UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES,
AND TOXIC SUBSTANCES

March 31, 2008

MEMORANDUM:

Subject: Formaldehyde: Toxicology Disciplinary Chapter for the Registration Eligibility Decision (RED) Document.

To: Sharon Carlisle, Chemical Review Manager
Antimicrobials Division

From: Timothy F. McMahon, Ph.D., Senior Toxicologist
Antimicrobials Division

Attached is the Toxicology disciplinary chapter for formaldehyde for the Reregistration Eligibility Decision Document.

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1.0 HAZARD CHARACTERIZATION

Technical grade formaldehyde (37% a.i.) has a moderate order of acute toxicity in experimental animals via the oral and dermal routes (Toxicity Categories II and III). Inhalation toxicity studies on formaldehyde are extensive and include both acute exposures and longer term exposures. Toxicity from acute exposures is characterized by pathology of the respiratory epithelium and has been observed in rats exposed for 4 hours to a concentration of 10 ppm (Bhalla, 1991), while longer term exposures of rats (3 ppm for 6 hours/day for 5 days) also results in respiratory tract lesions (Buckley et al., 1984). Repeated exposure to 40 ppm formaldehyde for 6 hours/day, 5 days/week for 13 weeks results in mortality in 80% of B6C3F1 mice whereas exposure to 20 ppm formaldehyde for the same time period produced no mortality (Maronpot et al., 1986). Formaldehyde is a severe eye and skin irritant (Toxicity Category I) and is positive for dermal sensitization.

In one repeated dose (90 day) oral toxicity study in the rat irritability, weight loss, hair loss, yellowing of teeth, and decreased food consumption were observed at 0.6% formaldehyde in male Hotzman rats. In another 90-day oral toxicity study in the rat (Johannsen et al.,1986), decreased body weight gain was observed at 100 mg/kg/day in male Sprague-Dawley rats.. In a 28-day drinking water toxicity study in rats (Til et al.,1988), decreased protein and albumin levels in blood plasma and histologic changes were observed at 125 mg/kg/day in rats.. In a 90-day oral toxicity study in non rodents (Johannsen et al., 1986), reduced body weight gain was observed in beagle dogs at 100 mg/kg/day.

In a 90-day inhalation toxicity study in the rat (Woutersen et al., 1987) a marked increase in the number of labeled nasal epithelial showing clear squamous metaplasia and hyperplasia was observed at 12 mg/m^3 formaldehyde administered 6 hours/day for 5 days/week. In another 90-day inhalation toxicity study conducted by the Chemical Industry Institute of Toxicology, formaldehyde was administered to 20 mice and rats at concentrations of 4, 12.7, or 38.6 ppm (4.96, 15.74 and 47.84 mg/m^3, respectively), for 6hrs/day, 5 days/week for 13 weeks. The systemic LOAEL was 12.7 ppm (15.74 mg/m^3), based on body weight decrease and nasal erosion.

Developmental toxicity of formaldehyde by inhalation has been examined in the open literature. Saillenfait et al. (1989) exposed Sprague-Dawley rats to 5, 10, 20, and 40 ppm formaldehyde 6 hours/day on gestation days (GDs) 6–10.Ddams exposed to 40 ppm exhibited reduced body weight, indicative of general toxicity. This exposure concentration also led to a significant decrease in fetal body weight (FBW). There was also a slight, albeit statistically significant, decrease (from 5.61 to 5.35 g/litter) in male FBW in dams exposed to 20 ppm formaldehyde. No other significant signs of fetal malformations were reported., In a similar study, Martin (1990) reported the effects of exposure of Sprague-Dawley rats to 2, 5, and 10 ppm 6 hours/day on GDs 6–15. Food consumption and dam weight gain were reduced significantly in dams exposed to 10 ppm formaldehyde. These studies indicate that inhalation of formaldehyde is unlikely to be teratogenic at maternally toxic doses, although high doses may generally be fetotoxic.

In a dermal developmental toxicity study by Overman (1984), pregnant Syrian hamsters were administered 0.5 mL formaldehyde (37% a.i.) 2 hrs/day from gestation day 8

through 11. Treatment had no effect on maternal weight gain. The treatment did not influence fetal C- R length. Mean fetal weight was slightly increased in experimental animals, but the difference was not statistically-significant. No skeletal malformations were found and no other malformations were observed.

Reproductive toxicity of formaldehyde was examined. In one study (MRID 00143291), formaldehyde (40% a.i.) was provided to Beagle dogs in the diet at concentrations of 0, 3.1 or 9.4 mg/kg/day on gestation days 4 through 56. There were no formaldehyde-related effects in any of the parameters other than pup weights, which were lower by group in litters of dams exposed to formaldehyde Cassidy et al. (1983) administered single oral doses of 100 or 200 mg/kg to five male Wistar rats/group. Testes from these animals and 20 controls were excised and examined for spermatogenic abnormalities 11 days after dosing. Although no significant toxicological effects of formaldehyde on total sperm counts were observed at either tested dose, an increased incidence (19%) of testicular sperm head counts was observed in rats exposed to 200 mg/kg-day formaldehyde. The percentage of abnormal sperm heads also significantly increased (5%) in the 200 mg/kg-day dose group compared to controls. These data suggest that formaldehyde can induce morphological abnormalities in the germ cells of male experimental animals at dose levels that did not significantly affect testis weights or sperm counts.

Chronic toxicity and carcinogenicity of formaldehyde has been examined in several studies. In one study (Kerns et al., 1983), groups of F344 rats and C57BL/6 x C3H F_1 ($B6C3F_1$) mice (approximately 120/sex/concentration) were exposed to 0, 2.0, 5.6, and 14.3 ppm formaldehyde gas, 6 hours/day, 5 days/week for 24 months. Lesions in the nasal cavity were the primary formaldehyde related effect in both mice and rats throughout the study. However, examination of the histopathology tables also suggested an increase in mouse lymphomas and rat leukemia in female animals.

In a chronic toxicity study conducted by Battelle, Pacific Northwest laboratories B6C3F1 mice (5/sex/group) were exposed to one of five concentrations of vaporized formaldehyde for a period of 6 hours per day at target concentrations of 15, 25, 50, 100, and 200 ppm (18.59, 30.98, 61.96, 123.93, and 247.85 mg/m^3). Mild supportive rhinitis was observed in the 18.59 mg/m^3 dose level dose level while necrosis and sloughing of the mucosa in the turbinates, trachea, and proximal bronchi were seen the 61.96 mg/m^3 animals. Male and females rats exposed at 15 ppm formaldehyde exhibited increased bone marrow hyperplasia beginning at 20 months It is not known if bone marrow hyperplasia may be indicative of a myeloproliferative or lymphoproliferative disorder, or a regenerative response to gross tissue injury at the point of entry. Squamous cell carcinoma (SCC) was also observed at the two highest dose levels in this study.

The Chemical Industry Institute of Toxicology (CIIT) performed a second bioassay on inhaled formaldehyde in 9-week-old male F344 (CDF[F344]/CrlBr) rats (Monticello et al., 1996). The rats were exposed 6 hours/day, 5 days/week for 24 months to 0, 0.7, 2.0, 6.0,

10.0, and 15.0 ppm. Nasal neoplasms included SCC and polypoid (transitional) adenomas and were similar in morphological characteristics to those described in the Kerns et al. (1983) chronic bioassay. The incidence of SCC was increased at 6 ppm and above, with a NOAEL of 2 ppm for this effect.

In a chronic toxicity study in the rat (Kamata et al., 1997), male Fischer 344 rats were exposed via the inhalation route to formaldehyde (37% a.i.) at concentrations of 0, 0.3, 2, or 15 ppm (0, 0.4, 2.5, or 19 mg/m^3), 6hr/day, 5 days/week. A highly nonlinear response for SCC and proliferative lesions in the nasal cavity was observed in animals exposed to 15 ppm formaldehyde, while animals in the 2 ppm group showed a statistically significant increase in some epithelial lesions.

In studies by Hauptmann et al. (2003, 2004), retrospective cohort mortality studies of U.S. workers involved in the production or use of formaldehyde was examined. These studies were large epidemiology studies, and provided individual quantitative exposure estimates for the workers. The NCI cohort consisted of 25,619 workers (88% male) employed in any of the 10 plants prior to 1966; the current follow-up analyzes 8,486 deaths (178 attributed to lymphohematopoietic malignancy and 9 to nasopharyngeal cancer). A detailed exposure assessment was conducted for each worker based on exposure estimates for different jobs held and tasks performed (Stewart et al., 1986). Exposure estimates were made using several different metrics - peak exposures, average intensity, cumulative exposure, and duration of exposure. Respirator use and exposures to formaldehyde particles and other chemicals were also considered. Significant increases in relative risk for lymphohematopoietic cancer were observed primarily for myeloid leukemia and Hodgkin's disease and for the peak exposure and average intensity exposure metrics. For the nasopharyngeal cancers, significant trends were observed for the cumulative and peak exposure metrics.

Based of the on going development of the science to predict carcinogenic potential of formaldehyde within EPA, OPP has decided to present the formaldehyde cancer risks for the pesticidal uses using both the existing 1991 IRIS cancer unit risk of 1.3 E-5 per ($\mu g/m^3$) and the CIIT biologically-based dose-response (BBDR) model until any new cancer estimates are fully peer reviewed. OPP also acknowledges the wide range in cancer risks using these approaches and will coordinate with other offices in EPA on the outcome of the upcoming peer review process on the carcinogenicity of formaldehyde. The formaldehyde IRIS assessment is scheduled to be begin internal review in May 2008 and is scheduled to start external peer review in January 2009. Because formaldehyde air concentrations approach those associated with ocular and respiratory tract irritation, the risk mitigation measures to be implemented in the meantime for the pesticidal uses will be based on mitigating the non-cancer effects at a limit of 0.01 ppm. It is believed that this level will reduce exposures sufficiently such that the cancer risks would not be of concern. The EPA process of regulating pesticides allows for reevaluation at any time if new information from the peer review process of the carcinogenic potential of formaldehyde warrants.

Formaldehyde's mutagenicity has been examined in a variety of in vitro and in vivo test systems. In a bacterial reverse mutation test (MRID 00132156), formaldehyde (2%) was tested at concentrations of 0.001, 0.01, 0.10, 1.0, or 5.0 μL and found to be negative. In a second submitted study (MRID 00132157), formaldehyde (2%) was tested at concentrations of 3.0, 15.0, 75.0, 150, or 300 μg/plate and found to be positive in the bacterial reverse mutation assay. Formaldehyde caused a positive response (3.2-fold increase) on tester strain TA98 without metabolic activation. A 1.9-fold increase was observed on TA98 with metabolic activation. Also, increases of 2.2-fold and 1.7-fold were observed on tester strain TA100 with and without activation, respectively. In an in vitro mammalian chromosome aberration test (MRID 00132168), formaldehyde (37% formalin), was tested on Chinese hamster ovary cells at concentrations of 28.43, 37.91, or 50.55 nL/mL. The test article caused a significant dose-dependant increase in the frequencies of chromosome aberrations in the Chinese Hamster Ovary cells, both with and without S-9 activation. One submitted study (MRID 00132169), tested formaldehyde (37%) for Unscheduled DNA synthesis (UDS) in Primary rat liver hepatocytes. The test material was tested at concentrations of 0.0005, 0.001, 0.005, 0.01, 0.02, or 0.04 μL/mL and found to cause no significant increase in UDS in rat hepatocytes.

In published studies, formaldehyde has shown both positive and negative results in the Ames Salmonella assay (Donovan et al., 1983; Connor et al., 1983, 1985; Frei et al., 1984; Fiddler et al., 1984; Oerstavik and Hongslo, 1985; Takahashi et al., 1985; Schmid et al., 1986; Zielenska and Guttenplan, 1988; Le Curieux et al., 1993; O'Donovan and Mee (1993) Watanabe et al., 1996; Dillon et al., 1998; Ryden et al., 2000; Wilcox et al., 1990; Jung et al., 1992; Marnett et al., 1985; Mueller et al., 1993).

Temcharoen and Thilly (1983) examined the capacity of formaldehyde to induce forward mutations to 8-azaguanine resistance in S. typhimurium TM 677, a his^+ revertant of TA 1535. Both toxicity and mutagenicity were obtained at formaldehyde concentrations of 0.17 mM in the absence of S9 and 0.33 mM in the presence of S9 Dillon et al. (1998) employed Salmonella strains TA102 and TA104 because they are more sensitive to oxidative mutagens. Formaldehyde was mutagenic in both strains, as well as in TA100. However, the authors reported that the mutagenic activity was not reduced in TA104 in the presence of S9 from either Aroclor-induced male Fischer F 344 rats or male $B6C3F_1$ mice.

In another study, formaldehyde induced forward mutations to trifluorothymidine resistance in mouse lymphoma L5178Y $tk^{+/-}$ cells both in the absence and presence of rat liver S9 (higher concentrations required for effect with S9). Both toxicity and mutagenicity were abolished when formaldehyde dehydrogenase was incorporated in the exposure medium (Blackburn et al., 1991).

Ross and Shipley (1980) used a [^{14}C]-thymidine-incorporated mouse L1210 cell line to monitor formaldehyde-induced DNA strand breaks and DPX. Single strand breaks (SSB) and DNA-protein cross links were induced by formaldehyde, with SSB at concentrations greater than 200 □M and a reduction of radiation-induced breaks (indirect measure of DPX) at 50 □M. Formaldehyde-induced DPX were repaired 24 hours after the compound was removed from the culture.

In vivo, no treatment-related increase in either micronuclei or chromosome aberrations were observed following intraperitoneal exposure to formaldehyde at 0, 6.25, 12.5, or 25 mg/kg. (Natarajan et al. (1983)). Similarly, chromosomal analysis of spermatocytes at metaphase I did not reveal any chromosomal lesions in Q strain mice injected intraperitoneally with 50 mg/kg of the compound (Fontignie-Houbrechts, 1981). Exposure of male and female Fischer F-344 rats to 0.5, 6, or 15 ppm (0.6, 7.4, 18.5 mg/m^3) formaldehyde by inhalation for 6 hours/day for 5 days showed no increases in either SCE or chromosome aberrations at any dose level (Kligerman et al. (1984)) .

In a neurotoxicity screening battery (Malek et al., 2003a), rats were exposed to 0, 1.0, 2.5, or 5.0 ppm (0, 1.23, 3.08, or 6.15 mg/m^3) formaldehyde for 2 hours and locomotor activity was assessed for 1 hour in an open field 2 and 24 hours after termination of formaldehyde exposure. Reductions in horizontal movements (crossed quadrants) were observed after two hours at 1.0 ppm. In another neurotoxicity study (Malek et al., 2003b), rats (10 per group) were exposed at 0, 0.1, 0.5, or 5.0 ppm (0, 0.123, 0.615, or 6.15 mg/m^3) formaldehyde for 2 hours and open field behavior tests were conducted on each animal 2 hours after formaldehyde exposure. Significant reductions in motor activity in males were observed at 1.0 ppm after 2 hours. In a third neurotoxicity study (Pitten et al., 2000), adult male Wistar rats were exposed to 0 ppm, 2.6 ppm (0.25% formaldehyde solution to yield 3.06 ± 0.77 mg/m3), or 4.6 ppm (0.70% formaldehyde solution to yield 5.55 ± 1.27 mg/m3) formaldehyde, 10 minutes/day, 7 days/week for 90 days. The animals were assessed for performance in the maze every seventh day, at least 22 hours after the exposure on the previous day. Neurotoxicity was observed at 2.6 ppm**,** based on statistically significant performance errors as compared to the control group and increased run times through the maze. In a behavioral and neurotoxicity study conducted by Boja et al., 1985, Sprague-Dawley rats were exposed to either air or formaldehyde at concentrations of 5, 10, or 20 ppm (6.20, 12.39, or 24.79 mg/m^3) via inhalation for 3 hours on two days. Exposure to 5 ppm (6.20 mg/m^3) formaldehyde resulted in statistically significant decreased motor activity within 15 minutes

From the ATSDR Toxicological review on formaldehyde (ATSDR, 1999), formaldehyde is rapidly metabolized primarily by formaldehyde dehydrogenase, a widely distributed enzyme present in all tissues, particularly nasal mucosa. Unmetabolized formaldehyde can form cross-links between proteins and between protein and DNA. Jeffcoat et al. (1983) examined disposition of dermally applied formaldehyde in rats, guinea pigs, and monkeys and observed between 5-8% excretion in urine of rats and guinea pigs and 0.7-1.5% excretion in feces. Excretion in monkeys was less than 1% of the applied dose by all routes. Trapped expired air constituted the largest percentage of excretion in rats and guinea pigs (21-24% of the administered dose).

2.0 TOXICOLOGY DATA

The available toxicology data for Formaldehyde is listed below.

Table 1. Toxicology Data for Formaldehyde

Test		Technical		
		MRID	Required	Satisfied
870.1100	Acute Oral Toxicity	00058054, 00065508	yes	yes
870.1200	Acute Dermal Toxicity	00058054, 00065508, 00159395	yes	yes
870.1300	Acute Inhalation	Open Literature	yes	yes
870.2400	Primary Eye Irritation	00058054, 00065508, 00159395	yes	yes
870.2500	Primary Dermal Irritation	00058054, 00065514, 00159392	yes	yes
870.2600	Dermal Sensitization	40161103, Open Literature	yes	yes
870.3100	90-day oral (Rodent)	00124677, 00134114, Open Literature	yes	yes
870.3150	90-day oral (Non-rodent)	Open Literature	yes	yes
870.3250	90-Day Dermal (Rat)	-	yes	no
870.3465	90-Day Inhalation (Rat)	00082134,	no	
		00149755, Open Literature	yes	yes
870.3700	Developmental Toxicity (Rat)	00082136, 00123769, 00123770, 00164652, Open Literature	yes	yes
870.3700	Developmental Toxicity (Rabbit)	-	yes	no
870.3800	Reproduction	00143291, Open Literature	yes	yes
870.4100a	Chronic (Rodent)	Open Literature	yes	yes
870.4100b	Chronic (Non-rodent)	-	no	
870.4200a	Carcinogenicity (Rat)	00143288, Open literature	yes	yes
870.4200b	Carcinogenicity (Mouse)	Open Literature	yes	yes
870.5100	Mutagenicity – Bacterial Reverse Gene Mutation assay	00132156, 00132157, Open Literature	yes	yes
870.5385	Mutagenicity – Bone marrow chromosome aberration test (Mouse)	-	yes	no
870.5550	Mutagenicity – Unscheduled DNA synthesis in primary rat hepatocytes	00132169	yes	yes

Test		Technical		
		MRID	Required	Satisfied
870.6200	90-day neurotoxicity (Mammal)	Open Literature	yes	yes
870.7485	Metabolism	Open Literature	yes	yes
870.7600	Dermal penetration	Open Literature	yes	yes

3.0 DATA GAPS

90-day dermal toxicity study (rodent)
Developmental toxicity in non –rodents
Mutagenicity – Bone marrow chromosome aberration test (mouse)

4.0 HAZARD ASSESSMENT

4.1 Acute Toxicity

Adequacy of database for Acute Toxicity: The acute toxicity database for formaldehyde is considered complete. Technical grade formaldehyde (37% a.i.) has a moderate order of acute toxicity in experimental animals via the oral and dermal routes (Toxicity Categories II and III). Inhalation toxicity studies on formaldehyde are extensive and include both acute exposures and longer term exposures. Toxicity from acute exposures is characterized by pathology of the respiratory epithelium and has been observed in rats exposed for 4 hours to a concentration of 10 ppm (Bhalla, 1991), while longer term exposures of rats (3 ppm for 6 hours/day for 5 days) also results in respiratory tract lesions (Buckley et al., 1984). Repeated exposure to 40 ppm formaldehyde for 6 hours/day, 5 days/week for 13 weeks results in mortality in 80% of B6C3F1 mice whereas exposure to 20 ppm formaldehyde for the same time period produced no mortality (Maronpot et al., 1986). Formaldehyde is a severe eye and skin irritant (Toxicity Category I and is positive for dermal sensitization.

The acute toxicity data for Formaldehyde is summarized below in Table 2.

Table 2. Acute Toxicity data for Formaldehyde technical a.i.

Guideline Number	Study Type/ Test substance (% a.i.)	MRID Number/ Citation	Results	Toxicity Category
870.1100 (§81-1)	Acute Oral – Guinea Pig Purity 37.3% - Formaldehyde	00058054	LD_{50} = 260 mg/kg	II
870.1200 (§81-2)	Acute Dermal – Rat Purity 37.3% - Formaldehyde	00058054	LD_{50} = 300 mg/kg	II

Table 2. Acute Toxicity data for Formaldehyde technical a.i.				
Guideline Number	**Study Type/ Test substance (% a.i.)**	**MRID Number/ Citation**	**Results**	**Toxicity Category**
870.1200 (§81-2)	Acute Dermal – Rat Purity 37.0% - Formaldehyde	00159395	LD_{50} > 4.9 mL/kg (4.53 g/kg)	III
870.1200 (§81-2)	Acute Dermal – Rabbit Purity 37.3% - Formaldehyde	00058054	LD_{50} = 240 mg/kg	II
870.1200 (§81-2)	Acute Dermal – Dog Purity 37.3% - Formaldehyde	00058054	LD_{50} = 550 mg/kg	II
870.1300 (§81-3)	Acute Inhalation – Mouse and Rat	See Open Literature studies in Toxicity Profile for Formaldehyde		
870.2400 (§81-4)	Primary Eye Irritation Purity 37.3% - Formaldehyde	00058054	Severe eye irritant	I
870.2500 (§81-5)	Primary Dermal Irritation Purity 37.3% - Formaldehyde	00058054	Formation of vesicles with superficial necrosis or nodules.	I
870.2600 (§81-6)	Dermal Sensitization – Guinea pigs Purity 40.0% - Formaldehyde	40161103	Extreme Sensitizer	NA

NA – Not Applicable

4.2 Subchronic Toxicity

Adequacy of database for Subchronic Toxicity: The database for subchronic toxicity consists of guideline and open literature studies.

870.3100 Subchronic (90-day oral) Toxicity – Rat

In one 90-day oral toxicity in the rat (MRID 00124677) formaldehyde (37% a.i.) was administered orally at 0, 0.3, 0.6, 1.2 or 2.4 % to 10 male Holtzman rats/dose. A **NOAEL was established at 0.3% while the LOAEL was 0.6%**, based on irritability, weight loss, hair loss, yellowing of teeth, and decreased food consumption.

Rats exposed to concentrations of 0.6% formaldehyde and higher exhibited dose-related increases in irritability, disability, hair loss, and yellowing of teeth and dose-related decreased food consumption. Growth rates significantly different from controls are expected at formaldehyde concentrations ≥ 0.50 %, believed due to decreased food consumption.

870.3100 Subchronic (28-day oral) Toxicity – Rat

In a 28-day oral toxicity study in the rat (MRID 00134114), formaldehyde (60% a.i.) was administered to 10 male Sprague-Dawley rats/dose at concentrations of 0, 79, 158 or 316 µg/kg/day, once daily, 5 days/week for four weeks.

Statistical evaluation of overall body weight gains and total food consumption revealed no significant differences between the control group and test groups. The appearance and behavior of the test rats were comparable to those of the control rats. No pathological findings associated with the oral administration of the test substance were observed.

One rat exposed to158 uL/kg/day died during the 4th week. Autopsy revealed a pale, mottled liver. Three rats receiving the high dose of formaldehyde showed slight salivation during the 4th week of the study.

870.3100 Subchronic (90-day oral) Toxicity – Rat

In an oral subchronic toxicity study conducted by Johannsen et al. (1986) Groups of albino Sprague-Dawley rats (15/sex) were administered the equivalent of 0, 50, 100, or 150 mg/kg-day paraformaldehyde (95 % a.i.) in their drinking water for 91 consecutive days. Rats were observed at frequent intervals for behavioral reactions. Body weights and food and water intake were recorded on a weekly basis. Hematology (HCT, Hb, total and differential leukocyte counts), clinical chemistry [blood sugar, BUN, ALP, glutamic oxalacetic transaminase (GOT),), and urine analyses (color, appearance, pH, specific gravity, sugar, protein, and microscopic elements) were conducted on 10 male and 10 female rats selected from each test group. Tissue weights were recorded for the adrenals, gonads, hearts, kidneys, livers, lungs, and thyroids. Histopathology was performed on a set of over 20 tissues and organs from rats in the high-dose and control groups.

No deaths or abnormal reactions were observed in rats administered formaldehyde for 90 days. Significant reductions in weight gain were observed in both sexes at 150 mg/kg and in male rats given 100 mg/kg. There was a dose-related decrease in liquid consumption in both male rats (9%, 18%, and 31%) and females (13%, 22%, and 30%) administered formaldehyde in their drinking water. There were no overall differences in mean food intake or feed efficiency in rats at any test level, thus reductions in body weight gain are considered to be a reflection of systemic effects of formaldehyde. No statistically-significant differences were observed in hematologic parameters in any treated rats. No specific treatment-related effects were observed on any organ or tissue, including possible target organs like the kidney, liver, and lung. Clinical chemistry and urinalysis studies failed to indicate any necrotic effects on muscle, kidney, liver, or heart. No differences were apparent between absolute or relative organ weights of treated rats. No treatment-related pathological changes were observed microscopically.

A s**ystemic toxicity NOAEL of 50 mg/kg/day for males and 100 mg/kg/day for females was established while the systemic NOAEL was set at 100 mg/kg/day for males and 150**

mg/kg/day for females, based on decreased body weight gain.

870.3100 Subchronic (90-day oral) Toxicity – Rat

Til et al. (1988) evaluated the oral toxicity of formaldehyde and acetaldehyde in a subacute study in Wistar (Cpb:WU; Wistar random) rats. Groups of rats (10/sex/dose) were exposed to acetaldehyde or formaldehyde at 0, 25, 125, and 625 mg/kg-day or 0, 5, 25, and 125 mg/kg-day in drinking water for 4 weeks. The control group was comprised of 20 rats of each sex, and, to account for potential effects of decreased water consumption in treated animals, an additional control group of 10 male and 10 female rats was given drinking water in an amount equal to the amount of liquid consumed by the group given the highest dose. Fresh solutions of test concentrations were prepared weekly and stored at 4 ºC until used. Endpoints examined included food and water intake, body weight, daily observations for condition and behavior, organ weights, hematological and clinical chemistry parameters, urinalysis, gross pathology, and histopathology. Weights were recorded for all major tissues. Histopathological examination was restricted to the established major target tissues: liver, kidneys, GI tract, and nose (six standard cross sections). Examination of the GI tract was performed in all dose groups and included the tongue, esophagus, and stomach. Histopathology for the other tissues was performed on high dose and control animals.

"The rats appeared to be healthy throughout the study, and no effects on growth occurred despite significant decreases in food and water intake that occurred at the high dose (125 mg/kg-day). Yellow discoloration of the fur occurred in the rats on the high dosage from week 3 onwards. There were no significant changes in hematology among the exposed groups except for slight (not statistically different) increases in packed cell volumes in the water-restricted group and in high-dose males. The high-dose groups of the formaldehyde-exposed and in the water-restricted controls had slightly increased urine density, but again this was not statistically significant. Plasma TP and ALB levels were decreased in the males of the highest-dose group. No changes in tissue organ weights occurred with the exception that relative kidney weights were slightly increased in the females of the high-dose group. Gross pathological findings were restricted to the GI tract and revealed a thickening of the limiting ridge of the forestomach in all animals exposed at the highest dose that was accompanied by a yellowish discoloration of the mucosa. These latter changes were not observed in the acetaldehyde-exposed animals. Treatment-related histopathological changes were seen in the GI tract only. As shown in Table 5-29, slight (8/20) or moderate (12/20) focal hyperkeratosis of the forestomach and slight focal atrophic gastritis occurred in animals of the high dose groups only. One female had moderate focal papillomatous hyperplasia. No histopathological changes were observed in any animals of the lower dose groups. The study established a LOAEL and NOAEL for epithelial changes in the GI tract of male and female Wistar rats exposed to formaldehyde in drinking water of 125 mg/kg-day and 25 mg/kg-day, respectively. "

870.3150 Subchronic (90-Day oral) Toxicity in nonrodents – Dog

In a 90-day oral toxicity study in non rodents (Johannsen et al., 1986), paraformaldehyde (95% a.i., aqueous) was administered to four Beagle dogs/sex/dose at doses of 0, 50, 75, or 100 mg/kg/day.

No deaths or abnormal reactions were observed. Significant reductions in weight gain were observed in both sexes at 100 mg/kg/day. Treated animals had reduced food consumption and feed efficiency even at the lower dosages (50 and 75 mg/kg/day) which did not depress weight gain. Hematological values from treated dogs fell within normal limits. No specific treatment-related effects were observed on any organ or tissue, including possible target organs like the kidney, liver, and lung.

The **NOAEL was 75 mg/kg/day while the LOAEL was established to be 100 mg/kg/day** for both males and females, based on reduced weight gain.

870.3465 Subchronic (90-Day Inhalation) Toxicity

An earlier cross-species study examined changes in lung tissue resulting from continuous exposure but did not evaluate effects on the nasal mucosa (Coon et al., 1970). Animals were exposed to 3.7 ppm formaldehyde (4.6 mg/m3) for 90 days. Five species of animals were studied: male and female Sprague-Dawley and Long-Evans derived rats (15), male and female Princeton-derived guinea pigs (15), male New Zealand albino rabbits (three), male squirrel monkeys (Saimiri sciureus) (three), and purebred male beagle dogs (two). Blood samples were taken for hemoglobin (Hb) concentration, hematocrit (HCT), leukocyte counts, and serum levels of blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and LDH. Sections of heart, lung, liver, kidney, and spleen were fixed and examined from each species (details of method not provided). Brain, spinal cord, and adrenal tissue also were examined in monkeys and dogs as well as thyroid from dogs. Liver and kidney sections were stained for reduced nicotinamide adenine dinucleotide, lactate, isocitrate, and ß-hydroxybutyrate. Tissue sections of the nasal mucosa were not examined in this study.

Hematological parameters were unaffected by formaldehyde treatment. The lung tissue of all species exhibited interstitial inflammation after 90 days of formaldehyde exposure (detailed description not provided). Formaldehyde-treated rats and guinea pigs also had focal chronic inflammation in heart and kidney tissue sections. However, the authors were uncertain whether the observed changes to heart and kidney were due to formaldehyde exposure.

870.3465 Subchronic (6-Week Inhalation) Toxicity

In a 6-Week inhalation toxicity study (MRID 00149755), Fisher 344 rats – 10/sex/dose; Syrian golden hamsters – 10/sex/dose; and Cynomolgous monkeys – 6 males/dose, were administered formaldehyde (4.96% a.i.) at concentrations of 0, 0, 0.20, 1.00, or 3.00 ppm equivalent to 0, 0, 0.19, 0.98 and 2.95 ppm, respectively, for 26 weeks.

Treatment-related effects during the study were not seen. Compared to controls, monkeys receiving 1.00 ppm showed increased incidence of dried material around the nose, increased incidences of hoarseness and congestion.

Body weight
Compared to controls, no significant body weight changes were seen for monkeys and hamsters throughout the study. The 3 ppm male and female rats showed significant differences ($p \leq 0.01$) from week 2-26 compared to controls.

Organ weight
Organ weights for monkeys and hamsters were not significantly different compared to controls. Male and female rats in the 0.2 ppm group had significant mean heart weight depression ($p \leq 0.01$) compared to the control. Males in the 3.0 ppm test group had significantly ($p \leq 0.01$) depressed mea absolute heart and kidney weights compared to the controls, but the relative weights of these same tissues were significantly increased for these same rats. Females in the 3.0 ppm test group had significantly ($p \leq 0.01$) depressed absolute heart weights with the mean relative heart weight significantly increased ($p \leq 0.01$). For the 3 ppm group, the mean absolute and relative liver weights were significantly depressed ($p \leq 0.01$) compared to the controls.

Gross and Microscopic Pathology
In monkeys, hamsters and rats, no abnormalities were seen in or attributable to formaldehyde vapors.

870.3465 Subchronic (90-Day Inhalation) Toxicity - Rat

In a 90-Day inhalation toxicity study (Woutersen et al., 1987), male and female albino SPF Wistar rats (10/group) were exposed to 0, 1, 10, or 20 ppm formaldehyde (0, 1.23, 12.3, or 24.6 mg/m3), 6 hours/day, 5 days/week for 13 weeks. Rats were checked daily and weighed weekly. During week 13, blood samples were taken for Hb, packed cell volume, RBC count, and a differential count of leukocytes. Urine samples were taken for density, volume, pH, protein, glucose, occult blood, ketones, and appearance. At sacrifice, blood samples were taken and analyzed for ALB, creatinine, glucose, TP, BUN, and the enzyme activities (AST, ALT, and ALP). GSH and protein content were determined in liver homogenates. Organs were examined and weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, and thyroid. Three longitudinal sections of lungs, trachea, and larynx and six standard cross sections of the nose were taken for microscopic examination. Two rats per exposure group were similarly treated for 3 days and sacrificed 18 hours later, and nasoturbinates were dissected to measure cell proliferation. Nasoturbinates were removed, cultured with [3H]-thymidine for 2 hours, and processed for autoradiography.

No gross pathological changes were seen upon autopsy, but body weights decreased in the both male and female rats at the 20 ppm treatment level. Of the organs weighed, 6 of 11 organs had significantly increased relative rates in male rats exposed to 20 ppm formaldehyde (not detailed). Relative brain weight was increased in female rats at the same

treatment level.

Clinical chemistry parameters of liver and kidney function and hematological parameters were also measured after the 13-week treatment by Woutersen et al. (1987). Compared to controls, activities of AST, ALT, and ALP were significantly elevated in plasma from the 20 ppm treated male rats (by 124%, 132%, and 126%, respectively, $p<0.05$). Total plasma protein was reduced to 95% of controls in the same animals. Although there was an observed increase in BUN in male rats treated with 1 ppm, this was not considered a treatment effect. Furthermore, no statistically significant differences were seen for these parameters in female rats at any concentration level. Likewise, plasma ALB, plasma creatinine, plasma glucose, total liver protein, GSH, and relative liver weight were unchanged in all treatment groups.

Cell proliferation was measured in the respiratory epithelium of rats treated for 3 successive days to the same treatment levels of formaldehyde, 0, 1, 10, and 20 ppm. There was an increase of 37.6% in [3H]-thymidine incorporation (indicating proliferation) in visibly metaplastic epithelium resulting from exposure to 20 ppm formaldehyde, compared to a level of 2.8% seen in unaffected tissue. However, there were only two rats per treatment level. Statistically significant increases were seen in focal respiratory epithelial hyperplasia and keratinization in both male and female rats at the highest treatment level (20 ppm). Male rats also had statistically significant increases in observed respiratory epithelial squamous metaplasia, focal olfactory epithelial thinning, and rhinitis. Both male and female rats treated with 10 ppm formaldehyde showed statistically significant increases in squamous metaplasia, hyperplasia, and keratinization of the respiratory epithelium.

Disarrangement of the respiratory epithelium was only significantly increased in female rats, but this change was observed at both the 10 ppm and 20 ppm treatment levels. Although some lesions were observed in animals treated with 1 ppm formaldehyde, their incidences were not statistically significant and the findings equivocal. A systemic toxicity **NOAEL was established at 1.2 mg/m^3 and the LOAEL was fixed at 12 mg/m^3,** based on a marked increase in the number of labeled cells, practically all of which were present in areas of the epithelium showing clear squamous metaplasia and hyperplasia.

870.3465 Subchronic (90-Day Inhalation) Toxicity

Appelman et al. (1988) studied the effects of bilateral intranasal electrocoagulation damage on susceptibility to inhaled formaldehyde in male SPF Wistar (Cpb: WU) rats. Rats were exposed 6 hours/day, 5 days/week for 13 or 52 weeks to 0, 0.1, 1.0, or 10 ppm (0, 0.12, 1.24, or 12.4 mg/m^3) formaldehyde. These concentrations were chosen because the various short-term studies performed in this laboratory showed that formaldehyde was non-cytotoxic to the nasal mucosa at levels of 0.3, 1.0, and 2.0 ppm, slightly cytotoxic at 3 and 4 ppm, and strongly cytotoxic at 10 and 20 ppm (Woutersen et al., 1987; Zwart et al., 1988; Wilmer et al., 1987). Further, because nasal tumors have only been found at exposure concentrations that also induced severe degenerative, hyperplastic, and metaplastic changes in the nasal epithelium (Squire and Cameron, 1984; Griesemer et al., 1985), Feron et al. (1984) and the investigators at the TNO-CIVO Toxicology and Nutrition Institute postulated that formaldehyde at a subcytotoxic concentration was only a very weak initiator without

promoting activity. Appelman et al. (1988) used an electrocoagulation method in this study to evaluate if damage to the mucosa followed by compensatory cell proliferation might render the epithelium vulnerable to subcytotoxic levels of formaldehyde. One half of the rats used in the study (10/group) were damaged bilaterally and then subjected to the first 6-hour exposure to formaldehyde at approximately 20–26 hours after the electrocoagulation procedure. Ten undamaged rats/group were also exposed at each concentration for either 13 or 52 weeks.

General condition and behavior was monitored daily. Individual body weights were recorded at the start of exposure, once weekly during the first month, and monthly thereafter. Blood samples were drawn on all interim sacrifice rats to measure plasma ALB and TP, and the activities of AST, ALT, and ALP. Blood samples drawn on rats of the 53-week sacrifice were examined for Hb, PCV, and differentials. Urine samples collected at the second sacrifice were examined for volume, density, pH, protein, glucose, occult blood, and appearance. The livers of all animals were weighed. The left lateral lobe of each liver was used to determine hepatic protein and GSH content in postmitochondrial fractions. Additional organ weights obtained for animals in the last sacrifice included adrenals, brain, heart, kidneys, lungs, pituitary, spleen, testes, thymus, and thyroid. Histopathological examination included six standard cross-section levels in the nose, livers of all rats killed at 14 weeks and of all control and 10 ppm exposed rats killed in week 53, larynxes, tracheas, and lungs of all rats of the control and 10 ppm exposed rats killed in week 53, and organs and tissues of control and 10 ppm exposed rats with an undamaged nasal mucosa killed in week 53.

Yellow discoloration of the fur occurred in all animals of the two highest exposure groups. Growth retardation was observed in the animals killed with or without damaged noses after 2 weeks exposure to 10 ppm formaldehyde. No toxicologically significant findings in the body weights or organ weights of any animals in the other exposure groups were observed. No relevant differences between groups were found in any of the hematological or urinary parameters with the exception of frequent oliguria ($p < 0.05$) in the top exposure group without nasal coagulation and killed in week 53. Three-way ANOVA revealed a significant increase in TP content of the liver in rats with damaged noses as compared to rats with undamaged noses, and there was a significant negative correlation between the formaldehyde exposure level and TP in these same rats. Hepatic GSH were positively correlated with both nasal damage and age of the animals.

The systemic toxicity **NOAEL was established at 1.24 mg/m^3 while the LOAEL was 12.4 mg/m^3,** based on body weight retardation, incidence of oliguria, and incidence of lesions of the respiratory and olfactory epitheliums for damaged and undamaged animals.

870.3465 Subchronic (90-Day Inhalation) Toxicity – Mice and Rat

In a 90-day inhalation toxicity study conducted by the Chemical Industry Institute of Toxicology, formaldehyde was administered to 20 mice and rats at concentrations of 4, 12.7, or 38.6 ppm (4.96, 15.74 and 47.84 mg/m^3, respectively), for 6hrs/day, 5 days/week for 13 weeks.

No adverse effects observed in the 4 ppm group. At 12.7 ppm, a decrease in body weight and evidence of nasal erosion in two exposed rats was observed. Ulceration and necrosis of the nasal mucosa seen at 38.6 ppm resulted in termination of exposure after 2 weeks. **The systemic NOAEL was 4 ppm (4.96 mg/m^3, LDT) while the systemic LOAEL was established to be 12.7 ppm (15.74 mg/m^3)**, based on body weight decrease and nasal erosion.

870.3465 Subchronic (90-Day Inhalation) Toxicity

In a 90-day inhalation toxicity study (Citation not available), formaldehyde was administered to 25 rats at concentrations of 0.0098, 0.028, 0.82 or 2.4 ppm (0.012, 0.035, 1.03 and 2.97 mg/m^3, respectively).

At 2.4 ppm there was a significant decrease in cholinesterase activity; at 2.4 and 0.82 ppm, there was proliferation of lymphocytes and histiocytes in the lungs and some peribronchial and perivascular hyperemia. There were no significant findings at the two lower concentrations. The **systemic activity NOAEL was 0.028 ppm (0.035 mg/m^3) and the Systemic LOAEL was 0.82 ppm (1.02 mg/m^3)**, based on proliferation of lymphocytes, histiocytes in the lungs, perivascular hyperemia. The **cholinesterase (ChE) NOAEL was 0.82 ppm (1.02 mg/m^3) while the ChE LOAEL was 2.4 ppm (2.97 mg/m^3),** based on a significant decrease in cholinesterase activity at this dose level.

4.3 Prenatal Developmental Toxicity

Adequacy of database for Prenatal Developmental Toxicity: The database for developmental toxicity of formaldehyde is considered incomplete with a developmental toxicity study in non-rodents being unavailable. The database consists of three submitted and two open literature studies in rodents.

870.3700a Prenatal Developmental Toxicity Study – Mouse

Marks et al. (1980) carried out a developmental toxicity study of formaldehyde in CD-1 mice in which 29-35 pregnant animals were gavaged on GDs 6-15 with aqueous formaldehyde (containing 10-15% methanol) at 74, 148, and 185 mg/kg-day. Seventy-six controls were gavaged with water alone. All dams were sacrificed on GD 18 and the number of implantation sites in each uterine horn were counted. The high-dose of formaldehyde was toxic to the dams, as indicated by the deaths of 22 of 34 mice before GD 18. Thus, the dose of 148 mg/kg-day was a NOAEL for maternal toxicity in this study. However, it is unclear to what extent an estimated concurrent dose of up to 75 mg/kg-day methanol may have contributed to this toxic response. To assess the developmental toxicity of formaldehyde, live fetuses were weighed individually, sexed, and examined for external, visceral, and skeletal malformations. Fetuses of surviving high-dose dams and of those of other groups did not show an increased incidence of malformations. Therefore, Marks et al. (1980) concluded that formaldehyde did not induce fetal abnormalities and that the 185 mg/kg-day dose level was a NOAEL for the developmental toxicity of formaldehyde. Neither were the fetotoxic effects

of methanol apparent under the subject experimental conditions.

The maternal toxicity NOAEL was established to be **0 mg/kg/day and the maternal toxicity LOAEL was 74 mg/kg/day, based on decreased body weight gain.**

870.3700a Prenatal Developmental Toxicity Study – Rat

Sallenfait et al. (1989) report a comprehensive and well-documented developmental study in Sprague-Dawley rats. Pregnant rats were exposed beginning on GD 6 in order to cover critical stages of development (e.g., implantation and major organogenesis). Female Sprague-Dawley rats (25/group) were exposed to 0, 5, 10, 20, or 40 ppm formaldehyde (0, 6.15, 12.3, 24.6, or 49.2 mg/m3), 6 hours/day on GDs 6–20. The onset of pregnancy was determined by the presence of sperm in a vaginal smear. Dams were exposed to formaldehyde in a dynamic flow chamber and formaldehyde concentrations were determined to be 5.17 ± 0.51, 9.92 ± 0.88, 20.04 ± 0.88, and 38.96 ± 3.70, respectively. Dams were weighed on GDs 0, 6, and 21 and sacrificed on day 21. Upon examination, uterine weights, fetal weights, sex ratio, and the number of implantation and resorption sites, live and dead fetuses were recorded. Fetuses were examined for external malformations and cleft palate. One half of viable fetuses were sectioned to assess soft-tissue alterations. The other half were fixed, stained with Alizarin Red S, and examined for skeletal alterations.

Body weight gain of dams and body weight of male and female fetuses were reduced by exposure to 40 ppm formaldehyde to 49%, 78%, and 81% of control values, respectively ($p<0.01$) (Saillenfait et al., 1989). Reduced weight gain in dams remained significantly decreased when uterine weight was accounted for ($p<0.01$). Mean fetal weight of male pups was reduced at maternal exposures of 20 and 40 ppm formaldehyde (5.53 g and 4.42 g versus 5.61 g in controls). Decreased fetal body weight in females was only seen at 40 ppm (4.27 g versus 5.24 g in controls.). All other pregnancy endpoints were unchanged by formaldehyde exposure (e.g., uterine weight, implantation and resorption sites, live fetuses, dead fetuses, and sex ratios). No major malformations were noted in fetuses. Some minor soft tissues and skeletal anomalies such as dilated ureter, missing sternebrae, extra fourteenth rib, and rudimentary thirteenth rib (statistics not given), were reported. However, these effects occurred at similar frequencies in control and treatment groups. The incidence of delayed ossification of the thoracic vertebrae was 8.7% in fetuses from the 40 ppm exposure group versus 1.8% in controls. However, this difference was not statistically significant. Overall, formaldehyde was not embryolethal or teratogenic in the studies and only exhibited fetotoxic effects at exposures of 20 ppm and above. These are levels where there was a significant decrease in fetal body weight.

The maternal toxicity **NOAEL was 24.8 mg/m^3 and the LOAEL was established to be 49.6 mg/m^3**, based on decreased body weight gain. The developmental toxicity **NOAEL was 12.4 mg/m^3 and developmental toxicity LOAEL was established to be 24.8 mg/m^3**, based on reduced fetal weight gain.

870.3700a Prenatal Developmental Toxicity Study – Hamster

Experiments by Overman (1985) were aimed at examining the compound's potential for inducing systemic effects in laboratory animals via the dermal route; in this case, the potential of topically applied formaldehyde to induce teratological effects in the progeny of pregnant Syrian golden hamsters. The shaved dorsal surfaces of lightly anesthetized dams were treated with a single dose of 0.5 mL 37% formaldehyde for a 2-hour period on any one of GDs 8, 9, 10, or 11, after which the skin was washed thoroughly with water to remove any remaining compound. Fetuses were dissected from sacrificed dams on GD 15, fixed in Bouin's solution, then examined for visceral malformations or skeletal abnormalities**.** Treatment had no effect on maternal weight gain. The treatment did not influence fetal C- R length. Mean fetal weight was slightly increased in experimental animals, but the difference was not statistically-significant. Two fetuses from the same litter after treatment on day 8 were significantly smaller than their litter mates (>3 SD below mean). The same was true for 2 fetuses from different litters after treatment on day 10. One fetus of normal size treated on day 10 had a subcutaneous hemorrhage in the dorsal cervical region. No skeletal malformations were found and no other malformations were observed.

4.4 Reproductive Toxicity

Adequacy of database for Reproductive: The database for reproductive toxicity of formaldehyde is considered complete with one study in the rat and one study in the dog.

870.3550 Reproductive Toxicity - Dog

In MRID (00143291) Hurni and Ohder tested the developmental toxicity of formaldehyde in 9 to10 pregnant beagle dogs who received the compound in the diet on GDs 4-56. Commercial grade formaldehyde (as a 40% solution) was sprayed on the pellets prior to feeding. Each animal was allotted a diet of 300 g of chow (reduced to 200 g 1 week prior to term) that was promptly consumed (within 5-10 minutes) before the formaldehyde volatilized appreciably. The concentrations of formaldehyde in the chow were 0, 125, or 375 ppm, equivalent to doses of 0, 3.1, and 9.4 mg/kg-day, respectively. Dams were allowed to deliver normally and weight gain, gestation length, the number of litters, litter size, number of live pups, number of pups surviving through weaning, and pup weights weekly for the first 8 weeks were monitored as indices of the potential reproductive/developmental toxicity of formaldehyde. There were no formaldehyde-related effects in any of the parameters other than progressive pup weights, which were lower by group in litters of dams exposed to formaldehyde. A developmental impact of formaldehyde was evident in this strain of dog under the conditions of the experiment. The pup weight decrements were 6.3% for the low-dose dams and 12% for the high-dose. However, no internal or skeletal malformations were observed in any of the 264 live-born and 20 still-born pups, suggesting that formaldehyde had no developmental toxicity in beagles at ingested concentrations as high as 9.4 mg/kg-day.

870.3550 Reproductive Toxicity – Rat

Cassidy et al. (1983) administered single oral doses of 100 or 200 mg/kg to five male Wistar rats/group. Testes from these animals and 20 controls were excised and examined for spermatogenic abnormalities 11 days after dosing. Although no significant toxicological effects of formaldehyde on total sperm counts were observed at either tested dose, an increased incidence (19%) of testicular sperm head counts was observed in rats exposed to 200 mg/kg-day formaldehyde. The percentage of abnormal sperm heads also significantly increased (5%) in the 200 mg/kg-day dose group compared to controls. These data suggest that formaldehyde can induce morphological abnormalities in the germ cells of male experimental animals at dose levels that did not significantly affect testis weights or sperm counts.

4.5 Chronic Toxicity

870.4100a Chronic Toxicity – Rodent

In a chronic toxicity study conducted by Battelle, Pacific Northwest laboratories, B6C3F1 mice (5/sex/group) were exposed to one of five concentrations of vaporized formaldehyde for a period of 6 hours per day for a total of ten exposures. The target concentrations were 15, 25, 50, 100, and 200 ppm (18.59, 30.98, 61.96, 123.93, and 247.85 mg/m^3).

Concentrations of 123.93 mg/m^3 or greater produced 100% mortality. The highly irritating nature of this chemical was evident microscopically in all dose levels examined, ranging from minimal to mild supportive rhinitis in the 18.59 mg/m^3 dose level dose level, to necrosis and sloughing of the mucosa in the turbinates, trachea, and proximal bronchi in the 61.96 mg/m^3 animals.

Differential weight gains of both male and female mice at 18.59, 30.98, and 61.96 mg/m^3 was significant as compared to the controls. At 123.93 and 247.85 mg/m^3, only female mice showed significant weight loss, as the early mortality of the males precluded obtaining any meaningful data.

870.4100a Chronic Toxicity – Rodent

Kamata et al. (1997) evaluated the effects of inhaled formaldehyde in male F344 (F344/DuCrj) rats (32/group) exposed for 28 months. Formaldehyde exposure was generated by metering 37% formalin (containing 10% methanol) into a sprayer in a glass bottle and diluting with room air. Concentration in the chamber was monitored twice daily by the acetyl acetone method. Exposures were for 6 hours/day, 5 days/week at nominal formaldehyde concentrations of 0, 0.3, 2.0, and 15 ppm. Actual levels (mean ± SD) were 0, 0.3 ± 0.07, 2.17 ± 0.32, and 14.85 ± 2.22 ppm. Rats in the 0 ppm group were noted to inhale methanol at the same concentration (4.2 ppm) as the 15 ppm group. A room control, no-exposure group was also included in the study. All animals were observed for clinical signs once a day during the study. Body weights and food consumption were recorded weekly. Five animals

per group were randomly selected at the end of 12, 18, and 24 months and surviving animals at 28 months were sacrificed for hematological measurements [Hb, RBCs, PCV, MCV, mean corpuscular hemoglobin (MCH), MCHC and WBCs], biochemical determinations (TP, ALB, BUN, ALP, AST, ALT, glucose, albumin/globulin ratio, phospholipids, triglycerides, and total cholesterol), and pathological examinations. Wet weights were taken on brain, heart, lungs, liver, kidneys, spleen, testes, and adrenal gland of each rat. Histopathology was performed on all moribund or dead animals and those at specified sacrifices on all gross lesions and the following tissues: pituitary, thyroid, nasal cavity, trachea, esophagus, stomach, small and large intestines, prostate gland, urinary bladder, muscle, femur, sciatic nerve, spinal cord, and mesenteric lymph nodes. Histopathological sections were obtained from five anatomical levels, but these did not correspond to the typical levels taken in other bioassays. Most notably, section Level B was anterior and not posterior to the incisor teeth. The incidence data for nasal histopathology were not reported with respect to section level location with the exception that the nonproliferative lesions and tumors reported were described to occur predominantly at Levels B and C.

Yellow discoloration of the coats occurred in animals exposed at the 2 and 15 ppm levels. Significant decreases in body weight and food consumption were observed in the high concentration (15 ppm) group throughout the exposure period. Mortality rates at the 28th month were 59.6% in the room control group, 45.5% in the 0 ppm group, 31.8% in the 0.3 ppm group, 55.9% in the 2 ppm group, and 88.3% in the 15 ppm group. In the 15 ppm group, the first death occurred after 6 months and a total of 20 rats died by the end of 24 months. Rats started to die from the 19th month in the 0 ppm group with a total of eight animals dying spontaneously in that group by the end of the study. No abnormal compound-related hematological findings were observed. A statistically significant decrease in triglycerides was observed at the 12-month sacrifice, and a similar trend (though not statistically significant) was observed in animals at the high concentration (15 ppm) at the 18- and 24-month sacrifices. Absolute liver weight was significantly reduced in these same animals and the relative liver weight was decreased at the 18th month sacrifice. Relative adrenal weights were increased in the animals exposed at the high concentration at the 12-month sacrifice. No other remarkable observations were made.

Macroscopic and histopathological findings were limited to the nasal cavity. Squamous cell metaplasia without epithelial cell hyperplasia was increased in animals exposed at the 2 ppm level and was likely masked by the proliferative lesions observed in the animals exposed at 15 ppm. It was difficult to discern a duration-related trend in any of the data due to the concurrent mortality. The total number (across all scheduled sacrifices and unscheduled deaths) of proliferative lesions observed with an increased incidence at the 15 ppm level included (* indicates significance at $p < 0.01$ level): squamous cell metaplasia with epithelial cell hyperplasia (29 %*), epithelial cell hyperkeratosis (26 %*), papillary hyperplasia (2%), SCC (13 %*), and squamous cell papillomae (3%). The total number of squamous cell metaplasia with epithelial cell hyperplasia was also increased (7 %*) in the 2 ppm group but by only 4% in the 0.3 ppm group(not statistically significant). These results are similar to those reported above for the CIIT 2-year bioassays performed in F344 rats and are consistent with those of Woutersen et al. (1989), who exposed Wistar rats for 28 months. A highly nonlinear response for SCC and proliferative lesions in the nasal cavity was observed in

animals exposed to 15 ppm formaldehyde, while animals in the 2 ppm group showed a statistically significant increase in some epithelial lesions.

The systemic toxicity **NOAEL was established to be 0.4 mg/m^3 and the LOAEL was established 2.5 mg/m^3.**

4.6 Carcinogenicity

Adequacy of database for Carcinogenicity: The database for carcinogenicity consists of one submitted and six open literature studies.

870.4200a Carcinogenicity – Rat

In a carcinogenicity study (MRID 00143288), rats were repeatedly subjected to subcutaneous injections of 1 cm^3 of an aqueous formaldehyde solution at 0.6% to 0.8%. With 0.4% to 0.5% aqueous formaldehyde solutions it was possible to inject subcutaneously once or twice a week. Subcutaneous injections of 1 cm^3 of a 0.4% aqueous formaldehyde solution were continued on 10 rats once a week for about 1 year and three months.

0.6% to 0.8%: Necrosis, formation of an ulcer, while the area around the injection spot formed a tuber which was very difficult to heal

0.4% - 0.5%: rare occurrence of an ulcer. After two to five months after having stopped the injections observations revealed the occurrence of sarcomas either at the injection spot or in the internal organs of 4 out of 10 of the rats.

870.4200a Carcinogenicity – Rat

In a carcinogenicity study in male Fischer Rats, Tobe et al. (1985), administered formaldehyde at concentrations of 0, 0.3, 2.0 or 15 ppm (0, 0.37, 2.48 or 18.59 mg/m^3, respectively) in aqueous solution methanol, 6 hours/day, 5 days/week for 28 months. The exposure at 15 ppm was tested for 24 months. A positive control – 3.3 ppm methanol and a nonexposure (NE) control were also used.

During the exposure running noses, running tears and crouching were seen in the 15 ppm dose group. These symptoms decreased as the number of exposures increased. Hair around the abdominal region was observed to be yellow in color and bleeding from the forelimbs was seen. Yellow discoloration of abdominal hair was also seen in the 2.0 ppm dose group although it was light. Significant suppression of weight gain and a decrease in the amount of food gain were seen in the 15 ppm dose group. 20 of 24 animals in the 15 ppm dose group died in the 24 month dosing period giving a high death rate of 88.3%.

Recognizable tumors were observed in the 15 ppm group from the 420th day onwards and tumors were recognized macroscopically in eight animals by the 24th month. Squamous cell carcinoma was recognized in 14 rats and pappiloma in 5 rats. Unclassified carcinoma was seen in 1 rat in the nonexposure group which died on the 825th day.

No neoplastic changes were seen in the 0.3 and 2.0 ppm and exposure control dose groups. Excessive secretion was seen in the nasal cavity, rhinitis accompanied by desquamation, squamous epithelial metaplasia and epithelial cell hyperplasia were recognized in the 0.3 and 2.0 ppm dose groups and these were significant in the 15 ppm dose group.

A decrease in the T-GLY and a decrease in liver weight, assumed to be changes accompanying decrease in food intake due to formaldehyde exposure were seen in the 15 ppm dose group. However, these changes were not accompanied by histological changes.

870.4200a Carcinogenicity – Rat

Takahashi et al. (1986) studied the effects of formaldehyde in an initiation-promotion model of stomach carcinogenesis in male outbred Wistar (Shizuoka Laboratory Center, Shizuoka) rats. Rats (n=17) were given 100 mg/L of N-methyl-N¹-nitro-N-nitrosoguanidine (MNNG) in drinking water and a diet supplemented with 10% sodium chloride for the first 8 weeks as an initiation phase. This was followed by 0.5% formalin in drinking water for 32 weeks as the promotion phase of the protocol. A comparison group (n=10) was given stock water and diet without any supplementation for the first 8 weeks followed by 0.5% formalin in drinking water for 32 weeks. Animals were observed daily and weighed once every 4 weeks. Histopathology was evaluated on the stomach and other tissues in the peritoneal cavity.

Body weight gain was reduced by exposure to MNNG with sodium chloride, and formaldehyde exposure during the promotion phase exacerbated this effect. Histopathological investigations were restricted to the GI tract. Formaldehyde was shown to statistically increase the incidence of lesions in the forestomach and stomach in the animals initiated with MNNG with sodium chloride as compared to controls receiving no initiation. Increases in papilloma in the forestomach, adenomatous hyperplasia in the fundus, and adenocarcinoma in the pylorus were observed. Histopathology in the animals receiving formaldehyde alone for weeks 9 through 32 showed only an increase in forestomach papillomas without any lesions reported for the glandular stomach. The adenomatous hyperplasia was defined as proliferative, noninvasive mucosal lesions, and the adenocarcinomas as well differentiated and composed of typical glandular structures demonstrating a tubular pattern and cellular or structural atypism without metastasis. No definition of criteria for papilloma diagnosis was provided. The findings in this study are inconsistent with those of Til et al. (1989) who found no evidence of carcinogenicity in a 2-year bioassay at comparable concentrations (assuming 37% formaldehyde in formalin results in 0.19% formaldehyde in this study). As discussed above, the differences may be due to differences in the strains of rat or in the diagnostic criteria. The lack of more than one test concentration precludes dose-response analysis of this study and provides only a stand-alone LOAEL of 0.2% formaldehyde in drinking water. The lack of consumption data precludes an estimation of dose in mg/kg-day.

870.4200b Carcinogenicity – Mouse

In a study conducted by Iversen (1986), the possible carcinogenic potency of formaldehyde in classical skin painting experiments (at concentrations comparable to those used in pathology laboratories) was evaluated.

Formaldehyde was topically applied to the back skin of hairless hr/hr Oslo mice. The animals were divided into five groups and the study design was as follows: Group I – 200 µL of a 1% solution of formaldehyde (40% a.i.) in water was applied twice weekly. Group II - 200 µL of a 10% solution of formaldehyde (40% a.i.) in water was applied twice weekly. Group III – 51.2 µg of DMBA (Dimethylbenz(*a*)anthracene) in 100 µL acetone was applied once initially and nine days later followed with 200 µL of a 10% solution of formaldehyde (40% a.i.) in water, the latter being applied twice weekly. Group IV - 51.2 µg of DMBA in 100 µL acetone was applied once initially and nine days later followed with 17 nmol of TPA (12-0-tetradecanoylphorbol-13-acetate), the latter being applied twice weekly. 16 male and 16 female mice were utilized for the first four groups and observed weekly for 60 weeks. Group V – 176 mice were treated with 51.2 µg DMBA once and given no further treatment. The animals were observed once weekly for 80 weeks.

All animals in groups II and III (10% formaldehyde – treated) were autopsied and all organs, including the brain, were inspected for any sign of pathology. Tissue pieces were fixed in buffered formaldehyde and histological sections of the brain, nasal mucosa and lungs were subsequently made. Any signs of carcinoma, papilloma or any other tumor were observed for. Lungs were observed for inflammatory lesions and the presence of any alveolar macrophages. The epithelium in the nasal mucosa was investigated for possible metaplasia or dysplasia.

All animals in group I survived the whole experiment with one of them developing an infected atheroma of the skin. Apart from this one observation, there were no pathological lesions, either macroscopically or microscopically. Animals in Group II generally had a slight hyperplasia of the epidermis. A few animals had small skin ulcers or scratches, and in two animals small nonspecific granulomas were observed in the lungs. There were no signs of lesions in the brain or any other organs in the animals belonging to this group. No signs of metaplasia or dysplasia were seen in the nasal mucosa either. In group III one animal died accidentally in week 26, but histologically only epidermal hyperplasia was found. Three animals in the group had lung adenomas and 11 of 32 animals had neoplastic growths on the skin, altogether 3 squamous cell carcinomas and 22 papillomas i.e. 25 skin tumors. The first three papillomas were observed in week 10 following treatment. There was no evidence of brain or other tumors, nasal dysplasia and no increase in number of alveolar macrophages. Group IV animals showed an increase in mortality. At 20 weeks there were 80% surviving animals which decreased to 40% by week 40. At termination (week 46), there were only 11 animals surviving of the original 32. At week 20, all the surviving animals in the group were papillomabearing. No carcinomas or sarcomas were observed. Almost all of the 176 animals in group V survived the experiment. Altogether 225 skin tumors developed on 85 animals, 6 of these being squamous cell carcinomas. Two animals exhibited lymphosarcomas at autopsy.

Formaldehyde alone gave no tumors while there was a significant enhancement effect of painting twice with 10% formaldehyde subsequent to painting once with DMBA as compared to DMBA alone. There was no significant difference between tumor yields in the two groups mentioned above. There was a significant difference between DMBA followed by TPA on the one hand, and the other two combined treated schedules on the other. Neither 1% nor 10% formaldehyde in water applied topically twice a week on the back skin of hairless mice had any observable carcinogenic effect on any of the observed organs of mice. Mice painted with 51.2 µg DMBA in acetone followed by 10% formaldehyde application, demonstrated a significant tumor enhancement and shortened latency times.

870.4200b Carcinogenicity – Mouse

This carcinogenicity study by Iversen (1988) is a repeat of an earlier study (Iversen, 1986) conducted by the same author but in this case topical applications of formaldehyde were evaluated using SENCAR mice, an animal model bred for maximum sensitivity to chemical tumorigenesis.

A group of 16 male and 16 female SENCAR mice (Group A) were topically administered 100 µL of reagent grade acetone on the skin of the back twice a week. Another group of 32 animals (Group B) was treated with 200 µL 4% formaldehyde (10% formalin) in water twice a week. A third group (Group C) was administered 51.2 µg DMBA in acetone once followed by 200 µL of 1% formaldehyde (2.5% formalin) twice weekly. A pooled group from previous studies (Group D – 176 mice) which had been treated with 51.2 µg DMBA was used as a historical control to compare with the SENCAR mice. A control group (Group E – 96 mice) was given a single application of 51.2 µg DMBA in 100 mL acetone. The last group (Group F) was administered 51.2 µg DMBA in acetone followed by 200 µL of 4% formaldehyde (10% formalin) twice weekly.

The animals were observed for 58 weeks for skin tumors and all animals were autopsied. Specimens were collected from all skin lesions and from obvious pathological lesions observed in the internal organs. Tissue pieces were fixed in 4% buffered formaldehyde and histological sections made. Statistical assessments were made on the basis of the crude incidence of skin tumors with the results being represented as tumor rates (the percentage of tumor-bearing animals in relation to the number of animals alive at the appearance of the first tumor related to time) and tumor yields (the cumulative occurrence of all skin tumors related to time) in all groups. Differences in tumor rates were assessed using the method of Peto (1974) and differences in tumor yields were assessed using the method of Gail et al. (1980). Final tumor yields were also assessed with the chi-square method, which was also utilized to compare differences between the groups with regards to lung adenomas and malignant tumors.

There was no difference in mortality rates among the treatment groups. There was a negligible tumor rate in SENCAR mice treated with acetone or with 4% formaldehyde alone (Groups A and B). Group C (DMBA followed by 1% formaldehyde) produced 26% tumor bearing animals and the time to tumors were slightly shorter (21 weeks versus 32 weeks

before 50% of the final tumor rates were reached) than those administered DMBA alone. Group D (single application of 51.2 µg DMBA) animals produced 42% tumor- bearing animals while Group F (DMBA followed by 4% formaldehyde) produced 44% tumor-bearing animals and the average time to tumor seemed to be somewhat shorter (20 versus 32 weeks) than seen in the group given DMBA alone. There was a significantly higher tumor crop in the SENCAR mice (Group E) than in the hr/hr Oslo mice (Group D). There was a very significant difference in tumor rates between Groups A, B and Group E while there was no difference in tumor rates between animals of Group E when compared separately with Group C and Group F animals. There was a very significant trend seen with DMBA followed by 4% formaldehyde as the most effective tumorigenic protocol.

Tumor yields were found to be highest for animals in Group F (DMBA followed by 4% formaldehyde) but were not statistically significant. Tumor yield for animals in Group E (96 mice) were adjusted to correspond with the number of mice in other groups and the results for tumor yields were similar to those observed for tumor rates. Final number of tumors was higher in Group F (51 tumors in 32 animals) compared to Group E (36 tumors in 32 animals). The difference however, was not significant according to the chi-square method ($\chi^2 = 1.7859$ with 1 DF, $0.20 > p > 0.10$).

Three dermal fibromas and one lymphocytic lymphoma were found in Group A, two lung adenomas occurred in Group B, one lung adenoma, one dermal sarcoma, one lymphoma and one adenocarcinoma of the breast were found in Group C, 14 squamous cell carcinomas of the skin per 32 animals were found in Group E, three lymphomas and one lung adenoma were found in Group F. The chi-square test showed only a suggestive difference between Groups E and F as regards to skin malignancies ($\chi^2 = 3.8065$, 1 DF, $0.10 > p > 0.05$). Hence, there was a slight tendency for formaldehyde to reduce the number of skin carcinomas provoked by DMBA.

The experiment with SENCAR mice gave results similar to those observed in the hr/hr Oslo strain (Iversen, 1986). Formaldehyde has no tumorigenic effect of its own, but may slightly enhance DMBA induced tumorigenesis, manifested in a tendency to shorten the latency period (for tumor rates) and provoking a slightly, but not convincingly higher number of tumors (tumor yield). In regard to malignant skin tumors, formaldehyde tended to be anticarcinogenic.

870.4200b Carcinogenicity – Mouse

In a study by Krivanek et al. (1983), formaldehyde in a 50:50 acetone: water vehicle was tested for its ability to initiate and/or promote tumorigenesis in CD-1 female mice. In addition, a repeated exposure skin irritation pretest to determine irritating and nonirritating doses of formaldehyde was also undertaken by the authors.

In the skin irritation pretest formaldehyde was applied to approximately 1 square inch of shaved skin on the back of 30 female CD-1 mice at concentrations of 0.1 (0.1%), 0.5 (0.5%), 1.0 (1%), 2.0 (2%), 5.0 (5%) or 10.0 (10%) mg/100 µL in a acetone: water mixture. The 0.1 and 0.5 mg/100 µL doses were applied daily, except for weekends, for three weeks while all

other doses were applied daily, except for weekends, for two weeks. Skin responses were observed daily on weekdays.

Initiation treatment consisted of a single application of 5 mg formaldehyde (10%) in 2.5 µg TPA (Phorbol myristate acetate) with the control for this treatment being 50 µL acetone/2.5 µg TPA. Two weeks subsequent, promotion treatments were initiated with the promoter potential of formaldehyde being tested at concentrations of 0.1, 0.5 and 1.0 mg/150 µg BaP (Benz (*a*)pyrene). The dose used to test formaldehyde as an initiator as well as promoter was 5 mg formaldehyde/1.0 mg formaldehyde. The control for this group was 5 mg formaldehyde/100 µL acetone. Promotion treatments were administered thrice weekly for 26 weeks to groups of 30 female CD-1 mice following which the animals were held for an additional 26-week recovery period. Body weight determinations were made biweekly during the promotion treatment and monthly during the recovery period. Skin test sites were observed daily during promotion and biweekly during the recovery period. Skin nodules were chartered monthly while mortality was recorded upon occurrence.

Observations made during the skin irritation pre-test showed that the 10 mg dose produced moderate skin irritation after 2-4 applications, characterized by drying and cracking of the skin (fissuring), sloughing and papules. The 2.0 and 5.0 mg doses produced mild to moderate skin irritation after 4-5 treatments while the 1.0 mg produced mild irritation during the second week of treatment. The 0.5 mg dose level exhibited slight skin irritation potential which was reversible after a brief rest period. No skin irritation was observed in the 0.1 mg dose group after three weeks of treatment. Based on the results of the skin irritation pre-test, doses of 0.1, 0.5 and 1.0 mg formaldehyde were selected for evaluating the promoter potential of formaldehyde.

Body weight curves for the initiation-promotion study indicate no significant differences between the four representative groups: acetone/TPA, BaP/TPA, 5 mg formaldehyde/TPA and 5 mg formaldehyde/1.0 mg formaldehyde. Mortality was low in all but the positive control group ranging from 2-5 per group. Eight positive control mice died or were sacrificed in extremis due to large ulcerated tumors. Skin nodules were seen in 3/27 mice in the acetone/TPA group, 5/28 mice in the formaldehyde/TPA group and comparison of these groups for initiator potential of formaldehyde revealed no significant difference. In the positive control group (BaP/TPA) 29/29 mice had at least one skin nodule. The incidence of nodules was 3/27 in the BaP/acetone group, 7/27 in the BaP/0.1 mg formaldehyde group, 2/28 in the BaP/0.5 mg of formaldehyde group and 1/25 in the Bap/1.0 mg formaldehyde. However, Fisher's exact test analysis indicated no statistical difference between these groups. There were no mice with skin nodules in the formaldehyde/formaldehyde and formaldehyde/acetone groups. In addition, the positive control group BaP/TPA exhibited the earliest time-to-nodule onset when compared to other groups. Only animals in the positive control group developed malignant tumors – squamous cell carcinomas. There were no statistically significant differences between the test and control groups as indicated by Fischer's Exact Test.

Minimally irritating solutions of formaldehyde do not initiate nor promote skin tumors. Formaldehyde does not act as a complete tumorigen when evaluated in the mouse skin painting test.

870.4300 Carcinogenicity- Rat and Mouse

Kerns et al. (1983) exposed about 120 animals/sex/species (Fischer 344 rats and B6C3F1 mice) to 0, 2, 5.6 or 14.3 ppm, 6 hours/day, 5 days/week for 24 months. Five animals per group were sacrificed at 6 and 12 months and 20 per group were killed at 18 months. At 24 and 27 months the number sacrificed is unclear. The studies were terminated at 30 months. From the 12th month on, male and female rats in the highest dose group (14.3 ppm) showed significantly increased mortality compared with controls. In the 5.6ppm group, male rats showed a significant increase in mortality from 17 months on. Female mice showed generally comparable survival across dose groups, as did male mice, but the male mice as a whole showed increased mortality because of housing problems. Squamous cell carcinomas were seen in the nasal cavities of 51/117 male rats and 52/115 female rats at 14.3 ppm (HDT) by experiment's end (as many as 35 carcinomas had been identified in males by month 18 based on EPA analysis notes and Kerns (Chart 8). At 5.6 ppm, 1/119 male rats and 1/116 female rats showed squamous cell carcinomas of the nasal cavity. No such tumors were seen at 0 or 2 ppm. Polypoid adenomas of the nasal mucosa were seen in rats at all doses (0 ppm: 1/118 M, 0/114 F; 2 ppm: 4/118 M, 4/118 F; 5.6 ppm: 6/119 M, 0/116 F; 14.3 ppm: 4/117 M, 1/115 F) in a significant dose-related trend, albeit one that falls off after a peak. Among the mice, squamous cell carcinomas were seen in two males at 14.3 ppm. No other lesions were noteworthy.

Non-guideline Carcinogenicity – Hamster

Dalbey (1982) examined the effects of inhaled formaldehyde alone for a lifetime or combined with diethylnitrosamine (DEN) in an initiation-promotion study design using male Syrian golden hamsters. The hamsters were housed continuously in stainless steel cages and exposed in whole body chambers, 5 hours/day, 5 days/week for a lifetime. Two groups of hamsters were included in the study of inhaled formaldehyde alone: 132 untreated controls and 88 male hamsters exposed to 10 ppm of formaldehyde. The study of formaldehyde and DEN interaction involved five different groups. DEN, administered subcutaneously, was used as the initiator or primary carcinogen. Formaldehyde was generated by paraformaldehyde sublimation and monitored by colorimetric analysis. Fifty animals served as controls; another 50 were exposed to 30 ppm formaldehyde, 5 hours/day, 1 day/week for life. The third group consisted of 100 hamsters treated only with DEN (0.5 mg) once per week for 10 weeks. The fourth group received an exposure to 30 ppm of formaldehyde 48 hours prior to each weekly injection of DEN for 10 weeks followed by weekly formaldehyde exposures (5 hours/week) for life. The fifth group was given weekly 5-hour formaldehyde exposures (30 ppm) for a lifetime but beginning two weeks after the last DEN injection. In the formaldehyde-only study, tissues were prepared by hematoxylin and eosin (H&E) staining and histopathological evaluations were carried out on two transverse sections of the nasal turbinates (otherwise not specified), longitudinal sections of larynx and trachea, and all lung lobes cut along the bronchus prior to embedding. Respiratory tract tissues in the combination study were stained with Wright's stain and rendered semitransparent by a clearing technique for subgross evaluation under a dissecting scope. Areas of dense nuclear staining were scored as tumors. Nasal turbinates were fixed, decalcified, cut, stained, and

cleared for subgross evaluation. At least 10 lesions observed on the subgross level in each tissue of each treatment group were examined microscopically.

In the formaldehyde-only (10 ppm) experiment, mortality was reduced in hamsters ($p < 0.05$) relative to unexposed controls. No tumors and little evidence of toxicity to the nasal epithelium were observed. There was no increase in rhinitis, and hyperplastic or metaplastic areas were each observed at an incidence of 5% relative to none in the controls. While these data are somewhat difficult to interpret given the lack of information on the location of the nasal sections, they do suggest that the hamster is less susceptible to inhaled formaldehyde since F344 rats chronically exposed to ≥10 ppm (Monticello et al., 1996) exhibited an increase in SCCs, and Wistar rats exposed to 10 ppm for 28 weeks exhibited significant epithelial disruption (Woutersen et al., 1989). Differences may be due to dosimetry, as is evident for mice (Chang et al., 1983), or tissue sensitivity, or both.

In the initiation-promotion protocol with DEN, weekly exposures to formaldehyde alone (30 ppm once per week) did not influence mortality. Treatment with DEN alone significantly ($p < 0.05$) increased mortality above that of untreated controls, and mortality was further elevated ($p < 0.05$) in the two groups exposed to both DEN and formaldehyde relative to DEN alone. Actual group size was 27 for concurrent DEN and formaldehyde treatment and 23 for formaldehyde given after DEN because of unplanned mortality resulting from an exposure accident at 48 weeks. No tumors were observed in untreated animals or those receiving only formaldehyde. DEN treatment alone resulted in a high incidence (77%) of tumors. All tumors observed were classified as adenomas. Lifetime exposure of animals treated with DEN and formaldehyde did not increase the number of TBA above those given DEN only. The number of tumors per tumor-bearing animal (TBA) was significant only in the group given formaldehyde prior to each DEN injection and only in the trachea and not in larynx or lung. An adjustment of the tumor incidence for age at death may have increased the statistical significance further. The author concluded that the relation of the time of exposures to formaldehyde to the DEN appeared to be significant, noting that the formaldehyde exposure resulted in an increase only when administered 48 hours prior to each injection. These findings may be due to a cytotoxic effect of formaldehyde on cell proliferation as later shown by Monticello et al. (1996) for rats.

870.4300 Combined Chronic Toxicity/Carcinogenicity – Rat and Mouse

In a combined chronic toxicity/carcinogenicity study (MRID 00143289), Rats (Fischer 344) and Mice ($B6C3F_1$) were administered 0, 2.0, 5.6, or 14.3 ppm (0, 2.5, 6.9, or 18 mg/m^3) formaldehyde via inhalation, 6 hrs/day, 5 days/week, for up to 24 months.

From exposure weeks 3 to 103, mildly (15 to35 g) decreased body weights ($p<0.05$) in male and female rats (6.9 and 18 mg/m^3) were observed. Animals in the 2.5 mg/m^3 exposure group had sporadically reduced body weights ($p>0.05$) throughout the exposure period. Male and female rats in the 18 mg/m3 exposure group exhibited significantly increased mortality

($p<0.001$) from the 12th month onward. Male rats in the intermediate exposure groups showed a statistically-significant concentration-dependent decrease in cumulative survival from 17 months onward.

In male mice, there were no differences in survival. The number of male mice surviving a minimum of 18 months were 41, 33, 32, and 25 for the 0, 2.5, 6.9, and 18 mg/m^3 exposure groups, respectively. There were no differences in cumulative survival among the female mice.

There were no alterations in the clinical pathology or ophthalmologic or neurofunctional data that were considered related to formaldehyde exposure.

Exposure to formaldehyde produced a concentration-dependent increase in yellow discoloration of the hair. Other significant macroscopic observations (at the 18 mg/m^3 group) included dypsnea, emaciation, and large facial swellings that were proliferative lesions (carcinomas) protruding from the nasal cavity. Neoplastic lesions were first observed clinically at Day 358 in females and Day 432 in males. Formaldehyde-induced microscopic lesions were confined to the nasal cavity and the proximal trachea.

Exposure to 18 mg/m^3 formaldehyde for 24 months produced a high incidence of nasal cancer in male and female rats. The tumors had a sharp concentration-response relationship, with the 2 carcinomas in the intermediate group identical to the 103 squamous cell carcinomas observed in rats exposed to 18 mg/m^3. Although the incidence of polyploid adenomas in the nasal cavity was not statistically significant, there was a positive concentration response for the occurrence of benign neoplasms in male rats. There was no evidence of progression of polyploid adenoma to squamous cell carcinoma.

Two male mice exposed to 18 mg/m^3 of formaldehyde developed squamous cell carcinomas in the nasal cavity similar to the neoplasms in the rats. Formaldehyde-induced lesions (squamous metaplasia and inflammation) in mice were much less severe than similar lesions in rats. The incidence of squamous cell carcinomas in mice exposed to 18 mg/m3 was similar to rats exposed to 6.9 mg/m^3.

Epidemiology Studies

The follow-up study by Hauptmann et al. (2003, 2004) of the National Cancer Institute (NCI) retrospective cohort mortality study of U.S. workers involved in the production or use of formaldehyde represents the best available data set for quantitative cancer risk assessments of lymphohematopoietic cancers and nasopharyngeal tumors based on human data. The NCI study is a large epidemiology study, and it provides individual quantitative exposure estimates for the workers.

The NCI cohort consisted of 25,619 workers (88% male) employed in any of the 10 plants prior to 1966; the current follow-up analyzes 8,486 deaths (178 attributed to lymphohematopoietic malignancy and 9 to nasopharyngeal cancer). A detailed exposure assessment was conducted for each worker based on exposure estimates for different jobs held and tasks performed (Stewart et al., 1986). Exposure estimates were made using several different metrics - peak exposures, average intensity, cumulative exposure, and duration of exposure. Respirator use and exposures to formaldehyde particles and other chemicals were also considered. Significant increases in relative risk for lymphohematopoietic cancer were observed primarily for myeloid leukemia and Hodgkin's disease and for the peak exposure and average intensity exposure metrics. For the nasopharyngeal cancers, significant trends were observed for the cumulative and peak exposure metrics.

With respect to the subtypes of lymphohematopoietic malignancy, the strongest exposure-response relationships were observed for Hodgkin's disease and myeloid leukemia for both the peak exposure and average intensity exposure metrics. The (all) lymphohematopoietic malignancies category also showed a highly significant trend for the peak exposure metric and a significant trend with the average intensity metric, and this was the category selected for the cancer risk analyses presented here. While other lymphohematopoietic cancer subtypes did not exhibit statistically significant increases, many did suggest increases in relative risk with formaldehyde exposure, and the subtype analyses were generally based on small numbers of cases (i.e., lower statistical power). Furthermore, as noted by the NCI investigators, "[a]lthough the accuracy of death certificates for lymphohematopoietic malignancies is generally high, classification of subtypes of leukemia and lymphoma from death certificates is less accurate than from hospital records." Finally, the all lymphohematopoietic cancer category contains the most data, and the results are more stable.

4.8 Mutagenicity

Adequacy of database for Mutagenicity Toxicity: The database for mutagenicity of formaldehyde is considered incomplete with the lack of a bone marrow chromosome aberration test in the mouse.

The mutagenicity data for Formaldehyde is summarized below in Table 3.

Table 3. Mutagenicity data for Formaldehyde

Guideline No./ Study Type	MRID No./ Reference Information/ Study Classification	Dosing and Animal Information	Results
Mutagenicity			
870.5100 Bacterial reverse mutation test	**MRID 00132156** Jagannath, D. (1978) Mutagenicity Evaluation of Dantoin DMDMH-55 40-697 737543 in the Ames Salmonella/Microsome Plate Test: LBI Project No. 20838. Final rept. (Unpublished study received May 9, 1983 under 38906-5; prepared by Litton Bionetics, Inc., sub-mitted by Glyco, Inc., Greenwich, CT; CDL:250313-A) **Supplementary**	0.001, 0.01, 0.10, 1.0, or 5.0 µL. Salmonella tester strains TA-98, TA-100, TA-1535 TA-I 537 and TA-1538. Saccharamyces indicator organisms, strain 04.	**Negative**
870.5100 Bacterial reverse mutation test	**MRID 00132157** Haworth, S.; Lawlor, T.; Burke, P.; et al. (1982). Salmonella/ Mammalian-microsome Preincubation Mutagenicity Assay (Ames Test): Test Article 447:34-3: Study No. T1804.502. (Unpublished study received May 9, 1983 under 38906-5; prepared by Microbiological Assoc., submitted by Glyco, Inc., Greenwich, CT; CDL:250313-B). **Acceptable**	Test material (447:34-3, MA #T1804) tested at concentrations of 3.0, 15.0, 75.0, 150, or 300 µg/plate. Tester strains TA98, TA100, TA1535, TA1357, TA1358 ± metabolic activation with araclor induced rat liver microsomes.	**Positive** Test article caused did cause a positive response (3.2-fold increase) on tester strain TA98 without metabolic activation. A 1.9-fold increase was observed on TA98 with metabolic activation. Also, increases of 2.2-fold and 1.7-fold were observed on tester strain TA100 with and without activation, respectively.

Table 3. Mutagenicity data for Formaldehyde			
Guideline No./ Study Type	**MRID No./ Reference Information/ Study Classification**	**Dosing and Animal Information**	**Results**
870.5100 Bacterial reverse mutation test	O'DONOVAN, MR AND MEE,CD; FORMALDEHYDE IS A BACTERIAL MUTAGEN IN A RANGE OF SALMONELLA AND ESCHERICHIA INDICATOR STRAINS; MUTAGENESIS 8(6):577-581, 1993 (NCEA) **Open Literature**	0-200 ug/plate, pre-incubation exposure and standard plate-incorporation assays S. typhimurium Strains TA1535, TA1537, TA1538, TA98, TA100, and TA102 and E.coli Strains WP2(pKM101) and WP2uvrA(pKM101) Purity: 37%	Pre-incubation exposure: positive for mutagenicity in TA98, TA100, and TA102 and both E.coli strains. Standard plate-incorporation assays: Consistent mutagenicity was seen only for TA100 and WP2uvrA (pKM101). No evidence of mutagenicity was seen for TA1535, TA1537, or TA1538 using either method of exposure.
870.5100 Bacterial reverse mutation test	Schmid, E., W. Goggelmann; and M. Bauchinger. (1986) Formaldehyde-induced Cytotoxic, Genotoxic, and Mutagenic Response in Human Lymphocytes and Salmonella typhimurium. Mutagenesis vol. 1 no. 6 p. 427-431. (NCEA) **Open Literature**	The tests were carried out using the plate incorporation assay and the pre-incubation method +/- S9 activation at doses of 0-1.5mM and 0-0.3mM formaldehyde, respectively. The incubation mixture consisted of 0.5 ml phosphate buffer or S9 mix and10 ul of an appropriate concentration of formaldehyde in water. S. typhimurium Strain TA100 (0.1 ml bacterial suspension of about 10*8 cells were used in the pre-incubation method) Purity: 37%	Plate Assay: weak positive response Pre-Incubation Method: Without S9 mix, a1.6-fold increase of revertant numbers over controls was induced. With S9 mix, a 2.7-fold increase of revertant numbers over controls was induced.

Table 3. Mutagenicity data for Formaldehyde			
Guideline No./ Study Type	**MRID No./ Reference Information/ Study Classification**	**Dosing and Animal Information**	**Results**
870.5100 Bacterial reverse mutation test	Temcharoen, P; Thilly, WG. (1983) Toxic and mutagenic effects of formaldehyde in Salmonella typhimurium. Mutat Res 119:89-93. (NCEA) **Open Literature**	The capacity of formaldehyde to induce forward mutations to 8-azaguanine resistance in was examined. Formaldehyde concentrations of 0.17 mM in the absence of S9 and 0.33 mM in the presence of S9. S. typhimurium TM 677, a his^+ revertant of TA 1535 Purity: 37%	Both toxicity and mutagenicity were obtained at formaldehyde concentrations of 0.17 mM in the absence of S9 and 0.33 mM in the presence of S9. The authors noted that, while the S9 might be enzyme inactivating formaldehyde, the binding of formaldehyde to amino groups of proteins in the S9 would effectively reduce the concentration of formaldehyde entering the cells.
870.5200 Mouse visible specific locus test	Mouse Lymphoma L5178Y Cell TK Locus Assay for Mutagenicity; A Study with Formaldehyde. (DuPont, 7/28/80, Haskell Laboratory Report No. 581-80). **Open Literature**	Doses of 0, 0.1, 0.5, 1, 5, 10 or20 ug/ml without activation only, four trials Mouse Lymphoma L5178Y Cell Purity: 37%	An increase in mutation frequency was reported, especially at 10 and 20 ug/ml.
870.5275 Sex-linked recessive lethal test in Drosophila melanogaster	Valencia, R., J.M. Mason, and S. Zimmering. (1989) Chemical Mutagenesis Testing in Drosophila. VI. Interlaboratory Comparison of Mutagenicity Tests After Treatment of Larvae. Environmental and Molecular Mutagenesis, v. 14, p. 238-244. **Open Literature**	Doses of 2,600 and 1,100 ppm D. melanogaster (Canton-S M and Basc F) Purity: 37%	**Positive**
870.5375 In vitro mammalian chromosome aberration test	**MRID 00132168** Thilagar, A.; Kumaroo, P.; Pant, K. (1982) Cytogenicity Study: Chi- nese Hamster	Test material (447:34-1) was tested at concentrations of 28.43, 37.91, or 50.55	**Positive** Test article caused a significant dose-dependant

Table 3. Mutagenicity data for Formaldehyde			
Guideline No./ Study Type	**MRID No./ Reference Information/ Study Classification**	**Dosing and Animal Information**	**Results**
	Ovary (CHO) Cells in vitro: Test Article 447:34-1: Study No. T1802.338. (Unpublished study received May 9, 1983 under 38906-5; prepared by Microbiological Assoc., submitted by Glyco, Inc., Greenwich, CT; CDL:250313-M) **Acceptable**	nL/mL. Chinese hamster ovary cells (cell repository number CCL, 61) Purity: 37% Formalin	increase in the frequencies of chromosome aberrations in the Chinese Hamster Ovary cells, both with and without S-9 activation.
870.5375 In Vitro mammalian chromosome aberration test	Natarajan, A.T. et al. (1983) Evaluation of the mutagenicity of formaldehyde in mammalian cytogenics assays in vivo and vitro. Mutation Research 122: 355-360. (NCEA) **Open Literature**	In Vitro CHO cells exposed to 0, 0.003, 0.006, 0.012, or 0.024 uL/mL paraformaldehyde In Vivo Mouse 0.4 mL paraformaldehyde injected intraperitoneally to achieve doses of 0, 6.25, 12.50, or 25.00 mg/kg	**In Vitro: Positive** Frequencies of chromosomal aberrations and SCEs increased with increasing dose. All classes of aberration, i.e. gaps, breaks, and exchanges, were induced by formaldehyde. All the aberrations were chromatid-type, indicating that formaldehyde acts as an S-dependent agent. The addition of mammalian metabolic activation system reduced the frequencies of formaldehyde-induced aberrations at all doses. Similarly, there was also a reduction in the frequencies of SCEs induced by formaldehyde, if the treatment was done in the presence of S9. **In Vivo: Negative** None of the concentrations used increased the frequencies of micronuclei over the control level. Formaldehyde was not effective in inducing chromosomal aberrations.
870.5380 Mammalian spermatogonial	Fontignie-Houbrechts, N. (1981) Genetic Effects of Formaldehyde in the Mouse.	Mice received an i.p. injection of 50 mg/kg formaldehyde	**Negative** No chromosomal lesions

Table 3. Mutagenicity data for Formaldehyde			
Guideline No./ Study Type	**MRID No./ Reference Information/ Study Classification**	**Dosing and Animal Information**	**Results**
chromosomal aberration test	Mutation Research, v. 88, p. 109-114. (NCEA) **Open Literature**	M Q Strain Mouse (200 spermatocytes/ animal) Purity: 35%	were revealed
870.5450 Rodent dominant lethal assay	Fontignie-Houbrechts, N. (1981) Genetic Effects of Formaldehyde in the Mouse. Mutation Research, v. 88, p. 109-114. (as cited in Ma and Harris) (NCEA) **Open Literature**	Mice received an i.p. injection of 50 mg/kg formaldehyde and 10 males were caged with 2 virgin females each for one week. The females were renewed each week during 7 weeks. M Q Strain Mouse Purity: 35%	Embryonic death or pre-post implantation death at 1 and 3 week periods
870.5450 Rodent dominant lethal assay	Odeigah, P.G.C. (1997) "Sperm Head Abnormalities and Dominant Lethal Effects of Formaldehyde in Albino Rats." Mutation Research 389: 141-148. **Open Literature**	Five daily interperitonial injections of 0.125, 0.250, and 0.6 mg/kg formaldehyde. Males were caged with 2 untreated virgin females which were replaced weekly for 3 consecutive weeks giving a total of 24 females for the periods 1-7, 8-14, and 15-21 days post-injection, respectively. All females were sacrificed 13 days after the mid-week of their caging. At autopsy, each female was scored for total implants. Albino Rats (12 M/group)	**Positive** The frequency of dominant lethal mutations in female rats sired by males exposed to formaldehyde was significantly higher than the control group.

Table 3. Mutagenicity data for Formaldehyde			
Guideline No./ Study Type	**MRID No./ Reference Information/ Study Classification**	**Dosing and Animal Information**	**Results**
		Purity: 37% solution (stabilized with 10% methanol)	
870. 5550 Unscheduled DNA synthesis in mammalian cells in culture	**MRID 00132169** Thilagar, A.; Pant, K. (1982) Unscheduled DNA Synthesis in Rat Hepatocytes: Test Article 447:34-1: Study No. T1802.380002. (Un-published study received May 9, 1983 under 38906-5; prepared by Microbiological Assoc., submitted by Glyco, Inc., Greenwich, CT; CDL:250313-N) **Acceptable**	Test material (447:34-1) was tested at concentrations of 0.0005, 0.001, 0.005, 0.01, 0.02, or 0.04 µL/mL. Primary rat liver hepatocytes – Sprague-Dawley rats, 2.5 x 10^5 HPC/plate Purity: 37% a.i.	**Negative** The test article did not cause a significant increase in UDS in rat hepatocytes.
870.5900 *In Vitro* Sister Chromatid Exchange Assay	A. Basler, W. v. d. Hude, and M. Scheutwinkel-Reich (1985) "Formaldehyde-Induced Sister Chromatid Exchanges in vitro and the Influence of the Exogenous Metabolizing Systems S9 Mix and Primary Rat Hepatocytes." Arch Toxicol 58: 10-13. **Open Literature**	The test compound was added to cell cultures 18 hours after seeding 5 x 10^5 cells per 25 cm^2 flask. The exposure time was 1, 2, 3, or 28 hours. In the experiments with short-term exposure (1-3 hours), the medium was removed after this treatment. The cells were restored in medium supplemented with 5-bromo-2-deoxyuridine (BrdU). The cells were cultured in the presence of BrdU (10^{-5} M) for 28 h, with colcemide (0.1 µg/ml) for the last 4 h. In the experiments with long-term exposure, the cells were cultured in the presence of BrdU and	There was a three- to four-fold increase in the SCE frequency at non-toxic doses. However, in the presence of an exogenous metabolizing system, the number of formaldehyde-induced SCE's decreased. S9 mix as well as hepatocytes reduced the SCE frequency to nearly that of the control range. It could be demonstrated that the reduction was not due to an unspecific binding of formaldehyde to macromolecules of the added S9 mix. The decrease in genotoxic effects, due to rapid metabolisation of formaldehyde in vitro and un vivo, explains the differences between results obtained in the in vitro experiments – performed without metabolizing systems – and in vivo results.

Table 3. Mutagenicity data for Formaldehyde			
Guideline No./ Study Type	**MRID No./ Reference Information/ Study Classification**	**Dosing and Animal Information**	**Results**
		the test compounds for 28 hr. In tests with S9 mix, the cells were incubated with 0.5 ml S9 mix per 25 cm^2 flask and 0.033, 0.067, 0.13, 0.2, 0.27, 0.4, and 0.54 mM formaldehyde for 3 h, followed by incubation for 28 hr in the presence of BrdU as described above. In tests with primary rat hepatocytes, 10^6 viable hepatocytes were added to the monolayer. After 2 hr, the nonattached hepatocytes were sucked off and the different concentrations of formaldehyde were added. The medium was complemented with BrdU and incubated for 28 h as above. S9 fraction was prepared from Aroclor 1254-induced male Wistar rats. Chinese Hamster V79 Cells Purity: 37% Formaldehyde/10% Methanol	
870.5915 In Vivo Sister Chromatid Exchange Assay	Kligerman,AD; Phelps, MC; Erexson, GL. (1984) Cytogenetic analysis of lymphocytes from rats following formaldehyde inhalation. Toxicol Lett 21:241-246.	Rats were exposed to 0.5, 6, or 15 ppm (0.6, 7.4, 18.5 mg/m^3) formaldehyde by inhalation for 6 hours/day for 5 days. Blood was obtained by cardiac puncture	There were no increases in either SCE or chromosome aberrations at any dose level.

Table 3. Mutagenicity data for Formaldehyde			
Guideline No./ Study Type	**MRID No./ Reference Information/ Study Classification**	**Dosing and Animal Information**	**Results**
	Open Literature	within 1 h of the last exposure and cultured with BrdU for sister chromatid exchange (SCE) analysis. M and F Fischer F-344 Rat Purity: 95% a.i.? (Not reported in study)	
In vitro human lymphoblasts	Craft, T.R., E. Bermudez, and T.R. Skopek (1987) Formaldehyde mutagenesis and formation of DNA-protein crosslinks in human lymphoblasts in vitro. Mutation Research 176: 147-155. **Open Literature**	0, 15, 30, 50, 125, or 150uM Human Lymphoblasts (4x 10^5 cells/mL) Formaldehyde (37% w/w 10-15% methanol)	**Positive** The 15, 30, and 50 uM treatments resulted in no significant difference in growth rate compared to control values; the 125 uM and150 uM treatments resulted in approximately 30% and 20% survival, respectively. Single treatments of various concentrations of formaldehyde resulted in a nonlinear increase in induced mutant fraction at the thymidine kinase locus with increasing slope above 125 uM. Concentrations ≥ 30 uM yielded statistically significant responses ($p<0.05$). Multiple treatments of 15, 30, and 50 uM also resulted in increases in mutant fractions. Lymphoblasts exposed repeatedly to these lower concentrations accumulate formaldehyde-induced mutations, but at a lower rate than if a single 150uM treatment was given at one time.
In vitro human	Liber, HL; Benforado, K;	Liber et al. (1989)	Northern blot analysis

Table 3. Mutagenicity data for Formaldehyde			
Guideline No./ Study Type	**MRID No./ Reference Information/ Study Classification**	**Dosing and Animal Information**	**Results**
lymphoblasts	Crosby, RM; et al. (1989) Formaldehyde-induced and spontaneous alterations in human hprt DNA sequence and mRNA expression. Mutat Res 226:31-37. **Open Literature**	followed up the findings of Crosby et al. (1988) by performing Northern blot and sequence analysis on the 16 induced and 10 spontaneous mutants not showing deletions. Human Lymphoblasts Purity: 37%	showed that the point mutations fall into four categories; normal size and amount of RNA, normal size but reduced amounts of RNA, reduced size and amounts of RNA, and no RNA. Sequence analysis of recombinant DNAs from HRPT mRNA in compound-induced mutants showed a preferential AT to CG transversion at a single site, with other changes represented to a lesser degree.
Other	Graves, RJ; Trueman, P; Jones, S; Green, T. (1996) DNA sequence analysis of methylene chloride-induced HPRT mutations in Chinese hamster ovary cells: Comparison with the mutation spectrum obtained for 1,2-dibromoethane and formaldehyde. Mutagenesis 11:229-233. **Open Literature**	DNA sequence analysis of formaldehyde-induced HRPT mutations Chinese Hamster Ovary Cell Purity: 40%	Single-base pair transversions, including three AT to TA at position 548 of exon 8, one GC to TA, and two AT to CG transversions at other sites.
Other	Blackburn, GR; Dooley, J, III; Schreiner, CA; et al. (1991) Specific identification of formaldehyde-mediated mutagenicity using the mouse lymphoma L5178Y TK positive negative assay supplemented with formaldehyde dehydrogenase. In Vitro Toxicology 4:121-132. **Open Literature**	Forward mutation assay Mouse lymphoma L5178Y tk+/- cells Purity: 37% (w/w) aqueous solution (formalin)	Formaldehyde induced forward mutations to trifluorothymidine resistance both in the absence and presence of rat liver S9 (higher concentrations required for effect with S9). Both toxicity and mutagenicity were abolished when formaldehyde dehydrogenase was incorporated in the exposure medium.

4.9 Neurotoxicity

Adequacy of database for Neurotoxicity: The database for neurotoxicity consists of four open literature studies.

870.6200 Neurotoxicity Screening Battery – Rat

Malek et al. (2003a) examined open field behavior of rats after acute formaldehyde exposures. Male and female LEW.1K rats (15/sex/group) were exposed to 0, 1.0, 2.5, or 5.0 ppm (0, 1.23, 3.08, or 6.15 mg/m3) formaldehyde for 2 hours. Formaldehyde was vaporized from aqueous solutions directly below the exposure chamber. Formaldehyde levels were checked 16 times throughout the 2-hour exposure periods. Mean formaldehyde levels of 1.01 ± 0.29 ppm, 2.51 ppm (standard deviation is missing) and 5.04 ± 0.27 ppm were achieved. Locomotor activity was assessed for 1 hour in an open field 2 and 24 hours after termination of formaldehyde exposure using an automated device to count the number of squares crossed. Other behaviors were noted, including grooming (face cleaning, fur licking, and scratching), rearing, sniffing (air and floor), wall climbing, and defecation.

The authors reported no signs of irritation or changes in activity or food or water intake during exposure (Malek et al., 2003a). In general, sniffing was increased after formaldehyde exposure and movement was decreased (crossed quadrants and climbing) in both male and female rats ($p<0.05$). Significant reductions in horizontal movements (crossed quadrants) were observed at all dose levels and were characterized by a U-shaped dose response. The lowest dose tested (1 ppm) demonstrated higher level of activity suppression than the two higher doses, but all groups were still suppressed relative to controls. Although female rats displayed a greater level of activity overall, a similar U-shaped dose-response pattern was also observed.

Activity in the same apparatus was reassessed 24 hours later. As expected, controls demonstrated habituation to the test apparatus exhibiting only 20% of the motor activity observed on day 1. In contrast, formaldehyde-treated animals failed to demonstrate the same degree of habituation. Activity levels for males observed on day 2 were 60-80% of the activity levels seen on day 1. Formaldehyde-treated females also failed to habituate and actually demonstrated increases in activity on day 2 relative to day 1 at all formaldehyde exposure levels.

The neurotoxicity LOAEL was established to be 1.0 ppm, 2 hours

A follow-up study by Malek et al. (2003b) further expanded the dose-response analysis for acute formaldehyde exposure. As described above, male and female LEW.1K rats (10 per group) were exposed at 0, 0.1, 0.5, or 5.0 ppm (0, 0.123, 0.615, or 6.15 mg/m3) formaldehyde for 2 hours. Formaldehyde levels were checked 16 times throughout the 2-hour exposure periods and mean values were found to be 0.13 ± 0.04, 0.48 ± 0.05, and 5.18 ± 0.66 ppm. Open field behavior tests were conducted on each animal 2 hours after formaldehyde exposure. The number of crossed quadrants for both controls and a 5 ppm group are comparable to those observed in the first study. Horizontal movement was decreased by formaldehyde exposure in a dose dependent manner with significant reductions in motor

activity as low as 0.1 ppm in males and 0.5 ppm in females. The consistency of the data across studies and between genders provides greater confidence in the effects of low level formaldehyde exposure on this standard test of neurotoxicity.

The neurotoxicity LOAEL (Males) was established to be 0.1 ppm, 2 hours

870.6500 Scheduled Control Operant Behavior – Rat

Pitten et al. (2000) evaluated the effects of very brief formaldehyde exposures (10 minutes) but prolonged duration (90 days) on previously learned performance in a land version of the labyrinth maze. Adult male and female Wistar rats were acclimated to the task for 14 days, 2 trials/day. Animals were required to make a series of five consecutive turns from the entrance of the maze to retrieve a piece of cheese placed in the goal box at the opposite end. Animals were guided by the experimenter through the maze during this acclimation phase until all subjects were able to retrieve the food without aid. A pretraining phase followed in which performance was assessed once daily for 11 days, and the latency to complete the maze, as well as the number of errors committed when traversing from the entrance to the goal box, were recorded. The maze was wiped clean between subjects to remove urine, boli and olfactory stimuli from the previous subject. Animals were then assigned to one of three dose groups (five to eight/sex/group) such that task performance was equivalent across groups prior to commencement of formaldehyde exposures. Animals were exposed to 0 ppm, 2.6 ppm (0.25% formaldehyde solution to yield 3.06 ± 0.77 mg/m3), or 4.6 ppm (0.70% formaldehyde solution to yield 5.55 ± 1.27 mg/m3) formaldehyde, 10 minutes/day, 7 days/week for 90 days. Animals were assessed for performance in the maze every seventh day, at least 22 hours after the exposure on the previous day. At the end of the 90-day exposure period, monitoring of maze performance continued once every 10 days for an additional 40 days. All rats were sacrificed at the end of the post-exposure trials and tissue sections were prepared for histological examination by light microscopy, including liver, trachea, lung, kidney, heart, spleen, pancreas, testicle, and brain. No changes in food or water consumption weight gain or in histological samples of lung and liver obtained at the termination of the experiment were observed.

The authors reported that no gender differences existed as a function of formaldehyde treatment; therefore, data were presented by combining sexes. Control rats showed no change in error rate but a slight decrease in running time through the maze during the course of the experiment. The formaldehyde-exposed groups began with a similar performance level and error rate as controls, but their performance degraded over the course of formaldehyde exposure. By the fourth week of exposure, increased numbers of errors were evident in both exposed groups relative to controls. This trend reached statistical significance by the thirteenth week for a greater than twofold increase in error rate ($p<0.05$). Formaldehyde-treated rats also tended to have increased run times through the maze ($p=0.04$), but no difference was seen by formaldehyde concentration. By 4 weeks after termination of exposure, no statistical differences among the three groups were evident, but the tendency for the two exposed groups to make more errors and have longer latencies remained. Since Pitten et al. (2000) tested animals after the task was acquired, these results indicate deficits in the retention of a previously learned task.

The neurotoxicity LOAEL was established to be 2.6 ppm, 10 min/90 days.

Non-Guideline Neurotoxicity

In a neurotoxicity study conducted by Boja (1985), male Sprague-Dawley rats were exposed to either air or formaldehyde at concentrations of 5, 10, or 20 ppm (6.20, 12.39, or 24.79 mg/m^3) via inhalation for 3 hours on two days

Exposure to 6.20 mg/m^3 formaldehyde resulted in statistically significant decreased motor activity within 15 minutes. At the beginning of day 2, all of the rats exposed to formaldehyde on day 1 displayed lower activity levels. Similar effects on motor activity were seen at the 12.39 mg/m^3 formaldehyde exposure level, whereas effects seen after 24.79 mg/m^3 exposure were reported to be "not readily interpretable" and were not shown. Exposure to 6.20 mg/m^3 formaldehyde statistically significantly increased concentrations of 5-hydroxyindoleacetic acid, 3,4-dihydroxyphenylacetic acid, and dopamine in the hypothalamus.

4.10 Metabolism and Pharmacokinetics

Adequacy of database for Metabolism and Pharmacokinetics: The database for metabolism of formaldehyde is considered complete with one submitted and two open literature studies in the rat.

870.7485 General Metabolism

In a metabolism study conducted by Casanova et al. (1989), rats (4/group) were exposed to formaldehyde (nose-only exposure) at concentrations of 0, 0.3, 0.7, 2, 6, or 10 ppm (0.37, 0.87, 2.5, 7.4, or 12 mg/m^3) for 6 hours.

DNA-protein crosslinking occurred at all concentrations. The formation of crosslinks was interpreted in terms of a nonlinear pharmacokinetic model incorporating oxidation of inhaled formaldehyde as a defense mechanism. The slope of the fitted concentration-response curve at 12 mg/m^3 is7.3-fold greater than at 0.37 mg/m^3, and the detoxification pathway is half-saturated at an airborne concentration of 3.2 mg/m^3.

870.7485 General Metabolism

In a metabolism study (Casanova-Schmitz et al., 1984), 14C and 3H-formaldehyde was administered at doses of 0, 0.3, 2, 6, 10, or 15 ppm (0, 0.37, 2.5, 7.4, 12, or 19 mg/m^3) for 6 hours.

The major route of nucleic acid labeling at all concentrations and in all tissues was metabolic incorporation; protein labeling in the respiratory mucosa was mainly due to covalent binding at the higher formaldehyde concentration. Incorporation of 14C- formaldehyde into DNA in

the respiratory mucosa was maximal at 7.4 mg/m^3 but decreased at higher concentrations, whereas labeling of DNA in the olfactory mucosa and bone marrow increased monotonically with concentration. Evidence for covalent binding of formaldehyde to respiratory mucosal DNA was obtained at formaldehyde concentrations equal to or greater than 2.5 mg/m^3. The concentration of formaldehyde covalently bound to DNA at 7.4 mg/m^3 was 10.5-fold higher than at 2.5 mg/m^3, indicating significant nonlinearity of DNA binding with respect to the inhaled formaldehyde concentration under these conditions. Covalent binding to proteins increased in an essentially linear manner with increases in the airborne concentration. No evidence was obtained for the formation of covalent adducts with macromolecules in the olfactory mucosa or bone marrow. The nonlinear increase in covalent binding to respiratory mucosal DNA with increasing formaldehyde concentrations may be explained either by a decrease in the efficiency of defense mechanisms or by an increase in the availability of reaction sites on the DNA resulting from increased cell turnover.

4.11 Special Studies

The special studies data for formaldehyde is summarized in Table 4 below:

Table 4. Special studies data for Formaldehyde

Guideline Number/ Study Type/ Test Substance (% a.i.)	MRID Number (Year)/ Citation/ Classification/ Doses	Results
Modeling	Conolly, R.B., et al. 2003. Biologically Motivated Computational Modeling of Formaldehyde Carcinogenicity in the F344 Rat. Toxicol. Sci. 75: 432–447. **Open Literature** 3-D F344 Rat Model Biologically based quantitative modeling of the relationship between formaldehyde inhalation and the development of nasal squamous cell carcinoma on the basis of the Kerns et al. (1983) and Monticello et al. (1996) data.	The analysis suggested evidence of: 1) a cytolethality-regenerative cellular proliferation (CRCP) mechanism with little or no involvement of direct mutagenesis; and 2) a J-shaped dose-response relationship between formaldehyde and squamous cell carcinoma.
Sensitization	Ohtsuka, R; Shuto, Y; Fujie, H; et al. (1997) Response of respiratory epithelium of BN and F344 rats to formaldehyde inhalation. Exp Anim 46:279-286. (NCEA) Ohtsuka, R; Shutoh, Y; Fujie, H; et al. (2003) Rat strain difference in histology and expression of Th1- and	Although no pulmonary measurements were made, the authors observed fewer clinical signs of respiratory irritation in the BN rats compared to F344 rats, such as abnormal respiration (three versus five) and nasal discharge (three versus five). Formaldehyde-treated F344 rats showed less body

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	Th2-related cytokines in nasal mucosa after short-term formaldehyde inhalation. Exp Toxicol Pathol 54:287-291. **Open Literature** 18 F344 and 18 Brown Norway (BN) Rats Rats were exposed to formaldehyde aerosol for 3 hours/day, 5 days/week for 2 weeks. The aerosol was generated from a 1% formaldehyde solution by a two-fluid atomizer and formaldehyde level maintained at 2 mg (1% sol.)/L (approximately 16 ppm or 20 mg/m^3), by adjusting the flow rate for formaldehyde solution to the atomizer.	weight gain over the 2-week treatment, resulting in lower body weight at week 1 and week 2 than F344 controls ($p<0.05$ and 0.01). BN rats were more resistant to epithelial cell damage than F344 rats, exhibiting milder lesions that impacted a smaller portion of the URT. Squamous metaplasias were present in the respiratory epithelium (Levels 1 and 2) in both strains in formaldehyde-treated rats. However, a distinct keratinized layer was noted in Level 1 epithelium of F344 rats, and the extent of lesions in Level 2 respiratory epithelium was much greater than that seen in BN rats. Additionally, the olfactory epithelium (Level 2) in formaldehyde-exposed F344 rats exhibited degeneration, necrosis, and desquamation not seen in BN rats. Mild squamous metaplasia was noted in Level 3 of the respiratory epithelium in the treated F344 rats but not the BN rats. The authors note that their earlier research indicated the BN rats have well-developed submucosal glands and that greater mucus flow may be partly responsible for the greater resistance of BN rats to the histological signs of formaldehyde toxicity. In a subsequent study in the same laboratory, Ohtsuka et al. (2003) compared cytokine profiles in the nasal mucosa of formaldehyde-treated F344 and BN rats. Formaldehyde aerosol was generated as above and rats (nine per group) were exposed 3 hours/day for 5 days to approximately 16 ppm of formaldehyde (20 mg/m^3). The incidence and severity of clinical signs in F344 rats was

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		greater than BN rats as previously observed (Ohtsuka et al., 1997). Also, lesions and neutrophil infiltrations were more severe in F344 formaldehyde-exposed rats compared to treated BN rats. F344 rats had various lesions in all three levels of epithelium examined, which impacted both respiratory and olfactory epithelium. Mucosal lesions in formaldehyde-treated BN rats only impacted the respiratory epithelium of Levels 1 and 2. Although changes in cytokine mRNA expression were modest, there was a depression of T-lymphocyte helper 1 (TH-1)-related cytokines in formaldehyde-treated BN rats (INF-g, Il-2) and a similar, although not statistically significant, decrease in TH-2 cytokines (IL-4, IL-5) compared to unexposed BN rats. There were no treatment differences in cytokine expression in F344 rats. Type 1 hypersensitivity reactions generally result in increased TH-2 cytokines. Therefore, although modest changes in cytokine profile were seen in formaldehyde-treated BN rats, they were not consistent with Type 1 hypersensitivity.
Sensitization Purity: 37% formalin	Biagini, RE; Moorman, WJ; Knecht, EA; et al. (1989) Acute airway narrowing in monkeys from challenge with 2.5 ppm formaldehyde generated from formalin. Arch Environ Health 44:12-17. (NCEA) **Open Literature** 9 Cynomolgus Monkeys known to be hyperreactive to methacholine (acetyl-β-methacholine chloride) The effect of a single pulmonary exposure on pulmonary mechanics including bronchial constriction (BC) was evaluated. Each monkey was	Methacholine challenge increased pulmonary flow resistance at increasing levels of methacholine (0.125, 0.5, 2.0, and 8.0 mg/mL) to 196 ± 16, 285 ± 57, 317 ± 64, and 461 ± 120 % of baseline levels respectively. Similarly, formaldehyde exposure increased pulmonary flow resistance from 11.3 ± 1.4 cm H2O prior to formaldehyde exposure, to 16.1 ± 2.1, 16.9 ± 2.8, and 20.0 ± 3.4 cm H2O, at 2, 5, and 10 minutes after formaldehyde exposure (with 142, 150, and 177% change, respectively). Although bronchial constriction, seen as increased

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	exposed separately to methacholine and formaldehyde in order to compare their formaldehyde response to an agent known to trigger BC. Monkeys were exposed to increasing levels of methacholine for 10 minutes (0, 0.125, 0.5, 2.0, and 8.0 mg/mL) as an aerosol (0.065 mL/min with a mean aerodynamic diameter of 1.0-1.5 μm). After a 2-week recovery period, pulmonary mechanics were measured before and after a 10-minute exposure to 2.5 ppm formaldehyde (2, 5, and 10 minutes post-exposure).	pulmonary flow resistance, was increased by both methacholine and formaldehyde, there was not a correlation between methacholine responsiveness and the magnitude of effect after formaldehyde exposure ($p>0.1$). Therefore although formaldehyde exposure stimulated BC similarly to a known direct stimulating agent, formaldehyde may not work through the same site of action as methacholine.
Sensitization	Fujimaki, H; Kurokawa, Y; Kunugita, N; et al. (2004) Differential immunogenic and neurogenic inflammatory responses in an allergic mouse model exposed to low levels of formaldehyde. Toxicology 197:1-13. (NCEA) **Open Literature** Mice were exposed to 0, 0.082, 0.393, or 1.87 ppm formaldehyde (0, 0.1, 0.48, or 2.3 mg/m^3), 16 hours/day, 5 days/week for 12 weeks. Six mice at each exposure level were given intraperitoneal injections of OVA plus adjuvant before the initial exposure and on weeks 3, 6, 9, and 11 of the experiment. Five mice at each formaldehyde-exposure level did not receive OVA injections. One day after the last exposure, spleens were collected and disaggregated and spleen cells harvested for cell culture. Lymphocyte proliferation in response to lipopolysaccharide (LPS), phytohemagglutinin A (PHA), or OVA was determined after 72 hours in culture. Splenocytes were cultured for 48 hours in the presence of LPS, PHA, and OVA (immunized mice only), and supernatants were collected for cytokine analysis (IL-4, IL-5, and INF-γ). Splenocytes were cultured for 24 hours in the presence or absence of	In nonimmunized mice, spleen weights were reduced by formaldehyde exposure from 152 mg in control to 128, 118, and 121 mg in mice exposed to 0.08, 0.40, and 1.8 ppm formaldehyde, respectively. However, spleen weights were unchanged by formaldehyde exposure in OVA-immunized mice. In immunized mice exposed to 1.8 ppm formaldehyde, the total number of BAL cells, MPs, and eosinophils were increased (9.65 versus 2.84, 7.22 versus 2.74, and 2.0 versus 0.02 ×104 cells, respectively). Levels of IL-1β in BAL of immunized mice were decreased by formaldehyde exposure ($p<0.05$ at 1.8 ppm formaldehyde). Immunization with OVA significantly increased the neuropeptide nerve growth factor (NGF) in BAL. However, this increase with OVA immunization was attenuated by 0.08 and 0.40 ppm formaldehyde exposure. A similar response was seen in blood plasma NGF levels, where the increase with OVA immunization was attenuated in mice exposed to 0.08 and 0.40 but not to 1.8 ppm formaldehyde. Plasma level of Substance P (a mediator of

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	OVA to assess chemokine production (MCP-1 and MIP1-α). Anti-OVA IgE, IgG1, IgG2, and IgG3 were quantified in blood plasma.	neurogenic inflammation) was increased by formaldehyde exposures in non-immunized mice. Although Substance P was increased by OVA immunization, this again seemed to be attenuated by formaldehyde exposure, reducing Substance P levels to undetectable levels. Formaldehyde exposure (1.8 ppm) increased INF-γ fourfold in LPS stimulated cultured spleen cells from non-immunized mice. OVA in vitro stimulation significantly increased the chemokines MIP-1 and MCP-1 for control and formaldehyde-treated OVA-immunized mice. The OVA stimulated release of MCP-1 in vitro was enhanced by formaldehyde exposure in a concentration dependent manner, increasing threefold and fourfold at 0.40 and 1.8 ppm, respectively. Anti-OVA IgG1 was slightly depressed in immunized mice exposed to 0.40 ppm formaldehyde, and anti-OVA IgG3 was depressed in immunized mice exposed to 0.08 and 0.40 ppm formaldehyde.
Pulmonary Hypersensitivity	**MRID 43167201** Burleigh- Flayer, H. D. and W.J. Kintigh (1992) Glutaraldehyde and Formaldehyde: Vapor Pulmonary Hypersensitivity Study in Guinea Pigs. Bushy Run Research Center (Export, PA), Union Carbide. Study ID 92U1123, dated February 28, 1992, Unpublished. **acceptable** Guinea Pig (8/group) Induction: 14 ppm (17 mg/m^3), 60 minutes, 5 consecutive days	Formaldehyde did not cause increased respiratory rate or altered respiratory waveform indicative of pulmonary hypersensitivity during the challenge exposures. No mortality, clinical signs, body weight effects, or gross lesions were observed

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	Challenge: 5 ppm (6.2 mg/m^3) for 60 minutes, at days 14, 21, and 35 following induction	
870.1300 Acute Inhalation Toxicity Purity: 95%	Dean et al. (1984) Studies of Immune Function and Host Resistance in B6C3F1 Mice Exposed to Formaldehyde. Toxicology and Applied Pharmacology, v.72, p. 519-529. **Open Literature** 255 Female (SPF) B6C3F1 Mice 21-Day (6 hr/day, 5 days/week) inhalation exposure to 18.59 mg/m^3 formaldehyde to test a series of immune function and host resistance parameters.	Decrease in the absolute number of monocytes. In the absence of a difference in recovery of peritoneal cells, the change in monocyte number may signal only a peripheral response to the local nasal inflammation and healing which occurs following HCHO exposure.
Inhalation	Casanova, Mercedes, et al. (1991) Covalent Binding of Inhaled Formaldehyde to DNA in the Respiratory Tract of Rhesus Monkeys: Pharmacokinetics, Rat- to-Monkey Interspecies Scaling, and Extrapolation to Man. Fundamental and Applied Toxicology 17: 409-428. **Open Literature** 9 Male Rhesus Monkey (*Macaca mulatta*) 14C-Formaldehyde was administered at 0, 0.7, 2, or 6 ppm (0, 0.87, 2.5, or 7.4 mg/m^3) for 6 hours	DNA protein cross-links were formed in the respiratory tract of rhesus monkeys exposed to formaldehyde. Concentrations of cross-links (pmol/mg DNA) were highest in the mucosa of the middle turbinates; lower concentrations were produced in the anterior lateral wall/septum and nasopharynx. Very low concentrations were found in the larynx/trachea/carina and in the proximal portions of the major bronchi of some monkeys exposed to 7.4 mg/m^3 but not to 9.87 mg/m^3. No cross-links were detected in the maxillary sinuses or lung parenchyma. The pharmacokinetics of cross-link formation in the nose were interpreted using a model in which the rate of formation is proportional to the tissue concentration of formaldehyde. Using this model, the concentration of cross-links formed in corresponding tissues of different species can be predicted by scaling the pharmacokinetic parameter

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		depending on minute volume and quantity of nasal mucosal DNA> The concentration-response curve for the average rate of cross-link formation in the turbinates, lateral wall, and septum of rhesus monkeys as predicted from that of F344 rats exposed to similar conditions. Concentrations of cross-links that may be produced in the nasal mucosa of adult men were predicted based on experimental data in rats and monkeys. The results suggest that formaldehyde would generate lower concentrations of cross-links in the nasal mucosa of humans than of monkeys, and much lower concentrations in humans than in rats. The rate of formation of DNA-protein cross-links can be regarded as a surrogate for the delivered concentration of formaldehyde.
Inhalation	D'A. Heck, Henry, and Merccedes Casanova (1987) Isotope Effects and Their Implications for the Covalent Binding of Inhaled (3H) and (14C) Formaldehyde in the Rat Nasal Mucosa. Toxicology and Applied Pharmacology 89: 122-134. **Open Literature** Male F-344 (CDF/ CrIBR) rats Isotopic effect on DNA-protein crosslinking by 3HCHO and H-14-CHO: Rat hepatic nuclei incubated with 3H and 14C formaldehyde Isotopic effect on the oxidation of 3HCHO and H14-CHO: homogenates of the rat nasal mucosa incubated with 3H and14C formaldehyde at total formaldehyde concentrations ranging from 0.1 to 11 uM, NAD+ (1 mM), GSH (15 mM), and pyrazole (1mM)	Isotopic effect on DNA-protein crosslinking by 3HCHO and H-14-CHO: A small (3.4 +- 0.9%) isotope effect was detected on this reaction, which slightly favored 3HCHO over H14CHO in binding to DNA. The magnitude of this isotope effect cannot account for the high isotope ratio observed in the crosslinked DNA in vivo. Isotopic effect on the oxidation of 3HCHO and H14-CHO: 3HCHO is oxidized significantly more slowly than H14CHO under these conditions. A similar isotope effect was observed in the absence of GSH, presumably due to the oxidation of 3HCHO and H14CHO, which can bind to DNA resulting in an isotope ratio higher than that of inhaled gas. The

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		isotope effect on the oxidation of 3HCHO and H14CHO suggests that previous estimates of the amount of formaldehyde covalently bound to nasal mucosal DNA may have been too large; especially at low airborne concentrations and that the shape of the concentration-response curve for DNA-protein cross linking is more nonlinear than reported previously.
Inhalation	Morgan, K. et al. (1986) Responses of the Nasal Mucociliary Apparatus of F-344 Rats to Formaldehyde Gas. Toxicology and Applied Pharmacology 82: 1-13. **Open Literature** Rat 0, 0.7, 2, 6, or 15 ppm (0, 0.62, 2.5, 7.4, or 19 mg/m^3) 6 hour exposures for up to 3 week duration	**NOAEL: 2.5 mg/m^3** **LOAEL: 7.4 mg/m^3** Rats exposed to 2.5, 7.4, or 19 mg/m^3 exhibited concentration-related evidence of eye and nose irritation, including ocular and nasal discharge, and reddish exudate in the nasal passages. Defects in mucociliary function in specific regions of the nose, such as cessation or severe slowing of mucus flow (mucostasis), loss of ciliary activity (ciliastasis), or altered mucus flow patterns, were readily detected. These changes were clearly related to formaldehyde concentration and duration exposure, and only minimal variation was observed between animals within each exposure group. Mucostasis was usually more extensive than ciliastatsis, but in some areas mucus was flowing over areas of inactivated cilia. Inhibition of mucociliaryfuction by 19 mg/m^3 formaldehyde was most frequently observed on the dorsal and medial aspects of the maxilloturbinate, especially the hook-like scroll of this turbinate (lateral scroll), the ridge dorsal to this scroll (lateral ridge), and the lateral wall. These changes were progressively more extensive with increasing number

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		of days of exposure and showed little or no evidence of recovery 18 hours after the last exposure. At 7.4 mg/m^3, the effects were much less extensive and they were minimal or absent at 2.5 mg/m^3. Localized inhibition of ciliary activity on the ventral margin of the nasoturbinate was observed in a few animals exposed to 2.5 mg/m^3 for 9 days. Slowing or cessation of mucus flow was detected in the more anterior regions of the maxilloturbinate following exposure for 1 day to 19 mg/m^3, and more posterior regions were affected after 9 days. In rats exposed to 7.4 mg/m^3 formaldehyde, no consistent effects on the mucus flow rate were observed except in areas exhibiting mucostasis. At 2.5 mg/m^3, there was no evidence of reduced mucus flow rate. In rats exposed to 19 mg/m^3 formaldehyde, there were lesions in the respiratory epithelium which became more extensive with increasing number of days of exposure. Lesions were most severe in the anterior nasal passages on the lateral, dorsal, and medial aspects of the maxilloturbinate, the lateral and ventral surfaces of the nasoturbinate, and the lateral wall. Exposure to 19 mg/m^3 for 6 hours produced minimal effects, characterized by separation of epithelial cells and intravascular margination and local tissue infiltration by neutrophils and monocytes in the regions which later exhibited severe, degenerative changes. Over affected areas, a layer of floccular material was covered by a continuous membrane. These layers were presumed to be coagulated mucus

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		and were not present elsewhere in the nose. The surface coagulum was absent in animals killed 18 hours after a single 6-hour exposure, and cilliated cells in affected areas were variably disintegrated while infiltrating phagocytes were more numerous. Following 2 days exposure to 19 mg/m^3, epithelial damage and inflammation were more severe and extensive with a serofibrinous exudate present over damaged areas. These changes were even more advanced after 4 days. Epithelial lesions had extended posteriorly along the lateral wall where exfoliating ciliated and non-ciliated cells were located frequently over areas of cellular proliferation and early squamous metaplasia. The distribution of epithelial lesions correlated with the areas of inhibition of the mucociliary function. No epithelial lesions were detected in areas exhibiting mucostasis without ciliastasis. Similar, but less severe changes were found in rats exposed to 7.4 mg/m^3. There was a good correlation between the distribution of epithelial lesions and inhibition of ciliary activity. No epithelial lesions were detected in rats exposed to 0.62 or 2.5 mg/m^3.
Inhalation Short and Intermediate term	Monticello, et al. (1991) Regional Increases in Rat Nasal Epithelial Cell Proliferation following Acute and Subchronic Inhalation of Formaldehyde. Toxicology and Applied Pharmacology 111: 409-421. **Open Literature** Rats (36/group) 0, 0.7, 2, 6, 10, or 15 ppm (0, 0.87, 2.5, 7.4, 12, or 19 mg/m^3), 6 hr/day for 1, 4, or 9 days, or 6 weeks (5	**NOAEL: 2.5 mg/m^3** **LOAEL: 7.4 mg/m^3** Animals exposed to 2.5 mg/m^3 or less had no microscopic evidence of formaldehyde-induced lesions. Formaldehyde-induced lesions at higher doses were confined to nasal passages primarily involving the cuboidal-transitional and respiratory epithelium. Light microscopic lesions were not observed in the trachea, carina, or more distal conducting airways.

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	days/week)	Lesions exhibiting an anterior-posterior severity gradient varied over exposure time and were concentration-dependent. For acute exposure (1 to 9 days), rats exposed to 12 or 19 mg/m^3 formaldehyde had nasal lesions which became more severe and extensive with increasing exposure time. Formaldehyde-induced lesions were more severe in the anterior nasal passages on the lateral aspect of the nasoturbinate, the lateral wall, and the lateral, dorsal, and dorsomedial aspects of the maxilloturbinates. Less severe formaldehyde-induced lesions were present on the midseptum at Levels II and III and the midlateral wall at Level III. More severe effects were observed at the higher dose. Following one 6-hour exposure to 10 or 15 ppm (12 or 19 mg/m^3) formaldehyde, lesions were characterized by epithelial cell vacuolar degeneration, individual cell necrosis, epithelial exfoliation, and multifocal erosions. There was also a mild mixed inflammatory cell infiltrate consisting primarily of neutrophils with fewer numbers of lymphocytes and plasma cells. Formaldehyde-induced lesions progressed by day 4 to erosions, locally extensive ulceration, and an increased neutrophilic infiltrate. There was evidence of early epithelial hyperplasia with karyomegaly. Following 9 days of exposure, epithelial hyperplasia and squamous metaplasia were also evident. These lesions extended posteriorly to include the midlateral walls and the midventral septum at Level III, and occasionally they included the ventral floor of the nasopharynx. Lesions induced by exposure to 7.4

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		mg/m^3 formaldehyde were much less severe than at higher concentrations, primarily confined to Site 1 of Level II. They were characterized by mild, multifocal, individual cell necrosis, a very mild neutrophilic infiltrate, mild, patchy, epithelial cell hyperplasia, and squamous metaplasia observed only after 9 days of exposure. For subchronic exposure (6 weeks), lesions in the 12 and 19 mg/m^3 groups consisted of epithelial hyperplasia, squamous metaplasia, and a mild neutrophilic cellular infiltrate. These lesions were located on the lateral wall, the midventral septum of Level II, and the lateral walls of Level III. Lesions were also present in the nasopharynx, characterized by mild epithelial hyperplasia and squamous metaplasia. For animals exposed to 7.4 mg/m^3, lesions were present at Level II, characterized by mild hyperplasia and squamous metaplasia of the lateral wall epithelium. There were no detected treatment-induced responses in cell proliferation indices in the two lowest formaldehyde concentration groups. Elevations in cell proliferation were first detected following 1 day of formaldehyde exposure in the 7.4, 12, and 19 mg/m^3 groups. Increases in the ULLI were present in every site except the nasal septum. Statistically significant elevations in cell proliferation following 6 weeks of exposure to 7.4 mg/m^3 were confined to the lateral wall and the maxilloturbinate of Level II only. The levels of cell proliferation at the lateral wall site decreased significantly ($p<0.05$) from Level II to Level III, demonstrating a clear anterior-

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		posterior response gradient. Statistically significant increases in the ULLI at Level III were present at Days 1, 4, and 9 for the lateral wall and at Days 4 and 9 for the septum, even though epithelial lesions were not observed by light microscopy in these locations. For the 12 and 19 mg/m^3 dose groups, statistically significant increases in the ULLI were observed at each site at days 1, 4, 6, 9, and 6 weeks, with the exception of the maxilloturbinate at day 1. At Level III following 6 weeks of exposure, the lateral wall site in both the 12 and 19 mg/m^3 groups had a greater magnitude increase in cell proliferation over controls, as compared to the Level II nasal spetal site. The anterior-posterior gradient of the cell proliferation response observed at 7.4 g/m^3, was not apparent at these higher concentrations.
Other – Sensory Irritation Purity: 37%	Kane, Laurel E.; and Alarie, Yves. (1977) Sensory Irritation to Formaldehyde and Acrolein During Single and Repeated Exposures in Mice. American Industrial Hygiene Association Journal, v.38, p. 509-522. **Open Literature** M SPF Swiss Webster Mouse (4/group) Mice were exposed via inhalation for 3 hours/day for 4 days to a concentration of formaldehyde that would be expected to produce a 30% decrease in respiratory rate within the first 10 minutes of exposure (as predicted by the concentration-response relationship) or to an atmosphere containing a concentration equal to 1/10 the RD50 for formaldehyde for 3 hr/day for 3	**RD_{50}: 3.84 mg/m^3**

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	days.	
Other - Sensory Irritation	Steinhagen, WH; Barrow, CS. (1984) Sensory irritation structure-activity study of inhaled aldehydes in B6C3F1 and Swiss-Webster mice. Toxicol Appl Pharmacol 72:495-503. (NCEA) **Open Literature** M Swiss Webster and B6C3F1 mice (3-4/dose) 10 minute head-only exposure to formaldehyde and other aldehydes	**RD50: 3.2 ppm (2.1–4.7 ppm) (Swiss Webster)** **RD50: 4.90 ppm (3.9–6.4 ppm) (B6C3F1)** The difference in results between strains was not statistically significant. On the average, α, β unsaturated aliphatic aldehydes and formaldehyde were approximately 2 orders of magnitude more potent than cyclic aldehydes and about 3 orders of magnitude more potent than acetaldehdye and other saturated aliphatic aldehydes. The authors hypothesized that the difference might be due to differences in the degree to which a particular aldehyde undergoes hydration and its subsequent hydrate dissociation constant (Khyd). This proposed mechanism could account for the difference in RD50 between acetaldehyde with a hydration of 49.7% and a Khyd value of 0.99 compared to formaldehyde with a hydration of >99.8% and a Khyd value of >100 (Schauenstein et al., 1977).
870.1300 Other - Sensory Irritation Purity: 5%	Gardner, RJ; Burgess, BA; Kennedy, GL, Jr. (1985) Sensory irritation potential of selected nasal tumorigens in the rat. Food Chem Toxicol 23:87-92. **Open Literature** 8-week-old Crl-CD male rats (4/group) The RD50 of eight chemicals was determined to determine whether there was a correlation between the ability of a chemical to produce sensory irritation and tumorigenic potency. Groups of rats were exposed for 15	**RD50: 13.8 ppm** Estimate was about threefold less than the 31.7 ppm reported for male F344 rats (Barrow et al., 1983). This may indicate differences in responsiveness to formaldehyde among different strains of rat. Concentrations of 5.5 ppm or more produced considerable depression in respiratory rate. The decrease was observed during the first minute of exposure and achieved a maximum at about 3 minutes. Some recovery was observed during exposure from 3 to 10 minutes after the start

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	minutes to various concentrations of formaldehyde ranging from 0.77 to 24.9 ppm after a 5-minute pretest exposure to control air.	but was incomplete during the first 5 minutes after exposure. Taking the results of the eight chemicals together, sensory irritation potency did not correlate with the carcinogenic potency indicated by long-term inhalation experiments.
870.1300 Other - Sensory Irritation Purity: 95% paraformaldehyde	Chang, JC; Barrow, CS. (1984) Sensory irritation tolerance and cross-tolerance in F-344 rats exposed to chlorine or formaldehyde gas. Toxicol Appl Pharmacol 76:319-327. **Open Literature** M Fischer 344 (CDF[F 344]Crl/Br) rats (4/group) Chang and Barrow (1984) determined whether tolerance would develop in rats exposed to formaldehyde. Tolerance was defined as return of respiratory rate to baseline levels following an initial decrease induced by test gas exposure. Groups of rats were exposed in double-chamber plethysmographs for 10 minutes after a 20-minute acclimation and a 5-minute baseline period. This measurement was performed 18 to 24 hours after any pretreatment. Pretreatment exposures were carried out in a glass chamber for 6 hours/day, 5 days/week, for various durations.	Exposure to formaldehyde at 15 ppm for 6 hours/day, 5 days/week failed to induce tolerance. However, tolerance was observed following exposure to 28 ppm formaldehyde for 4 days. The concentration-response curve in these animals was significantly different than that of naïve animals, with an increase in RD50 estimate for this exposure duration from 31.7 to 70.2 ppm.
870.1300 Other - Sensory Irritation Purity: 90%-92% Paraformaldehyde	Cassee, FR; Arts, JH; Groten, JP; et al. (1996) Sensory irritation to mixtures of formaldehyde, acrolein, and acetaldehyde in rats. Arch Toxicol 70:329-337. (NCEA) **Open Literature** M Wistar rats (4/dose) Cassee et al. (1996) determined the RD50 values for formaldehyde, acetaldehyde, and acrolein as a result	**RD50: 10.0 (95% CI 4.7–13.7)**

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	of a 30-minute nose-only exposure.	
870.1300 Other - Sensory Irritation Purity: 95% Paraformaldehyde	Kulle, TJ; Cooper, GP. (1975) Effects of formaldehyde and ozone on the trigeminal nasal sensory system. Arch Environ Health 30:237-243. (NCEA) **Open Literature** Adult M Sprague-Dawley rats (5/experiment) The effects of formaldehyde on trigeminal nerve afferent activity in rats was investigated. Electrodes were implanted through a dissection in the right eye orbit. The authors state that because the ethmoid nerve and trigeminal nerve responded similarly the experiments were performed with the nasopalatine nerve to eliminate potential contribution from the mechanoreceptor fibers in the ethmoid nerve. The sensory threshold was determined by extrapolation from the measured nerve response to a range of formaldehyde concentrations (0.5–2.5 ppm) or ozone (5.0–29 ppm) for an exposure duration of 2 minutes. Amyl alcohol exposure (0.3–10.0 ppm) was for 25 seconds. Kulle and Cooper (1975) also investigated the effects of prolonged exposure on trigeminal nerve activity using the in situ preparation described above. Formaldehyde (0, 0.5, 1.0, 1.5, or 2.0 ppm) was presented continuously for 1 hour. Pre-exposure responsiveness was determined to a test series of amyl alcohol (0.3, 0.7, 1.0, 3.3, 6.7, or 10.0 ppm). After exposure to formaldehyde and a 10-minute recovery period of exposure to control air, the amyl alcohol series was repeated to evaluate reversibility. If formaldehyde produced any depression or enhancement of nerve activity as evidenced by the amyl alcohol test series, another recovery	**The mean thresholds were 0.25 ppm for formaldehyde**, 5.0 ppm for ozone, and 0.30 ppm for amyl alcohol. There was a progressive depression in response to amyl alcohol with increasing stimulus of formaldehyde concentration [$p < 0.01$, analysis of variance (ANOVA)]. The effects of exposure to 2.0 ppm were similar regardless of whether it was presented immediately as a separate exposure or as the final concentration of a progressively increasing series. The response to amyl alcohol did not fully recover within the 1-hour extended recovery period. Thus it appeared that the afferent function depression was not due to receptor adaptation or insufficient time for formaldehyde diffusion away from receptor sites.

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	period of control air ensued and the test was repeated. Control tests with amyl alcohol were run for 8 hours to establish that there were no significant changes in response to prolonged exposures to the referent gas. It was also determined if there was a difference when the formaldehyde concentration was progressively increased to 2.0 ppm in a series of exposures at the concentrations above or presented separately at 2.0 ppm.	
870.1300 Other - Sensory Irritation	Tsubone, H; Kawata, M. (1991) Stimulation to the trigeminal afferent nerve of the nose by formaldehyde, acrolein, and acetaldehyde gases. Inhal Toxicol 3:211-222. **Open Literature** M Wistar Rat (6/group) The afferent activity of the surgically isolated ethmoidal nerve (a branch of the trigeminal nerve) during delivery of formaldehyde (0.32–4.7 ppm) into the cannulated URT of rats at a flow rate of 200 mL/minutes for 22 seconds was recorded. Each exposure was repeated two to four times at different concentrations.	The vapor concentration associated with a 50% increase in nerve activity over the level of control gas was calculated as approximately 1.8 ppm for formaldehyde.
Sensory Irritation Formaldehyde, Lot No. 420807, 10 %a.i. (methanol free)	**MRID No. 43170601** Werley et al. (1994). Glutardehyde and Formaldehyde: Sensory Irritation Study in Swiss-Webster Mice. Union Carbide Lab Project No. 91U0123. **Supplementary** Male Swiss-Webster ND4 mice (40-55 days old at start of study), 4 animals/dose 0, 0.34, 1.4, 6.9, 18.8, or 80.0 ppm (0, 0.42, 1.73, 8.55, 23.3, or 99.1 mg/m^3), 30 min, head-only chambers	No treatment related mortality was observed. Mice exposed to formaldehyde showed no treatment-related clinical findings. All mice exposed to formaldehyde at 99.1mg/m3 showed increased lacrimation and periocular wetness. Slight reductions in body weight were observed in some of the mice at the highest exposure doses for formaldehyde.

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
Immunologic Sensitization	Tarkowski, M. and Gorski, P. 1995. Increased IgE antiovalbumin level in mice exposed to formaldehyde. Int. Arch. Allergy Immunol. 106: 422–424. **Open Literature** F Balb/c Mouse Groups were exposed to 2 mg/m^3 formaldehyde either 6 hours/day for 10 days, or to 6 hours/day once a week for 7 weeks. Then all mice were sensitized intranasally with ovalbumin.	Following sensitization, titer of serum anti-ovalbumin IgE antibodies were significantly higher in mice exposed to formaldehyde 6 hours/day for 10 days, compared to mice exposed 6 hours/week for 7 weeks or untreated. The authors concluded that formaldehyde facilitates animal sensitization to ovalbumin through histological changes occurring in the upper respiratory tract.
Immunologic Sensitization	Riedel, F., et al. C.H.L. 1996. Formaldehyde Exposure Enhances Sensitization in the Guinea Pig. Allergy 51: 94–99. **Open Literature** Guinea Pig (12/group) Animals were exposed to formaldehyde concentrations of 0 (controls), 160 or 310 ug/m^3 (0.13 and 0.25 ppm) for 5 days, followed by sensitization to inhaled ovalbumin at days 5 and 19. On day 26, a bronchial provocation test with ovalbumin was performed, followed by repeated lung function measurements to monitor bronchial obstruction. Also, blood samples were taken on day 0 (before formaldehyde exposure) and day 25 (before bronchial provocation test) and tested for anti-ovalbumin IgG1 antibodies.	Following ovalbumin challenge, 10/12 animals exposed to 310 ug/m^3 showed bronchial obstruction, compared with 3/12 control animals ($p<0.01$); animals exposed to 160 ug/m^3 were not significantly different from controls. Anti-ovalbumin IgG antibodies were not detectable (<10 ELISA units or EU) in any animal at day 0, but were detectable in 0/12 controls, 3/12 animals exposed to 160 ug/m^3, and 6/12 animals exposed to 310 ug/m^3 at day 25.
Immunological	Jakab, GJ. (1992) Relationship between carbon black particulate-bound formaldehyde, pulmonary antibacterial defenses, and alveolar macrophage phagocytosis. Inhal Toxicol 4:325-342.	Mice exposed to 15 ppm formaldehyde for the 4 hours following bacterial infection (Regimen A) had approximately an 8% increase in bacteria, indicating decreased host resistance

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	Open Literature White Female Swiss Mouse Mice were exposed to formaldehyde after bacterial infection (Regimens A and C), before bacterial infection (Regimen B), or before and after infection (Regimen D). In the first trial mice were exposed to 0, 1.0, 5.0, 10.0, or 15.0 ppm formaldehyde (0, 1.2, 6.2, 12.3, or 18.5 mg/m^3). The remaining mice were exposed to 0, 0.5, or 1.0 ppm formaldehyde (0, 6.2, or 1.2 mg/m^3). A 30-minute exposure to an infectious aerosol of S. aureus deposited 2x105 staphylococci in the lungs. Bacterial loading was determined in homogenized lung tissue by culturing diluted aliquots for an estimate of bacteria present immediately after loading and 4 hours later.	(p=0.006). Pre-infection exposure to 0.5 or 1.0 ppm did not change bacterial loading 4 hours after infection (Regimen B). However, combining an 18-hour pre-infection formaldehyde exposure with a 4-hour post-infection 1 ppm formaldehyde exposure increased pulmonary bacterial loading by approximately 6.5% ($p<0.05$). Increased bacterial loading indicates that formaldehyde exposure (Regimens A and D) reduced pulmonary bacterial resistance. This is in apparent contradiction to the findings of increased host resistance by Dean et al. (1984). However, there are important differences between the studies. The studies by Jakab (1992) are acute studies examining effects at the respiratory tract where direct effects are possible. Additionally, in some cases, the exposures were concurrent with bacterial infection, and it is difficult to distinguish the potential for formaldehyde effects directly on the mucociliary apparatus as a barrier to infection.
Other	Adams, D.O. et al. (1987) The Effect of Formaldehyde Exposure upon the Mononuclear Phagocyte System of Mice. Toxicology and Applied Pharmacology 88: 165-174. **Open Literature** Female Mouse 15 ppm (19 mg/m^3), 6 hr/day, 5 days/week. 3 weeks	Exposure of mice to 19 mg/m^3, 6 hr/day, 5 days/week for 3 weeks did not appreciably alter the number of resident macrophages in the peritoneal cavit+y or that elicited in response to MVE-2.
Other	Bartnik, F.G., Gloxhuber Chr., and Zimmermann V. (1985) Percutaneous Absorption of Formaldehyde in Rats. Toxicology Letters. v. 25. p. 167 - 172.	Under non –occlusive conditions, absorption of radiolabeled formaldehyde in the cosmetic cream preparation was published as 6.1% in males and 9.2% in

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	Open Literature 10 Male/4 Female Rat [14C] Formaldehyde as a tracer and non-labeled formaldehyde were incorporated into a cream and dermally applied at 200 mg to occluded and nonocclusive dosing areas for 48 hours	females. Occlusive conditions reported absorption as 3.4% in males.
Other	Hester, et al. (2003) Formaldehyde-Induced Gene Expression in F344 Rat Nasal Respiratory Epithelium. Toxicology 187: 13-24. (NCEA) **Open Literature** 8 F344 Male Rats 40 ul aliquots of water or formaldehyde (400 mM) were instillled into nostrils using a pipette. Twenty-four hours after treatment, nasal epithelium was recovered from which total RNA was used to generate cDNA probes.	Siginificance analysis of microarrays (SAM) hybridization data revealed that 24 of the 1185 genes queried were significantly up-regulated and 22 genes were significantly downregulated. The identified genes with FA-induced change in expression belong to the functional gene categories xenobiotic metabolism, cell cycle, apoptosis, and DNA repair. These data suggest that multiple pathways are dysregulated by formaldehyde exposure, including those involved in DNA synthesis/repair and regulation of cell proliferation.
870.3100 Other Purity: 28.44%	Vargova M, Wagnerova J, Liskova A, et al. (1993) Subacute immunotoxicity study of formaldehyde in male rats. Drug Chem Toxicol 16:255-275. **Open Literature** Male Wistar Rat Formaldehyde was administered by gavage at doses of 0, 20, 40, and 80 mg/kg/day for 4 weeks, 5 days/week, 1x/day	**NOAEL: 40 mg/kg/day** **LOAEL: 80 mg/kg/day** for an increase in the incidence of hepatocelluar vacuolization
870.3465 Other Purity: 14-C Paraformaldehyde	Casanova, Mercedes, et al. (1994) DNA-Protein Cross-links and Cell Replication at Specific Sites in the	**NOAEL: 2.5 mg/m^3** **LOAEL: 7.4 mg/m^3**

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
(97.3-99%), Unlabeled paraformaldehyde (95%)	Nose of F344 Rats Exposed Subchronically to Formaldehyde. Fundamental and Applied Toxicology 23: 525-536. **Open Literature** Rats inhalation (20 rats/group, 10 of which are preexposed (PE), 10 not (N)) 0.7, 2, 6, 10, or 15 ppm (0, 0.87, 2.5, 7.4, 12.4, or 18.6 mg/m^3) Preexposed animals whole-body exposed 6 hr/day, 11 weeks +4 days) On the 5th day of the 12th week, animals exposed once nose-only for 3 hours H14-CHO using nominal concentrations DPX estimation: 6 ppm or 10 ppm (7.4 or 12.4 mg/m^3) On 5th day of 12th week, exposed to unlabeled formaldehyde once nose-only for 3 hours using nominal concentration	Visible lesions were only observed in animals exposed to 18.6 mg/m^3 for 12 weeks. No formaldehyde-induced lesions were observed in the squamous and olfactory regions of the nose. Rats exposed to 0.87 or 2.5 mg/m^3 were indistinguishable from controls. At 7.4 mg/m^3, lesions were confined to multifocal epithelial hypertrophy, hyperplasia, and squamous metaplasia of the lM, while the rest of the nose was unaffected. At 12.39 mg/m^3, the most characteristic response was squamous metaplasia of the transitional epithelial lining of the LM and medial maxilloturbinate, and mild epithelial hyperplasia of the midseptum with generally mild inflammatory cell infiltration. At 18.6 mg/m^3, formaldehyde-induced lesions were more severe than all other exposure groups. Rats exposed for 12 weeks exhibited extensive damage to the lining of the LM (high tumor site) with epithelial erosions, transitional epithelial hyperplasia, squamous metaplasia, intraluminal and mucosal infiltration by inflammatory cells, and keratinizing epithelial plaques associated with subepithelial inflammation. Animals exposed to 18.6 mg/m^3 also exhibited thickening of the periosteum of bones adjacent to severe epithelial damage, and moderate degrees of edema and hyperemia of the lamina propria in these regions. At 7.4 and 18.6 mg/m^3, significantly ($p<0.01$) greater incorporation of 14C into DNA occurred in the lateral meatus of preexposed rats. Significantly ($p<0.01$) greater incorporation also occurred in the medial and posterior meatuses of preexposed rats at 18.6 mg/m^3.

Table 4. Special studies data for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**

5.0 Toxicity Endpoint Selection

5.1 See Section 7.1, Summary of Toxicological Doses and Endpoint Selection

5.2 Dermal Absorption

Only two studies from the open literature were located that examined dermal absorption of formaldehyde (Jeffcoat et al., 1983; Bartnik et al., 1985). In the Bartnik et al. study, a cosmetic cream containing 0.1% formaldehyde was applied an 8 cm^2 area of the shaved dorsal skin of male and female rats under non-occlusive and occlusive conditions. Urine and feces were collected up to 48 hours post-dose. Under non –occlusive conditions, absorption of radiolabeled formaldehyde in the cosmetic cream preparation was published as 6.1% in males and 9.2% in females. Occlusive conditions reported absorption as 3.4% in males.
In the study be Jeffcoat et al., rats, guinea pigs, and monkeys were used in experiments to determine dermal absorption of 0.1 and 2.0 mg doses of radiolabelled formaldehyde from application to a 2 cm^2 area for 24 hours. In rats, between 6-9% of a dose of 0.1 or 2.0 mg formaldehyde was absorbed, while in guinea pigs results were similar. In monkeys, less than 1% was absorbed.

5.3 Classification of Carcinogenic Potential

The Agency is currently reevaluating the carcinogenic potential of formaldehyde. The historical and ongoing development of an inhalation unit risk value to assess the carcinogenic potential of formaldehyde is briefly summarized below. Contributors to this summary included scientists from several EPA program offices (Office of Pesticide Programs [OPP], Office of Pollution, Prevention, and Toxics [OPPT], Office of Research and Development,/National Center for Environmental Assessment [ORD/NCEA], Office of Research and Development/National Health Effects Exposure Research Laboratory [ORD/NHEERL], and Office of Air and Radiation [OAR]).
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- In 1991 the EPA's Integrated Risk Information System (IRIS) published a weight-of-evidence characterization for carcinogenicity of formaldehyde, classifying formaldehyde as a B1 probable human carcinogen with a potency factor of 1.3 E-5 per ($\mu g/m^3$) on the basis of squamous cell nasal tumors observed in a two-year study in rats (Kerns et al., 1983).

- In 1999 the Chemical Industry Institute of Toxicology (CIIT) developed a health risk assessment for formaldehyde based upon animal toxicity data (CIIT, 1999). This document presented the dose-response modeling of these data in two distinct parts: 1). based upon a biologically-based dose response (BBDR) model , 2) benchmark dose models that were based upon point of departures at various response levels of the tumor and precursor data. Both these approaches made extensive use of the available time-to-tumor and mechanistic information. The 1999 assessment was subsequently published in various articles in peer-reviewed journals (Kimbell et al., 2001; Schlosser et al., 2003; Conolly et al., 2002, 2003, 2004).

- In 1999, the U.S. EPA's Office of Air and Radiation and Office of Research and Development, in conjunction with Health Canada, conducted an external peer review workshop for the CIIT BDDR model as well as an external written peer review and public comment period for their assessments. While the review was largely positive on the overall approach in the assessment, reviewers also pointed to the potential for significant uncertainty due to model mis-specification and uncertainties in key parameters involved in the BBDR model

- Based on the peer review of the CIIT model, OAR determined in 2004 that the CIIT model was the most appropriate tool for risk assessment for formaldehyde. OAR has subsequently used the the CIIT model for a number of risk assessments involving formaldehyde emissions to the atmosphere such as the Plywood and Composite Wood Products National Emission Standard for Hazardous Air Pollutants (final rule 2004, reconsidered final rule 2006, remanded to EPA by court 2007); Control of Hazardous Air Pollutants from Mobile Sources (Final Rule 2007); and Proposed Rule for National Emission Standard for Combustion Turbines (2004). Health Canada, Australia, the World Health Organization, and the German MAK Commission have also used the CIIT model. Model strengths include consideration of the mode of action data for formaldehyde and an approach to account for potential direct DNA interaction and mutation induction. Model uncertainties include variability for some of the parameters of the model (e.g., cell proliferation) which can affect predictions of risk (Subramanian et al 2007; 2008 [in press]).

- In 2004, NCEA convened a panel of experts, including scientists from CIIT, to provide advice on these and other critical biological and statistical uncertainties. The strength of the CIIT model is its consideration of mode of action and extensive mechanistic information.

- Although current OAR assessments still use the CIIT model, these assessments now acknowledge previously unknown uncertainties with the CIIT model when characterizing the risk results.

- In 2004, the International Agency for Research on Cancer (IARC) characterized formaldehyde as a human carcinogen based on their review of the current literature (IARC, 2004), including data in humans on nasopharyngeal cancer, cancer of the

nasal cavity and paranasal sinuses, and leukemia (Hauptmann et al., 2003, 2004). It should be noted that some epidemiology studies did not find a reported association between formaldehyde exposure and carcinogenicity. For example, Coggon et al, 2003 studied over 14,000 workers exposed to formaldehyde in industrial workplaces and reported no excesses of either leukemia or nasal and nasopharyngeal cancer.

- In 2005, the Scientific Review Panel (SRP) of the California Office of Environmental Health Hazard Assessment (OEHHA) responded to the CA Air Resources Board request to reevaluate the carcinogenic potential of formaldehyde. The SRP noted in this 2005 review that OEHHA's November 2002 evaluation of a petition had included the 1999 report on the CIIT model and other information, and that California's OEHHA had concluded that "*the evidence…(1) did not change the determination that formaldehyde is a carcinogen; (2) presented information that considered the possibility of non-linear dose response relationships, but presented no clear grounds to review the original "no threshold" determination; and (3) did not provide any new epidemiology or bioassays supporting a change in potency. In addition, there was insufficient information to fully evaluate the CIIT model, issues such as model uncertainty were not adequately addressed….*" The Scientific Review Panel's overall conclusion in 2005 was, *"there was not sufficient new data to support the petition to review the [OEHHA's earlier 1992] formaldehyde risk assessment. In addition, the newly published studies represented relevant new information, but they did not allow determination of a causal relationship between formaldehyde exposure and leukemia. These studies deserve further evaluation over time given their potential importance."* Froines (2005).

- EPA is currently completing a new IRIS assessment that will include a cancer unit risk value for formaldehyde; the reassessment is scheduled to start internal peer review in May 2008 and begin independent external peer review in January 2009 (http://cfpub.epa.gov/ncea/iristrac/index.cfm?fuseaction=viewChemical.showChemical&sw_id=1031). EPA anticipates that the peer review of the formaldehyde assessment will not be finished before EPA completes the reregistration process for formaldehyde pesticidal uses, scheduled to conclude in September 2008.

Based on the on going re-evaluation of the science to predict carcinogenic potential of formaldehyde, OPP has decided to present the formaldehyde cancer risks for the pesticidal uses using both the existing 1991 IRIS cancer unit risk of 1.3 E-5 per ($\mu g/m^3$) and the CIIT BBDR model until any new cancer estimates are fully peer reviewed. OPP also acknowledges the wide range in cancer risks using these approaches and will coordinate with other offices in EPA on the outcome of the upcoming peer review process on the carcinogenicity of formaldehyde. Because formaldehyde air concentrations approach those associated with ocular and respiratory tract irritation, the risk mitigation measures to be implemented in the meantime for the pesticidal uses will be based on mitigating the non-

cancer effects at a limit of 0.01 ppm. It is believed that this level will reduce exposures sufficiently such that the cancer risks would not be of concern. The EPA process of regulating pesticides allows for reevaluation at any time if new information from the peer review process of the carcinogenic potential of formaldehyde warrants.

6.0 FQPA Considerations

Under the Food Quality Protection Act (FQPA), P.L. 104-170, which was promulgated in 1996 as an amendment to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug and Cosmetic Act (FFDCA), the Agency was directed to "ensure that there is a reasonable certainty that no harm will result to infants and children" from aggregate exposure to a pesticide chemical residue. The law further states that in the case of threshold effects, for purposes of providing this reasonable certainty of no harm, "an additional tenfold margin of safety for the pesticide chemical residue and other sources of exposure shall be applied for infants and children to take into account potential pre- and post-natal toxicity and completeness of the data with respect to exposure and toxicity to infants and children. Notwithstanding such requirement for an additional margin of safety, the Administrator may use a different margin of safety for the pesticide residue only if, on the basis of reliable data, such margin will be safe for infants and children."

Although formaldehyde has no food tolerances and the registered antimicrobial uses do not involve dietary exposure, the database with respect to determining susceptibility to infants and children shows no increased susceptibility, based on results of developmental and reproductive toxicity testing. Assessments of the reproductive and developmental toxicity of formaldehyde conducted by the Australian government (www.nicnas.gov.au) as well as the Agency for Toxic Substances and Disease Registry (ATSDR, 1999)_support this conclusion.

7.0 Summary of Toxicological Doses and Endpoints for Formaldehyde for use in Risk Assessment

Table 5 Summary of Toxicological Doses and Endpoints for Formaldehyde			
Exposure Scenario	**Dose Used in Risk Assessment (mg/kg/day)**	**Target MOE, UF, Special FQPA SF* for Risk Assessment**	**Study and Toxicological Effects**
Dietary Risk Assessments			
Acute Dietary (general population including infants and children)	An acute dietary assessment is not needed for the registered antimicrobial uses of formaldehyde.		
Chronic Dietary (all populations)	A chronic dietary assessment is not needed for the registered antimicrobial uses of formaldehyde.		
Non-Dietary Risk Assessments			
Incidental Oral	An incidental oral risk assessment is not required for the registered antimicrobial uses of formaldehyde.		
Dermal (all durations)	A dermal risk assessment is not required for the registered antimicrobial uses of formaldehyde.		
Inhalation (all durations)	**NOAEL** (human) = 0.1 ppm	**UF = 1 (occupational)** **UF = 10 (residential)**	ACGIH 2001 publication on formaldehyde Horvath, E.P. et al. (1986): JAMA 259(5): 701-707. Based on complaints of eye, nose, and throat irritation in particle board workers at concentrations of formaldehyde from 0.4 – 1.0 ppm. Redden, J. (2005): Section 18 Emergency Exemption for the use of Paraformaldehyde: U.S. Army Medical Research Institute of Infectious Diseases.
Cancer	Formaldehyde is currently classified as a B1 (probable human carcinogen) in EPA's IRIS assessment. IARC has classified formaldehyde as "carcinogenic to humans." The Agency has decided to present the formaldehyde cancer risks for the pesticidal uses using both the existing 1991 IRIS cancer unit risk of 1.3 E-5 per ($\mu g/m^3$) and the CIIT BBDR model until any new cancer estimates are fully peer reviewed		

8.0 Toxicity Profile Tables

8.1 Acute Toxicity Profile Table - (See Section 4.1, Acute Toxicity, Table 2).

8.2 Subchronic, Chronic and Other Toxicity Profiles Table (Table 6)

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
Subchronic Toxicity		
870.3100 90-Day oral toxicity in rodents Purity: 37% a.i.	**MRID 00124677** Driedger, A.; Walker, J.; Galloway, F. (1973) Letter sent to C. Smart dated Oct 1, 1973: "Rat tolerance to Dietary Formaldehyde: Reference No. AD-114-73, JRW-341-73." (Unpublished study; submitted by Celanese Chemical Co., Dallas, TX; CDL:094622-H) 10 male Holtzman rats/dose 0, 0.3, 0.6, 1.2, or 2.4 % formaldehyde	**NOAEL: 0.3%** formaldehyde **LOAEL: 0.6%** formaldehyde, based on irritability, weight loss, hair loss, yellowing of teeth, and decreased food consumption Rats exposed to concentrations of 0.6% formaldehyde and higher exhibited dose-related increases in irritability, disability, hair loss, and yellowing of teeth and dose-related decreased food consumption. Growth rates significantly different from controls are expected at formaldehyde concentrations ≥ 0.50 %, believed due to decreased food consumption.
870.3100 28-Day oral toxicity in rodents Purity: 60% a.i.	**MRID 00134114** Viguera, C.; Kundzins, M. (1960) "28-Day Oral Administration--Rats: U.F. Concentrate-85|." (Unpublished study; prepared by Hazleton Laboratories, Inc.; CDL: 105284-C) 10 Male Sprague-Dawley rats/dose 0, 79, 158, or 316 uL/kg/day, once daily, 5 days/ week, 20 doses	Statistical evaluation of overall body weight gains and total food consumption revealed no significant differences between the control group and test groups. The appearance and behavior of the test rats were comparable to those of the control rats. No pathological findings associated with the oral administration of the test substance were observed. One rat exposed to 158 uL/kg/day

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		died during the 4th week. Autopsy revealed a pale, mottled liver. Three rats receiving the high dose of formaldehyde showed slight salivation during the 4th week of the study.
870.3100 90-Day oral toxicity in rodents Purity: 95% a.i., aqueous paraformaldehyde	Johannsen, F.R., G.J. Levinskas A.S. Tegris (1986) Effects of Formaldehyde in the Rat and Dog following Oral Exposure. Toxicology Letters 30: 1-6. (NCEA) **Open Literature** Sprague-Dawley Albino Rat (15/sex/dose) Formaldehyde was administered in the drinking-water at target doses of 0, 50, 100, or 150 mg/kg bw/d for 13 weeks (91 consecutive days)	**NOAEL: 50 mg/kg/day (M), 100 mg/kg/day (F)** **LOAEL: 100 mg/kg/day (M), 150 mg/kg/day (F)**, based on decreased body weight gain No deaths or abnormal reactions were observed in rats administered formaldehyde for 90 days. Significant reductions in weight gain were observed in both sexes at 150 mg/kg and in male rats given 100 mg/kg. There was a dose-related decrease in liquid consumption in both male rats (9%, 18%, and 31%) and females (13%, 22%, and 30%) administered formaldehyde in their drinking water. There were no overall differences in mean food intake or feed efficiency in rats at any test level, thus reductions in body weight gain are considered to be a reflection of systemic effects of formaldehyde. No statistically-significant differences were observed in hematologic parameters in any treated rats. No specific treatment-related effects were observed on any organ or tissue, including possible target organs like the kidney, liver, and lung. Clinical chemistry and urinalysis studies failed to indicate any necrotic effects on muscle, kidney, liver, or heart. No differences were apparent between absolute or relative organ weights of treated rats. No treatment-related pathological changes were observed microscopically.

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
870.3100 28-Day oral toxicity in rodents Paraformaldehyde (95% a.i., aqueous)	Til, H.P., et al. (1988) Evaluation of the Oral Toxicity of Acetaldehyde and Formaldehyde in a 4-week Drinking Water Study in Rats. Fd. Chem. Toxic. 26(5): 447-452. (NCEA) **Open Literature** Rat (10/sex/dose) 0, 5, 25, or 125 mg/kg/day; a water-restricted group (10/sex) received the same amount of water as liquid consumed by the high-dose groups	**NOAEL= 25 mg/kg/day** **LOAEL = 125 mg/kg/day**, based on yellowish fur from week 3 onward, decreased food intake, decreased protein and albumin levels in blood plasma, and histologic changes. There were no deaths and the rats appeared healthy throughout the study. The fur of the rats receiving 125 mg/kg/day showed a yellowish discoloration from week 3 onwards. Food intake of animals receiving the high dose was significantly lower, whereas females receiving the low- and mid- dose groups had increased food intake. There were no significant changes in hematology among the test groups. Total protein and albumin levels in the blood plasma were decreased in males in the high dose. Relative kidney weights were increased at 125 mg/kg/day ($p>0.05$). Histologic examination of test groups revealed: focal hyperkeratosis of the forestomach (20/20); Focal gastritis (3/10 males, 3/10 females); submucosal mononuclear-cell infiltrate (1/10 males); focal papillomatous hyperplasia (1/10 females); and polymorphonuclear leukocytic infiltration (1/10 females). The water- restricted group had slightly higher blood cell values in males. Clinical chemistry and blood plasma changes observed include: increased urea in males and females; decreased bilirubin levels and increased chloride and sodium levels in males and decreased sodium, calcium, and phosphorus in females. Increased relative organ weights were observed in male gonads ($p<0.01$),

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		brains (males: $p<0.05$, females: $p<0.01$), male hearts ($p<0.01$), kidneys ($p<0.01$), and in the liver (males: $p<0.01$, females: $p<0.05$). Histopath examination revealed dilated fundic glands (2/10) in males.
870.3150 90-Day oral toxicity in nonrodents Purity: Paraformaldehyde (95% a.i., aqueous)	Johannsen, F.R., G.J. Levinskas, A.S. Tegris. (1986). Effects of Formaldehyde in the Rat and Dog following Oral Exposure. Toxicology Letters 30: 1-6. (NCEA) **Open Literature** Beagle Dog (4/sex/dose) 0, 50, 75, or 100 mg/kg/day in drinking water for 90 days	**NOAEL: 75 mg/kg/day (M/F)** **LOAEL: 100 mg/kg/day (M/F)**, based on reduced weight gain No deaths or abnormal reactions were observed. Significant reductions in weight gain were observed in both sexes at 100 mg/kg/day. Treated animals had reduced food consumption and feed efficiency even at the lower dosages (50 and 75 mg/kg/day) which did not depress weight gain. Hematological values from treated dogs fell within normal limits.No specific treatment-related effects were observed on any organ or tissue, including possible target organs like the kidney, liver, and lung.
870.3465 90-Day inhalation toxicity	**MRID 00082134** Coon, R.A. et al. (1970) Animal Inhalation Studies on Ammonia, Ethylene Glycol, Formaldehyde, Dimethylamine, and Ethanol. Toxicology and Applied Pharmacology 16: 646-655. (NCEA) 15 Sprague-Dawley and Long-Evans rats (M/F), 15 Princeton-derived guinea pigs (M/F), 3 New Zealand rabbits (M), 3 squirrel monkeys (M), 2 Beagle dogs (M) Formaldehyde Continuous exposure to 4.6 mg/m^3, 8 hours/day, 5 days/week, 6 weeks	One of the 15 rats died; none of the other animals showed signs of illness or toxicity. Hematologic values were normal. On histopathologic examination, the lungs of all species consistently showed varying degrees of interstitial inflammation. The hearts and kidneys from guinea pigs and rats showed focal chronic inflammatory changes.

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
870.3465 6-Week inhalation toxicity Purity = 4.96%	**MRID 00149755** Rusch, G.; Rinehart, W. (1980) A 26 Week Inhalation Toxicity Study of Formaldehyde in Monkey, Rat, and Hamster: Project No. 79- 7259. Unpublished study prepared by Bio/dynamics Inc. 184 p. Fisher 344 rats – 10/sex/dose; Syrian golden hamsters – 10/sex/doseand Cynomolgous monkeys – 6 males/dose Test material (Formaldehyde, Lot #0611N-79) was administered at 0, 0, 0.20, 1.00, or 3.00 ppm equivalent to 0, 0, 0.19, 0.98 and 2.95 ppm, respectively, for 26 weeks.	Treatment-related effects during the study were not seen. Compared to controls, monkeys receiving 1.00 ppm showed increased incidence of dried material around the nose, increased incidences of hoarseness and congestion. <u>Body weight</u> Compared to controls, no significant body weight changes were seen for monkeys and hamsters throughout the study. The 3 ppm male and female rats showed significant differences ($p \leq 0.01$) from week 2-26 compared to controls. <u>Organ weight</u> Organ weights for monkeys and hamsters were not significantly different compared to controls. Male and female rats in the 0.2 ppm group had significant mean heart weight depression ($p \leq 0.01$) compared to the control. Males in the 3.0 ppm test group had significantly ($p \leq 0.01$) depressed mea absolute heart and kidney weights compared to the controls, but the relative weights of these same tissues were significantly increased for these same rats. Females in the 3.0 ppm test group had significantly ($p \leq 0.01$) depressed absolute heart weights with the mean relative heart weight significantly increased ($p \leq 0.01$). For the 3 ppm group, the mean absolute and relative liver weights were significantly depressed ($p \leq 0.01$) compared to the controls. <u>Gross and Microscopic Pathology</u> In monkeys, hamsters and rats, no abnormalities were seen in or attributable to formaldehyde vapors.
870.3465	Woutersen, R.A. et al. (1987)	**NOAEL: 1.2 mg/m^3**

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
90-Day inhalation toxicity Purity: 97-99% a.i.	Subchronic (13-week) Inhalation Toxicity Study of Formaldehyde in Rats. Journal of Applied Toxicology, 7(1): 43-49. **Open Literature** Rats (10/sex/dose) 0, 1.0, 10, or 20 ppm (0, 1.2, 12, or 24 mg/m^3), 6 hr/day, 5 days/week	**LOAEL: 12 mg/m^3** In the high-dose group, uncoordinated locomotion, and climbing of the cage walls were observed only during the 1st 30 minutes of each exposure period. Statistically-significant growth retardation occurred in males and females of the high-dose group. Treatment-related changes were not observed in the autopsy, except for a yellowish fur of mid- and high-dose animals. No relevant differences were found in the hematological and urinary parameters measured. Dose-related histopathologic changes in the nose were observed in the mid- and high-dose groups. Half of the 24 mg/m^3 male rats showed squamous metaplasia, occasionally accompanied by keratinization, of the epithelium lining the vocal cord region of the larynx. The nasal turbinates of rats exposed to12 or 24 mg/m^3 formaldehyde exhibited a marked increase in the number of labeled cells, practically all of which were present in areas of the epithelium showing clear squamous metaplasia and hyperplasia.
870.3465 90-Day inhalation toxicity	Appelman, L.M. et al. (1988) One-year Inhalation Toxicity Study of Formaldehyde in Male Rats with a Damaged or Undamaged Nasal Mucosa. Journal of Applied Toxicology, 8(2): 85-90. **Open Literature** Male albino Wistar rats (Cpb:WU) - 40/dose Formaldehyde exposure via inhalation route for 6 hr/day, 5 days/week, 13 or 52 weeks at concentrations of 0.1, 1, or 10 ppm (0, 0.12, 1.24, or 12.4	**NOAEL = 1.24 mg/m^3** **LOAEL = 12.4 mg/m^3**, based on body weight retardation, incidence of oliguria, and incidence of lesions of the respiratory and olfactory epitheliums for damaged and undamaged animals The nose damaged by electrocoagulation is more susceptible to cytotoxic action of formaldehyde than the undamaged nose. 8 animals (7 with damaged and 1 with undamaged nose) randomly

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	mg/m^3), 1/2 with bilaterally damaged nasal mucosa	distributed among control and test groups, had to be killed in extremis or were found dead. Growth retardation was observed in animals with or without a damaged nose after 2 weeks exposure to 12.4 mg/m^3 formaldehyde. No relative differences were found between the hematological and urinary parameters, with the exception of frequent oliguria ($p<0.05$) in the high-dose group without nasal coagulation killed in week 53. 13 weeks: Histopathologic examination revealed focal squamous metaplasia and focal basal cell hyperplasia ($p<0.01$) and focal rhinitis ($p<0.05$) in the respiratory epithelium of the undamaged 12.4 mg/m^3 dose group. In the damaged 12.4 mg/m^3 dose group, focal thinning/ disarrangement of the olfactory epithelium was identified ($p<0.05$). 52 weeks: The undamaged 0.12 mg/m^3 and 1.24 mg/m^3 dose groups displayed squamous metaplasia of the respiratory epithelium ($p<0.05$). At 12.4 mg/m^3, the undamaged group had squamous metaplasia, basal cell hyperplasia, and focal rhinitis ($p<0.05$) of the respiratory epithelium. The 12.4 mg/m^3 damaged dose group displayed thinning/disarrangement and loosely arranged submucosal connective tissue ($p<0.01$) in the olfactory epithelium and squamous metaplasia ($p<0.05$) of the respiratory epithelium.
870.3465 90-Day inhalation toxicity	Chemical Industry Institute of Technology 20 Mice and Rats Test material administered at	**NOAEL = 4 ppm (LDT)** **Systemic LOAEL = 12.7 ppm,** based on body weight decrease and nasal erosion.

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	concentrations of 4, 12.7, or 38.6 ppm for 6 hours each day, five days a week for 13 weeks.	No adverse effects observed in the 4 ppm group. At 12.7 ppm, a decrease in body weight and evidence of nasal erosion in two exposed rats was observed. Ulceration and necrosis of the nasal mucosa seen at 38.6 ppm resulted in termination of exposure after 2 weeks.
870.3465 90-Day inhalation toxicity Purity	Test material administered at concentrations of 0.0098, 0.028, 0.82, or 2.4 ppm for 3 months. 25 Rats	**Systemic NOAEL = 0.028 ppm** **Systemic LOAEL = 0.82 ppm,** base don proliferation of lymphocytes, histiocytes in the lungs, perivascular hyperemia. **ChE NOAEL = 0.82 ppm** **ChE LOAEL = 2.4 ppm** At 2.4 ppm there was a significant decrease in cholinesterase activity; at 2.4 and 0.82 ppm, there was proliferation of lymphocytes and histiocytes in the lungs and some peribronchial and perivascular hyperemia. There were no significant findings at the two lower concentrations.
870.3465 90-Day inhalation toxicity	Dubreuil, A., G. Bouley, J. Godin, and C. Boudène. (1976). Continuous inhalation of low-level doses of formaldehyde: Experimental study on the rat. Eur. J. Toxicol. 9:245-250. **Open Literature** 25 rats Test material administered at concentrations of 1.6, 4.55, or 8.07 ppm for 45-90 days.	**NOAEL = 1.6 ppm** The only adverse effect seen at 1.6 ppm was discoloration of hair. The 4.55 ppm group was exposed for 45 days and had a decrease in rate of weight gain. The 8.07 ppm was exposed for 60 days and has respiratory and eye irritation, a decrease in food consumption, and a decease in liver weight.
Developmental Toxicity		
870.3700a Prenatal Developmental Toxicity Purity: 35% a.i.	**MRID 00082136, 00123770** Schnurer, Lars- Bentil (1963) Maternal and Fetal Responses to Chronic Stress in Pregnancy: A Study	Formalin exposure resulted in small, subcutaneous necroses. No differences in smear cytology were noted between the pregnant treated

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	in Albino Rats. Acta Endocrinologica, Supplement 80: 1-96. Rats (56, 67, 50, 44/group, respectively) Subcutaneous injection, Pregnant rats exposed to 0.25 mL of 2% solution, pregnant control rats, non-pregnant rats exposed to 0.25 mL of 2%, control non- pregnant rats; 2x/day, GD 2-19 to 22	and pregnant control rats. No stress-induced changes of the gastric mucosa were seen. The following treatment- related organ weight changes were observed: thyroid weight was significantly lower in formaldehyde-exposed non-pregnant rats; adrenal weights increased significantly in exposed non-pregnant rats. Formaldehyde- exposed pregnant rats yielded 56 litters, totaling 551 fetuses. Pregnant controls yielded 67 litters, 662 fetuses. Formaldehyde-exposed rats had heavier fetuses than controls. No instances of malformed limbs or cleft palate were observed. Fetal thyroid and adrenal weight reductions may be due to passage of corticosteroids from exposed mothers to fetuses.
870.3700a Prenatal Developmental Toxicity (rodent) Purity: Fischer certified ACS solution, contains 12-15% methanol	**MRID 00164652** Marks, Thomas A. et al. (1980) Influence of Formaldehyde and Sonacide (Potentiated Acid Glutaraldehyde) on Embryo and Fetal Development in Mice. Teratology 22: 51-58. Oral gavage (76/29/35/34 animals/dose) 0, 74, 148, or 185 mg/kg/day, GD 6-15 Female CD-1 Mice	Maternal Toxicity: **NOAEL = 0 mg/kg/day** **LOAEL = 74 mg/kg/day**, based on decreased body weight gain. The 185 mg/kg/day dose of formaldehyde was clearly toxic; 22 of the 34 pregnant mice died before day 18. Methanol, 12-15% of the original solution, may have contributed to this toxicity. There was also a significant decrease in average weight gain during pregnancy at 74 mg/kg/day. The test solution did not have a significant effect in the incidence of malformed mouse fetuses. Doses of 148 and 74 mg/kg/day had no significant effect on the unborn offspring or on the pregnant dam.
870.3700a	Saillenfait, A.M., et al (1989) The	Maternal Toxicity:

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
Prenatal Developmental Toxicity Purity: 37% a.i.	effects of maternally inhaled formaldehyde on embryonal and foetal development in rats. Fd. Chem. Toxic. 27(8): 545-548. (NCEA) **Open Literature** Female Sprague-Dawley rats (25/dose) 0, 5, 10, 20, or 40 ppm (0, 6.2, 12.4, 24.8, or 49.6 mg/m^3) for 6 hr/day, GD 6-20	**NOAEL = 24.8 mg/m^3** **LOAEL = 49.6 mg/m^3**, based on decreased body weight gain Offspring Toxicity: **NOAEL = 12.4 mg/m^3** **LOAEL = 24.8 mg/m^3**, based on reduced fetal weight gain Not teratogenic, slightly fetotoxic without overt signs of maternal toxicity. There were no significant differences between groups in the numbers of implantations, number of resorptions and the stage of gestation at which they occurred, or the numbers of dead or live fetuses. Exposure to formaldehyde had no detectable adverse influence on the incidence of pregnancy or the fetal sex ratio. External, visceral and skeletal examination of the fetuses did not reveal any major abnormalities. The only outward sign of a fetal response was a significant concentration-related reduced in fetal body weight gain (fetal body weight was 5% less at 24.8 mg/m^3 and 21% less at 49.6 mg/m^3).
870.3700a Prenatal Developmental Toxicity (rodent) Purity: 37% a.i.	Overman, D.O. (1984) Absence of Embryotoxic Effects of Formaldehyde after Percutaneous Exposure in Hamsters. Toxicology Letters 24: 107-110. **Open Literature** Pregnant Charles river Lak:LVG (SYR) Golden strain hamsters – Number of animals not reported 0.5 mL, 2 hours/day, GD 8-11	Treatment had no effect on maternal weight gain. The treatment did not influence fetal C-R length. Mean fetal weight was slightly increased in experimental animals, but the difference was not statistically-significant. Two fetuses from the same litter after treatment on day 8 were significantly smaller than their litter mates (>3 SD below mean). The same was true for 2 fetuses from different litters after treatment on day 10. One fetus of normal size

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		treated on day 10 had a subcutaneous hemorrhage in the dorsal cervical region. No skeletal malformations were found and no other malformations were observed.
Reproductive Toxicity		
870.3800 Reproduction and fertility effects Purity: 40% a.i.	**MRID 00143291** Hurni, H. and H. Odher (1972) Reproduction Study with Formaldehyde and Hexamethylenetetr-amine in Beagle Dogs. Fd. Cosmet. Toxicol. 11: 459-462. 51 female Beagle dogs 0, 3.1, or 9.4 mg/kg/day	The study revealed no teratogenic action. The treatments did not affect the pregnancy rate. The body weight increased regularly during pregnancy in all groups and the duration of gestation was unaffected by the treatments. The mean litter size was within the normal range for all groups, demonstrating that fecundity was not affected by treatment. Neither the adult dogs nor their litters showed any signs of physiological or skeletal abnormalities or disorders of reproduction.
870.3800 Reproduction and fertility effects Purity: 40% a.i.	Cassidy, S.L., K.M. Dix, and T. Jenkins (1983) Evaluation of a testicular sperm head counting technique using rats exposed to dimethoxyethyl phthalate (DMEP), glycerol a-monochlorohydrin (GMCH), epichlorohydrin (ECH), formaldehyde (FA), or methyl methanesulphonate (MMS). Arch. Toxicol. 53:71-78. **Open Literature** Male Wistar rats (5/group for treatment, 20 controls) Treatment groups were dosed once orally with 100 or 200 mg/kg formaldehyde and killed 11 days after dosing	200 mg/kg: A statistically significant increase in total sperm heads per gram testis, as well as an increase in percentage of abnormal sperm heads. Data indicated that "the induction of increased levels of abnormal sperm may be a measurable index of the mutagenic potential of a chemical for mammalian germ cells".
Chronic Toxicity		

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
870.4100a Chronic Toxicity Purity: 9.20%	Battelle, Pacific Northwest Laboratories. (1980) "Tracor Jitco Inhalation Carcinogenesis Bioassay: Repeated Dose Study Report on Formaldehyde." **Open Literature** B6C3F1 Mouse (5/sex/group) Mice were exposed to one of five concentrations of vaporized formaldehyde for a period of 6 hours per day for a total of ten exposures. The target concentrations were 15, 25, 50, 100, and 200 ppm (18.59, 30.98, 61.96, 123.93, and 247.85 mg/m^3).	Concentrations of 123.93 mg/m^3 or greater produced 100% mortality. The highly irritating nature of this chemical was evident microscopically in all dose levels examined, ranging from minimal to mild supportive rhinitis in the 18.59 mg/m^3 dose level dose level, to necrosis and sloughing of the mucosa in the turbinates, trachea, and proximal bronchi in the 61.96 mg/m^3 animals. Differential weight gains of both male and female mice at 18.59, 30.98, and 61.96 mg/m^3 was significant as compared to the controls. At 123.93 and 247.85 mg/m^3, only female mice showed significant weight loss, as the early mortality of the males precluded obtaining any meaningful data.
870.4100a Chronic Toxicity Purity: 37% a.i.	Kamata, Eiichi et al. (1997) Results of a 28-month Chronic Inhalation Toxicity Study of Formaldehyde in Male Fischer-344 Rats. The Journal of Toxicological Sciences 22(3): 239-254. **Open Literature** Male Fischer 344 rats (32/dose) 0, 0.3, 2, or 15 ppm (0, 0.4, 2.5, or 19 mg/m^3), 6hr/day, 5 days/week via inhalation	**NOAEL: 0.4 mg/m^3** **LOAEL: 2.5 mg/m^3** Nasal tumors were macroscopically evident in the 19 mg/m^3 group from the 14th month. Histopathological examination revealed squamous cell papillomas and carcinomas. No nasal tumors were observed in the lower exposure groups (0.4 and 2.5 mg/m^3 groups). In the high-dose group, frequent face washing, coughing and/or crouching position, lacrimation, nasal discharge, and yellow discoloration of the haircoat were observed. Significant decreased food consumption was observed and 20 rats died by the 24th month. Reduced triglyceride levels and liver weights, presumably related to reduced food intake, were also seen in the 19 mg/m^3 group. Epithelial cell hyperplasia, hyperkeratosis, and squamous

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		metaplasia were apparent in all exposure groups. Inflammatory cell infiltration, erosion, or edema was apparent in all exposure groups, including the controls. The benchmark dose for squamous metaplasia and epithelial hyperplasia were 0.30 and 0.31 mg/m^3, respectively.
Carcinogenicity		
870.4200a Oncogenicity (Rat)	**MRID 00143288** Watanabe, F., et al. (1954) Study on the Carcinogenicity of Aldehyde. 1st Report. Experimentally Produced Rat Sarcomas by Repeated Injections of Aqueous Solution of Formaldehyde. Two unpublished translations of Japanese article published in Gann 45(2-3):451-452. Rat Repeated subcutaneous injections of 1 cc of an aqueous formaldehyde solution at 0.6% to0.8%. With 0.4% to 0.5% aqueous formaldehyde solutions it was possible to inject subcutaneously once or twice a week. Subcutaneous injections of 1 cc of a 0.4% aqueous formaldehyde solution were continued on 10 rats once a week for about 1 year and three months.	0.6% to 0.8%: necrosis, the formation of an ulcer, while the area around the injection spot formed a tuber which was very difficult to heal 0.4% - 0.5%: rare occurrence of an ulcer. After two to five months after having stopped the injections observations revealed the occurrence of sarcomas either at the injection spot or in the internal organs of 4 out of 10 of the rats.
870.4200a Oncogenicity (Rat)	Tobe, M., T. Kaneko, Y. Uchida, et al. 1985. Studies of the inhalation toxicity of formaldehyde. National Sanitary and Medical Laboratory Service (Japan). p. 1-94. **Open Literature** 32 Male Fischer 344 rats/dose Test material was administered at concentrations of 0, 0.3, 2.0 or 15 ppm in aqueous solution methanol, 6 hours/day, 5 days/week for 28 months.	During the exposure running noses, running tears and crouching were seen in the 15 ppm dose group. These symptoms decreased as the number of exposures increased. Hair around the abdominal region was observed to be yellow in color and bleeding from the forelimbs was seen. Yellow discoloration of abdominal hair was also seen in the 2.0 ppm dose group although it was light. Significant suppression of weight gain and a decrease in

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	The exposure at 15 ppm was tested for 24 months. A positive control – 3.3 ppm methanol and a nonexposure (NE) control were also used.	the amount of food gain were seen in the 15 ppm dose group. 20 of 24 animals in the 15 ppm dose group died in the 24 month dosing period giving a high death rate of 88.3%. Recognizable tumors were observed in the 15 ppm group from the 420th day onwards and tumors were recognized macroscopically in eight animals by the 24th month. Squamous cell carcinoma was recognized in 14 rats and pappiloma in 5 rats. Unclassified carcinoma was seen in 1 rat in the nonexposure group which died on the 825th day. No neoplastic changes were seen in the 0.3 and 2.0 ppm and exposure control dose groups. Excessive secretion was seen in the nasal cavity, rhinitis accompanied by desquamation, squamous epithelial metaplasia and epithelial cell hyperplasia were recognized in the 0.3 and 2.0 ppm dose groups and these were significant in the 15 ppm dose group. A decrease in the T-GLY and a decrease in liver weight, assumed to be changes accompanying decrease in food intake due to formaldehyde exposure were seen in the 15 ppm dose group. However, these changes were not accompanied by histological changes.
870.4200a Oncogenicity (Rat)	Takahashi et al. (1986) Effects of Ethanol, Potassium Metabisulfate, Formaldehyde, and Hydrogen Peroxide on Gastric Carcinogenesis in Rats after Initiation with N- methyl-N'nitro-N'nitrosoguanidine. Jap. J. Cancer Res. 77: 118-124. **Open Literature**	Formalin did not produce malignant tumors when given alone. Forestomach papillomas occurred in 8/10 animals administered formalin alone. In the group administered both MNG and formalin, forestomach papillomas occurred in 15/17

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	Male Wistar rats A two-stage carcinogenesis bioassay was conducted in which N-methyl-N'nitro- N'nitrosoguanidine was administered at 100 mg/l in the drinking water for the first 8 weeks of the study, followed by administration of formalin (dose not specified).	animals, adenocarcinoma of the pylorus in 4/17, preneoplastic hyperplasia of the pylorus in 7/17, and adenocarcinoma of the duodenum in 1/17.
870.4200b Oncogenicity (Mouse) Purity: 1% and 10%	Iversen, Olav Hilmar. (1986) Formaldehyde and Skin Carcinogenesis. Environ Int 12:541-544. **Open Literature** Hairless mice of the hr/hr Oslo Strain (16/sex) Topical application of 200 ug formaldehyde in water on the back skin twice a week for 60 weeks	Nonspecific granulomas in the lung; slight hyerplasia of the epidermis, small skin ulcers
870.4200b Oncogenicity (Mouse) Purity: 10%	Krivanek, N.D., N.C. Chromey and J.W. McAlack, "Skin initiation-promotion study with formaldehyde in CD-1 mice", E.I. du Pont de Nemours & Company, Inc. In: Formaldehyde: Toxicology, Epidemiology, and Mechanisms, Clary, J.J., J.E. Gibson, and R.S. Waritz, Eds., N.Y., Marcel Dekker, Inc., 1983. **Open Literature** Female CD-1 Mouse Mice were treated on shaved dorsal skin with up to 10 mg formaldehyde, followed by repeated doses. Formaldehyde was also applied once at 5 mg/mouse to assess initiation potential. Promoter potential was tested at 0.1, 0.5, and1.0 mg/mouse, applied 3 times/wk for 26 wk. Positive controls [150 mg benzo(a)pyrene (BaP) as initiator, 2.5 mg 12-O-	Repeated doses of 2-5 mg caused mild to moderate skin irritation, whereas 1 mg caused only mild irritation. As expected, BaP/TPA gave a high tumor yield (28/29 mice, 9 of which had malignant tumors. Benign test site tumors were keratoacanthomas or squamous papillomas. No other combinations gave yields significantly different from controls. Thus the test is negative under study conditions, with the caveat that one cannot be certain whether formaldehyde underwent significant degradation to formic acid or other products.

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	tetradecanoylphorbol-13-acetate(TPA) as promoter], or negative control (acetone) were used.	
870.4200b Oncogenicity (Mouse) Purity: 3.7%-4%	Spangler, F. and J.M. Ward, "Skin initiation/promotion study with formaldehyde in Sencar mice". Study location: Microbiological Associates (Bethesda, MD) in conjunction with NCI. In: Formaldehyde: Toxicology, Epidemiology, and Mechanisms, Clary, J.J., J.E. Gibson, and R.S. Waritz, Eds., N.Y., Marcel Dekker, Inc., 1983. **Open Literature** Female Sencar Mice (30/group) Mice were treated in various combinations with or without an initiator (DMBA) or promoter [12-O-tetradecanoylphorbol-13- acetate (TPA)]. All test compounds were applied to back skin of mice with acetone, which was used as a negative control in some treatment combinations Formaldehyde was tested for initiating and promoting capability. In all cases, formaldehyde was applied in acetone; however the amount of this solution applied was not specified. All tests of initiators (including formaldehyde, when tested for such potential) were as a single dose. Promoters (including formaldehyde, when tested for such potential) were applied once or twice a week. This is an interim report, relating counts of skin papillomas as of the first 48 weeks of the study.	Study found no evidence of formaldehyde as an initiating agent, nor as a complete carcinogen, however investigators considered there to be "a slight possibility that formaldehyde may be a very, very weak promoting agent", based on a very small tumor yield when formaldehyde was tested as a promoter in mice treated with DMBA.
870.4200 Oncogenicity Purity: Not reported	Dalbey, W.E. (1982). Formaldehyde and tumors in hamster respiratory tract. Toxicology. 24: 9-14. **Open Literature** 88 male Syrian golden hamsters	Lifetime exposure to formaldehyde reduced survival time ($P < 0.05$) relative to unexposed controls. No tumors were observed in the respiratory tract of non-exposed hamsters or of those exposed to formaldehyde. There was,

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	Test material was administered at a 10 ppm concentration 5 times/week for lifetime.	therefore, no evidence of carcinogenic activity of formaldehyde under the given exposure conditions. Little evidence of toxicity from formaldehyde exposure was observed in the nasal epithelium, expected to be a prime target issue. There was no increase in the incidence of rhinitis related to exposure (observed in 31% of untreated animals and 24% of the formaldehyde-exposed hamsters). Hyperplastic and metaplstic areas were each observed in the nasal epithelium of 5% of hamsters exposed to formaldehyde while none were observed in control animals.
870.4300 Chronic/ Oncogenicity Purity: Not Reported	**MRID 00143289** Kerns, W.D. et al. (1983) Carcinogenicity of Formaldehyde in Rats and Mice after Long-Term Inhalation Exposure. Cancer Research 43: 4382-4392. Rat (Fischer 344) and Mice ($B6C3F_1$) - approx 120/sex/dose 0, 2.0, 5.6, or 14.3 ppm (0, 2.5, 6.9, or 18 mg/m^3), 6 hrs/day, 5 days/week, up to 24 months	From exposure weeks 3 to 103, mildly (15 to35 g) decreased body weights ($p<0.05$) in male and female rats (6.9 and 18 mg/m^3) were observed. Animals in the 2.5 mg/m^3 exposure group had sporadically reduced body weights ($p>0.05$) throughout the exposure period. Male and female rats in the 18 mg/m3 exposure group exhibited significantly increased mortality ($p<0.001$) from the 12th month onward. Male rats in the intermediate exposure groups showed a statistically-significant concentration-dependent decrease in cumulative survival from 17 months onward. In male mice, there were no differences in survival. The number of male mice surviving a minimum of 18 months were 41, 33, 32, and 25 for the 0, 2.5, 6.9, and 18 mg/m^3 exposure groups, respectively. There were no differences in cumulative survival among the female mice.

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		There were no alterations in the clinical pathology or ophthalmologic or neurofunctional data that were considered related to formaldehyde exposure. Exposure to formaldehyde produced a concentration-dependent increase in yellow discoloration of the hair. Other significant macroscopic observations (at the 18 mg/m^3 group) included dypsnea, emaciation, and large facial swellings that were proliferative lesions (carcinomas) protruding from the nasal cavity. Neoplastic lesions were first observed clinically at Day 358 in females and Day 432 in males. Formaldehyde-induced microscopic lesions were confined to the nasal cavity and the proximal trachea. Exposure to 18 mg/m^3 formaldehyde for 24 months produced a high incidence of nasal cancer in male and female rats. The tumors had a sharp concentration-response relationship, with the 2 carcinomas in the intermediate group identical to the 103 squamous cell carcinomas observed in rats exposed to 18 mg/m^3. Although the incidence of polyploid adenomas in the nasal cavity was not statistically significant, there was a positive concentration response for the occurrence of benign neoplasms in male rats. There was no evidence of progression of polyploid adenoma to squamous cell carcinoma. Two male mice exposed to 18 mg/m^3 of formaldehyde developed squamous cell carcinomas in the nasal cavity similar to the neoplasms in the rats.

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		Formaldehyde-induced lesions (squamous metaplasia and inflammation) in mice were much less severe than similar lesions in rats. The incidence of squamous cell carcinomas in mice exposed to 18 mg/m3 was similar to rats exposed to 6.9 mg/m^3.
Neurotoxicity		
870.6200 Neurotoxicity screening battery Purity: (2003a) - 37% stock solution was used to prepare solutions of 0.5%, 1%, and 2.5% (2003b) – 0.1%, 0.2%, and 1%	Malek, FA; Moritz, KU; Fanghanel, J. (2003a) Formaldehyde inhalation and open field behaviour in rats. Ind J Med Res 118:90-96. (NCEA) Malek, FA; Moritz, K-U; Fanghaenel, J. (2003b) A study on specific behavioral effects of formaldehyde in the rat. J Exp Anim Sci 42:160-170. **Open Literature** Male and Female LEW.1K Rat Malek et al. (2003a): Rats were exposed to 0, 1.0, 2.5, or 5.0 ppm (0, 1.23, 3.08, or 6.15 mg/m^3) formaldehyde for 2 hours. Mean formaldehyde levels of 1.01 ± 0.29 ppm, 2.51 ppm (standard deviation is missing) and 5.04 ± 0.27 ppm were achieved. Locomotor activity was assessed for 1 hour in an open field 2 and 24 hours after termination of formaldehyde exposure. Malek et al. (2003b): Rats (10 per group) were exposed at 0, 0.1, 0.5, or 5.0 ppm (0, 0.123, 0.615, or 6.15 mg/m^3) formaldehyde for 2 hours. Open field behavior tests were conducted on each animal 2 hours after formaldehyde exposure.	Malek et al. (2003a): **LOAEL = 1.0 ppm, 2 hours** In general, sniffing was increased after formaldehyde exposure and movement was decreased (crossed quadrants and climbing) in both male and female rats ($p<0.05$). Significant reductions in horizontal movements (crossed quadrants) were observed at all dose levels and were characterized by a U-shaped dose response. The lowest dose tested (1 ppm) demonstrated higher level of activity suppression than the two higher doses, but all groups were still suppressed relative to controls. Although female rats displayed a greater level of activity overall, a similar U-shaped dose-response pattern was also observed. After 24 hours, as expected, controls demonstrated habituation to the test apparatus exhibiting only 20% of the motor activity observed on day 1. In contrast, formaldehyde-treated animals failed to demonstrate the same degree of habituation. Activity levels for males observed on day 2 were 60-80% of the activity levels seen on day 1. Formaldehyde-treated females also failed to habituate and actually demonstrated increases in activity on day 2 relative to day 1 at all formaldehyde exposure levels.

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		Malek et al. (2003b): **LOAEL (M) = 0.1 ppm, 2 hours** The number of crossed quadrants for both controls and a 5 ppm group are comparable to those observed in the first study. Horizontal movement was decreased by formaldehyde exposure in a dose dependent manner with significant reductions in motor activity as low as 0.1 ppm in males and 0.5 ppm in females. The consistency of the data across studies and between genders provides greater confidence in the effects of low level formaldehyde exposure on this standard test of neurotoxicity.
870.6500 Schedule-controlled operant behavior Purity: Not reported	Pitten, FA; Kramer, A; Herrmann, K; et al. (2000) Formaldehyde neurotoxicity in animal experiments. Pathol Res Pract 196:193-198. **Open Literature** Adult Male and Female Wistar Rat (5 to 8/sex/group) Pitten et al. (2000) evaluated the effects of very brief formaldehyde exposures (10 minutes) but prolonged duration (90 days) on previously learned performance in a land version of the labyrinth maze. Rats were acclimated to the task for 14 days, 2 trials/day. Animals were required to make a series of five consecutive turns from the entrance of the maze to retrieve a piece of cheese placed in the goal box at the opposite end. Animals were exposed to 0 ppm, 2.6 ppm (0.25% formaldehyde solution to yield 3.06 ± 0.77 mg/m3), or 4.6 ppm (0.70% formaldehyde solution to yield 5.55 ± 1.27 mg/m3) formaldehyde, 10 minutes/day, 7 days/week for 90 days. Animals were assessed for performance in the maze every	**LOAEL: 2.6 ppm, 10 min/90 days** The authors reported that no gender differences existed as a function of formaldehyde treatment; therefore, data were presented by combining sexes. Control rats showed no change in error rate but a slight decrease in running time through the maze during the course of the experiment. The formaldehyde-exposed groups began with a similar performance level and error rate as controls, but their performance degraded over the course of formaldehyde exposure. By the fourth week of exposure, increased numbers of errors were evident in both exposed groups relative to controls. This trend reached statistical significance by the thirteenth week for a greater than twofold increase in error rate ($p<0.05$). Formaldehyde-treated rats also tended to have increased run times through the maze ($p=0.04$), but no difference was seen by formaldehyde

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	seventh day, at least 22 hours after the exposure on the previous day. At the end of the 90-day exposure period, monitoring of maze performance continued once every 10 days for an additional 40 days.	concentration. By 4 weeks after termination of exposure, no statistical differences among the three groups were evident, but the tendency for the two exposed groups to make more errors and have longer latencies remained. Since Pitten et al. (2000) tested animals after the task was acquired, these results indicate deficits in the retention of a previously learned task.
Other Purity: 96%	Boja JW, Nielsen JA, Foldvary E, et al. (1985) Acute Low-Level Formaldehyde Behavioural and Neurochemical Toxicity in the Rat. Prog Neuro-Psychopharmacol Biol Psychiat 9:671-674. **Open Literature** 88 M Sprague-Dawley Rat Rats were exposed to either air or formaldehyde at concentrations of 5, 10, or 20 ppm (6.20, 12.39, or 24.79 mg/m^3) via inhalation for 3 hours on two days	Exposure to 6.20 mg/m^3 formaldehyde resulted in statistically significant decreased motor activity within 15 minutes. At the beginning of day 2, all of the rats exposed to formaldehyde on day 1 displayed lower activity levels. Similar effects on motor activity were seen at the 12.39 mg/m^3 formaldehyde exposure level, whereas effects seen after 24.79 mg/m^3 exposure were reported to be "not readily interpretable" and were not shown. Exposure to 6.20 mg/m^3 formaldehyde statistically significantly increased concentrations of 5-hydroxyindoleacetic acid, 3,4-dihydroxyphenylacetic acid, and dopamine in the hypothalamus.
Metabolism		
870.7485 General Metabolism	Casanova, Mercedes, Donald F. Deyom and Henry D'A. Heck (1989) Covalent Binding of Inhaled Formaldehyde to DNA in the Nasal Mucosa of Fischer 344 Rats: Analysis of Formaldehyde and DNA by High-Performance Liquid Chromatography and Provisional Pharmacokinetic Interpretation. Fundamental and Applied Toxicology 12: 397-417. **Open Literature**	DNA-protein crosslinking occurred at all concentrations. The formation of crosslinks was interpreted in terms of a nonlinear pharmacokinetic model incorporating oxidation of inhaled formaldehyde as a defense mechanism. The slope of the fitted concentration-response curve at 12 mg/m^3 is7.3-fold greater than at 0.37 mg/m^3, and the detoxification pathway is half-saturated at an airborne concentration of 3.2

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
	Rat (4/group), nose-only exposure 0, 0.3, 0.7, 2, 6, or 10 ppm (0.37, 0.87, 2.5, 7.4, or 12 mg/m^3) for 6 hours	mg/m^3.
870.7485 General Metabolism	Casanova-Schmitz, Mercedes, Thomas B. Starr, and Henry D'A. Heck (1984) Differentiation between Metabolic Incorporation and Covalent Binding in the Labeling of Macromolecules in the Rat Nasal Mucosa and Bone Marrow by Inhaled (14C)- and (3H) Formaldehyde. Toxicology and Applied Pharmacology 76: 26-44. **Open Literature** Rats (4/group) 14C and 3H- formaldehyde was administered at doses of 0, 0.3, 2, 6, 10, or 15 ppm (0, 0.37, 2.5, 7.4, 12, or 19 mg/m^3) for 6 hours	The major route of nucleic acid labeling at all concentrations and in all tissues was metabolic incorporation; protein labeling in the respiratory mucosa was mainly due to covalent binding at the higher formaldehyde concentration. Incorporation of 14C- formaldehyde into DNA in the respiratory mucosa was maximal at 7.4 mg/m^3 but decreased at higher concentrations, whereas labeling of DNA in the olfactory mucosa and bone marrow increased monotonically with concentration. Evidence for covalent binding of formaldehyde to respiratory mucosal DNA was obtained at formaldehyde concentrations equal to or greater than 2.5 mg/m^3. The concentration of formaldehyde covalently bound to DNA at 7.4 mg/m^3 was 10.5-fold higher than at 2.5 mg/m^3, indicating significant nonlinearity of DNA binding with respect to the inhaled formaldehyde concentration under these conditions. Covalent binding to proteins increased in an essentially linear manner with increases in the airborne concentration. No evidence was obtained for the formation of covalent adducts with macromolecules in the olfactory mucosa or bone marrow. The nonlinear increase in covalent binding to respiratory mucosal DNA with increasing formaldehyde concentrations may be explained either by a decrease in the efficiency of defense mechanisms or by an increase in the availability of reaction sites on

Table 6. Subchronic, Chronic and Other Toxicity Profiles for Formaldehyde		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/ Classification/ Doses**	**Results**
		the DNA resulting from increased cell turnover.

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