] DPX concentrations were multiplied by 5/7 (see Section 5.2.2).
[c] Effective numbers, based on recommendation of the Interagency Risk Management Council (IRMC, 1984).

Results of Linearized Multistage Procedure (GLOBAL86), Goodness of fit test, p = 0.07

Maximum Likelihood Estimate of Lifetime Cancer Risk:

$$P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2)],$$

with $q_0 = 0.0$,

$q_1 = 0.0$,

$q_2 = 1.0 \times 10^{-5}$ (pmol/mg DNA/day)$^{-2}$

Upper Bound on Lifetime Cancer Risk Estimate (based on 95% animal upper bound):

$$P^*(d) = 1 - \exp[-(q_0^* + q_1^* d + q_2^* d^2)],$$

with $q_0^* = 0.0$

$q_1^* = 1.6 \times 10^{-4}$ (pmol/mg DNA/day)$^{-1}$

$q_2^* = 1.0 \times 10^{-5}$ (pmol/mg DNA/day)$^{-2}$

Table 5-2 must be used for DPX levels corresponding to exposures greater than 0.7 ppm, owing to the nonlinear relationship between DPX formation and tumor response.

To calculate expected human risks from this relationship for formaldehyde, it is necessary to relate the concentration in air to the expected DPX level in human nasal tissue. There are two alternatives. First, one can use the relationship of DPX to air concentration in rats to estimate the human DPX from a given inhalation exposure. This alternative addresses only the high-to-low dose nonlinearity of the air concentration DPX/relationship. Second, one could use the DPX curve in monkeys to derive the human estimate. Since monkeys are more like humans in their anatomy, airflow patterns, and cell distribution characteristics in the nose, this alternative aims at allowing for interspecies difference as well as air concentration differences in the level of DPX formation.

Estimates of expected human DPX levels corresponding to ambient exposures of 0.1, 0.5 and 1.0 ppm were calculated from either the monkey or the rat DPX data by using the equations for monkeys and rats in Table 5-1 (see Table 5-3). Next, to obtain a DPX estimate for a human with continuous exposure to that air concentration, the concentration of DPX formed in 6 hours was multiplied by 4. Then the full model was used to calculate the risks associated with lifetime exposure to continuous concentrations of 0.1, 0.5 and 1.0 ppm (see Table 5-4).

For applying the q_1^* to human exposures below 0.7 ppm (and for comparison with the 1987 unit risk), the dose units in (pmol/mg DNA)/day are converted to ppm units of continuous exposure. For rat dosimetry, the unit risk becomes:

$$\frac{1.6 \times 10^{-4}}{(\text{pmol/mg DNA})/\text{day}} \times \frac{4.4\ (\text{pmol/mg DNA})/\text{day}}{\text{ppm}} \times \frac{24\ \text{hr}}{6\ \text{hr}} = 2.8 \times 10^{-3}\ \text{per ppm};$$

the 4.4 pmol/mg DNA comes from the first linear relationship for rats in Table 5-1. The unit risk based on monkey dosimetry is calculated similarly:

$$\frac{1.6 \times 10^{-4}}{(\text{pmol/mg DNA})/\text{day}} \times \frac{0.51\ (\text{pmol/mg DNA})/\text{day}}{\text{ppm}} \times \frac{24\ \text{hr}}{6\ \text{hr}} = 3.3 \times 10^{-4}\ \text{per ppm};$$

the 0.51 pmol/mg DNA comes from the first linear relationship for monkeys in Table 5-1. It should be noted that these unit risks are applied only to continuous exposure or to low exposures that have been prorated over a lifetime.

Table 5-3

Interpolated Delivered-Dose Concentrations Corresponding to Selected Human Environmental Exposures

	Delivered-Doses (pmol/mg DNA/day)[a] at Selected Human Environmental Exposures		
	0.1 ppm	0.5 ppm	1.0 ppm
Rats:			
6 hours daily	0.43	2.6	7.7
Continuous daily[b]	1.72	10.2	30.8
Monkeys:			
6 hours daily	0.051	0.26	0.88
Continuous daily[b]	0.204	1.04	3.52

[a] Derived using the relationships in Table 5-1.
[b] 6 hour daily exposures multiplied by (24 hours/6 hours) = 4.

Table 5-4

Comparison of Estimates of Upper Bounds (and Maximum Likelihood Estimates) of Human Lifetime Carcinogenic Risk Associated with Lifetime Continuous Daily Exposure to Formaldehyde

Exposure Rate (ppm)	1987 Risk Estimates[a]	1991 Risk Estimates[b]	
		Monkey-based	Rat-based
0.1	2 E-3[c] (5 E-7)	3 E-5 (4 E-7)	3 E-4 (3 E-5)
0.5	8 E-3 (5 E-4)	2 E-4 (1 E-5)	3 E-3 (1 E-3)
1.0	2 E-2 (1 E-2)	7 E-4 (1 E-4)	1 E-2 (1 E-2)

[a] Estimated using 1987 inhalation unit risk 1.6 x 10^{-2} per ppm.
[b] Incorporated monkey or rat dosimetry data (see Section 5.3.3).
[c] 2 E-3 = 2 x 10^{-3}.

5.4 Comparison with Other Formaldehyde Risk Estimates

5.4.1 Comparison with 1987 Unit Risk Estimate

There is an approximately 6-fold difference between the 1987 and the 1991 rat dosimetry-based risk estimates (2.8×10^{-3} per ppm vs 1.6×10^{-2} per ppm). An approximate 2.5-fold difference is due to use of DPX as an internal dosimeter in rats. This reflects accommodation of the high-to-low dose nonlinearity in the relation of air concentration to tissue exposure. As discussed in Section 5.1, a 2.5-fold difference from EPA's 1987 assessment is due to a change in EPA's policy for interpreting the linearized multistage procedure, resulting in the choice of a model of different order.

Use of the monkey nasal DPX data as a surrogate for human delivered dose further lowers the estimated risk to humans at low exposures about another 9-fold, yielding an overall 50-fold reduction of unit risk estimates compared to the 1987 unit risk (3.3×10^{-4} per ppm vs 1.6×10^{-2} per ppm).

5.4.2 Quantitative Assessment by Starr (1990)

Starr (1990) calculated cancer risks based on the DPX experiments of Casanova et al. (1989) in rats, and of Heck et al. (1989) in monkeys. As in the EPA assessment, he made the species equivalence assumption that a "given level of formaldehyde covalently bound to DNA would pose the same lifetime cancer risk to both rats and monkeys." All cancer risks were based on the response data in the Kerns et al. (1983) bioassay, using a slightly different accounting of tumor incidence than EPA used, namely 0/160, 0/160, 2/160, and 87/160, in the 0, 2.0, 5.6, and 14.3 ppm groups, respectively. Using GLOBAL82 (ICF Clement Associates, Ruston, Louisiana), Starr fit a "three-stage" model using rat DPX levels interpolated from the DPX experiment to correspond to the bioassay exposures. Predicted risks corresponding to 0.1, 0.5, and 1.0 ppm formaldehyde in air, based on the DNA-binding data for both rats and monkeys are reproduced in Table 5-5. Starr also did not address the non-nasal DPX observed in monkeys in making his calculations.

Starr concluded that point estimates of human risk (also called maximum likelihood estimates, or MLEs) based on DPX in monkeys were lower than those based on airborne concentrations to rats (the basis of EPA's 1987 unit risk), by as much as 150,000-fold. Starr calculated a MLE risk of 3×10^{-7} based on the air concentration of 0.1 ppm administered to rats, while the corresponding MLE human risk based on monkey DPX was 2×10^{-12}. The differences between upper bounds on risk were less dramatic, the largest difference being 25-fold between an upper bound rat dosimetry-based risk of 2×10^{-4} and an upper bound monkey

Table 5-5

Comparison of Risk Estimates from Starr (1990), Upper Bounds and Point (Maximum Likelihood) Estimates[a]

Air Conc. (ppm)	Upper Bound and (MLE) Estimates of Risk From Several Formaldehyde Exposure Measures		
	Rat/1983[b]	Rat/1989[c]	Monkey/1989[d]
0.1	2 E-4[e] (3 E-7)	7 E-5 (2 E-9)	8 E-6 (2 E-12)
0.5	8 E-4 (3 E-5)	4 E-4 (3 E-7)	4 E-5 (3 E-10)
1.0	2 E-3 (3 E-4)	1 E-3 (6 E-6)	1 E-4 (1 E-8)

[a] Continuous lifetime average exposure adjustment not used.
[b] Kerns et al. (1983) exposure concentrations (ppm).
[c] DNA-protein cross-links (pmol/mg DNA) from Casanova et al. (1989).
[d] Using the 1989 rat DNA-binding data for the dose-response relationship, and the Heck et al. (1989) DNA-protein cross-links for delivered dose at 0.1, 0.5, and 1.0 ppm.
[e] 2 E-4 = 2×10^{-4}

dosimetry-based risk of 8×10^{-6} at an air concentration of 0.1 ppm.

The 1991 upper bound risk estimates (unadjusted, as listed in Table 5-4), based on either rat or monkey DPX data, are greater than those estimated by Starr (1990) by a factor of 4, reflecting the continuous exposure adjustment in the 1991 EPA estimates. The MLE estimates, however, differ dramatically, with the 1991 point estimates higher than Starr's by 3 to 4 orders of magnitude. The significance of these differences is only slight, owing to the instability of the maximum likelihood estimates for such nonlinear dose-response patterns.

The EPA generally does not compare point estimates of risk based on animal data, particularly in instances where such estimates are based on very small numbers of animals responding, which are expected to provide high variability in the MLE coefficients. A reliable procedure for calculating point estimates of risk does not yet exist, as is stated in the EPA Guidelines for Carcinogen Risk Assessment (USEPA, 1986).

5.5 Discussion of Uncertainties

By the arguments discussed in section 5.2, use of DPX as a dosimeter should avoid some uncertainties associated with using administered dose, the dosimeter used in the 1987 assessment, because the DPX should be more closely proportional to the target dose than the ambient exposure. Uncertainties specific to the 1991 risk assessment are discussed below.

5.5.1 Uncertainties Associated with Use of DPX

There are a number of uncertainties associated with risk estimates based on rat carcinogenicity data using dosimetry data, i.e. DPX, from the rat and monkey. Human risk estimates based on the rat dosimeter may be too high at a given exposure concentration, owing to differences in breathing patterns resulting in different exposure of the target tissue. The observed rat DPX concentrations best predict the rate of nasal tumors, albeit in rats. Since the DPX were lower in the monkey nasal mucosa, use of the rat dosimetry-based estimates may overestimate the probability of nasal tumors in primates. On the other hand, the monkey dosimetry-based estimates may be too low because the DPX levels measured in the nasopharynx of monkeys, corresponding to a site having a possible association of tumor incidence in humans, are not considered in the derivation of risk estimates. The dynamics of carcinogenesis in these different respiratory regions in different species have not been adequately examined.

Although there is evidence of a dose-rate effect in short-term experiments examining cell proliferation, nonmalignent and

nonmalignent changes, it is not possible to incorporate this information more explicitly into the risk assessment at this time, principally because the current studies were not carried out for a long enough period of observation to assess quantitatively the comparable consequences on tumor incidence. If dose-rate could be taken into account in the risk calculations, then there is a suggestion that, for some human exposure conditions, such as those of the workplace, the cancer risk could be higher than these which rely on time-weighted averages, although the amount of any increase cannot be predicted at this time.

It should also be noted that the experimental conditions for measuring DPX did not duplicate the conditions of the chronic assay, in which the cell type that developed cancer (squamous cells) was not seen following acute exposures, and the dynamics of cell proliferation and cytotoxicity were not assessed. Also, if continued exposure to formaldehyde stimulates cell proliferation, a time when more DNA is produced, then chronically exposed animals could have a lower concentration of DPX than those that were exposed for one session, if respiratory surface area and dynamics determine the amount of formaldehyde absorption. On the other hand, the amount of available DNA could be the determining factor in the amount of formaldehyde absorbed by the respiratory mucosa. These factors could have a greater impact at high concentrations (those above 6 ppm) based on available dose-rate effect information.

5.5.2 Concordance with Epidemiologic Evidence

As mentioned above, it was acknowledged in 1987 that human-based risk estimates could not be calculated, owing principally to imprecise exposure estimates. Nevertheless, some rough comparisons (Figure 7-1; USEPA, 1987) suggested that animal-based risk estimates using airborne concentrations were concordant with observed human risks.

The Blair et al. (1986) study was selected in this comparative assessment since it provides the most thoroughly characterized exposure data. The authors reported less than two excess nasopharyngeal cancer deaths for each of the three exposure level categories. The predicted excess cancer deaths based on rat carcinogenicity data, using rat and monkey DPX dosimetry, were on the order of 10^{-2} to 10^{-1}, and 10^{-3} to 10^{-2}, respectively (See Appendix B for details of the analysis).

A number of factors may contribute to the differences between the predicted animal-based risk estimates and the observed risks in the Blair et al. (1986) study. The oronasal pattern of respiration in humans and monkeys would be expected to reduce the dose of formaldehyde received by the nose and the nasopharynx, and to increase the dose delivered to the oral

cavity and upper respiratory tract, relative to the rat's strictly nasal pattern. This is supported by the observation of a lower rate of DPX formation but more widespread distribution in the respiratory tract of monkeys, particularly with higher level exposure. Since DPX are being used as a surrogate for the agent(s) inducing tumors, the possible contribution of non-nasal DPX in monkeys should be noted. The rat-tumor-based model, however, depends only on nasal DPX levels, first in rats, for the model derivation, then in monkeys, for translation across species. Consequently, its use in confirming the experience reported in the Blair et al. study might somewhat underestimate total respiratory cancer at all anatomical sites.

Without carcinogenic data in monkeys, it is not possible to assess how susceptible monkeys are to formaldehyde-induced carcinogenesis. For noncancer effects, the monkeys appear to be more susceptible to formaldehyde toxicity than rats, based on an observed induction of more widespread histologic lesions in the respiratory tract of monkeys at equivalent exposure concentrations (6 ppm). It is difficult to evaluate the full impact of these observations because, as stated before, no consistent correlation has yet evolved between histopathological changes and tumor induction. DNA binding also occurs in the lower respiratory tract of monkeys, so that risk estimates based only on cross-links data that correspond to those in rats may underestimate human risk.

Several exposure measurement issues further complicate a direct comparison of human- and animal-based risks. First, the human exposures reported in Blair et al. (1986) included particles onto which formaldehyde adsorbed, or which may have contained releasable formaldehyde. The particles may have caused some irritation which could potentiate the effect of the formaldehyde actually delivered. Therefore, formaldehyde exposure for these situations could have been underestimated, tending to underestimate the number of expected deaths.

Second, human exposures in Blair et al. (1986) were reported as cumulative exposures. As seen from animal data (Wilmer et al., 1989; Feron et al., 1988), dose/exposure rate may be more predictive of cancer incidence than cumulative exposure. This is based on the observation that noncancer effects appeared to be correlated with exposure rate more than with cumulative exposure. When used for comparison with the animal model predictions, which are based on dose rate, cumulative exposures averaged over length of employment probably lead to underestimates of effective exposure, and consequently, underestimates of cancer risk. Individual records of length of employment and occurrence of peak exposures would need to be evaluated and incorporated for a more accurate estimation of dose rate to use with an animal model.

It is not clear what the relative contributions of these two aspects may be to an accurate risk estimation. As a rough estimate, each probably affects the exposure measures by less than an order of magnitude on average, but their relative impacts on individual exposures and consequent health effects are much harder to assess. Individual exposures would need to be reassessed for a more parallel comparison of animal and human risks.

5.6 Summary

Recently published research concerning formaldehyde toxicity has potential for refining the formaldehyde level estimates associated with carcinogenesis. These data favor the use of formaldehyde DNA-protein cross-links as an appropriate measure of formaldehyde exposure associated with the incidence of squamous cell carcinomas. DNA-protein cross-link data for rats were used in the linearized multistage procedure, and estimates of risk to humans have been calculated using DNA-protein cross-link data from rats or monkeys.

6.0 NONCARCINOGENIC HAZARD EFFECTS

6.1 Review of New Animal Studies

6.1.1. Respiratory Effects

Animal studies reviewed in EPA (1987) demonstrated that formaldehyde causes cellular effects in the nasal cavity of rats and monkeys, with the extent and severity dependent on both concentration and duration of exposure. A NOEL of 1.0 ppm for squamous metaplasia was determined in Cynomolgus monkeys (Rusch et al., 1983) and rats (Kerns et al., 1983) following 26 weeks and 24 months of exposure, respectively. The cellular effects of formaldehyde are confirmed in later studies in mice (Maronpot et al., 1986), rats (Zwart et al., 1988; Woutersen et al., 1987; Monticello and Morgan, 1989; Monticello, 1990) and monkeys (Monticello et al, 1989). However, the NOEL for cellular changes in rats were not consistent among these studies.

The pathologic effects of formaldehyde were determined in male and female B6C3F1 mice exposed to 0, 2, 4, 10, 20, or 40 ppm for 6 hr/day, 5 days/week for 13 weeks. Squamous metaplasia and rhinitis were induced in the nasal tissues and larynx at 20 ppm, and in the nasal tissues at 10 ppm. Minimal squamous metaplasia was detected in the nasal mucosa of 1/20 mice exposed to 4 ppm. No lesions were detected in mice exposed to 0 or 2 ppm. The cellular effects on the respiratory system of mice were more prevalent in males than in females (Maronpot et al., 1986).

To study the cellular effects of low concentrations of formaldehyde, Zwart et al. (1989) exposed male and female albino

Wistar rats to 0, 0.3, 1, or 3 ppm formaldehyde vapor for 6 hr/day, 5 days/week for 13 weeks. Compound-related histopathologic nasal changes varying from epithelial disarrangement to epithelial hyperplasia and squamous metaplasia were found in the 3 ppm group. These changes were not observed in the other low dose groups, indicating a NOEL of 1.0 ppm, which is consistent with a NOEL of 1.0 ppm determined in a chronic study (Kerns et al., 1983) as reported previously in EPA (1987).

In contrast, in another 13 week inhalation study in male and female albino Wistar rats (exposed to 0, 1, 10, or 20 ppm formaldehyde) conducted by the same laboratory, minimal focal hyperplasia or squamous metaplasia was found in the respiratory epithelium lining in the nasal septum and maxillary turbinates of 3 of 20 rats exposed to 1 ppm formaldehyde. Squamous metaplasia were induced in the nasal tissues and larynx at 20 ppm, and in the nasal tissues at 10 ppm. No lesions were found in control animals (Woutersen et al., 1987).

A concentration-dependent response in formaldehyde-induced cellular changes was also observed in a recent chronic study in rats (Monticello, 1990). Male F344 rats were exposed to 0, 0.7, 2, 6, 10 or 15 ppm formaldehyde for up to 18 months. However, only the highest two concentrations of formaldehyde (10 and 15 ppm) induced nasal epithelial cell necrosis, inflammation, hyperplasia and metaplasia. The lack of response at 6 ppm in this study is inconsistent with the positive effects found in a previous chronic study in F344 rats by Kerns et al. (1983). The reasons for the discrepant results from these two studies are not known.

Monticello and colleagues compared the results in monkeys with those observed in F344 rats exposed under similar conditions (Monticello et al., 1989; Monticello and Morgan, 1989). Groups of three rhesus monkeys were exposed to 0 or 6 ppm formaldehyde vapor 5 days/week for 1 or 6 weeks. Groups of 6 male F344 rats were exposed under a similar test regimen. In the rat, formaldehyde-induced lesions were detected only in the anterior portion of the nose. These lesions were characterized as epithelial degeneration, hyperplasia and squamous metaplasia. In contrast, lesions in monkeys were more widespread, being detectable as deep as the trachea and bronchi. Slight progression of histologic lesions occurred between 1 and 6 weeks of exposure, and the percent of nasal surface affected was greater in monkeys for 6 weeks than in monkeys exposed for 1 week.

6.1.2 Contact Sensitization

In a study by Lee et al. (1984), formaldehyde was tested in guinea pigs with inhalation, dermal and injection exposure in order to assess the route of exposure most likely to cause

sensitization in animals and to evaluate the potency of formaldehyde as a sensitizing chemical. In this study, formaldehyde induced contact sensitivity by all three routes of exposure. Dermal and injection exposure routes were more potent than the inhalation route; as the dose of formaldehyde was increased, both the degree of sensitization and the percentage of animals sensitized increased by either route. Contact sensitivity was detected only at 24 hours post-challenge in 2/4 animals exposed via inhalation to 10 ppm for 8 hours/day for 5 days. Animals exposed to either 6 or 10 ppm for 6 hours/day for 5 days did not exhibit evidence of either contact sensitivity or antibody production.

Animals injected with an emulsion of formaldehyde and Freund adjuvant responded to topical challenge with formaldehyde by displaying extensive dermal reactions that were maximal 24 hours after challenge. Antibodies were detected in low titer in 2/4 animals in this group. All animals in this group displayed extensive dermal sensitivity when challenged. The degree of contact sensitivity was determined to be dose-dependent and increased doses resulted in a greater percentage of animals becoming sensitized.

6.1.3 Pulmonary Sensitization

Formaldehyde did not produce pulmonary sensitivity (Lee et al., 1984). However, the authors urge caution in concluding that formaldehyde does not cause pulmonary sensitization, because the protocol used in this study was developed for toluene diisocyanate and the latent period for eliciting a sensitization response to formaldehyde may be different. Guinea pigs induced by inhalation did not demonstrate either immediate or delayed onset respiratory reactions when challenged by bronchial provocation of either 2 ppm formaldehyde for 1 hour or 4 ppm for 4 hours.

Animals sensitized by dermal application of 74 mg formaldehyde on each of 2 days did not give any evidence of a respiratory response when challenged with inhalation exposure to either 4 ppm formaldehyde for 4 hours or 2 ppm for 1 hour. No cytophilic antibodies were detected in the blood of these animals.

6.1.4 Immunotoxicologic Studies

No papers evaluating the role of the immune system in formaldehyde cytotoxicity were discussed in the EPA (1987). One recent, well-designed study focuses on the role of macrophage development and function and suggests a new mechanism through which formaldehyde-associated cytotoxicity might be induced.

The systemic effects of formaldehyde inhalation exposure on macrophage development and function were studied (Adams et al., 1987) to determine the potential role of formaldehyde in altering the mononuclear phagocyte system (MPS). Intraperitoneal macrophages were selected to measure effects on the MPS that were not simply due to local irritation and inflammation. Female pathogen-free B6C3F1 mice were exposed to either 0 or approximately 15 ppm of formaldehyde for 6 hr/day, 5 days/week for 3 weeks. Peritoneal leukocytes were collected by peritoneal lavage from control and exposed mice 2-3 days after final exposure and tested to determine whether formaldehyde exposure altered macrophage activation, produced by the prototype activator, pyran copolymer (MVE-2). Macrophage phagocytosis was determined using labelled sheep red blood cells. Binding and lysis of target cells to macrophage monolayers were also quantified. Hydrogen peroxide (H_2O_2) was measured to test the capacity of macrophages to secrete reactive oxygen intermediates (ROI), a response usually elicited by cells exposed to an irritant, an inflammatory agent or mycobacteria. The enzyme activity of leucine aminopeptidase (LAP) was quantified to distinguish inflammatory from activated macrophages.

Exposure of mice to 15 ppm formaldehyde for 5 days/week for 3 weeks did not alter the number of resident macrophages appreciably in the peritoneal cavity or the number elicited in response to MVE-2. Overall, comparison of the functional stage of macrophage activation between exposed and control animals indicated that the peritoneal population of macrophages was in the basal or resident stage of development, and was not in the activated stage. The macrophages from formaldehyde-exposed mice did have a lower content of the enzyme leucine aminopeptidase. However, there was no other evidence to suggest that resident macrophages were activated. Thus, formaldehyde exposure did not induce significant systemic maturation of resident tissue macrophages. Similarly, formaldehyde neither inhibited the development of activated macrophages (induced by exposure to pyran (MVE-2)), nor did it alter the two acquired functions of these macrophages: binding of tumor cells and cytolysis.

Primed macrophages acquired competence for release of hydrogen peroxide, which was significantly increased in macrophages from formaldehyde-exposed mice. Thus, formaldehyde exposure can, on a systemic basis, significantly enhance macrophage competence to release ROI. The biological impact of a change in the potential of mononuclear phagocytes to release ROI may be either beneficial or detrimental. An earlier study has documented that exposure to formaldehyde enhances host resistance to challenge with the microbe Listeria monocytogenes. A mechanism proposed for this effect was macrophage competence for release of ROI because it is strongly correlated with macrophage activation for destruction of many facultative or obligate intracellular microbes. It has also been hypothesized that

macrophages might induce genotoxic damage of significant mutagenic potential in bystander eukaryotic cells. Released ROI from these macrophages, at concentration levels comparable to those induced in this study, might contribute to the genotoxicity and lesion induction of formaldehyde.

6.1.5 Summary and Conclusions

Results from recent animal studies and those reviewed in EPA (1987) indicate that formaldehyde is capable of inducing cellular changes in the respiratory tract of mice, rats and monkeys. The affected site(s) and severity of formaldehyde-induced lesions are dependent on both concentration and duration of exposure. At low concentrations (≤3 ppm) lesions are confined to the anterior region of the nose of mice, rats, and monkeys. However, at high concentrations (≥6 ppm), lesions may extend beyond the nasal turbinates though the lesions at distal sites are generally less extensive. There are clear differences in the distribution of formaldehyde induced lesion between rats and monkeys at high concentration, i.e., 6 ppm. Lesions are more widespread extending to the bronchi whereas in rats, cellular changes are confined to the anterior portion of the nasal passages. Based on available data, it can be determined that a NOEL for formaldehyde induced cellular changes is 2.0 ppm in mice, 1.0 ppm in monkeys, and ranges from 0.3 - 1.0 ppm in rats.

Dermal sensitization induced by direct skin contact with formaldehyde is well documented. Limited available information indicate that inhalation exposure to formaldehyde may induce contact sensitivity in guinea pigs. However, there is no evidence that formaldehyde elicits pulmonary sensitization in laboratory animals.

The study on macrophage function by Adams et al. (1987) suggests that altered macrophage competence may play a role in lesion induction after formaldehyde exposure. However, this hypothesis is highly speculative. Neither the role of the immune system in the induction of cellular and neoplastic changes during inhalation exposure to formaldehyde, nor the effects of formaldehyde inhalation exposure on pulmonary antibacterial defenses, are well understood. These areas require further elucidation.

6.2 Review of Noncancer Human Studies

This section reviews the major studies published since the release of EPA (1987) for noncancer effects in humans associated with inhaled formaldehyde. The effects investigated in these studies included irritation of the eyes and upper respiratory tract, lower airway symptoms, changes in lung function, cellular changes in the nasal tissues, and immunologic and neurologic alterations.

The reports under review included studies of occupational populations (Alexandersson and Hedenstierna, 1988; Alexandersson and Hedenstierna, 1990; Holness and Nethercott, 1989; Horvath et al., 1988; Imbus and Tochilin, 1988; Kilburn et al., 1988; Malaka and Kodama, 1990; Nunn et al., 1990; Uba et al., 1989), residents in mobile homes or urea-formaldehyde-foam-insulated (UFFI) houses (Broder et al., 1988a, 1988b, 1988c; Liu et al., in press, personal communication; Ritchie and Lehnen, 1987) and controlled human exposures (Green et al., 1987; Sauder et al., 1986, 1987; Schachter et al., 1986, 1987; Witek et al., 1986, 1987). Three studies examined cellular changes in the nasal turbinates by biopsy of workers (Boysen et al., 1990; Edling et al., 1988; Holmstrom et al., 1989). Several studies examined either immunologic (Pross et al., 1987; Thrasher et al., 1987, 1988, 1989; 1990; Wilhelmsson and Holmstrom, 1987) or neurologic (Kilburn et al., 1987) responses. Most of these studies looked at a variety of endpoints; the context and details of the study's design are given at its' first discussion.

6.2.1 Eye and Upper Respiratory Tract Effects

6.2.1.1 Irritant Symptoms

Results from studies of residential, occupational, and controlled exposures supported the 1987 assessment that sensory irritation of the eyes, nose, and throat occurred at mean formaldehyde concentrations of 0.1 to 3.0 ppm. In general, the newer studies showed that eye and upper airway irritant effects have been transient and of mild to moderate intensity.

Three prevalence studies of residential exposures (Broder et al. 1988a, 1988b, 1988c; Ritchie and Lehnen, 1987; Liu et al., in press) examined eye and upper airway irritant effects. Broder et al. (1988a, 1988b, 1988c) conducted a health survey of 1726 residents in 571 UFFI houses and compared health outcomes to a control group of 720 residents in 231 homes. UFFI-homes were identified from a Canadian Government-sponsored registry (Consumer and Corporate Affairs). The UFFI-house group was subdivided into three categories depending on their intention to remove the insulation, take other remedial action, or take no action. Controls were recruited by letters to homes selected at random from streets adjacent to consenting UFFI-households. Cases and controls were similar in their distributions of race, sex, and occupation. The control population, however, was younger, had fewer exsmokers, and spent fewer hours at home. The refusal rate was 5% among UFFI-home residents and 80% among controls.

All homes and occupants were examined on two occasions separated by a 12-month interval, during which two-thirds of the UFFI-homes performed remedial work. At the first visit, mean formaldehyde levels in the three groups of UFFI homes fell

between 0.039 ppm (other remedial action group) and 0.045 ppm (no action group) (range: 0.01-0.23 ppm), and for control homes, 0.035 ppm (range: 0.01-0.11 ppm). Pooled subjects from all UFFI homes showed statistically significant positive exposure-response relationships between exposure level and the prevalence at the first visit of throat discomfort, eye irritation, and nasal problems (either runny nose, stuffy nose, sneezing, nasal discomfort, or nose bleeds), but the relationships were largely dependent on a small group whose mean indoor formaldehyde levels were greater than 0.12 ppm. These logistic regression analyses adjusted for time of year, sex, age, race, smoking, and number of hours spend in the house per week. Objective indicators of health status (nasal airway resistance and detection of pyridine odor) did not demonstrate differences between UFFI-home and control groups.

Repeated participation rates at the second survey were 84% for controls and 91% for the pooled UFFI-home group. Of the pooled UFFI-home group, 90% of the intended removal, 74% of the no action, and 66% of the other remedial action houses had performed remedial work. Although for all three exposure subgroups statistically significant declines were seen between the two surveys in the prevalence of throat discomfort and nasal symptoms, the pooled-UFFI group continued to show at the second visit a small but statistically significantly greater amount of eye irritation and nasal problems compared to controls. The prevalence of sense of smell problems and results of nasal airway resistance remained constant between visits for all groups and, with respect to the second visit, between the pooled-UFFI and control groups.

Mean formaldehyde levels remained constant between the first and second visits. Exposure-response analyses which controlled for age, sex, smoking experience, total hours spent in the house per week, race, and outside temperature, however, showed formaldehyde level at the second survey as a statistically significant predictor of nasal problems. No relationships were noted, as in the first visit, with other upper respiratory symptoms.

In this study, the presence of respondent bias is a possibility due to the nature of the self-selected study population. Such bias would tend to obscure any exposure-response relationship. However, the presence of exposure-response relationships diminishes our belief in the likelihood of respondent bias.

Ritchie and Lehnen (1987) studied 2000 residents with concerns about formaldehyde exposure who lived in 397 mobile and 494 conventional homes. The Minnesota Department of Health provided names of study participants given by referring physicians. In an analysis examining formaldehyde concentration

($\leq$0.1 ppm, 0.1-0.3 ppm, and $\geq$0.3 ppm) and eye and nose/throat irritation, the prevalences of response were statistically significantly associated with exposure level. For each residence type, 90% of occupants exposed to 0.3 ppm or greater and 1-2% exposed to $\leq$0.1 ppm reported eye irritation. For the 0.1 to 0.3 ppm group, between 12% (conventional home residents) and 21% (mobile home residents) reported eye irritation. For nose/throat irritation, depending on age and smoking status, 74% and 99% of respondents reported the symptoms at the high concentration range ($\geq$0.3 ppm); 12% and 36% at the middle level (0.1-0.3 ppm); 0 and 11% at levels $\leq$0.1 ppm. The authors pointed out that the results could have been biased by self-selection. The inclusion of some subjects might have been related to the existence of symptoms and approximate knowledge about the level of formaldehyde exposure, but the extent of such bias was difficult to assess.

Liu et al. (in press) conducted a prevalence study of randomly-selected non-complaint mobile homes in the State of California using a house-age-stratified sampling scheme. Among 2203 letters mailed to recruit participants, a total of 663 homes with 1394 residents completed the summer phase and 523 homes with 1096 residents finished the winter phase of the survey; 472 of the latter homes were also part of the summer study. Approximately 60% of the homes were manufactured between 1981 and 1983. The large sample size allowed for control of potential confounders such as age, sex, smoking status, time spent at home, and the effect of formaldehyde level on people with chronic respiratory illnesses and allergy problems.

Formaldehyde concentrations varied from below the limit of detection (0.01 ppm) to 0.46 ppm; the mean formaldehyde concentration was 0.089 ppm in the summer and 0.088 ppm in the winter. The authors found that cumulative exposure (ppm-hours) better described the health effect relationships rather than concentration (ppm). All analyses, thus, were based on cumulative exposure as categorized into three groups: <7.0 ppm*hours, 7.0-12.9 ppm*hours, and >12.0 ppm*hours.

Logistic regression analyses showed that complaints of burning/tearing eyes (summer and winter), stinging/burning skin (summer), dizziness (winter), and sleeping problems (winter) were significantly ($p<0.05$) associated with cumulative formaldehyde exposure after adjusting for identified possible confounders. Response for burning/tearing eyes during the summer and winter phases for <7.0 ppm*hours was 10.8% to 13.3%; for 7.0 to 12.0 ppm*hours, 14.7% to 17.1%; and for >12.0 ppm*hours, 20.6% to 21.4%. The limitations associated with this study are similar to those of Broder et al. (1988a, 1988b, 1988c) and Ritchie and Lehnen (1987). The low participation rate (about 52%) and the self-selection of participants enhance the potential for respondent bias. The presence of exposure-response relationships diminishes this possibility, however.

Five studies of occupationally-exposed populations (Uba et al., 1989; Horvath et al., 1988; Alexandersson and Hedenstierna, 1988; and Holness and Nethercott, 1989) noted increased reporting of upper irritant effects. All studies except Uba et al. (1989) were of the cross-sectional (prevalence) design. Uba et al. (1989) assessed acute and persistent respiratory effects in a well-conducted prospective study of 107 medical students exposed to formaldehyde during a 7-month gross anatomy laboratory. The occurrence of acute symptoms (upper respiratory tract and lower airway effects) was measured by the American Thoracic Society (ATS) Epidemiology Standardization Project respiratory questionnaire in 81 students after a gross anatomy laboratory in which there was formaldehyde exposure, and after a microanatomy laboratory in which no formaldehyde was used. Responses at the beginning of the school year were compared to subjects' responses at the end of the study to give estimates of the incidence of persistent (chronic) effects (See Section 6.2.2.1 for lower airway effects).

For acute effects, these investigators found statistically significant elevations in the occurrences of eye, nose, and throat irritation during gross anatomy laboratory than during microanatomy laboratory. The time-weighted averages (TWA) of formaldehyde in the gross anatomy laboratory ranged from 0.16-0.93 ppm; peak exposures ranged from 0.1 to 5.0 ppm, with a mean of 1.9 ppm.

Horvath et al. (1988) evaluated upper airway effects in 109 workers exposed to formaldehyde (mean=10 years) at a plant producing particleboard or molded products, and in 254 control subjects from food-processing plants. Controls were similar to exposed on most demographic descriptors except the control group contained more females than males. Subjects completed a modified ATS questionnaire before and after the monitored work shift, in addition to estimating the intensity of symptoms by a visual analog scale. Personal monitoring showed formaldehyde levels (TWA) in the molded products/particleboard processes ranged from 0.17- 2.93 ppm, with a mean of 0.69 ppm. The molded products and particleboard processes also contained nuisance particulates; approximately one-third were of respirable sizes. The mean level at the control food processing plants was 0.05 ppm.

In this well-conducted study, these investigators showed statistically significantly higher prevalences of eye irritation (46% exposed vs 24% controls) and irritation of the nose and throat (35% vs 13%) among exposed subjects compared to controls. Exposed positive responders reported moderate intensity of burning eyes; however, considerable variability existed between individuals. It is difficult to draw comparative inferences regarding intensity since no such assessment was made in the nonexposed control population. Logistic regression analysis showed formaldehyde concentration to be a statistically

significant predictor of the following symptoms: burning eyes, itchy eyes, burning nose, stuffy nose, itchy nose, and sore throat. While this study is limited by possible respondent bias, The authors believed the exposure-response relationships demonstrated a qualitative hazard.

Alexandersson and Hedenstierna (1988) investigated respiratory effects associated with formaldehyde exposure among 38 workers involved in painting work using acid-hardening lacquers and in 18 nonexposed controls. These investigators found statistically significantly increased prevalences for irritation of the eyes (66% versus 17%) and of the nose/throat (39% versus 0%). Mean formaldehyde concentrations for exposed averaged 0.32 ppm (range of 0.12-2.08 ppm), with peaks up to 0.56 ppm. Formaldehyde-exposed subjects also had exposures to low concentrations of solvents, i.e., 10 to 100 lower than the TWA.

The exposed population could be considered a survivor group since participants were chosen if they were working at the time of the study and had been exposed during the preceding 12 months. The mean number of years worked, 7.8, is not surprising given the eligibility criteria. These investigators do not describe selection criteria for controls. Cases and controls appeared similar with respect to age; however, the control population contained fewer smokers. No information was provided on sex.

Holness and Nethercott (1989) studied 84 funeral service workers exposed to formaldehyde and 38 controls. Funeral service workers were selected from a funeral directors' association list of homes expressing interest in the study. Among funeral service workers, 66% were active licensed embalmers, 20% were currently inactive licensed embalmers, and 14% were apprentices. Inactive embalmers were older and had worked a longer number of years than active embalmers. Apprentices were younger than active workers, and had just started embalming regularly during the past year. Controls were recruited from a large service organization and from a group of paid student volunteers. Exposed and controls were similar in age and sex; however, the exposed population had accumulated more pack-years smoked and had higher body weight. All analyses controlled for the effects of smoking. These investigators obtained information on respiratory complaints using the ATS questionnaire. The mean formaldehyde level measured during the period of study was 0.36 ppm with a range from 0.08-0.81 ppm. Average formaldehyde level for controls was 0.02 ppm.

These investigators observed statistically significant increases in the prevalence of nose and of eye irritation over controls (44% vs 16%; 42% vs 21%, respectively) in logistic regression analyses which accounted for smoking. Exposed subjects also reported throat irritation more often than controls (17% vs 5%), but this difference was not statistically

significant ($p=0.10$). No differences in irritative symptoms existed between apprentices and active licensed embalmers; however, active members reported eye irritation more frequently (73% vs 71%, $p<0.05$) than inactive embalmers.

Upper respiratory symptoms and eye irritation symptoms have also been reported in a number of controlled-exposure studies where subjects were exposed to formaldehyde at 2 ppm for 40 minutes (Schachter et al., 1986, 1987; Witek et al., 1986, 1987) or 3 ppm for 1 (Green et al., 1987) or 3 hours (Sauder et al., 1986, 1987). Schachter et al. (1986, 1987) and Witek et al. (1986, 1987) each exposed healthy volunteers and/or asthmatics to 0 or 2 ppm formaldehyde in a double-blind random sequence for 40 minutes on four separate days, two days at rest and two days with exercise. Subjects served as their own controls. Study participants completed a symptom question upon entering the exposure chamber, 30 minutes into the exposure, and 4, 8, and 24 hours after exposure cessation.

In their 1986 study, Schachter et al. noted the most common irritative symptoms reported by 15 nonsmoking healthy volunteers exposed to formaldehyde at either rest or during exercise were unusual taste and odor, sore throat, nasal discharge or stuffiness, and eye irritation. These investigators did not perform statistical testing to determine whether the differences in response between formaldehyde exposure and room air were statistically significant. For the symptoms of odor, taste, nasal discharge, and eye irritation, participants reported the highest severity upon first entering the chamber, with decreasing severity by 30 minutes. The opposite was seen for sore throat, where symptom score was higher at 30 minutes. Upper airway irritant complaints were nonexistent after exposure cessation and were not affected by exercise.

In a subsequent study of 15 laboratory workers, Schachter et al. (1987) observed a greater number of subjects responding with eye, nose, and throat irritation, and with odor and taste perception during exposure to 2 ppm formaldehyde when compared to response a 0 ppm. These investigators did not perform statistically testing to determine whether the differences in response between formaldehyde exposure and room air were statistically significant. The intensity of response was also judged by participants to be greater during formaldehyde exposure. Participants perceived the severity associated with all symptoms except odor as mild or moderate. Exercise did not modify the incidence of response nor its severity. The exception was for odor, where the mean severity score associated with formaldehyde exposure was higher during exercise than at rest. These laboratory workers had frequent exposure to formaldehyde in their work environment. Three subjects were current smokers and all participants had a mean of duration of formaldehyde exposure of six years (range, 1-21 years).

Using a similar study design as Schachter et al. (1986, 1987), Witek et al. (1986) found more frequent reporting of irritation of the eyes, nose, and throat and odor perception with formaldehyde exposure than with room air among 15 healthy and 15 asthmatic subjects. Asthmatics generally had a higher responses to these symptoms than healthy subjects, although both groups had similar perceptions of severity. In a subsequent study of only asthmatics (n=15), Witek et al. (1987) noted that unusual taste, odor, nasal discomfort, and eye irritation were commonly reported complaints, and were of mild to moderate severity. As in the reports of Schachter et al. (1986, 1987), the investigators did not perform statistical testing to determine whether the differences in response between formaldehyde exposure and room air were statistically significant.

In a series of studies, Sauder et al. (1986, 1987) and Green et al. (1987) exposed healthy nonsmoking or asthmatic participants to 3 ppm formaldehyde for three hours. Each subject served as his or her own control and received clean air for three hours. Participants in both Sauder (1986) and Green et al. (1987) performed exercise during exposure. Symptom questionnaires in all studies assessed the presence and severity of odor, nose or throat irritation, and eye irritation.

These studies found statistically significant increases in upper airway complaints. Sauder et al. (1986) observed statistically significant increases in response for odor, nose and throat irritation, and eye irritation in their study of 9 healthy nonsmoking participants during formaldehyde exposure than during exposure to room air. The individual severity scores ranged from none to moderate. Likewise, Sauder et al. (1987) noted in 9 asthmatic volunteers statistically significant increases in nose and throat irritation and in eye irritation. with formaldehyde exposure than during exposure to clean air. Study participants reported irritation of the nose and throat after 30 minutes, whereas, increased response to eye irritation did not occur until one hour after start of exposure. In a study of 22 healthy nonsmoking and 16 asthmatics subjects, Green et al. (1987) reported statistically significant increases in perceived odor, nose and throat irritation, and eye irritation. The severity of response appeared highest after each exercise stint.

In summary, the newer studies demonstrated that the eye and upper airway irritation occurs with formaldehyde exposure. Results from studies of mobile home populations indicated that eye and upper respiratory tract irritation complaints increase at formaldehyde concentrations above 0.1 ppm, as reported previously. Some studies reported higher prevalences or incidences of eye irritation whereas other studies report nose and throat irritation as the more frequent sensory irritant effects of formaldehyde. Different categorizations of symptoms by individual investigators and the characteristics of the

studied population (i.e., healthy volunteers or self-selected individuals) may account for these disparate observations. The finding of dose-response relationships for eye as well as for nose and throat irritation supports a tenet that complaints of irritant symptoms are exposure related, and not an artifact of reporting bias.

6.2.1.2 Nasal Cellular Changes

Three prevalence studies of formaldehyde-exposed occupational populations (Edling et al., 1988; Holmstrom et al., 1989; Boysen et al., 1990) and one prevalence study of UFFI-house occupants examined cellular alterations in tissue from the nasal turbinates. Edling et al. (1988) assessed histopathologic changes in nasal biopsies of 75 male workers at two particleboard processing plants and one laminate plant. Seventy-two percent of the total eligible workforce participated in this study. Of participating workers, mean exposure duration was 11 years. Histological findings from the formaldehyde-exposed workers were compared with a referent group of 25 men, matched for age and smoking habits, who had no industrial exposure to formaldehyde. Concurrent formaldehyde levels in the three plants ranged from 0.08 ppm to 0.88 ppm (TWA) with peaks up to 4 ppm. Higher exposure had occurred in the past. Exposure to wood dust was also likely in the particleboard plants, but not in the laminant plant, where only formaldehyde exposure occurred. Concurrent wood dust levels ranged from 0.6 to 1.1 mg/m^3.

Gross examination revealed normal nasal mucosa in 75% of the exposed, whereas 25% had swollen and/or dry changes of the nasal mucosa. Histological abnormalities were noted in 96% of all exposed. The histologic grading, using a scale from 0 (normal) to 8 (carcinoma), showed a higher score among exposed when compared to unexposed (2.9 vs 1.8, $p<0.05$). The histologic examination of exposed showed 4% with normal epithelium, 11% with loss of cilia and goblet cell hyperplasia, 56% with squamous metaplasia, and 8% with mild or moderate dysplasia. These investigators also assessed the effect of wood dust and formaldehyde in comparison to only formaldehyde. No difference in average histopathological score was found between particleboard workers and laminate workers. No relationship was observed with duration of exposure. Duration of exposure may be a poor surrogate for exposure; the investigators pointed out that dose rate may be more important and may account for the lack of an observed exposure-response relationship.

Holmstrom et al. (1989) investigated histologic effects in nasal turbinate biopsies associated with formaldehyde exposure alone and combined with wood dust. Two groups of formaldehyde-exposed workers were examined; one group of 70 chemical manufacturing workers exposed only to formaldehyde or formaldehyde resins (FA) and another group of 100 workers from

furniture factories with exposure to formaldehyde resins and wood dust (WD-FA). Histological scores of these workers were compared to 36 controls, primarily clerks, with no occupational formaldehyde exposure. All study participants had long employment durations (means ranged from 9 to 15 years). Average formaldehyde levels among exposed were 0.04 to 0.40 ppm (median 0.24 ppm), with frequent peaks over 0.80 ppm, for the FA group, and around 0.16 to 0.20 ppm, with levels never exceeding 0.40 ppm, for the WD-FA group. Mean wood dust levels for the WD-FA group ranged between 1 to 2 mg/m^3, with the majority of the particles being of respirable size. Controls had exposure to formaldehyde at background levels, with a measured mean of 0.07 ppm.

These investigators observed higher mean histologic scores for exposed, FA, 2.2 (range 0-4) and WD-FA, 2.1 (range 0-6), compared to controls, 1.6 (range 0-4), on a scale of 0 (normal) to 8 (carcinoma). The difference between exposed and controls was statistically significant for the exposed groups combined and for the FA group. In addition, these investigators noted that loss of cilia, goblet cell hyperplasia, and cuboidal and squamous cell metaplasia replacing columnar epithelium were more frequent in the group exposed to FA than in controls. The mean histologic score of the WD-FA group alone was not statistically significantly different from the control group. However, higher mean scores appeared for WD-FA workers grinding wood for more than four hours a day when compared to whose grinding for less than one hour daily, although the difference was not statistically significant ($p>0.05$). Two subjects (2%) of the WD-FA group had mild or moderate dysplasia (a score of 6); both workers ground wood for more than 4 hours daily.

These investigators did not find a relationship between mean histologic score and levels of exposure to formaldehyde (ppm) or wood dust, of duration of exposure, or of cumulative formaldehyde exposure (ppm-year). In addition, no relationship was observed with smoking.

Boysen et al. (1990) evaluated histopathologic changes in biopsy samples of the nasal turbinates from 37 workers producing formaldehyde and formaldehyde resins and 37 age-matched referents. In addition to the histologic examination, rhinoscopic examinations and nasal complaints were also assessed. Referents were selected from the office staff of two chemical industries and from outpatients at an ear, nose, and throat department of a local hospital. Exposed workers represented one-half of the current workforce. Exposure levels were estimated from recent measurements, from knowledge of the production process, and from previous and present subject-reported symptoms. Boysen et al. grouped exposed workers into two exposure categories, 0.5 to 2 ppm and >2 ppm; however, all analyses were performed using the aggregated exposure group.

The histologic classification was based on a 5 point system, with dysplasia ranked the highest; 92% of exposed had scores higher than 0 (normal epithelium) compared to 86% of controls. More pronounced metaplastic alterations were found among exposed workers than among controls (scores of 1.9, range 0-5, and 1.4, range 0-3, respectively), but the difference was not statistically significant ($p>0.05$). Three exposed workers (8%) who were categorized into the 0.5 to 2 ppm category had squamous cell epithelial dysplasia; none of the referents had dysplasia.

The rhinoscopic findings appeared to show a higher prevalence of hyperplastic nasal mucosa in the exposed than in the control group, but the difference was not statistically significant. These investigators showed statistically significant increases in the prevalence of subjective nasal complaints (the identify of which was not described by the investigators) among exposed workers. The authors pointed out that although metaplastic changes are generally considered as non-specific, by being induced by a number of factors including temperature, humidity, dust, and age, the finding of dysplasia among exposed individuals was noteworthy.

Broder et al. (1988b, 1988c) assessed possible cellular changes of the nasal cavity of UFFI-house occupants and controls. Swab samples of the nasal epithelial surface were acquired from the nostril and not from any internal structure (e.g., the turbinate) as obtained in the occupational studies. The intended UFFI-removal group, but not other UFFI-house residents, showed a statistically significant small magnitude increase in the mean number of squamous metaplastic cells per subject relative to the controls. In the follow-up survey one year later (Broder et al., 1988c), all groups (exposed: UFFI-removal, other action, and no action; and controls) showed a statistically significant decrease in total cells recovered in the swab and in squamous metaplastic cells. No differences, however, existed between the exposed groups and controls.

In summary, results of the newer studies indicate that formaldehyde exposure along with other exposures encountered in the studied occupational settings may enhance the severity of cellular damage. The major cellular changes in the nasal epithelium of formaldehyde-exposed workers were loss of cilia, squamous metaplasia, and mild or moderate dysplasia. The magnitude of the squamous metaplastic changes was more pronounced among exposed than among the referent populations. A low prevalence of dysplasia also existed among exposed workers which were absent among referents.

These findings are based upon few individuals, and this limits the conclusions. In addition, the prevalent design of these studies may over- or under-estimate the effects. The lack of exposure-response relationships in these studies may also be a

reflection of the prevalent study design; the studied populations could be considered survivor populations. In addition, exposure misclassification may also be present, thus, limiting the ability to detect exposure-response relationships.

6.2.2 Lower Airway Effects

6.2.2.1 Subject-Reported Symptoms

Lower airway irritation is characterized by cough, phlegm, wheezing, and chest discomfort, and has been reported often in people exposed to 5-30 ppm formaldehyde (EPA, 1987). Several recent studies suggested increased prevalences of lower airway symptoms at lower exposure levels than reported in EPA (1987).

Two studies of residential exposures (Broder et al., 1988b, 1988c; Liu et al., in press) evaluated lower airway irritant effects. Broder et al. (1988b) showed statistically significant positive relationships between formaldehyde level and the prevalence in the first survey of cough and sputum in UFFI-house occupants (mean formaldehyde levels of 0.039 to 0.045 ppm) using logistic regression analyses. These relationships remained even when the subgroup exposed to levels greater than 0.06 ppm were deleted. The UFFI-house group was subdivided into three categories depending on their intention to remove the insulation, take other remedial action, or take no action. In the logistic regression analyses, the respondent's intent to take remedial action and formaldehyde level together produced a statistical interaction in which the estimated prevalence of cough was larger for both variable combined than for only formaldehyde level by itself. Results from a repeat survey one year later (Broder et al., 1988c) showed a decrease in symptoms among only the subgroup of UFFI subjects who had undertaken remedial work, however, the positive relationship ($p<0.05$) remained between formaldehyde level and cough. No statistically interaction such as that observed for cough in the first survey was noted in the follow-up survey. Formaldehyde levels between these surveys did not change (0.040 to 0.044 ppm).

Liu et al. (in press), using logistic regression analyses, did not find any relationship between cumulative formaldehyde exposure (ppm-hour) and either cough or wheezing. Rather, these symptoms were related to smoking, sex, and presence of chronic disease. Mean formaldehyde exposures in this study were higher, 0.89 ppm, than in Broder et al. (1988b, 1988c).

Six studies of occupational populations (Horvath et al., 1988; Malaka and Kodama, 1990; Uba et al., 1989; Alexandersson and Hedenstierna, 1988; Holness and Nethercott, 1989; Nunn et al., 1990) evaluated lower airway irritant effects. Horvath et al. (1988) observed statistically significantly increased prevalences of cough (35% vs 19%), phlegm (27% vs 10%),

and chest discomfort (9% vs 2%) in particleboard and molded products workers exposed to formaldehyde when compared to prevalences in workers from food-processing plants (Horvath et al., 1988). In addition, these investigators observed statistically significant dose-response relationships for cough, chest complaints, and phlegm. The severity of chest symptoms among positive responders was mild and was accompanied by considerable individual variation. Formaldehyde levels (TWA) in the molded products/particleboard processes ranged from 0.17 to 2.93 ppm, with a mean of 0.69 ppm, and in food processing, 0.05 ppm (mean). The molded products and particleboard processes also contained nuisance particulates, approximately one-third were of respirable sizes.

Another study (Malaka and Kodama, 1990) also reported statistically significant increases in the prevalences of cough and phlegm among 93 formaldehyde-exposed Indonesian male plywood workers compared to 93 non-exposed working referents. These results were obtained using logistic regression analyses which controlled for age, smoking status, and dust exposure. Referents were age, sex, and smoking-habit-matched workers not occupationally exposed to formaldehyde. Mean formaldehyde levels across the plywood plant ranged, for exposed, from 0.22 to 3.48, and for referents, from level of detection to 0.07 (background levels). Both formaldehyde-exposed subjects and referents had respirable dust exposures above the threshold limit value. The increased prevalence of lower airway symptoms is difficult to interpret. This population also had a high prevalence of chronic upper respiratory infectious diseases which may have contributed to the lower airway symptoms, but due to the cross-sectional design of this study, it is not known whether formaldehyde may have aggravated any preexisting condition.

In a prospective study of medical students currently enrolled in a gross anatomy laboratory course (Uba et al., 1989), cough, wheezing, and dyspnea among 81 students completing an ATS respiratory questionnaire were not statistically significantly related to formaldehyde. However, the prevalence of chest tightness was of borderline significant (5% vs 0%, $p=0.05$). Questionnaires were administered once after a gross anatomy laboratory in which there was formaldehyde exposure (TWA ranged from 0.16 to 0.93 ppm) and once after a control microanatomy laboratory in which no formaldehyde was used.

These investigators also assessed persistent symptoms among 103 students associated with prolonged exposure to formaldehyde over a 7-month period. Medical students reported cough more often ($p<0.05$) at the end of the 7-month exposure than at the beginning (8% vs 1%). Conversely, wheezing both with and without dyspnea were reported more frequently ($p<0.05$) at the beginning than at the end of the anatomy course. Formaldehyde concentration (TWA) was highest at the beginning of the study

period (up to 0.8 ppm) and declined steadily over the 7-month period to an average of 0.1 ppm.

In 38 workers exposed to formaldehyde in acid-curing paints and lacquers when compared to 18 nonexposed controls, Alexandersson and Hedenstierna (1988) noted a higher prevalence of dyspnea during work (11% vs 0%), "chest oppression" (11% vs 0%), and cough (5% vs 0%), but the difference was not statistically significant ($p>0.01$). Mean formaldehyde concentrations for exposed averaged 0.32 ppm (range of 0.12 to 2.08 ppm), with peaks averaging 0.56 ppm. Formaldehyde-exposed subjects also had exposures to low concentrations of solvents, (10 to 100 lower than the TWA).

In a study by Holness and Nethercott (1989), no statistically significant differences existed in the prevalence of cough, sputum presence, and chest tightness among 84 funeral service workers (active and retired embalmers, and apprentices) when compared to 38 controls. Formaldehyde-exposed funeral home workers, however, more frequently ($p<0.05$) reported chronic bronchitis and shortness of breath. Mean formaldehyde level for the funeral home workers was 0.36 ppm with a range from 0.08 to 0.81 ppm. The average formaldehyde level for control workers was 0.02 ppm.

Nunn et al. (1990) did not find any differences in the prevalence of lower airway irritant symptoms (chronic bronchitis, shortness of breath or wheezing) among 125 chemical workers with free formaldehyde exposure over a six-year period when compared to 95 other workers at this plant who did not have free formaldehyde exposure. The prevalence in free formaldehyde-exposed and nonexposed workers with self-reported symptoms was similar; 12% and 16% reported breathlessness on hurrying and 26% and 20% for wheezing, respectively. None of the formaldehyde exposed, but 4% of the control group, had chronic bronchitis. Mean (geometric) formaldehyde concentration as measured semiannually from routine industrial hygiene monitoring over the 6 year study period were less than 1 ppm, except one measurement which was approximately 1.4 ppm. Peak levels were as high as 5.4 ppm.

Workers included in this study were participants in an annual health assessment for workers exposed to free formaldehyde and workers exposed to other materials such as phenolic resins, epoxy resins, and carbon fibers (the controls). Workers were identified in 1980 and included 164 workers exposed to formaldehyde and 129 nonexposed personnel. During the six year study period, workers had either died (5 exposed, 1 nonexposed) or left employment (39 exposed, 26 nonexposed). Among those workers who had left employment, only 9 exposed and four nonexposed attended the final examination. Results from 10

workers (4 exposed, 6 nonexposed) with unacceptable spirometric traces were excluded from the analyses.

Although this study identifies workers with exposure six year earlier, follow-up was limited mainly to workers currently employed. Only 9 out of 39 exposed workers who had left employment during this study participated in the final examination. Workers with adverse respiratory effects associated with formaldehyde may have left employment early so that the remaining workers would be considered a survivor group. Nunn et al. presented data that suggested this bias was present. Of 12 men in the exposed group not seen six years later, the FEV_1 at the start of the study was less than predicted in 75% compared to 36% of the 117 who were followed over the six years. In addition, use of chemical workers as a non-formaldehyde referent group may introduce another source of bias since respiratory health in this group may be compromised by exposure to potentially harmful agents. This referent population had annual respiratory screening because of their exposure to phenolic resins, epoxy resins, and carbon fibers. These two limitations would make it difficult to see differences between exposed and controls.

None of the controlled-exposure studies of formaldehyde exposure to 2 ppm for 40 minutes (Witek et al., 1986, 1987; Schachter et al., 1986) or 3 ppm for 3 hours (Sauder et al., 1986; Green et al., 1987), at rest and with exercise, reported symptoms of lower airway effects.

In summary, data from controlled-exposure studies showed that acute exposures to formaldehyde up to 3 ppm did not elicit symptoms of lower airway effects. Four studies (Broder et al., 1988b, 1988c; Horvath et al., 1988; Uba et al. 1989; Malaka and Kodama, 1990) documented increased reporting of lower airway irritation with longer-term formaldehyde exposure (occupational and UFFI-house) in the presence of other exposures (for example, particulates of wood dust or resins in the occupational studies). Other studies (Alexandersson and Hedenstierna, 1988; Holness and Nethercott, 1989; Nunn et al., 1990) examining formaldehyde exposures at levels less than 5 ppm did not show any associations. The small number of exposed subjects examined limits the ability to detect small differences between exposed and controls. In addition, these studies were unable to examine all workers who had left employment (i.e., Nunn et al., 1990), increasing the possibility of underestimating any underlying exposure-related-effects since workers who left employment may be considered a more sensitive subpopulation.

Despite these limitations of the overall data base, it is concluded that some exposed subjects will experience lower airway effects but the precise level at which the effects are initiated is uncertain. The levels in which lower airway symptoms have

been observed are lower than the lower limit previously reported with formaldehyde-associated lower airway effects, 5.0 ppm (EPA, 1987). In the occupational studies reporting lower airway symptoms, the mean level over a workshift was reported as <1.0 ppm. The range of exposure concentrations in the newer studies, however, were between 0.17 and 3.48 ppm, and the frequency of peak exposures above 1.0 ppm was not provided. The number of excursions above 1.0 ppm during a working day may influence the prevalence of lower airway irritation complaints.

6.2.2.2 Lung Function Measurements

Lung function measurements as objective health status indicators of formaldehyde effects on the lower airways were assessed in a number of studies. Overall, chronic decrements do not appear to be associated with formaldehyde exposure, although small transient decreases were observed at formaldehyde levels around 1.0 ppm and above.

Broder et al. (1988b) found no differences in pulmonary function between UFFI-home occupants and their referents after adjustment for age and other covariates in their two surveys separated by a year later. Formaldehyde levels in this study were very low, from 0.039 to 0.045 ppm.

Imbus and Tochilin (1988) observed no differences between pulmonary function tests conducted in 99 particleboard workers before the work shift and five hours into the shift. These workers had exposure to formaldehyde gas (<0.02-0.06 ppm), phenol-formaldehyde resins, and wood dust. It is not known whether the baseline spirometry measurements in these workers' values were within or deviated from normal limits since no comparison with a standard population was made. In addition, these investigators did not state whether they followed ATS-or other board-recommended procedures. This limits interpretation of the observed results.

Holness and Nethercott (1989) did not observe any differences in preshift (baseline) or cross-shift changes in lung function of 22 embalmers compared to controls. Lung function was assessed by FVC, FEV_1, $FEV_{1.0}/FVC$, and $FEF_{50\%}$, $FEF_{75\%}$, nitrogen washout, and closing volume. Spirometry tests were administered to embalmers prior to and following an embalming, and for controls two times during a three-hour period. The mean formaldehyde level in this study measured 0.36 ppm with a range from 0.08 to 0.81 ppm.

A longitudinal study of 125 (116 currently-employed) workers exposed daily to free formaldehyde during the production of urea-formaldehyde resin found no evidence of a formaldehyde-related decline in FEV_1 over a six year period (Nunn et al., 1990). Smoking explained the expected rate of decline in FEV_1 among

nonexposed. The mean rate of decline in the exposed group was similar, however, between smokers and nonsmokers. This finding suggested that some other factor besides smoking was responsible for the observed decrease in FEV_1. This study included few nonsmokers, and results from this subgroup are subject to large statistical variation. These investigators, in addition, did not find any association among exposed between the rate of decline in FEV_1 and level or duration of formaldehyde exposure.

Lung function measurements (FEV_1 and FVC) were part of the annual health assessment for workers exposed to free formaldehyde and workers exposed to other materials such as phenolic resins, epoxy resins, and carbon fibers (the controls). These investigators did not present results on FVC in the paper. Mean (geometric) formaldehyde concentrations as measured from biyearly routine industrial hygiene monitoring (i.e., accidents and spill situations were not included) were less than 1 ppm except for one with a TWA of approximately 1.4 ppm. Peak exposures were as high as 5.4 ppm. The possibility that the observed results might reflect bias which was discussed in Section 6.2.2.1 (survivor population and inappropriate control population) limits the ability to draw strong inferences from this study.

Occupational exposures at higher levels (around 1.0 ppm and above) appeared to produce transient decreases in volume and/or flow parameters. Preshift (baseline) pulmonary function studies were considered to reflect chronic effects of long-term exposure to formaldehyde and post- or cross-shift changes were considered acute effects. Three studies (Alexandersson and Hedenstierna, 1988; Kilburn et al., 1989; Malaka and Kodama, 1990) observed differences in preshift tests, but not in post-shift tests, while three others (Horvath et al., 1988; Uba et al., 1989; Alexandersson and Hedenstierna, 1989) observed the opposite, post-shift changes but not preshift changes.

Alexandersson and Hedenstierna (1988) observed statistically significant decreases in baseline lung function (FVC and FEV_1) among 38 formaldehyde-exposed acid-hardening lacquer workers compared with sex-, age-, and height-adjusted reference values as assessed before work on Monday after 2 days of no exposure. Nonsmokers had larger decreases than smokers. No changes were noted in volume or flow parameters over a work shift.

The deviations in preshift FVC and FEV_1 were not correlated to either peak formaldehyde exposure or mean formaldehyde exposure/length of employment period. Mean formaldehyde concentrations for exposed averaged 0.32 ppm (range of 0.12 to 2.08 ppm), with peaks averaging 0.56 ppm. Formaldehyde-exposed subjects also had exposures to low concentrations of solvents (10 to 100 lower than the TWA). These investigators were uncertain as to the exact nature of the functional impairments, but noted that the degree of functional disturbance was small and should

not cause any measurable reduction of physical capacity. They further noted that since impairment was observed after a 2-day nonexposure interval, the possibility existed for a short-term or chronic functional impairment. This latter possibility is important since the cross-sectional design of the study may have underestimated effects of formaldehyde exposure because the most sensitive workers may have left employment and would not have been included.

Kilburn et al. (1989) observed steeper age-related decrements in vital capacity and flows (FVC, FEV_1, and $FEF_{25\%-75\%}$) in a cross-sectional study of 280 non-smoking female histology technicians compared to the expected values derived from a random sample of the female population in Michigan. These analyses adjusted for the effects of age and height. In addition, non-smoking histology technicians had lower flows ($FEF_{25\%-75\%}$ and $FEF_{75\%-85\%}$) than the corresponding stratified random sample of Michigan women. Information on individual study participants regarding the specific levels of formaldehyde exposure was not provided. Formaldehyde levels as assessed through industrial hygiene measurements obtained from a nonrepresentative sample of laboratories ranged from 0.2 to 1.9 ppm with peaks up to 5 ppm. Solvents such as xylene, toluene, and chloroform were also present.

Malaka and Kodama (1990) also observed statistically significant differences in preshift spirometric tests (FEV_1, FEV_1/FVC, and $FEF_{25\%-75\%}$) among 93 formaldehyde-exposed Indonesian plywood workers than among 93 non-exposed Indonesian plywood workers. No post-shift changes were observed. Spirometric procedures recommended by the ATS were followed. Formaldehyde exposure among exposed ranged from 0.22 to 3.48 ppm, and for the non-exposed, from 0.003 to 0.07 ppm. Formaldehyde-exposed also had respirable dust exposures (i.e., to wood dust and resins).

These investigators also observed a positive exposure-response relationship between cumulative formaldehyde (ppm-year) and FEV_1, FEV_1/FVC, and $FEF_{25\%-75\%}$. The predicted decrements for these parameters per unit of cumulative exposure were small. As mentioned previously, the high prevalence of abnormal chest X-rays among study subjects indicated chronic upper respiratory tract infection, the decrements in lung function may reflect the overall poorer health status of these workers.

Horvath et al. (1998) observed post-shift decrements in both volume (FVC) and flow parameters ($FEF_{25\%-75\%}$, $FEF_{50\%}$, $FEF_{75\%}$) among particleboard manufacturing workers with no changes observed among unexposed workers employed in food-processing facilities. In addition, these investigators observed statistically significant positive exposure-response relationships between formaldehyde level and post-shift changes in FEV_1/FVC, $FEF_{25\%-75\%}$, $FEF_{50\%}$, and $FEF_{75\%}$. The correlation coefficients for these

relationships, however, were small. No differences were observed in preshift lung function parameters between formaldehyde-exposed and controls and duration of formaldehyde exposure was not a statistically significant predictor of baseline lung function among exposed. Analyses adjusted for the effects of age, sex, height, smoking, mobile home residency, and workshift.

The authors also observed higher prevalences of workers exhibiting the greatest post-shift declines in lung function (more than one standard deviation greater than the mean changes as calculated from controls) among formaldehyde-exposed than among the controls. Horvath et al. termed this group "reactors". Further analyses showed that formaldehyde concentration was a statistically significant predictor of reactor status for FEV_1/FVC, $FEF_{25\%-75\%}$, $FEF_{50\%}$, and $FEF_{75\%}$.

In this well-conducted study, investigators followed ATS testing procedures and methodology throughout this study. Formaldehyde levels (TWA) in the particleboard processes ranged from 0.17 to 2.93 ppm, with a mean of 0.69 ppm, and in food processing, 0.05 ppm (mean). The molded products and particleboard processes also contained nuisance particulates, approximately one-third were of respirable sizes. Based upon the study's results, Horvath et al. (1988) concluded that formaldehyde, perhaps combined with other airborne substances, might exert a small effect on the airways as measured by post-shift tests, but no evidence existed for permanent respiratory impairment.

Uba et al. (1989) also found among 96 medical students enrolled in a gross anatomy laboratory class a statistically significant larger post-shift decrease in FVC with formaldehyde exposure than the post-shift change observed in spirometry testing conducted at the beginning of the school term (a period of no formaldehyde exposure). No chronic decrements were noted, however, over a 7-month laboratory period. Spirometry was conducted on three occasions: before start of the laboratory session, 2 weeks into the laboratory session, and at the end of 7-month laboratory period. On each test day, spirometry was performed before the start of laboratory and at the end, 4 hours later. Testing procedures conformed with ATS recommendations. Formaldehyde concentration (TWA) during the 7-month laboratory period ranged from limit of detection (0.05 ppm) to 0.93 ppm, with peaks up to 5.0 ppm (mean=1.9 ppm) during dissection. The authors did not consider the crossshift decreases in FVC to be physiologically significant because of their small size, and that the observation may represent an acute irritant response which was not accentuated by repeated exposure.

Alexandersson and Hedenstierna (1989) observed statistically significant small changes in post-shift lung function (FEV_1/FVC, CV%) among 21 formaldehyde-exposed woodworkers compared to sex-,

age-, height,- and weight-adjusted reference values of normal individuals. These effects were mainly confined to a subgroup of 10 nonsmokers. In addition, nonsmokers also showed a statistically significant small decrease in $FEF_{25\%-75\%}$ when compared to reference values. The lung function decrements did not appear to persist during a period of no formaldehyde exposure. After a 4-week period of work interruption, lung function among nonsmokers was similar to that expected based upon reference values, with more moderate improvement was shown by smokers.

In addition to the cross-shift changes, these investigators demonstrated statistically significant greater annual age-adjusted decreases in $FEF_{25\%-75\%}$ for the whole group (smokers and nonsmokers) and in FEV_1/FVC for nonsmokers. Significant correlations between the mean level of formaldehyde exposure and decrements in preshift lung function parameters were also observed by the authors for several variables (FEV_1/FVC, FEV_1, and $FEF_{25\%-75\%}$ for nonsmokers; FEV_1/FVC and $FEF_{25\%-75\%}$ for smokers).

The 21 studied workers were part of a larger group of 34 gluers manufacturing kitchen cabinets and 18 local controls who had been previously studied by Alexandersson and Hedenstierna (1982). In their 1982 study, Alexandersson and Hedenstierna also observed lung function changes decrements over a workshift among 47 gluers, but not among 20 non-formaldehyde-exposed local controls, when compared to national reference values. Of the original 47 gluers, 13 had transferred to another process and no longer had formaldehyde exposure. Average daily formaldehyde levels as assessed by personal monitoring remained similar between the two studies, 0.34 ppm at the first examination and 0.40 ppm at the second examination. The high peak exposures noted in the first examination (>1.0 ppm) had sharply declined in the second examination (mean=0.55 ppm) due to process changes and automation.

Post-shift declines in lung function has also been assessed in controlled-exposure studies. Sauder et al. (1986) noted statistically significant decreases in two lung function parameters ($FEV_{1.0}$ and $FEF_{25\%-75\%}$) at 30 minutes into a 3 hour exposure scenario in a study of nine healthy nonsmokers exposed to 3 ppm formaldehyde. These decreases were not present at 60 or 180 minutes, and pulmonary function was normal at 24 hours. Similar findings were reported in a study by Green et al. (1987). Healthy subjects (n=22) performing heavy exercise during exposure for 1 hour to 3 ppm formaldehyde showed small but statistically significant decrements in $FEV_{1.0}$, FVC, and $FEV_{3.0}$. A similar pattern of decrease in $FEF_{25\%-75\%}$ was observed, but the change was not statistically significant. Three other controlled-exposure studies (Witek et al., 1986; Schachter et al., 1986; Schachter et al., 1987) did not find any measurable deficits in lung function. Exposures in these studies were lower (2 ppm) and for a shorter time (40 minutes).

In summary, formaldehyde, possibly along with other agents, appears to produce transient lung function changes, but does not appear to produce any chronic decrements. Five of the newer studies (Horvath et al., 1988; Uba et al., 1989; Alexandersson and Hedenstierna, 1989; Sauder et al., 1987; Green et al., 1987) demonstrated transient lung function changes after short-term controlled exposures or over a working shift. Although deficits in volume parameters (FEV or FVC) were noted in all four studies, no consistent deficit in a single parameter (e.g., FEV) was noted across the studies. In those studies reporting statistically significant deficits in lung function, exposure levels were higher in the controlled-exposure studies (3 ppm) than in the occupational studies where average values were approximately 1 ppm, with higher peak exposures. The populations studied by Horvath et al. (1988) and Alexandersson and Hedenstierna (1989) also had exposures to particles from wood dust. Exposure to particles in combination with formaldehyde may be important in initiating the post-shift changes in lung function observed at the lower formaldehyde levels, however, the role of combined exposures cannot be determined from these studies.

Three studies of occupationally-exposed populations (Malaka and Kodama, 1990; Kilburn et al., 1989; Alexandersson and Hedenstierna, 1988) reported declines in baseline tests. It is difficult, however, to draw firm conclusions from these observations since the better designed and conducted studies did not demonstrate any chronic deficits. Other factors may explain the observations. It is difficult to separate the effects of formaldehyde from any possible contribution from other exposures, such as solvents for the studies of Kilburn et al. (1989) and Alexandersson and Hedenstierna (1988). In addition, the study population in Malaka and Kodama (1990) had a high prevalence of respiratory infectious diseases which may have accounted for some of the lung function decrement. Due to the cross-sectional design of the study, it cannot be determined whether formaldehyde may have enhanced any lung function effects related to preexisting chronic disease.

6.2.3 Respiratory Effects in Asthmatics

Information on possible formaldehyde-related respiratory effects in asthmatics was derived from controlled-exposure studies and one occupational study. Asthmatics were studied to determine whether they may be a more sensitive subpopulation. Asthmatics are known to be more sensitive to other environmental agents such as SO_2 (Witek et al., 1986).

In the prospective study of medical students (Uba et al., 1989), 12 of the 103 subjects identified themselves as having a previous history of asthma, but no clinical verification was performed. Asthmatics reported a statistically significantly greater prevalence of burning and watering eyes with acute formaldehyde exposure when compared self responses in the absence of formaldehyde exposure. No differences existed between

asthmatics and nonasthmatics regarding the prevalence of eye irritation associated with acute exposure, nor were differences noted in the development of respiratory symptoms from chronic exposure over a 7-month period to formaldehyde. With respect to lung function testing, no clear evidence of a bronchoconstrictive response existed for asthmatics. As these investigators noted, the absence of clinically significant bronchoconstriction among the 12 asthmatic subjects does not provide strong evidence regarding the bronchoconstrictive effects of formaldehyde since the number of asthmatics in the study was small and the diagnoses were not verified.

Asthmatic subjects in controlled-exposure studies exposed to 2 ppm formaldehyde for 40 minutes at rest and with exercise (Witek et al., 1986, 1987), 3 ppm formaldehyde for 1 hour at rest or with exercise (Green et al., 1987), or 3 ppm formaldehyde for 3 hours at rest (Sauder et al., 1987) reported upper respiratory symptoms and eye irritation, however, the incidence of response was not greater for asthmatics than for healthy individuals.

Witek et al. (1986, 1987), Sauder et al. (1987), and Green et al. (1987) observed that asthmatic individuals exposed to formaldehyde at 3 ppm for 1 or 3 hours or 2 ppm for 40 minutes had common irritative symptoms as that reported previously (Section 6.2.1) for healthy individuals, i.e., unusual odor, sore throat, nasal discharge or stuffiness, and eye irritation. The incidence of nose/throat irritation and eye irritation as reported by Sauder et al. (1987) and by Green et al. (1987) was statistically significantly elevated. Likewise, lower respiratory symptoms were either mild or absent (Witek et al., 1987). In comparisons with healthy subjects, no difference existed in the incidence of response for eye and upper respiratory tract irritation between asthmatics and nonasthmatics (Witek et al., 1987; Green et al., 1987).

Acute formaldehyde exposure did not appear to induce acute airway obstruction or bronchoconstriction as measured by lung function testing during or following exposure (Witek et al., 1986, 1987; Sauder et al., 1987; Green et al., 1987). Witek et al. (1987) in a study of 15 asthmatics did not observe any deficits in either volume or flow parameters. Rather, formaldehyde exposure appeared to lower the dose of methacholine necessary to cause a 20% drop in FEV_1, as assessed in the methacholine inhalation challenge (MIC) test, in eight of the 12 subjects. Witek et al. (1987) concluded that formaldehyde exposure may alter nonspecific airway hyperresponsiveness in asthmatics, but does not cause any functional impairment.

Sauder et al. (1987) observed similar results from spirometry testing with exposure to 3 ppm formaldehyde for 3 hours at rest, i.e., no deficits in lung function were detected. Unlike Witek et al. (1987), however, Sauder et al. (1987) did not observed any statistically significant changes in lung function parameters for airway reactivity (MIC). In addition, Green et

al. (1987), in a study comparing the responses of 16 asthmatics and 22 nonasthmatics to 3 ppm formaldehyde for one hour during mild and heavy exercise, reported no differences in severity of effects for lung function parameters and upper respiratory symptoms. These investigators also did not find any changes in nonspecific airway reactivity.

In summary, results from these studies indicate that asthmatics, like healthy individuals, experienced eye and upper respiratory tract irritant symptoms. Formaldehyde exposure does not appear to exaggerate the incidence of response. Further, these studies do not provide evidence that asthmatics are more sensitive than nonasthmatics to any formaldehyde-related effects on the lower respiratory tract.

6.2.4 Immunologic Effects

The presence of IgG antibodies against formaldehyde-human serum albumin conjugates and human serum albumin (HSA) was described previously in EPA (1987). Several reports published since 1987 measured antibodies to formaldehyde-HSA in mobile home dwellers and in workers with potential exposure to formaldehyde. Cell-mediated immunity alterations were also observed in residents of mobile, UFFI, or recently redecorated homes.

Wilhelmsson and Holmstrom (1987) examined IgE levels in 30 workers in a factory producing formaldehyde. Roughly 40% of these workers had nasal symptoms such as rhinitis, nasal obstruction, and nasal discharge. Immunoglobulins other than IgE were not assayed in this study. These investigators observed that two of the 30 workers had elevated levels of IgE to formaldehyde-HSA. Both these workers had complaints of nasal symptoms clearly associated with the workplace. Wilhelmsson and Holmstrom (1987) believed these two cases indicated that long-term inhalation exposure to formaldehyde may cause sensitization and trigger a classical IgE-mediated allergic reaction.

In a series of reports, Thrasher et al. (1987, 1988, 1989, 1990) examined immunologic parameters among patients with symptoms which the investigators believed were related to multiple chemical sensitivity. In the latest report, Thrasher et al. (1990) assessed immune activation and autoantibodies in four groups of subjects which included previously studied subjects (Thrasher et al., 1987, 1988, 1989), in addition to other symptomatic individuals. A control group, composed of asymptomatic subjects, had acute exposure to formaldehyde some 12 months previously. The investigators considered the symptomatic subjects in the other four groups to represent chronic formaldehyde exposure. The five groups were: 28 asymptomatic chiropractic students (controls) exposed to formaldehyde for 13 hours/week for 28 weeks one year prior to the study; 19 current mobile symptomatic home residents, some of which were studied by Thrasher et al. (1987); 21 office workers with multiple health complaints, some were studied by Thrasher et al. (1989); 21

patients with multiple symptoms who had been removed from mobile homes for at least one year; and eight occupationally-exposed patients, 6 of whom were studied by Thrasher et al. (1988). Formaldehyde levels were directly measured for two groups, in current mobile home residents (0.05 to 0.5 ppm) and in the removed mobile home residents (0.14 to 0.81 ppm), and were inferred from other monitoring data for office workers (0.01 to 0.77 ppm) and for controls (0.43 ppm).

Thrasher et al. (1990) observed a statistically significantly greater proportion of positive anti-formaldehyde-HSA titers (either IgM, IgE, or IgG) in each of the symptomatic groups (75 to 100%) when compared to asymptomatic controls (39%). This finding was also reported in the earlier studies (Thrasher et al., 1987, 1988, 1989). The number of Ta1 cells was statistically significantly increased in all four symptomatic groups compared to the controls, whereas only two of the four symptomatic groups showed statistically significant increases in IL2+ receptor (acute antigenic stimulation) (mobile home residents and removed mobile home patients) and number of B cells (office workers and removed mobile home patients). Previous reports showed increased numbers and percent of Ta1 cells among occupationally-exposed subjects (Thrasher et al., 1988) and no difference in B cell number or blastogenesis in current mobile home dwellers (Thrasher et al., 1987). Of note, even though differences existed between symptomatic groups and controls, all values for IL2+ and B cells fell within the expected reference ranges. With respect to autoantibodies, antiparietal cell was the most frequently found autoantibody in all groups, with the lowest percentage in controls.

Pross et al. (1987) in a controlled-exposure study examined the effects of UFFI off-gassing products (free-formaldehyde) on hematologic and immunologic parameters in 23 asthmatics living in UFFI-homes and four asthmatics from conventional homes. Asthmatics were considered to be a possibly sensitive subpopulation. The subjects, who served as their self control, received exposure in an environmental chamber to room air, formaldehyde gas (1.0 ppm for 3 hours), and formaldehyde-free UFFI off products (0.5 particles/ml, 4 um, 3 hours) in sequence. Blood samples for testing were obtained before the exposure series, one day after completion, and 7 days later. The hematologic and immunologic parameters examined by these investigators included: complete blood count and differential, erythrocyte sedimentation rate, lymphocyte subpopulations (E-rosetting, T_3, T_4, T_8, FcR, and F_c receptor positive lymphocytes and large granular lymphocytes), lymphocyte response to phytohemagglutinin and formalin-treated red blood cells (Form-RBC), serum antibody against the Thomsen-Friedenreich RBC antigen and against Form-RBC, natural killer (NK), interferon-boosted NK, and antibody-dependent cell-mediated cytotoxicity.

These investigators found that long-term chronic exposure to UFFI off-gassing products had no effect on the immunologic

parameters studied, i.e., no differences existed between groups in the preexposure blood samples. Rather, short-term acute exposure resulted in minor immunologic changes among the UFFI-insulated home subjects. Analyses comparing before and after UFFI chamber exposure demonstrated small but statistically significant increases in the percentage of eosinophils, basophiles, and T_8 positive cells in the UFFI-insulated home group only. The authors commented that the mechanism of the eosinophilia in chronic allergic states has not been established, and the observed levels were not remarkable compared to levels reported in patients with chronic hypersensitivity reactions. They also noted that the small change (10% increase) in T_8 positive cells may not be biologically relevant.

These studies are inadequate for determining whether the immunologic changes are formaldehyde related. First, certain antibodies to formaldehyde appear in the sera of exposed individuals, but not always the same antibodies. Some exposed subjects had elevations in IgE levels whereas other exposed subjects had elevations in IgG. Second, it cannot be determined from these studies whether the changes in immunologic parameters resulted from the health effects, whether they represent only markers of exposure, or whether they are part of the mechanism in the production of chemical hypersensitivity. Third, none of the reports provide information concerning exposure levels at which immunologic responses might have been initiated. Fourth, three (Wilhelmsson and Holmstrom, 1987; Thrasher et al., 1988, 1989) did not contain comparison groups. The lack of comparison groups makes it difficult to determine the magnitude of any observed effect.

6.2.5 Central Nervous System Effects

Reports in the literature link formaldehyde with a number of behavioral and physiological effects such as thirst, dizziness and apathy, inability to concentrate, and sleep disturbances (EPA, 1987). Only one report published since 1987 assessed neurological effects and formaldehyde exposure. Kilburn et al. (1987) studied 305 histology technicians attending two annual conferences. Attendance at either of the two conferences made the technician eligible for inclusion in this study. Increased daily hours of exposure to formaldehyde, as assessed over the duration of the technician's employment in any histology laboratory, were correlated with poor performance on several indicators of neurological functioning which reflected dexterity, balance, coordination, and choice performance. While age was also related to poor performance on these indicators, performance was not related to years of cigarette smoking, educational level, or solvent exposure.

These investigators did not believe that solvent exposure was responsible for the observed deficits in psychological parameters attributable to formaldehyde, although solvent exposure and formaldehyde exposure were individually

statistically significantly associated with memory loss. The effect of solvent exposure on memory loss was not as great as the formaldehyde exposure contribution.

The Kilburn et al. (1987) study is inadequate for hazard identification. First, neurological functioning was not assessed in an unexposed group. Second, although the researchers do not believe that solvent exposure is as important as formaldehyde, it is still difficult to separate the effects related to solvent exposure from those of formaldehyde exposure. Third, this prevalence study may be biased by self-selection. Reasons for volunteering for this study are not known. Fourth, the formaldehyde levels at which decrements in neurological functioning are observed cannot be determined from this study. Formaldehyde was measured in only four histology laboratories. Levels found in these laboratories may not be characteristic of all formaldehyde exposures encountered by these histology technicians.

6.2.6 Limitations

The data on the irritant effects of formaldehyde came from controlled-exposure studies, prospective (cohort) studies, prevalence (cross-sectional) studies or case reports. Case reports such as Thrasher et al. (1987, 1988, 1989, 1990) are often used to generate hypotheses, but they have serious limitations for making causal inferences. Case-reports lack information with which to decide the magnitude of the reported symptom or any association with exposure. In this situation, the size and characteristics of the population at risk, from which the case was drawn, are not known, neither how it differs from a population without risk.

The majority of the studies were designed as cross-sectional studies, also known as surveys (Alexandersson and Hedenstierna, 1988; Holness and Nethercott, 1989; Horvath et al., 1988; Imbus and Tochilin, 1988; Kilburn et al., 1988; Malaka and Kodama, 1990; Broder et al., 1988a, 1988b, 1988c; Liu et al., in press, personal communication; Ritchie and Lehnen, 1987; Boysen et al., 1990; Edling et al., 1988; Holmstrom et al., 1989; Wilhelmsson and Holmstrom, 1987; Kilburn et al., 1987). In these studies, study individuals are not followed over a period of time and health effects are assessed at the same time as exposure. It is difficult to determine whether exposure produced the observed effect. In addition, the study of Wilhelmsson and Holmstrom (1987) did not incorporate an unexposed comparison or control group. Without a control group, the attributable magnitude of a reported symptom cannot be determined.

Prospective studies (Uba et al., 1989; Nunn et al., 1990; Alexandersson and Hedenstierna, 1990) and controlled-exposure studies (Green et al., 1987; Sauder et al., 1986, 1987; Schachter et al., 1986, 1987; Witek et al., 1986, 1987; Pross et al., 1987) follow a group of individuals, thus, disease development can be

inferred subsequent to exposure. These later studies carry the greatest weight in an assessment of causality.

There is uncertainty regarding whether the statistically significant elevations in an observed effect is real or simply reflecting statistical variability related to multiple testing (i.e., the observation is a false positive). The majority of the reviewed studies assessed several different endpoints such as eye and upper tract respiratory effects, lower airway symptoms, and lung function. Due to the many statistical comparisons, a larger probability exists that several of these relationships will be falsely positive, but their identify can not be determined.

Confounding and bias introduce other uncertainties in these studies. Wood dust and particulates were found simultaneously with formaldehyde in several studies ((Alexandersson and Hedenstierna, 1988; Alexandersson and Hedenstierna, 1990; Horvath et al., 1988; Imbus and Tochilin, 1988; Malaka and Kodama, 1990; Boysen et al., 1990; Edling et al., 1988; Holmstrom et al., 1989; Wilhelmsson and Holmstrom, 1987). Unless noted, results were not adjusted for these exposures and an observed effect may not be due to formaldehyde alone.

With respect to non-occupational factors such as sex, age, and smoking, only the studies of Liu et al. (in press) and Broder et al. (1988a, 1988b, 1988c) adjusted for potentially confounding effects in their statistical analyses. In addition, these factors are not considered problems in studies where subjects served as served as their own control (Uba et al., 1989; Nunn et al., 1990; Alexandersson and Hedenstierna, 1990; Green et al., 1987; Sauder et al., 1986, 1987; Schachter et al., 1986, 1987; Witek et al., 1986, 1987; Pross et al., 1987).

Bias may have be introduced in the controlled-exposure studies since the steady subjects studied were presumably self-selected and may not be representative of the general population. Likewise, the cross-sectional studies of occupational populations (i.e, Nunn et al., 1990), by nature of their design, did not include individuals who left employees. Survivor bias can not be completely ruled out in these studies.

6.2.7 Summary and Conclusions

The irritative effects of formaldehyde on the eyes and upper respiratory tract are well-documented (EPA, 1987); a large number of observations of people from controlled-exposure and observational settings support a conclusion that the generally observed range over which most people experience irritation of the eyes and upper airways is 0.1-3.0 ppm of formaldehyde. The studies published since 1987 added further evidence of acute sensory irritation of the eye and upper respiratory airways at levels identified previously. In addition, exposure-response relationships for eye, throat, or nose irritation were present in three studies (Ritchie and Lehnen, 1987; Horvath et al., 1988;

Liu et al., in press), and provided support that formaldehyde induces these effects. These findings corroborated the exposure-response gradients observed in several studies reviewed earlier by EPA (1987). See Section 7 for further analysis of the dose-response relationships.

Inhalation exposure to formaldehyde may also cause increased severity of histological changes (metaplasia and dysplasia) in the nasal epithelial mucosa of occupationally-exposed workers compared to non-formaldehyde exposed occupational controls (Edling et al., 1988; Holmstrom et al., 1989; Boysen et al., 1990). Formaldehyde levels in these exposed populations ranged from 0.04-2.0 ppm. In addition to formaldehyde, these occupational populations had exposure to particles from formaldehyde resins or from wood dust. No exposure-response relationship was noted between histologic score and duration of employment. Duration of exposure is often an imprecise surrogate with which to examine exposure trends. Caution must be employed when making inferences from these observations due to biases introduced by the small number of exposed persons, the cross-sectional design of the studies (essentially survivor populations were studied and the most susceptible individuals were not available), and the inability to identify a possible contribution from the presence of particles.

Four of the reviewed studies (Broder et al., 1988b, 1988c; Horvath et al., 1988; Uba et al. 1989; Malaka and Kodama, 1990) reported lower airway irritation symptoms at mean exposure levels less than 1 ppm. These effects were reported among particleboard workers, medical students in anatomy laboratory, and UFFI-home populations. The occupationally-exposed groups also experienced peak exposures to concentrations greater than 1 ppm, but less than 5 ppm. Other cross-sectional studies of chronic exposure and controlled-exposure studies of acute exposures to 2 ppm formaldehyde for 40 minutes or 3 ppm formaldehyde for 3 hours did not produce symptoms of lower airway effects. Results from these studies may be limited by the small number of subjects studied and by selection bias. In addition, exposures in some of the cross-sectional studies were close to background, 0.05 ppm. Overall, the results of studies published to date suggest that onset of symptoms of lower airway irritation due to formaldehyde exposure may occur at concentrations ranging somewhere in the range of 1 ppm and 5 ppm. These exposure levels are lower than had been reported previously (EPA, 1987).

Small transient (acute) effects on volume and flow lung function parameters were suggested in four studies (Horvath et al., 1988; Uba et al., 1989; Sauder et al, 1987; Green et al., 1987). These more recent observations appeared consistent with those observed in previously reviewed studies (EPA, 1987). Two studies of chronic exposure, a prospective study of medical students (Uba et al., 1989) and a prevalence study of particleboard workers (Horvath et al., 1988), observed statistically significant decreases in FVC (both studies) and FEF

(Horvath et al., 1988) between pre-exposure and post-exposure tests. Two acute exposure studies (the controlled-exposure studies Sauder et al. (1987) and Green et al. (1987)) observed statistically significant deficits in FEV (both studies), FEF (Sauder et al., 1986), and FVC (Green et al., 1987) between pre-exposure and post-exposure tests. Mean formaldehyde levels in the Uba et al. (1989) and Horvath et al. (1988) were around 1 ppm, with peaks up to 5 ppm (Uba et al., 1989). Subjects in the controlled-exposure studies received 3 ppm formaldehyde for 3 hours.

EPA weighed the above four studies more heavily due to their better designs (except for Horvath et al. (1988), all were controlled-exposure or prospective studies) and their careful conduct (i.e., use of ATS-recommended procedures and control for possibly confounding variables). The transient effects on lung function have been slight, possibly explaining why other cross-sectional studies reporting upper respiratory effects did not observe these effects. In addition, these other studies contained limitations related to fewer numbers of subjects, exposures close to background, or selection bias, which may partly explain the differences in observations. There was no evidence for chronic decrements in pulmonary function as reflected by no observed changes in pre-shift lung function measurements in occupationally-exposed populations. The reviewed studies, however, are limited in their ability to adequately assess any possible relationship.

Data examining possible associations between immunological changes and poorer neurological functioning and inhalational formaldehyde exposure were considered inadequate for hazard identification.

7.0 ESTIMATES OF UPPER RESPIRATORY AND EYE IRRITATION RISKS

In 1987, the EPA reviewed several studies for determining the dose-response characteristics for upper respiratory and eye irritation in populations exposed to formaldehyde. The upper respiratory system is a likely target of formaldehyde's effects since inhalation is a primary route of exposure. At that time, response over a range of formaldehyde levels was estimated for four studies (Hanrahan et al., 1985; Andersen and Molhave; 1984; Bender et al, 1983; Kulle, 1985) using logistic regression analyses. None of these studies proved adequate data to quantify population risks for the irritant effects of formaldehyde. These studies were limited due to possible selection bias, their design (some measured only prevalence) and to small numbers of participants. These studies, however, provided a qualitative estimate of population response over a wide exposure range and quantitative estimates of response for very select populations.

7.1 Incorporation of New Data

Three studies (Ritchie and Lehnen, 1987; Horvath et al., 1988; Liu et al, in press) released since 1987 demonstrate dose-response relationships between formaldehyde exposure and the prevalence of irritation of the eyes and/or nose and throat. Prevalence is the number of cases existing with the outcome at a single point in time.

Ritchie and Lehnen (1987) examined health complaints in a study of mobile and convention home residents. Study participants were identified from complaints the Minnesota Department of Health received from physicians. These investigators observed positive dose-response relationships between formaldehyde concentration and eye irritation, nose/throat irritation, headaches, and skin rash. In these analyses, formaldehyde concentration was categorized as $\leq$0.1 ppm, 0.1-0.3 ppm, and $\geq$0.3 ppm. Age, sex, and smoking (either active smoking or exposure through passive smoke) were identified as statistically significant confounders, and were controlled in the analyses.

The Horvath et al. (1988) study of particleboard workers observed a concentration (ppm)-dependent relationship between formaldehyde and the prevalence of burning of the nose. These workers also had exposures to nuisance particles, which included respirable particles; nuisance particles were not adjusted for in the analyses. Eight-hour time weighted averages (TWA) for formaldehyde ranged from 0.2-2.93 ppm (mean 0.69 ppm). The mean respirable dust level was 0.11 mg/m^3 (range of 0.025-1.06 mg/m^3).

Liu et al. (in press) observed a positive exposure-response relationship between tearing and/or burning eyes and cumulative formaldehyde exposure (ppm-hour) among occupants from mobile homes in California. Mobile homes in this study were randomly selected from a list of all mobile homes registered by the California Department of Housing and Community Development. Approximately 60% of the home were manufactured after 1980. Average formaldehyde concentration in this study was around 0.09 ppm (range: level of detection, 0.01 ppm,-0.46 ppm). Additional data provided to EPA by Liu (personal communication with C. Siegel Scott, 14 February 1991) suggested a concentration-dependent effect (Figure 7-3). Statistical tests for linear trend were not statistically significant ($p\geq0.05$), however.

These authors accounted for two possibly confounding exposures in their logistic regression analysis examining cumulative exposure; sex and previous chronic illness were statistically significantly associated with burning eyes. Since the prevalence of response was higher in summer than in winter, season, also, appears to be a modifying factor. The analyses performed by EPA examining the relationship with concentration did not adjust for these confounders and are limited by this fact.

Figures 7-1 through 7-7 present the dose-response relationships observed in Ritchie and Lehnen (1987) (Figures 7-1 and 7-2), Horvath et al. (1988) (Figure 7-3), and Liu et al. (in press; personal communication) (Figures 7-4 and 7-7). The dose-response relationships estimated for the data of Horvath et al. (1988) was obtained using logistic regression analyses, and presented by the authors. Estimated prevalences of response simultaneously taking into account possibly confounding factors such as sex, gender, and smoking were not presented by Ritchie and Lehnen or by Liu et al. (in press; personnel communication), even though these investigators discussed results of regression analyses controlling for these possibly confounding factors. The figures associated with these two studies (Figures 7-1, 7-2, 7-4, and 7-5), therefore, show the unadjusted data for the study participants.

One can relate responses in the newly reviewed studies to those predicted from the data identified in Section 8 of EPA (1987). It must be acknowledged that this comparison contains limitations since each study characterized endpoints differently, although all endpoints can be broadly defined as irritation of mucous linings. As identified in EPA (1987), Hanrahan et al. (1984) presented data for eye discomfort only, both Andersen and Molhave (1984) and Kulle (1985) assessed the combined symptoms of eye, nose, and throat irritation, and Bender et al. (1983) measured eye irritation.

Response for irritation of the eye and of the nose/throat as reported by Ritchie and Lehnen (1987) (Figures 7-1 and 7-2) show a marked increase as exposure increases from $\leq$0.1 ppm to $\geq$0.3 ppm. The prevalence of eye irritation and nose/throat irritation associated with exposure between 0.1-0.3 ppm appears to be similar to the predicted prevalence at around 0.2 ppm reported by Hanrahan et al. (1984) for burning eyes and by Andersen and Molhave (1984) for the combined effects of eye, nose, and throat irritation. In contrast, the slope of the curve of Horvath et al. (Figure 7-3) for burning nose is not as steep, i.e., response associated with a particular exposure level is lower, as curves identified in Section 8 of EPA (1987) for Hanrahan et al. (1984), Andersen and Molhave (1984), or Bender et al. (1983). Rather, response in Horvath et al. is more similar to that of Kulle (1985) for the combination of eye, nose, and throat irritation.

Liu et al. (in press) found that cumulative exposure (ppm-hour) (Figure 7-4) best described the relationship between burning and/or tearing eyes. Due to differences in exposure units, however, a strict comparison can not be made with the other studies. It is best to base a comparison on common exposure units, concentration (ppm), as shown in Figure 7-5. The data of Liu et al. (personal communication) can only be compared to the data of Ritchie and Lehnen (1987) since exposure levels in these two studies roughly overlap. All the other studies examined irritant effects associated with higher levels of formaldehyde exposure. For exposure <0.10 ppm, Liu et al.

Figure 7-1. Health Effects by HCHO in Complaint Mobile Homes

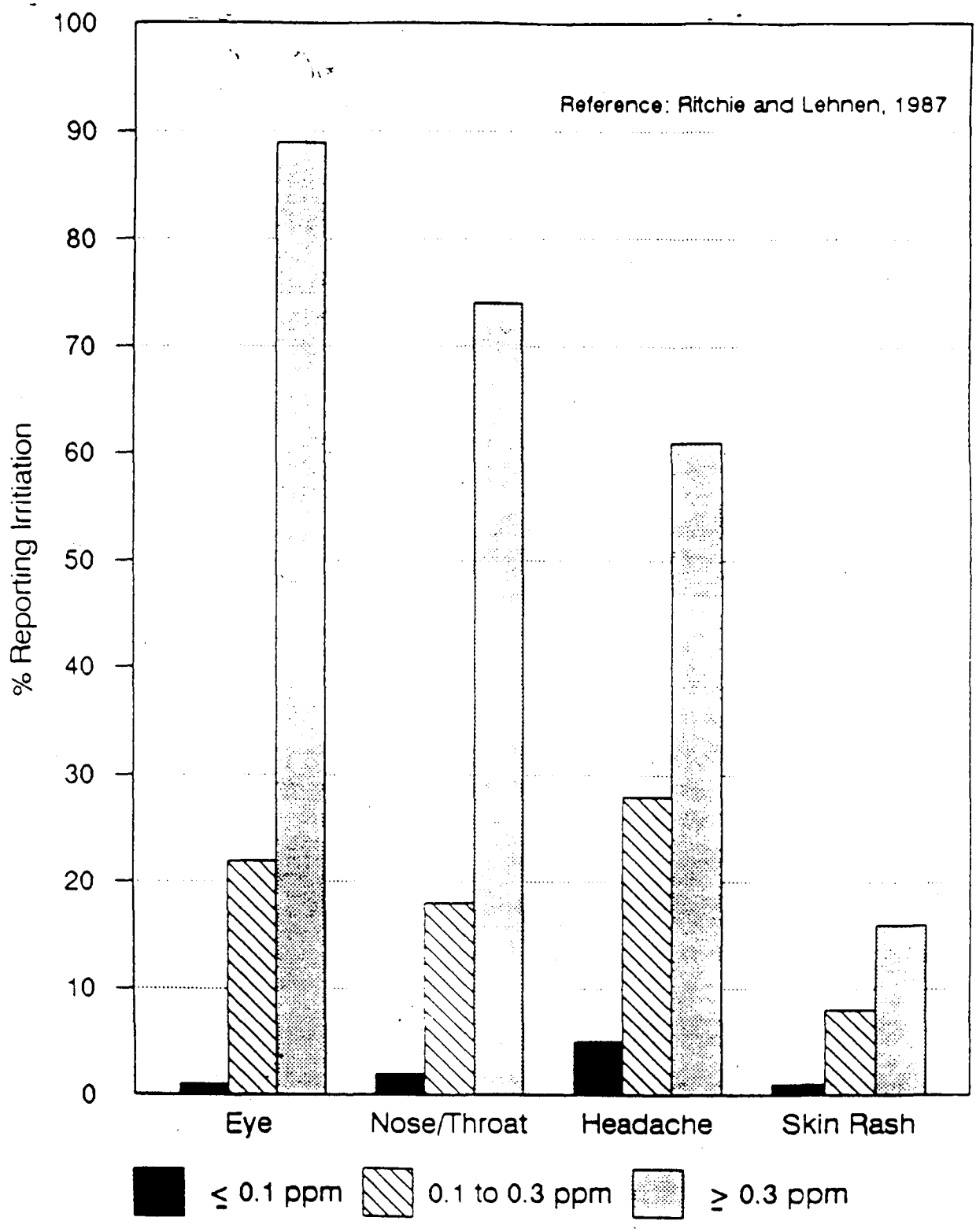


Figure 7-2. Health Effects by HCHO in Complaint Conventional Homes

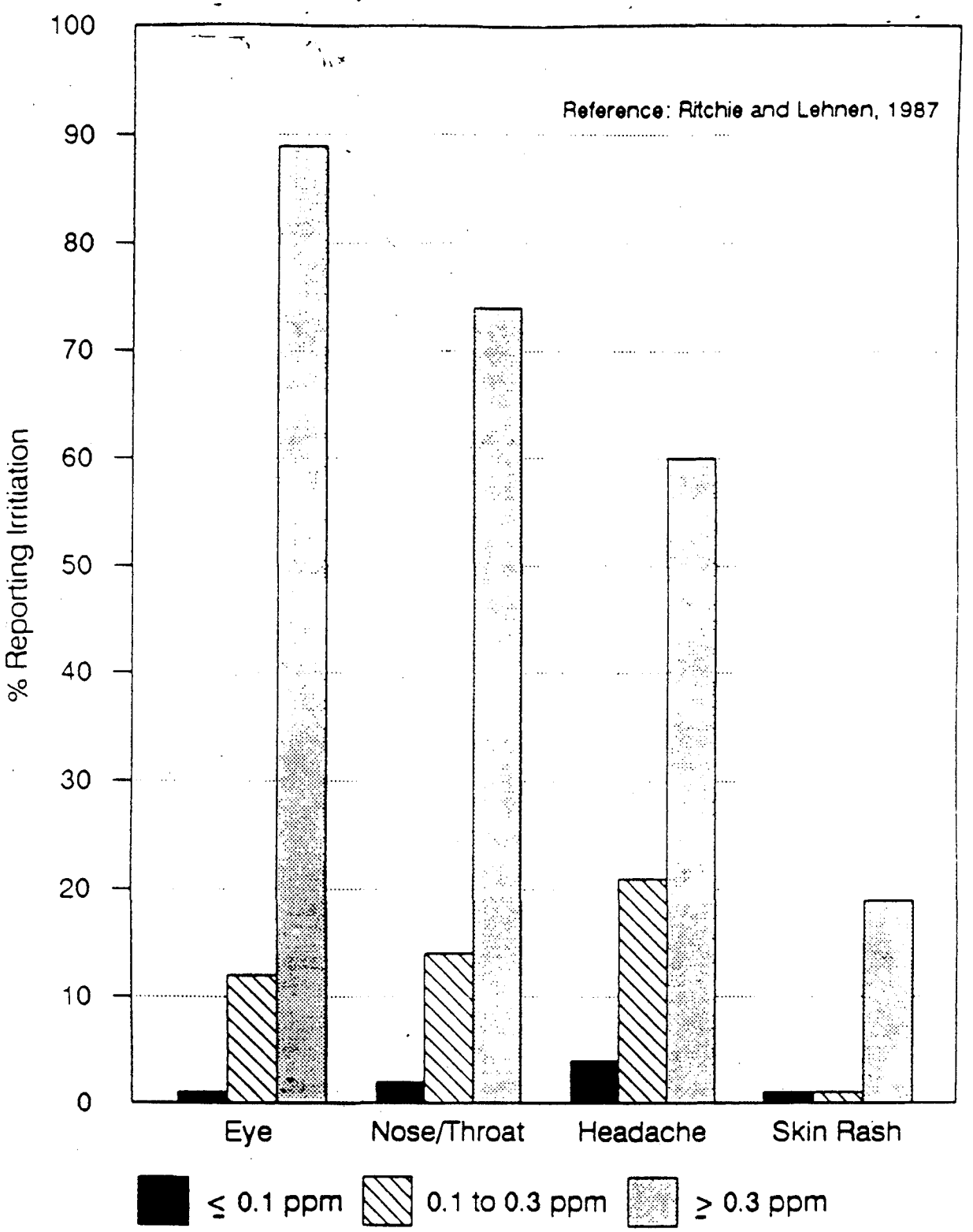


Figure 7-3. Predicted prevalence of burning nose over range of formaldehyde concentrations in ppm

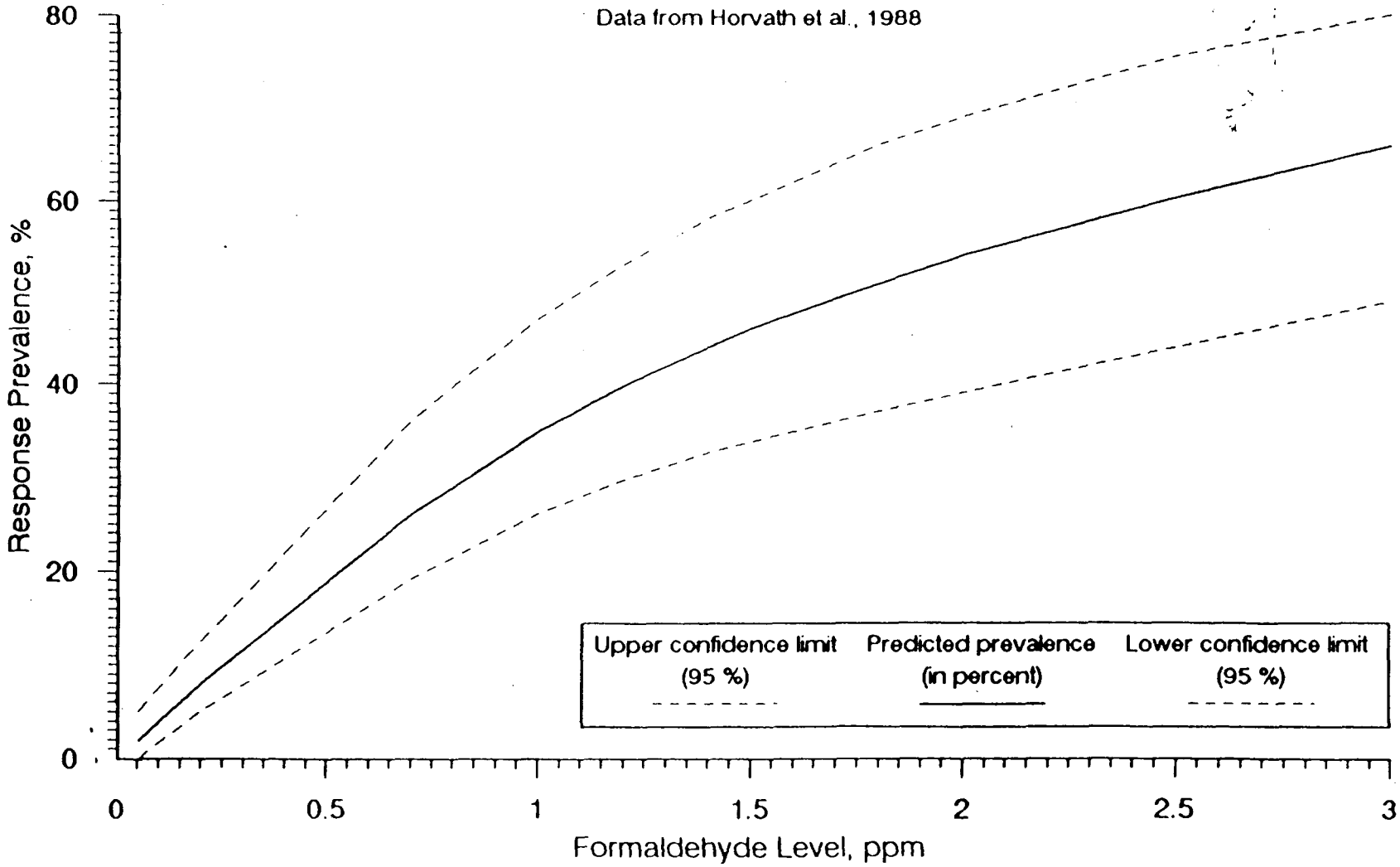


Figure 7-4. Observed burning/tearing eye irritation response over a range of HCHO levels

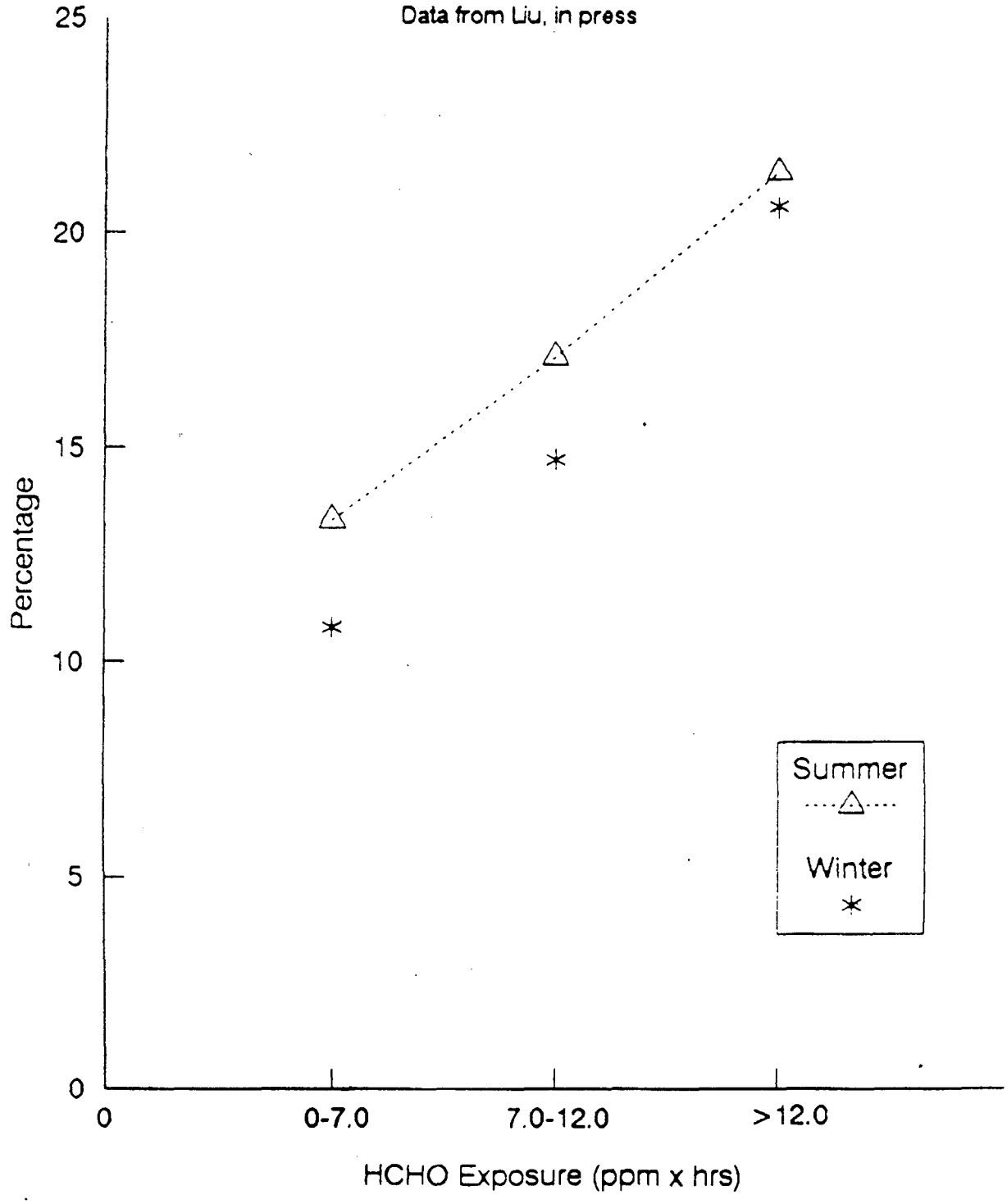
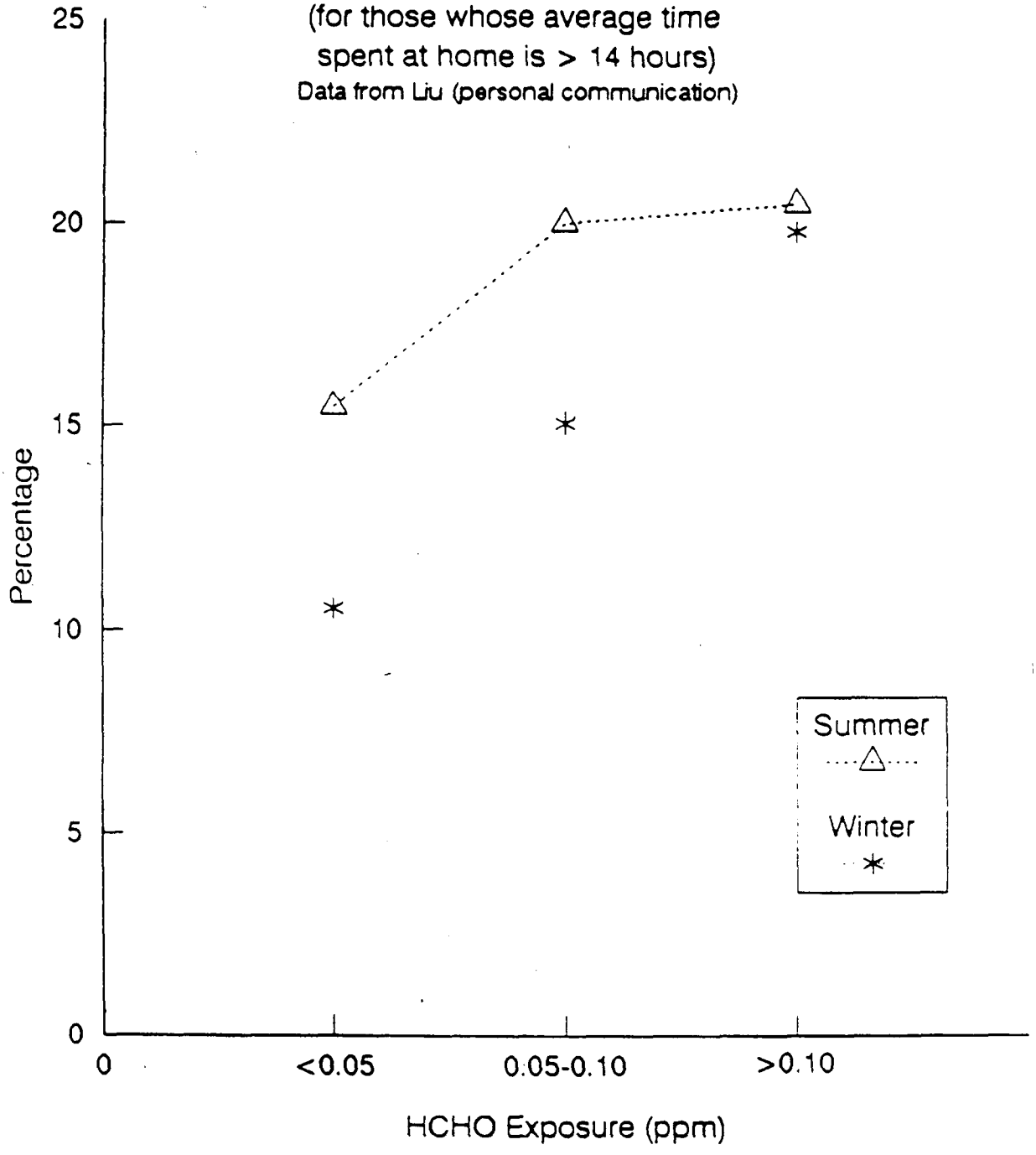


Figure 7-5. Observed burning/tearing eye irritation response over a range of HCHO levels



(Figure 7-5) reported a higher prevalence of burning/tearing eye irritation than that reported by Ritchie and Lehnen (1987) for eye irritation.

7.2 Discussion of the Overall Evidence

A range of predicted responses for a given formaldehyde concentration is obtained when the seven studies (Bender et al., 1983; Hanrahan et al., 1984; Horvath et al., 1988; Kulle, 1985; Liu et al., in press; Molhave and Andersen, 1984; and Ritchie and Lehnen, 1987) are examined comparatively. This may be due to several limitations which prevent the use of these studies to infer the magnitude of general population risks. Three of these studies (Kulle, 1984; Bender et al., 1983; Andersen and Molhave, 1984) were of controlled-exposures. These studies did not utilize a randomization scheme. Study participants were self-selected and may not be representative of the general population.

Confounding may influence the magnitude of response. Only the studies of Ritchie and Lehnen (1987) and Liu et al. (in press) were able to account for possibly two confounding variables, smoking and gender. Unfortunately, predictions of response in analyses controlling for these confounding variable could not be obtained from either study. In addition, Liu et al. further suggest that season and average time spent at home may be important covariates. None of the other studies accounted for these factors.

The population studied by Horvath et al. (1988) had nuisance dust exposure, and it is not known how this may confound response. The response predicted by the Horvath et al. data, however, is not as steep as that predicted from the data of Hanrahan et al. (1984) and Andersen and Molhave (1984). Nuisance dust particles, therefore, does not appear to greatly influence the prevalence of irritation response.

Most important, a wide variation in response across all studies is not uncommon since these studies did not incorporate similar categorizations of symptoms. Each study identifies eye or upper respiratory tract involvement, however, each study defines and groups these effects differently. For example, response to tearing and/or burning of the eyes may be quite different than for only burning eyes. Also, these studies report subjective and self-reported symptoms. These symptoms have not been medically verified, and thus, results may be biased by over- or under-reporting.

In conclusion, caution must be taken in inferring the results in EPA (1987) and in Figures 7-1 through 7-5 to the general population. Limitations in these studies at the present prevent the inference of eye and upper respiratory risks. None of the studies reviewed in this document and in EPA (1987) provide adequate data to precisely quantify general population risks for eye and upper respiratory effects associated with a

specific formaldehyde concentration. Nevertheless, these studies document eye and upper respiratory tract effects at levels previously identified, 0.1 ppm to 3 ppm. Even though the prevalence of exposure can not be precisely estimated for a given formaldehyde concentration, these studies support the conclusion that the number of individuals responding in a population will increase with increasing formaldehyde concentration.

8.0 RISK CHARACTERIZATION

This section presents the major conclusions of EPA's risk assessment of formaldehyde. Some subsections have been taken from the 1987 assessment document and modified as indicated by subsequent developments. The current document reviews the underlying scientific foundation for the findings, describes the strengths and weaknesses of the supporting data, and discusses the uncertainties attending EPA's interpretation of the data and projection of risk. The risk characterization discusses the qualitative aspects of the risk assessment and the quantitative risk estimations at exposure levels relevant for the population of concern.

8.1. Noncancer Effects

The major noncancer effects posed by exposure to formaldehyde are due to the irritating nature of the chemical. These effects include sensory irritation which is readily perceived by the exposed individual; inhibitory effects on the mucociliary system and cellular changes of the nasal cavity, which in turn may result in increased susceptibility to respiratory infections. Newer studies have failed to provide evidence linking formaldehyde exposure to either immunological, neurologic, or pulmonary sensitization effects. This document, however, has not included an exhaustive examination of the total spectrum of health points of possible concern.

8.1.1. Sensory Irritation

The well-documented health effects from acute and repeated inhalation exposures to formaldehyde are concentration dependent. These effects include irritation of the eyes, nose, throat and lower airways, the intensity of which is dependent upon the extent and duration of exposure, and may result in extreme discomfort and inability to function normally at work or in routine daily activities.

Dose-response relationships for irritation effects obtained from studies of selected populations cannot be generalized to give the probability of response in larger populations. However, qualitative evidence supports the expectation that the number of persons who respond in a population will increase with increasing concentrations of formaldehyde. A large number of observations of people in various clinical and nonclinical settings support a conclusion that the generally observed range over which most

people experience irritation of the eyes and upper airway is 0.1-3.0 ppm of formaldehyde.

Symptoms of eye, nose and throat irritation above background incidence frequently occur at about 0.1 ppm and become widespread at concentrations near 3.0 ppm. Exposures greater than 3.0 ppm are generally intolerable for more than short periods. Lower airway irritation has been noted at formaldehyde concentrations that range from around 1 ppm to below 5 ppm. The latter irritation effects were transient and there were no indications of chronic lower airway impairment. Tolerance to low levels of formaldehyde can occur in individuals after 1-2 hours of exposure, but symptoms can return if exposure is interrupted and then resumed. These acute irritation effects are usually reversible upon removal of the source of exposure.

8.1.2. Mucociliary Clearance Effects

A major function of the nose is to prepare the inhaled air for the lungs. This includes warming, moistening, and filtering the inspired air. Dust and many bacteria found in the inspired air are precipitated in the mucus that bathes the mucous membrane and are moved outward by the action of the cilia of the nasal passage. Research indicates that formaldehyde has a number of effects on the workings of this mucociliary apparatus.

Very limited data in humans have shown that nasal mucociliary function is inhibited by formaldehyde exposure at levels as low as 0.3 ppm (Anderson and Molhave, 1983). The stopping of mucous flow (mucostasis) followed by cessation of ciliary activity (ciliastasis) was clearly shown in male rats following repeated exposure to 15 ppm formaldehyde; only slight effects were noted in animals exposed at lower concentrations; and at 0.5 ppm no effects were observed (Morgan et al., 1983, 1986).

Overall, the animal data show that at low formaldehyde concentrations (somewhere less than 2 ppm), the mucus layer of the nasal cavity can trap and remove a large proportion of inhaled formaldehyde. At higher formaldehyde concentrations (especially above 6 ppm), there is a pronounced inhibition of mucociliary activity. The results obtained indicate an apparent nonlinear dose-dependent on the rat mucociliary apparatus which suggests possible saturation. Once the mucus layer is saturated, the mucociliary clearance system may be seriously compromised and allow a greater amount of formaldehyde to reach the nasal epithelium.

A reduction in the efficient operation of the mucociliary system can increase the risk of persons exposed to formaldehyde to develop respiratory disease since it is an important defense mechanism in the removal of foreign particles and bacteria.

8.1.3. Cellular Changes

The primary point of contact of formaldehyde upon exposure by inhalation is the internal lining of the nose. It appears that inhalation of formaldehyde above some threshold level, which varies from person to person, causes a number of cellular effects which can impair the normal functioning of the nose and are dependent on the concentration and duration of exposure. Cellular changes considered in this analysis include cytotoxicity, cell proliferation, and histological changes.

Evidence of cellular damage in humans is limited to results of biopsies of the nasal turbinates. Several studies (Edling et al., 1988; Edling et al., 1985; Holmstrom et al., 1989; Boysen, et al., 1990) in which humans were occupationally exposed from nine to twenty years to formaldehyde in the range of 0.04-2 ppm, time-weighted average (TWA) concentration with peak exposures up to 4 ppm, showed loss of cilia, development of squamous metaplasia, and a few workers exposed for the longest durations showed cellular dysplasia. These findings are consistent with the known irritating properties of formaldehyde. Overall, the severity of the squamous metaplasia induced in these workers was only sligthly greater than that observed in control groups. The incidence of cellular dysplasia-- a lesion that may be considered precursor to tumor development-- was low and it showed no consistent correlation with either concentration or duration of exposure. Finally, caution must be used when generalizing observations from these studies because of the small number of exposed persons examined and the possibility of confounding exposure to wood dust and resin particles.

The cumulative evidence from animal studies indicates that the effect of formaldehyde on cell proliferation in nasal passages is time and concentration dependent. Short-term formaldehyde exposure (6 ppm) of rhesus monkeys causes significant increases in the rates of cell proliferation in the damaged nasal regions (squamous metaplasia); cell proliferation rates remain elevated at the end of 6 weeks of exposure which is a departure from the results obtained in the rat. There was no apparent effect of formaldehyde in the maxillary sinuses of the monkey. A lower degree of cell proliferation and tissue damage also occur in the trachea and bronchi in monkeys exposed to formaldehyde at this same concentration (6 ppm).

In chronic studies, cellular effects such as rhinitis (inflammation of the nasal mucosa), epithelial dysplasia (displacement of one cell type with another one), and squamous metaplasia (replacement of normal mucosal cells with squamous cells), developed in the nasal cavities of rats and monkeys after exposures for 12 months and 26 weeks, respectively, to 2-3 ppm of formaldehyde. After 24 months of exposure, the incidence of squamous metaplasia in rats increased to nearly 100 percent. In contrast to the rat, where formaldehyde-induced lesions were confined to the anterior nasal passages, these lesions were more

widespread in the nasal passages of the monkey. In both rats and monkeys, a NOEL (no-observed-effect level) of 1.0 ppm for squamous metaplasia was determined, with a LOEL (lowest observed effect level) of 2.0 ppm in rats and 3.0 ppm in monkeys.

8.2. Carcinogenic Effects

EPA has classified formaldehyde as a "Probable Human Carcinogen" (Group B1) under its Guidelines for Carcinogen Risk Assessment. This classification is based on:

- limited evidence of carcinogenicity in humans;
- sufficient evidence of carcinogenicity in animals; and
- additional supportive evidence (i.e., mutagenicity and structure activity and mechanistic considerations)

8.2.1. Studies of Humans

In 1987 the EPA examined 28 epidemiologic studies relevant to formaldehyde. Based upon a review of these studies, EPA concluded that "limited" evidence existed that formaldehyde may be a carcinogen in humans. Eleven additional studies were reviewed in the present assessment. These newer studies support the conclusions drawn in 1987 regarding "limited" evidence. The evidence for potential human carcinogenicity associated with formaldehyde exposure rests heavily on associations with cancers of the nasal cavity and sinus and of the nasopharynx, and to a small degree on observations of elevated risks between lung cancer and combined formaldehyde and particulate exposures.

Epidemiologists use several criteria for judging, in a collection of studies, whether exposure represents a carcinogenic hazard to humans. These criteria include: 1) antecedent exposure (disease occurs after exposure), 2) strength of the association 3) coherence of the observations across different studies and exposure assessments, 4) specificity of the association (i.e., whether elevated relative risks are confined to specific disease subcategories or exposure subgroups), and 5) consistency with existing knowledge of the biological behavior of the agent. Antecedent exposure is satisfied because all the epidemiologic studies reviewed in the 1987 and in this document examined health consequences of previous exposure.

With regard to the strength of the association, the associations observed in the epidemiologic studies between formaldehyde exposure and either cancers of the sinonasal cavity, nasopharynx, or lung have not been large. The question of whether bias may influence the magnitude of risk becomes important. Bias is not thought to artificially inflate the observed relative risks. Several studies (Vaughan et al., 1986, Roush et al., 1987) took into consideration such factors as age, smoking, sex, or socioeconomic status. Statistically significant elevations in risks between nasopharyngeal cancer and

formaldehyde exposure were noted after taking into account some of these possibly confounding factors. Three other studies that adjusted for simultaneous wood dust exposure (Hayes et al., 1986; Olsen and Asnaes, 1986; Olsen et al., 1984) observed apparently elevated, but not statistically significant, risks between sinonasal cavity cancer and formaldehyde exposure. Smoking is not considered a factor in one other study (Blair et al., 1986, 1987) since examination of sites most likely associated with smoking did not show a consistent pattern of elevations.

In contrast, the excesses in leukemia and brain cancer observed among populations that are occupationally exposed to formalin are most likely related to exposure other than formaldehyde. Elevations in these site-specific risks have not been observed in studies of industrially-exposed populations. Viruses and solvents were also potential exposures for the formalin-exposed, and may be important with respect to the development of leukemia and brain cancer.

The observation of similar results across different studies or across different exposure assessments can support a causal association between exposure and disease. Increased nasopharyngeal cancer risks have been observed in four studies (Vaughan et al., 1986a, 1986b; Blair et al., 1986, 1987; Roush et al., 1987; and Malker et al., 1990) that were carried out under differing study designs and exposure scenarios. Exposure-response gradients were observed in two of these studies (Vaughan et al., 1986a, 1986b); Blair et al., 1987), and suggested in another (Roush et al., 1987). In addition, one reanalysis (Collins et al., 1988), which adopted a different exposure assessment appears to support formaldehyde and particulates together as risk factors for nasopharyngeal cancer.

Two studies (Hayes et al., 1986; Olsen et al., 1984) reviewed in the 1987 document reported an association between sinonasal cavity cancer and formaldehyde and wood dust exposure. Similar trends in risk were noted in analyses that controlled for wood dust exposure, however, these risks were not statistically significant. In addition, risk appeared to be specific for the histologic type, squamous cell carcinoma (Hayes et al., 1986; Olsen and Asnaes, 1986). Two studies (Roush et al., 1987; Stern et al., 1987) did not observed any associations between cancer of the nasal cavity and sinuses and formaldehyde exposure.

Not all of the reviewed studies reported elevated cancer risks due to sinonasal cavity or nasopharyngeal cancer. Limitations in these remaining studies tended to prevent the results from revealing differences from the referent population (a conservative bias).

Elevations in lung cancer relative risks have been observed in several studies (Blair et al., 1986; Sterling and Weinkam, 1989; Acheson et al., 1984; Stayner et al., 1988; Bertazzi et al, 1986; Partanen et al, 1984; and Gerin et al, 1989). Only the

elevations in Blair et al., Acheson et al, and Bertazzi et al. were statistically significant; exposure in these studies were to formaldehyde and particulates. Because the elevations in lung cancer risk have not been consistently observed across the studies, these observations do not carry great weight.

A finding of the highest risk among those with the longest latency and the highest occupation exposure suggests that an occupational agent, here most likely formaldehyde, is reasonable for the observed increased cancer risk. First, elevated risks for nasopharyngeal, and nasal cavity and sinus cancers are observed among various subgroups: those who are older, those with the greatest latency since first exposure, those with the highest duration or level of exposure, and those with occupational particulate exposure. Second, relative risks for cancer of the nasal cavity and sinuses associated with formaldehyde exposure are specific for one histologic type. Two studies (Hayes et al., 1986; Olsen et al., 1984) observed the largest risks for squamous cell carcinoma with formaldehyde exposure. Wood dust does not enter into this observation since wood dust exposure was associated only with adenocarcinoma and not with squamous cell carcinoma.

The associations between formaldehyde and cancers of the nasopharynx and sinonasal cavity are persuasive in light of a biological interpretation of how formaldehyde may be acting. Experimental data of Kerns et al. (1983) indicate that formaldehyde reacts with biological material at the point of contact. Human exposure to formaldehyde is predominately by inhalation, thus, sites along the respiratory tract are expected to be "at risk". The observation of dysplasia in nasal biopsies among formaldehyde-exposed workers (Edling et al., 1988; Holmstrom et al, 1989; Boysen., 1990) adds further biological support for a possible association between formaldehyde and cancer of the nasal cavity and sinuses.

The relevance of combining cancers of the nasal cavity with those of the sinuses in studies of humans for hazard identification has been questioned. Formaldehyde DNA-protein adducts have been detected in the monkey in the upper respiratory tract (nose, nasopharynx, larynx, trachea, and carina) and, to a lesser degree, in major intrapulmonary airways greater than 2 mm diameter (Heck et al., 1989). Experimental conditions in monkeys, however, do not show evidence of formaldehyde DNA-protein adducts (Heck et al., 1989) or of cellular proliferation (Monticello and Morgan, 1989) in the maxillary sinuses.

Insufficient information is presented in the reviewed studies to examine whether or not formaldehyde exposure is associated with cancers of the nasal sinuses. The epidemiologic studies can neither support nor reject this hypothesis. Any bias which would be introduced by grouping cancer sites unrelated to exposure with those that are would be similar to the bias introduced by misclassification of exposure; it would more likely

influence the risk towards seeing no association between exposure and disease. Several well-conducted case-control studies examining nasal cavity and sinus cancer have detected elevated risks in cancer at this aggregated site with formaldehyde exposure, although it is recognized these elevations could always reflect random events. Therefore, these studies are considered pertinent to hazard identification.

In summary, the studies released since 1987 support the conclusions drawn by EPA (1987) and do not alter the evaluation that "limited" evidence exists for an association between formaldehyde and human cancer according to the EPA's cancer assessment guidelines (EPA, 1986). Although the common exposure in the reviewed studies was formaldehyde, the epidemiologic evidence does not conclusively demonstrate a causal relationship; possible exposure to other agents may have confounded the findings of excess site-specific cancers. In addition, excesses in nasopharyngeal and nasal and sinus cavity cancers are based on a small number of deaths. It is for this reason that the evidence is called "limited"[1] rather than "sufficient".

8.2.2. Studies in Animals

Based upon a review of studies conducted by the Chemical Industry Institute of Toxicology (CIIT) (Kerns et al., 1983) and by Albert et al. (1982) and Tobe et al. (1985), EPA (1987) concluded that there is "sufficient" evidence of carcinogenicity[2] of formaldehyde in animals. This finding is based on the induction by formaldehyde of an increased incidence of a rare type of malignant tumor (i.e., nasal squamous-cell carcinoma) in both sexes of rats, in multiple inhalation experiments, and in multiple species (i.e., rats and mice). In these long-term laboratory studies, tumors were not observed beyond the initial site of nasal contact nor have other mammalian in vivo tests shown effects at distant sites. A subsequent report by CIIT (Monticello, 1990) lends further support for the carcinogenicity of formaldehyde in rats.

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[1] EPA's Guidelines for Carcinogen Risk Assessment define limited evidence of carcinogenicity in humans as indicating that "...a causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding, could not adequately be excluded."

[2] EPA's Guidelines for Carcinogen Risk Assessment define sufficient evidence of carcinogenicity from studies in experimental animals as indicating that "...there is an increased incidence of malignant and benign tumors: (a) In multiple species or strains; or (b) in multiple experiments, preferably with different routes of administration or using different dose levels); or (c) to an unusual degree with regard to incidence, site or type of tumor, dose-response effects, as well as information from short-term tests or on chemical structure."

In contrast to the inhalation data, results obtained from several carcinogenicity studies with formaldehyde given to rats in drinking water provide only suggestive evidence for carcinogenic potential via the oral route. The target tissue in these studies was the forestomach, which is consistent with observations from the inhalation studies in that tumors develop at the site of initial contact. In recent tumor promotion studies, formaldehyde enhanced the tumor response in mouse skin, rat trachea, and rat stomach (indicating that formaldehyde has tumor promotion potential at least in some tissues) but not in hamster respiratory tract-- consistent with the observed lack of carcinogenic response to formaldehyde in hamsters reported by Dalbey (1982) and discussed in EPA (1987).

8.2.3. Additional Supportive Evidence

Tests for point mutations, numerical and structural chromosome aberrations, DNA damage/repair, and in vitro cell transformation provide evidence for the potential mechanisms of carcinogenicity. A battery of tests which measure different endpoints helps to characterize the chemical's response spectrum. In general, the wider the range and the greater the intensity of response of a substance in applicable short-term tests, the more likely it is that the substance may cause cancer.

Formaldehyde is mutagenic in numerous bacterial test systems and test systems using fungi and insects (Drosophila). It also transforms cells in culture and causes DNA cross-linking, sister chromatid exchanges (SCE) and chromosome aberrations. In addition, formaldehyde has been shown to bind with DNA and with proteins in both in vivo and in vitro test systems. Its ability to interfere with DNA repair in human cells has also been shown. Mutagenicity data obtained since the 1987 assessment confirm the original conclusions.

8.3. Quantitative Risk Assessment

The risk assessment has identified two biological effects for which the data are sufficient for quantitative examination. These are sensory irritation and cellular effects of the upper respiratory tract and cancer. Results obtained from studies in animals and humans were used to assess the sensory irritation and cellular effects. Cancer risk estimates were derived by modeling data obtained from studies in animals.

This assessment is focused on the population constituted by persons who reside in mobile and conventional homes constructed using "significant amounts" of urea-formaldehyde (UF) pressed-wood (i.e, homes in which UF pressed wood is used for floor underlayment and, in some cases, for wall paneling). The exposure scenarios on which the quantitation is based are those described in the 1987 risk assessment document.

8.3.1. Noncancer Risk Assessment

Table 8-1 illustrates the relationship between the dose levels associated with sensory irritation and cellular effects in the nasal cavity and the exposure levels for a number of population groups. In keeping with Agency practice, instead of using high-to-low-dose extrapolation models, the degree of concern from these effects is approximated by comparing existing exposures to no-observed-effect-level (NOEL) or lowest-observed-effect-level (LOEL) values. Generally, little risk is expected in cases where exposures are ten-fold less than a NOEL, or one hundred-fold less than a LOEL when based on human observations. An additional uncertainty factor of ten is applied to account for interspecies differences in response if animal-derived NOEL and LOEL values are used.

The results shown in Table 8-1 illustrate the spectrum of responses that humans and animals exhibit on exposure to formaldehyde. Human mucociliary function may be inhibited at formaldehyde concentrations of 0.3 ppm and above; comparable effects are reported in animals, with a NOEL of 0.5 ppm in rats (Table 8-1). The NOEL for nasal squamous metaplasia in rats and monkeys is 1.0 ppm for both species, with LOELs of 2.0 ppm (rats) and 3.0 ppm (monkeys). Similarly, studies of humans showed nasal cavity effects in some persons exposed in the range of 0.08-0.9 ppm (Edling, et al., 1985, 1988; Holmstrom et al., 1989) with reported peak exposures of >4 ppm. The lack of adequate exposure data in the epidemiologic studies as well as confounding exposure to wood dust and resin particles do not allow a conclusive determination of the exposure level(s) eliciting human response relative to animal observations.

From the above, it appears that humans and animals may respond similarly to the cellular effects of formaldehyde in the nose. Formaldehyde exposures in mobile and conventional homes (0.1-0.2 ppm with peak exposures up to 0.4 ppm) fall below the NOELs and LOELs for cellular effects determined from studies in animals (Table 8-1). Since the anticipated exposures in the identified populations are close-- within a factor of ten-- to those associated with effects in humans occupationally exposed and animals, home residents are likely to be be at some risk of experiencing these cellular effects.

From the data in Table 8-1, it is apparent that there is no margin between existing exposures and levels of formaldehyde that are associated with irritation of the eye and upper airway in some humans. Since the available data do not allow the development of a well-defined dose-response relationship for these irritation effects, only qualitative assertions may be made with regard to population response. The people at greatest risk of experiencing discomfort due to formaldehyde-induced irritation would be homeowners during the first year of occupancy, particularly under conditions of high temperature and humidity which favor elevated levels of formaldehyde in these homes.

TABLE 8-1

HEALTH EFFECTS AND ASSOCIATED FORMALDEHYDE EXPOSURE CONCENTRATIONS

Formaldehyde Concentration (ppm)	Health Effects	Exposure Comments
<0.05		Indoor ambient background
0.1	Eye irritation observed in some people	New mobile homes 10-year average concentration
0.3	Upper airway irritation; most people experience eye irritation; human mucociliary inhibition	HUD[a] standard = 0.4 ppm
≥1.0	Lower airway irritation reported; nasal squamous metaplasia NOEL[b] (rat and monkey)	Current OSHA PEL= 1.0 ppm (8 hr TWA)
2.0	Rat squamous metaplasia and mucociliary system LOEL[c]	
3.0	Human (most) experience nose and throat irritation	
5.0	Rat observed 1% cancer incidence	Highest recorded level in homes[d]
15.0	Rat observed 50% cancer incidence; Mouse observed 1% cancer incidence	

[a]House and Urban Development.
[b]NOEL= no observed effect level.
[c]LOEL= lowest observed effect level.
[d]Urea-formaldehyde (UFFI) foam insulated home.

Although quantitative estimates of risk are not possible, the frequency and severity of response are concentration related. Fewer responses are expected to be associated with less frequent and less intense exposure. In addition, both sensory irritation and cellular effects are expected to be reversible once formaldehyde exposure is reduced.

8.3.2. Cancer Risk Assessment

In principle, data from studies of humans are preferred for derivation of numerical risk estimates. However, the available epidemiologic data on formaldehyde were not suitable for low-dose quantitative cancer risk estimation, mainly because of a lack of adequate exposure information in the studies. Accordingly, results from studies in animals have been used to estimate low-dose human cancer risk.

8.3.2.1. Dose-Response Assessment

As detailed in EPA (1987), of the carcinogenicity studies with formaldehyde in animals, EPA selected the CIIT study in rats (Kern et al., 1983) as the best study for cancer risk extrapolation. This study was well designed, well conducted, included multiple doses, and used a large number of animals per dose.

Data relevant to selecting a model for extrapolation of cancer risk associated with exposure to formaldehyde were reviewed; some of the biological information support a direct relationship between exposure and carcinogenicity while other data are consistent with a nonlinear response. The Agency, however, concludes that insufficient information is available at present to propose an extrapolation model for formaldehyde different from the one recommended by EPA's Guidelines for Carcinogen Risk Assessment (i.e., linearized multistage procedure). The Agency presented various other models in its 1987 assessment for comparative purposes.

Biologic evidence on mechanism of action, which can aid in model selection, largely is inferred from a variety of types of studies. These are limited and suggestive of several mechanisms for formaldehyde. Thus, the ability of formaldehyde to cause point mutations, chromosome aberrations and DNA damage is consistent with the chemical's ability to initiate the carcinogenic reaction. Mutagenicity studies suggest a direct relationship (i.e., a linear one) between exposure to formaldehyde and carcinogenicity. The steep curvilinearity of the rat nasal carcinoma dose-response data in the CIIT study (Kerns et al., 1983) suggests, however, that cancer development is limited at low dose levels and greatly accentuated above certain concentrations.

The CIIT also conducted molecular dosimetry experiments attempting to relate ambient exposures to formaldehyde with tissue-specific levels of formaldehyde-DNA adducts as DNA-protein cross-links (DPX). In its previous analysis, EPA (1987) used the administered dose to calculate carcinogenic risk from formaldehyde exposure because of perceived deficiencies in experimental design in the DPX dosimetry approach. However, recent evidence with an improved technique indicates that the use of DNA-protein cross-links as a measure of intracellular dose may provide a better indicator of target tissue exposure than would airborne formaldehyde levels. The use of an intracellular dosimeter (DPX) in the derivation of risk estimates would reflect the impact of both mucociliary clearance and metabolism, whose combined influence effectively reduces the amount of formaldehyde available to the nasal epithelial cells. Particularly at low dose levels, much of the inhaled formaldehyde does not reach nasal tissues, as it is rapidly cleared by the mucociliary system and oxidative metabolism. At sufficiently high exposure concentrations the detoxification mechanisms become overwhelmed, making a greater amount of formaldehyde available for interactions with DNA and other cellular macromolecules. DPX formation increases nonlinearly with increasing airborne exposure concentrations in a manner similar to the observed carcinogenic response in the rat cancer bioassay. These findings suggest possible saturation of detoxification processes at high formaldehyde concentration. The conclusion derived from the above discussion is that the use of airborne concentration in the derivation of risk estimates would, by ignoring important contributions from detoxifying processess, lead to an overestimate of exposure and concomitant risk.

While formation of DPX may lead to a number of genotoxic effects, its role--if any-- in the induction of nasal cancer is not completely understood. The Agency recognizes that the available DNA-binding data are derived from acute or subacute exposure. The latter may either underestimate or overestimate binding levels under chronic exposure conditions where cell proliferation may be significant, particularly at high exposure concentrations. The argument for the use of DPX as a surrogate dose is that this measure provides an index of the area under the curve of a reactive formaldehyde species in the target cells, both in rats and other species. This argument applies whether DPX are mechanistically involved in the carcinogenic process or are simply an indicator of intracellular exposure.

Although toxicological studies on formaldehyde have shown that adverse effects are a function of exposure, there is increasing evidence that the exposure parameter that more closely associates with observed toxicities is airborne formaldehyde concentration rather than total--cumulative-- daily dose (the latter is used in the 1987 quantitative estimates of cancer risk). Studies in rats by various authors have measured cell

turnover using constant cumulative daily dose (ppm-hr) but varying the concentration of formaldehyde and duration of exposure. Cell turnover rates are found to increase significantly with increasing concentrations at nasal regions where neoplasms develop. In contrast, in certain nasal sites, cell proliferation appears to be a function of total daily dose. The combined results of several toxicologic studies further support the importance of airborne formaldehyde concentration in the induction of toxic effects. Inhalation exposure to a low level of formaldehyde for a long duration of daily exposure (1 ppm for 22 hours) over the course of 6 months does not cause lesions in the rat nose. In contrast, subchronic or chronic inhalation exposure to higher formaldehyde concentrations (2-4 ppm) but with a shorter duration of daily exposure (6 hours) produces varying degree of nasal damage in rats. Similar observations are found in a recent subchronic study showing that cell proliferation and nasal damage are only seen with the animal group treated intermittently to high formaldehyde concentration (intermittent exposure for a total of 4 hours at 4 ppm). No responses are found in animals receiving the same total daily dose at a lower concentration (2 ppm for 8 hours). All these findings emphasize that airborne formaldehyde concentration is an important exposure parameter and imply that the utilization of lifetime average daily concentrations for risk quantification purposes may overestimate risk potential by arbitrarily lowering the exposure concentration at which adverse effects are expected.

In sum, a number of modifications, relative to the 1987 assessment, have been incorporated in the current quantitative risk assessment. These include the following: (1) the use of GLOBAL86 (as opposed to GLOBAL83) which contains EPA's current interpretation of the linearized multistage procedure; (2) the omission of lifetime average daily exposure adjustments to arrive at daily levels prior to dose-response modelling; this lifetime adjustment was used in the 1987 risk assessment; and (3) the use of an intracellular dosimeter- DPX binding data, instead of airborne concentrations of formaldehyde.

8.3.2.2 Cell Proliferation and Carcinogenesis

As discussed in section 4.5.2, the results of chronic studies point to cell proliferation is a contributor to the observed nonlinearity in nasal cancer induction; thus, the postulated cancer risk for low exposures to formaldehyde may have been overestimated by not incorporating cell proliferation in the derivation of such estimates. However, a number of data important for factoring cell proliferation into quantitative cancer risk assessment are still lacking; the potential magnitude of the cell proliferation effect on formaldehyde carcinogenesis cannot be determined at this time. For example, a clear relationship between formaldehyde-induced regional cell proliferation and tumor formation has not been established. The

causes for the discrepancy in cytotoxicity/cell proliferation and carcinogenic responses at the medial aspect of the maxilloturbinate need to be explored. As the nasal respiratory epithelium comprises numerous cell types, the cell type of origin of the squamous cell carcinomas and cell proliferation remains to be determined. Such information is important for the quantitative assessment of dose to critical target cell populations. Furthermore, the relative roles of cytotoxicity/cell proliferation and mutation in the multistep process of formaldehyde carcinogenesis is still unknown. Whether formaldehyde affects mutation rate and increases initiated cells or promotes spontaneous mutation without involving direct DNA reactivity (or both) has not been studied in the rat nasal cavity. Quantitative data on mutation rate per unit of DPX of the nasal cavity at different stages of the carcinogenic process and at different concentrations of formaldehyde are needed. In addition, information on the effects of formaldehyde exposure on cell cycle length (kinetics of cell proliferation) in control, non-neoplastic, pre-neoplastic and neoplastic tissues of the nasal cavity is also needed for meaningful modelling of cancer risk. There are ongoing studies at CIIT to generate the necessary data for the development of biologically-based risk assessment models for formaldehyde. This biologically-based risk assessment approach should improve the human risk assessment of formaldehyde.

8.3.2.3 Discussion and Conclusions

Formaldehyde is a component of normal metabolism in the biosynthesis of cellular molecules including amino acids, lipids, and nucleotides. Because of its high chemical reactivity and rapid rate of metabolism, inhaled formaldehyde is essentially not transported into the body from the site of contact, at least at environmental concentrations. Enzyme participation in the biological disposition of formaldehyde is only evident in a detoxication step, the GSH-dependent formaldehyde dehydrogenase.

Toxic effects occurring upon exposure to formaldehyde appear to be attributable primarily to the interactions of the chemical molecule with contact tissue. The absence of enzyme participation in transformations that may potentially lead to toxic events (DNA binding, tissue irritation, etc.) introduces an element of predictability in the biological behavior that this chemical may exhibit in different species. There is site concordance among mice, rats and monkeys in the lesions inflicted by formaldehyde under inhalation exposure conditions; there is similarity between rats and monkeys in the shape of the dose response curve for both cellular effects and rate of DPX formation. Comparable human data are more limited but do provide indications that the behavior of formaldehyde is consistent with the chemical characteristics elucidated in animal experiments. Interactions of formaldehyde with cells result in cellular

irritation and destruction, DNA damage and possible mutations. These mutations along with cellular proliferation could lead to the development of cancer, an endpoint that is observed in test animals and for which there is limited evidence in humans.

The mechanisms of formaldehyde induced-carcinogenesis are not completely understood; there is, however, knowledge about some factors which correlate with tumor response and which have plausible roles in cancer development. There is ample evidence that formaldehyde is a mutagen, which is one possible component to the carcinogenicity of formaldehyde. The focus of recent studies on formaldehyde has been on the effects of concentration, total daily dose, and the length of exposure period on various biological endpoints-- mucociliary clearance, cell proliferation, cytotoxicity, DNA-protein binding, and pathology-- that may help to explain the pronounced nonlinear carcinogenic response observed in the cancer studies in rats.

As stated earlier, the epidemiologic data base on formaldehyde does not provide information sufficient to calculate cancer dose-response. An attempt was made to project limits based on human data that could put risk estimates derived from animal data into perspective, however. The study by Blair et al. (1986) was used to gauge the behavior of the current risk estimates while recognizing that the exposure data are inadequate as a basis for unit risk estimation. The excess deaths attributed to nasopharyngeal cancer are approximately one for each exposure category (range 0.05- to 89-ppm-year) defined by Blair et al. The corresponding upper bound expected excess lifetime cancer deaths obtained by using the 1991 animal-based unit risk, derived from either rat or monkey dosimetry, are in the range of 10^{-3} to 10^{-1}. A number of issues, including the use of cumulative exposure categories, confounding exposure to other carcinogenic agents, and anatomical and physiological differences among species, modulate the magnitude of this difference. The concentration(s) at which these toxic effects become evident cannot be firmly established at this time, although it can be offered that based on the chemical characteristics of formaldehyde and dose response data in animals the dose response curve for humans is expected to be similar in shape, i.e., relatively shallow at low concentrations followed by a steep increase after an undetermined concentration. The information currently available do not allow a reasonable estimate of the latter. As mentioned earlier, the EPA recognizes the need to develop quantitative risk assessment procedures which further attempt to incorporate biologic data, particularly with regard to pharmacokinetics and mechanism, that can yield estimates of risk that appear to conform better to all facets of the information in hand.

In this document, human risk estimates, as stated earlier, are based on animal data. Dosimetry data obtained in rats and monkeys were used as alternative means to adjusting exposure

concentrations for human subjects to better reflect exposure of the target cells in the nasal epithelium. The Agency supports the use of monkeys as a more suitable animal model than rats for human extrapolation since monkeys have similar anatomic and morphologic structures and breathing pattern to humans (see Appendix C). Anatomical and physiological similarities that support the use of the monkey as a model for dosimetry estimates for humans include the oronasal mode of breathing; comparable relative nasal surface area; mucociliary clearance routes; and inspiratory airflow routes (see Appendix C for a complete description). The rat is an obligate nose breather, exhibits a greater relative nasal surface area than humans (or monkeys), shows differences in mucociliary clearance routes, and a different proportion distribution in nasal surface area covered by different epithelia. Anatomical and physiological differences are expected to influence both the rate and pattern of distribution of inhaled formaldehyde. This observation is of particular relevance when applied to formaldehyde which, as described earlier, is expected to react chemically at the site of contact. Thus, the breathing mode and large relative nasal surface area in the rat would be expected to lead to a high accumulation of formaldehyde in this area, which is consistent with the experimental findings. The oronasal pattern of respiration in humans and monkeys would be expected to reduce the dose of formaldehyde received by the nose and the nasopharynx, and to increase the dose delivered to the oral cavity and upper respiratory tract. This is supported by the observation of a lower DPX formation but more widespread distribution in the respiratory tract of monkeys.

The risk estimates for the linearized multistage procedure upper bound (UB) at various exposure levels are presented in Table 8-2. These risk estimates are derived utilizing the monkey-dosimetry unit risk. Risks at any exposure level may range from the upper bound to zero. An established procedure does not yet exist for making "most likely" or "best" estimates of risk within the range of uncertainty defined by the upper bound and zero. The upper bound estimate for excess lifetime risk of developing cancer is 5×10^{-6} [Group B1] for residents of mobile homes who are exposed for 10 years to an average level of 0.20 ppm; and 2×10^{-6} [Group B1] for residents of some conventional homes who are exposed for 10 years to an average level of 0.07 ppm. For residents of older, conventional homes exposed for 10 years to an average level of 0.05 ppm (upper limit indoor level) the projected cancer risk is 1×10^{-6}. The formaldehyde levels in the first two settings are expected to decline and eventually approach background levels ($\leq$ 0.05 ppm).

TABLE 8-2

SUMMARY OF CANCER RISK ESTIMATES ASSOCIATED WITH FORMALDEHYDE EXPOSURE UNDER SELECTED INDOOR ENVIRONMENTS

Population Segment (Exposure Level)	Projected Upper Bound Lifetime Excess Individual Risk[a]
Mobile Home Residents (0.20 ppm 10-yr average)	5×10^{-6}
Conventional Home[b] Residents (0.07 ppm 10-year average)	2×10^{-6}
Older Homes/Indoor Ambient Background Upper Limit (0.05 ppm 10 year)	1×10^{-6}

[a]1991 Unit Risk based on monkey dosimetry: 3.3×10^{-4}/ppm. Exposures were assumed to cover 14 hr/day and were adjusted to 70-year lifetime.

[b]For homes containing substantial amounts of urea-formaldehyde pressed wood (e.g., floor underlayment and/or paneling)

The human carcinogenic risks estimates based on the monkey DPX data are approximately ten-fold lower than corresponding risks based on rat DPX data at a given formaldehyde exposure concentration. The analysis based on rat DPX data can be interpreted as forgoing the use of the DPX dosimeter for cross-species extrapolation on the grounds that rat-human differences may be poorly illuminated by reference to rat-monkey differences. That is, the unit risk based on rat DPX reflects a correction only for high-to-low dose differences in tissue exposure that results from the apparent saturation of detoxification processes at the high exposure concentrations used in the bioassay.

The use of the monkey DPX data in quantitative risk estimation aims at taking into account not only high-to-low nonlinearity in tissue dose levels, but also dosimetry differences between rodents and primates. Incorporating these dosimetry differences, however, leaves open the question of relative sensitivity of rodents and primates to the toxic effects of formaldehyde. In the absence of any carcinogenic data in monkeys, it is not known how susceptible monkeys are to formaldehyde-induced carcinogenesis. For noncancer effects, the monkeys appear to be more susceptible to formaldehyde toxicity than rats, as shown by an induction of more widespread histologic lesions in the respiratory tract at equivalent exposure concentrations (6 ppm), however, the relevance of this observation to cancer development is uncertain since not all such lesions develop into tumors. In addition, it is also true that a gradient of effects is observed with decreasing exposure concentrations where at 3 ppm cellular changes are confined to the nasal cavity, and are not observed at concentrations of 1 and 0.2 ppm. This gradient of effects has been reported for both rats and monkeys, and a similar profile is anticipated in humans. Cross-sectional studies of workers in formaldehyde-related industries show higher histological scores (more metaplasia and dysplasia) with longer exposure; unfortunately, only nasal turbinates could be examined. It is difficult to evaluate the full impact of these observations because as stated before, no consistent correlation has yet evolved between nonneoplastic changes and tumor induction. DNA binding also occurs in the lower respiratory tract of monkeys at 6 ppm but it is not measurable at lower exposure concentrations with exception of the nasal area.

The forgoing argument is based on estimates of risk to the nasal epithelium only. Monkeys, as stated above, show DPX formation deeper in the respiratory tract in regions for which rats show neither DPX nor tumor response. Whether these further DPX engender additional risk, and whether humans are subject to such risk from formaldehyde inhalation is not considered in the present analysis. It is possible that basing risk estimates only on cross-links data corresponding to those in rats for the nose may lead to an underestimate of risk in humans. Epidemiologic information provides limited evidence for an association between

formaldehyde exposure and cancer of the nasal cavity and nasopharynx but a weaker case is available for the lung. Under the anticipated human exposure scenarios in the current assessment (airborne concentrations of about 0.3 ppm and lower), the cumulative evidence indicates that areas of the respiratory tract that come in most immediate contact with inhaled formaldehyde are the most likely targets for formaldehyde-induced toxicity. In conclusion, EPA believes that the predicted cancer risk estimates depicted in this document provide a plausible description of the actual risks for low level exposures to formaldehyde, as encountered in new mobile and conventional homes.

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APPENDIX A:

Summary of Epidemiologic Studies Cited

in

1987 and 1990 EPA Formaldehyde Risk Assessments

Table A-1

Risks Observed in SMR Studies

Study	Size	Cancer Site	Observed	Expected	SMR	95% Confidence Limits Lower	Upper
FORMALIN-EXPOSED COHORTS							
Matanoski (1982)	1336	buccal cavity and pharynx	NG[a]	NG	---	---	---
pathologists		lung	NG	NG	---	---	---
		colon	NG	NG	---	---	---
. no exposure data		brain	5	1.7[b]	296	96	686
		lymphopoietic	7	9.5[b]	74	30	152
		leukemia	NG	NG	---	---	---
		nasal	0	NG	---	---	---
pathologists	1439	buccal cavity and pharynx	NG	NG	---	---	---
		lung	6	8.1[b]	74	27	161
. no exposure data		colon	NG	NG	---	---	---
		brain	1	1.2[b]	82	2	464
		lymphopoietic	5	6.2[b]	81	26	188
		leukemia	NG	NG	---	---	
		nasal	0	NG	---	---	
Harrington and Shannon (1975)	2079	buccal cavity and pharynx	NG	NG	---	---	---
pathologists		lung	11	27.9	*39	22[c]	65[d]
		colon	NG	NG	---	---	---
. no exposure data		brain	NG	NG	---	---	---
		lymphopoietic	8	4.0	*200	100[c]	361[d]
		leukemia	1	1.6	63	3[c]	296[d]
		nasal	0	NG	---	---	---
Harrington and Oakes (1984)	2307	buccal cavity and pharynx	NG	NG	---	---	---
male pathologists		lung	9	22.0	*41	21[c]	71[d]
		colon	NG	NG	---	---	---
. no exposure data		brain	4	1.2	*331	114[c]	763[d]
		lymphopoietic	2	3.0	67	12[c]	210[d]
		leukemia	1	1.1	91	5[c]	431[d]
		nasal	0	0.1[e]	---	---	2996[d]
Levine et al. (1984)	1477	buccal cavity and pharynx	1	2.1	48	1	265
morticians		lung	19	20.2	94	57	147
		colon	NG	NG	---	---	---
. TWA: 0.02 ppm (1980's)		brain	3	2.6	115	24	337
. Mean TWA while embalming;		lymphopoietic	8	6.5	124	53	243
0.3 - 0.9 ppm (1980's)		leukemia	4	2.5	160	44	410
. Peak: 0.4 - 2.1 ppm (1980's)		nasal	0	0.2	---	---	1844

Table A-1

Risks Observed in SMR Studies

Study	Size	Cancer Site	Observed	Expected	SMR	95% Confidence Limits Lower	Upper
Stroup (1984)	2239	buccal cavity and pharynx	1	6.8	*15	<1	82
anatomists		lung	12	43.0	*28	14	49
		colon	20	18.5	108	66	167
. 1 ppm - 3 ppm (1974-1980)		brain	10	3.7	*271	130	497
. Higher peak exposure		lymphopoietic	18	14.4	125	74	198
		leukemia	10	6.7	148	72	274
		nasal	0	0.4	---	---	922
		brain	11	1.9[b]	*579	289	1036
		leukemia	8	3.8[b]	212	91	415
INDUSTRIAL COHORTS							
Stayner et al. (1988)	11030	buccal cavity and pharynx	6	3.9	155	68[c]	307[d]
garment workers		buccal cavity	4	1.2	*343	118[c]	786[d]
		pharynx	2	1.8	113	20[c]	359[d]
Concurrent:		lung	39	34.1	114	86[c]	149[d]
. Average TWA, 0.15 ppm		colon	NG	NG	---	---	---
(1981-1984)		brain	5	7.0	71	28[c]	149[d]
. Higher past exposures		lymphopoietic	18	19.8	91	59[c]	135[d]
		leukemia	9	7.9	114	60[c]	200[d]
		connective tissue	4	1.1	*364	123[c]	825[d]
		nasal	0	0.6	---	---	499[d]
Acheson et al. (1984)	7716	buccal cavity and pharynx	5	4.3	116	38	271
formaldehyde and resin		lung	205	196.0	105	91	120
production workers		colon	NG	NG	---	---	---
		brain	5	12.5	40*	13	94
. particulates present		lymphopoietic	20	26.3	76	46	117
. < 0.1 ppm - > 2.0 ppm		leukemia	9	11.4	79	36	150
		nasal	0	1.1	---	---	335
BIP plant (subset of cohort)		lung	166	141.0	118*	100	137
		nose	0	0.7	---	---	3527

. > 2.0 ppm
. particulates

Table A-1 (continued)

Risks Observed in SMR Studies

Study	Size	Cancer Site	Observed	Expected	SMR	95% Confidence Limits Lower	Upper
Bertazzi et al. (1985, 1986) formaldehyde-exposed resin workers	4462	buccal cavity and pharynx	NG	NG	---	---	---
		lung	5	3.7	136	44	315
		colon	NG	NG	---	---	---
		brain	NG	NG	---	---	---
		lymphopoietic	3	1.1	273	56	797
Mean:		leukemia	NG	NG	---	---	---
		nasal	0	NG	---	---	---
• 0.13 ppm - 2.5 ppm (1974-1979)							
Range:							
• 0.5 ppm - 9.8 ppm (1974-1979)							
• particulates present							
Blair et al.[9] (1986, 1987) formaldehyde and resin production workers (white males)	26561						
• ≥ 0.1 ppm, TWA (average exposure)		buccal cavity and pharynx	18	19	96	57	150
		lung	201	192	111	96	127
		colon	42	48	87	63	117
		brain	17	21	81	47	130
		lymphopoietic	56	62	91	62	184
		leukemia	19	24	80	48	124
		nasal	2	2.2	91	11	328
• < 0.5 ppm-yr - > 5.5 ppm-yr (cumulative exposure)		buccal cavity and pharynx	19	22	86	52	135
		nasopharynx	6	2	300*	110	653
		lung	236	214	110	97	125
		colon	55	57	96		
		brain	NG	NG	---	---	---
		lymphopoietic	64	73	88	68	112
		leukemia	23	28	82	52	123
with ≥ 20 years latency (subset of exposure group)		lung	146	109	133*	113	157
• simultaneous particulate exposure		nasopharynx					
0			---	---	---	---	---
< 0.5 ppm-yr			1	0.5	192	5	1114
0.5 - < 5.5 ppm-yr			2	0.5	403	48	1445
≥ 5.5 ppm-yr			2	0.3	746	81	2408

Table A-1 (continued)

Risks Observed in SMR Studies

Study	Size	Cancer Site	Observed	Expected	SMR	95% Confidence Limits Lower	Upper
Collins et al. (1988) (based on Blair et al. 1986, 1987)	7 case subset	nasopharynx					
	0		2	.9	215	25	802
	< 0.5 ppm-yr + particulates		2	.6	343	37	1204
	0.5 - ≤ 5.5 ppm-yr + particulates		1	.5	216	3	1113
	≥ 5.5 ppm-yr + particulates		2	.2	826	112	3611
Sterling and Weinkam - personal communication (based on Blair et al. 1986)	280 case subset	lung					
	< 0.1 ppm-yr				1.00[b]	--	--
	0.1 - 0.5 ppm-yr				1.12[b]	0.76[b]	1.84[b]
	0.5 - 2 ppm-yr				1.14[b]	0.73[b]	1.77[b]
	2+ ppm-yr				1.46[b]	0.87[b]	2.49[b]
Blair et al. (1990a) (based on Blair et al. 1986)	212 case subset	lung					
• exposure to formaldehyde only							
	> 0 - <0.5 ppm-yr		40	36.2	110	80	149
	0.5 - 5.5 ppm-yr		35	31.8	110	78	151
	≥ 5.5 ppm-yr		13	17.5	74	41	124
• exposure to formaldehyde and other agents							
	> 0 - <0.5 ppm-yr		39	25.2	154*	110	212
	0.5 - 5.5 ppm-yr		43	36.3	118	87	158
	≥ 5.5 ppm-yr		42	27.2	154*	113	207

Table A-1 (continued)

Risks Observed in SMR Studies

Study	Size	Cancer Site	Observed	Expected	SMR	95% Confidence Limits Lower	Upper
Stern et al. (1987)	9,365	buccal cavity and pharynx	1	NG	--	--	--
chrome leather		lung	24	34.3	70	45	105
tannery workers		colon	NG	NG	--	--	--
		brain	NG	NG	--	--	--
• 0.5 ppm - 7 ppm (1981),		lymphopoietic	14	13.2	106	58	178
finishing department		leukemia	7	5.6	125	50	258
• Mean TWA, 2.5 ppm (1981)		nasal	1[g]	.4[g]	250	6	1393
Malker et al. (1990)							
• no exposure data	471 incident cases	nasopharnyx					
	cases from Swedish	fiber board					
	Environment-Cancer	manufacture	4	1.0	*3.9[i]	1.08[f]	10.24[f]
	Registry	textile workers	1	2.5	0.4[i]	0.01[f]	2.23[f]
		furniture workers	3	3.8	0.8[i]	0.16[f]	2.31[f]
		chemical workers	1	1.7	0.6[i]	0.01[f]	3.27[f]
		shoe repair	5	1.3	*4.0[i]	1.24[f]	8.97[f]

Footnotes:

* $p \leq 0.05$.

[a] NG, observed or expected number of deaths not given in paper.

[b] Age-specific mortality rates of psychiatrists used as the comparison group.

[c] Lower 90% confidence interval.

[d] Upper 90% confidence interval.

[e] As described in Levine et al. (1984).

[f] Observed and expected numbers are for workers in the finishing department.

[g] Given in text.

[h] Odds ratio.

[i] Standardized incidence ratio.

Cohorts studied by Wong (1983) and Tabershaw Associates (1982) are included in the Blair et al. (1986) study and thus, are not included in this table.

Confidence intervals were obtained from the published papers. In those cases where this information was lacking, EPA caluculated the confidence interval.

Table A-2

PMR Studies

Study	Size	Cancer Site	Observed	Expected	PMR	95% Confidence Limits Lower	Upper
FORMALIN - EXPOSED:							
Walrath and Fraumeni (1983)	1132	buccal cavity and pharynx	8	7.1	113	56[a]	203
NY embalmers		lung	72	66.8	108	88	131
and funeral		colon	29	20.3	*143	102	194
directors		brain	9	5.8	156	81	271
		lymphopoietic	25	20.6	121	84	170
Presumed exposure levels		leukemia	12	8.5	140	81	229
• Peaks: up to 5.3 ppm (1975)		nasal	0	0.5[c]	---	---	599[b]
• Average: 0.25 - 1.4 ppm (1975)							
• Average concentration: 0.25 - 1.4 ppm (1975)							
Walrath and Fraumeni (1984)	1050	buccal cavity and pharynx	8	6.1	131	65[a]	237[b]
California		lung	41	42.9	96	72[a]	124[b]
embalmers		colon	30	16.0	*187	135[a]	254[b]
		brain	9	4.7	*193	100[a]	334[b]
Presumed exposure levels		lymphopoietic	19	15.5	123	170[a]	180[b]
• Peaks: up to 5.3 ppm (1975)		leukemia	12	6.9	*175	80[a]	282[b]
• Average: 0.25 - 1.4 ppm (1975)		nasal	0	0.6[d]	---	100[a]	499
• Average concentration: 0.25 - 1.4 ppm (1975)							
Hayes et al. (1990)	3649	buccal cavity and pharynx	26	21.8	119	78	174
embalmers and funeral		nasopharynx	3	1.6	189	39	548
directors (white)		lung	285	294.0	97	86	109
		colon	95	80.5	118	95	144
		brain	24	19.4	123	80	184
Presumed exposure levels		lymphopoietic	100	76.4	131	106	259
• Average: 0.98 ppm, 3.99 ppm		leukemia	NG	NG	---	---	605
• Total dust: 0.07 - 0.78 mg/m^3		nasal	NG	NG	---	88	161
INDUSTRIAL EXPOSED:							
Delzell and Grufferman	4462	buccal cavity and pharynx	18	18.0	100	59	158
textile		lung	106	117.8	90	73	108
workers		colon	115	115	100	83	120
		brain	17	18.9	90	52	144
		lymphopoietic	121	64.2	188	156	225
• exposure data lacking		leukemia	45	37.5	120	144	605
		nasal	NG	NG	---	88	161

Footnotes:

* $p \leq 0.05$

[a] Lower 90% confidence interval.

[b] Upper 90% confidence interval.

[c] As published in Levine et al. (1984).

The individuals studied by Marsh (1983) and Liebling et al. (1984) are included in the Blair et al. (1986) study, and thus are not listed separately here.

Table A-3

Odds Ratios Observed in Case-Control Studies

Study	Size	Cancer Site	Exposure Ratio = P_0	Odds Ratio	95% Confidence Limits Lower	Upper
Hardell et al. (1982) nasal and nasopharyngeal cases in Sweden . exposure levels lacking	44 cases/ 541 controls	nasal cavity	particleboard production: males (0.8%)	5.8*	1.2	25.9
Olsen et al. (1984) nasal and nasopharyngeal cancer cases in Denmark . exposure levels lacking	839 cases/ 2465 controls	nasal cavity and sinuses	formaldehyde: females (0.1%)	2.8	0.5[a]	14.3[b]
			males (4.2%)	2.8*	1.3[a]	4.3[b]
		nasopharynx	formaldehyde: females (0.1%)	2.6	0.3[a]	21.9[b]
			males (4.2%)	0.7	0.3[a]	1.7[b]
Hayes et al. (1984, 1986) nasal and nasal sinus cases in the Netherlands . exposure levels lacking	144 cases/ 353 controls (1:2 match)	nasal cavity and sinuses	formaldehyde: males with no or low level wood dust exposure: classification A (6.2%)	2.5*	1.2[a]	5.0[b]
			classification B (27.3%)	1.6	0.9[a]	2.8[b]
			males with high level wood dust exposure: classification A (47.1%)	1.9	0.7[a]	5.5[b]
			classification B (91.2%)	--	--	--
			formaldehyde: males, controlled for smoking: classification A	2.2*	1.1[a]	4.6[b]
			classification B	1.6	0.9[a]	2.8[b]

Table A-3 (continued)

Study	Size	Cancer Site	Exposure Ratio = P_0	Odds Ratio	95% Confidence Interval Lower	Upper
Fayerweather et al. (1982) cancer deaths in chemical workers	481 cases/ 481 controls (1:1 match)	lung, bronchus and trachea	formaldehyde: male workers intermittant level #1 (10%) (<2.0 ppm)	0.9	0.4	2.0
			intermittant level #2 (13%) (≥2.0 ppm)	0.9	0.5	6.4
			continuous level #1 (2%) (<0.1 ppm)	1.7	0.3	10.9
			continuous level #2/#3 (7%) (0.1 ppm - 2.0 ppm)	1.1	0.4	2.6
Partenen et al. (1985) nested respiratory cancer case-control study	55 cases/ 169 controls	respiratory system	formaldehyde: ever exposed (26.6%)	1.4	0.7[a]	3.0[b]
			level of exposure 0.1 - 1.0 ppm (16%)	1.5	0.7[a]	3.4[b]
			> 1.0 ppm (7.7%)	1.4	0.4[a]	5.3[b]
Partenen et al. (1990) nested respiratory cancer case-control study	136 cases/ 408 controls	respiratory system	formaldehyde: >3 ppm-months[c]	1.4	0.7[a]	4.1[b]
	118 cases/ 354 controls	lung	formaldehyde: >3 ppm-months[c]	0.9	0.3[a]	3.0[b]
Vaughan et al. (1986 a,b) sinonasal and pharyngeal cases in Washington State exposure levels lacking	53 cases/ 552 controls	nasal cavity and sinuses	occupational formaldehyde: cumulative exposure 5 - 9 years (6.3%)	0.7	0.3	1.4
			10 + years (10.9%)	0.3	0.1	1.9
			occupational: resins, glues and adhesives low exposure (6.5%)	2.0	0.7	5.6
			high exposure (2.3%)	3.8*	1.1	12.9
			domestic: mobile home residence 1 + years (12.0%)	0.6	0.2	1.7
	27 cases/ 552 controls	nasopharyngeal	occupational formaldehyde: no. of years exposed 1 - 9 (25.0%)	1.2	0.5	3.1
			10 + (10.3)	1.6	0.4	5.8
			domestic: mobile home residence 1 - 9 years (12.0%)	2.1	0.7	6.6
			10 + years (3.7%)	5.5*	1.6	19.4

Table A-3 (continued)

Study	Size	Cancer Site	Exposure Ratio = P_o	Odds Ratio	95% Confidence Interval Lower	Upper
	174 cases/ 552 controls	oro-hypo-pharyngeal	occupational formaldehyde: no. of years exposed			
			1 - 9 (25.0%)	0.6	0.3	1.0
			10 + (10.3%)	1.2	0.7	2.5
			occupational: resins, glues, adhesives			
			low exposure[c]	1.3	0.6	3.0
			high exposure[c]	3.9*	1.5	10.1
			domestic: mobile home residence			
			1 - 9 years (12.0%)	0.9	0.5	1.8
			10 + years (3.7%)	0.8	0.2	2.7
Gerin et al.(1989) cancer cases among males aged 35-70 in Montreal	875 cases/ 1523 controls	adenocarcinoma of the lung	formaldehyde: long duration -			
			low level	0.5	0.2	1.3
			medium level	1.0	0.4	2.5
			high level (TWA <1.0 ppm)	2.2	0.7	7.6
Roush et al. (1987) nasal sinus and nasopharyngeal cancer cases in Connecticut	371 cases/ 605 controls	nasal sinus	formaldehyde: high levels, ≥1 ppm, at 20+ years prior to death (3.3%)	1.5	0.6	3.9
		nasopharyngeal	high level, ≥ 1 ppm, at 20+ years prior to death (3.3%)	2.3	0.9	6.0

* $p \leq 0.05$, except as noted.

[a] Lower 90% confidence interval.

[b] Upper 90% confidence interval.

[c] Prevalence of the exposure among the controls not cited by Partenen et al. (1990) nor Vaughan et al. (As reported in SAIC, 1986).

Confidence intervals were obtained from the published papers. In those cases where this information was lacking, EPA caluculated the confidence interval.

APPENDIX B

Comparison of Animal Model Risk Predictions with Epidemiologic Evidence

An analysis was carried out to compare the epidemiologic data base and the 1990 animal-based risk estimates, to see if light can be shed on the procedure used to derive the 1990 risk estimates. An analogous comparison was carried out in 1987. In that comparison, site-specific excess risks were calculated by multiplying the excess risk observed in the epidemiologic studies, that is the excess above a risk of one, by the site-specific mortality ratios in the concurrent general population. These lifetime excess risks were then compared to upper bound lifetime excess risks calculated by applying the 1987 unit risk to representative exposure levels from the epidemiologic studies. Risks were found to be comparable by the two approaches, with rat-based excess risks tending to be within an order of magnitude lower than the corresponding epidemiologically based excess risks.

Such comparisons are approximate, and do not require the epidemiologic data to be of sufficient quality to derive risk estimates. Nevertheless, the data affording the most information on exposure groupings were selected. Blair et al. (1986, 1987) give information on relative cumulative exposure, and on observed and expected numbers of nasopharyngeal cancer cases.

The current comparison was carried out following the approach of Tollefson et al. (1990). This approach is equivalent to the 1987 approach, but focuses on numbers of excess nasopharyngeal cancer deaths, rather than on risk of nasopharyngeal cancer. The steps are outlined in Table B-1, and are described below.

- Steps a - c. Excess nasopharyngeal cancer deaths observed in the Blair et al. (1986) study were calculated as the difference between the observed deaths (step a in Table B-1) and the deaths expected based on 1980 US rates for the period covered by the study (step b).
- Under the hypothesis that the animal-based 1991 unit risk estimates apply, the excess nasopharyngeal cancer risks to be expected in the Blair cohort were predicted by applying the monkey dosimetry unit risk, 3.3×10^{-4}/ppm, and the rat dosimetry unit risk, 2.8×10^{-3}/ppm, to the exposures reported in Blair et al. (1986):
  - Step d. Cumulative exposures were reported in intervals, $\leq$ 0.5, 0.5-5.5, and $\geq$ 5.5 ppm-yr. Representative values for the first 2 intervals were taken to be the upper limits 0.5 and 5.5 ppm-yr, while the median of the range for the $\geq$ 5

ppm-yr interval (range 5 - 89 ppm-yr), 12.6 ppm-yr, was used. These are conservative representations of exposure, tending to elevate the risk predictions from animal data.

- Steps e, f. These representative cumulative exposures were converted to average exposure rates by dividing each by the average employment duration of the employees in each exposure category. This approach ignores periods of zero exposure before, and perhaps after, occupational exposure. Accounting for these periods would tend to lower the animal-based risk predictions.

- Steps g, j. The average exposure rates were multiplied by the 1991 unit risks, adjusted for 8-hour occupational exposures. This yielded excess lifetime nasopharyngeal cancer risks for each exposure category.

- Steps h, k. Total excess nasopharyngeal cancer deaths were predicted by applying these animal-based risk estimates to the total number of employees in each exposure category. This total represents the situation in which nasopharyngeal cancer would lead to early enough death that all such cases would have been observed during the period of this study.

- Steps i, l. Excess nasopharyngeal cancer deaths for the period this study covered were predicted by adjusting the total deaths expected by the proportion of the study group which was expected to die during that time, based on concurrent age-specific US mortality rates. Overall, approximately 11% of the entire study group was expected to have died by the time the study was concluded. Implicit in this approach is the assumption that the rate of nasopharyngeal cancer incidence is constant over lifespan.

The results are listed in Table B-1. The excess deaths observed in the study due to nasopharyngeal cancer were approximately 1 in each exposure category. The predicted upper bounds on expected cancer deaths were on the order of 10^{-3} to 10^{-2} deaths.

Table B-1: Comparison of Excess Nasopharyngeal Cancer (NPC) Deaths Observed in Blair et al. (1986), with those Expected Using 1991 Animal Models

	Exposure Category (ppm-yr)			
	Unexposed	≤ 0.5	0.5-5.5	5.5-89
Excess human deaths observed in Blair et al. (1986):				
a Observed NPC deaths	1	2	2	2
b No. NPC deaths expected (US rates)	0.2	0.7	0.8	0.5
c **Observed excess deaths** (a - b)	**0.8**	**1.3**	**1.2**	**1.5**
Exposure rate calculations for use with 1991 unit risks:				
d Cumulative Exposure Level used for Comparison (ppm-yr)	0	0.5	5.5	12.6[a]
e Average employment duration (years)	14.0	11.8	15.7	17.9
f Average Exposure Rate (ppm) (d/e)	0.0	0.042	0.350	0.704
Excess predicted deaths using 1991 monkey dosimetry unit risk:				
g Individual lifetime excess risk (f x 8/24 x 3.3×10^{-4})	0	5×10^{-4}	4×10^{-3}	8×10^{-3}
h Total number predicted NPC deaths (g x N)	0	0.08	0.4	0.3
i **Number predicted excess NPC deaths** (h x D/N)	**0.0**	**0.004**	**0.04**	**0.05[b]**
Excess predicted deaths using 1991 rat dosimetry unit risk:				
j Individual lifetime excess risk (f x 8/24 x 2.8×10^{-3})	0	4×10^{-3}	3×10^{-2}	7×10^{-2}
k Total number predicted NPC deaths (j x N)	0	0.7	3.4	2.5
l **Number predicted excess NPC deaths** (k x D/N)	**0.0**	**0.05**	**0.5**	**0.5[b]**
Characteristics of Blair et al. (1986) cohort:				
N - Number of employees	2638	17769	10484	3767
D - Expected deaths, all causes (US rates)	353	1254	1414	783

[a] 12.6 was the median exposure level (from transcripts of 1987 OSHA formaldehyde hearing).

[b] Using the upper limit of the highest exposure range, the number of predicted excess NPC deaths would be (average continuous exposure rate = 89/17.9 x 8/24 = 1.66)

Monkey dosimetry - Total excess NPC deaths: $1.66 \times 3.3 \times 10^{-4} \times 3767 = 2.1$
Among expected deaths: $1.66 \times 3.3 \times 10^{-4} \times 783 = 0.4$

Rat dosimetry - Total excess NPC deaths: $1.66 \times 2.8 \times 10^{-3} \times 3767 = 17.5$
Among expected deaths: $1.66 \times 2.8 \times 10^{-3} \times 783 = 3.6$

Appendix C

The Anatomy and Physiology of the Nasal Passages of Humans, Monkeys, and Rats

ANATOMY AND PHYSIOLOGY

Overview of the Upper Respiratory Region

The respiratory system consists of a specialized interface for exchange of gases between the blood and air, and a series of passageways leading from the interface to the ambient air. Clinically, the respiratory system is often divided into upper and lower respiratory regions based upon the gross anatomical location of the structures. While this division has been criticized as being vague when attempting to study deposition of particulates in various portions of the airways (Bates et al., 1966), it is easily visualized. Figure 1 illustrates the various components of the respiratory system. As depicted in figure 1, those structures contained within the thorax compose the lower respiratory region; extrathoracic structures constitute the upper respiratory region.

The upper respiratory airways serve as a conduit from the ambient air to the lungs. The particular anatomical territories included in the upper airways are the nasal passages, pharynx, larynx, and trachea. In higher primates, including humans (figure 2) and monkeys (figure 3), the arrangement of the upper airways is "L" shaped (Schreider, 1986). This arrangement is due to the orientation of the face, which is perpendicular relative to the body, causing the upper respiratory airways to exhibi a 90 degree turn in the pharynx (Patra, 1986). In this configuration, the larynx is inferior to the oral cavity, and the epiglottis (a cartilaginous flap that is attached to the anterior wall of the larynx and is used to seal off the trachea during swallowing) does not appose the soft palate. Consequently, since both the oral cavity and nasal passages open into the pharynx, higher primates are able to breathe through both their mouths and their noses (oronasal breathers [Negus, 1958; Reznik,1990]).

In the rat (figure 4), the layout of the upper respiratory structures is tandem such that the air could travel in a nearly linear fashion from the nose to the bifurcation of the trachea (Schreider, 1986). The linear arrangement of the upper airways allows the larynx of rats to lie close to the posterior edge of the oral and nasal cavities. In this condition, the epiglottis lies against the soft

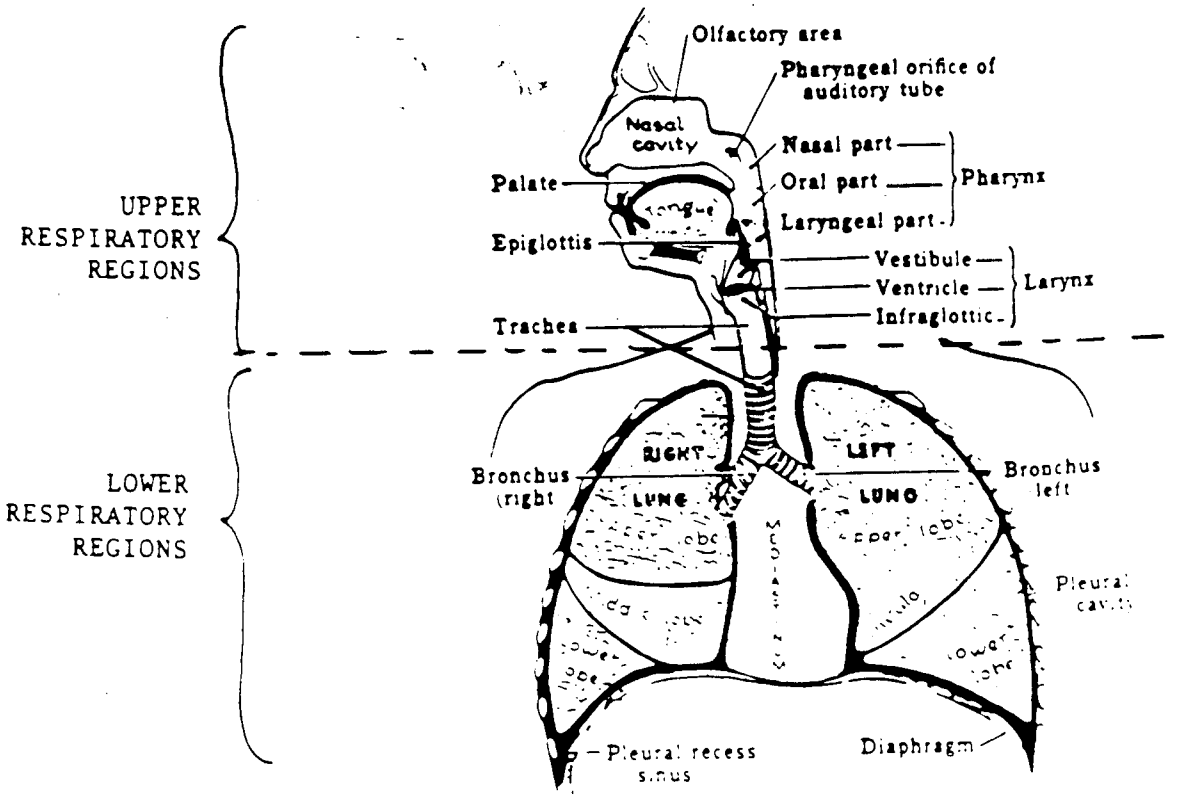
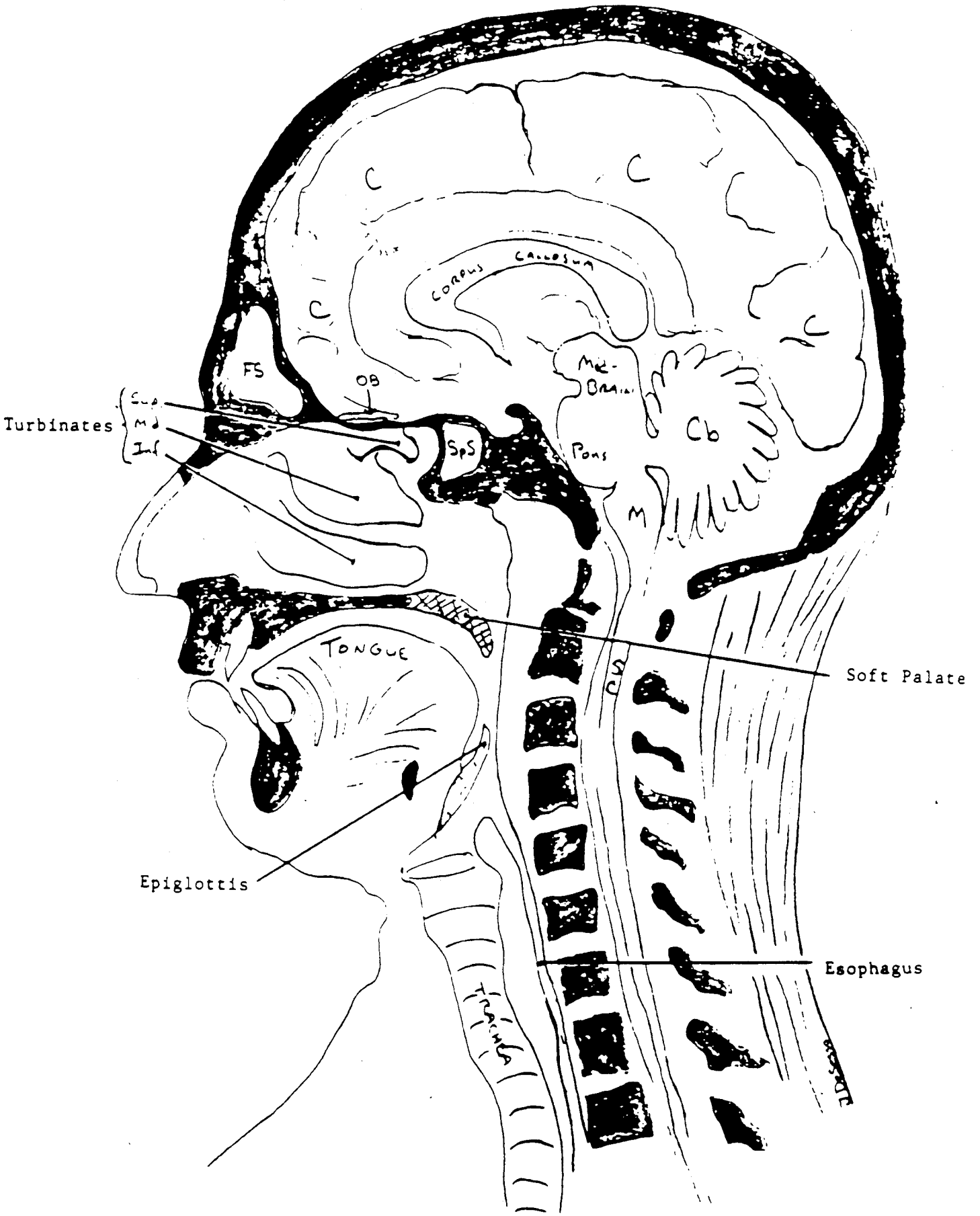


Figure 1. Diagram of the human respiratory system, illustrating the major gross anatomical structures and the division of these structures into upper and lower respiratory regions. (Modified from Anderson, 1978).

Figure 2. Sagittal section through the head of a human. Within the cranial cavity, note the small olfactory bulb (OB), which receives and processes nervous stimuli from the olfactory nerves in the roof of the nasal passage. The cerebral cortex (C) is extremely large. The external nose is outlined in white. Note that most of the nasal passages is located within the skull (as opposed to the external nose). The nasal septum has been removed to reveal structures on the lateral wall of the nasal cavity. The three turbinate bones are simple in structure and appear as vertical shelves. The more caudal respiratory structures make a right angle turn inferiorly in the pharynx. The epiglottis is associated with the base of the tongue and does not approach the soft palate. Consequently, the oral and nasal cavities are in open communication with the pharynx, thereby permitting oronasal breathing. Dashed line represents the plane of section through nasal cavity in Figure 5. C = cerebral cortex; Cb = cerebellum; FS = frontal sinus; M = medulla oblongata; OB = olfactory bulb; SC = spinal cord; SpS = sphenoid sinus.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CORPUS CALLOSUM
FS
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Mid-Brain
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Turbinates
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Epiglottis
Esophagus
TRACHEA

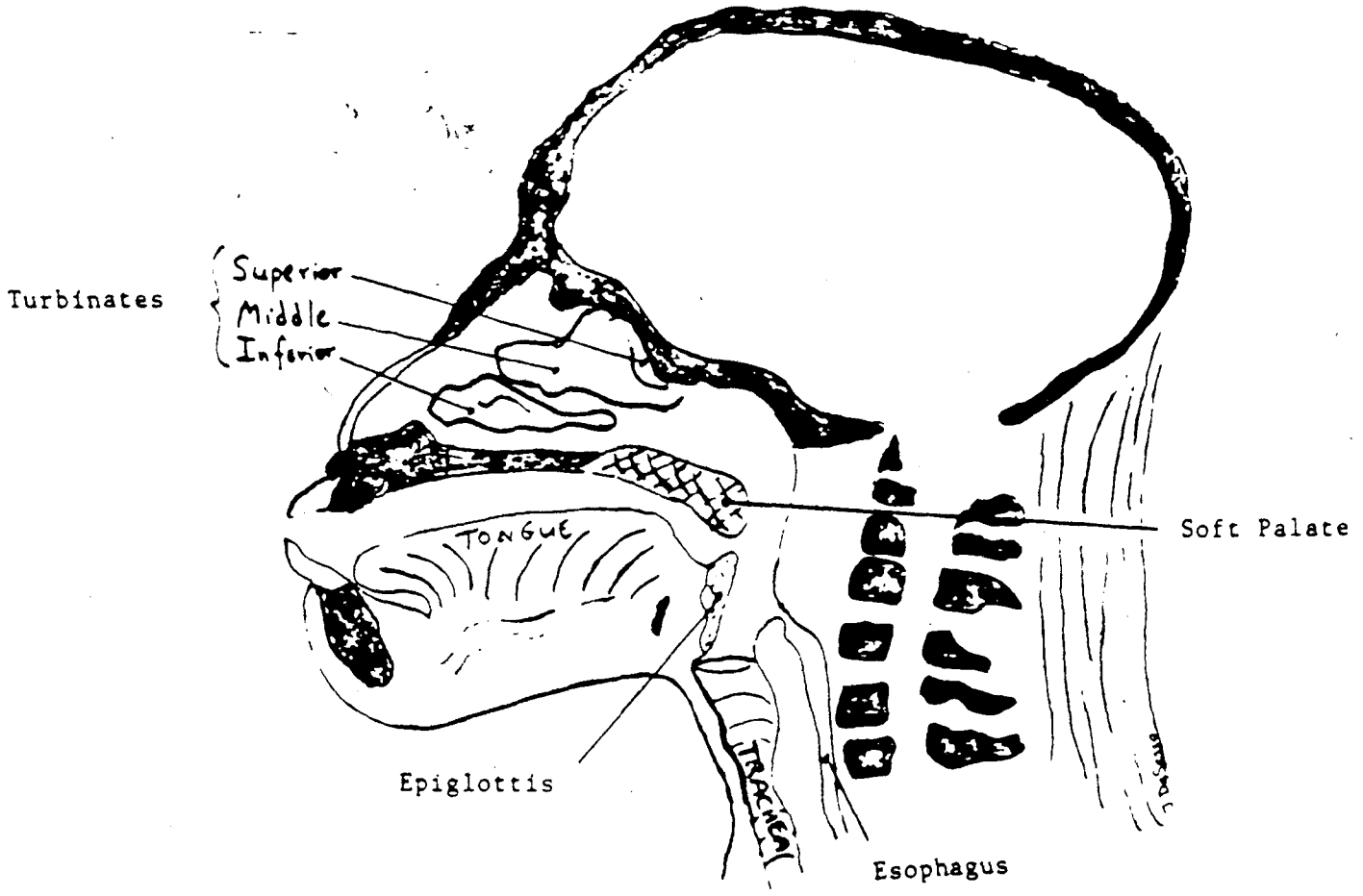

Figure 3. Sagittal section through the head of a monkey with the nasal septum removed to reveal structures of the lateral nasal wall. The shape of the nasal cavity is similar to that of the human. The three turbinate bones appear rather simple in structure; the middle turbinate is plow shaped, protruding towards the nostrils; the superior turbinate is small. The caudal upper respiratory structures make a virtual right angle turn at the region of the pharynx; note the inferior position of the trachea relative to the pharynx and the separation between the epiglottis and soft palate, thereby allowing oronasal breathing as in humans. Dashed line represents the plane of section through nasal cavity in Figure 5.

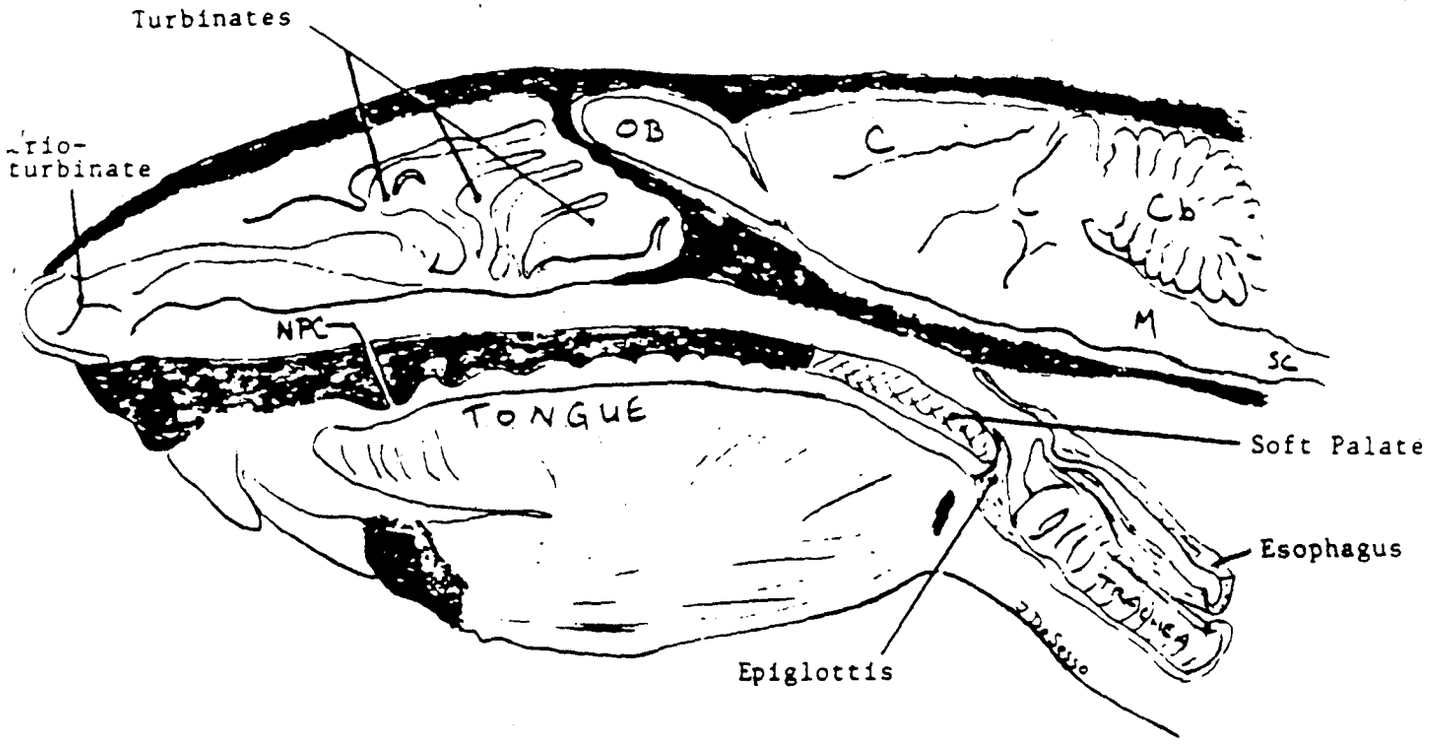

Figure 4. Sagittal section through the head of rat with the nasal septa removed to reveal structures on the lateral walls of the nasal cavity. The cranial cavity is proportionately smaller than that of the human. Despite that, the olfactory bulb (OB) is large especially when it is compared to the cerebral cortex (C). The nasal cavity is elongated. The major turbinate bones appear complex in structure; note also the presence of the anteriorly placed atrioturbinate bone. Note the nearly tandem arrangement of the respiratory structures and the consequent apposition of the epiglottis to the soft palate, closing off the oral cavity from the pharynx and making the rat an obligatory nose breather. Dashed line represents the plane of section through nasal cavity in Figure 5. C = cerebral cortex; Cb = cerebellum; M = medulla oblongata; NPC = Nasopalatine Canal; OB = olfactory bulb; SC = spinal cord.

palate. The apposition of the epiglottis to the soft palate in the resting condition isolates the oral cavity from the respiratory airways and makes the rat an obligatory nose breather (Negus, 1958; Proctor and Chang, 1983).

Nasal Passages

While the overall arrangement of the upper respiratory airways differs among these species in that the rat is an obligate nose breather whose design is nearly linear whereas higher primates are oronasal breathers whose upper airways exhibit a right angle turn, even greater differences occur in the anatomy of the nasal passages. Despite the differences in nasal anatomy, the nasal passages of all three species serve as the site for olfactory stimulation and for conditioning air (i. e., humidifying, warming, removal of inhaled particulate matter [Negus, 1958; Proctor and Chang, 1983; Schreider, 1986]) prior to its access to the gaseous exchange interface in the lungs. The following paragraphs will outline the common features of the nasal passages shared by all three species and will point out the major anatomical differences. The anatomical features are depicted in figures 2 (human), 3 (monkey), and 4 (rat).

In all three species, the nose contains a pair of openings (nares) leading into the vestibule of the nasal cavity. The vestibule is relatively large in humans, and entry into the nasal cavity proper in humans occurs through the nasal valve, a narrow opening flanked by cartilage and muscle (Proctor and Chang, 1983; Reznik, 1990). A homologous structure does not exist in the rat. While the rat does not possess a muscular nasal valve, it does exhibit small cartilaginous projections into the vestibule just inside the nares. These projections are the atrioturbinates (Proctor and Chang, 1983; Hebel and Stromberg, 1986). Similar projections do not exist in the vestibules of the primates. The rat also possesses a small diameter, patent nasopalatine canal that connects the nasal cavity and the oral cavity (Negus, 1958). While the higher primates do possess nasopalatine canals as embryos, they give rise to the incisive canals in adults (Warwick and Williams, 1973). The incisive canals of higher primates are covered by mucosa and are not patent conduits between the oral and nasal cavities (Negus, 1958).

The nasal cavity proper is divided into paired passageways by a vertical cartilaginous and bony septum. The nasal passages extend into the head, superior to the roof of the mouth, posteriorly as far as the pharynx. Only a portion of the nasal passages are contained in the external nose; the remainder is located within the skull and is not readily visible. When viewed from the front (see figure 5), a coronal section through the head reveals each of the nasal passages to be nearly triangular in shape with the vertex oriented superiorly. The floor of the nasal passage (made up of the hard and soft palates, which separate the oral and nasal cavities) and the medial wall (the nasal septum) are relatively flat, with no remarkable surface features. In the rat and other rodents, the inferior aspect of the nasal septum contains structures termed swell bodies that are composed of several vascular spaces subjacent to the epithelium (Proctor and Chang, 1983; Reznik, 1990). These swell bodies are capable of rapid engorgement with blood (Sorokin, 1988). When the swell bodies are in a collapsed condition, the nasal septum exhibits a flat, vertical profile and the nasal passage is maximally open. This allows inspired air to take a rather direct course through the nasal passage, which is especially important when the animal is physically active. When the swell bodies are engorged, the inferior aspect of the nasal septum encroaches on, and nearly obliterates, the inferior portion of the nasal passage. In this condition, air is forced into the upper portion of the nasal airway, which houses the olfactory apparatus. Swell bodies do not exist in the higher primates (Negus, 1958; Reznik, 1990).

In contrast to the relatively featureless contours of the septum and floor of the nasal cavity, the lateral walls of the nasal passages possess a series of bony processes that project into the passageway. The processes are termed turbinate bones or conchae; there are three major turbinates. While the specific names differ among species, they will be referred to according to the human nomenclature for ease of comparison. The three turbinates are designated the inferior, middle, and superior according to their positions relative to the floor of the nasal passages and each other.

The most striking anatomical differences can be seen in the structure of the major turbinate bones (Negus, 1958; Schreider, 1986). When examining the sagittal sections of the human and monkey, three turbinates are readily discerned. The rat, however, appears to exhibit additional

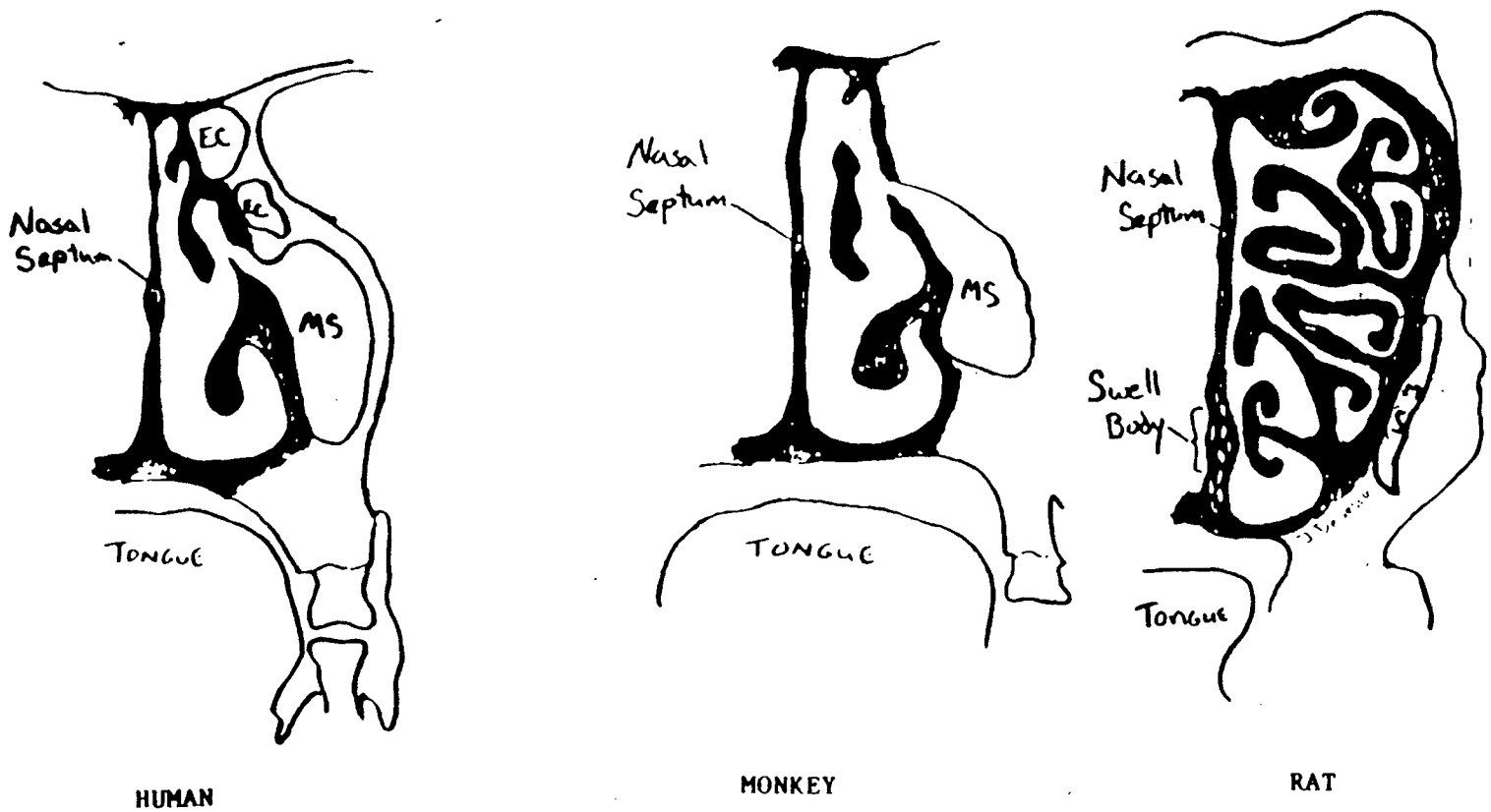

Figure 5. Coronal (frontal) sections through the left nasal passage of human, monkey, and rat. Planes of section are denoted by dashed lines on Figures 2, 3, and 4, respectively. Note the simple, comma-shaped profile of the turbinate bones in human and monkey. The rat turbinate bones are complex, exhibiting a "t" shaped profile with scroll-like foldings at the ends. Note the comparable size of the maxillary sinus (MS) in human and monkey; the MS of rat is much smaller. Only humans possess ethmoid sinuses (EC). Note also the position of the (collapsed) swell body on the ventral aspect of the nasal septum of rat. Primates do not possess septal swell bodies.

turbinates. What appears to be additional turbinates in the rat is caused by the existence of dorsal and ventral lamellae for each turbinate (Hebel and Stromberg, 1986). The complexity of the nasal turbinate bone in the rat can best be appreciated by studying diagrams of coronal (frontal) sections taken midway through the nasal cavities of humans, monkeys, and rats (figure 5). In each diagram, only the left nasal passage is depicted. The plane of section is denoted by the dashed vertical lines on the sagittal section of the appropriate species (figures 2-4). In all three species, the nasal septa are thin with no prominent surface features and the lateral walls possess turbinate bones that protrude into the nasal passage. In the monkey and human, the turbinate bones exhibit simple "comma" shaped profiles that extend inferiorly into the nasal passage (Negus, 1958; Warwick and Williams, 1973; Anderson, 1978). Both primate species possess large maxillary sinuses (MS) that open into the middle meatus (inferior to the middle turbinate bone). Humans possess small sinuses (ethmoid air cells [EC]) in the ethmoid bone forming part of the roof of the nasal passage; corresponding structures do not exist in either monkeys or rats. Due to the plow shaped structure of the middle turbinate of the monkey (see figure 4; Harkema et al., 1987; Harkema, 1990), the attachment site of the middle turbinate to the lateral wall is not depicted in this diagram. In contrast to the primates, the nasal passage of the rat exhibits elaborate turbinate bones that exhibit "t" shaped profiles when viewed in cross section (Hebel and Stromberg, 1986; Schreider, 1986). The distal edges of the "t" often appear scrolled. The effect of this arrangement of turbinate bones in the rat is to greatly increase the surface area of the nasal passage compared to monkey and human. The maxillary sinus (MS) of rats is very small (Hebel and Stromberg, 1986).

Morphometry

The differences in anterior-posterior extent and complexity of the turbinates in the three species result in differences in the amount of surface area that lines the nasal passages. Table 1 presents morphometric data for each species concerning the nasal cavity volumes, surfaces of the nasal passages and other upper airways, as well as body weights, body surface areas, and parameters of pulmonary physiology.

Table 1. Morphometric and Physiological Data for the Nasal Passages, Upper Respiratory Tract, and Pulmonary Systems of Humans, Monkeys, and Rats

Species	Body Weight (Kg)	Body Surface Area (m^2)	Nasal Cavity		Relative Nasal Surface Area [1]	Upper Respiratory Surface Areas (cm^2)[2]			Pulmonary Physiology		
			Volume (cm^3)	Surface Area (cm^2)		Pharynx	Larynx	Trachea	Tidal Volume (cm^3)	Breaths per minute	Minu Volu (L/mi
Human	70[3]	1.85[4]	25[5]	160[3]	6.4	46.6	29.5	82.5	800[6] 750[3]	15[6] 12[3]	12[6] 9[3]
Rhesus Monkey	7[2]	0.35	8[2]	62[7]	7.75	--	--	--	70	34[6]	2.4[8]
Rat	0.25[4]	0.045[4]	0.26[9]	13.44[9]	51.7	1.2	0.17	3	2[6]	120[6]	0.24[9]

[1] Relative Surface Area of nasal passage = Surface Area of nasal cavity + Volume of nasal cavity

[2] Calculated from data in Patra, 1986

[3] Snyder et al., 1975

[4] Calabrese, 1983

[5] Harkema, 1990

[6] Alarie, 1982

[7] Schrieder, 1986

[8] Heck et al., 1989

[9] Gross et al., 1982

The human nasal passage proper has a volume of 25 cm^3 (Harkema, 1990) with a surface area of 160 cm^2, excluding the vestibule (Snyder, et al., 1975). The human vestibule has a surface area of 40 cm^2 (Snyder et al., 1975); an estimate of the vestibular volume has not been published. Because the human vestibule is so large compared to those of rat and monkey, and because it shares so much in common with the skin of the face, the vestibule was not considered as part of the nasal passages proper for the interspecies comparisons in this paper. The values for surface areas and volumes for the rat and monkey include the vestibule. The vestibules of these animals are considerably smaller than that of the human, and specific surface area and volume data have not been published.

The absolute volume and surface area of the nasal passages in humans are, not unexpectedly, larger than those of both the rat and the monkey. In an attempt to compare these data among all three species, relative nasal surface area ratios were calculated by dividing the nasal surface area by the nasal cavity volume for each species. The relative nasal surface areas of humans and monkeys were nearly equal (6.4 and 7.75, respectively). The relative nasal surface area of the rat (51.7) was eight-fold larger than that of humans. This means that the rat has eight times more surface area per unit volume in the nose than either of the primate species. Inspection of figure 5 reveals that this is not an unexpected finding.

It is interesting to note that the surface area of the nasal passage is larger than that of the trachea. In humans, the nasal passages exhibit twice the surface area of the trachea. In rats, due to their elaborate turbinate structure, the surface area of the nasal passages is 4.5 times that of the trachea.

Patra et al., (1986) performed a morphometric analysis of the nasopahryngeal airways of rat, rhesus monkey, baboon, beagle, guinea pig, and a human child. Using nasal casts and literature values, the authors calculated the cross sectional areas and perimeters of the airways at various distances from the nares. The data was normalized to correct for differences in body size and compared graphically. The graphs of the monkey and the baboon most closely resembled those of the child. The graphs of the rat and the guinea pig were similar to each other, but were significantly

different from the primates. The graphs of the beagle differed from those of both the primates and the rodents. From this analysis of normalized data it can be concluded that, while the geometry of the nasal passages and upper airways within taxonomic orders are similar, there are significant differences in nasal cavity geometry (beyond mere size) when one compares rats and humans.

Mucosa

In all three species, the nasal passages are lined by a well vascularized mucous membrane, or mucosa, that serves as a barrier to the entry of inhaled materials into the nasal walls. The mucosa is composed of an epithelial sheet, lying on a basement membrane and underlain with a thin layer of loose connective tissue that contains blood and lymphatic vessels. The mucosa is named after the type of overlying epithelium.

Epithelia. There are three major types of epithelia in the nasal passages proper: squamous, respiratory, and olfactory (Bloom and Fawcett, 1968; Gross et al., 1982; Sorokin, 1988). The entry to the nose is the vestibule, which is covered by modified skin (keratinized, stratified squamous epithelium) containing sebaceous glands and hairs. Within the nasal cavity proper, the epithelial covering becomes progressively more delicate, in terms of its ability to respond to stress, and more specialized, in terms of its functions of conditioning air and olfaction.

Beyond the vestibule, the anterior nasal passages are covered by a moist stratified squamous epithelium, consisting of multiple layers of cells that become thin and plate-like as they approach the surface. While the surface of this epithelium is covered by mucus secretions emanating from the adjacent respiratory epithelium, the squamous epithelium itself is non-secretory. Moist stratified squamous epithelium is not as resilient as the modified skin of the vestibule. The number of layers of cells decreases, and the squamous epithelium thins, as one travels deeper into the nasal passages. At approximately the anterior end of the turbinate bones, the epithelium changes to respiratory epithelium, which is more delicate than the moist squamous epithelium. The respiratory epithelium is a mucus-secreting, ciliated, pseudostratified columnar epithelium with goblet cells. The underlying

connective tissue contains numerous muco-serous glands. The secretions of these glands together with that of the respiratory epithelium itself serve to keep the nasal passages moist. In the roof of the nasal cavity, the mucous membrane is covered by the highly specialized olfactory epithelium. Olfactory epithelium appears yellow in the fresh condition. It is a tall pseudostratified columnar epithelium that is higher than the respiratory epithelium and contains neither cilia nor goblet cells. The olfactory cells are bipolar neurons that exhibit very long, nonmotile processes (stereocilia) that lie on the epithelial surface and house the receptors for olfaction. The olfactory cells are surrounded by sustentacular cells which exhibit numerous apical microvilli and continuously secrete a serous fluid. The glands in the connective tissue are also serous glands.

The approximate distribution of these epithelia within the nasal passages of the human, monkey, and rat is depicted in figure 6. While relatively defined lines of demarcation between areas of the nasal passages covered by the various epithelia have been published (Hebel and Stromberg, 1986; Harkema, 1990), the zone of transition between adjacent areas of squamous and respiratory epithelia is usually not abrupt and the transitional epithelia in these zones share the characteristics (in graded fashion) of the adjacent epithelial types (Menco, 1983; Harkema et al., 1987; Morgan and Monticello, 1990).

Under conditions of physiological stress, the more delicate respiratory epithelia can be replaced by a more hardy epithelium. This change usually involves the replacement of the respiratory epithelium with moist, stratified squamous epithelium and is termed squamous metaplasia (Bloom and Fawcett, 1968).

Measurements of the surface area of the nasal passages covered by the three major epithelia in rats have been made by Gross et al. (1982). Such measurements for humans have not been made except for the olfactory epithelium (Negus, 1958; Bloom and Fawcett, 1968). No surface area measurements could be found for the monkey. The available surface area data for nasal epithelia are presented in table 2. Based upon the percentage of total nasal epithelium that is olfactory, animals such as the rat (50 percent olfactory epithelium) have been designated macrosmatic, whereas primates

DISTRIBUTION OF EPITHELIA

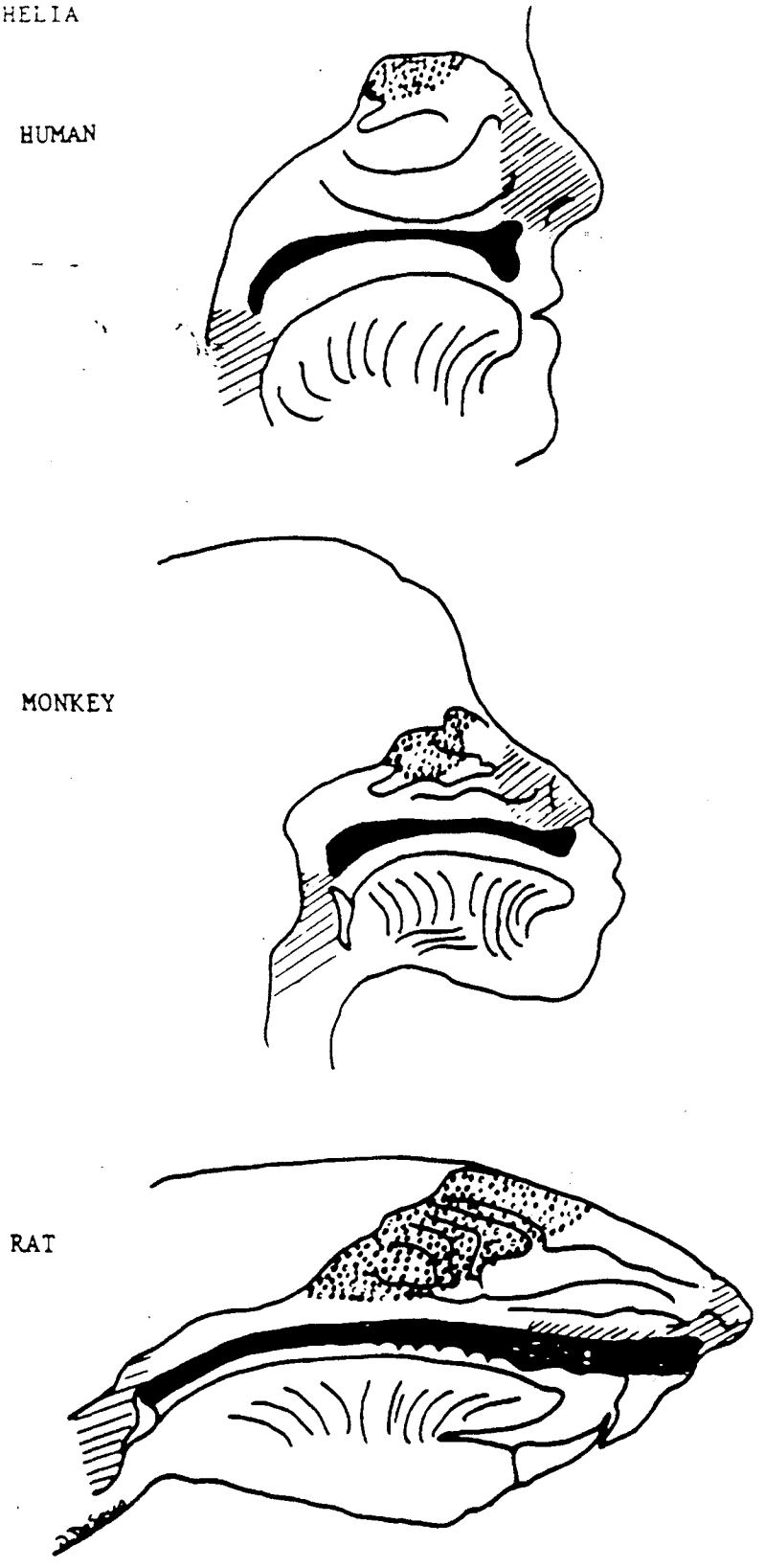

Figure 6. Sagittal sections through the heads of human, monkey, and rat to illustrate the extent of the major types of epithelia in the nasal passages. Note the relatively large olfactory area in rat compared to human and monkey. Stippled area = olfactory; clear = respiratory; striped = squamous.

Table 2. Epithelial Surface Areas of the Nasal Passages in Rats and Humans

Species	Total Surface Area (cm^2)	Olfactory Area (cm^2)	%	Respiratory Area (cm^2)	%	Squamous Area (cm^2)	%
Rat	13.44[1]	6.75[1]	50	6.23[1]	47	0.44[1]	3
Human	160[2]	12.5[3]	8	NF	--	NF	--

(1) Gross et al., 1982

(2) Snyder et al., 1975

(3) Negus, 1958

NF = not found

including humans (8 percent olfactory epithelium) and monkeys are classified as microsmatic (Negus, 1958; Reznik,1990). The relative importance of olfaction to rats versus humans can perhaps be appreciated by comparing the size of the olfactory bulb (OB) to the cerebral cortex (C) in each of the species as depicted in figure 2 (human) and figure 4 (rat).

Vascular Supply. The mucosa of the nasal passages contains a rich vascular supply. An unexpected feature of the arterial supply is that the olfactory and respiratory mucosae receive blood from different sources (Warwick and Williams, 1973; Sorokin, 1988). The olfactory mucosa is supplied by the posterior and anterior ethmoidal arteries, which are very small calibre branches of the ophthalmic artery. The respiratory mucosa receives arterial supply from the sphenopalatine artery, which is the terminal branch of the maxillary artery (the great artery of the face), as well as the greater palatine artery, another branch of the maxillary. Both of these arteries are of greater calibre than the ethmoidal arteries. Based on the arterial diameters, the blood supply to the respiratory epithelium appears to be richer than that of the olfactory epithelium. This may be related to the secretory rates of these epithelia, as discussed later. The epithelium of the vestibule and anterior nasal passages receive blood supply from the nasal branches of the superior labial artery (a branch of the facial artery).

The swell bodies located on the ventral aspect of the nasal septum in rats are formed by a superficial plexus of thin walled veins that resemble erectile tissue, except that they lack muscular septa (Negus, 1958). While the higher primates do not possess swell bodies, thin walled venous plexuses are present on the anterior aspect of the inferior and (sometimes) middle turbinates (Proctor and Chang, 1983; Sorokin, 1988). Distension and collapse of these vascular structures can occur extremely quickly (Negus, 1958) and, due to their location, these structures can alter both the course of airflow through the nasal passages (Proctor and Chang, 1983) and the volume of the airway in the nasal passages (Guilmette et al., 1989).

Nasal Secretions. Since the functions of the nasal passages include warming and humidification of air, removal of particulate matter, and olfaction, it is not surprising that the nasal mucosae are covered with secretions which are continuously produced, transported through the nasal

passages, and removed. In humans, it has been estimated that the thickness of nasal secretions on the mucosa is 0.5 mm (Snyder et al., 1975); that secretions are replaced every 10 minutes over the posterior two-thirds of the nasal passages and once an hour over the anterior one-third (Negus, 1958); and that the daily production volume of nasal secretions is 0.5-1.0 liters, most of which is transported to the nasopharynx and swallowed (Snyder et al., 1975).

The great majority of the nasal passage secretions is produced by the respiratory epithelium. While this secretion is frequently referred to as "mucus", the secretion really consists of two distinct components (Proctor and Chang, 1983). Immediately adjacent to the epithelial surface and extending above it 5-30 μm is a layer of periciliary fluid. This periciliary fluid is a transudate of plasma (Sorokin, 1988) that provides a low viscosity medium in which the cilia of the respiratory can beat in a regular and coordinated manner, thereby creating a current for the transport of secretions. The mucous secretions of the goblet cells and mixed sero-mucous glands of the lamina propria are deposited on the surface of the periciliary fluid, where they form a continuous layer in the nasal passages (Proctor and Chang, 1983).

The course of transport by the mucociliary activity of the respiratory epithelium in humans (Negus, 1958; Proctor and Chang, 1983), monkeys (Lucas, 1932), and rats (Negus, 1958; Morgan et al., 1986a) are illustrated in figure 7. In both monkeys and humans, the predominant direction of transport is posteriorly towards the nasopharynx. A small amount of secretion is transported toward the nostril, but the rate of transport is very slow (1-2 mm per hour in the anterior nasal cavity versus 8-10 mm per minute in the posterior region [Snyder et al., 1975]) allowing dehydration of the secretion and eventual removal from the nose through nose blowing or by digital cleaning. In the case of the rat, a much greater amount of secretion is directed towards the nostril. Morgan et al. (1986a) measured the rate of transport of particles in various parts of the rat nasal cavity. While their measurements are not directly comparable to those given for humans, the rat did not show such a great disparity in rates of transport when comparing anterior and posterior directions. It has been suggested that the anterior mucociliary clearance in the rat allows more rapid transport of particulate

ROUTES OF MUCUS FLOW

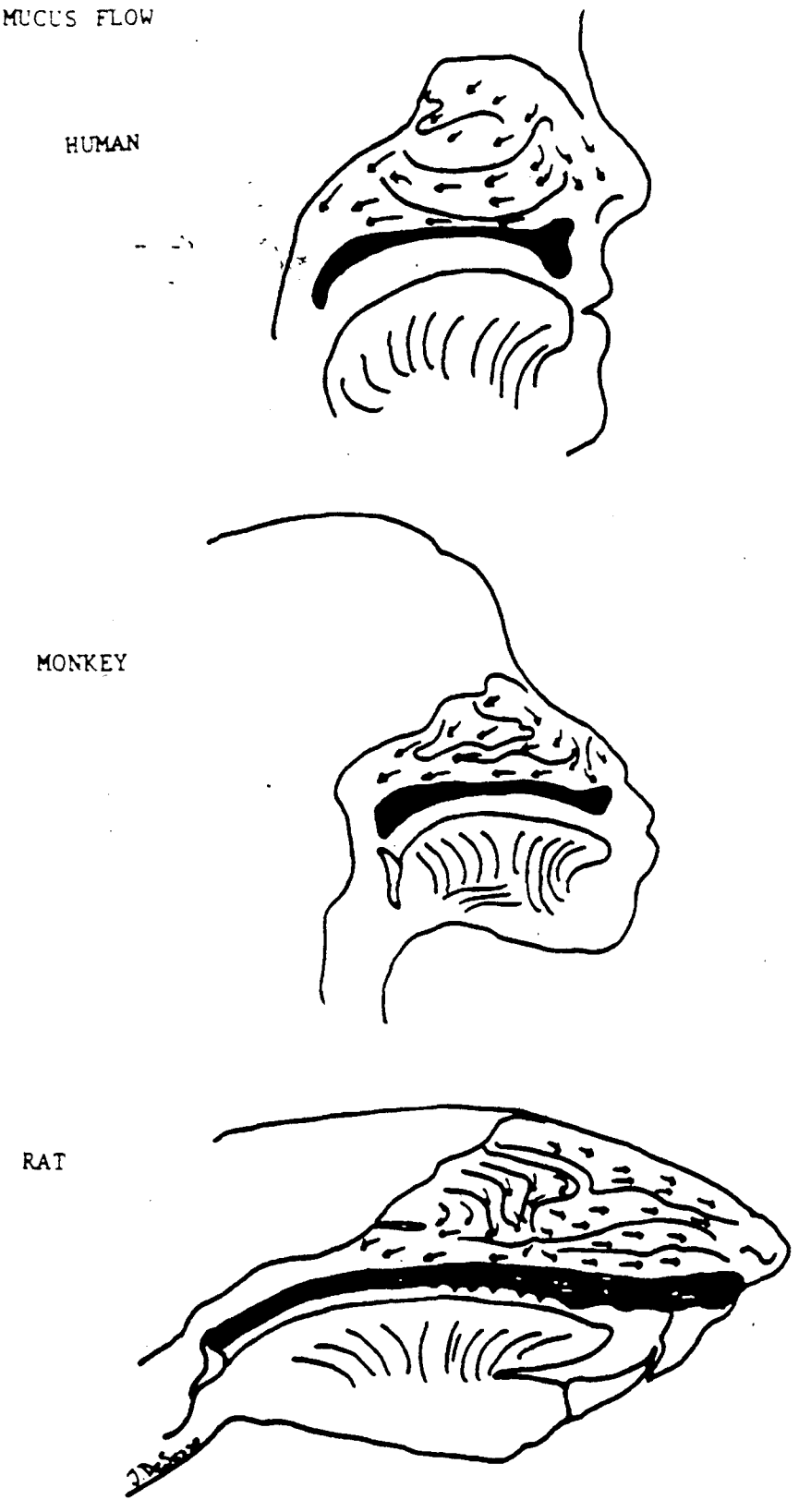

Figure 7. Sagittal sections through the heads of human, monkey, and rat to illustrate the flow of mucus in the nasal passages. The predominant direction of mucus flow in human and monkey is toward the posterior of the nasal passage. In rat, a greater amount of mucus flow is directed anteriorly along the lateral nasal wall and septum toward the nostril.

laden secretions to the vestibule where the secretions may be removed by licking or sneezing (Proctor and Chang, 1983).

It should be noted that the secretions of the respiratory mucosa form a physiological barrier which inhaled foreign materials (dust particles, aerosol droplets, vapors, etc.) must traverse before they can interact with the cells of the nasal mucosa. The nasal secretions are aqueous fluids that contain a continuous external layer of mucus, which is a glycoprotein. Aqueous soluble, reactive toxicants are likely to be dissolved in, and to react with, the secretions. Such toxicants are unlikely to gain access to the nasal mucosae until the potential targets in the nasal secretions are overwhelmed. Consequently, the large daily volume of nasal secretions are an important part of the nasal cavity's defense mechanism, providing a physical threshold which must be surpassed before exposure can occur.

The secretions of the olfactory region are serous (watery) and primarily serve as a solvent for odiferous materials that enables interaction between the material and the chemoreceptors of the olfactory cells. In contrast to the respiratory region, there are no ciliated cells to rapidly transport the secretions away from their site of origin, although they follow the same transport pathways described above. Not surprisingly, the rate of secretion in the olfactory region is not particularly high.

It is interesting to note that the arterial vascular supplies to the olfactory and respiratory mucosae differ in a way that is consistent with the differences in secretory volumes of the respective mucosae. The respiratory mucosa, which produces the majority of the nasal secretions, receives the larger blood supply (via the large calibre sphenopalatine artery). Not only does the respiratory mucosa receive a greater volume of blood than the olfactory mucosa, but also the superficial capillaries of the respiratory region are fenestrated, thereby further facilitating transudation of plasma, which is the source of the periciliary fluid (Sorokin, 1988).

Airflow

Due to the complex bony framework surrounding and protruding into the nasal cavity, it is difficult to describe the airways of the nasal passages and associated structures in words. Despite the complex folding of the surface of the lateral walls of the nasal passages, several regions have been described by relating the position of the airway to nearby structures such as the turbinate bones and nasal septum (Proctor and Chang, 1983). Figure 8 depicts a coronal section through a generalized human nasal passage to reveal four regions: main (respiratory) airway, olfactory airway, meatuses, and paranasal sinuses. The clear regions between the nasal septum and both the lower portion of the middle turbinate (MT) and the inferior turbinate (IT) are the main airways. The stippled region in the superior regions are the olfactory airways; the horizontally striped territories are the meatuses. Note the overlap of olfactory region with meatus in the vicinity of the superior turbinate (ST). The cross-hatched regions lateral and superior to the nasal cavity are the paranasal sinuses.

During nasal inspiration in humans, it has been determined that most air traverses a corridor between the medial edges of the middle and inferior turbinates and the nasal septum (Negus, 1958; Proctor and Chang, 1983). This corridor corresponds to the main (respiratory) airway in figure 8. The rate of airflow through both the olfactory airway and the meatuses is considerably less than that of the main airway. The rate of air exchange through the openings into the paranasal sinuses is so slight that it can considered to be nonexistent (Negus, 1958).

The regions in the nasal cavities of rats and monkeys are named by similar relationships, however, differences exist in the ancillary structures. Neither monkeys nor rats possess the sphenoid sinuses (SpS) and the ethmoid air cells (EC), and the rat possesses a very small maxillary sinus (MS) (Negus, 1958; Hebel and Stromberg, 1986; Reznik, 1990). During nasal inspiration in the monkey, air traverses corridors that are similar to the human (Negus, 1958; Proctor and Chang, 1983). The situation is more complex in the rat due to the presence of the atrioturbinates and the swell bodies which deflect inspired air upward and laterally into the meatuses and the olfactory airway.

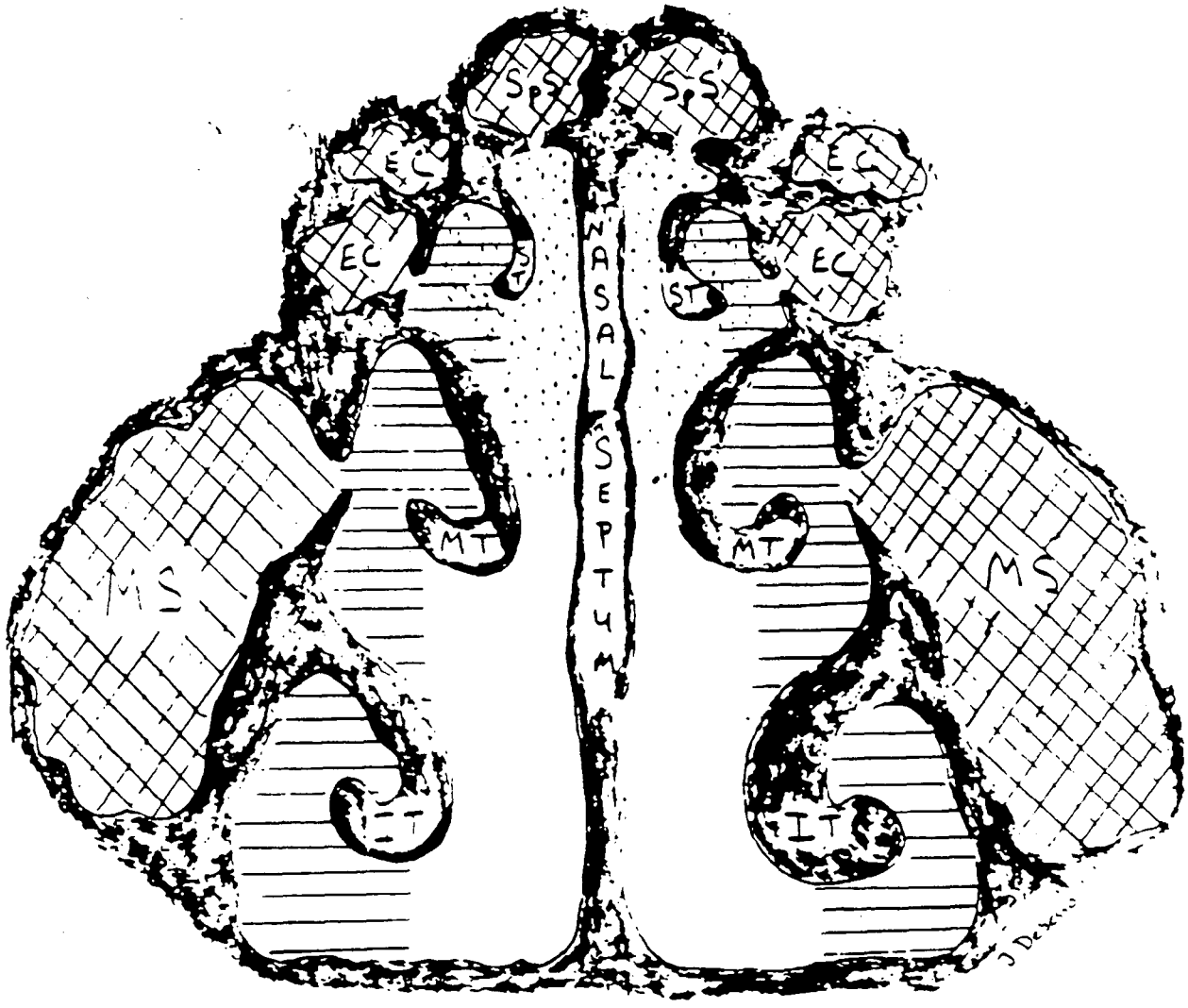

Figure 8. Diagram illustrating the regions of the generalized arrangement of airways in the nasal cavity and ancillary territories. The clear regions are the main airways. The stippled region in the superior regions are the olfactory airways; the horizontally striped territories are the meatuses. The cross-hatched regions lateral and superior to the nasal cavity are the paranasal sinuses. EC = ethmoid air cells; IT = inferior turbinate; MS = maxillary sinus; MT = middle turbinate; SpS = sphenoid sinuses; ST = superior turbinate.

Figure 9 depicts the anterior-posterior routes of airflow through the nasal passages of humans, monkeys, and rats during normal nasal inspiration. In humans, air enters the nasal vestibule travelling cephalically and turns approximately 60 degrees as it enters the nasal cavity proper to traverse the major nasal passage. The air remains close to the floor of the nasal passage between the inferior turbinates and the nasal septum (respiratory region) until it reaches the pharynx where it strikes the posterior wall of the nasopharynx, makes a right angle turn inferiorly, and passes into the larynx and trachea. In monkeys, air enters the nasal passage in a nearly horizontal orientation. The first major structure encountered is the anterior end of the middle turbinate, which projects towards the anterior end of the nose. As in humans, the air traverses the nasal passage close to the floor and between the middle and inferior turbinates. The air strikes the posterior wall of the nasopharynx and turns sharply downward to enter the larynx and trachea. In rats, air enters the nose in a nearly horizontal orientation, but strikes the atrioturbinates just inside the nostril. The atrioturbinates deflect the air laterally and superiorly so that the air traverses the olfactory region of the nasal cavity including the lateral meatus and the upper lamellae of the superior and middle turbinates. At the posterior end of the nasal passage, the air strikes the oblique wall of the respiratory pharynx and makes a turn of about 45 degrees to enter the larynx and trachea.

From the diagrams, it can be predicted that toxicants inspired by humans will first experience a major interaction with tissues at the posterior of the nasal passages in the nasopharynx. Some interaction is also likely to occur with the edge of the inferior turbinate as the toxicants traverse the main airway. In monkeys, the anterior protrusion of the middle turbinate provides the first site of interaction for toxicants As in the human, the nasopharynx will also be a major site for toxicant-tissue interaction. In the case of the rat, the deflection of the inspired air by the atrioturbinates results in turbulence close to the entry into the nasal cavity proper. The toxicant-tissue interaction is likely to occur near the transition from squamous to respiratory epithelium at the region of the lateral meatus.

Recent studies by Morgan et al. (1986b), Heck et al. (1989), and Monticello (1990) provide limited agreement with the predictions of the preceding paragraphs. In these studies, rats and monkeys were exposed for six hours by inhalation to varying concentrations of ^{14}C-labelled

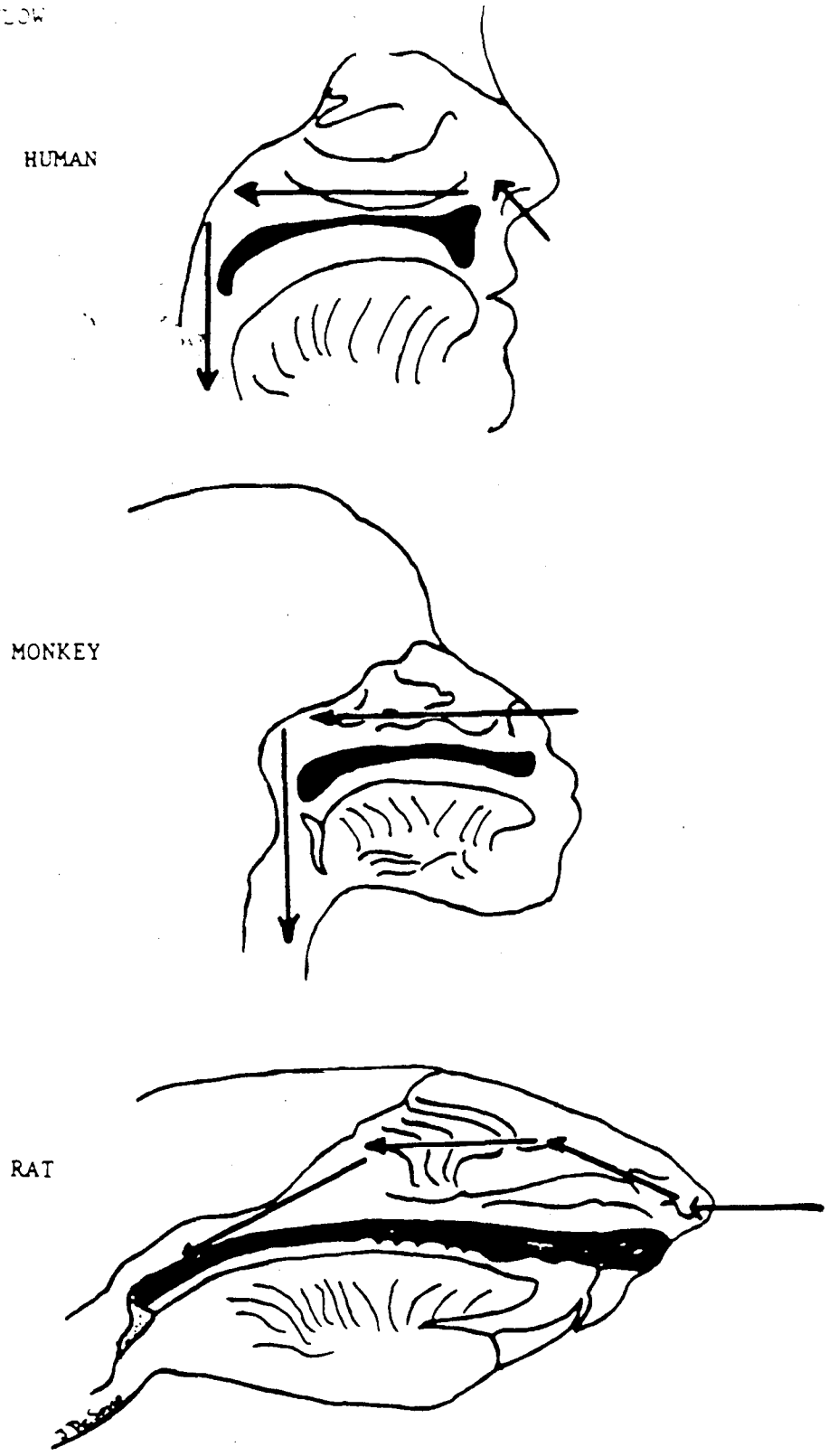

Figure 9. Diagrams illustrating the major inspiratory flow routes of air during normal (nasal) breathing in humans, monkeys, and rats. The airflow in monkeys and humans is predominantly across the lower portions of the nasal passages. In contrast, airflow in the rat is deflected by the atrioturbinates to the superior (olfactory) airway.

formaldehyde, a water soluble toxicant. Following exposure regions of the upper respiratory airways were examined for DNA-protein cross-links as indications for the sites of interaction between tissue and formaldehyde. Briefly, the results showed that, in the monkey, the major sites of formaldehyde-tissue interaction were the anterior portion of the middle and inferior turbinate, and, to a lesser degree, the nasopharynx. In the rat, the major site of formaldehyde-tissue interaction was in anterior portion of the inferior turbinate near the lateral meatus. In both species, the amount of formaldehyde-tissue interaction was a nonlinear function of the exposure concentration. This could be interpreted to mean that the physical barrier of nasal secretions were sufficient to protect both species from formaldehyde at low exposure concentrations, but the barrier was overwhelmed at higher concentrations allowing the interactions to occur.

Data reported by Torjussen et al. (1979) on histopathological changes in the nasal cavities from workers in the nickel refining industry also lend credence to the airflow predictions. They reported a variety of histopathological changes on the anterior curvature of the middle turbinate in active and retired nickel workers. These changes included nasal carcinoma (3/343) and epithelial dysplasia (45/343), which are relatively rare among the general population (control incidences were 0/57 and 1/57, respectively).

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