4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Toxicokinetics*

(*a*) *Humans*

In humans, as in other animals, formaldehyde is an essential metabolic intermediate in all cells. It is produced endogenously from serine, glycine, methionine and choline, and it is generated in the demethylation of *N*-, *O*- and *S*-methyl compounds. It is an essential intermediate in the biosynthesis of purines, thymidine and certain amino acids (Neuberger, 1981).

The endogenous concentration of formaldehyde, determined by GC–MS (Heck *et al.*, 1982), in the blood of human subjects not exposed to formaldehyde was 2.61 ± 0.14 μg/g blood (mean ± standard error [SE]; range, 2.05–3.09 μg/g) (Heck *et al.*, 1985), i.e. about 0.1 mmol/L (assuming that 90% of the blood volume is water and the density of human blood is 1.06 g/cm^3 (Smith *et al.*, 1983)). This concentration represents the total concentration of both free and reversibly bound endogenous formaldehyde in the blood.

The concentration of formaldehyde measured in the blood of six human volunteers immediately after exposure by inhalation to 1.9 ppm [2.3 mg/m^3] for 40 min was 2.77 ± 0.28 μg/g, which did not differ from the pre-exposure concentration due to metabolically formed formaldehyde (see above). The absence of an increase is explained by the fact that formaldehyde reacts rapidly at the site of contact and is swiftly metabolized by human erythrocytes (Malorny *et al.*, 1965), which contain formaldehyde dehydrogenase (FDH) (Uotila & Koivusalo, 1987) and aldehyde dehydrogenase (ALDH) (Inoue *et al.*, 1979).

Overton *et al*. (2001) developed a mathematical model to predict interactions of formaldehyde in the respiratory tract, based on a one-dimensional equation of mass transport to each generation of an adult human, symmetric, bifurcating Weibel-type respiratory tract anatomical model, augmented by an upper respiratory tract. This predicted that over 95% of inhaled formaldehyde would be retained by the respiratory tract and that the flux is over 1000 times higher in the first tracheobronchial region compared with the first pulmonary region with no flux in the alveolar sacs.

A GC method was used to examine the urinary excretion of formate by veterinary medical students who were exposed to low concentrations of formaldehyde, in order to determine whether monitoring of formate is a useful biomarker for human exposure to formaldehyde (Gottschling *et al.*, 1984). The average baseline level of formate in the urine of 35 unexposed subjects was 12.5 mg/L, but this varied considerably both within and

among subjects (range, 2.4–28.4 mg/L). No significant changes in concentration were detected over a 3-week period of exposure to formaldehyde at a concentration in air of less than 0.5 ppm [0.61 mg/m^3]. The authors concluded that biological monitoring of formic acid in the urine to determine exposure to formaldehyde is not a feasible technique at this concentration.

(*b*) *Experimental systems*

(i) In vivo

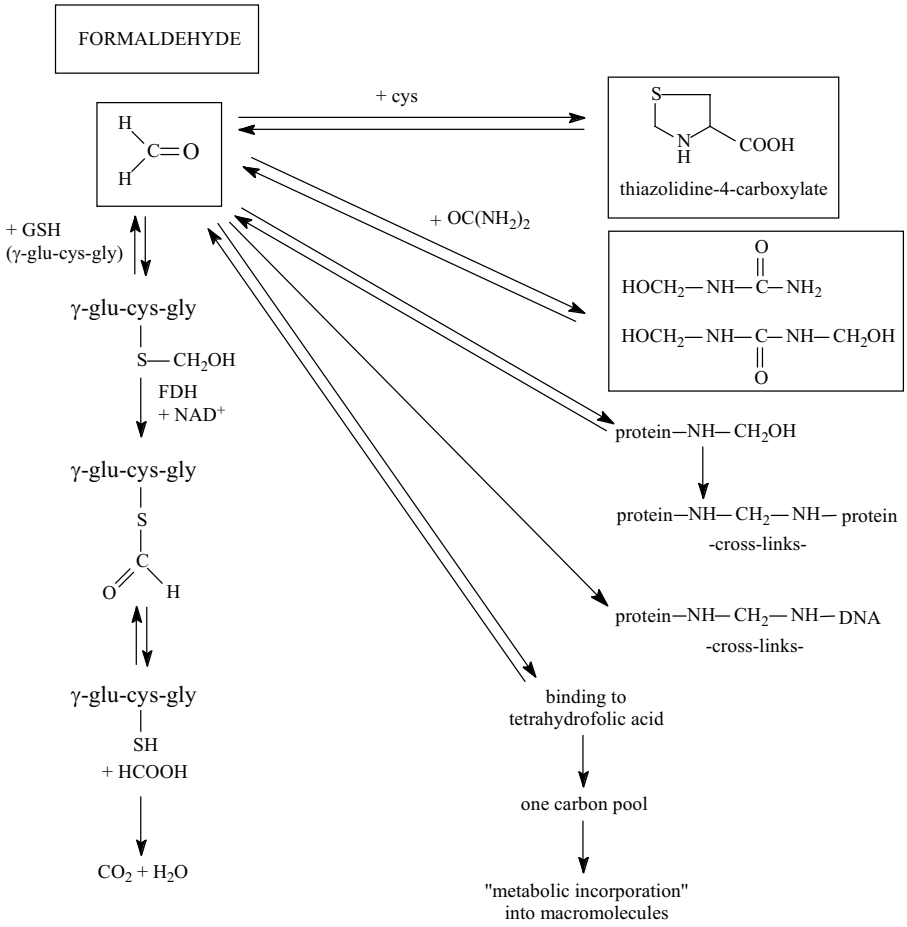
The steady-state concentrations of endogenous formaldehyde have been determined by GC–MS (Heck *et al.*, 1982) in the blood of Fischer 344 rats (2.24 ± 0.07 μg/g of blood (mean ± SE)) (Heck *et al.*, 1985) and three rhesus monkeys (2.04 ± 0.40 μg/g of blood; range, 1.24–2.45 μg/g) (Casanova *et al.*, 1988). These concentrations are similar to those measured in humans by the same method (see Section 4.1.1(*a*)). The blood concentrations of formaldehyde immediately after a single exposure of rats to 14.4 ppm [17.6 mg/m^3] (for 2 h) or subacute exposure of monkeys to 6 ppm [7.3 mg/m^3] (for 6 h per day on 5 days per week for 4 weeks) were indistinguishable from those before exposure.

More than 93% of a dose of inhaled formaldehyde was absorbed readily by the tissues of the respiratory tract (Kimbell *et al*., 2001a). In rats, formaldehyde is absorbed almost entirely in the nasal passages (Chang *et al.*, 1983; Heck *et al.*, 1983). In rhesus monkeys, absorption occurs primarily in the nasal passages but also in the trachea and proximal regions of the major bronchi (Monticello *et al.*, 1989; Casanova *et al.*, 1991). The efficiency and sites of formaldehyde uptake are determined by nasal anatomy, which differs greatly among species (Schreider, 1986). The structure of the nose gives rise to complex airflow patterns, which have been correlated with the location of formaldehyde-induced nasal lesions in both rats and monkeys (Morgan *et al.*, 1991).

The mucocilliary apparatus is an important defence system in the respiratory tract and may provide protection of the underlying epithelium from gases and vapours. Schlosser (1999) performed limiting-case calculations to determine the significance of convective mucus transport and chemical reaction to formaldehyde in rat nasal epithelial mucus. Less than 4.6% of absorbed formaldehyde can be bound to amino groups (serum albumin) after 20 min of exposure; therefore, at the slowest mucus flow rates measured in rats (~1 mm/min), a fluid element of mucus could travel more than 2 cm before binding 5% of the absorbed formaldehyde by which time the element would probably have left the nose (site of toxic response). Given the solubility of formaldehyde in mucus (water) and estimates of total mucus flow, as much as 22–42% of inhaled formaldehyde may be removed by mucus flow.

After exposure by inhalation, absorbed formaldehyde can be oxidized to formate and carbon dioxide or can be incorporated into biological macromolecules via tetrahydrofolate-dependent one-carbon biosynthetic pathways (see Fig. 1). The fate of inhaled formaldehyde was studied in Fischer 344 rats exposed to [^{14}C]formaldehyde (at 0.63 or 13.1 ppm [0.8 or 16.0 mg/m^3]) for 6 h. About 40% of the inhaled ^{14}C was eliminated as

Figure 1. Biological reactions and metabolism of formaldehyde



Adapted from Bolt (1987)

expired [^{14}C]carbon dioxide over a 70-h period; 17% was excreted in the urine, 5% was eliminated in the faeces and 35–39% remained in the tissues and carcass. Elimination of radioactivity from the blood of rats after exposure by inhalation to 0.63 ppm or 13.1 ppm [^{14}C]formaldehyde is multiphasic. After inhalation, the terminal half-time of the radioactivity in the plasma was approximately 55 h (Heck *et al.*, 1983). Analysis of the time course of residual radioactivity in plasma and erythrocytes after inhalation or intravenous injection of [^{14}C]formaldehyde or intravenous injection of [^{14}C]formate showed that the radioactivity is due to incorporation of ^{14}C (as [^{14}C]formate) into serum proteins and erythrocytes and subsequent release of labelled proteins and cells into the circulation (Heck *et al.*, 1983). The half-time of formaldehyde in rat plasma after intravenous administration is reported to be approximately 1 min (Rietbrock, 1965).

The fate of [^{14}C]formaldehyde after dermal application to Fischer 344 rats, Dunkin–Hartley guinea-pigs and cynomolgus monkeys was described by Jeffcoat *et al.* (1983). Aqueous formaldehyde was applied to a shaven area of the lower back, and the rodents were placed in metabolism cages for collection of urine, faeces, expired air and [^{14}C]formaldehyde evaporated from the skin. Monkeys were seated in a restraining chair and were fitted with a plexiglass helmet for collection of exhaled [^{14}C]carbon dioxide. The concentrations of ^{14}C in the tissues, blood and carcass of rodents were determined at the end of the experiment. Rodents excreted about 6.6% of the dermally applied dose in the urine over 72 h, while 21–28% was collected in the air traps. It was deduced that almost all of the air-trapped radioactivity was due to evaporation of formaldehyde from the skin, since less than 3% of the radioactivity (i.e. 0.6–0.8% of the applied ^{14}C) was due to [^{14}C]carbon dioxide. Rodent carcass contained 22–28% of the ^{14}C and total blood about 0.1%; a substantial fraction of ^{14}C (3.6–16%) remained in the skin at the site of application. In monkeys, only 0.24% of the dermally applied [^{14}C]formaldehyde was excreted in the urine, and 0.37% was accounted for as [^{14}C]carbon dioxide in the air traps; about 0.015% of the radioactivity was found in total blood and 9.5% in the skin at the site of application. Less than 1% of the applied dose was excreted or exhaled, in contrast to rodents in which nearly 10% was eliminated by these routes. Coupled with the observation of lower blood levels of ^{14}C in monkeys than in rodents, the results suggest that the skin of monkeys may be less permeable to aqueous formaldehyde than that of rodents.

Formaldehyde is absorbed rapidly and almost completely from the rodent intestinal tract (Buss *et al.*, 1964). In rats, about 40% of an oral dose of [^{14}C]formaldehyde (7 mg/kg) was eliminated as [^{14}C]carbon dioxide within 12 h, while 10% was excreted in the urine and 1% in the faeces. A substantial portion of the radioactivity remained in the carcass as products of metabolic incorporation. In another study in which Sprague-Dawley rats were injected intraperitoneally with 4 or 40 mg/kg bw [^{14}C]formaldehyde, a portion of the injected material (about 3–5% of a dose of 40 mg/kg bw) was excreted unchanged in the urine within 12 h (Mashford & Jones, 1982).

(ii) In vitro

Since formaldehyde can induce allergic contact dermatitis in humans (see Section 4.2.1), it can be concluded that formaldehyde or its metabolites penetrate human skin (Maibach, 1983). The kinetics of this penetration was determined *in vitro* using an excised full-thickness human skin sample mounted in a diffusion cell at 30 °C (Lodén, 1986). The rate of 'resorption' of [^{14}C]formaldehyde (defined as the uptake of ^{14}C into phosphate-buffered saline, pH 7.4, that flowed unidirectionally beneath the sample) was 16.7 μg/cm^2/h when a 3.7% solution of formaldehyde was used, and increased to 319 μg/cm^2/h when a 37% solution was used. The presence of methanol in both of these solutions (at 1–1.5% and 10–15%, respectively) may have affected the rate of uptake, and it is unclear whether the resorbed ^{14}C was due only to formaldehyde. Skin retention of formaldehyde-derived radioactivity represented a significant fraction of the total amount of formaldehyde absorbed.

(iii) *Predictive models to analyse the effects of formaldehyde in the respiratory tract*

Computational fluid dynamic models

Anatomically accurate, three-dimensional computational fluid dynamics (CFD) models of formaldehyde in the nasal passages of rat, monkeys and humans have been developed to aid the understanding of absorption and mechanisms of action and risk assessment (Conolly *et al*., 2000; Kimbell *et al*., 2001a,b; Georgieva *et al*., 2003). Conolly *et al*. (2000) sought to increase confidence in predictions of human DNA–protein cross-links (see Fig. 1) by refining earlier models of formaldehyde deposition and DNA–protein cross-links in nasal mucosa. Anatomically accurate CFD models of nasal airways of Fischer 344 rats, rhesus monkeys and humans that were designed to predict the regional flux of formaldehyde were linked to a model of tissue disposition of formaldehyde and kinetics of DNA–protein cross-links. Statistical optimization was used to identify parametric values, and good simulations were obtained. The parametric values obtained for rats and monkeys were used to extrapolate mathematically to the human situation. The results showed that the levels of nasal mucosal DNA–protein cross-links in rats, rhesus monkeys and humans varied with the concentration of formaldehyde inhaled and the predicted DNA–protein cross-link dose–response curves for the three species were similar, in spite of the significant interspecies differences in nasal anatomy and breathing rates.

Kimbell *et al*. (2001a) used anatomically accurate three-dimensional CFD models of nasal air flow and transport of formaldehyde gas in Fischer 344 rats, rhesus monkeys and humans to predict local patterns of wall mass flux (pmol/(mm^2 h–ppm)). The nasal surface of each species was partitioned by flux into smaller regions (flux bins), each of which was characterized by surface area and an average flux value. Flux values higher than half the maximum flux value were predicted for nearly 20% of human, 5% of rat and less than 1% of monkey nasal surfaces at resting inspiratory flow rates. Human nasal flux patterns shifted distally and the percentage of uptake decreased as inspiratory flow rate increased.

Kimbell *et al*. (2001b) used anatomically accurate three-dimensional CFD models of nasal passages in Fischer 344 rats, rhesus monkeys and humans to estimate and compare the regional patterns of uptake of inhaled formaldehyde that were predicted among these species. Maximum flux values, averaged over one breath, in non-squamous epithelium were estimated to be 2620, 4492 and 2082 pmol/(mm^2 h–ppm) in rats, monkeys and humans, respectively. At sites where cell proliferation rates had been measured and found to be similar in rats and monkeys, predicted flux values were also similar, as were predicted fluxes in a region of high tumour incidence in the rat nose and anterior portion of the human nose.

Thermodynamic model: exposure to particle-adsorbed formaldehyde

The possibility that gaseous formaldehyde may be adsorbed to respirable particles, inhaled and subsequently released into the lung has been examined in a thermodynamic model based on measurable physicochemical properties of particles and volatile pollutants. In this model, analysis of the adsorption of formaldehyde to and its release from respirable

carbon black particles showed that only 2 ppb [0.0025 mg/m^3] of an airborne formaldehyde concentration of 6 ppm [7.4 mg/m^3] would be adsorbed to carbon black (Risby *et al.*, 1990).

4.1.2 *Biomonitoring and metabolism of formaldehyde*

(*a*) *Humans*

(i) *Biological monitoring*

A quantitative method that was developed for the determination of formaldehyde in biological tissues used stable-isotope dilution combined with GC–MS. Multilabelled formaldehyde that contained 90 atom-% ^{13}C and 98 atom-% deuterium (^{2}H) was used as the isotopic diluent, which was added to homogenized tissue. Derivatization was conducted *in situ* with pentafluorophenylhydrazine, followed by extraction and analysis of the pentafluorophenylhydrazone by selected ion monitoring. With this method, endogenous formaldehyde could be analysed quantitatively in tissue samples as small as 20 mg wet wt (Heck *et al.*, 1982).

Blood

Concentrations of formaldehyde were determined by the method described above in samples of venous blood that were collected before and after exposure of six human volunteers to 1.9 ppm [2.3 mg/m^3] formaldehyde by inhalation for 40 min. Average concentrations of formaldehyde were 2.61 ± 0.14 μg/g blood before and 2.77 ± 0.28 μg/g blood after exposure. These values were not significantly different. However, the subjects exhibited significant inter-individual variation with respect to their blood concentrations of formaldehyde, and some showed significant differences — either an increase or a decrease — before and after exposure, which suggests that individual blood concentrations of formaldehyde vary with time. The results are consistent with the assumption that toxicity due to exposure to formaldehyde is unlikely to occur at sites that are distant from the portal of entry (Heck *et al.*, 1985). The absence of an exposure-related increase in blood concentration of formaldehyde in this study can be explained by the fact this chemical is rapidly metabolized by human erythrocytes (Malorny *et al.*, 1965), which contain FDH (Uotila & Koivusalo, 1987) and ALDH (Inoue *et al.*, 1979) (see also Section 4.1.1(*a*)).

DNA–protein cross-links in blood leukocytes were used as a marker for exposure to formaldehyde in a study that involved 12 workers (Anatomy Department and Pathology Institute; duration of exposure, 2–31 years) and eight controls. Protein-bound DNA was separated from protein-free DNA by precipitation with sodium dodecylsulfate (Zhitkovich & Costa, 1992). A significantly higher (p = 0.03) level of cross-links was measured among exposed workers (mean ± SD, 28 ± 5%; min., 21%; max., 38%) than among the unexposed controls (mean ± SD, 22 ± 6%; min., 16%; max., 32%). Of the 12 exposed workers, four (33%) showed cross-link values above the upper range of the controls. The level of cross-links was generally higher in workers exposed for longer periods, and was reported not to be influenced by tobacco smoking. The data suggest that

DNA–protein cross-links can be used as a method for the biological monitoring of exposure to formaldehyde (Shaham *et al.*, 1996a).

Urine

Exposure to formaldehyde was monitored in a group of 35 students of veterinary medicine who were enrolled for 3 consecutive weeks in a functional anatomy class, where they worked extensively with animal tissue preserved in formalin. The biological monitoring was based on the known metabolism of formaldehyde to formic acid, which may then be excreted in the urine. Urinary formic acid was converted to methyl formate and measured by GC. The average concentration of formate in the urine of the subjects before the class was 12.5 mg/L, and considerable variation was observed both within and among subjects (range, 2.4–28.4 mg/L). No significant changes in concentration were detected over the 3-week period of exposure to formaldehyde at a concentration in air of < 0.4 ppm [0.5 mg/m^3]. Biological monitoring of exposure to formaldehyde by measurement of urinary formic acid does not appear to be a suitable method at these levels of exposure (Gottschling *et al.*, 1984) (see also Section 4.1.1(*a*)).

Respiratory tract: animal models and extrapolation to humans

The pharmacokinetics of formaldehyde-induced formation of DNA–protein cross-links in the nose was investigated by use of a model in which the rate of formation is proportional to the tissue concentration of this chemical. The model includes both saturable and non-saturable elimination pathways and regional differences in DNA binding are attributed to anatomical rather than biochemical factors. Using this model, the concentration of DNA–protein cross-links formed in corresponding tissues of different species can be predicted by scaling the pharmacokinetic parameter that depends on minute volume and quantity of nasal mucosal DNA. The concentration–response curve for the average rate of formation of cross-links in the turbinates, lateral wall and septum of rhesus monkeys was predicted from that of Fischer 344 rats that were exposed under similar conditions. A significant overlap was observed between predicted and fitted curves, which implies that minute volume and nasal mucosal DNA are major determinants of the rate of formation of DNA–protein cross-links in the nasal mucosa of different species. Concentrations of DNA–protein cross-links that may be produced in the nasal mucosa of adult humans were predicted on the basis of experimental data in rats and monkeys. The authors suggested from their results that formaldehyde would generate lower concentrations of DNA–protein cross-links in the nasal mucosa of humans than that 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 (Casanova *et al.*, 1991).

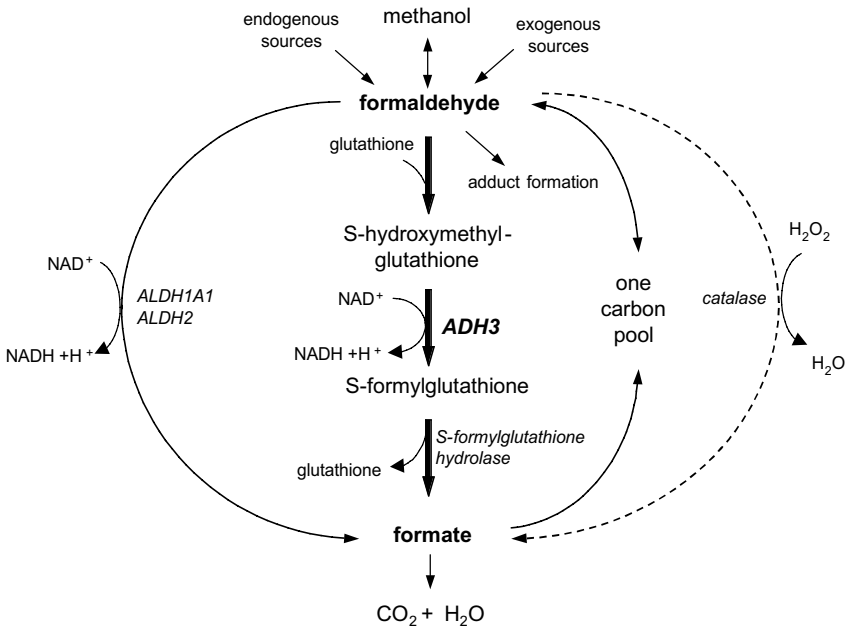
(ii) *Metabolism of formaldehyde*

Formaldehyde is an endogenous metabolic product of *N*-, *O*- and *S*-demethylation reactions in the cell (Neuberger, 1981). During exogenous exposure, gaseous formaldehyde — which is highly soluble in water — is virtually completely removed by the nose during

nasal respiration (Ballenger, 1984). In recent years, the metabolic capacities of nasal cavity tissues have been investigated extensively in mammals, including humans. ALDHs, cytochrome P450 (CYP)-dependent monooxygenases, glutathione (GSH) transferases (GSTs), epoxide hydrolases, flavin-containing monooxygenases and carboxyl esterases have all been reported to occur in substantial quantities in the nasal cavity. The contributions of some of these enzyme activities to the induction of toxic effects from volatile chemicals have been the subject of numerous studies (see reviews by Dahl & Hadley, 1991; Thornton-Manning & Dahl, 1997). The two main enzymes involved in the metabolism of formaldehyde in humans — a dehydrogenase and a hydrolase — are discussed in detail below.

Formaldehyde is detoxified by oxidation to formate by different enzyme systems (Fig. 2; from Hedberg *et al*., 2002). The primary and generally most important system initially involves GSH-dependent FDH (Uotila & Koivusalo, 1974), which is identical to alcohol dehydrogenase 3 (ADH3; Koivusalo *et al*., 1989), that oxidizes *S*-hydroxymethylglutathione (the thiohemiacetal that is formed spontaneously from formaldehyde and GSH) to *S*-formylglutathione. The systematic name for the enzyme is formaldehyde:NAD+ oxidoreductase (glutathione-formylating) (CAS Registry No. 9028-84-6, IUBMB code EC1.2.1.1). (Other names for this enzyme are formaldehyde dehydrogenase, formaldehyde dehydrogenase (glutathione), NAD-linked formaldehyde dehydrogenase and formic dehydrogenase.) This intermediate is then further metabolized by *S*-formylglutathione

Figure 2. Fate and metabolism of formaldehyde



Adapted from Hedberg *et al*. (2002)

hydrolase to yield formate and reduced GSH (Uotila & Koivusalo, 1997). Analyses across species and among various mammalian tissues and cell types imply that ADH3 represents a ubiquitous enzyme, and that it is the ancestral form of ADH from which other types of vertebrate ADH have evolved (Estonius *et al*., 1996; Hedberg *et al*., 2000; Jörnvall *et al*., 2000; Hedberg *et al*., 2001; Höög *et al*., 2001, 2003). The activities of ADH3 are two to three orders of magnitude lower than those of *S*-formylglutathione hydrolase and thus the ADH3-catalysed step is rate-limiting (Uotila & Koivusalo, 1997). Formation of *S*-hydroxymethylglutathione efficiently counteracts the existence of free formaldehyde, a reaction that is determined by the fact that cellular GSH is an abundant molecule and is often present in millimolar concentration levels (Meister & Anderson, 1983). The equilibrium constant (K_{eq}) for the formation of this adduct — under conditions of excess GSH — was determined to be 1.77 ± 0.13 mM (Naylor *et al.*, 1988; Sanghani *et al.*, 2000). Furthermore, when formaldehyde is reacted with the thiols, cysteine and cysteinylglycine, it can function as an alternative substrate to *S*-hydroxymethylglutathione for ADH3, but at a far lower turnover (Holmquist & Vallee, 1991). The mechanism for the ADH3-dependent step is identical to any alcohol oxidation by ADH enzymes; it requires catalysis by zinc and uses NAD^+/NADH as the electron acceptor and donor, respectively (Höög *et al*., 2001). The second enzyme system is the ALDHs — class 1 (cytosolic ALDH; ALDH1A1) and class 2 (mitochondrial ALDH; ALDH2) — which have an affinity for free formaldehyde. Because the Michaelis-Menten constant (K_m) is less than micromolar for aldehyde substrates, they are called low-K_m ALDHs (Petersen & Lindahl, 1997), except for free formaldehyde for which the K_m values are high (in the range of 0.6 mM; Mukerjee & Pietruszko, 1992). This value is about 100-fold higher than the K_m displayed by ADH3 for *S*-hydroxymethylglutathione (4 μM) (Holmquist & Vallee, 1991; Uotila & Koivusalo, 1997; Hedberg *et al*., 1998). Therefore, ADH3 is probably the predominant enzyme responsible for the oxidation of formaldehyde at physiologically relevant concentrations, while low-K_m ALDHs contribute increasingly when the concentrations of formaldehyde increase (Dicker & Cederbaum, 1986). Catalase may also contribute to the oxidation of formaldehyde to formate, but only under circumstances in which hydrogen peroxide is formed (Jones *et al*., 1978; Uotila & Koivusalo, 1989). Formaldehyde may also be reduced to methanol and then be re-converted to formaldehyde, when ADH1 is involved (Pocker & Li, 1991). Many endogenous and exogenous factors can contribute to the generation of formaldehyde in cells (Grafström, 1990). Both formaldehyde and formate contribute to the 'one-carbon pool', which involves the folic acid metabolic pathway for synthesis of certain nucleic acids and amino acids, and, eventually, cellular macromolecules (Neuberger, 1981). *S*-Hydroxymethylglutathione and *S*-formylglutathione are among the most notable components of this pool, which also includes the tetrahydrofolate adducts, N^5-hydroxymethyl-, N^5,N^{10}-methylene, and N^5- and N^{10}-formyl-tetrahydrofolate, serine, methionine, histamine and methylamine, other reversible amine adducts and hydrated formaldehyde.

ADH3 is the only ADH that can participate in the cellular detoxification of formaldehyde. The nomenclature recommended by Duester *et al*. (1999) defines the protein as

ADH3 and the gene as *ADH3*, whereas previous nomenclature used χ (chi) for the protein and *ADH5* for the gene (Jörnvall & Höög, 1995). The older nomenclature used *ADH3* to define the gene that codes for *ADH1C*, which is important for the oxidation of ethanol, and studies of the latter protein, including the role of polymorphisms in the causation of cancer (Schwartz *et al*., 2001; Yokoyama *et al*., 2002), should not be confused with the enzyme that is involved in the oxidation of formaldehyde. *S*-Nitrosoglutathione constitutes an excellent substrate for reductive cleavage by ADH3, and, accordingly, ADH3 is also termed *S*-nitrosoglutathione reductase (Jensen *et al*., 1998; Liu *et al*., 2001). Comparative analysis indicates that ADH3 oxidizes GSH-conjugated formaldehyde (i.e. *S*-hydroxymethylglutathione) and reduces *S*-nitrosoglutathione with catalytic efficiencies of 58 000 $min^{-1}.mM^{-1}$ and 90 000 $min^{-1}.mM^{-1}$, respectively (Hedberg *et al*., 2003). Considering both reactions, exposure to formaldehyde would naturally favour the oxidant activity of ADH3, whereas nitrosative stress leading to the formation of *S*-nitrosoglutathione would favour reductive activity (Liu *et al*., 2001; Hedberg *et al*., 2003). The cellular levels of NAD^+ are normally two orders of magnitude higher than those of NADH and thus, under physiological conditions, the oxidation of *S*-hydroxymethylglutathione would be favoured rather than the reduction of *S*-nitrosoglutathione (Svensson *et al*., 1999; Molotkov *et al.*, 2002; Höög *et al*., 2003). Although it has a lower efficiency than other ADH enzymes, ADH3 also mediates oxidation of all-*trans*-retinol to retinal, and may also oxidize ethanol to acetaldehyde at levels of exposure that are reached during alcohol intoxication (Svensson *et al*., 1999; Molotkov *et al.*, 2003). ADH3 also shows oxidizing activity towards longer-chain primary alcohols, methylglyoxal, ketoxal, hydroxypyruvaldehyde, 20-hydroxy-leukotriene B_4 and ω-hydroxy fatty acids, e.g. 12-hydroxydodecanoic acid (Wagner *et al.*, 1984; Gotoh *et al.*, 1990; Jörnvall *et al*., 2000).

The physiological relevance of these activities remains unclear, since the catalytic efficiencies for these substrates are considerably lower than those of *S*-hydroxymethylglutathione and *S*-nitrosoglutathione. However, all of these substrates compete with *S*-hydroxymethylglutathione and *S*-nitrosoglutathione for the active site of ADH3, and thereby influence the catalytic activity for formaldehyde scavenging. The *ADH3* gene promoter sequence shows several polymorphisms that might influence *ADH3* transcription, but such polymorphisms were not observed in exons that code for the *ADH3* gene (Hedberg *et al*., 2001). In contrast, polymorphisms in *ALDH*, including *ALDH2*, may reduce the capacity for the oxidation of formaldehyde to less than half of that of the wild-type activity (Wang *et al*., 2002). Transcription of *ADH3* is coupled to proliferative states in human oral keratinocytes where *ADH3* mRNA exhibits a short half-life (7 h); when the protein is formed, it remains highly stable and metabolically active during cellular life expectancy (Hedberg *et al*., 2000; Nilsson *et al*., 2004). Formaldehyde and *S*-hydroxymethylglutathione regulate *ADH3* transcription in the bacterium *Rhodobacter sphaeriodes*, and wounding and growth hormones regulate ADH3 in the plant *Arabidopsis*, which provides examples of ADH3 regulation through feed-back control and from both external and internal signals (Barber & Donohue, 1998; Diaz *et al*., 2003). [The Working Group did not find evidence of such regulation in humans.] The biochemistry that underlies the metabolism of formaldehyde

indicates that changes in glutathione levels, NAD^+:NADH ratios, other redox changes and oxidant stress in general, including from nitric oxide and *S*-nitrosoglutathione, protein S-nitrosothiols and intermediates of the folic acid metabolic pathway should be considered in the assessment of the consequences of exposure to formaldehyde.

(*b*) *Experimental systems*

The distribution of selected metabolizing enzymes in the respiratory tract was compared with that in the liver of beagle dogs. Four beagle dogs (two males, two females; 13–18 kg, 13–130 months of age) were euthanized, the liver and respiratory tract were removed and tissues were prepared for isolation of metabolizing enzymes, including infusion of cold agarose into a lung lobe. The respiratory tract was separated into respiratory nasal epithelium, olfactory nasal epithelium, tracheal epithelium, epithelium from proximal middle and distal bronchus and lung parenchyma. The cytosolic fraction was isolated and the activity of sulfite oxidase, ADH, ALDH, FDH, GST and protein contents were quantified. Sulfite oxidase with sulfite as the substrate had greatest activity in the liver (9.95 nkat/mg protein); in the respiratory tract, the greatest activity was in the lung parenchyma (4.62 nkat/mg protein) and activity in the rest of the respiratory tract was below 4 nkat/mg protein. FDH with formaldehyde as the substrate had greatest activity in the nose (10.5 nkat/mg protein) while the liver (2.28 nkat/mg protein) had less activity than any site in the respiratory tract. ALDH with formaldehyde as the substrate had greatest activity in the trachea (1.28 nkat/mg protein), and the rest of the respiratory tract and liver had levels of activity below 1 nkat/mg protein. ADH with ethanol as the substrate had greatest activity in the liver (4.40 nkat/mg protein), and the respiratory tract had levels below 1 nkat/mg protein. All but one of the 1-chloro-2,4-dinitrobenzene-related GSTs had greatest activity in the liver parenchyma, which ranged from 50 to 300% greater than that in the respiratory tract. The cytosolic fraction of the epoxy-3-(*para*-nitrophenoxy)-propane-related GST had greatest activity in the trachea (3.94 nkat/mg protein); activity in the liver (0.24 nkat/mg protein) was less than 20% of that in any part of the respiratory tract on average. In general, metabolizing enzymes are spread throughout the respiratory tract and can have a metabolic capacity the same as or greater than the liver (Maier *et al*., 1999).

4.2 Toxic effects

4.2.1 *Humans*

(*a*) *Irritation*

There is extensive literature on domestic exposures to products that contain formaldehyde, such as plywood, and to urea–formaldehyde foam (see Section 1). Irritative effects have consistently been reported after exposure to formaldehyde; these have also been observed in children and a wide variation in susceptibility has been reported. Because these domestic exposures also involve exposure to other agents, this literature is not reviewed in

detail here because the findings do not generally add any information on the specific mode of action of formaldehyde as a potential carcinogen.

(i) *Experimental studies of acute effects*

Airborne formaldehyde irritates the eyes, nose and throat in healthy humans who had clinical disease. Chamber studies and the symptoms reported therein are summarized in Table 30.

Dose–response irritative effects were investigated (Kulle *et al.*, 1987; Kulle, 1993) in nonsmokers who received 3-h exposures to 0, 0.5, 1, 2 and 3 ppm [0, 0.61, 1.22, 2.44 and 3.66 mg/m^3] formaldehyde. The results are shown in Figure 3. Eye irritation increased linearly at doses of 0.5–3 ppm and was the dominant symptom; no effect was observed with 0.5 ppm; 21% of the volunteers had mild eye irritation with 1 ppm and 35% with 2 ppm. Of the volunteers, 40% could smell formaldehyde at 0.5 ppm (5% when no formaldehyde was present). Nose and/or throat irritation was the least sensitive response with an estimated threshold of 1 ppm.

Adult asthmatics

Green *et al*. (1987) exposed 22 healthy subjects and 16 asthmatics to 3 ppm formaldehyde for 1 h during exercise. In the healthy group, 32% of the subjects reported moderate-to-severe nose and throat irritation and 27% moderate-to-severe eye irritation. In the asthmatics, these percentages were 31% and 19%, respectively.

Unsensitized adult asthmatics

The effects of inhaled formaldehyde on the airways of healthy people and unsensitized asthmatics have been reviewed (Smedley, 1996; Krieger *et al.*, 1998; Suh *et al.*, 2000; Bender, 2002; Liteplo & Meek, 2003).

Bender (2002) reviewed nine controlled chamber studies of exposure to formaldehyde of asthmatic subjects (Reed & Frigas 1984; Sauder *et al*., 1986; Green *et al*., 1987; Kulle *et al*., 1987; Schachter *et al*., 1987; Witek *et al*., 1987; Harving *et al*., 1990; Pazdrak *et al*., 1993; Krakowiak *et al*., 1998). Exposure to 2–3 ppm formaldehyde for up to 3 h did not provoke asthma in unsensitized asthmatics. There was no asthmatic response to 0.1–3 ppm formaldehyde in 11 women and two men who reported chest tightness, cough and wheeze attributed to exposure to formaldehyde at home or at work (Reed & Frigas, 1984). Similar conclusions were reached in the review of Liteplo and Meek (2003) who included three further studies (Day *et al*., 1984; Schachter *et al*., 1986; Sauder *et al*., 1987).

Studies on nasal lavage

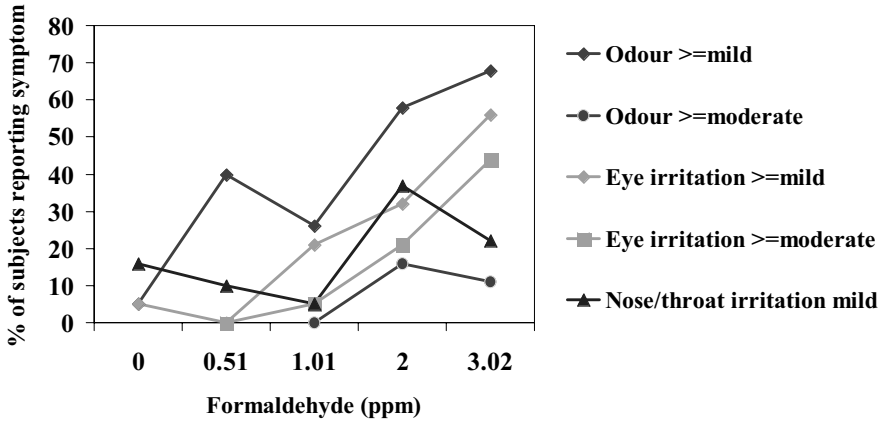
Two studies have investigated the effect of exposure to formaldehyde on nasal lavage. Krakowiak *et al.* (1998) reported on 10 healthy subjects and 10 asthmatics who reported nasal or respiratory symptoms from formaldehyde at work (nurses, textile and shoe workers), who received a single, blind exposure to clean air or 0.5 mg/m^3 formaldehyde (range, 0.2–0.7 mg/m^3) for 2 h. Nasal washings were performed before and 0, 0.5, 4 and 24 h after exposure. During exposure to formaldehyde, all subjects developed sneezing,

Table 30. Studies of acute exposure to formaldehyde that reported irritant-type symptoms

Subjects (no.)	Exposure to formaldehyde	Effect	Reference
Normal (22) Asthmatics (16)	3 ppm [3.7 mg/m^3] for 60 min	Moderate/severe symptoms Eye, 27%; nose/throat, 32% Eye, 19%; nose/throat, 31%	Green *et al.* (1987)
Normal with no exposure (10) Asthmatics with allergic symptoms due to exposure to formaldehyde (10)	0.5 mg/m^3 for 2 h	Scored on 0–7 point scale Nasal itching and congestion in all Score 4.3/7 in normal subjects Score 4.6/7 in asthmatics	Krakowiak *et al.* (1998)
Healthy nonsmokers (19)	3 ppm for 3 h	See Figure 3	Kulle *et al.* (1987); Kulle (1993)
Healthy (11) Formaldehyde contact dermatitis (9)	0.5 mg/m^3 [0.41 ppm] for 2 h	Scored on 0–7 point scale (sneezes, nasal itching and congestion) Mean nasal score 4/7 at 10 min for allergic and healthy subjects	Pazdrak *et al.* (1993)
Healthy nonsmokers (9)	3 ppm for 3 h	Mean symptom scores (air/formaldehyde) Throat/nose, 0.22/1.33 Eye irritation, 0/0.78	Sauder *et al.* (1986)
Asthmatic nonsmokers (9)	3 ppm for 3 h	Eye and nose irritation started after 2 min	Sauder *et al.* (1987)
Normal formaldehyde-exposed (15)	2 ppm [2.5 mg/m^3] for 40 min	Odour, 12/15 (80%) Sore throat, 0/15 (0%) Nasal irritation, 0/15 (0%) Eye irritation, 7/15 (47%)	Schachter *et al.* (1987)
Asthmatics (15)	2 ppm for 40 min	Odour, 15/15 (100%) Sore throat, 5/15 (33%) Nasal irritation, 7/15 (47%) Eye irritation, 11/15 (73%)	Witek *et al.* (1987)
Normal (9), asthmatics with UFFI symptoms (9)	3 ppm for 2 h (1 ppm for 90 min and 2 ppm UFFI for 30 min)	Eye irritation, 15/18 Nasal congestion, 7/18 Throat irritation, 5/18 Same in UFFI symptomatic and normal groups	Day *et al.* (1984)

UFFI, urea–formaldehyde foam insulation

Figure 3. Symptoms reported by 19 nonsmoking healthy adults exposed to formaldehyde for 3 h



Adapted from Kulle (1993)

itching and congestion, with substantial resolution by 4 h. An increase in total leukocytes and eosinophils was observed immediately after exposure to formaldehyde that was similar in both groups of subjects, with resolution after 4 h. An increase was also observed in the albumin:total protein ratio with a similar time course that was interpreted as an increase in nasal mucosal permeability. No significant increases in tryptase or eosinophil cationic protein were found in either group. An earlier study by the same group (Pazdrak *et al.*, 1993) that used a similar methodology investigated nine workers who had skin hypersensitivity (positive patch test) to formaldehyde and 11 healthy men who had negative formaldehyde patch tests. Nasal lavage was performed at 0, 10 min, 3 (or 4) and 18 h after exposure. Eosinophils increased in the nasal lavage and were maximal immediately after exposure, but were still increased at 4 and 18 h; the percentage of epithelial cells was reduced. Albumin levels were increased in the lavage and albumin:total protein ratios were increased only immediately after exposure. The group that had dermatitis and the normal group had similar responses. The authors concluded that the irritative effects of formaldehyde were confirmed, and also suggested a non-specific, non-allergic pro-inflammatory effect when formaldehyde was inhaled at a low dose (0.5 mg/m^3).

An in-vitro study of human nasal ciliated epithelial cells showed reduced frequency of ciliary beat after exposure to 5000 µg/m^3 formaldehyde for 2 h, but no effect after exposure to 5000 µg/m^3 for 1 h or 500 µg/m^3 for 2 h (Schäfer *et al.*, 1999).

(ii) *Residential exposure*

Studies have been conducted among residents who were exposed to formaldehyde in the home and school children who were exposed to formaldehyde in classrooms (Broder *et al.*, 1991; Wantke *et al.*, 1996a). These studies are difficult to interpret because they did

not control for confounding factors, particularly lack of ventilation and other allergens. It is possible that co-exposures to irritants and allergens increases the risk for allergen sensitization, but this has not been shown reliably for exposures to formaldehyde. Some studies looked for IgG and/or IgE antibodies to formaldehyde/human serum albumin conjugates, but the results were inconsistent (Patterson *et al.*, 1989; Wantke *et al.*, 1996a; Kim *et al.*, 2001; Doi *et al.*, 2003).

(*b*) *Pulmonary function: effect of chronic occupational exposure*

Kriebel *et al*. (1993) noted that epidemiological studies of irritants are difficult to perform using standard epidemiological methods because of the reversible nature of the health outcomes, the selection of sensitive individuals from the study population and the wide heterogeneity of normal responses to irritants. Twenty-four physical therapy students, who were exposed to formaldehyde during dissection for 3 h per week over 10 weeks with breathing zone exposures to formaldehyde of 0.49–0.93 ppm, were included in the study. Peak expiratory flow and a symptom questionnaire were completed before and after each exposure, and again before and after 3-h laboratory sessions after several months with no exposure to formaldehyde. Symptoms increased following exposure (eyes, +43%; nose, +21%; throat, +15%; breathing, +20%; and cough, +5%). The intensity of symptoms tended to decrease over time [this might be due to a repeated questionnaire effect with weekly questionnaires]. The peak expiratory flow declined by an average of 4.8 L/min during the morning (pre-dissection to midday) and by 10 L/min before exposure over the 10 weeks of exposure (2% of baseline) with recovery after 12 weeks with no exposure (daily measurements before exposure). The study group included five asthmatics whose peak expiratory flow after exposure fell by 37 L/min (non-asthmatics, 3.9 L/min). [The Working Group noted the lack of controls in this study, to adjust for any seasonal effects over time. The Working Group also noted that this is a difficult study to interpret. The time of day of the dissection sessions is not given, and the peak expiratory flow would be expected to increase for the first 4–10 h after waking, so that the time of day may have affected the outcome of the measurements.]

Akbar-Khanzadeh and Mlynek (1997) compared 50 nonsmoking first-year medical students exposed to breathing zone levels of 1.36–2.58 ppm [1.66–3.2 mg/m^3] formaldehyde with 36 second-year physiotherapy students who had no exposure to formaldehyde. Lung function was measured before exposure, 1 h after exposure and at midday. An increase in lung function was observed in both exposed and unexposed groups during the morning (exposed forced expiry volume in 1 sec (FEV_1), +1.2%; control, +2.1% at 1 h; exposed FEV_1, + 2.4%; control, +6.2% at 3 h). In the exposed group, 82% reported nose irritation, 76% reported eye irritation (18% wore goggles), 36% reported throat irritation and 14% reported airway irritation. The authors concluded that the reduced increase in lung function during the morning in the exposed group was probably due to the exposure to formaldehyde.

The same group reported a similar study of 34 medical students and instructors who were exposed to 0.07–2.94 ppm (mean, 1.24 ppm) [0.08–3.6 mg/m^3 (mean, 1.22 mg/m^3)]

formaldehyde and 12 control students and instructors who had no exposure to formaldehyde (Akbar-Khanzadeh *et al.*, 1994). Pre- and post-morning sessions showed a 0.03% fall in FEV_1 in the formaldehyde-exposed group compared with a 1% increase in the controls.

(*c*) *Effect of chronic exposure on nasal mucosa*

(i) *Occupational exposure*

The possibility that formaldehyde may induce pathological or cytogenetic changes in the nasal mucosa has been examined in subjects exposed either in residential environments or in occupational settings.

Cell smears were collected by a swab that was inserted 6–8 cm into the nose of 42 workers who were employed in two phenol–formaldehyde plants and 38 controls who had no known exposure to formaldehyde. The concentrations of formaldehyde in the plants were 0.02–2.0 ppm [0.02–2.4 mg/m^3], with occasional peaks as high as 9 ppm [11.0 mg/m^3], and the average length of employment in the plants was about 17 years. Atypical squamous metaplasia was detected as a function of age > 50 years, but no association was found with exposure to formaldehyde (Berke, 1987).

Biopsy samples were taken from the anterior edge of the inferior turbinate of the nose of 37 workers in two particle-board plants, 38 workers in a laminate plant and 25 controls of similar age. The concentrations of formaldehyde in the three plants were 0.1–1.1 mg/m^3, with peak concentrations of up to 5 mg/m^3. Simultaneous exposure to wood dust occurred in the particle-board plants but not in the laminate plant. The average length of employment was 10.5 years. Exposure to formaldehyde appeared to be associated with squamous metaplasia and mild dysplasia, but no concentration–response relationship was observed, and the histological score was not related to number of years of employment. No difference was detected in the nasal histology of workers exposed to formaldehyde alone or to formaldehyde and wood dust (Edling *et al.*, 1987b, 1988).

Biopsy samples were collected from the medial or inferior aspect of the middle turbinate, 1 cm behind the anterior border, from 62 workers who were engaged in the manufacture of resins for laminate production, 89 workers who were employed in furniture factories and who were exposed to particle-board and glue and 32 controls who were mainly clerks in a local government office. The concentrations of formaldehyde in the resin manufacturing plant were 0.05–0.5 mg/m^3, with frequent peaks of over 1 mg/m^3, and those in the furniture factories were 0.2–0.3 mg/m^3, with rare peaks up to to 0.5 mg/m^3; the latter workers were also exposed to wood dust (1–2 mg/m^3). The control group was exposed to concentrations of 0.09–0.17 mg/m^3 formaldehyde. The average length of employment was about 10 years. The histological scores of workers who were exposed to formaldehyde alone were slightly but significantly higher than those of controls, but the histological scores of workers who were exposed to formaldehyde and wood dust together did not differ from those of controls. No correlation was found between histological score and either duration or concentration of exposure (Holmström *et al.* (1989a). [The possible effect of age on nasal cytology, as noted by Berke (1987), was not determined.]

A nasal biopsy sample was taken from the anterior curvature of the middle turbinate of 37 workers who were exposed at a chemical company where formaldehyde resins were produced and 37 age-matched controls. The concentrations of formaldehyde in the company ranged from 0.5 to > 2 ppm [0.6–> 2.4 mg/m^3], and the average length of employment was 20 years. Hyperplasia and squamous metaplasia were more common among the exposed workers than among the controls, but the difference was not significant. The histological scores increased with age and with concentration and duration of exposure, but the changes were not significant (Boysen *et al.*, 1990).

Histopathological abnormalities of respiratory nasal mucosa cells were determined in 15 nonsmokers (seven women, eight men) who were exposed to formaldehyde that was released from a urea–formaldehyde glue in a plywood factory. Each subject was paired with a control who was matched for age and sex. The mean age of the controls was 30.6 ± 8.7 years and that of exposed workers was 31.0 ± 8.0 years. The mean levels of exposure to formaldehyde (8-h TWA) were about 0.1 mg/m^3 in the sawmill and shearing-press department and 0.39 mg/m^3 in the warehouse area. Peak exposure levels were not given. Concurrent exposure to low levels of wood dust (respirable mass, 0.23 mg/m^3 in the warehouse, 0.73 mg/m^3 during sawing and 0.41 mg/m^3 in shearing-press) occurred. Nasal respiratory cell samples were collected from near the inner turbinate with an endocervical cytology brush. The exposed group had chronic inflammation of the nasal respiratory mucosa and a higher frequency of squamous metaplasia than the controls (mean scores, 2.3 ± 0.5 in the exposed group; 1.6 ± 0.5 in the control group; $p < 0.01$, Mann–Whitney U test) (Ballarin *et al.*, 1992).

(ii) *Residential exposure*

Samples of cells were collected by a swab that was inserted 2–3 cm into the nostrils of subjects who lived in urea–formaldehyde foam-insulated homes and of subjects who lived in homes without this type of insulation and were examined cytologically. Small but significant increases were observed in the prevalence of squamous metaplastic cells in the samples from the occupants of urea–formaldehyde foam-insulated homes (Broder *et al.*, 1988a,b,c). A follow-up study 1 year later (Broder *et al.*, 1991) showed a decrease in nasal symptoms that was unrelated to any decrease in levels of formaldehyde.

The effects of formaldehyde, other than cancer, on the nasal mucosa are summarized in Table 31.

(*d*) *Sensitization to formaldehyde*

Formaldehyde is a well recognized cause of allergic contact dermatitis and an occasional cause of occupational asthma. Provided that suitable control exposures for the affected patient are performed and that similar exposures of unsensitized asthmatics do not provoke asthma, cause and effect can reasonably be implied. The studies on asthma are summarized in Table 32.

Hendrick and Lane (1975) found a late asthmatic reaction following exposure to formaldehyde in a nurse who had no reaction after a control exposure. An asthmatic patho-

Table 31. Findings in the nasal mucosa of people who had occupational exposure to formaldehyde

Industry	Concentration of formaldehyde (mg/m^3)	No. of exposed	No. of controls	Method	Findings	Reference
Formaldehyde (laminate plant)	0.5–1.1	38	25	Nasal biopsy	Histological score: exposed, 2.8; controls, 1.8 ($p < 0.05$). Four exposed men had mild dysplasia.	Edling *et al*. (1987b)
Formaldehyde Wood dust (laminated particle-board)	0.1–1.1 (peaks to 5) 0.6–1.1	75	25	Nasal biopsy	Histological score: exposed, 2.9; controls, 1.8 ($p < 0.05$). Six men had mild dysplasia.	Edling *et al*. (1988)
Formaldehyde (phenol?) (laminate)	0.02–2.4 (peaks to 11–18.5)	42	38	Swab smears	No positive correlation between exposure to formaldehyde and abnormal cytology	Berke (1987)
				Clinical examination	More mucosal abnormalities in non-smoking exposed workers ($p = 0.04$)	
Formaldehyde (production of formaldehyde and formaldehyde resins)	0.6–> 2.4	37	37	Nasal biopsy	Histological score: exposed, 1.9; controls, 1.4 ($p > 0.05$). Three exposed and no controls had dysplasia.	Boysen *et al*. (1990)
Formaldehyde (resins for laminate production)	0.05–0.5 (peaks to > 1)	62	32	Nasal biopsy	Histological score: exposed, 2.16; controls, 1.56 ($p < 0.05$). No case of dysplasia	Holmström *et al*. (1989a)
Formaldehyde Wood dust (plywood factory)	0.1–0.39 0.23–0.73	15	15	Nasal scrapes	Micronuclei in nasal mucosal cells: exposed, 0.90; controls, 0.25 ($p < 0.010$). Cytological score: exposed, 2.3; controls, 1.6 ($p < 0.01$). One exposed had mild dysplasia.	Ballarin *et al*. (1992)

Table 32. Studies of occupational asthma/dermatitis

Sex	Route	Concentration of formaldehyde	Duration	Effects	Reference
Men (aged 39 years)	Inhalation	0.06 ppm, max. 0.13 ppm 0.01 ppm 0.5 ppm	6 months 20 min 20 min	Asthma None Late asthmatic reaction, IgE negative	Kim *et al*. (2001)
Men and women	Skin (patch test)	1% in water	Unspecified	Case series of 280 health care workers; 13.9% positive patch test to formaldehyde, little cross reaction with glutaraldehyde (12.4%) and glyoxal (1.9%)	Kiec-Swierczynska *et al*. (1998)
Men and women	Inhalation	2.3 mg/m^3 4.8 mg/m^3 31 mg/m^3 4.8 mg/m^3	30 min 30 min 7 min 30 min	Late asthmatic reaction Dual immediate and late asthmatic reaction Irritant asthmatic reaction No reaction in unsensitized asthmatics	Burge *et al*. (1985)
Women	Inhalation	5 ppm 3 ppm	15 min 5 min	Late asthmatic reaction Late asthmatic reaction No reaction in controls	Hendrick & Lane (1975, 1977); Hendrick *et al*. (1982)
Unspecified	Inhalation			Immediate and late reactions in 2 workers	Popa *et al*. (1969) [few details]
Men and women	Inhalation	1.2 mg/m^3 2.5 mg/m^3	30 min 30 min	1 worker, early response 11 workers, 6 late response 12/230 exposed symptomatic workers + specific challenge to formaldehyde negative in 218, 71 with NSBR	Nordman *et al*. (1985)
Men	Inhalation (cutting and preparing brain specimens)		2 h	Acute pneumonitis; breath smelled of formaldehyde; resolved	Porter (1975)

NSBR, non-specific bronchial hyper-reactivity

logist had no reaction following a similar 60-min exposure to 5 ppm [6 mg/m^3] formaldehyde. A later study (Hendrick *et al*., 1982) repeated the original exposures, which were reported to be 5 ppm. A second report (Hendrick & Lane, 1977) mentioned a second case who reacted to exposure to 3 ppm [3.6 mg/m^3] formaldehyde for 5 min.

Burge *et al.* (1985) reported the results of challenge tests in 15 workers who were exposed to formaldehyde. Three had late asthmatic reactions that suggested sensitization; one reacted following exposure to 2.3 mg/m^3 and two after exposure to 4.8 mg/m^3. Control asthmatics had no asthma attack provoked by exposures to 4.8 mg/m^3; one asthmatic exposed to a sheep dip that contained formaldehyde had an irritant reaction following a 7-min exposure to 31 mg/m^3.

Nordman *et al.* (1985) reported the results of inhalation challenges in 230 workers who were investigated at the Finnish Institute of Occupational Medicine. One worker reacted to 1.2 mg/m^3 formaldehyde and 11 reacted to 2.5 mg/m^3 formaldehyde. No reaction was observed at 2.5 µg/m^3 among the remaining 218 workers, 71 of whom had non-specific hyper-responsiveness.

Kim *et al.* (2001) reported on a worker who made crease-resistant trousers and who had a late asthmatic reaction 5 h after exposure to 0.5 ppm [0.6 mg/m^3], but not to 0.01 ppm [0.01 mg/m^3] formaldehyde (20-min exposures). His IgE to formaldehyde conjugates was negative.

One patient developed anaphylaxis during dialysis with a dialyser that had been sterilized with formaldehyde and contained 5–10 µg/mL residual formaldehyde. She had previously been sensitized to formaldehyde on the skin through contact dermatitis. IgE antibodies to formaldehyde/human serum albumin were strongly positive (Maurice *et al.* (1986).

One case of toxic pneumonitis following a 2-h exposure to a concentration of formaldehyde that was sufficient to be smelled on the breath has been reported (Porter, 1975).

(*e*) *Oral poisoning*

Two reports have been made of three patients who were poisoned following ingestion of formaldehyde solution. All three patients died (Eells *et al.*, 1981; Köppel *et al.*, 1990). Two patients had ingested unknown amounts of formalin that had not been contaminated with methanol. Both developed acidosis with raised plasma formic acid levels (6.09 and 4.57 mmol/L) and additional lactic acidosis. Both survived the initial necrosis of the gut mucosa and renal failure, but died from late acute respiratory distress syndrome and cardiac failure at 3 weeks and 8 weeks after ingestion, respectively (Köppel *et al.*, 1990). The third patient had ingested 120 mL (37% w/v) formaldehyde that contained 12.5% v/v methanol. Initial blood levels 30 min after ingestion were 0.48 µg/dL formaldehyde, 44 mg/dL formate and 42 mg/dL methanol. She died after 28 h (Eells *et al.*, 1981).

4.2.2 *Experimental systems*

(*a*) *In-vitro studies*

(i) *Cytotoxicity*

The toxicity of formaldehyde was assessed in cultured human bronchial epithelial cells under defined serum- and thiol-free exposure conditions (Grafström *et al.,* 1996). Results obtained from studies of 1-h exposures showed that 0.2 mM and 0.6 mM formaldehyde inhibited cell growth by 50% as measured by loss of clonal growth rate and colony-forming efficiency, respectively. Membrane integrity, i.e. exclusion of trypan blue and cellular uptake of neutral red, an energy-dependent lysosomal accumulation of the dye, was decreased by 50% at concentrations of 2 mM and 6 mM formaldehyde, respectively. Inhibition of growth was also associated with significant decreases in GSH, which occurred without concomitant formation of oxidized GSH and with no alteration of the levels of protein thiols. This result indicated that exposure to formaldehyde was associated with the expected formation of thiohemiacetal, but not with overt oxidative stress as assessed by thiol status, in bronchial cells. Extensive loss of intracellular GSH coincided with loss of membrane integrity, which implies that plasma membrane leakage may have contributed to the effect. Moreover, active re-reduction of oxidized GSH to GSH by GSH reductase could potentially have masked an oxidant effect. Formaldehyde-dependent decreases in thiols provide a mechanism for formaldehyde-induced inhibition of growth, since minor decreases in GSH levels are known to inhibit cell growth efficiently (Atzori *et al.*, 1989, 1994). The steady-state concentration of intracellular Ca^{2+} was increased by 50% within a few minutes after treatment with 0.5 mM formaldehyde, and transient increases were recorded at lower concentrations (Grafström *et al.*, 1996). The toxicity of formaldehyde to keratinocytes is manifested as aberrant induction of terminal differentiation, i.e. increases in involucrin expression and formation of cross-linked envelopes. This cellular response is probably linked to the noted increases in cellular Ca^{2+}, which activates transglutaminase-dependent cross-linking of various proteins, including involucrin, into the cross-linked envelope (Rice & Green, 1979). Various types of genetic damage and mutation are caused by formaldehyde at levels as low as 0.1 mM (see Section 4.4), and may also underlie some of the cytotoxic actions of formaldehyde. Inhibition of DNA repair was shown in bronchial cells following treatment with 0.1–0.3 mM formaldehyde, which implies that inhibition of enzyme function might be an essential aspect of the toxicity of formaldehyde. In this respect, enzymes that carry a thiol moiety in their active site may be particularly sensitive (Grafström *et al.*, 1996).

The toxicity of formaldehyde was evaluated in isolated rat hepatocytes (Teng *et al*., 2001); exposure to 4 mM for 2 h caused 50% cell lysis. Toxicity was associated with a dose-dependent loss of GSH and mitochondrial membrane potential and, moreover, was associated with inhibition of mitochondrial respiration and the formation of reactive oxygen species. Higher doses were associated with lipid peroxidation. Depletion of GSH and inhibition of ADH and ALDH activities increased the toxicity of formaldehyde, whereas antioxidants such as butylated hydroxytoluene and iron chelators such as desferoxamine

protected against toxicity. Prevention of toxicity by cyclosporine or carnitine, agents that prevent the opening of the mitochondrial transition pore, provided evidence that formaldehyde targets mitochondria.

(ii) *Proliferation and apoptosis*

The toxicity of formaldehyde and the influences of exogenous and endogenous thiols were studied in cultured human oral fibroblasts and epithelial cells (Nilsson *et al*., 1998). The presence of serum and cysteine counteracted the toxicity of formaldehyde, and lower levels of intracellular thiols, including GSH, in fibroblasts (relative to epithelial cells) were associated with greater toxicity. The results emphasize the high thiol-reactivity of formaldehyde and the central role of cellular thiols in the scavenging of formaldehyde. The toxicity of formaldehyde was compared in human dental pulp fibroblasts, buccal epithelial cells and HeLa cervical carcinoma cells (Lovschall *et al*., 2002). In assessments of proliferation (bromodeoxyuridine incorporation), methylthiazole tetrazolium conversion and neutral red uptake, both of the normal cell types were shown to be more sensitive than the carcinoma cells to the toxicity of formaldehyde. Formaldehyde, applied at concentrations of 0.1–10 mM to HT-29 colon carcinoma and normal endothelial cell cultures, stimulated proliferation at 0.1 mM, inhibited proliferation and induced apoptosis at 1 mM and induced cell lysis at 10 mM (Tyihák *et al*., 2001). The authors concluded that formaldehyde may either stimulate or inhibit proliferation or induce overt toxicity, depending on the dose. Apoptosis was also induced in rat thymocytes by concentrations of 0.1 mM formaldehyde or more after a 24-h exposure (Nakao *et al*., 2003). The proliferation-enhancing effects of formaldehyde are supported by studies of gene expression in mouse fibroblast C3H/10T1/2 cells (Parfett, 2003). Formaldehyde at 0.05–0.1 mM, concentrations that are known to induce cell transformation in this system, increased the transcription of proliferin, a response that is also shared by other transformation-promoting agents.

Formaldehyde at doses of 1 ng/mL to 1 μg/mL induced expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in human microvascular endothelial cells of the nasal mucosa (Kim *et al*., 2002). Exposures to formaldehyde were also associated with increased adhesiveness of the endothelial cells to eosinophils. The authors concluded that the noted effects and changes in gene expression of intercellular and vascular cell adhesion molecules might underlie the irritant effects of formaldehyde in the nasal mucosa.

(iii) *Effect on the mucociliary apparatus of the respiratory mucosa*

In an ex-vivo study, dose-dependent changes in the mucociliary apparatus of frog respiratory mucosa during exposure to formaldehyde in aqueous solutions were examined. Frog palates (10 per group) were removed and were refridgerated for 2 days to allow the mucous to clear. The palates were then immersed in Ringer solution alone or with 1.25, 2.5 or 5.0 ppm formaldehyde solution. The frequency of ciliary beat, as a measure of the fluctuation of light patterns during the ciliary wave over time, and mucociliary clearance, as a function of the time it took a mucous plug to travel 6 mm of palate,

were quantitated. Measurements were taken at time 0, before immersion in formaldehyde solution, and then every 15 min for a total of four measurements over 60 min of exposure at 20 °C and 100% humidity. The later time-points were all compared with their respective 0-time measurement to give a percentage of the baseline measurement. A dose- and time-dependent decrease was observed in both mucociliary clearance and frequency of ciliary beat (Flo-Neyret *et al*., 2001).

(*b*) *In-vivo studies*

(i) *Acute effects*

Irritation

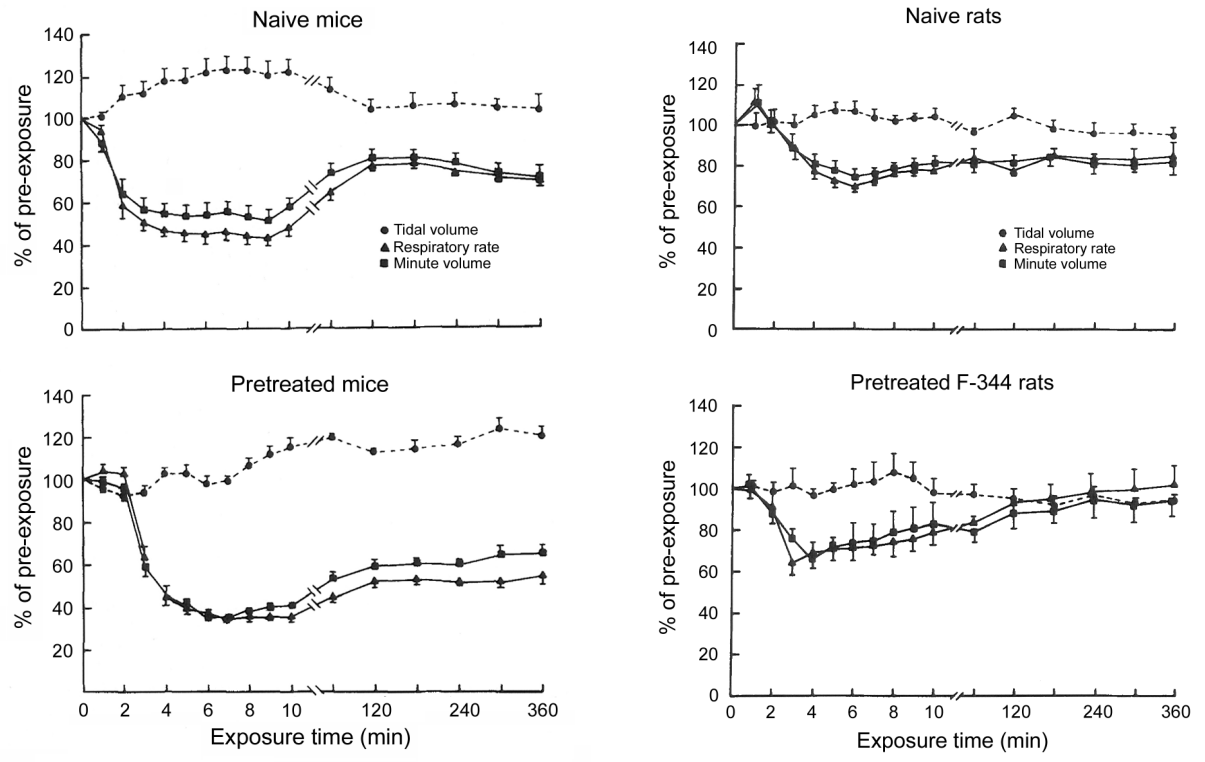
A quantitative measure of sensory irritation in rodents is provided by the reflex decrease in respiratory rate of mice or rats that is caused by stimulation of trigeminal nerve receptors in the nasal passages. In comparison with other aldehydes (Steinhagen & Barrow, 1984), formaldehyde is a potent respiratory tract irritant, and elicits a 50% decrease in respiratory frequency in $B6C3F_1$ mice at 4.9 ppm [6.0 mg/m^3] and in Fischer 344 rats at 31.7 ppm [38.7 mg/m^3] (Chang *et al.*, 1981). Swiss-Webster mice exposed for 5 days (6 h per day) to formaldehyde at a concentration that elicits a 50% decrease in respiratory frequency (3.1 ppm [3.8 mg/m^3]) developed mild histopathological lesions in the anterior nasal cavity, but no lesions were found in the posterior nasal cavity or in the lung (Buckley *et al.*, 1984).

In addition to decreasing the respiratory rate, formaldehyde may also alter the tidal volume, which results in a decrease in minute ventilation. Exposure to formaldehyde over a 10-min test period induced prompt reductions in both respiratory rates and minute volumes in mice and rats, whether or not they were exposed before testing to 6 ppm [7.4 mg/m^3] formaldehyde for 6 h per day for 4 days (Fig. 4). These effects were observed at lower concentrations of formaldehyde in mice than in rats (Chang *et al.*, 1983). A similar effect has been demonstrated in $C57Bl6/F_1$ mice and CD rats (Jaeger & Gearhart, 1982).

Rats exposed to 28 ppm [34.2 mg/m^3] formaldehyde for 4 days developed tolerance to its sensory irritancy, but those exposed to 15 ppm [18.3 mg/m^3] for 1, 4 or 10 days did not (Chang & Barrow, 1984).

Sensory irritation to formaldehyde, acrolein and acetaldehyde vapours and their mixture was studied in groups of male Wistar rats weighing 240–300 g (four per group) that were exposed to concentrations of the aldehyde vapours up to a level that decreased respiratory frequency by 50%. Formaldehyde vapours were produced by thermal depolymerization of paraformaldehyde in water and evaporation into the in-flow airstream. The maximal decrease in breathing frequency was observed within 3 min of exposure; desensitization occurred within a few minutes after maximal decrease in breathing frequency and only partial recovery was achieved within 10 min after exposure. The authors proposed that the decreased breathing frequency within the first few minutes of exposure is due to trigeminal nerve stimulation from the irritant effect of formaldehyde. The level of exposure to formaldehyde that resulted in a 50% respiratory depression was 10 ppm

Figure 4. Time–response curves for respiratory rate, tidal and minute volume from naive and formaldehyde-pretreated mice and rats (6 ppm, 6 h/day, 4 days) during a 6-h exposure to 6 ppm formaldehyde



Adapted from Chang *et al.* (1983)
Data shown are $\bar{x}$ ± SE for each time point; n = 6 for each group

[12.3 mg/m^3]. Since this was a study of the mixture, the authors also found that the reduction in breathing frequency after exposure to the mixture was always greater than after exposure to the same concentration of any of the components alone (Cassee *et al*., 1996a).

The retinal toxicity of a single dose of formaldehyde, methanol and formate was investigated in male albino rabbits weighing 2.1–2.3 kg (four per group) by injection into the vitreous cavity of 100 μL of a buffered (pH 7.4) phosphate-saline solution that contained 1% methanol, 0.1% or 1.0% formaldehyde or 1% formate. The final vitreous concentrations were 700 μg/mL methanol, 70 μg/mL formaldehyde, 700 μg/mL formaldehyde and 700 μg/mL formate. The eyeballs were examined with a biomicroscope and ophthalmoscope before treatment and 1, 2, 7, 14 and 30 days after treatment. After 30 days, the rabbits were euthanized and the eyeballs were fixed in formalin for microscopic examination of the retina, choroid, sclera, optic disc and optic nerve. No lesions were observed in the methanol- or formate-treated eyes at any time-point. The eyes treated with formaldehyde had ophthalmoscopic alterations at all time-points and at both doses; the higher dose induced more severe alterations including subcapsular cataract, retinal vessel dilatation and juxtapapillary retinal haemorrhages. Histologically, the eyes treated with 0.1% formaldehyde had disorganization of the ganglion cell and outer nuclear layers of the retina; these symptoms were more severe in eyes treated with 1.0% formaldehyde. The optic nerves had vacuolization after treatment with either dose of formaldehyde (Hayasaka *et al*., 2001).

Pulmonary hyper-reactivity

Formaldehyde induced pulmonary hyper-reactivity in guinea-pigs: exposure to 0.3 ppm [0.37 mg/m^3] for 8 h caused transient bronchoconstriction and hyper-reactivity to infused acetylcholine; exposure to higher concentrations (> 9 ppm [> 11 mg/m^3]) for 2 h induced bronchoconstriction. No evidence of tracheal epithelial damage was observed after exposure to 3.4 ppm [4.2 mg/m^3] for 8 h. However, the mechanism by which these effects occur is unknown (Swiecichowski *et al.*, 1993).

The effects of formaldehyde (vaporized formalin) on pulmonary flow were determined in cynomolgus monkeys that were tranquilized before exposure and received an endotracheal tube transorally. Pulmonary flow resistance was increased after exposure to formaldehyde at a concentration of 2.5 ppm [3.0 mg/m^3] for 2, 5 and 10 min. Narrowing of the airways by formaldehyde was not correlated with methacholine reactivity (Biagini *et al.*, 1989). [The Working Group questioned the relevance of these findings, in view of the method of administration.]

Male Sprague-Dawley rats, 6 weeks of age, were exposed to formaldehyde (10 ppm [12.2 mg/m^3]), ozone (0.6 ppm [1 mg/m^3]) (with coefficients of variation of less than 12% and 5%, respectively) or a combination of the two during exercise on a treadmill that was modified to deliver a stream of control air or air that contained formaldehyde. The rats were exercised at a moderately fast-walking gait of 15 m/min on a 20% gradient for 3 h, which raised metabolic gas exchange over the resting rate by a factor of at least two. Resting exposure was conducted in a nose-only inhalation tube. Histology, including labelling indices, was examined quantitatively for the nose and lung. Formaldehyde increased labelling in the

transitional epithelium in the nose after individual or mixed exposure at rest or after exercise but ozone alone did not. Formaldehyde and ozone after exercice either individually or as a mixture produced injury in the trachea; the mixture caused greater (approximately additive) injury than either of the chemicals alone. The pulmonary parenchyma was unaffected by exposure to formaldehyde. Functional changes included decreased breathing frequency during exposure to formaldehyde; the response to the mixture was lower. Tidal volume decreased after treatment with ozone or formaldehyde but was increased after exposure to the mixture. Formaldehyde produced slow and shallow breathing which resulted in depressed minute ventilation (Mautz, 2003).

Sensitizing effect and inflammatory response

In order to investigate the induction of sensitization to formaldehyde, undiluted formalin was painted on shaven and epilated dorsal sites of guinea-pigs; a second application was administered 2 days later at naive sites, to give a total dose of 74 mg/animal. Other animals received diluted formalin at doses of 0.012–9.3 mg/animal. All animals that received 74 mg formalin developed skin sensitivity when tested 7 days after exposure. A significant dose–response relationship was observed for the degree of sensitization and for the percentage of animals that were sensitized; however, pulmonary sensitization was not induced when formaldehyde was administered dermally, by injection or by inhalation, and no cytophilic antibodies were detected in the blood (Lee *et al.*, 1984).

In the guinea-pig maximization test, 10 guinea-pigs (five per exposure group) received six intradermal injections of saline or 0.25% [2.5 mg/mL] formaldehyde solution followed 6–8 days later by an occlusive dressing of a patch soaked in 10% [100 mg/mL] formaldehyde for 48 h. After an additional 12–14 days, another occlusive dressing was applied for 24 h; 24 h after removal, the size of the area of the erythema was measured. A similar study was conducted in guinea-pigs but only occlusive dressings were used. Both studies resulted in a positive response (Hilton *et al.*, 1996).

The local lymph node assay was conducted in BALB/c mice that received daily applications of 25 μL of 10%, 25% or 50% w/v solutions of formaldehyde on the pinna of both ears for 3 consecutive days. Five days after the initiation of exposure, all mice were injected with tritiated methyl thymidine and were killed 5 h later. The lymph nodes adjacent to the ear were removed and labelled cells were counted to measure the rate of cell proliferation. Formalin induced a strong proliferative response that was considered to be positive for a contact allergen (Hilton *et al.*, 1996).

The mouse IgE test is thought to identify allergens that have the potential to cause sensitivity in the respiratory tract by stimulating a significant increase in serum IgE concentration. This test was conducted in groups of six BALB/c mice that received 50 μL of a 10%, 25% or 50% formalin solution (37% formaldehyde dissolved in dimethylformamide) on an occlusive dressing applied to the shaved flank. Seven days later, 25 μL of the solution of formaldehyde at half the concentration used previously was applied to the dorsum of both ears. Fourteen days after the initial exposure, the mice were exsanguinated and blood was

collected for analysis of IgE. There was no significant change in levels of circulating IgE after treatment with formaldehyde (Hilton *et al.*, 1996).

Production of the cytokines interferon-γ (IFN-γ) and interleukin (IL)-10 was measured in draining lymph nodes of the skin of the flanks and the ears of 10 BALB/c mice that had received skin applications of 10%, 25% or 50% formalin solutions (37% formaldehyde in dimethylformamide) on the shaved flank twice a day for 5 days followed by 3 days on the dorsum of the ears. Thirteen days after the initiation of exposure, draining auricular lymph nodes were removed. Lymphocytes were isolated and cultured for up to 120 h, and the supernatant was collected and analysed for IFN-γ and IL-10. The levels of IFN-γ were significantly increased after all concentrations of formaldehyde but not those of IL-10. The authors suggested that formaldehyde causes a contact sensitization reaction in the skin (mediated by Th1-type lymphocytes that secrete IFN-γ) but not sensitization of the respiratory tract (Hilton *et al*., 1996).

Eighteen 8-week-old male BN/Crj and Fischer 344/DuCrj rats were exposed to an aerosol of 1% formaldehyde solution (equivalent to 15–20 ppm) for 3 h per day for 5 days in a whole-body inhalation exposure system; another nine animals were exposed to an aerosol of ion-exchanged water only. After death by exsanguination, the nose was examined histologically or the nasal epithelium was removed by scraping and used to isolate RNA. Measurements of cDNA were made by reverse-transcriptase polymerase chain reaction (RT-PCR) for quantitative real-time analysis of *IFN-γ*, *IL-2*, *IL-4* and *IL-5* mRNA levels. All samples were examined for levels of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA and quantities of gene were presented as the ratio to *GAPDH*. The histological lesions were consistent with those reported previously and were of greater severity in Fischer 344 than in BN rats (Ohtsuka *et al.*, 1997). Compared with *GAPDH*, *IFN-γ* and *IL-2* (Th1-related cytokines involved in non-allergic inflammation) were expressed at significantly lower levels in BN rats treated with formaldehyde. None of the other mRNA levels were statistically significant, although the expression of *IL-4* and *IL-5* (Th2-related cytokines) in BN rats and that of *IL-2* and *IL-5* in Fischer 344 rats were lower than the levels observed in controls (Ohtsuka *et al*., 2003).

Cytotoxicity and cell proliferation in the respiratory tract

The acute and subacute effects of formaldehyde in experimental animals are summarized in Table 33. A critical issue for the mechanism of carcinogenesis is whether low concentrations of formaldehyde increase or decrease the rate of cell turnover in the nasal epithelium. Subacute exposure to a low concentration of formaldehyde (1 ppm [1.2 mg/m^3]; 6 h per day for 3 days) has been reported to induce a small, transient increase in nasal epithelial cell turnover in Wistar rats (Zwart *et al.*, 1988), but this statistically significant increase was not confirmed in later studies (Reuzel *et al.*, 1990). Other investigators did not detect an increase in cell turnover in the nasal epithelium of Fischer 344 rats exposed to 0.7 or 2 ppm [0.9 or 2.4 mg/m^3] (6 h per day for 1, 4 or 9 days) (Monticello *et al.*, 1991) or to 0.5 or 2 ppm [0.6 or 2.4 mg/m^3] (6 h per day for 3 days) (Swenberg *et al.*, 1983). Low concentrations of formaldehyde (0.5 or 2 ppm; 6 h per day for 1, 2, 4, 9 or 14 days) also did not

Table 33. Cytotoxicity and cell proliferation induced by acute and subacute exposure to formaldehyde

Strain, species, sex	Exposure	Effects	Reference
Fischer 344 rat, male; $B6C3F_1$ mouse, male	0, 0.6, 2.4, 7.4, 18.5 mg/m^3, 6 h/day, 3 days	0.6, 2.4: no increase in cell replication rate in nasal mucosa; 7.4: increased cell turnover (rats only); 18.5: cell proliferation (rats and mice)	Swenberg *et al*. (1983)
Fischer 334 rat, male; $B6C3F_1$ mouse, male	0, 18.5 mg/m^3, 6 h/day, 1 or 5 days	18.5: cell proliferation induced in nasal mucosa of both species; rat responses exceeded those of mice.	Chang *et al*. (1983)
Fischer 344 rat, male	3.7 mg/m^3 × 12 h/day, 7.4 mg/m^3 × 6 h/day, 14.8 mg/m^3 × 3 h/day ($C \times t$ = 44 mg/m^3–h/day), 3 or 10 days	Cell proliferation related more closely to concentration than to time; less proliferation after 10 than after 3 days of exposure, indicating adaptation	Swenberg *et al*. (1983)
Fischer 344 rat, male	0, 0.6, 2.4, 7.4, 18.5 mg/m^3, 6 h/day, 1, 2, 4, 9 or 14 days	0.6: no effects on mucociliary function; 2.4: minimal effects; 7.4: moderate inhibition; 18.5: marked inhibition	Morgan *et al*. (1986b)
Fischer 344 rat, male	0, 2.4, 18.5 mg/m^3, 10, 20, 45 or 90 min or 6 h	2.4: no effect on mucociliary function; 18.5: inhibition of mucociliary function, marked recovery 1 h after exposure	Morgan *et al*. (1986c)
Fischer 344 rat, male	0, 0.6, 2.4 mg/m^3, 6 h/day, 1 or 4 days; 7.4 mg/m^3, 6 h/day, 1, 2 or 4 days; 18.5 mg/m^3, 6 h/day, 1 or 2 days	0.6, 2.4: no lesions; 7.4, 18.5: non-cell-specific, dose-related injury, including hypertrophy, non-keratinized squamous cells, nucleolar segregation	Monteiro-Riviere & Popp (1986)
Wistar rat, male	0, 6.2 mg/m^3 × 8 h/day, 12.3 mg/m^3 × 8 h/day ($C \times t$ = 49 or 98 mg/m^3–h/day); 12.3 mg/m^3 × 8 × 0.5 h/day, 25 mg/m^3 × 8 × 0.5 h/day ($C \times t$ = 49 or 98 mg/m^3–h/day), 5 days/week, 4 weeks	Labelling index increased at all concentrations; cell proliferation more closely related to concentration than to total dose	Wilmer *et al*. (1987)
Wistar rat, male and female	0, 0.37, 1.2, 3.7 mg/m^3, 6 h/day, 3 days	Significant, transient increase in cell turnover at 1.2 and 3.7 mg/m^3 but not confirmed in later studies at concentration of 1.2 ppm (Reuzel *et al*., 1990)	Zwart *et al*. (1988)

Table 33 (contd)

Strain, species, sex	Exposure	Effects	Reference
Rhesus monkey, male	0, 7.4 mg/m^3, 6 h/day, 5 days/week, 1 or 6 weeks	Lesions similar to those in rats (Monticello *et al*., 1991) but more widespread, extending to trachea and major bronchi; increased cell replication in nasal passages, trachea and carina; percentage of nasal surface area affected increased between 1 and 6 weeks.	Monticello *et al*. (1989)
Wistar rat, male	0, 0.37, 1.2, 3.7 mg/m^3, 22 h/day, 3 days Also investigated effect of simultaneous exposure to 0.4, 0.8 or 1.6 mg/m^3 ozone	0.37, 1.2: either no increase in or inhibition of cell proliferation; 3.7: increased cell replication; 0.8 mg/m^3 ozone + 1.2 or 3.7 mg/m^3 formaldehyde: synergistic increase in cell turnover; 1.6 mg/m^3 ozone + 1.2 mg/m^3 formaldehyde: inhibition of cell turnover	Reuzel *et al*. (1990)
Fischer 344 rat, male	0, 0.86, 2.4, 7.4, 12.3, 18.5 mg/m^3, 6 h/day, 5 days/week, 1, 4, or 9 days or 6 weeks	0.86, 2.4: no effect on cell turnover; 7.4, 12.3, 18.5: concentration- and site-dependent cell proliferation induced at all exposure times	Monticello *et al*. (1991)
Wistar rat, male	0, 4.43 mg/m^3; 8 h followed by 4 h no exposure for 6 consecutive 12-h cycles Also investigated effect of simultaneous exposure to 0.4 ppm ozone.	4.43 mg/m^3 resulted in increased GSH peroxidase in nasal respiratory tissue and increased PCNA expression by immunohistochemistry.	Cassee & Feron (1994)
Wistar rat, male	0, 1.23, 3.94, 7.87 mg/m^3, 6 h/day, 1 or 3 days Also investigated effect of simultaneous exposure to acetaldehyde (750, 1500 ppm) and acrolein (0.25, 0.67 ppm).	The mixture resulted in more extensive and severe histopathology of the nose than the individual exposure.	Cassee *et al*. (1996b)
BN/Crj rat, male; Fischer 344/DuCrj rat, male	0 or 18.45–24.6 mg/m^3 (estimate), 3 h/day, 5 days	Nasal respiratory degeneration and necrosis more severe in Fischer 344 rats	Ohtsuka *et al*. (1997)

C, concentration; *t*, time; PCNA, proliferating cell nuclear antigen

inhibit mucociliary function in the nasal passages of Fischer 344 rats (Morgan *et al.*, 1986b,c), and no injury to the nasal epithelium of rats of this strain was detected ultrastructurally after exposure to 0.5 or 2 ppm (6 h per day for 1 or 4 days) (Monteiro-Riviere & Popp, 1986).

Wistar rats exposed to 3 ppm [3.7 mg/m^3] (6 h per day for 3 days (Zwart *et al.*, 1988) or 22 h per day for 3 days (Reuzel *et al.*, 1990)) had a transient increase in cell replication. Higher concentrations of formaldehyde (≥ 6 ppm [7.3 mg/m^3]) induced erosion, epithelial hyperplasia, squamous metaplasia and inflammation in a site-specific manner in the nasal mucosa of Wistar rats (Monticello *et al.*, 1991). Mice are less responsive than rats, probably because they are better able than rats to reduce their minute ventilation when exposed to high concentrations of formaldehyde (Chang *et al.*, 1983; Swenberg *et al.*, 1983). Fischer 344 rats exposed to 6, 10 or 15 ppm [7.3, 12.2 or 18.3 mg/m^3] (6 h per day for 1, 4 or 9 days, or 6 h per day on 5 days per week for 6 weeks) had an enhanced rate of cell turnover (Monticello *et al.*, 1991). The severity of nasal epithelial responses at 15 ppm was much greater than that at 6 ppm (Monteiro-Riviere & Popp, 1986). Rhesus monkeys exposed to 6 ppm (6 h per day for 5 days) developed similar nasal lesions to rats. Mild lesions, characterized as multifocal loss of cilia, were also detected in the larynx, trachea and carina (Monticello *et al.*, 1989).

The relative importance of concentration and total dose on cell proliferation was examined in Fischer 344 and Wistar rats exposed to a range of concentrations for various lengths of time, such that the total inhaled dose was constant. Exposures were for 3 or 10 days (Swenberg *et al.*, 1983) or 4 weeks (Wilmer *et al.*, 1987). All of the investigators concluded that concentration, not total dose, is the primary determinant of the cytotoxicity of formaldehyde. A similar conclusion was reached when rats were exposed for 13 weeks (Wilmer *et al.*, 1989).

Ten 8-week-old male BN/Crj or Fischer 344/DuCrj rats were exposed to 100 L/min (2 mg 1% formaldehyde solution/L air, equivalent to 15–20 ppm) aerosol or water for 3 h per day on 5 days per week for 2 weeks. Clinical signs were monitored and light and scanning electron microscopy were performed. Both strains of rat had abnormal respiration, nasal discharge and sneezing following treatment with formaldehyde; the Fischer 344 rats had a more severe response. Lesions were present only in the nose and trachea from Fischer 344 rats and nose from BN rats; again, Fischer 344 rats were more severely affected. The typical lesions of squamous metaplasia, respiratory hyperplasia and degeneration and necrosis in the nose that were described with Fischer 344 rats affected all sections of the nose examined, whereas BN rats only had squamous metaplasia in the ventral portion of level II. Epithelial hyperplasia was present in the trachea of Fischer 344 rats. By scanning electron microscopy, squamous epithelial-like changes were seen in the anterior nose after treatment with formaldehyde in both rat strains. BN rats were less sensitive to exposure to formaldehyde than Fischer 344 rats (Ohtsuka *et al*., 1997).

Co-exposure with other agents

Ozone

The effects of simultaneous exposure to formaldehyde and ozone were investigated in Wistar rats exposed to 0.3, 1 or 3 ppm [0.37, 1.2 and 3.7 mg/m^3] formaldehyde, 0.2, 0.4 or 0.8 ppm [0.4, 0.8 or 1.6 mg/m^3] ozone or mixtures of 0.4 ppm ozone with 0.3, 1 or 3 ppm formaldehyde or 1 ppm formaldehyde with 0.2, 0.4 or 0.8 ppm ozone (22 h per day for 3 days). Both formaldehyde (3 ppm) and ozone (0.4 or 0.8 ppm) induced cell proliferation in the most anterior region of the respiratory epithelium. In a slightly more posterior region, ozone had no effect on cell replication, but formaldehyde either enhanced cell proliferation (3 ppm) or appeared to inhibit it slightly (0.3 or 1 ppm). Combined exposures to low concentrations (0.4 ppm ozone and 0.3 ppm formaldehyde, 0.4 or 0.8 ppm ozone and 1 ppm formaldehyde) induced less cell proliferation than ozone alone; however, more than additive increases in cell proliferation were detected in the anterior nose after exposure to 0.4 ppm ozone in combination with 3 ppm formaldehyde, and in a slightly more posterior region after exposure to 0.4 ppm ozone with 1 or 3 ppm formaldehyde. The results suggested to the authors a complex response of the nasal epithelium to low (non-irritating) concentrations of these irritants but a synergistic increase in cell proliferation at irritating concentrations. To induce a synergistic effect on cell proliferation, at least one of the compounds must be present at a cytotoxic concentration (Reuzel *et al.*, 1990).

The pathophysiology of nasal alterations was investigated in 80 8-week-old male Wistar rats (20 per group) after nose-only exposure to formaldehyde or ozone, or their mixture for 8 h followed by 4 h of no exposure for six consecutive 12-h cycles. The formaldehyde was generated from paraformaldehyde by thermal depolymerization in water and evaporation into the air stream at a concentration of 3.6 ± 0.1 ppm or 3.5 ± 0.1 ppm [4.3 mg/m^3] in the individual or ozone mixture exposure groups, respectively (concentration of ozone, 0.4 ppm [0.8 mg/m^3]). After euthanasia, the respiratory portion of the nasal epithelium was collected on ice from a subset of the rats and another subset [numbers not specified] was used for microscopic examination of the nose with haematoxylin and eosin, periodic acid Schiff and proliferating cell nuclear antigen immunohistochemistry. The nasal respiratory epithelial samples were pooled (six rats), homogenized and centrifuged for extraction of the supernatant. The enzyme activities of GST, GSH peroxidase, glucose-6-phosphate dehydrogenase, GSH reductase, ADH and FDH were measured. In addition, GSH and protein levels were quantified. All animals lost weight during the exposure period and weight loss was significantly greater in the treated animals compared with controls. Ozone alone resulted in degenerative changes in the respiratory epithelium but formaldehyde alone or in combination with ozone induced necrosis in the respiratory epithelium. Rhinitis was induced by all three treatments but was more severe in rats treated with formaldehyde than in those treated with ozone and was most severe after exposure to the mixture. Cell proliferation was increased after all treatments compared with controls and a uniformly greater increase was observed in rats exposed to formaldehyde combined with ozone compared with those exposed to ozone alone. Rats treated with formaldehyde alone had proliferative rates in most

of the measured areas equivalent to those of the ozone–formaldehyde-exposed rats. However, rats that received formaldehyde only had greater proliferation indices in the septum and lateral wall of the posterior section than rats exposed to the mixture. Only GSH peroxidase activity was increased after exposure to formaldehyde and only GST activity was decreased after exposure to the mixture. Ozone alone did not alter any enzyme activities significantly. The authors suggested that there appeared to be no major role for GSH or GSH-dependent enzymes in the pathogenesis of toxicity induced by formaldehyde and/or ozone (Cassee & Feron, 1994).

Other aldehydes

The histological and biochemical effects of exposure to formaldehyde, acetaldehyde and acrolein individually or as mixtures were examined in rats. Groups of male Wistar rats, 8 weeks of age, were exposed to formaldehyde at 0, 1.0, 3.2 or 6.4 ppm [0, 1.23, 3.94 or 7.87 mg/m^3] for 6 h per day for 1 or 3 days in nose-only inhalation chambers. Additional rats were exposed to mixtures of 1.0 ppm formaldehyde and 0.25 ppm acrolein, 1.0 ppm formaldehyde, 0.25 ppm acrolein and 750 ppm acetaldehyde, or 3.2 ppm formaldehyde, 0.67 ppm acrolein and 1500 ppm acetaldehyde. After euthanasia, five or six treated animals per exposure group and a total of 19 control animals from all substudies were used for histology (haematoxylin and eosin and proliferating cell nuclear antigen immunohistochemistry) and nine animals from each exposure group were used for biochemical studies. Respiratory and olfactory epithelium were removed separately and homogenized on ice for extraction of the cytosolic fraction and analysis of GSH peroxidase, GST, GSH reductase, ADH, FDH and total amount of protein and non-protein sulfhydryl groups. No histological or biochemical alterations were observed after 1 day of exposure to formaldehyde at any concentration. Only acrolein or the high-dose mixture of all three chemicals induced a biochemical change after 1 day (a decrease in GSH reductase activity). Acetaldyde induced a dose-dependent increase in non-protein sulfhydryls. After 3 days of exposure to 1.0 ppm formaldehyde, no histological alterations were present. Only the group exposed to 3.2 ppm formaldehyde had histopathology that was characterized by degeneration and necrosis of the respiratory epithelium with basal-cell hyperplasia. The lesions were most pronounced along the lateral walls of the naso- and maxilloturbinates. An associated significant increase in cell proliferation was also observed after exposure to 3.2 ppm formaldehyde for 3 days. After 3 days of exposure to 3.2 or 6.4 ppm formaldehyde, only GSH peroxidase activity had increased statistically significantly. Three days of exposure to 1.4 ppm acrolein resulted in a decrease in GST and ADH and an increase in FDH activities while the groups of rats exposed to the mixtures of all three compounds had increased GST and GSH peroxidase activities. Histological alterations of the nasal epithelium were more severe after exposure to the mixture than after exposure to any of the components alone at comparable concentrations and duration of exposure. The distribution of the lesions induced by formaldehyde was different from that produced by acetaldehyde or acrolein, in that lesions produced by formaldehyde were concentrated in the respiratory epithelium (Cassee *et al*., 1996b).

Enzyme induction

No increase in the activities of FDH or ALDH was seen in the nasal mucosa of Fischer 344 rats exposed to 15 ppm [18.3 mg/m^3] formaldehyde (6 h per day on 5 days per week for 2 weeks) (Casanova-Schmitz *et al.*, 1984a). A large increase in the concentration of rat pulmonary CYP was seen, however, after exposure to 0.5, 3 or 15 ppm formaldehyde [0.6, 3.7 or 18.3 mg/m^3] (6 h per day for 4 days) (Dallas *et al.*, 1989), although Dinsdale *et al.* (1993) could not confirm these results in the same strain of rat and found no increase in pulmonary concentration of CYP after exposure to 10 ppm [12.3 mg/m^3] formaldehyde (6 h per day for 4 days).

The relative contribution of three isoforms of ADH to ethanol, formaldehyde and retinoic acid acute toxicity was examined in knockout mice that had induced deletions of *Adh1*, *Adh3* or *Adh4* genes, which make the enzymes non-functional. The comparison with formaldehyde was based on the LD_{50} after intraperitoneal injection of a 10% formalin solution that resulted in a dose range of 0.09–0.22 g/kg bw formaldehyde. A lethal dose to wild-type mice resulted in death within 90 min. It was assumed in the interpretation of these studies that formaldehyde and not a metabolite was the ultimate toxicant. Mice that had *Adh3* knocked out required significantly lower levels of formaldehyde (0.135 g/kg) than the wild-type control (0.2 g/kg) to achieve an LD_{50} whereas there was no difference between wild-type and mice that had *Adh1* and *Adh4* knocked out. These studies showed that *Adh3* is responsible for the clearance of formaldehyde but does not play a role in the clearance of ethanol or retinoic acid. *Adh1* and *Adh4* demonstrate overlapping functions in the metabolism of ethanol and retinol *in vivo*. *Adh3* is conserved across most levels of biological organization including all mammalian species, invertebrates and plants (Deltour *et al*., 1999).

Other effects

The nephrotoxicity of formaldehyde after intravascular injection was studied in male Sprague-Dawley rats (eight animals per group) that weighed 200 g. The rats were injected through the tail vein with 7.6 or 38 μM of a saline solution of formaldehyde or normal saline. Blood samples were taken for determination of blood urea and creatinine levels 24 and 48 h after injection of the test solution. Urine was also collected 24 h after injection and analysed for lactate dehydrogenase (LDH) and protein. All rats were killed 48 h after the single injection and the kidneys were removed for histological examination. No histological alterations were present in the kidney. No statistical change in urinary protein or LDH levels nor in blood creatinine was observed. A small but statistically significant increase in blood urea was reported at 24 h (5.23 ± 0.3 versus 4.13 ± 0.5 for control [units not reported]) after treatment which returned to normal levels at 48 h. These data suggested that renal toxicity does not occur after acute intravascular exposure to formaldehyde (Boj *et al*., 2003).

Twenty-one male (weighing 250–280 g) and 19 female (weighing 180–200 g) 16-week-old Wistar rats were trained over 14 days to find food in a maze and were then tested in the maze after exposure to formaldehyde. The number of mistakes and the length of time to find

the food were counted daily for 11 days as a baseline. The animals were exposed to aqueous formaldehyde solutions of either 0.25% (equivalent to 3.06 ± 0.77 mg/m^3; 2.6 ppm) or 0.7% (equivalent to 5.55 ± 1.27 mg/m^3; 4.6 ppm) for 10 min per day on 7 days per week for 90 days. Every 7th day, the animals were tested in the maze prior to exposure. After 90 days of exposure, the animals were then allowed a 30-day recovery and were tested in the maze every 10th day. At the end of the study, the animals were killed and the liver, trachea, lungs, kidneys, heart, spleen, pancreas, testicles, brain and spinal cord were collected and fixed in formalin. Both formaldehyde-exposed groups made more mistakes and took longer to complete the maze than the controls but no difference was observed between the exposed groups. None of the groups differed from one another after the recovery period. There were no treatment-related histological lesions (Pitten *et al*., 2000). [There is no evidence that the changes seen in this study are due to formaldehyde-induced neurotoxicity, and could have just as easily have been from loss of olfactory capacity and visual difficulties from irritant effects to the cornea which would have improved after the treatment was stopped.]

Lewis rats were exposed to formaldehyde vapours and placed in a water maze to test the effect of formaldehyde on learning. A total of 120 male and female LEW.1K rats, 110–130 days of age, were separated into four groups and exposed to distilled water or 0.1 ± 0.02, 0.5 ± 0.1, or 5.4 ± 0.65 ppm [0.12 mg/m^3, 0.62 mg/m^3 or 6.64 mg/m^3] volatilized formaldehyde for 2 h per day for 10 days. Two hours after exposure, the rats were subjected to the water maze test. The length of time taken to complete the maze and the number of errors while attempting this were measured. At the conclusion of the study, the animals were killed and the lungs, heart, thymus, kidneys, liver, pancreas, skeletal muscle and spleen were examined microscopically. All rats exposed to formaldehyde made more errors in completing the maze, but no difference was observed between exposure groups or sexes. After 10 days, only the 0.5- and 5.4-ppm groups took longer to complete the water maze; no difference was observed between sexes. No histological lesions were found in the tissues examined. The authors suggested that formaldehyde vapours had a central effect on learning and memory. However, none of the tissues sampled were target organs for formaldehyde toxicity in the rat except at very high and prolonged exposure concentrations. [The nose was not examined in this study. Formaldehyde is a surface irritant which would cause degeneration and necrosis of the olfactory epithelium as well as the surface epithelium lining the cornea. The complications of blurry vision and loss of olfactory cues was not controlled for in this study, which suggests that an effect on the central nervous system may not have resulted in the treatment-related response] (Malek *et al*., 2003).

Forty-two male Wistar rats (weighing approximately 250 g) were divided into six groups (seven rats per group) and exposed by inhalation to 0, 6.1 mg/m^3 or 12.2 mg/m^3 formaldehyde for 8 h per day on 5 days per week for subacute (4 weeks) or subchronic (13 weeks) periods. At the end of the exposure period, the animals were killed and the brains were removed for analysis of zinc, copper and iron levels (mg metal/kg parietal cortex). Both zinc and copper increased in concentration with increasing dose whereas iron decreased in concentration. The increase in copper concentration and decrease in iron concentration were both time-dependent. Exposure to formaldehyde altered the trace

element level of copper, zinc and iron in the brain. The greatest change was a 54% increase in levels of zinc after 4 weeks of exposure to the high dose which fell to a 33% increase over controls after 13 weeks (Özen *et al*., 2003).

Forty male albino Charles Foster rats, weighing 147–171 g, were given daily intraperitoneal injections of 0, 5, 10 or 15 mg/kg bw formaldehyde in saline for 30 days. On day 31, the animals were anaesthetized for blood collection then killed and the thyroid glands were collected, weighed and processed for histology. Thyroid weights were significantly decreased after treatment with 10 and 15 mg/kg per day and histological changes of follicular degeneration, atrophy and epithelial size were observed. Triiodothyronine and thyroxine were significantly decreased and thyroid-stimulating hormone was increased after doses of 10 and 15 mg/kg per day. After treatment with 5 mg/kg per day, triiodothyronine was decreased and thyroid-stimulating hormone was increased but thyroxine was unchanged and the thyroid glands did not differ histologically from those of controls (Patel *et al*., 2003).

Levels of serum corticosterone were examined in Sprague-Dawley rats (weighing 260–280 g) exposed by inhalation to 0.7 or 2.4 ppm [0.86 or 2.95 mg/m^3] formaldehyde for 20 or 60 min per day on 5 days per week for 2 or 4 weeks. Treatment had no effect after short-term exposure; however, rats exposed to 0.7 ppm for 4 weeks had increased baseline serum corticosterone. Rats treated with 2.4 ppm formaldehyde for 2 or 4 weeks had increased levels of serum corticosterone after a formaldehyde challenge (Sorg *et al*., 2001).

(ii) *Chronic effects*

Cytotoxicity and cell proliferation in the respiratory tract

The subchronic and chronic effects of formaldehyde in different animal species exposed by inhalation are summarized in Table 34. No increase in cell turnover or DNA synthesis was found in the nasal mucosa after subchronic or chronic exposure to concentrations of ≤ 2 ppm [≤ 2.4 mg/m^3] formaldehyde (Rusch *et al.*, 1983; Maronpot *et al.*, 1986; Zwart *et al.*, 1988; Monticello *et al.*, 1993; Casanova *et al.*, 1994). Small, site-specific increases in the rate of cell turnover were noted at 3 ppm [3.7 mg/m^3] (6 h per day on 5 days per week for 13 weeks) in Wistar rats (Zwart *et al.*, 1988) and in the rate of DNA synthesis at 6 ppm [7.3 mg/m^3] (6 h per day on 5 days per week for 12 weeks) in Fischer 344 rats (Casanova *et al.*, 1994). At these concentrations, however, an adaptive response occurred in rat nasal epithelium, as cell turnover rates after 6 weeks (Monticello *et al.*, 1991) or 13 weeks (Zwart *et al.*, 1988) were lower than those after 1–3 or 4 days of exposure. Monticello *et al.* (1996) detected no increase in cell turnover in the nasal passages of Fischer 344 rats exposed to 6 ppm [7.3 mg/m^3] formaldehyde for 3 months (6 h per day on 5 days per week). However, as already noted, Casanova *et al.* (1994) detected a small increase in DNA synthesis under these conditions, but after 12 weeks of treatment. Large, sustained increases in cell turnover were observed at 10 and 15 ppm [12.2 and 18.3 mg/m^3] (6 h per day on 5 days per week for 3, 6, 12 or 18 months) (Monticello & Morgan, 1994; Monticello *et al.*, 1996). The effects of subchronic exposure to various concentrations of formaldehyde on DNA synthesis in the rat nose are illustrated in Figure 5.

Table 34. Cytotoxicity and cell proliferation induced by subchronic and chronic exposures to formaldehyde

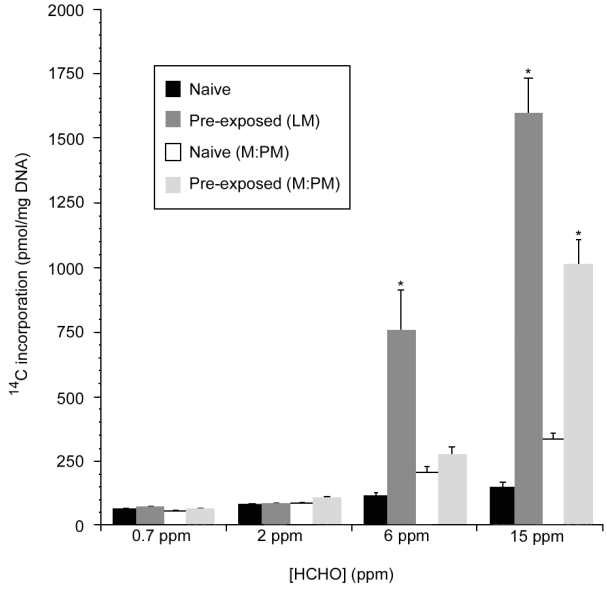
Strain, species, sex	Exposure	Effects	Reference
Fischer 344 rat, Syrian hamster, male and female; cynomolgus monkey, male	0, 0.25, 1.2, 3.7 mg/m^3, 22 h/day, 7 days/week, 26 weeks	Rat: squamous metaplasia in nasal turbinates at 3.7 mg/m^3 only; hamster: no significant toxic response; monkey: squamous metaplasia in nasal turbinates at 3.7 mg/m^3 only	Rusch *et al*. (1983)
B6C3F1 mouse, male	0, 2.5, 4.9, 12.3, 24.6, 49.2 mg/m^3, 6 h/day, 5 days/week, 13 weeks	2.5, 4.9: no lesion induced; 12.3, 24.7, 49.2: squamous metaplasia, inflammation of nasal passages, trachea and larynx; 80% mortality at 49.2 mg/m^3	Maronpot *et al*. (1986)
Wistar rat, male and female	0, 0.37, 1.2, 3.7 mg/m^3, 6 h/day, 5 days/week, 13 weeks	0.37, 1.2: no increase in cell replication; 3.7: increased cell turnover in nasal epithelium but cell proliferation lower than after 3 days of exposure	Zwart *et al*. (1988)
Wistar rat, male and female	0, 1.2, 12.3, 24.7 mg/m^3, 6 h/day, 5 days/week, 13 weeks	1.2: results inconclusive; 12.3, 24.7: squamous metaplasia, epithelial erosion, cell proliferation in nasal passages and larynx; no hepatotoxicity	Woutersen *et al*. (1987)
Wistar rat, male	0, 0.12, 1.2, 12.3 mg/m^3, 6 h/day, 5 days/week, 13 or 52 weeks Nasal mucosa of some rats injured by bilateral intranasal electrocoagulation to induce cell proliferation	0: electrocoagulation induced hyperplasia and squamous metaplasia, still visible after 13 weeks but slight after 52 weeks; 0.12, 1.2: focal squamous metaplasia after 13 or 52 weeks; no adverse effects in animals with undamaged nasal mucosa; 12.3: squamous metaplasia in respiratory epithelium (both intact and damaged nose); degeneration with or without hyperplasia of olfactory epithelium (damaged nose only)	Appelman *et al*. (1988)

Table 34 (contd)

Strain, species, sex	Exposure	Effects	Reference
Wistar rat, male	0, 1.2 mg/m^3 × 8 h/day, 2.4 mg/m^3 × 8 h/day ($C \times t$ = 9.8 or 19.7 mg/m^3–h/day), 5 days/week, 13 weeks; 2.4 mg/m^3 × 8 × 0.5 h/day, 4.9 mg/m^3 × 8 × 0.5 h/day ($C \times t$ = 9.8 or 19.7 mg/m^3–h/day), 5 days/week, 13 weeks	1.2, 2.5: no observed toxic effect; 4.9: epithelial damage, squamous metaplasia, occasional keratinization; concentration, not total dose, determined severity of toxic effect	Wilmer *et al*. (1989)
Fischer 344 rat, male	0, 0.86, 2.5, 7.4, 12.3, 18.5 mg/m^3, 6 h/day, 5 days/week, 6 weeks	0.86, 2.5: no increase in cell replication detected; 7.4: increase in cell proliferation; 12.3, 18.5: sustained cell proliferation	Monticello *et al*. (1991)
Fischer 334 rat, male	0, 0.86, 2.5, 7.4, 18.5 ppm, 6 h/day, 5 days/week, 12 weeks	0.86, 2.5: DNA synthesis rates in nasal mucosa similar in naive (previously unexposed) and subchronically exposed rats; 7.4, 18.5: DNA synthesis rates higher in subchronically exposed than in naive rats, especially at 18.5 mg/m^3	Casanova *et al*. (1994)
Fischer 344 rat, male	0, 0.86, 2.5, 7.4, 12.3, 18.5 mg/m^3, 6 h/day, 5 days/week, 3 months	0.86, 2.5, 7.4: no increase in cell replication detected; 12.3, 18.5: sustained cell proliferation. Site-specific increase in cell replication corresponded to location of squamous-cell carcinomas.	Monticello *et al*. (1996)

C, concentration; *t*, time

Figure 5. Cell turnover in the lateral meatus (LM) and medial and posterior meatuses (M:PM) of pre-exposed and naive (previously unexposed) Fischer 344 rats, as measured by incorporation of ^{14}C derived from inhaled [^{14}C]formaldehyde (HCHO) into nucleic acid bases (deoxyadenosine, deoxyguanosine and thymidine) and thence into DNA, during a single 3-h exposure to 0.7, 2, 6 or 15 ppm [0.86, 2.5, 7.4 or 18.5 mg/m^3] formaldehyde



Reproduced, with permission, from Casanova *et al.* (1994)
Pre-exposed rats were exposed subchronically to the same concentrations of unlabelled formaldehyde (6 h per day on 5 days per week for 11 weeks and 4 days), while naive rats were exposed to room air. The exposure to [^{14}C]formaldehyde occurred on the 5th day of the 12th week. The asterisk denotes a significant difference between pre-exposed and naive rats.

Additional studies have shown the importance of increased cell turnover in the induction of rat nasal tumours (Appelman *et al.*, 1988; Woutersen *et al.*, 1989). The nasal mucosa of Wistar rats was damaged by bilateral intranasal electrocoagulation and the susceptibility of the rats to inhalation of formaldehyde at concentrations of 0.1, 1 or 10 ppm [0.1, 1.2 or 12.2 mg/m^3] (for 6 h per day on 5 days per week for 13 or 52 weeks (Appelman *et al.*, 1988), 28 months or 3 months followed by a 25-month observation period (Woutersen *et al.*, 1989)) was evaluated. In rats with undamaged mucosa, the effects of exposure were seen only at 10 ppm; these effects were limited to degenerative, inflammatory and hyperplastic changes, and were increased by electrocoagulation. In the group exposed to 10 ppm for 28 months, nasal tumours were induced in 17/58 rats. No compound-related tumours were induced at 0.1 or 1 ppm. It was concluded that the damaged mucosa was more susceptible to the cytotoxic effects of formaldehyde and that severe damage contributes to the induction of nasal tumours.

The time-dependent development of formaldehyde-induced lesions was examined in 180 male Fischer 344 rats (six per exposure group), 8–9 weeks of age, that were exposed by inhalation to nominal concentrations of 0, 0.7, 2, 6, 10 or 15 ppm [0, 0.86, 2.46, 7.38, 12.3 or 18.45 mg/m^3, respectively] formaldehyde [thermal depolymerization of paraformaldehyde, purity unspecified] for 6 h per day on 5 days per week for 3, 6, 12, 18 or 24 months. Five days before necropsy, the rats were anaesthetized and received an implant of an osmotic minipump that contained 2mCi [methyl-^{3}H]thymidine for incorporation of the thymidine into DNA during S-phase to allow subsequent quantitation of cell proliferation. A detailed morphological analysis of the nasal cavity of each rat was made in order to collect information on the development and distribution of lesions in seven separate regions of the nose (anterior lateral meatus, posterior lateral meatus, anterior mid-septum, posterior mid-septum, anterior dorsal septum, anterior medial maxilloturbinate and maxillary sinus). The unit length labelling index method was used to establish the proliferation index in each area of the nose. The number of cells in each of the designated areas was also determined through a combination of actual cell counts and estimates of numbers using surface area measurements and a CFD model of the rat nose. A population-weighted unit length labelling index was calculated for direct comparison across time and dose. Survival was not different or greater in controls than in rats treated with 10 ppm formaldehyde or less. Survival was decreased in rats treated with 15 ppm formaldehyde. Formaldehyde-induced lesions were primarily confined to areas lined by respiratory and transitional epithelium and were more prevalent and severe in the anterior portion of the nose. The severity of the lesions was dose-dependent, with only minimal lesions present after exposure to 6 ppm and none after exposure to 2 ppm or 0.7 ppm. The predominant non-neoplastic formaldehyde-induced lesions were epithelial hypertrophy and hyperplasia, squamous metaplasia and inflammatory cell infiltration. The majority of formaldehyde-induced neoplasms were squamous-cell carcinomas with much lower incidences of polypoid adenomas, adenocarcinomas and rhabdomyosarcomas. The 10- and 15-ppm groups had parallel cumulative incidence curves, although the 10-ppm group had a later time to onset of tumours. The squamous-cell carcinomas appeared to arise from regions lined by transitional and respiratory epithelium and were most common in the lateral meatus and mid-septum, the incidence was higher in the more anterior portions (Table 35). Significant increases in the unit length labelling index were only observed in the 10- and 15-ppm groups with the greatest increase in the more anterior portion of the nose where the tumour response was greatest. An elevated unit length labelling index developed in the anterior dorsal septum later in the course of the exposure. This belated elevation in the more posterior nose of animals exposed to the high dose may have been secondary to changes in airflow patterns and resultant local formaldehyde concentations associated with growth of lesions and distortion of the airspace in the nose. The mapping of cell numbers per area showed significant differences in the total populations of nasal epithelium at risk in the different areas counted for this study. An additional method of examining the association between cells at risk and the labelling index used in this study was the population-weighted unit length labelling index which is the product of the total numbers of cells in the specified

Table 35. Incidences of nasal squamous-cell carcinomas in male Fischer 344 rats exposed by inhalation to formaldehyde

Concentration of formaldehyde (ppm)	No. of nasal cavities examined	Nasal location							No. of animals with squamous-cell carcinoma[a]
		Anterior lateral meatus	Posterior lateral meatus	Anterior mid-septum	Posterior mid-septum	Anterior dorsal septum	Anterior medial maxillo-turbinate	Maxillary sinus	
0	90	0	0	0	0	0	0	0	0
0.7	90	0	0	0	0	0	0	0	0
2	96	0	0	0	0	0	0	0	0
6	90	1	0	0	0	0	0	0	1
10	90	12	2	0	0	0	0	0	20
15	147	17	9	8	1	3	4	0	69

From Monticello *et al*. (1996)

[a] Total number of animals with squamous-cell carcinoma, including those too large to allocate and those located in a site not listed in this table

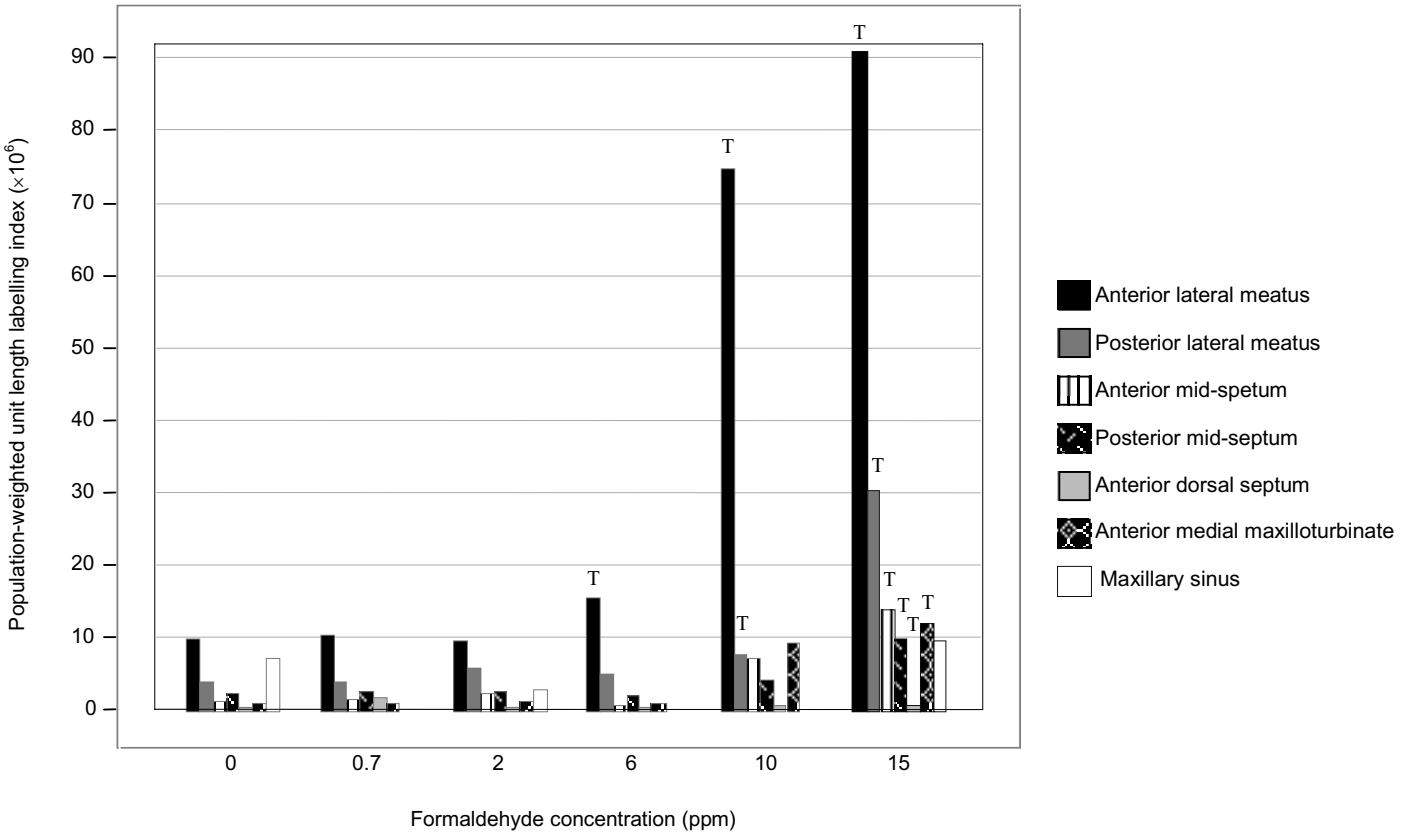
area and the unit length labelling index. This product corresponded very closely with tumour development at all sites in the nose (Figs 6 and 7) (Monticello *et al*., 1996).

A rat CFD model was used to test whether the distribution of formaldehyde-induced squamous metaplasia was related to the location of high-flux regions posterior to the squamous epithelium. Histological sections of nose corresponding to level 6 (Mery *et al*., 1994) from male Fischer 344 rats exposed by whole-body inhalation to nominal concentrations of 0, 0.7, 2, 6, 10 and 15 ppm [0, 0.86, 2.46, 7.38, 12.3 and 18.45 mg/m^3] formaldehyde were examined. Distribution of squamous epithelium within 20 subset areas within the section was mapped. Squamous metaplasia was considered to be present when ≥ 50% of the epithelium within a subsection was of the squamous type. The regions were then ranked by presence of squamous metaplasia by dose group. Inspiratory airflow and formaldehyde uptake were simulated based on a minute volume of 288 mL/min for a 315-g rat. Steady-state simulations were performed using air concentrations of 6, 10 or 15 ppm formaldehyde. Only these three concentrations were used because no squamous metaplasia was present in sections of nose from rats exposed to 2 ppm or less. Squamous metaplasia was present on the lateral and medial walls of the airway after exposure to 10 or 15 ppm; the highest incidence was on wells of the lateral meatus in all three groups (6, 10 and 15 ppm). The distribution of formaldehyde-induced squamous metaplasia was consistent with the location of high formaldehyde flux in rat noses after exposure to 10 or 15 ppm for 6 months. The data were inconclusive for the 6-ppm group, probably due to the insufficient number of rats examined (Kimbell *et al*., 1997).

A larger percentage of the nasal mucosal surface area of rhesus monkeys exposed to 6 ppm [7.3 mg/m^3] formaldehyde (6 h per day on 5 days per week) was affected after 6 weeks of exposure than after 5 days. Cell proliferation was detected in the nasal passages, larynx, trachea and carina, but the effects in the lower airways were minimal in comparison with the effects in the nasal passages (Monticello *et al.*, 1989). Other studies showed that Fischer 344 rats exposed to 1 ppm [1.2 mg/m^3] (22 h per day on 7 days per week for 26 weeks) formaldehyde did not develop detectable nasal lesions (Rusch *et al.*, 1983), but that rats exposed to 2 ppm [2.4 mg/m^3] (6 h per day on 5 days per week for 24 months) developed mild squamous metaplasia in the nasal turbinates (Kerns *et al.*, 1983b). Although the total dose received by the former group was 1.5 times higher than that received by the latter group, the incidence and severity of lesions was smaller, which demonstrates the greater importance of concentration than total dose (Rusch *et al.*, 1983).

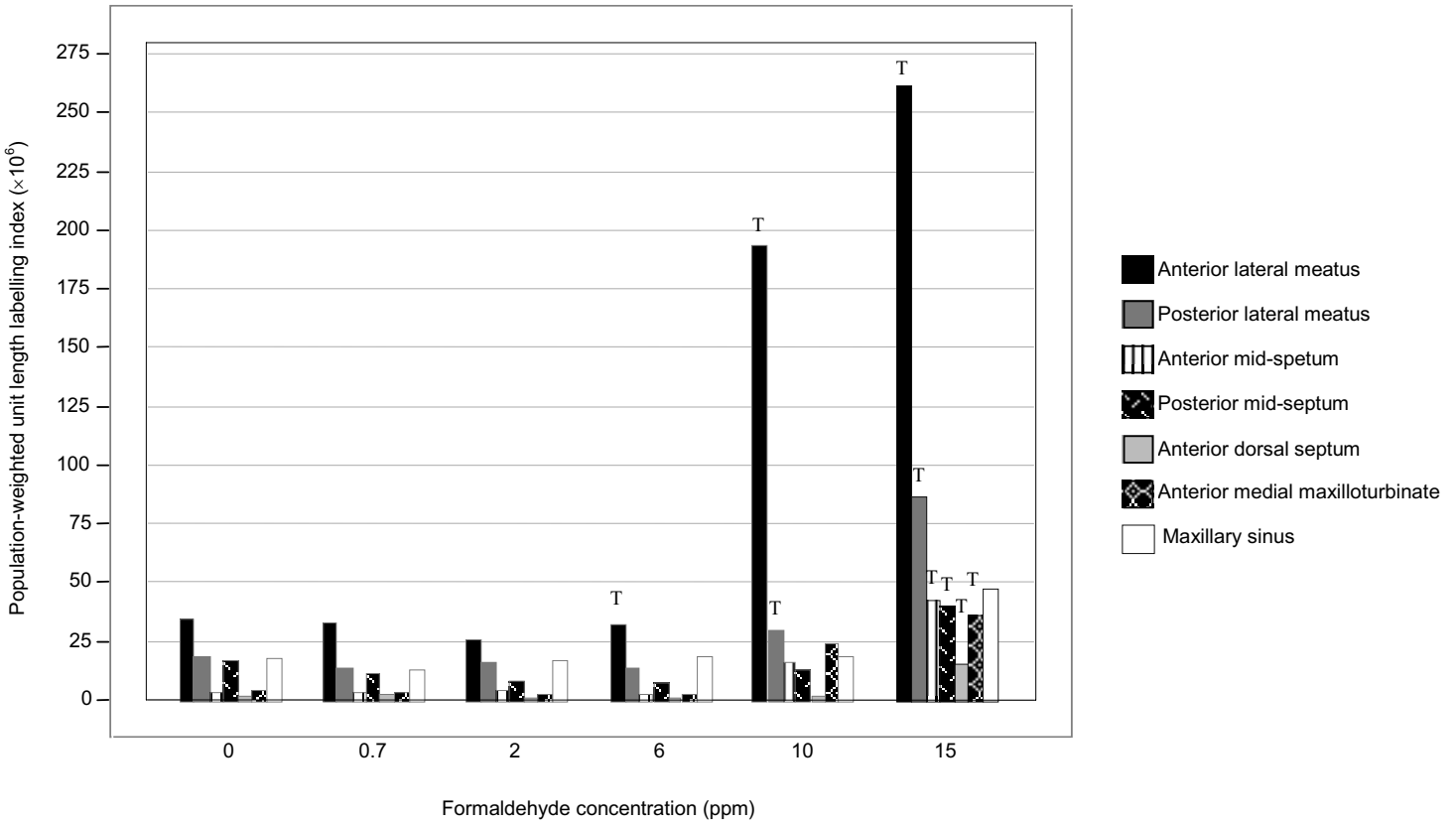
A computer simulation of the relationship between airflow and the development of formaldehyde-induced lesions was developed for rhesus monkeys. A three-dimensional computer model was developed using video image analysis of serial coronal sections from an 11.9-kg, 7-year-old male rhesus monkey. Coordinates were taken for every 0.1 mm over the 83 mm long nasal passage. Eighty cross sections were used for the final model and spanned 75 mm of airway. Values for airflow simulation were estimated by allometric scaling, using body weight and a calculated minute volume of 2.4 L/min and half maximum nasal airflow calculated to be 3.8 L/min. Simulations were performed using airflow parameters of 0.63–3.8 L/min. The nasal cavity of rhesus monkeys has two air-

Figure 6. Calculated total proliferative population in areas of the nose of rats after exposure to formaldehyde for 3 months and T-site where tumours developed



Adapted from Monticello *et al.* (1996)

Figure 7. Sum of calculated total proliferative population in areas of the nose of rats after exposure to formaldehyde over time and T-site where tumours developed



Adapted from Monticello *et al.* (1996)

ways that are separated by a septum that extends approximately 71.5 mm from the anterior tip of the nares to the nasopharynx. The nose has three distinct regions: the nasal vestibule, the central nasal passage and the nasopharynx. The nasal vestibule extends from the tip of the nostrils to the anterior margin of the middle turbinate. Airflow enters the nasal vestibule in an undeviated pattern and flows along the septal wall to the ventral medial, middle medial and dorsal airways in the central nasal passage. The central nasal passage begins at the anterior margin of the middle turbinate, extends to the middle and ventral turbinates and has a streamlined airflow in the middle portion of the nose which is slower than that of the nasal vestibule. The nasopharynx extends from the posterior nasal passage at the point where the middle turbinate attaches to the nasal wall dorsally to the soft palate and has streamlined airflow in the anterior portion which increases in velocity toward the posterior after the end of the dorsal meatus. The model predicted that 90% of gas uptake would be from the nostrils to the end of the septal wall. In general, the regions where mass flux was predicted to be high (nasal vestibule, mid-septum, floor of the anterior meatus, medial inferior turbinate and the middle turbinate) are also areas where formaldehyde-induced lesions occurred (Monticello *et al.*, 1989). Areas with low mass flux (dorsal meatus and the wall of the ventral lateral meatus) did not develop formaldehyde-induced lesions. An exception was the lateral wall where lesions occurred but mass flux was predicted to be low (Kepler *et al*., 1998).

Toxicity in the gastrointestinal tract after oral administration

The toxic effects of formaldehyde administered orally have been reviewed (Restani & Galli, 1991).

Formaldehyde was administered orally to rats and dogs at daily doses of 50, 100 or 150 mg/kg bw (rats) or 50, 75 or 100 mg/kg bw (dogs) for 90 consecutive days. Significant changes in body weight were observed at the higher doses, but clinical and pathological studies revealed no specific treatment-related effects on the kidney, liver or lung, which were considered to be possible target organs, or on the gastrointestinal mucosa (Johannsen *et al.*, 1986).

Formaldehyde was administered in the drinking-water to male and female Wistar rats for up to 2 years. In the chronic portion of the study, the mean daily doses of formaldehyde were 1.2, 15 or 82 mg/kg bw (males) and 1.8, 21 or 109 mg/kg bw (females). Controls received drinking-water either *ad libitum* or in an amount equal to that consumed by the highest-dose group, which had a marked decrease in water consumption. Pathological changes after 2 years were essentially restricted to the highest-dose group and consisted of a thickened and raised limiting ridge of the forestomach and gastritis and hyperplasia of the glandular stomach. The no-adverse-effect level was estimated to be 15 mg/kg bw per day (males) or 21 mg/kg bw per day (females) (Til *et al.*, 1989). In a 4-week study, the effects of formaldehyde that were also observed only in the highest-dose group (125 mg/kg bw) were thickening of the limiting ridge and hyperkeratosis in the forestomach and focal gastritis in the glandular stomach (Til *et al.*, 1988).

In another experiment in which formaldehyde was administered in the drinking-water to male and female Wistar rats, fixed concentrations (0, 0.02, 0.1 and 0.5%) were given for up to 2 years. Estimated from the water intake and body weight, these concentrations corresponded, on average, to 0, 10, 50 and 300 mg/kg bw per day. All rats that received the highest dose died during the study. The lesions induced in the stomach were similar to those reported by Til *et al.* (1988, 1989). No treatment-related tumour was found. The no-effect level was estimated to be 0.02% (10 mg/kg bw per day), as forestomach hyperkeratosis was observed in a small number of rats (2/14) that received 0.1% formaldehyde (50 mg/kg bw per day) (Tobe *et al.*, 1989).

(iii) *Immunotoxicity*

The possibility that formaldehyde may induce changes in the immune response was examined in $B6C3F_1$ mice exposed to 15 ppm [18.3 mg/m^3] formaldehyde (6 h per day on 5 days per week for 3 weeks). A variety of tests of immune function revealed no significant changes, except for an increase in host resistance to challenge with the bacterium, *Listeria monocytogenes*, which implied an increased resistance to infection. Exposure did not alter the number or impair the function of resident peritoneal macrophages, but increased their competence for release of hydrogen peroxide (Dean *et al.*, 1984; Adams *et al.*, 1987). Formaldehyde enhanced the anti-ovalbumin IgE titre after pre-exposure of BALB/c mice to 2 mg/m^3 formaldehyde for 6 h per day for 10 days (Tarkowski & Gorski, 1995) but did not enhance the IgG1 response of ICR mice to a mite allergen in the respiratory tract after exposure to an aerosol of 0.5% formaldehyde saline solution (Sadakane *et al*., 2002).

Sprague-Dawley rats were exposed to 12.6 ppm [15.5 mg/m^3] formaldehyde (6 h per day on 5 days per week for 22 months), then vaccinated with pneumococcal polysaccharide antigens and tetanus toxoid and were tested 3–4 weeks later for the development of antibodies. An IgG response to pneumococcal polysaccharides and to tetanus toxoid and an IgM response to tetanus toxoid were found in both exposed and control groups. No evidence was obtained that long-term exposure to a high concentration of formaldehyde impairs B-cell function, as measured by antibody production (Holmström *et al.*, 1989c).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

A variety of epidemiological studies are available that have evaluated the reproductive effects of occupational exposures to formaldehyde both directly and indirectly. The outcomes examined in these studies include spontaneous abortions, congenital malformations, birth weight and infertility.

The incidence of spontaneous abortion was studied among hospital staff in Finland who used ethylene oxide (see IARC, 1994b), glutaraldehyde and formaldehyde to sterilize instruments. Potentially exposed women were identified in 1980 with the help of supervising nurses at all of the approximately 80 general hospitals of the country. An equal number of control women were selected by the supervising nurse from among nursing

auxiliaries in the same hospitals who had no exposure to sterilizing agents, anaesthetic gases or X-rays. Study subjects were administered a postal questionnaire which requested personal data and information on tobacco smoking habits, intake of alcohol, reproductive history, including the number of pregnancies and their outcome, and occupation at the time of each pregnancy. Information on exposure to chemical sterilizing agents was obtained from the supervising nurses. The crude rates of spontaneous abortion were 16.7% for sterilizing staff who were considered to have been exposed during the first trimester of pregnancy, 6.0% for sterilizing staff who left employment when they learned that they were pregnant (the difference being significant) and 10.6% for controls. When adjusted for age, parity, decade of pregnancy, tobacco smoking habits and alcohol and coffee consumption, the rate associated with exposure to ethylene oxide, with or without other agents, was 12.7%, which was significantly increased ($p < 0.05$), whereas that associated with formaldehyde, with or without other agents, was 8.4%, which was comparable with the reference level of 10.5% and was thus not significantly correlated with spontaneous abortions (Hemminki *et al.*, 1982).

In a nationwide record linkage study in Finland, all nurses who had been pregnant between 1973 and 1979 and who had worked in anaesthesia, surgery, intensive care, operating rooms or internal departments of a general hospital (and in paediatric, gynaecological, cancer and lung departments for the part of the study that was concerned with malformations) were identified. Each of the 217 women treated for spontaneous abortion according to the files of the Finnish hospital discharge register and the 46 women notified to the Register of Congenital Malformations was individually matched on age and hospital with three control women, who were selected at random from the same population of nurses and matched for age and hospital where they were employed. Information was obtained from supervising nurses by postal questionnaires on the exposure of cases and controls to sterilizing agents (ethylene oxide, glutaraldehyde and formaldehyde), anaesthetic gases, disinfectant soaps, cytostatic drugs and X-radiation. Exposure to formaldehyde during pregnancy was reported for 3.7% of the nurses who were later treated for spontaneous abortion and for 5.2% of their controls, yielding a crude odds ratio of 0.7 [95% CI, 0.28–1.7]. Exposure to formaldehyde was also reported for 8.8% of nurses who gave birth to a malformed child and 5.3% of matched controls, to give an odds ratio of 1.74 [95% CI, 0.39–7.7]; the latter analysis was based on eight exposed subjects (Hemminki *et al.*, 1985). [The Working Group noted that these numbers appear to be recalculated from published reports.]

The occurrence of spontaneous abortions among women who worked in laboratories in Finland, and congenital malformations and birth weights of the children were investigated in a matched retrospective case–control study using a case–referent design. The final population in the study of spontaneous abortion was 206 cases and 329 controls; that in the study of congenital malformations was 36 cases and 105 controls. Information on occupational exposure, health status, medication, contraception, tobacco smoking and alcohol consumption during the first trimester of the pregnancy was collected by postal questionnaire. The exposure to individual chemicals was estimated on the basis of a

reported frequency of chemical use. An occupational hygienic assessment was conducted and an exposure index was calculated. The odds ratio for spontaneous abortion was increased among women who had been exposed to formalin for at least 3 days per week (odds ratio, 3.5; 95% CI, 1.1–11.2). A greater proportion of the cases (8/10) than the controls (4/7) who had been exposed to formalin had been employed in pathology and histology laboratories. Most of the cases (8/10) and controls (5/7) who were exposed to formalin were also exposed to xylene (see IARC, 1989). The authors stated that the results for individual chemicals should be interpreted with caution because laboratory personnel are often exposed to several solvents and other chemicals simultaneously. No association was observed between exposure to formalin and congenital malformations [data not shown] (Taskinen *et al.*, 1994).

Reduced fertility was investigated in a retrospective study of time to pregnancy that was conducted among female wood workers who were exposed to formaldehyde and who had given birth between 1985 and 1995 (Taskinen *et al*., 1999). Time to pregnancy was analysed using a discrete proportional hazards regression approach. Study criteria included women who had worked in the wood-processing industry for at least 1 month and for whom employment in wood-related work had started at least 6 months before pregnancy. Exposure assessment was based on responses from a detailed questionnaire that asked women to describe their occupational title and their work tasks. Women estimated the number of hours they spent in various types of factories/enterprises in the industry and the number of hours they were exposed to various chemicals including formaldehyde, organic solvents, wood preservatives, glues or wood-protecting chemicals. The questionnaire also collected information on the use of personal protective equipment, and exposure to welding fumes, exhaust gases, pesticides and tobacco smoke. An occupational hygienist assessed the exposures and calculated a daily exposure index. The authors used workplace exposure measurements to support these exposure estimates. Among the 699 female wood workers, exposure to formaldehyde was significantly associated with delayed conception density, as assessed by an adjusted fecundability density ratio, which was 0.64 (95% CI, 0.43–0.92). When no gloves were used during high levels of exposure, the fecundability density ratio was 0.51 (95% CI, 0.28–0.92). Exposure to phenols, dusts, wood dusts or organic solvents was not related to the time to pregnancy. All women exposed to phenols were also exposed to formaldehyde but the opposite was not true.

Although the study focus was time to pregnancy, other analyses of these workers showed an increased odds ratio for spontaneous abortion (52 pregnancies) of 3.2 (95% CI, 1.2–8.3) in the high-exposure and 2.4 (95% CI, 1.2–4.8) in the low-exposure categories. Exposure to formaldehyde at high levels was also associated with an increased risk (odds ratio, 4.5; 95% CI, 1.0–20.0) for endometriosis (Taskinen *et al.*, 1999).

A meta-risk analysis conducted by Collins *et al.* (2001b) noted that, of the 11 epidemiological studies that they reviewed for their evaluation of reproductive effects among workers exposed to formaldehyde, nine evaluated spontaneous abortions. Four of these studies reported significantly higher rates of spontaneous abortion among women who were occupationally exposed to formaldehyde (Axelsson *et al*., 1984; John *et al*., 1994; Taskinen

et al., 1994, 1999). Four other studies did not find an increased association (Hemminki *et al*., 1982, 1985; Stücker *et al*., 1990; Lindbohm *et al*., 1991). One study did not report relative risks but showed no increased risk for spontaneous abortion (Shumilina, 1975). Collins *et al*. (2001b) suggested that there is a reporting bias against negative studies and described some of the difficulties in conducting studies of spontaneous abortions.

Collins *et al.* (2001b) discussed the four epidemiological studies that used a case–control design to evaluate congenital defects among children of women exposed to formaldehyde (Axelsson *et al*., 1984; Ericson *et al*., 1984; Hemminki *et al*., 1985; Taskinen *et al*., 1994).

Three epidemiological studies evaluated birth weights. Neither Taskinen *et al*. (1994) nor Axelsson *et al*. (1984) reported associations between exposure to formaldehyde and decreased birth weight among the 500 and 968 births examined, respectively. A study in Russia by Shumilina (1975) reported an elevated number of births of babies that weighed less than 3000 g among 81 newborns of women who were potentially exposed to formaldehyde; however, if the cut-point of below 2500 g is used (more traditional definition of low birth weight), then these increases disappear.

It is important to note that most of the epidemiological studies reported in this section were not designed to evaluate exposures to formaldehyde specifically. For example, the studies by Taskinen *et al.* (1994), Hemminki *et al.* (1982, 1985), Ericson *et al.* (1984) and Axelsson *et al.* (1984) were designed to investigate pregnancy outcomes in laboratory workers and that of John *et al.* (1994) to investigate pregnancy outcomes in cosmetologists. All of these studies are confounded by significant co-exposures and, in general, have directed research to examine specific exposures in follow-up studies.

Other studies of reproductive effects in humans have investigated sperm abnormality. Eleven hospital autopsy service workers and 11 matched controls were evaluated for sperm count, abnormal sperm morphology and the frequency of one or two fluorescent F-bodies. Subjects were matched for age and use of alcohol, tobacco and marijuana; additional information was collected on health, medications and other exposure to toxins. Exposed and control subjects were sampled three times at 2–3-month intervals. Ten exposed subjects had been employed for 4.3 months (range, 1–11 months) before the first sample was taken, and one had been employed for several years. Exposure to formaldehyde was intermittent, with a time-weighted average of 0.61–1.32 ppm [0.75–1.6 mg/m^3] (weekly exposure, 3–40 ppm·h [3.7–48.8 mg/m^3·h]). No significant difference was observed between the exposed and control groups with regard to sperm parameters (Ward *et al.*, 1984).

4.3.2 *Experimental systems*

The reproductive and developmental toxicity of formaldehyde has been reviewed (Feinman, 1988; WHO, 1989; Collins *et al*., 2001b).

Numerous studies have been performed to examine the potential effects of formaldehyde on pregnancy and fetal development in rats, mice, hamsters, rabbits and dogs.

Routes of exposure have included inhalation, oral gavage, administration in the drinking-water and dermal application. The inhalation studies (Gofmekler, 1968; Gofmekler *et al.*, 1968; Pushkina *et al.*, 1968; Gofmekler & Bonashevskaya, 1969; Shevelera, 1971; Kilburn & Moro, 1985; Saillenfait *et al.*, 1989; Martin, 1990) and studies of dermal exposure (Overman, 1985) use relevant routes of exposure for evaluation. Studies conducted before 1970 (Gofmekler, 1968; Gofmekler *et al.*, 1968; Pushkina *et al.*, 1968; Gofmekler & Bonashevskaya, 1969) reported a prolongation of pregnancy, changes in fetal organ weight and a variety of clinical and biochemical changes in the spleen, liver, kidney, thymus and lymphocytes in rats (Thrasher & Kilburn, 2001; Collins *et al.*, 2001b). Thrasher and Kilburn (2001) reviewed studies of embryotoxicity and teratogenicity (Katakura *et al.*, 1990, 1991, 1993) and reported that [^{14}C]-labelled formaldehyde crossed the placenta and entered fetal tissues at levels greater than those in the dam. Embryotoxic and teratogenic outcomes were a function of the exposure regimen. Rats exposed before mating had increased embryo mortality and those exposed during mating had increased fetal anomalies. [The Working Group agreed with the authors' suggestion that this [C^{14}]-labelling would be consistent with the entry of the [^{14}C]-label from formaldehyde into the one-carbon pool.]

Groups of 25 pregnant Sprague–Dawley rats were exposed by inhalation to formaldehyde (0, 5, 10, 20 or 40 ppm [0, 6.2, 12.3, 24.6 or 49.2 mg/m^3]) for 6 h per day on days 6–20 of gestation. On day 21, the rats were killed and maternal and fetal parameters were evaluated. The authors concluded that formaldehyde was neither embryolethal nor teratogenic when administered under these conditions. The mean fetal body weight at 20 ppm was 5% less than that of controls ($p < 0.05$) in males but was not reduced in females; at 40 ppm, mean fetal body weight was about 20% less than that in controls ($p < 0.01$) in both males and females. The decrease in fetal weight in the group given the high dose was attributed to maternal toxicity. However, the authors stated that the significant reductions in fetal body weight observed at 20 ppm did not cause overt signs of maternal toxicity (Saillenfait *et al.*, 1989). [The Working Group noted that 20-ppm exposures in other studies would be considered to be 'toxic' doses.]

Groups of 25 mated female Sprague–Dawley rats were exposed by inhalation to formaldehyde (0, 2, 5 or 10 ppm [2.5, 6.2 or 12.3 mg/m^3]) for 6 h per day on days 6–15 of gestation. At 10 ppm, there was a significant decrease in maternal food consumption and weight gain. None of the parameters of pregnancy, including numbers of corpora lutea, implantation sites, live fetuses, dead fetuses and resorptions or fetal weights, were affected by treatment. An increased incidence of reduced ossification was observed at 5 and 10 ppm in the absence of maternal toxicity (10 ppm) (Martin, 1990). The author of this study noted that the effects at both 5 and 10 ppm were attributed to larger litter sizes which could have reduced fetal body weights (Martin, 1990).

Formaldehyde was applied topically to pregnant Syrian hamsters on day 8, 9, 10 or 11 of gestation by clipping the hair on the dorsal body and applying 0.5 mL formalin (37% formaldehyde) with a syringe directly onto the skin. In order to prevent grooming, the animals were anaesthetized with nembutal (13 mg intraperitoneally) during the 2-h treat-

ment. On day 15, fetuses were removed from four to six hamsters per group and examined. The number of resorptions was increased, but no teratogenic effects or effects on fetal weight or length were detected. The authors suggested that the increase in resorptions may have been caused by stress, as females were anaesthetized during formaldehyde exposures. No effect on maternal weight gain was observed (Overman, 1985).

In a study of post-implantation effects, 27 mature female Wistar rats were exposed by inhalation to 0.5 mg/m^3 or 1.5 mg/m^3 formaldehyde every day for 4 h for up to 4 months. On day 120, treated females were mated with untreated males. The effect of formaldehyde was evaluated in developing embryos at the 2nd and 3rd day after mating. The authors reported a significant increase in the number of degenerating embryos only from pregnant females who had been exposed to 1.5 mg/m^3. The impaired embryonic morphology was reported as structural impairment in blastomers. In a cytogenetic analysis, no increase was found in the number of embryos with chromosomal aberrations in comparison with the controls (Kitaeva *et al.*, 1990).

Reproductive effects were observed in a study of sperm head abnormalities and dominant lethal mutations. Male albino rats (six per group) received five daily intraperitoneal injections of 37% formaldehyde solution to provide 0.125, 0.25 or 0.5 mg/kg bw based on the LD_{50} and a lethal dose of 2 mg/kg bw to examine sperm head abnormalities 3 weeks after the last injection. A separate group of 12 male albino rats was injected intraperitoneally with the same doses, then housed with two untreated virgin female rats that were replaced weekly for 3 weeks to provide 24 females for each treatment group. Mating was detected by the presence of vaginal plugs. All females were killed and necropsied 13 days after the midweek of housing with the males. Total implant scores per female were collected and a dominant lethal mutation index was calculated based on the formula [1–(live implants treated/live implants control)] × 100. Formaldehyde induced sperm head abnormalities at all doses tested, which included pinhead, short hook, long hook, hook at wrong angle, unusual head and wide acrosome; short and long hook were the most common. Only total numbers of abnormalities were compared statistically, and only the incidence of wide acrosome was much greater than that in controls at the lowest dose tested (46 versus 0). In general, there was a decrease in sperm count with increasing dose of formaldehyde. A lower frequency of fertile matings was observed in females within the first 2 weeks after treatment, and the severity of effect was greater when mating took place earlier after treatment. The highest dose with the shortest time between final treatment and mating had the most severe effect and showed a dose- and time-dependent response. By 3 weeks after the last treatment, there was no longer a difference from controls (Odeigah, 1997). Morphological changes in sperm from mice were also identified after five daily intraperitoneal injections of 4, 10 or 30 mg/kg bw formaldehyde. Sperm counts were decreased after 10 and 30 mg/kg bw and deformed sperm were present after all doses (Yi *et al.*, 2000). [The Working Group noted that, because of the reactivity of formaldehyde, the positive results seen after intraperitoneal injection are of questionable biological significance.]

4.4 Genetic and related effects

The genotoxicity of formaldehyde has been reviewed extensively (IARC, 1982, 1987a; Ma & Haris, 1988; WHO, 1989; Feron *et al*., 1991; Monticello & Morgan, 1994; IARC, 1995; Conaway *et al*., 1996; Mathison *et al*., 1997; Monticello & Morgan, 1997; Bolt, 2003; Liteplo & Meek, 2003).

4.4.1 *Humans*

(*a*) *DNA–protein cross-links*

The induction of DNA–protein cross-links due to exposure to formaldehyde was studied in humans (Shaham *et al*., 1996a,b, 2003) (see Table 36). The number of DNA–protein cross-links and the amount of p53 protein, both pantropic (wild-type + mutant) and mutant, were measured in peripheral blood lymphocytes and serum, respectively, of 399 workers from 14 hospital pathology departments, 186 of whom were exposed to formaldehyde (59 men and 127 women), and 213 control workers (127 men and 86 women) from the administrative section of the same hospitals. The mean period of exposure to formaldehyde was 15.9 years (range, 1–51 years). The exposed group was divided into two subgroups: (1) low-level exposure (mean, 0.4 ppm [0.5 mg/m^3]; range, 0.04–0.7 ppm [0.5–0.62 mg/m^3]); and (2) high-level exposure (mean, 2.24 ppm [2.75 mg/m^3]; range, 0.72–5.6 ppm [0.88–6.9 mg/m^3). Before comparing the results obtained in the exposed and the unexposed group, adjustment was made for age, sex, origin and education. The amount of DNA–protein cross-links was expressed as a ratio to total DNA. The adjusted mean number of DNA–protein cross-links was significantly higher ($p < 0.01$) in all exposed subjects (adjusted mean, 0.21; SE, 0.006) compared with that in all unexposed subjects (adjusted mean, 0.14; SE, 0.006). It was also significantly higher ($p < 0.01$) in the subgroups of exposed men (adjusted mean, 0.21; SE, 0.011) and women (adjusted mean, 0.20; SE, 0.008) compared with that of unexposed men and women (adjusted mean, 0.15 and 0.12; SE, 0.008 and 0.008, respectively). Age, tobacco smoking habits, years of education and origin were not significant confounders. The study population was divided into those who had levels of pantropic p53 protein above or below 150 pg/mL. High levels of p53 (> 150 pg/mL) were more prevalent in the exposed group than in the unexposed (44.1% and 36.3%, respectively). The difference between high and low p53 was significant among exposed men, and exposure to formaldehyde was associated with a higher level of pantropic p53 (> 150 pg/mL). In the exposed group, a significantly ($p < 0.05$) higher proportion of p53 > 150 pg/mL was found among workers with DNA–protein cross-links above the median (0.19). Studies have shown elevated serum levels of p53 protein years before the diagnosis of malignant tumours such as lung cancer (Luo *et al.*, 1994; Hemminki *et al*., 1996). [The Working Group noted that the reported increases in p53 occurred in the serum and its relationship to the toxicity of formaldehyde is not known.]

Table 36. Genetic effects of formaldehyde in humans

Target tissue	End-point	Response	Comments	Reference
Peripheral blood lymphocytes	Chromosomal aberrations Micronuclei Sister chromatid exchange	+ ($p < 0.01$) + ($p < 0.01$) + ($p < 0.05$)	Exposed, 13; unexposed, 10; chromosomal aberrations included breaks and gaps, which renders interpretation difficult.	He *et al*. (1988)
Nasal mucosa	Micronuclei	+ ($p < 0.01$)	Exposed, 15; unexposed (control), 15; concurrent exposure to wood dust; no dose–response	Ballarin *et al*. (1992)
Peripheral blood lymphocytes	Chromosomal aberrations	–	Exposed, 20; unexposed (control), 19; high frequency in controls	Vargova *et al*. (1992)
Peripheral blood lymphocytes	DNA–protein cross-links	+ ($p = 0.03$)	Exposed, 12; unexposed, 8; pilot study	Shaham *et al*. (1996a)
Oral mucosa Nasal mucosa	Micronuclei	+ ($p = 0.007$) + (NS)	Exposed, 28; pre- versus post-exposure; no details on tobacco smoking habits	Titenko-Holland *et al*. (1996)
Peripheral blood lymphocytes	Sister chromatid exchange	+ ($p = 0.05$)	Exposed, 13; unexposed, 20; linear relationship between years of exposure and mean number of sister chromatid exchanges	Shaham *et al*. (1997)
Nasal mucosa Oral mucosa Peripheral blood lymphocytes	Micronuclei	+ ($p < 0.001$) + ($p < 0.01$) + (NS)	Exposed, 25; pre- versus post-exposure; control for age, sex and tobacco smoking habits questionable	Ying *et al*. (1997)
Peripheral blood lymphocytes	Sister chromatid exchange	–	Exposed, 23; pre- versus post-exposure	Ying *et al*. (1999)
Nasal mucosa	Micronuclei	+ ($p < 0.01$)	Exposed, 23; unexposed, 25; no dose–response	Burgaz *et al*. (2001)

Table 36 (contd)

Target tissue	End-point	Response	Comments	Reference
Oral mucosa	Micronuclei	+ ($p < 0.05$)	Exposed: 22 variable exposures, 28 exposed to formaldehyde; unexposed, 28; unexposed (control), 18; correlation with duration of exposure only in group with variable exposures (not exposed to formaldehyde)	Burgaz *et al*. (2002)
Peripheral blood lymphocytes	Sister chromatid exchange	+ ($p < 0.01$)	Exposed, 90; unexposed, 52; no dose–response relationship	Shaham *et al.* (2002)
Peripheral blood lymphocytes Serum	DNA–protein cross-links p53 protein	+ ($p < 0.01$) + ($p < 0.01$)	Exposed, 186; unexposed, 213; high levels of p53 protein	Shaham *et al*. (2003)

NS, not significant

(*b*) *Chromosomal effects*

The effects of formaldehyde on the frequencies of chromosomal aberrations and sister chromatid exchange in peripheral blood lymphocytes and micronuclei in nasal mucosa cells from workers exposed to formaldehyde have been reviewed previously (IARC, 1987a, 1995). Since that time, several further studies have assessed the induction of micronuclei, chromosomal aberrations and sister chromatid exchange in workers exposed to formaldehyde (see Table 36).

In a study of exposure to formaldehyde in a factory that manufactured wood-splinter materials, short-term cultures of peripheral lymphocytes were examined from a group of 20 workers (10 men and 10 women) aged 27–57 years (mean, 42.3 years) who were exposed to 8-h TWA concentrations of 0.55–10.36 mg/m^3 formaldehyde for periods of 5–≥ 16 years. The unexposed group consisted of 19 people [sex and age unspecified] who were employed in the same plant and whose habits and social status were similar to those of the exposed group. No significant difference was observed between control and exposed groups with respect to any chromosomal anomaly (including chromatid and chromosome gaps, breaks, exchanges, breaks per cell, percentage of cells with aberrations) scored in the study (controls: 3.6% aberrant cells, 0.03 breaks per cell; exposed: 3.08% aberrant cells, 0.045 breaks per cell). The authors noted that the frequency of aberrations in the control group was higher than that seen in the general population (1.2–2% aberrant cells) (Vargová *et al*., 1992). [The Working Group noted that, although the text states that there were 20 people in the exposed group, Table II of the paper gives a figure of 25. The Group also noted the lack of detail on tobacco smoking habits of the subjects.]

In the study of Ballarin *et al*. (1992), the frequency of micronuclei in respiratory nasal mucosa cells was investigated in 15 nonsmokers who were exposed to formaldehyde in a plywood factory. Mean exposure levels were 0.1–0.39 mg/m^3, with simultaneous exposure to wood dust at a very low level (about one tenth below the threshold limit value). At least 6000 cells from the nasal turbinate area from each individual were scored for micronuclei. A significant increase in the incidence of micronucleated cells was seen in the exposed group (mean percentage of micronucleated cells in the exposed group, 0.90 ± 0.47; range, 0.17–1.83; in controls, 0.25 ± 0.22; range, 0.0–0.66; Mann–Whitney U test, $p < 0.01$). No dose–response relationship between exposure to formaldehyde and the frequency of micronuclei was found. Concurrent exposure to wood dust could have contributed to the increased incidence of micronucleated cells seen in the exposed group.

Burgaz *et al*. (2001) studied the frequency of micronuclei in cells of the nasal mucosa of 23 individuals (11 women and 12 men) who were exposed to formaldehyde in pathology and anatomy laboratories and 25 healthy men who were not exposed to formaldehyde. The mean age of the exposed group was lower than that of the controls (mean ± SD, 30.56 ± 5.52 and 35.42 ± 9.63 years, respectively). More smokers were included in the control group ($n = 19$) than in the exposed group ($n = 9$). Mean duration of exposure to formaldehyde was 5.06 years (range, 1–13 years). From each individual, 3000 cells were scored for micronuclei. The mean frequency of micronuclei was significantly higher

($p < 0.01$) in the exposed group than in the control group (mean ± SD, 1.01 ± 0.62‰ and 0.61 ± 0.27‰, respectively). No significant difference in the mean frequency of micronuclei was observed between smokers and nonsmokers in the controls or in the exposed group ($p > 0.05$), but significantly higher ($p < 0.01$) mean frequencies of micronuclei were found in unexposed than in exposed smokers (1.18 ± 0.47‰ and 0.63 ± 0.29‰, respectively). No significant difference in the mean frequency of micronuclei was observed between men and women in the exposed group. The air concentration of formaldehyde was 2 ppm [2.4 mg/m^3] in the breathing zone of the pathology laboratory workers and 4 ppm [4.9 mg/m^3] in that of the anatomy laboratory workers. No dose–response was found between years of exposure and the frequency of micronuclei.

In another study, Burgaz *et al*. (2002) compared the frequency of micronuclei in buccal cells in three groups: group I, 22 workers (all men) from a shoe factory who were exposed to *n*-hexane, toluene and methyl ethyl ketone; group II, 28 workers (15 men and 13 women) who were pathologists or staff in pathology or anatomy laboratories and were exposed to formaldehyde; and group III, 18 unexposed workers (controls), none of whom had been occupationally exposed to potential genotoxic chemicals. The mean duration of exposure to formaldehyde was 4.7 ± 3.33 years (range, 1–13 years). Both exposed and control groups included smokers, most of whom were moderate smokers. There were no significant differences in mean age and smoking habits between the controls and the exposed groups. From each individual, 3000 cells were scored for micronuclei. The concentration of formaldehyde in the breathing zone of the laboratory workers was 2–4 ppm [2.4–4.9 mg/m^3]. A significant increase in the frequency of micronucleated cells ($p < 0.05$) was seen in the exposed groups (mean ± SD for workers in group I, group II and controls, 0.62 ± 0.45%, 0.71 ± 0.56% and 0.33 ± 0.30%, respectively). Analysis of variance indicated that only occupational exposure, but not smoking habits or sex, was associated with an increased frequency of micronuclei in groups I and II ($p < 0.05$). Duration of exposure was significantly associated with the frequency of micronuclei only in group I who were not exposed to formaldehyde ($p < 0.05$).

Titenko-Holland *et al*. (1996) assessed the induction of micronuclei in exfoliated buccal and nasal cells from 28 mortality science students who were exposed to embalming fluid that contained formaldehyde. The original study population included 35 students (seven women and 24 men [specifications of the additional four subjects not given]). Seven were excluded. The students were mainly nonsmokers. Previously unstained and unanalysed slides from the participants in a study by Suruda *et al*. (1993) were used. Each student was sampled before and after the 90-day embalming class. Exposure to formaldehyde was estimated for the 7–10 days before the post-exposure sample in order to correct for the possibility of exposure misclassification. The mean exposure to formaldehyde for the 19 subjects who had data on buccal cell micronuclei was 14.8 ± 7.2 ppm–h [18.2 ± 8.8 mg/m^3–h] for the entire 90-day period and 1.2 ± 2.1 ppm–h [1.5 ± 2.6 mg/m^3–h] for the 7–10 days before the post-exposure sample. For the 13 subjects who had data on nasal cell micronuclei, the mean exposure to formaldehyde was 16.5 ± 5.8 ppm–h [20.3 ± 7.1 mg/m^3–h] for the entire 90-day period and 1.9 ± 2.5 ppm–h [2.3 ± 3 mg/m^3–h] during the 7–10 days before the post-exposure sampling. Air samples of glutaraldehyde, phenol, methanol and isopropanol

revealed undetectable or very low exposure below the Occupational Safety and Health Administration permissible exposure limits. Quantification of micronuclei was performed by fluorescent in-situ hybridization with a centromeric probe. The mean total frequency of micronuclei was significantly increased in the buccal mucosa (mean ± SD per 1000 epithelial cells, 0.6 ± 0.5 pre-exposure, 2.0 ± 2.0 post-exposure; Wilcoxon rank sum test two-tailed $p = 0.007$), whereas in nasal cells, it was almost the same (2.0 ± 1.3 and 2.5 ± 1.3, respectively; Wilcoxon rank sum test two-tailed $p = 0.2$). Cells with multiple micronuclei were present only in buccal cell samples after exposure to embalming fluid, while in the nasal cell samples, nearly all cells with micronuclei had only one micronucleus per cell in both pre- and post-exposure samples. A weak statistical association (assessed by a Spearman rank order correlation) between cumulative exposure to embalming fluid (90 days) and the change in total micronucleus frequency was observed only in buccal cells ($r = 0.44$; $p = 0.06$). The authors suggested that the main mechanism of micronucleus induction by formaldehyde is due to chromosome breaks.

The frequency of micronuclei in cells of the nasal mucosa, oral mucosa and lymphocytes was evaluated for 25 students (13 men and 12 women; average age, 18.8 ± 1.0 years; all nonsmokers) from anatomy classes. The duration of the anatomy classes was 3 h, three times a week for a period of 8 weeks. The TWA concentrations (mean ± SD) of exposure during the anatomy classes and in the dormitories were 0.508 ± 0.299 mg/m^3 (range, 0.071–1.284 mg/m^3) and 0.012 ± 0.0025 mg/m^3 (range, 0.011–0.016 mg/m^3), respectively. Samples of nasal and oral mucosa cells and venous blood were taken before the beginning of the first class and after the end of the last class, so that every student served as his/her own control. A significant difference (paired t-test $p < 0.001$) was found in the mean frequency of micronuclei in the nasal and oral mucosa (mean ± SD per 1000 cells): 1.2 ± 0.67 versus 3.84 ± 1.48 (paired t-test, $p < 0.001$) and 0.568 ± 0.317 versus 0.857 ± 0.558 (paired t-test $p < 0.01$), respectively. The mean frequency of micronuclei in lymphocytes was higher after exposure (1.11 ± 0.543) than before exposure (0.913 ± 0.389), but this difference was not significant (Ying *et al*., 1997). [The Working Group noted that there were no data related to the possible influence of factors such as age, sex and smoking on the results.]

He *et al*. (1998) studied the frequency of sister chromatid exchange, chromosomal aberrations and micronuclei in peripheral blood lymphocytes of 13 students from an anatomy class. The duration of the anatomy classes was 10 h per week for 12 weeks. Average exposure to formaldehyde (from breathing-zone air samples) during dissection procedures was 2.37 ppm [3.17 mg/m^3]. The unexposed group included 10 students. The sex and age of the two groups were similar and all were nonsmokers. The mean frequency of micronuclei and chromosomal aberrations in the exposed group was significantly higher ($p < 0.01$) than that in the control group (6.38 ± 2.50‰ versus 3.15 ± 1.46‰ and 5.92 ± 2.40% versus 3.40 ± 1.57%, respectively). The main types of chromosomal aberration in the exposed group were chromatid breakages and gaps. A significantly higher ($p < 0.05$) frequency of sister chromatid exchange was observed in the exposed students (5.91 ± 0.71/cell) than in the controls (5.26 ± 0.51/cell). A correlation between micronuclei and chromosomal aberrations was observed. [The Working Group noted that the

evaluation of chromosomal aberrations included chromosomal breaks and gaps together, which makes the results difficult to interpret. In addition, the baseline frequencies of sister chromatid exchange and micronuclei in the controls were unusually low.]

Ying *et al*. (1999) studied the frequency of sister chromatid exchange in peripheral blood lymphocytes of 23 students from an anatomy class (11 men and 12 women), all of whom were nonsmokers. The duration of the anatomy classes was 3 h, three times a week for 8 weeks. Peak exposure was during cadaver dissection. TWA (mean ± SD) concentrations of formaldehyde were 0.508 ± 0.299 mg/m^3 (range, 0.071–1.284 mg/m^3) in the laboratory rooms compared with 0.012 ± 0.0025 mg/m^3 (range, 0.011–0.016 mg/m^3) in the dormitories. Blood samples were taken at the beginning of the anatomy classes and again after 8 weeks. No significant difference was found in the mean frequency of sister chromatid exchange before and after exposure, either in the total population (6.383 ± 0.405 versus 6.613 ± 0.786, respectively) or in the subgroups of men and women.

In a pilot study, Shaham *et al*. (1997) studied the frequency of sister chromatid exchange in the peripheral blood lymphocytes of 33 workers, including 13 from a pathology institute who were exposed to formaldehyde and 20 unexposed controls. The mean age of the exposed workers was 42 ± 10 years and that of the control group was 39 ± 14 years. The range of concentrations of formaldehyde was 1.38–1.6 ppm [1.7–2 mg/m^3] in the rooms and 6.9 ppm [8.5 mg/m^3] in the laminar flow. Personal samples showed a range of 2.8–3.1 ppm [3.5–3.7 mg/m^3] formaldehyde at the period when most of the work was in progress and 1.46 ppm [1.8 mg/m^3] at midday, when most of the work had already been carried out. In order to score sister chromatid exchange, cells that had 44–48 clearly visible chromosomes were examined and the sister chromatid exchange count was normalized to the frequency expected for 46 chromosomes. The mean numbers of cells scored per individual were 28 for the exposed group (range, 25–32; SD, 2.36) and 32 for the controls (range, 25–34; SD, 2.0). A significant difference ($p = 0.05$) was found between the mean number of sister chromatid exchanges per chromosome in the exposed workers (mean ± SD, 0.212 ± 0.039) and the controls (mean ± SD, 0.186 ± 0.035). A significant difference ($p < 0.05$) was found between the mean number of sister chromatid exchanges per chromosome of nine exposed and six unexposed nonsmokers and those of three exposed and two unexposed smokers. The group of smokers who were exposed to formaldehyde had the highest mean number of sister chromatid exchanges per chromosome, and a linear relationship was reported between years of exposure and the mean number of sister chromatid exchanges per chromosome.

In a second study, Shaham *et al*. (2002) evaluated the frequency of sister chromatid exchange in peripheral blood lymphocytes of pathology staff exposed to formaldehyde compared with that in unexposed workers. The study population included 90 workers (25 men and 65 women) from 14 hospital pathology departments who were exposed to formaldehyde (mean age, 44.2 ± 8.5 years) and 52 unexposed workers (44 men and eight women) from the administrative section of the same hospitals (mean age, 41.7 ± 11.4 years). Tobacco smoking habits did not differ significantly between the study groups. The exposed group was divided into two subgroups according to levels of exposure to

formaldehyde (low-level exposure: mean, 0.4 ppm [0.5 mg/m^3]; range, 0.04–0.7 ppm [0.05–0.86 mg/m^3]; high-level exposure: mean, 2.24 ppm [2.8 mg/m^3]; range, 0.72–5.6 ppm [0.9–6.9 mg/m^3]). The mean duration of exposure to formaldehyde was 15.4 years (range, 1–39 years). The results on the frequency of sister chromatid exchange were expressed in two variables: (*a*) mean number of sister chromatid exchanges per chromosome; and (*b*) proportion of high-frequency cells (namely, the proportion of cells with more than eight sister chromatid exchanges). A high correlation between these two variables ($rs = 0.94$; $p < 0.01$) was found in the study population and in each of the two subgroups (exposed $rs = 0.79$; $p < 0.01$; unexposed $rs = 0.92$; $p < 0.01$). Before the results obtained from the exposed and the unexposed groups were compared, adjustment was made for age, sex, origin, education and tobacco smoking. The adjusted mean number of sister chromatid exchanges per chromosome was significantly higher ($p < 0.01$) among the exposed group (0.27; SE, 0.003) than the unexposed group (0.19; SE, 0.004). The adjusted mean of the proportion of high-frequency cells was also significantly higher ($p < 0.01$) among the exposed group (0.88; SE, 0.01) than the controls (0.44; SE, 0.02). After adjustment for potential confounders, the adjusted mean of the two variables of sister chromatid exchange were similar for the two periods of exposure (up to 15 years and more than 15 years). Tobacco smoking was found to be a significant confounder. With regard to levels of exposure, both variables of sister chromatid exchange were similar in the low- and high-level exposure subgroups. However, among smokers, both variables of sister chromatid exchange were higher in the high-exposure subgroup than in the low-exposure subgroup.

(*c*) *DNA repair*

Hayes *et al.* (1997) studied the effect of formaldehyde on DNA repair capacity by assessing the activity of O^6-alkylguanine–DNA alkyltransferase (AGT), which was found to be involved in the repair of DNA damage due to exposure to formaldehyde *in vitro*. AGT activity was measured in peripheral blood lymphocytes of 23 mortuary science students (seven women and 16 men), of whom 17 were nonsmokers and six were smokers. Blood samples were taken before the beginning of the course and after 9 weeks of practice. The number of embalmings that the students experienced varied, both before and during the course. The average air concentration of formaldehyde during embalming was about 1.5 ppm and, during some peak exposures, was three to nine times that of the corresponding TWA. Measurements of glutaraldehyde and phenol were below the limit of detection. Total exposure to formaldehyde during the study period, including embalming carried out outside the school, was within the range of 5.7–82.0 ppm–h [7–100 mg/m^3–h] (mean ± SD, 18.4 ± 15.6 ppm [22.5 ± 19.2 mg/m^3]). The total number of embalmings was correlated with the estimated total exposure to formaldehyde ($r = 0.59$; $p < 0.01$). Students who had had previous experience of embalming had greater estimated exposure to formaldehyde during the study period ($p < 0.05$), and their DNA repair capacity at baseline was reduced ($p = 0.08$). No exposure–response relationship was found between the number of embalmings during the 90 days before the course and the AGT activity ($r = –0.29$; $p = 0.19$). Sex,

age and tobacco smoking were not clearly related to pre-exposure DNA repair activity. Post-exposure and pre-exposure AGT activity were correlated ($r = 0.42$; $p < 0.05$). At the end of the course, a reduction in DNA repair capacity was found in 17 students and an increase in DNA repair capacity in six ($p < 0.05$). These findings were confirmed after analysis of variance, including adjustment for age, sex and tobacco smoking status. Among the eight students who had no embalming experience during the 90 days before the study, seven (88%) had decreased and only one had increased AGT activity during the study period ($p < 0.05$). Of the 15 students who had had previous embalming experience, 10 (67%) had decreased and five (33%) had increased AGT activity ($p > 0.05$). No relation was found between the extent of the decrease in AGT activity and the levels of exposure to formaldehyde throughout the 9-week study period or during the last 28 days. As was also noted by the authors, the limitation of the study was the small study population and the fact that many of the students had previous embalming experience.

Schlink *et al*. (1999) studied the activitity of the DNA repair enzyme, O^6-methylguanine–DNA methyltransferase (MGMT). The study population included 57 medical students from two universities who were exposed to formaldehyde during anatomy courses. Blood samples of 41 students from the first university were collected before (day 0, control group No. 1), during (day 50) and after (day 111) the course, which lasted 111 days; two 3-h courses were held per week. Additional blood samples from 16 students from the second university were taken at the end of their course, namely 98 days after the start, and an additional 10 blood samples were taken from unexposed students (control group No. 2). The first group of 41 students was exposed to levels of formaldehyde between 0.14 mg/m^3 and 0.3 mg/m^3 (mean ± SD, 0.2 ± 0.05 mg/m^3). The mean MGMT activity (± 95% CI) was 133.2 ± 14.9 fmol MGMT/10^6 cells. No significant difference was observed before and after 50 days of exposure or after 111 days of exposure. Age, sex, cigarette smoking, alcohol consumption and allergic disease had no influence on MGMT activities in either the exposed group or the controls. The exposure level of the second group of 16 students was 0.8 ± 0.6 mg/m^3. No significant difference in MGMT activity was observed between this exposed group and the controls (146.9 ± 22.3 fmol MGMT/10^6 cells and 138.9 ± 22.1 fmol MGMT/10^6 cells, respectively). In addition, the activity of MGMT of students at the second university who were exposed to a higher level of formaldehyde than those at the first university was not statistically significant from that of control group No. 1, and very similar results were obtained in both control groups.

(*d*) *Urinary mutagenicity*

Hospital autopsy service workers in Galveston, TX (USA) (15 men and four women), aged < 30–> 50 years, and a control group from the local medical school (15 men and five women), who were in the same age range and were matched for consumption of tobacco, marijuana, alcohol and coffee, were studied for urinary mutagenicity (Connor *et al.*, 1985). Individuals were sampled three times at approximately 2-month intervals. The TWA exposures to formaldehyde in the work areas were estimated to be 0.61–1.32 ppm [0.73–1.58 mg/m^3]. Urine (150–200 mL from each subject) was treated with β-glucuronidase and

passed through an XAD-2 column, which was then washed with water. The fraction that eluted with acetone was assayed for mutagenicity in *Salmonella typhimurium* TA98 and TA100 in the presence and absence of an exogenous metabolic activation system from the livers of Aroclor-1254-induced rats. No increase in mutagenicity was seen in the autopsy workers compared with the control group.

4.4.2 *Experimental systems*

(*a*) *DNA–protein cross-links*

(i) *In-vitro studies*

Formaldehyde induces DNA–protein cross-links in animal and human cells *in vitro* (see Table 37). The precise nature of these cross-links is unknown.

(ii) *In-vivo studies in animals*

Groups of four male Fischer 344 rats were exposed for 6 h to 0.3, 0.7, 2, 6 or 10 ppm [0.37, 0.9, 2.4, 7.4 or 12.3 mg/m^3] [^{14}C]formaldehyde in a nose-only inhalation chamber. Individual male rhesus monkeys (*Macaca mulatta)* were exposed for 6 h to 0.7, 2 or 6 ppm [^{14}C]formaldehyde in a head-only inhalation chamber. DNA–protein cross-links induced by exposure to formaldehyde were measured in the nasal mucosa of several regions of the upper respiratory tract of exposed animals. The concentrations of cross-links increased non-linearly with the airborne concentration in both species, but those in the turbinates and anterior nasal mucosa were significantly lower in monkeys than in rats. Cross-links were also formed in the nasopharynx and trachea of monkeys, but were not detected in the sinus, proximal lung or bone marrow. The authors suggested that the differences between the species with respect to DNA–protein cross-link formation may be due to differences in nasal cavity deposition and in the elimination of absorbed formaldehyde (Heck *et al.*, 1989; Casanova *et al*., 1991).

In order to determine whether the yields of DNA–protein cross-links after chronic and acute exposures are equivalent and to locate the site of DNA–protein cross-links and of DNA replication in the rat nasal respiratory mucosa in relation to tumour incidence, groups of rats were exposed (whole body) to concentrations of 0.7, 2, 6 or 15 ppm [0.8, 2.4, 7.4 or 18.5 mg/m^3] unlabelled formaldehyde for 6 h per day on 5 days per week for 11 weeks and 4 days (pre-exposed rats) while other groups were exposed to room air (naive rats). On day 5 of week 12, the pre-exposed and naive rats were simultaneously exposed (nose only) for 3 h to [^{14}C]-labelled formaldehyde at the same concentrations as those used for pre-exposure to quantitate the acute yield of DNA–protein cross-links and to measure cell proliferation in specific sites of the nose. Alternatively, in order to determine the cumulative yield of DNA–protein cross-links in comparison with unexposed rats, rats that were pre-exposed to 6 or 10 ppm formaldehyde and naive rats that were exposed to room air were simultaneously exposed for 3 h to the same concentration of unlabelled formaldehyde on day 5 or at week 12. The cumulative yield of DNA–protein cross-links was measured immediately after exposure. Nasal mucosal DNA was extracted

from proteins, and the percentage of interfacial DNA (an indicator for the concentration of DNA–protein cross-links) was determined. The percentage of interfacial DNA was also determined in a group of unexposed rats. Increases in the percentage of interfacial DNA, namely, decreases in the extractability of DNA from proteins in the exposed rats, was found by the authors to correlate highly with the yield of DNA–protein cross-links (Casanova-Schmitz *et al.*, 1984b). The amount of [^{14}C] incorporation into DNA was an indicator of cell replication. Comparison of cell replication rates in the lateral meatus with those in the medial and posterior meatus showed no significant difference at 0.7 and 2 ppm formaldehyde, but this difference became significant at 6 and 15 ppm formaldehyde in pre-exposed rats ($p \leq 0.02$, Scheffé's test). At 6 and 15 ppm formaldehyde, a significantly ($p \leq 0.01$, Scheffé's test) greater amount of [^{14}C] was incorporated into the DNA in the lateral meatus of pre-exposed rats compared with naive rats and, at 15 ppm, into the DNA in the medial and posterior meatus of pre-exposed rats compared with naive rats. The acute yield of DNA–protein cross-links increased non-linearly with concentrations and, in naive rats, was approximately sixfold greater in the lateral than in the medial and posterior meatus at all concentrations. At 0.7 and 2 ppm, the acute yield was not significantly different between naive and pre-exposed rats. However, at both 6 and 15 ppm formaldehyde, a greater acute yield of DNA–protein cross-links was found in the lateral meatus of naive rats than in that of pre-exposed rats, a difference that was significant at 15 ppm formaldehyde ($p = 0.028$, paired *t* test). Based on histopathological evidence, this difference can be attributed to an increase in the quantity of DNA due to an increase in the number of cells in the nasal mucosa of the lateral meatus at high concentrations, which results in a dilution of the DNA–protein cross-links. No significant difference was found between naive and pre-exposed rats in relation to acute yield of DNA–protein cross-links in the medial and posterior meatus at any concentration of formaldehyde. Since measurement of the percentage of interfacial DNA does not require the use of [^{14}C]formaldehyde, this parameter was used to investigate whether DNA–protein cross-links accumulated during subchronic, whole-body exposure to formaldehyde. The percentage of interfacial DNA following acute exposure (3-h nose-only exposure) to formaldehyde increased significantly ($p < 0.02$, Scheffé's test) at 6 and 10 ppm. However, at these levels, the percentage of interfacial DNA was lower in pre-exposed rats than in naive rats, a difference that was significant at 10 ppm formaldehyde ($p = 0.01$, Scheffé's test). The authors suggested that the cumulative and acute yields of DNA–protein cross-links in rats exposed to formaldehyde are essentially identical (Casanova *et al.*, 1994). [The Working Group noted that there is doubt about the adequacy of the methods that use interfacial DNA to detect DNA–protein cross-links; the connection between DNA yield needs to be clarified.]

Table 37. Genetic and related effects of formaldehyde in experimental systems and animals

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Misincorporation of DNA bases into synthetic polynucleotides *in vitro*	+	NT	30	Snyder & Van Houten (1986)
pUC13 plasmid DNA bound to calf-thymus histones, DNA–protein cross-links	+	NT	0.0075	Kuykendall & Bogdanffy (1992)
Escherichia coli PQ37, SOS repair test, DNA strand breaks, cross-links or related damage	+	NT	20	Le Curieux *et al.* (1993)
Escherichia coli K12 (or *E. coli* DNA), DNA strand breaks, cross-links or related damage; DNA repair	+	NT	60	Poverenny *et al.* (1975)
Escherichia coli K12, DNA strand breaks, cross-links or related damage; DNA repair	+	NT	600	Wilkins & MacLeod (1976)
Escherichia coli K12 KS160-KS66 *pol*AI, differential toxicity	+	NT	60	Poverenny *et al.* (1975)
*Escherichia coli pol*A^{+}/W3110 and *pol*A^{-} p3478, differential toxicity (spot test)	+	NT	10 µL of pure substance	Leifer *et al.* (1981)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	60 µg/plate	Gocke *et al.* (1981)
Salmonella typhimurium TA100, reverse mutation	–	+	10 µg/plate	Haworth *et al.* (1983)
Salmonella typhimurium TA100, reverse mutation	(+)	+	30 µg/plate (toxic above 125 µg/plate)[c]	Connor *et al.* (1983)
Salmonella typhimurium TA100, reverse mutation	+	+[d]	9 µg/plate	Pool *et al.* (1984)
Salmonella typhimurium TA100, reverse mutation	(+)	NT	51 µg/plate[c]	Marnett *et al.* (1985)
Salmonella typhimurium TA100, reverse mutation	+	NT	6 µg/plate	Takahashi *et al.* (1985)
Salmonella typhimurium TA100, reverse mutation	(+)	+	3	Schmid *et al.* (1986)
Salmonella typhimurium TA100, reverse mutation	+	NT	9.3	O'Donovan & Mee (1993)
Salmonella typhimurium TA100, TA104, reverse mutation	+	+	6.25–50 µg/plate	Dillon *et al.* (1998)
Salmonella typhimurium TA102, TA104, reverse mutation	+	NT	21 µg/plate[c]	Marnett *et al.* (1985)

Table 37 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA102, reverse mutation	+	NT	10	Le Curieux *et al.* (1993)
Salmonella typhimurium TA102, reverse mutation	+	NT	25 µg/plate	O'Donovan & Mee (1993)
Salmonella typhimurium TA102, reverse mutation	+	NT	0.1–0.25 µg/plate	Chang *et al*. (1997)
Salmonella typhimurium TA102, reverse mutation	+	?	6.25–50 µg/plate	Dillon *et al*. (1998)
Salmonella typhimurium TA1535, TA1537, reverse mutation	–	–	100–200 µg/plate	Haworth *et al.* (1983)
Salmonella typhimurium TA1535, reverse mutation	NT	–[d]	18 µg/plate	Pool *et al.* (1984)
Salmonella typhimurium TA1535, TA1537, TA1538, reverse mutation	–	NT	100 µg/plate	O'Donovan & Mee (1993)
Salmonella typhimurium TA98, reverse mutation	+	NT	12 µg/plate	Marnett *et al.* (1985)
Salmonella typhimurium TA98, reverse mutation	–	(+)	10 µg/plate	Haworth *et al.* (1983)
Salmonella typhimurium TA98, reverse mutation	(+)	(+)	30 µg/plate (toxic above 100 µg/plate)	Connor *et al.* (1983)
Salmonella typhimurium TA98, reverse mutation	NT	(+)[d]	9 µg/plate	Pool *et al.* (1984)
Salmonella typhimurium TA98, reverse mutation	+	NT	12.5 µg/plate	O'Donovan & Mee (1993)
Salmonella typhimurium (other miscellaneous strains), reverse mutation	–	–	100 µg/plate (toxic at 250 µg/plate)	Connor *et al.* (1983)
Salmonella typhimurium TM677, forward mutation to 8-azaguanine	+	+	5; 10[e]	Temcharoen & Thilly (1983)
Salmonella typhimurium TA97, reverse mutation	+	NT	12 µg/plate[c]	Marnett *et al.* (1985)
Salmonella typhimurium TA7005 (*his*$^+$), reverse mutation	+	NT	1.5 µg/plate	Ohta *et al*. (2000)
Escherichia coli K12, forward or reverse mutation (*gpt* locus)	+	NT	120	Crosby *et al.* (1988)
Escherichia coli K12, forward or reverse mutation	+	NT	60	Zijlstra (1989)
Escherichia coli K12, forward or reverse mutation	+	NT	18.8	Graves *et al.* (1994)

Table 37 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli WP2 *uvr*A, reverse mutation	+	NT	15	Takahashi *et al.* (1985)
Escherichia coli WP2 *uvr*A (pKM101), reverse mutation	+	NT	12.5 µg/plate	O'Donovan & Mee (1993)
Escherichia coli WP2, reverse mutation	+	NT	1200	Nishioka (1973)
Escherichia coli WP2, reverse mutation	+	NT	60	Takahashi *et al.* (1985)
Escherichia coli WP2 (pKM101), reverse mutation	+	NT	25 µg/plate	O'Donovan & Mee (1993)
Escherichia coli (other miscellaneous strains), reverse mutation	+	NT	100	Demerec *et al.* (1951)
Escherichia coli (other miscellaneous strains), reverse mutation	+	NT	900	Panfilova *et al.* (1966)
Escherichia coli (other miscellaneous strains), reverse mutation	+	NT	30	Takahashi *et al.* (1985)
Escherichia coli WP3104P, reverse mutation	(+)	NT	5 µg/plate	Ohta *et al*. (1999)
Escherichia coli WP3104P, reverse mutation	+	NT	~2 µg/plate	Ohta *et al*. (2000)
Saccharomyces species, DNA strand breaks and DNA repair	+	NT	990	Magaña-Schwencke *et al.* (1978)
Saccharomyces species, DNA strand breaks, DNA–protein cross links or related damage	+	NT	500	Magaña-Schwencke & Ekert (1978); Magaña-Schwencke & Moustacchi (1980)
Saccharomyces cerevisiae, gene conversion	+	NT	540	Chanet *et al.* (1975)
Saccharomyces cerevisiae, homozygosis by mitotic recombination or gene conversion	+	NT	18.5	Zimmermann & Mohr (1992)
Neurospora crassa heterokaryons H-12 strain, forward mutation	(+)	NT	250	de Serres *et al.* (1988); de Serres & Brockman (1999)

Table 37 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Neurospora crassa heterokaryons H-59 strain, forward mutation	+	NT	100	de Serres *et al.* (1988); de Serres & Brockman (1999)
Neurospora crassa, reverse mutation	–	NT	732	Dickey *et al.* (1949)
Neurospora crassa, reverse mutation	+	NT	300	Jensen *et al.* (1951)
Neurospora crassa, reverse mutation	–	NT	300	Kölmark & Westergaard (1953)
Agaricus bisporus, Glycine max, Lycopersicon esculentum, P. americana, Pinus resinosa, Pisum sativum, Populus × euramericana, Vicia faba, Zea mays, DNA damage	+	NT	3.7% solution, pH 3 and 7, 37 000 µg/mL	Douglas & Rogers (1998)
Plants (other), mutation	+	NT	NG	Auerbach *et al.* (1977)
Tradescantia pallida, micronucleus formation	+	NT	250 ppm [250 µg/mL], 6 h	Batalha *et al*. (1999)
Drosophila melanogaster, genetic crossing over or recombination	+		1260	Sobels & van Steenis (1957)
Drosophila melanogaster, genetic crossing over or recombination	+		420	Alderson (1967)
Drosophila melanogaster, genetic crossing over or recombination	+		2500	Ratnayake (1970)
Drosophila melanogaster, sex-linked recessive lethal mutations	+		1000	Kaplan (1948)
Drosophila melanogaster, sex-linked recessive lethal mutations	+		1800	Auerbach & Moser (1953)
Drosophila melanogaster, sex-linked recessive lethal mutations	+		1260	Sobels & van Steenis (1957)
Drosophila melanogaster, sex-linked recessive lethal mutations	+		420	Alderson (1967)
Drosophila melanogaster, sex-linked recessive lethal mutations	+		420	Khan (1967)
Drosophila melanogaster, sex-linked recessive lethal mutations	(+)		2000	Ratnayake (1968)
Drosophila melanogaster, sex-linked recessive lethal mutations	+		250	Stumm-Tegethoff (1969)

Table 37 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Drosophila melanogaster, sex-linked recessive lethal mutations	+		2200	Ratnayake (1970)
Drosophila melanogaster, heritable translocation	+		420	Khan (1967)
Drosophila melanogaster, heritable translocation	+		2500	Ratnayake (1970)
Drosophila melanogaster, dominant lethal mutation	+		1800	Auerbach & Moser (1953)
Drosophila melanogaster, dominant lethal mutation	+		1300	Šrám (1970)
Caenorhabditis elegans, recessive lethal mutations	+		700	Johnsen & Baillie (1988)
DNA strand breaks, DNA–protein cross-links or related damage, mouse leukaemia L1210 cells *in vitro*	+	NT	6	Ross & Shipley (1980)
DNA–protein cross-links, mouse leukaemia L1210 cells *in vitro*	+	NT	3.75	Ross *et al.* (1981)
DNA single strand breaks, DNA–protein cross-links or related damage, sarcoma rat cell line *in vitro*	+	NT	7.5	O'Connor & Fox (1987)
DNA single strand breaks or related damage, rat hepatocytes *in vitro*	+	NT	22.5	Demkowicz-Dobrzanski & Castonguay (1992)
DNA–protein cross-links, Chinese hamster ovary cells *in vitro*	+	NT	7.5	Olin *et al*. (1996)
DNA–protein cross-links, male B6C3F$_1$ mouse hepatocytes *in vitro*	+	NT	15	Casanova *et al*. (1997)
DNA–protein cross-links, Chinese hamster V79 lung fibroblast cells *in vitro*	+	NT	4	Merk & Speit (1998, 1999)
Unscheduled DNA synthesis, Syrian hamster embryo cells *in vitro*	+	NT	3	Hamaguchi *et al*. (2000)
Gene mutation, Chinese hamster V79 cells, *Hprt* locus	+	NT	9	Grafström *et al.* (1993)
Gene mutation, Chinese hamster V79 cells, *Hprt* locus	–	NT	15	Merk & Speit (1998, 1999)
Gene mutation, mouse lymphoma L5178Y cells, *Tk*$^{+/-}$ locus *in vitro*	NT	+	24	Mackerer *et al*. (1996)
Gene mutation, mouse lymphoma L5178Y cells *in vitro*	+	NT	> 2	Speit & Merk (2002)
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	NT	1	Obe & Beek (1979)
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	3.2	Natarajan *et al.* (1983)

Table 37 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	–	2	Basler *et al.* (1985)
Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	+	NT	4	Merk & Speit (1998, 1999)
Micronucleus formation, Chinese hamster V79 cells *in vitro*	+	NT	4	Merk & Speit (1998, 1999)
Chromosomal aberrations, Chinese hamster cells *in vitro*	+	NT	18	Ishidate *et al.* (1981)
Chromosomal aberrations, Chinese hamster cells *in vitro*	+	+	6.3	Natarajan *et al.* (1983)
Cell transformation, C3H10T1/2 mouse cells	+[f]	NT	0.5	Ragan & Boreiko (1981)
DNA single strand breaks, DNA–protein cross-links, human bronchial cells *in vitro*	+	NT	24	Fornace *et al.* (1982)
DNA single strand breaks, DNA–protein cross-links or related damage, human bronchial and skin cells *in vitro*	+	NT	3	Grafström *et al.* (1984)
DNA single strand breaks, DNA–protein cross-links or related damage, human bronchial cells *in vitro*	+	NT	3	Saladino *et al.* (1985)
DNA single strand breaks, DNA–protein cross-links or related damage, human bronchial cells *in vitro*	+	NT	3	Grafström *et al.* (1986)
DNA strand breaks, cross-links or related damage, human fibroblast cells *in vitro*	+	NT	3	Snyder & Van Houten (1986)
DNA strand breaks, DNA–protein cross-links or related damage, human lymphoblast cells *in vitro*	+	NT	1.5	Craft *et al.* (1987)
DNA strand breaks, DNA–protein cross-links or related damage, human bronchial cells *in vitro*	+	NT	12	Grafström (1990)
DNA–protein cross-links, human fibroblasts *in vitro*	+	NT	7.5	Olin *et al*. (1996)
DNA–protein cross-links, human white blood cells *in vitro*	+	NT	3	Shaham *et al*. (1996)
DNA–protein cross-links, EBV-BL human lymphoma cells *in vitro*	+	NT	37 × 18 h	Costa *et al*. (1997)

Table 37 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA double-strand breaks, human lung epithelial (A549) cells *in vitro*	+	NT	30 (8, 24, 72 h)	Vock *et al*. (1999)
DNA–protein cross-links, Comet assay, human gastric mucosa cells *in vitro*	+	NT	30	Blasiak *et al*. (2000)
DNA–protein cross-links, human lymphocytes *in vitro*	+	–	3	Andersson *et al*. (2003)
DNA repair exclusive of unscheduled DNA synthesis, human bronchial and skin cells *in vitro*	+	NT	6	Grafström *et al.* (1984)
DNA repair, human MRC5CV1 normal cell line, XP12 ROSV cell line, GMO6914 FA cell line, *in vitro*	+	NT	3.75	Speit *et al*. (2000)
Unscheduled DNA synthesis, human bronchial epithelial cells *in vitro*	-	NT	3 (> 3 was lethal)	Doolittle *et al.* (1985)
Gene mutation, human lymphoblasts TK6 line (*TK* locus) *in vitro*	+	NT	3.9	Goldmacher & Thilly (1983)
Gene mutation, human fibroblasts *in vitro*	+	NT	3	Grafström *et al.* (1985)
Gene mutation, human lymphoblasts TK6 line (*TK* locus) *in vitro*	+	NT	0.9	Craft *et al.* (1987)
Gene mutation, human lymphoblasts TK6 line (*HPRT* locus) *in vitro*	+	NT	4.5, 2 h, × 8 times	Crosby *et al.* (1988)
Gene mutation, human lymphoblasts TK6 line (*HPRT* locus) *in vitro*	+	NT	4.5, 2 h, × 8 times	Liber *et al.* (1989)
Gene mutation, human bronchial fibroblasts (*HPRT* locus) *in vitro*	+	NT	3	Grafström (1990)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	5	Obe & Beek (1979)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	5	Kreiger & Garry (1983)
Sister chromatid exchange, human lymphocytes *in vitro*	+	+	3.75	Schmid *et al.* (1986)
Micronucleus formation, human MRC5CV1 normal cell line, XP12 ROSV cell line, GMO6914 FA cell line, *in vitro*	+	NT	3.75	Speit *et al*. (2000)
Chromosomal aberrations, human fibroblasts *in vitro*	+	NT	60	Levy *et al.* (1983)

Table 37 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	10	Miretskaya & Shvartsman (1982)
Chromosomal aberrations, human lymphocytes *in vitro*	+	+	7.5	Schmid *et al.* (1986)
Chromosomal aberrations, premature chromosome condensation technique, human lymphocytes *in vitro*	+	NT	3.75	Dresp & Bauchinger (1988)
DNA–protein cross-links, rat respiratory and olfactory mucosa and bone marrow *in vivo*	+		2 ppm [2.5 mg/m^3], 6 h	Casanova-Schmitz *et al.* (1984b)
DNA–protein cross-links, rat nasal mucosa *in vivo*	+		6 ppm [7.4 mg/m^3], 6 h	Lam *et al.* (1985)
DNA–protein cross-links, rat respiratory mucosa *in vivo*	+		2 ppm [2.5 mg/m^3], 3 h	Heck *et al.* (1986)
DNA–protein cross-links, rat respiratory and olfactory mucosa and bone marrow *in vivo*	+		2 ppm [2.5 mg/m^3], 3 h	Casanova & Heck (1987)
DNA–protein cross-links, rat tracheal implant cells *in vivo*	+		50 ppm [50 µg/mL], instil.	Cosma *et al.* (1988)
DNA–protein cross-links, rat nasal respiratory mucosa *in vivo*	+		0.3 ppm [0.4 mg/m^3], inhal. 6 h	Casanova *et al.* (1989)
DNA–protein cross-links, rhesus monkey nasal turbinate cells *in vivo*	+		0.7 ppm [0.9 mg/m^3], inhal. 6 h	Heck *et al.* (1989)
DNA–protein cross-links, rhesus monkey nasal turbinate cells *in vivo*	+		0.7 ppm [0.9 mg/m^3], inhal. 6 h	Casanova *et al.* (1991)
Gene mutation, rat cells *in vivo* (*p53* point mutations in nasal squamous-cell carcinomas)	+		15 ppm [18.45 mg/m^3], inhal. 6 h/d, 5 d/wk, 2 y	Recio *et al.* (1992)
Mouse spot test	–		15 ppm [18 mg/m^3], inhal. 6 h/d × 3	Jensen & Cohr (1983) [Abstract]

Table 37 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, rat cells *in vivo*	–		15 ppm [18.45 mg/m^3], inhal. 6 h/d × 5	Kligerman *et al.* (1984)
Micronucleus formation, mouse bone-marrow cells *in vivo*	–		30 ip × 1	Gocke *et al.* (1981)
Micronucleus formation, mouse bone-marrow cells *in vivo*	–		25 ip × 1	Natarajan *et al.* (1983)
Micronucleus formation, rat gastrointestinal tract *in vivo*	+		200 po × 1	Migliore *et al.* (1989)
Micronucleus formation, newt (*Pleurodeles waltl*) *in vivo*	–		5 µg/mL, 8 d	Siboulet *et al.* (1984)
Chromosomal aberrations, mouse bone-marrow cells *in vivo*	–		25 ip × 1	Natarajan *et al.* (1983)
Chromosomal aberrations, rat bone-marrow cells *in vivo*	+		0.4 ppm [0.5 mg/m^3] inhal. 4 h/d, 4 mo	Kitaeva *et al.* (1990)
Chromosomal aberrations, rat bone-marrow cells *in vivo*	–		15 ppm [18.45 mg/m^3], inhal. 6 h/d × 5, 8 wk	Dallas *et al.* (1992)
Chromosomal aberrations, rat leukocytes *in vivo*	–		15 ppm [18.45 mg/m^3], inhal. 6 h/d × 5	Kligerman *et al.* (1984)
Chromosomal aberrations, mouse spermatocytes treated *in vivo*, spermatocytes observed	–		50 ip × 1	Fontignie-Houbrechts (1981)
Chromosomal aberrations, mouse spleen cells *in vivo*	–		25 ip × 1	Natarajan *et al.* (1983)
Chromosomal aberrations, rat pulmonary lavage cells *in vivo*	+		15 ppm [18.45 mg/m^3] inhal. 6 h/d × 5	Dallas *et al.* (1992)

Table 37 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Dominant lethal mutation, mouse	–		20 ip × 1	Epstein & Shafner (1968)
Dominant lethal mutation, mouse	–		20 ip × 1	Epstein *et al.* (1972)
Dominant lethal mutation, mouse	(+)		50 ip × 1	Fontignie-Houbrechts (1981)
Dominant lethal mutation, rat	(+)		1.2 ppm [1.5 mg/m^3] inhal. 4 h/d, 4 mo	Kitaeva *et al.* (1990)

EBV, Epstein–Barr virus; BL, Burkitt lymphoma; XP, xeroderma pigmentosum; FA, Fanconi anaemia

[a] +, positive; (+) weak positive; –, negative; NT, not tested; ?, inconclusive (variable response in several experiments within an adequate study)

[b] In-vitro tests, µg/mL; in-vivo tests, mg/kg bw; d, day; inhal., inhalation; instil., instillation; ip, intraperitoneal; mo, month; NG, not given; po, oral; wk, weeks; y, year

[c] Estimated from the graph in the paper

[d] Tested with exogenous metabolic system without co-factors

[e] LED with exogenous metabolic system is 0.33 mM [10 µg/mL].

[f] Positive only in presence of 12-*O*-tetradecanoylphorbol 13-acetate

(*b*) *Mutation and allied effects* (see also Table 37)

(i) *In-vitro studies*

Formaldehyde induced mutation and DNA damage in bacteria, mutation, gene conversion, DNA strand breaks and DNA–protein cross-links in fungi and DNA damage in plants. In *Drosophila melanogaster*, administration of formaldehyde in the diet induced sex-linked recessive lethal mutations, dominant lethal effects, heritable translocations and crossing-over in spermatogonia. In a single study, it induced recessive lethal mutations in a nematode. It induced chromosomal aberrations, sister chromatid exchange, DNA strand breaks and DNA–protein cross-links in animal cells and, in single studies, gene mutation, sister chromatid exchange and micronuclei in Chinese hamster V79 cells and transformation of mouse C3H10T1/2 cells *in vitro.* Formaldehyde induced DNA–protein cross-links, chromosomal aberrations, sister chromatid exchange and gene mutation in human cells *in vitro.* Experiments in human and Chinese hamster lung cells indicate that formaldehyde can inhibit repair of DNA lesions caused by the agent itself or by other mutagens, such as *N*-nitroso-*N*-methylurea or ionizing radiation (Grafström, 1990; Grafström *et al*., 1993).

(ii) *In-vivo studies in animals*

Formaldehyde induces cytogenetic damage in the cells of tissues of animals exposed either by gavage or by inhalation. Groups of five male Sprague–Dawley rats were given 200 mg/kg bw formaldehyde orally, were killed 16, 24 or 30 h after treatment and were examined for the induction of micronuclei and nuclear anomalies in cells of the gastrointestinal epithelium. The frequency of mitotic figures was used as an index of cell proliferation. Treated rats had significant (greater than fivefold) increases in the frequency of micronucleated cells in the stomach, duodenum, ileum and colon; the stomach was the most sensitive, with a 20-fold increase in the frequency of micronucleated cells 30 h after treatment, and the colon was the least sensitive. The frequency of nuclear anomalies was also significantly increased at these sites. These effects were observed in conjunction with signs of severe local irritation (Migliore *et al.*, 1989).

Male Sprague–Dawley rats were exposed by inhalation to 0, 0.5, 3 or 15 ppm [0, 0.62, 3.7 or 18.5 mg/m^3] formaldehyde for 6 h per day on 5 days per week for 1 and 8 weeks. No significant increase in chromosomal abnormalities in the bone-marrow cells of formaldehyde-exposed rats was observed relative to controls, but the frequency of chromosomal aberrations was significantly increased in pulmonary lavage cells (lung alveolar macrophages) from rats that inhaled 15 ppm formaldehyde. Aberrations, which were predominantly chromatid breaks, were seen in 8.0 and 9.2% of the scored pulmonary lavage cells from treated animals and in 3.5 and 4.4% of cells from controls after 1 and 8 weeks, respectively (Dallas *et al*., 1992).

In a second in-vivo study on bone-marrow cytogenetics, Wistar rats were exposed by inhalation to formaldehyde (0.5 or 1.5 mg/m^3) every day for 4 h (except for non-working days) for 4 months (Kitaeva *et* al., 1990). The concentration of 1.5 mg/m^3 formaldehyde caused diverse effects on germ cells that the authors correlated with their subsequent studies that showed impairment of early embryonic development (see Section 4.3.2). Both

concentrations (0.5 and 1.5 mg/m^3) caused a significantly increased number of chromosomal aberrations in the bone marrow that were of the chromatid (at 0.5 mg/m^3) and chromosomal (at 1.5 mg/m^3) type. The number of hypoploidal and hyperploidal cells was increased. The increase in aneuploidy was due only to chromosomal loss, not to chromosomal gain. At the higher exposure, chromosomal type aberrations were observed. [The Working Group noted that this was the only study that evaluated bone-marrow cytogenetics. Since chromosomal loss can frequently occur as an artefact of sample preparation, these studies should be repeated.] The study showed that exposure to formaldehyde at the lower dose was cytotoxic and mutagenic to bone-marrow cells. In an in-vitro part of this study, exposure to 0.5 mg/m^3 formaldehyde caused a decrease in the mitotic index in bone-marrow cells while, at 1.5 mg/m^3, the mitotic index was increased (Kitaeva *et al.*, 1990). [The Working Group noted that this is the only in-vivo study that showed positive cytogenetic effects of formaldehyde in the bone marrow.]

(*c*) *Mutational spectra*

(i) *In-vitro studies* (see also Table 37 and references therein)

The spectrum of mutations induced by formaldehyde was studied in human lymphoblasts *in vitro*, in *Escherichia coli* and in naked pSV2*gpt* plasmid DNA (Crosby *et al.*, 1988). Thirty mutant TK6 X-linked *HPRT*$^-$ human lymphoblast colonies induced by eight repetitive treatments with 150 µmol/L [4.5 µg/mL] formaldehyde were characterized by southern blot analysis. Fourteen (47%) of these mutants had visible deletions of some or all of the X-linked *HPRT* bands, indicating that formaldehyde can induce large losses of DNA in human TK6 lymphoblasts. The remainder of the mutants showed normal restriction patterns, which, according to the authors, probably consisted of point mutations or smaller insertions or deletions that were too small to detect by southern blot analysis.

Sixteen of the 30 formaldehyde-induced human lymphoblast TK6 X-linked *HPRT* mutants referred to above that were not attributable to deletion were examined by southern blot, northern blot and DNA sequence analysis (Liber *et al.*, 1989). Of these, nine produced mRNA of normal size and amount, three produced mRNA of normal size but in reduced amounts, one had a smaller size of mRNA and three produced no detectable mRNA. Sequence analyses of cDNA prepared from *HPRT* mRNA were performed on one spontaneous and seven formaldehyde-induced mutants indicated by normal northern blotting. The spontaneous mutant was caused by an AT→GC transition. Six of the formaldehyde-induced mutants were base substitutions, all of which occurred at AT base-pairs. There was an apparent hot spot, in that four of six independent mutants were AT→CG transversions at a specific site. The remaining mutant had lost exon 8.

In *E. coli*, the mutations induced by formaldehyde were characterized with the use of the xanthine guanine phosphoribosyl transferase gene as the target gene. Exposure of *E. coli* to 4 mmol/L [120 µg/mL] formaldehyde for 1 h induced large insertions (41%), large deletions (18%) and point mutations (41%). DNA sequencing revealed that most of the point mutations were transversions at GC base-pairs. In contrast, exposure of *E. coli* to

40 mmol/L formaldehyde for 1 h produced 92% point mutations, 62% of which were transitions at a single AT base-pair in the gene. Therefore, formaldehyde produced different genetic alterations in *E. coli* at different concentrations. When naked pSV2*gpt* plasmid DNA was exposed to 3.3 or 10 mmol/L [99 or 300 μg/mL] formaldehyde and used to transform *E. coli*, most of the resulting mutations were frameshifts that resulted from the addition or deletion of one base, which again suggests a different mechanism of mutation.

The potential of formaldehyde to induce mutation was determined in mouse lymphoma L5178Y cells treated for 2 h with different concentrations (62.5–500 μM [1.9–15 μg/mL]). The mouse lymphoma assay detects gross alterations as large deletions and rearrangements. Treated cells showed a concentration-related increase in mutation frequency. While the frequency of small colonies was increased, only a marginal increase was observed in the frequency of large colonies. The extent of loss of heterozygosity was studied at five polymorphic markers — D11Agll, D11Mit67, D11Mit29, D11Mit21 and D11Mit63 — all of which are equally distributed along chromosome 11. The analysis showed increased loss of heterozygosity at the marker D11Agll, which is located in the tirosine kinase gene. The authors suggested that the main mechanism involved in the mutagenesis of formaldehyde in the mouse lymphoma assay is the production of small-scale chromosomal deletion or recombination (Speit & Merk, 2002).

Exposure of Chinese hamster V79 cells to formaldehyde did not induce gene mutation at the *Hprt* locus, while DNA–protein cross-links, sister chromatid exchange and micronuclei were induced (Merk & Speit, 1998). Formaldehyde induced G:C→T:A transversion in *E. coli* Lac^+ WP3104P and in *S. typhimurium* His^+ TA7005 (Ohta *et al.*, 1999, 2000).

Specific locus mutations at two closely linked loci in the *adenine-3* (*ad-3*) region were compared in two strains of *Neurospora crassa* (H-12, a DNA repair-proficient heterokaryon; and H-59, a DNA repair-deficient heterokaryon) exposed to formaldehyde. The majority (93.2%) of formaldehyde-induced *ad-3* mutations in H-12 resulted from gene/point mutations and only 6.8% resulted from multilocus deletion mutations. In contrast, a greater percentage of formaldehyde-induced *ad-3* mutations (62.8%) observed in H-59 resulted from multilocus deletion mutations. The distribution of *ad-3* mutation in this mutational spectra is highly significantly different ($p << 0.001$). While formaldehyde induced only a 12.8-fold increase in the frequency of *ad-3* mutations over that which occurs spontaneously in H-12, it induced a 412-fold increase in H-59. Formaldehyde induced a 3.4-fold higher ($p << 0.001$) *ad-3B*^{R}/*ad-3A*^{R} ratio in H-12 than in H-59. According to the authors, these results indicated that there was a quantitative and qualitative strain difference in formaldehyde-induced mutagenesis (de Serres *et al.*, 1988; de Serres & Brockman, 1999).

(ii) *In-vivo studies in animals*

DNA sequence analysis of PCR-amplified cDNA fragments that contain the evolutionarily conserved regions II–V of the rat *p53* gene was used to examine *p53* mutations in 11 primary nasal squamous-cell carcinomas induced in rats that had been exposed by inhalation to 15 ppm [18.5 mg/m^3] formaldehyde for up to 2 years. Point mutations at GC base-pairs in the *p53* complementary DNA sequence were found in five of the tumours

(Table 38). The authors pointed out that all five human counterparts of the mutated *p53* codons listed in Table 38 have been identified as mutants in a variety of human cancers; the CpG dinucleotide at codon 273 (codon 271 in the rat) is a mutational hot spot that occurs in many human cancers (Recio *et al.*, 1992).

Table 38. DNA sequence analysis of *p53* cDNA (PCR fragment D) from squamous-cell carcinomas of nasal passages induced in rats by formaldehyde

DNA sequence[a]	Mutation (codon)[b]		Equivalent human *p53* codon no.	Location in conserved region
$_{396}$C→A	TT<u>C</u>→TT<u>A</u> (132)	phe→leu	134	II
$_{398}$G→T	T<u>G</u>C→T<u>T</u>C (133)	cys→phe	135	II
$_{638}$G→T	A<u>G</u>C→A<u>T</u>C (213)	ser→ile	215	
$_{812}$G→A	C<u>G</u>T→C<u>A</u>T (271)	arg→his	273	V
$_{842}$G→C	C<u>G</u>G→C<u>C</u>G (281)	arg→pro	283	V

From Recio *et al.* (1992)
[a] The A in the start codon is designated as base position 1.
[b] The start codon ATG is designated as codon 1.

An immunohistochemical technique was used to assess the presence of p53 protein, a marker of cell proliferation (proliferating cell nuclear antigen) and tumour growth factor-α in the histopathological sections of the above tumours. In addition to the positive p53 immunostaining in squamous-cell carcinomas, especially in cells with keratization, p53-positive immunostaining was also observed in preneoplastic hyperkeratotic plaques while normal nasal mucosa immunostained negatively. A correlation was found between both the pattern and the distribution of immunostaining of proliferating cell nuclear antigen and p53 (Wolf *et al.*, 1995).

Cell lines were established from the above set of squamous-cell carcinomas and, when injected into nude mice, two of them that contained the *p53* mutation were tumorigenic while the two cell lines that contained wild-type *p53* were not. The author suggested that these findings indicate that *p53* mutation occurs in the development of certain rodent nasal squamous-cell carcinomas and that it is associated with tumorigenicity (Recio, 1997). All the mutated *p53* codons that were identified in formaldehyde-induced rat squamous-cell carcinomas have also been found to be mutated in a variety of human cancers (for example, rat codon 271, human codon 273). *P53* is mutated in various human squamous-cell carcinomas including those of the respiratory tract, skin and oesophagus. It was suggested that these data indicate that the development of certain human and rat squamous-cell carcinomas share a molecular change. The author proposed a possible mechanistic explanation, which is based on the fact that the induction of nasal squamous-cell carcinomas by formal-

dehyde is accompanied by cytotoxicity and inflammation followed by cell proliferation that may induce genetic alterations.

Formaldehyde is also known to be genotoxic; a number of lesions have been proposed to explain this genotoxic effect, including DNA–protein cross-links (reviewed in IARC, 1995). Studies on the *HPRT* mutation spectrum in formaldehyde-exposed human cells revealed that 50% of the mutations are deletions, while 50% are due to point mutation at the A:T base-pair (Liber *et al.*, 1989). The finding of deletions as part of the formaldehyde mutation spectrum may explain the homozygous nature of pair mutations observed in *p53* in formaldehyde-induced squamous-cell carcinomas. However, there is an inconsistency with regard to the base-pair that is found to be A:T in *HPRT* in human (Liber *et al.*, 1989) and mammalian cell lines (Graves *et al.*, 1996) and G:C in *p53* in formaldehyde-induced squamous-cell carcinomas. The author suggested that an indirect mechanism of genotoxicity and not a direct effect of formaldehyde on the genome is involved in the carcinogenic process of formaldehyde (Recio, 1997).

In order to evaluate transcriptional changes involved with acute exposure to formaldehyde, two groups of male Fischer 344 rats received either 40 μL distilled water or formaldehyde (400 mM [12 mg/mL]) instilled into each nostril. Twenty-four hours after this treatment, total RNA was extracted from the nasal epithelium to generate cDNA. Adjusted signal intensity was obtained from image analysis of 1185 genes from samples from four controls and three formaldehyde-treated rats. Significance analysis of microarray hybridization data using Clontech™ Rat Atlas 1.2 arrays revealed that 24/1185 genes studied were significantly up-regulated and 22 genes were significantly down-regulated. Ten of the most highly expressed genes for formaldehyde-treated animals included the *N*-methyl-D-aspartate receptor, inducible nitric oxide synthase, macrophage inflammatory protein 1 α and macrophage inflammatory protein 2, Wilms tumour protein homologue 1, tumour necrosis factor ligand, methyl-CpG-binding protein 2, GABA receptor, Fos-responsive gene 1 and presomatotrophin. Of the 24 significantly up-regulated genes, six were receptors, six were involved in extracellular cell signalling and four were oncogene/tumour-suppressor genes, all of which are pathways that could be affected by treatment with formaldehyde. Of the 22 significantly down-regulated genes, five were involved in ion channel regulation and four were involved in protein turnover which suggests that an early response to treatment with formaldehyde may be impaired ion channel function and interruption of protein processing. Many phase I and phase II genes that regulate xenobiotic metabolism and oxidative stress — the CYP and glutathione family, respectively — were altered in treated but not in controls rats. In order to validate the direction of gene expression observed on the microarray, results for 10 of the differentially expressed genes were confirmed by quantitative real-time PCR. Eight of the 10 gene expression values were in positive agreement. No significant difference was found between formaldehyde-treated and control groups in relation to the expression of apoptosis genes. However, in comparison with the control group, the formaldehyde-treated group had a general trend of lower levels of expression of nine apoptic genes that represent three of the major apoptosis regulation pathways, including receptor-mediated, caspase and the mitochondrial-

associated *bcl2* family of genes. The authors suggested that their results indicated that exposure to formaldehyde can cause alteration in the levels of expression of genes that are involved in several functional categories including xenobiotic metabolism, cell cycle regulation, DNA synthesis and repair, oncogenes and apoptosis (Hester *et al*., 2003).

4.5 Mechanistic considerations

4.5.1 *Deposition (airway deposition models)*

Anatomically accurate CFD models have been applied to study the role of nasal airflow and formaldehyde flux in rats, monkeys and humans in order to achieve more accurate risk assessments (Kimbell & Subramaniam, 2001; Kimbell *et al.*, 2002). The use of DNA–protein cross-links as a measure of internal dose, as opposed to the use of air concentration, coupled with the use of monkey-based as opposed to rat-based predictions, resulted in a 50-fold reduction in risk for humans (McClellan, 1995).

Using the Fischer 344 rat CFD model, a sharp anterior to posterior gradient of formaldehyde deposition in the rat nose is predicted, which agrees with observed patterns of formation of DNA–protein cross-links and effects on cell replication (Monticello *et al*., 1996; Cohen Hubal *et al.*, 1997). In another study, predictions of formaldehyde dosimetry in the non-squamous epithelium in the rat CFD model were used to analyse the role of local flux on the distribution of squamous metaplasia induced by 10 ppm and 15 ppm formaldehyde (Kimbell *et al*., 1997). Squamous metaplasia is a useful indicator for formaldehyde-induced squamous-cell carcinoma in rodents due to its temporal relevance and consistency (McMillan *et al*., 1994). Predictions obtained with the model illustrate how, as squamous metaplasia develops, the sites of maximum absorption of formaldehyde move further back in the nose.

In the rhesus monkey, a CFD model of the nasal passages was used to facilitate species-to-species extrapolation of data on the toxicity of formaldehyde (Kepler *et al.*, 1998). As with rats, an anterior to posterior gradient in formaldehyde deposition was predicted and a strong correlation was observed between patterns of airflow and the occurrence of nasal lesions.

In a comparison of Fischer 344 rats, rhesus monkeys and humans, the role of mucous- and non-mucous-coated tissue on formaldehyde uptake was analysed (Kimbell *et al*., 2001b). Flux values for rats and monkeys were found to be very similar at sites where the effects of formaldehyde on cell proliferation had previously been observed. Similar flux values were predicted in a region of the nasal passage where a high incidence of tumours had been observed in rats and in an anterior region of the human nasal passage. It was suggested that a similar formaldehyde-induced response in rats might be expected in humans when regional fluxes are similar.

CFD models of Fischer 344 rat, rhesus monkey and human nasal passages were also used to predict patterns of wall mass flux in order to reduce the uncertainty in interspecies extrapolation of dose within the nasal passages (Kimbell *et al*., 2001a). In all cases, steep

anterior-to-posterior gradients of formaldehyde deposition were predicted. The surface area and average flux were estimated for the flux bins at different airflow rates. At a minute volume of 15 L/min, approximately 20% of the surface area of the human nasal passages had a predicted flux value greater than the median flux value. Increasing airflow in humans was also associated with a distal shift in flux patterns and a reduced percentage of uptake in the nasal passages. Differences across species in nasal anatomy and associated differences in flux were also suggested to play a potentially large role in the occurrence of local adverse effects due to the non-linear relationships of flux with DNA–protein cross-links and with cell proliferation. The adsorption of formaldehyde on inhaled particles does not appreciably alter the patterns of formaldehyde deposition associated with the inhalation of formaldehyde vapour (Rothenberg *et al.*, 1989; Risby *et al.*, 1990).

4.5.2 *Metabolism*

Formaldehyde is an endogenous metabolite that is formed by demethylation reactions and used in the biosynthesis of macromolecules. Average concentrations, including bound and free forms, in cells and biological fluids are reported to be about 0.1 mM (Heck *et al.*, 1985). Formaldehyde readily combines with GSH to form hydroxymethylglutathione, which is converted to formate in a two-step enzymatic reaction that initially involves ADH3. The K_m for the initial binding of hydroxymethylglutathione with ADH3 is in the order of 4 μM and the level of free formaldehyde is probably even lower (Holmquist & Vallee, 1991; Uotila & Koivusalo, 1997; Hedberg *et al.*, 1998). The half-life of formaldehyde in plasma is very short, and is approximately 1 min in rats exposed intravenously (Rietbrock, 1965).

4.5.3 *Genotoxicity*

Formaldehyde induced DNA–protein cross-links *in vitro* in human bronchial epithelial cells, fibroblasts and lymphocytes, in Chinese hamster ovary cells and in non-transformed human fibroblasts (Grafström *et al*., 1984; Olin *et al*., 1996; Shaham *et al*., 1996a,b). The formation of DNA–protein cross-links showed a consistent dose–effect relationship and was induced at concentrations of formaldehyde that allowed survival of up to 75% of the cells (Merk & Speit, 1998, 1999). A range of half-lives for the removal of DNA–protein cross-links from the cells has been reported (3–66 h), but most of the values are in the vicinity of 24 h (Gräfstrom *et al*., 1984; Merk & Speit, 1998, 1999; Quievryn & Zhitkovich, 2000).

Several studies that compared the kinetics of the formation and removal of DNA–protein cross-links in a variety of cell types reported no differences among normal and DNA repair-deficient cell types (Grafström *et al*., 1984; Speit *et al*., 2000). DNA single-strand breaks and/or alkali-labile sites are generated from the repair of formaldehyde damage in normal cells (Grafström *et al*., 1984). Formaldehyde is more toxic to Xeroderma Pigmentosum cells, which also show a higher percentage of micronuclei than normal cells. The data

suggested that neither defective nucleotide excision repair (Xeroderma Pigmentosum cells) nor defective cross-link repair (Fanconi anaemia cells) seem to delay the removal of formaldehyde-induced DNA–protein cross-links significantly and that more than one repair pathway may be involved in the removal of cross-links (Grafström *et al*., 1984; Speit *et al*., 2000). Human peripheral blood lymphocytes lost DNA–protein cross-links at a significantly slower rate than established cell lines (Quievryn & Zhitkovich, 2000). Repair of formaldehyde-induced DNA–protein cross-links was not changed significantly due to loss of GSH. Additional studies have indicated that the active removal of DNA–protein cross-links from cells may involve proteolytic degradation of cross-link proteins (Quievryn & Zhitkovich, 2000). Several studies indicate that formaldehyde may also interfere with the processes of repair of DNA by (*a*) inhibiting DNA repair enzymes, (*b*) inhibiting the removal of DNA lesions and (*c*) altering gene expression (Grafström *et al*., 1996).

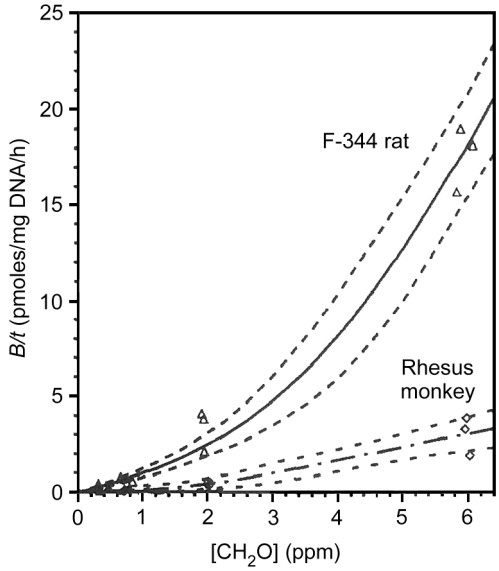
Assessment of DNA–protein cross-links and sister chromatid exchange in peripheral lymphocytes from formaldehyde-exposed workers demonstrated an association with overall exposure (Shaham *et al*., 1996a,b, 2003). It remains unclear to what extent these endpoints contribute to the mutagenesis and carcinogenicity of formaldehyde (Recio *et al.*, 1992; Merk & Speit, 1998; Speit *et al*., 2000; Liteplo & Meek, 2003).

Studies in humans have reported both positive and negative results for the incidence of micronuclei in nasal and buccal mucosa in workers exposed to formaldehyde (see Table 36). In one study of both buccal and nasal mucosa, a greater increase was observed in centromere-negative micronuclei than centromere-positive micronuclei, which implicates chromosome breakage as the major mechanism responsible for these effects (Titenko-Holland *et al.*, 1996). Although micronuclei are correlated with risk for cancer in animals, the association between micronuclei and cancer in humans requires clarification because of the high degree of inter-individual variability within the human population (Fenech *et al*., 1999).

The formation of DNA–protein cross-links in the nasal respiratory mucosa of rats after inhalation exposure to 6 ppm [7.4 mg/m^3] formaldehyde and higher has been demonstrated by a variety of techniques, including decreased extractability of DNA from proteins (Casanova-Schmitz & Heck, 1983), double-labelling studies with [^{3}H]- and [^{14}C]formaldehyde (Casanova-Schmitz *et al.*, 1984b; Casanova & Heck, 1987; Heck & Casanova, 1987) and isolation of DNA from respiratory mucosal tissue and quantification by HPLC of formaldehyde released from cross-links after exposure to [^{14}C]formaldehyde (Casanova *et al.*, 1989, 1994). The formation of DNA–protein cross-links is a non-linear function of concentration (Casanova & Heck, 1987; Casanova *et al.*, 1989, 1994; Heck & Casanova, 1995; see Fig. 8) and correlates with the site-specificity of tumours (Casanova *et al*., 1994). Cross-links were not detected in the olfactory mucosa or in the bone marrow of rats (Casanova-Schmitz *et al.*, 1984b; Casanova & Heck, 1987).

DNA–protein cross-links were also measured in the respiratory tract of groups of three rhesus monkeys immediately after single 6-h exposures to airborne [^{14}C]formaldehyde (0.7, 2 or 6 ppm [0.9, 2.4 or 7.4 mg/m^3]) (Casanova *et al.*, 1991). The concentrations of cross-linked formaldehyde in the nose of monkeys decreased in the order: middle

Figure 8. Average concentration of DNA–protein cross-links formed per unit time in the turbinates and lateral wall/septum of Fischer 344 rats and rhesus monkeys versus the airborne concentration of formaldehyde (CH_2O)



Adapted from Casanova *et al.* (1991)
All animals were exposed to formaldehyde for 6 h. Data in rats are taken from Casanova *et al.* (1989). (Results obtained in rats at 10 ppm are omitted for clarity.) Dashed lines are the 95% confidence limits about the mean for each species.

turbinates > lateral wall–septum > nasopharynx, which is consistent with the location and severity of lesions in monkeys exposed to 6 ppm (Monticello *et al.*, 1989). Very low levels of cross-links were also found in the trachea and carina of some monkeys. The yield of cross-links in the nose of monkeys was an order of magnitude lower than that in the nose of rats, due largely to species differences in minute volume and quantity of exposed tissue (Casanova *et al.*, 1991; Fig. 8). A pharmacokinetic model based on these results indicated that the concentrations of DNA–protein cross-links in the human nose would be lower than those in the noses of monkeys and rats (Casanova *et al.*, 1991).

In order to determine whether DNA–protein cross-links accumulate with repeated exposure, the yield of formaldehyde from cross-links was investigated in rats that were exposed either once or subchronically to unlabelled formaldehyde (6 or 10 ppm [7.4 or 12.3 mg/m^3]) for 6 h per day on 5 days per week for 11 weeks and 4 days (Casanova *et al.*, 1994). The yield was not higher in pre-exposed than in naive rats, which suggests that no accumulation had occurred in the former. The results also suggest that DNA–protein cross-links in the rat nasal mucosa are removed rapidly.

Conolly *et al*. (2000) described a model of tissue disposition of formaldehyde, which included CFD and measurements of mucosal epithelial thickness in order to refine previous predictions of the occurrence of DNA–protein cross-links in rats, monkeys and humans. The thickness of the epithelial tissue is approximately 4.5 times greater in humans than in rats, which results in a lesser net dose of formaldehyde in humans at concentrations that saturate the metabolic capacity of rats (Schlosser *et al*., 2003). Good correlations of model predictions and data of cross-links were obtained in association with the regions of high and low tumour response in the rat nose (Conolly *et al.*, 2000).

Merk and Speit (1998) evaluated the significance of DNA–protein cross-links for mutagenesis in V79 Chinese hamster cells. Formaldehyde was seen to induce DNA–protein cross-links, sister chromatid exchange and micronuclei in conjunction with a reduction in relative cloning efficiency. However, no gene mutations were observed in the *Hprt* test. It was concluded that DNA–protein cross-links were related to chromosomal effects, but not directly to gene mutations.

Shaham *et al*. (2003) reported that the formation of DNA–protein cross-links in peripheral lymphocytes in exposed workers was associated with increased serum p53 protein, including wild-type and mutant forms. Recio *et al*. (1992) found *p53* point mutations in 5/11 squamous-cell carcinomas in formaldehdye-exposed rats. Studies have shown (Luo *et al.*, 1994; Hemminki *et al.*, 1996) that higher levels of p53 protein are found in serum years before the diagnosis of malignant tumours such as lung cancer. [The Working Group felt that there may be an association, although further studies are needed to clarify the association between DNA–protein cross-links and effects on the state of p53.]

4.5.4 *Cytotoxicity and cell proliferation*

Formaldehyde is well established as a toxicant at the site of contact. Following inhalation exposure, cytotoxicity was evident in the nasal passages of rats and mice (Chang *et al*., 1983). Toxicity was greatest in the respiratory epithelium of rats, and the median septum and nasoturbinates were the sites most affected. At 15 ppm [18.45 mg/m^3], necrosis and sloughing of respiratory epithelium was evident immediately after a single 6-h exposure and early hyperplasia was present 18 h after a single exposure. Mice exhibited lower toxicity in the nasal epithelium associated with reduced exposure due to reduced minute volume. Severe ulcerative rhinitis, inflammation, epithelial hyperplasia and increased cell proliferation were observed in rats exposed to 15 ppm formaldehyde for 5 days (6 h per day). A second study examined cell proliferation and histopathology in rats exposed to 0, 0.7, 2, 6, 10 or 15 ppm [0, 0.86, 2.46, 7.38, 12.3, 18.45 mg/m^3] formaldehyde. Increased cell proliferation was evident at 6 and 15 ppm, but not at 2 ppm or lower following 9 days or 6 weeks of exposure (Monticello *et al*., 1991).

In a mechanistic 24-month carcinogenesis bioassay with interim sacrifices at 1, 4 and 9 days, 6 weeks, and 3, 6, 12 and 18 months, increased cell proliferation was demonstrated at all time-points in rats exposed to 6, 10 and 15 ppm (Monticello *et al*., 1991, 1996).

Monticello *et al*. (1996) evaluated the relationship of regional increases in nasal epithelial cell proliferation with nasal cancer in rats. Sublinear increases in both cell proliferation and squamous-cell carcinoma were observed with increasing concentration of formaldehyde. A good correlation was observed between site-specific tumour occurrence and the population-weighted unit length labelling index, which incorporates information on both the rate of cell replication and the numbers of cells at specific sites. It was therefore suggested that not only the rate at which cells divided but also the size of the target cell population that underwent DNA replication were crucial factors in the development of formaldehyde-induced nasal squamous-cell carcinoma. Both the rate of cell division and the number of cells in the proliferative pool are thought to increase the chance for mutation at a given dose of formaldehyde. Monticello *et al*. (1996) stated that, although there is evidence to suggest that concentration is more important than duration of exposure in determining the extent of formaldehyde-induced nasal epithelial damage, the development of formaldehyde-induced nasal squamous-cell carcinoma probably requires repeated and prolonged damage to the nasal epithelium. Sustained cellular proliferation, which was seen at 6, 10 and 15 ppm, is thought to play a crucial role in carcinogenesis through the transformation of cells with damaged DNA to mutated cells and the clonal expansion of the mutated cell population (McClellan, 1995).

Studies of cell cultures provide some support that proliferation is stimulated after exposure to formaldehyde (Tyihák *et al.*, 2001). Transcription of *ADH3* was associated with proliferative states (Nilsson *et al.*, 2004), formaldehyde and formate were shown to serve as donors to the one-carbon pool synthesis of macromolecules and, in one study, exposure to formaldehyde was shown to increase cell proliferation (Tyihák *et al*., 2001).

4.5.5 *Cancer*

Nasal squamous-cell carcinomas have been observed in toxicological studies of rats exposed to concentrations of at least 6 ppm formaldehyde (Swenberg *et al*., 1980; Kerns *et al*., 1983a,b). Epidemiological studies have also suggested increases in risk for sinonasal carcinomas and particularly for nasopharyngeal carcinoma in humans following exposure to formaldehyde. The exact biological mechanism by which exposure to formaldehyde may cause cancer is currently unknown (Conolly, 2002; Liteplo & Meek, 2003); however, formaldehyde causes formation of DNA–protein cross-links and increases cellular proliferation in the upper respiratory tracts of rats and monkeys.

However, most epidemiological studies of sinonasal cancer have not distinguished tumours that arise in the nose from those that develop in the nasal sinuses. Thus, any effect on the risk for nasal cancer specifically would tend to be diluted if there were no corresponding effect on the risk for cancer in the sinuses, and could easily be undetectable through the lack of statistical power.

4.5.6 *Computational dose–response modelling*

A theoretical model for DNA replication in the presence of DNA cross-links was developed by Heck and Casanova (1999). This model assumes that DNA–protein cross-links are formed randomly in the DNA and that replication can advance up to but not past a DNA–protein cross-link. This analysis is consistent with the experimental observation of decreased cell proliferation in the nasal epithelium of rats exposed to concentrations of 0.7 and 2.0 ppm [0.86 and 2.46 mg/m^3] formaldehyde.

A biologically based model of carcinogenesis of formaldehyde was described by Conolly (2002), which included a two-stage clonal growth model and used the incorporation of both DNA–protein cross-links and cell proliferation, together with linked regional dosimetry predictions, into the model to predict tumour incidence. Through inclusion of this information, the author suggested that a smaller degree of uncertainty was associated with the model.

Since both squamous-cell carcinoma and preneoplastic lesions develop in a characteristic site-specific pattern, Conolly *et al*. (2003) used anatomically accurate three-dimensional CFD models to predict the site-specific flux of formaldehyde from inhaled air into the tissues in rats. Flux into tissues was used as a dose metric for two modes of action: direct mutagenicity and cytolethality–regenerative cellular proliferation (CRCP). The two modes were linked to key parameters of a two-stage model of clonal growth. The direct mutagenic mode of action was represented by a low linear dose–response model of DNA–protein cross-link formation. An empirical J-shaped dose–response model and a threshold model fit to experimental data were used to describe CRCP. The J-shaped dose–response for CRCP provided a better description of the data on squamous-cell carcinoma than the threshold model. Sensitivity analysis indicated that the rodent tumour response is primarily due to the CRCP mode of action and the direct mutagenic pathway has little or no influence. The study suggests a J-shaped dose–response for formaldehyde-mediated nasal squamous-cell carcinoma in Fischer 344 rats. [The Working Group felt that uncertainty remains on some components and parameters of this model, which can affect both predictions of risk and qualitative aspects of model behaviour, and warrants further developmental work.]

Gaylor *et al*. (2004) conducted an analysis of the concentration–response relationship for formaldehyde-induced cell proliferation in rats using statistical methods designed to identify J-shaped concentration–response curves. Such J-shaped curves demonstrate an initial decline in response, followed by a return to background response rates at the zero equivalent dose and then an increase above background response at concentrations above the zero equivalent dose. This analysis demonstrated a statistically significant reduction in cytotoxicity at concentrations below the zero equivalent dose. The authors suggested that, at low doses, the increased risk for nasal cancer due to DNA damage may be offset by a reduction in cell proliferation, a postulate that is consistent with the threshold-like behaviour of the concentration–response curve observed for nasal cancer in rats.

Schlosser *et al*. (2003) described a dose–response assessment of formaldehyde that used a combination of benchmark dose and pharmacokinetic methods. Following the identification of points of departure for both tumours and cell proliferation in rats by analysis of the benchmark dose, extrapolation to humans was performed using either a CFD model alone to predict formaldehyde flux or a CFD model in combination with a pharmacokinetic model to predict the tissue dose and the formaldehyde-induced DNA–protein cross-links as a dose metric. Risk predictions obtained with the benchmark dose model are similar to earlier risk predictions obtained with previous benchmark dose models. The benchmark dose risk predictions are substantially higher than those obtained from the clonal growth time-to-tumour model (Liteplo & Meek, 2003).

4.5.7 *Summary of experimental data*

Formaldehyde is an essential metabolic intermediate in all cells, and average concentrations in the blood of unexposed subjects has been reported to be about 0.1 mmol/L. It is a potent nasal irritant, is cytotoxic at high doses and induces nasal cancer in rats exposed to high airborne concentrations. Nasal squamous-cell carcinomas have been observed in toxicological studies of rats exposed to concentrations of at least 6 ppm; the incidence of these tumours increases sharply with further increases in concentration (Monticello *et al.*, 1996). In addition, at concentrations of 2 ppm or less, no histological changes have been observed in rodents, although changes at these concentrations have been seen in humans (IARC, 1995; Liteplo & Meek, 2003).

Early assessments estimated the risk for cancer from exposure to low concentrations of formaldehyde using linear extrapolations from data on high concentrations in animals. However, linear low-dose extrapolation does not account for the sublinearities in the observed concentration–response relationship (Casanova *et al*., 1994; Monticello *et al*., 1996; Bolt, 2003). It was suggested that the risk for cancer in humans was probably overestimated by linear low-dose extrapolation from rats at concentrations that do not increase cell proliferation or the size of the cell population at risk (Monticello *et al*., 1996). Bolt (2003) recommended that predictions of risk at low (≤ 2 ppm) versus high (≥ 6 ppm) concentrations be made separately.

Formaldehyde demonstrates positive effects in a large number of in-vitro tests for genotoxicity, including bacterial mutation, DNA strand breaks, chromosomal aberrations and sister chromatid exchange. Studies in humans showed inconsistent results with regard to cytogenetic changes (micronuclei, chromosomal aberrations and sister chromatid exchange). The frequency of DNA–protein cross-links was found to be significantly higher in peripheral lymphocytes from exposed workers (Shaham *et al*., 1996, 2003).

In-vitro studies have shown that 0.1 mM [3 μg/mL] formaldehyde increases cell proliferation without detectable cytotoxicity (Tyihák *et al.*, 2001). Formaldehyde also induces genetic damage at concentrations as low as 0.1 mM and inhibits DNA repair in bronchial cells at 0.1–0.3 mM [3–9 μg/mL], although it does not appear to be associated with oxidative stress. Formaldehyde inhibits cell growth at 0.2 mM [6 μg/mL] and 0.6 mM

[18 μg/mL], as expressed by loss of clonal growth rate and colony-forming efficiency, respectively. It alters membrane integrity, which leads to increases in cellular Ca^{2+} and could affect cell death rates by accelerating terminal differentiation (Grafström *et al.*, 1996). Apoptosis has been demonstrated in rat thymocytes at concentrations at or above 0.1 mM (Nakao *et al.*, 2003). HeLa cervical carcinoma cells have been shown to be fairly resistant to the toxic effects of formaldehyde in comparison with untransformed human dental pulp fibroblasts and buccal epithelial cells (Lovschall *et al.*, 2002). In contrast, formaldehyde is only weakly genotoxic in in-vivo tests.

Using a variety of techniques, inhalation of formaldehyde has been shown to induce the formation of DNA–protein cross-links in nasal tissue from rats and monkeys, including decreased extractability of DNA from proteins, labelling studies and isolation of DNA by HPLC. The formation of DNA–protein cross-links is a non-linear function of formaldehyde concentration, described by an initial linear component with a shallow slope and a second linear component with a steeper slope that follows a significant decrease in survival of the cells and depletion of GSH at about 0.1 mM (Merk & Speit, 1998).

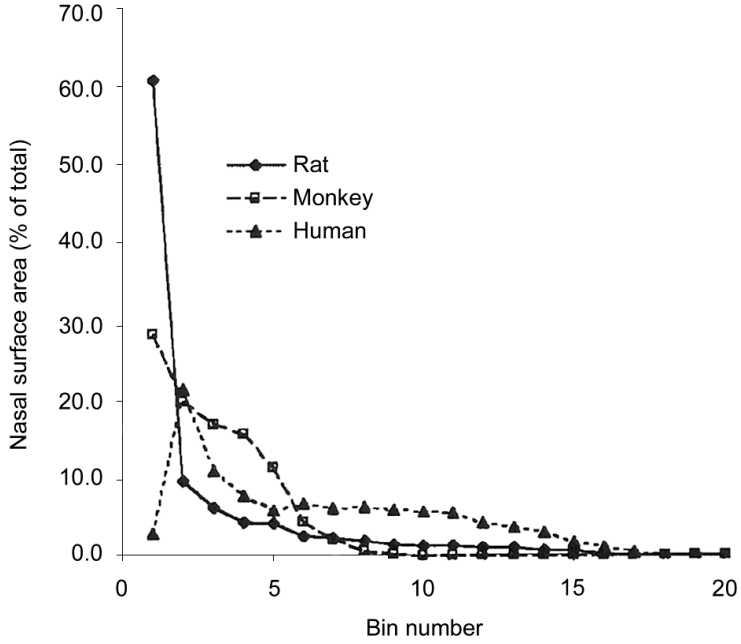
Species-specific differences in the rate of formation of DNA–protein cross-links, in the prevalence of squamous metaplasia (McMillan *et al.*, 1994) and in the occurrence of nasal cancer have also been observed at given concentrations of formaldehyde in rats, mice and monkeys (McMillan *et al.*, 1994; McClellan, 1995). Differences in nasal anatomy and regional airflow as well as differences in the absorptive properties of the nasal lining between species have been suggested to influence greatly the dose of formaldehyde that reaches different anatomical regions and subsequent formaldehyde-induced lesions at a given exposure concentration (Kimbell *et al*., 1997; Kepler *et al*., 1998; Zito, 1999; Kimbell *et al*,. 2001b; Conolly, 2002).

Anatomically accurate CFD models have been developed to describe the nasal uptake of formaldehyde in rats, monkeys and humans (Conolly, 2000). Interspecies differences in formaldehyde-induced DNA–protein cross-links and nasal lesions appear to be related to species-specific patterns in formaldehyde flux in different regions within the nasal passages (see Fig. 9; Kimbell *et al*., 2001a). CFD modelling predicts anterior-to-posterior gradients of formaldehyde deposition in the noses of rats, rhesus monkeys and humans (Kimbell *et al*., 2001a). This might suggest that formaldehyde is more likely to cause cancer of the nose than of the nasopharynx in humans.

Although formaldehyde is mainly deposited in the upper respiratory tract, the distribution of the deposition is expected to vary by species (Conaway *et al*., 1996; Liteplo & Meek, 2003). The lack of parallel data on the formation of nasal mucosal DNA–protein cross-links and on cell proliferation in humans complicates the extrapolation of the animal data, in particular rodent data, to humans (Conolly *et al*., 2000; Liteplo & Meek, 2003).

The current data indicate that both genotoxicty and cytoxicity play important roles in the carcinogenesis of formaldehyde in nasal tissues. DNA–protein cross-links provide a potentially useful marker of genotoxicity. The concentration–response curve for the formation of DNA–protein cross-links is bi-phasic, and the slope increases at concentrations of formaldehyde of about 2–3 ppm [2.5–3.7 mg/m^3] in Fischer 344 rats. Similar results have been

Figure 9. Allocation of nasal surface area to flux bins in rats, monkeys and humans



Adapted from Kimbell *et al.* (2001a)
Low bin numbers are associated with low flux values. Inspiratory airflow rates were 0.576 L/min in rats, 4.8 L/min in monkeys and 15 L/min in humans.

observed in rhesus monkeys, although the dose–response curve for DNA–protein cross-links is less well defined in this species. Cellular proliferation, which appears to amplify the genotoxic effects of formaldehyde greatly, increases considerably at concentrations of formaldehyde of about 6 ppm [7.4 mg/m^3], and results in a marked increase in the occurrence of malignant lesions in the nasal passages of rats at concentrations of formaldehyde above this level (Monticello *et al.*, 1996). Recent evidence suggests that cytotoxicity may demonstrate a J-shaped concentration–response curve, with a significant reduction in cellular proliferation rates at concentrations of 0.7–2 ppm [0.86–2.5 mg/m^3] (Conolly & Lutz, 2004; Gaylor *et al.*, 2004).

4.5.8 *Leukaemia*

The epidemiological evidence that formaldehyde may cause acute myelogenous leukaemia raises a number of mechanistic questions, including the processes by which inhaled formaldehyde may reach a myeloid progenitor (Heck & Casanova, 2004). The possibility that formaldehyde causes leukaemia is supported by the detection of cytogenetic abnormalities and an increase in the fraction of DNA–protein cross-links in circulating lymphocytes following occupational exposure to formaldehyde, as well as chromosomal aberrations in

bone marrow in a single inhalation study in rats (Kitaeva *et al.*, 1990, 1996; Shaham *et al.*, 1996a,b, 1997, 2003).

It is possible that formaldehyde itself can reach the bone marrow following inhalation, although the evidence is inconsistent (Kitaeva *et al.*, 1990; Dallas *et al*., 1992; Heck & Casanova, 2004). The relatively rapid rate of metabolism of formaldehyde by circulating red blood cells suggests that little if any inhaled formaldehyde could reach any tissue beyond the respiratory tract. [The Working Group noted that a clastogenic product of formaldehyde could conceivably be formed in the blood and circulate to the bone marrow, although this has not been suggested in the literature. Alternatively, it is possible that circulating myeloid progenitor stem cells could be the source of leukaemia. Such stem cells are known to be present in the blood and plausibly could be exposed to formaldehyde in the respiratory tract vasculature; however, there is currently no known prototype for such a mechanism of leukaemogenesis.]

Another mechanistic issue is the relation of cellular background levels of formaldehyde, as part of the one-carbon pool, to any risk from exogenous formaldehyde. Cellular background concentrations of formaldehyde are about 0.1 mM. In one study in humans, inhalation of 1.9 ppm [2.3 mg/m^3] formaldehyde for 40 min did not alter blood concentrations (Heck *et al.*, 1985). Elevated concentrations of formaldehyde were not observed in the blood of rats and rhesus monkeys exposed to formaldehyde at concentrations of up to 14.4 ppm [17.7 mg/m^3] (Heck *et al.*, 1985; Casanova *et al.*, 1988); nor were protein adducts or DNA–protein cross-links observed in the bone marrow of rats or rhesus monkeys exposed to formaldehyde at concentrations of up to 15 or 6 ppm [18.4 or 7.4 mg/m^3], respectively (Casanova-Schmitz *et al.*, 1984b; Heck *et al.*, 1989).

Known human myeloid leukaemogens include ionizing radiation, benzene and chemotherapeutic agents such as alkylators, DNA topoisomerase inhibitors and DNA-complexing agents. All of these agents produce overt bone marrow toxicity, including pancytopenia and aplastic anaemia in both laboratory animals and humans. Agents known to cause leukaemia in humans and animals are also known to induce chromosomal aberrations in peripheral lymphocytes. The absence of clear evidence of bone-marrow toxicity, even at high doses, indicates that, if formaldehyde is a human myeloid leukaemogen, its mechanism of action differs from those by which the known myeloid leukaemogens noted above operate. This is not inconceivable, because there is epidemiological evidence that butadiene and ethylene oxide are probably human leukaemogens, although neither is a classic bone-marrow toxicant.

Studies of workers exposed to benzene and of individuals who received cancer chemotherapy suggest that the induction period for acute myeloid leukaemia is less than 20 years, and is usually in the range of 2–15 years (Goldstein, 1990). This relatively short lag time for carcinogenesis reflects the intrinsic biology of the myeloid progenitor cell, irrespective of the mechanism that caused the initial cancer mutation. The epidemiological reports that suggest an association of formaldehyde with myeloid leukaemia have not clearly defined an induction period for the effect.

[The Working Group could not identify any rodent model for acute myeloid leukaemia. The lack of evidence of increased risk for leukaemia in long-term studies of formaldehyde in rodents is not informative with respect to myeloid leukaemogenesis in humans. The Working Group felt that, based on the data available at this time, it was not possible to identify a mechanism for the induction of myeloid leukaemia in humans by formaldehyde.]