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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 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.

The endogenous concentration of formaldehyde, determined by gas chromatography–mass spectrometry (Heck *et al.*, 1982) in the blood of human subjects not exposed to formaldehyde, was 2.61 ± 0.14 μg/g of blood (mean ± 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 endogenous formaldehyde in the blood, both free and reversibly bound.

The possibility that gaseous formaldehyde may be adsorbed to respirable particles, inhaled and subsequently released into the lung has been examined. Risby *et al.* (1990) developed and validated a model to describe the adsorption of formaldehyde to and release from respirable carbon black particles. They concluded that of an airborne concentration of 6 ppm [7.4 mg/m^3], only 2 ppb [0.0025 mg/m^3] would be adsorbed to carbon black. Rothenberg *et al.* (1989) investigated the adsorption of formaldehyde to dust particles in homes and offices and concluded that, even with a concentration of 1 ppm formaldehyde (1.2 mg/m^3), the particle-associated dose to the pulmonary compartment of an adult human would be approximately 0.05 μg/h, whereas the dose of vapour-phase formaldehyde delivered to the upper respiratory tract would be 500 μg/h, i.e. four orders of magnitude larger.

Since formaldehyde can induce allergic contact dermatitis in humans (section 4.2.1), it can be concluded that formaldehyde or its metabolites penetrate human skin (Maibach, 1983). The kinetics of this penetration were determined *in vitro* using a full-thickness 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, flowing unidirectionally beneath the sample) was 16.7 μg/cm^2 per h when a 3.7% solution of formaldehyde was used, and increased

to 319 μg/cm^2 per h when a 37% solution was used. The presence of methanol in both of these solutions (at 3.3–4.9% and 10–15%, respectively) may have affected the uptake rate, and it is unclear whether the resorbed ^{14}C was due only to formaldehyde. Skin retention of formaldehyde represented a significant fraction of the total amount of formaldehyde absorbed.

The concentration of formaldehyde was 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. The measured value was 2.77 ± 0.28 μg/g, which was not different from the pre-exposure concentration due to metabolically formed formaldehyde (see above). The absence of an increase is understandable, since formaldehyde is rapidly metabolized by human erythrocytes (Malorny *et al.*, 1965), which contain formaldehyde dehydrogenase (Uotila & Koivusalo, 1987) and aldehyde dehydrogenase (Inoue *et al.*, 1979).

A gas chromatographic method was used to examine the urinary excretion of formate by veterinary medical students 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 the level varied considerably both within and among subjects (range, 2.4–28.4 mg/L). No significant changes in concentration were detected over a three-week period of exposure to formaldehyde at a concentration in air of less than 0.4 ppm [0.5 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.

4.1.2 Experimental systems

The steady-state concentrations of endogenous formaldehyde have been determined by gas chromatography–mass spectrometry (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). The blood concentrations of formaldehyde immediately after exposure of rats once to 14.4 ppm [17.6 mg/m^3] (2 h) or exposure of monkeys subacutely to 6 ppm [7.3 mg/m^3] (6 h/day, five days/week, four weeks) were indistinguishable from those before exposure.

As reported in an abstract, more than 93% of a dose of inhaled formaldehyde was absorbed readily by the tissues of the respiratory tract (Patterson *et al.*, 1986). 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).

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 Figure 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 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. The terminal half-time of the radioactivity was approximately 55 h (Heck *et al.*, 1983), but the half-time of formaldehyde in rat plasma after intraperitoneal administration is reported to be approximately 1 min (Rietbrock, 1965). 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).

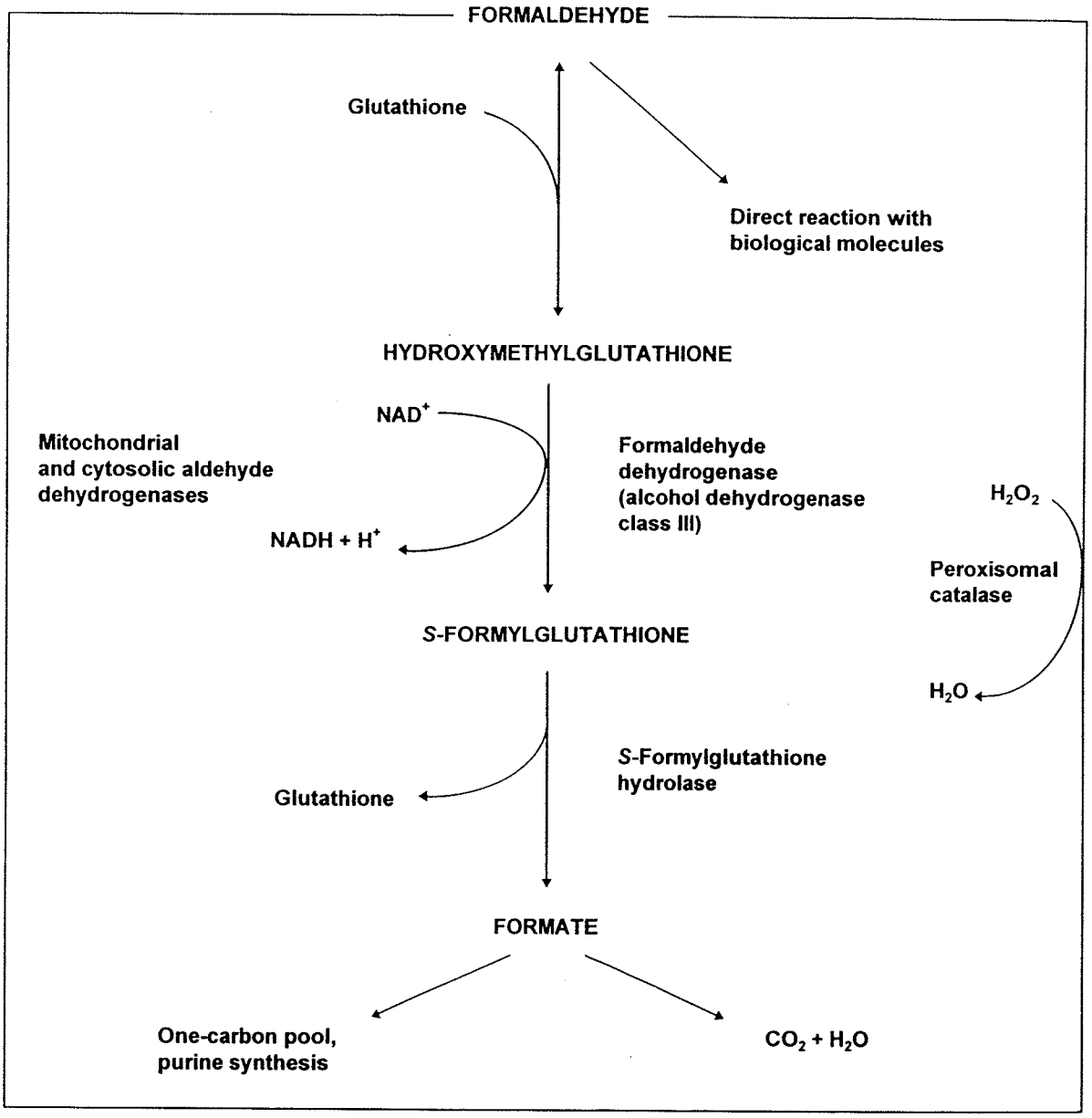
The fate of ^{14}C-formaldehyde after topical application to Fischer 344 rats, Dunkin–Hartley guinea-pigs and cynomolgus monkeys was described by Jeffcoat *et al.* (1983). Aqueous formaldehyde was applied to a shaven area of the lower back, and the rodents were placed in metabolism cages for collection 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 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.

Formaldehyde reacts rapidly with glutathione, forming a hemithioacetal, *S*-hydroxymethylglutathione, which is a substrate for the cytosolic enzyme, formaldehyde dehydrogenase [formaldehyde:NAD$^+$ oxidoreductase (glutathione-formylating), EC 1.2.1.1] (Uotila & Koivusalo, 1974a). With NAD$^+$ as a cofactor, this enzyme catalyses the oxidation of *S*-hydroxymethylglutathione to *S*-formylglutathione. The latter compound is hydrolysed to formate by

S-formylglutathione hydrolase [EC 3.1.2.12], regenerating free glutathione (Uotila & Koivusalo, 1974b).

Figure 1. Metabolism and fate of formaldehyde



Formaldehyde dehydrogenase has been identified in a number of tissues in several species (Koivusalo *et al.*, 1982). The activity of formaldehyde dehydrogenase is similar in the respiratory and olfactory mucosa of rats (Casanova-Schmitz *et al.*, 1984a; Bogdanffy *et al.*, 1986; Keller *et al.*, 1990). This enzyme is structurally identical to another well-characterized enzyme, class III alcohol dehydrogenase [alcohol:NAD^+ oxidoreductase, EC 1.1.1.1] (Kaiser *et al.*, 1991; Danielsson & Jörnvall, 1992), which catalyses the oxidation of long-chain primary

alcohols to aldehydes; in contrast to the well-characterized class I alcohol dehydrogenase, however, it has low affinity for ethanol and is not inhibited by 4-methylpyrazole. Class III alcohol dehydrogenase does not require glutathione when catalysing the oxidation of primary alcohols, but a thiol group is essential for the oxidation of formaldehyde, presumably because a hemithioacetal is formed which is structurally similar to a primary alcohol (Holmquist & Vallee, 1991). Numerous other thiols perform this function at nearly the same rate as glutathione (Holmquist & Vallee, 1991). Aldehydes other than formaldehyde are not oxidized by the enzyme.

Because formaldehyde dehydrogenase and class III alcohol dehydrogenase are identical, it cannot be concluded that the normal function of 'formaldehyde dehydrogenase' *in vivo* is solely to catalyse the oxidation of formaldehyde. Lam *et al.* (1985) and Casanova and Heck (1987) found that depletion of glutathione, either by inhalation of acrolein or by intraperitoneal injection of phorone, increased the amount of DNA–protein cross-links in the nasal mucosa of rats exposed to formaldehyde, implying that formaldehyde oxidation (detoxification) was partially inhibited. The authors postulated that depletion of glutathione had decreased the concentration of *S*-hydroxymethylglutathione, resulting in an increase in the tissue concentration of formaldehyde. Dicker and Cederbaum (1985, 1986) showed, however, that phorone not only depletes glutathione but can also inhibit a mitochondrial low-K_m aldehyde dehydrogenase, which may also be important for the oxidation of formaldehyde. The low-K_m mitochondrial aldehyde dehydrogenase [aldehyde:NAD^+ oxidoreductase, EC 1.2.1.3] catalyses the oxidation of both formaldehyde and acetaldehyde, although acetaldehyde is the preferred substrate of both. This enzyme is strongly inhibited by cyanamide, which acts by inhibiting the uptake and oxidation of formaldehyde by mitochondria and isolated rat hepatocytes (Dicker & Cederbaum, 1984). Inhibition of formaldehyde oxidation in hepatocytes was incomplete, however, presumably because formaldehyde was also being oxidized by the cytosolic formaldehyde dehydrogenase. The authors concluded that both formaldehyde dehydrogenase and the low-K_m mitochondrial aldehyde dehydrogenase contribute to the overall metabolism of formaldehyde in isolated rat hepatocytes, but, as the two enzymes have different K_m values, the importance of each is dependent on the formaldehyde concentration (Dicker & Cederbaum, 1986).

The experiments of Dicker and Cederbaum (1984, 1985, 1986) are useful for understanding the metabolism of formaldehyde in general and in hepatocytes in particular, but their relevance to the toxicology of inhaled formaldehyde is uncertain. Although aldehyde dehydrogenase activity was identified in rat nasal mucosa (Casanova-Schmitz *et al.*, 1984a; Bogdanffy *et al.*, 1986), it is not known whether this activity is due to the low-K_m mitochondrial aldehyde dehydrogenase. Moreover, the subcellular location of the low-K_m enzyme within the mitochondria might restrict its accessibility to exogenous formaldehyde and, therefore, impair its ability to metabolize the compound. Thus, the role of this dehydrogenase in the detoxification of inhaled formaldehyde is presently unknown.

Oxidation of formaldehyde to formate may also be mediated by catalase, which is located in peroxisomes. In this reaction, formaldehyde acts as a hydrogen donor for the peroxidative decomposition of the catalase–hydrogen peroxide complex. This reaction contributes less to the overall metabolism of formaldehyde in isolated, perfused rat liver than other pathways, owing to

the rate-limiting generation of hydrogen peroxide (Waydhas *et al.*, 1978). The latter compound is also decomposed by the glutathione peroxidase system, resulting in depletion of glutathione and the production of oxidized glutathione. In hepatocytes in which glutathione has been depleted, hydrogen peroxide production is increased, which may result in increased metabolism of formaldehyde via catalase (Jones *et al.*, 1978).

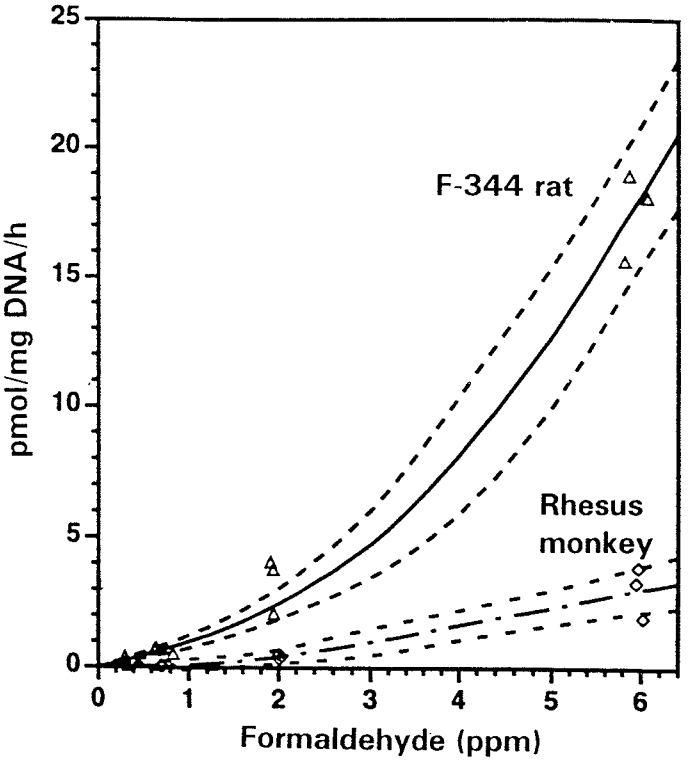
Incubation of formaldehyde with human nasal mucus *in vitro* resulted in the reversible formation of protein adducts, primarily with albumin, suggesting that a portion of the inhaled formaldehyde is retained in the mucous blanket (Bogdanffy *et al.*, 1987). No adducts were found in high relative-molecular-mass glycoproteins. Absorbed formaldehyde may react with nucleophiles (e.g. amino and sulfhydryl groups) at or near the absorption site, or it can be oxidized to formate and exhaled as carbon dioxide or incorporated into biological macromolecules via tetrahydrofolate-dependent one-carbon biosynthetic pathways.

Several of the urinary excretion products of formaldehyde in rats have been identified after intraperitoneal administration of ^{14}C-formaldehyde. After injecting Wistar rats with 0.26 mg/kg bw, Hemminki (1984) detected formate and a sulfur-containing metabolite (thought to be a derivative of thiazolidine-4-carboxylic acid) and products presumed to result from one-carbon metabolism. Thiazolidine-4-carboxylate, which is formed via the nonenzymatic condensation of formaldehyde with cysteine, was not detected in urine.

After Sprague–Dawley rats were injected intraperitoneally with 4 or 40 mg/kg bw of ^{14}C-formaldehyde, formate (80% of the total radioactivity in urine), *N*-(hydroxymethyl)- and *N,N'*-bis(hydroxymethyl)urea (15% of urinary radioactivity) (which appeared to have resulted from the condensation of formaldehyde with urea) and an unidentified product (5% of the total) were identified (Mashford & Jones, 1982). As the urine of the Sprague–Dawley rat contains little, if any, cysteine, formation of thiazolidine-4-carboxylate is precluded and urea-containing adducts can be formed. The existence of these adducts suggests that, at least in Sprague–Dawley rats administered large doses of formaldehyde, a portion of the injected material (about 3–5% at a dose of 40 mg/kg bw) is excreted unchanged in the urine. After exposure by inhalation, however, it is questionable whether a significant amount of formaldehyde is excreted unchanged in the urine, since such high dose levels are not attainable by this route.

The formation of DNA–protein cross-links by formaldehyde in the nasal respiratory mucosa of rats after exposure to 6 ppm [7.3 mg/m^3] and more 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 of cross-links by high-performance liquid chromatography after exposure to ^{14}C-formaldehyde (Casanova *et al.*, 1989, 1995). The formation of DNA–protein cross-links is a nonlinear function of concentration (Casanova & Heck, 1987; Casanova *et al.*, 1989, 1995; Heck & Casanova, 1995; see Figure 2). Cross-links were not detected in the olfactory mucosa or in the bone marrow of rats (Casanova-Schmitz *et al.*, 1984b; Casanova & Heck, 1987).

Figure 2. Concentration of DNA–protein cross-links formed per unit time in the turbinates and lateral wall/septum of Fischer 344 rats and rhesus monkeys in relation to airborne formaldehyde concentration



Reproduced, with permission, from Casanova *et al.* (1991)
All animals were exposed for 6 h. Dashed lines are the 95% confidence limits around the mean for each species.

DNA–protein cross-links were also measured in the respiratory tracts 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 7.3 mg/m^3]) (Casanova *et al.*, 1991). The concentrations of cross-links in the nose of monkeys decreased in the order: middle turbinates > lateral wall–septum > nasopharynx, and this order 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, but none were detected in the maxillary sinus. The yield of cross-links in the nose of monkeys was approximately 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; Figure 2). 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).

The yields of DNA–protein cross-links produced in rats exposed to formaldehyde (at 0.7, 2, 6 or 15 ppm [0.9, 2.4, 7.3 or 18.3 mg/m^3] for 6 h/day, five days/week for 11 weeks and four days) were compared with those produced in naive (previously unexposed) rats (Casanova *et al.*,

1995). The acute yields of cross-links (pmol/mg DNA) were determined in the lateral meatus (susceptible tumour site; see section 3.1 (Morgan *et al.*, 1986a)) and in the medial and posterior meatuses (low susceptibility site (Morgan *et al.*, 1986a)) after a single 3-h exposure of pre-exposed and naive rats to the same concentration of ^{14}C-formaldehyde. At 0.7 and 2 ppm, the acute yields of cross-links in the lateral meatus of pre-exposed rats were indistinguishable from those of naive rats; at 6 and 15 ppm, the acute yields in pre-exposed rats were approximately half those of naive rats, and the difference was significant (Figure 3). Pre-exposed animals had lower concentrations of cross-links than naive rats at 6 and 15 ppm partly because of an increase in total DNA in the target tissue caused by cell proliferation (Heck & Casanova, 1995; see section 4.2.2). The acute yields of DNA–protein cross-links in the medial and posterior meatuses were similar in pre-exposed and naive rats at all concentrations and were lower than the acute yields in the lateral meatus. This result is consistent with the location and severity of lesions in the rat nose (Morgan *et al.*, 1986a).

In order to determine whether DNA–protein cross-links accumulate with repeated exposure, the cumulative yield was investigated using reduced DNA extractability as a measure of cross-linking. Rats were exposed subchronically to unlabelled formaldehyde (6 or 10 ppm [7.3 or 12.2 mg/m^3]; 6 h/day, five days/week, 11 weeks and four days) (Casanova *et al.*, 1995), and the cumulative yields of DNA–protein cross-links in the nasal mucosa of pre-exposed rats were compared with those in naive rats after a single 3-h exposure to the same concentration of unlabelled formaldehyde. A concentration-dependent increase in the yield of DNA–protein cross-links over that in unexposed controls was seen in both pre-exposed and naive rats. The yield was not higher in pre-exposed than in naive rats, suggesting that no accumulation had occurred in pre-exposed rats. The results suggest that DNA–protein cross-links in the rat nasal mucosa are rapidly repaired.

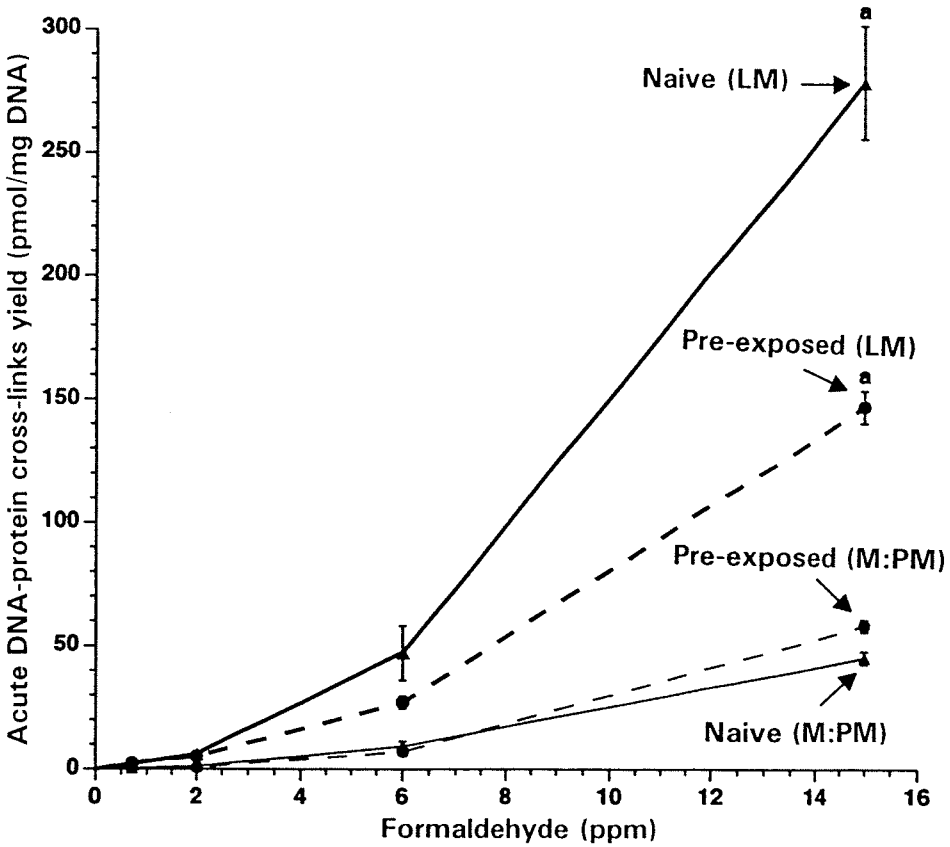
An anatomically based pharmacokinetic model was developed for determining the site-specificity of cross-link formation in the nasal mucosa of Fischer 344 rats (Heck & Casanova, 1995) and rhesus monkeys (Casanova *et al.*, 1991). The model is based on the assumption that the site-specificity of cross-links is due to nasal airflow and absorption patterns, rather than to site-specific differences in metabolism (Casanova *et al.*, 1991; Heck & Casanova, 1995). Parameter estimation indicates that at concentrations of less than about 3 ppm [3.7 mg/m^3], about 90% of a dose of inhaled formaldehyde is eliminated by saturable metabolism, 10% is eliminated by nonsaturable pathways and only $1/10^6$ (i.e. 10^{-4} %) exists as DNA–protein cross-links immediately after exposure. The amount bound to DNA increases sublinearly with respect to concentration but linearly with respect to time during exposure (Heck & Casanova, 1995). Computer simulations of nasal airflow and formaldehyde absorption patterns at specific sites in the nose of rats are generally consistent with the experimental results on the site-specificity of DNA–protein cross-links (Kimbell *et al.*, 1993).

4.2 Toxic effects

The toxicity of formaldehyde in humans and experimental systems has been reviewed (IARC, 1982; Heck & Casanova-Schmitz, 1984; Feinman, 1988; WHO, 1989; Heck *et al.*, 1990;

American Conference of Governmental Industrial Hygienists, 1991; Bardana & Montanaro, 1991; Restani & Galli, 1991; Vaught, 1991; Leikauf, 1992).

Figure 3. Acute yields of DNA–protein cross-links (mean ± SE) in the lateral meatus (LM) and medial and posterior meatuses (M:PM) of pre-exposed and naive (previously unexposed) Fischer 344 rats immediately after a single 3-h exposure to ^{14}C-formaldehyde



Adapted, with permission, from Casanova *et al.* (1995)
Pre-exposed rats were exposed subchronically to the same concentrations of unlabelled formaldehyde (6 h/day, five days/week, for 11 weeks and four days), while naive rats were exposed to room air. Exposure to ^{14}C-formaldehyde occurred on the fifth day of the twelfth week, and the acute yields pertain to the DNA–protein cross-links produced at that time.

4.2.1 Humans

(a) Acute effects

(i) Odour detection

The threshold for detection of formaldehyde odour was determined among 22 nonsmokers and 22 aged-matched, heavy smokers (all female) (Berglund & Nordin, 1992). Odour was detected at 25–144 ppb (31–177 μg/m^3) by nonsmokers and at 20–472 ppb (25–581 μg/m^3) by smokers ($p < 0.01$).

(ii) Irritation

The following studies of healthy humans given short-term exposures to formaldehyde under controlled conditions indicate that the irritation threshold for eyes, nose and throat is 0.5–1 ppm (0.6–1.2 mg/m^3).

Irritation thresholds were determined in subjects exposed to steadily increasing (0–3.2 ppm [0–3.9 mg/m^3] over 37 min) or to constant formaldehyde concentrations (0, 1, 2, 3 or 4 ppm [0, 1.2, 2.4, 3.7 or 4.9 mg/m^3], 1.5 min per exposure). The thresholds for eye and nose irritation were between 1 and 2 ppm (1.2–2.5 mg/m^3); the threshold for throat irritation was > 2 ppm (Weber-Tschopp *et al.*, 1977)..

Workers exposed to 0.35–1.0 ppm [0.43–1.2 mg/m^3] for 6 min had a significant irritation response at 1.0 ppm; nonsignificant responses were reported at 0.7 and 0.9 ppm [0.9 and 1.1 mg/m^3] (Bender *et al.*, 1983).

Among nonsmokers exposed to 0.5–3.0 ppm [0.6–3.7 mg/m^3], some subjects reported eye irritation at 1.0 ppm, and one reported nose and throat irritation at 0.5 ppm (Kulle *et al.*, 1987). Tolerance to the irritating effects of formaldehyde developed during prolonged exposure to concentrations above 1 ppm (Andersen & Mølhave, 1983).

Respiratory and ocular irritation has been reported by occupants of mobile homes (see section 1) and offices where there are low levels of formaldehyde (Hanrahan *et al.*, 1984; Bracken *et al.*, 1985; Ritchie & Lehnen, 1987; Broder *et al.*, 1988a,b,c; Liu *et al.*, 1991) and by medical students, histology technicians and embalmers, who may be exposed briefly to higher concentrations (Kilburn *et al.*, 1985; Holness & Nethercott, 1989; Uba *et al.*, 1989). In general, the reported thresholds for irritation in uncontrolled environments are lower than those in controlled exposures. The answers to a questionnaire indicated that a few individuals experienced sensory irritation at concentrations as low as 0.1 ppm [0.12 mg/m^3]; however, the contribution of other substances is unknown.

(iii) Pulmonary function

Fifteen healthy nonsmokers and 15 asthmatic subjects were exposed to 2 ppm [2.4 mg/m^3] formaldehyde for 40 min to determine whether acute exposures could induce asthmatic symptoms (Schachter *et al.*, 1986; Witek *et al.*, 1987). On separate days, the subjects either remained at rest or engaged in moderate exercise, and pulmonary function was measured before, during, immediately after or 24 h after exposure. No significant airway obstruction or changes in pulmonary function were noted. Neither healthy nor asthmatic subjects had bronchial hyperreactivity, as shown by responsiveness to methacholine.

Similar observations were made on a group of 15 hospital laboratory workers who had been exposed to formaldehyde (Schachter *et al.*, 1987). The subjects were exposed in an environmental chamber to 2.0 ppm [2.4 mg/m^3] for 40 min on four occasions, during two of which the subjects were at rest and during two of which they performed moderate exercise. Lung function was unaltered on all four days, and there were no delayed obstructive changes or increased reactivity to methacholine.

Healthy nonsmokers (nine subjects for 3 h, 22 for 1 h) and asthmatic subjects (nine subjects for 3 h, 16 for 1 h) were exposed to 3.0 ppm [3.7 mg/m^3] formaldehyde, either at rest or when

engaged in intermittent heavy exercise. Pulmonary function and nonspecific airway reactivity were assessed before, during and up to 24 h after exposure. No significant changes were observed among asthmatic subjects. Small decreases (< 5%) in pulmonary function (forced expiratory volume at one second, forced vital capacity) were observed in healthy nonsmokers exposed to formaldehyde while engaging in heavy exercise. Two normal and two asthmatic subjects had decrements greater than 10% at two times. There were no changes in nonspecific airway reactivity (as judged by the methacholine challenge test) (Sauder *et al.*, 1986; Green *et al.*, 1987; Sauder *et al.*, 1987).

Healthy nonsmokers were exposed for 3 h at rest to 0, 0.5, 1.0, 2.0 or 3.0 ppm [0, 0.6, 1.2, 2.4 or 3.7 mg/m^3] formaldehyde; they were also exposed to 2.0 ppm while exercising. Nasal flow resistance was increased at 3.0 ppm but not at 2.0 ppm. There was no significant decrement in pulmonary function or increase in bronchial reactivity to methacholine with exposure to 3.0 ppm at rest or to 2.0 ppm with exercise (Kulle *et al.*, 1987).

A group of 24 healthy nonsmokers were exposed while engaged in intermittent heavy exercise for 2 h to formaldehyde at 3 ppm [3.7 mg/m^3] or to a mixture of formaldehyde and 0.5 mg/m^3 of respirable carbon aerosol, in order to determine whether adsorption of formaldehyde on respirable particles elicits a pulmonary response. Small (< 5%) decreases were seen in forced vital capacity and forced expiratory volume, but these effects were not considered to be clinically significant (Green *et al.*, 1989). As noted previously, Risby *et al.* (1990) and Rothenberg *et al.* (1989) estimated that the amount of formaldehyde adsorbed onto carbon black or dust particles and delivered to the deep lung by particle inhalation is minuscule in relation to the amount that remains in the vapour phase and is adsorbed in the upper respiratory tract.

In a study of controlled exposure to formaldehyde, 18 subjects, nine of whom had complained of adverse effects from urea–formaldehyde foam insulation installed in their homes, were exposed to 1 ppm [1.2 mg/m^3] formaldehyde or to off-gas products of urea–formaldehyde foam insulation containing 1.2 ppm [1.5 mg/m^3] formaldehyde, for 90 min (Day *et al.*, 1984). No statistically or clinically significant change in pulmonary function was seen either during or 8 h after exposure, and no evidence was obtained that urea–formaldehyde foam insulation off-gas acts as a lower airway allergen. When 15 asthmatic subjects were exposed for 90 min to concentrations of 0.008–0.85 mg/m^3 formaldehyde, no change in pulmonary function was seen, and there was no evidence of an increase in bronchial reactivity (Harving *et al.*, 1990).

(b) Chronic effects

(i) Effects on the nasal mucosa

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. Samples of cells were collected with a swab inserted 2–3 cm into the nostrils of subjects living in urea–formaldehyde foam-insulated homes and of subjects living 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 one year later (Broder *et al.*, 1991) showed a decrease in nasal signs that was unrelated to any decrease in formaldehyde levels.

Cell smears were collected with a swab inserted 6–8 cm into the nose from 42 workers employed in two phenol–formaldehyde plants and 38 controls with no known exposure to formaldehyde. The formaldehyde concentrations 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, but there was no association 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 ages. The formaldehyde concentrations in the three plants were 0.1–1.1 mg/m^3, with peak concentrations 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 years of employment. There was no detectable difference in the nasal histology of workers exposed to formaldehyde alone and 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 engaged in the manufacture of resins for laminate production, 89 workers employed in furniture factories who were exposed to particle-board and glue, and 32 controls, who were mainly clerks in a local government office. The formaldehyde concentrations in the resin manufacturing plant were 0.05–0.5 mg/m^3, with frequent peaks over 1 mg/m^3. The concentrations in the furniture factories were 0.2–0.3 mg/m^3, with rare peaks to 0.5 mg/m^3; these workers were also exposed to wood dust (1–2 mg/m^3). The control group was exposed to concentrations of formaldehyde of 0.09–0.17 mg/m^3. The average length of employment was about 10 years. The histological scores of workers exposed to formaldehyde alone were slightly but significantly higher than those of controls, but the histological scores of workers 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.* (1989b). [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 from 37 workers exposed at a chemical company where formaldehyde resins were produced and from 37 age-matched controls. The formaldehyde concentrations 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 commoner among the exposed workers than the controls, but the difference was not significant. The histological scores increased with age and with exposure concentration and duration, 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 released from a urea–formaldehyde glue in a plywood factory. Each subject was paired with a control 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 time-weighted) 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. There was 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). 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).

The effects of formaldehyde, other than cancer, on the nasal mucosa are summarized in Table 20.

(ii) Pulmonary function

Pulmonary function has been assessed in residents of mobile and conventional homes (Broder *et al.*, 1988a,b,c) and mobile offices (Main & Hogan, 1983) exposed to concentrations of 0.006–1.6 ppm [0.007–2.0 mg/m^3]. No changes were seen in pulmonary function or airway resistance.

Lung function tests were performed on particle-board and plywood workers (Holmström & Wilhelmsson, 1988; Horvath *et al.*, 1988; Imbus & Tochilin, 1988; Malaka & Kodama, 1990), workers using acid-hardening paints (Alexandersson & Hedenstierna, 1988, 1989), embalmers (Levine *et al.*, 1984b; Holness & Nethercott, 1989), urea–formaldehyde resin producers (Holmström & Wilhelmsson, 1988; Nunn *et al.*, 1990), medical students (Uba *et al.*, 1989) and anatomy and histology workers (Khamgaonkar & Fulare, 1991). These groups were often exposed to formaldehyde in combination with other substances. The formaldehyde concentrations were < 0.02–> 5 ppm [< 0.02–> 6.0 mg/m^3]. In most of the studies, formaldehyde alone or in combination with other agents caused transient, reversible declines in lung function, but there was no evidence that formaldehyde induces a chronic decrement in lung function.

(iii) Effects on the skin

Formaldehyde is a skin irritant and can cause allergic contact dermatitis. It is difficult to distinguish between these two effects (Maibach, 1983). The estimated percentages of people with positive reactions in patch tests were 8.4% in the United States, 7.4% in Saskatoon, Canada, 9.2% in Cologne, Germany, and 5.5% of men and 12.4% of women in Hamburg, Germany (Cronin, 1991). Maibach (1983), however, indicated that these estimates may be considerably inflated, as they are usually uncorrected for the 'excited skin state' and are often unconfirmed. He estimated that the results of more than 40% of patch tests are unreproducible, especially for substances such as formaldehyde, as the concentrations that evoke an allergic response and an irritant response are similar.

In order to determine whether specific immunoglobulin (Ig) E antibodies are involved in contact dermatitis after exposure to formaldehyde, 23 patients with a history of a positive epicutaneous test to formaldehyde were studied. Fifteen (65%) showed a positive reaction on re-testing. The findings do not support the hypothesis that specific IgE antibodies are active in the

Table 20. Findings in nasal mucosa of people with occupational exposure to formaldehyde

Reference	Industry	Concentration of formaldehyde (mg/m^3)	No. of exposed	No. of controls	Method	Findings
Edling *et al.* (1987b)	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.* (1988)	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
Berke (1987)	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
					Clinical examination	More mucosal abnormalities in non-smoking exposed workers ($p = 0.004$)
Boysen *et al.* (1990)	Formaldehyde (production of formaldehyde and formaldehyde resins)	0.6–> 2.4	37	37	Nasal biopsy	Histological score: exposed 1.9, controls, 1.7 ($p > 0.05$) Three exposed and none of the controls had dysplasia
Holmström *et al.* (1989b)	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
Ballarin *et al.* (1992)	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

pathogenesis of contact sensitivity to formaldehyde, in either atopic or nonatopic patients (Lidén *et al.*, 1993).

Contact urticaria has also, but rarely, been associated with exposure to formaldehyde. Cases have been reported in a nonatopic histology technician (Rappaport & Hoffman, 1941), a worker exposed through contact with formaldehyde-treated leather (Helander, 1977) and a worker in a pathology laboratory (Lindskov, 1982). Information about the mechanisms of contact urticaria is limited (Maibach, 1983).

(c) Allergy

Immunological tests were performed on 23 asthmatic subjects who lived in urea–formaldehyde foam-insulated homes and on four asthmatic subjects living in conventionally insulated homes. The authors concluded that long-term exposure to formaldehyde had not affected the six immune parameters measured, but that short-term acute exposure resulted in minor immunological changes (Pross *et al.*, 1987).

No IgE-mediated sensitization could be attributed to formaldehyde in 86 individuals at risk of exposure to formaldehyde (Kramps *et al.*, 1989), and none of 63 practising pathologists had allergen-specific IgE directed against formaldehyde, although 29 subjects complained of sensitivityy to formaldehyde (Salkie, 1991).

The immune responses of a large number of people exposed to formaldehyde were investigated, including people living in mobile homes or working in buildings insulated with urea–formaldehyde foam, patients undergoing haemodialysis with formaldehyde-sterilized dialysers, physicians and dialysis nurses exposed to formaldehyde, histology technicians, medical and pathology students, and workers in an aircraft factory who were exposed to formaldehyde and other substances (including phenol and solvents) (Patterson *et al.*, 1989; Grammer *et al.*, 1990; Dykewicz *et al.*, 1991). The authors of the last paper stated that none of their studies indicated an immunological basis for respiratory or conjunctival symptoms (conjunctivitis, rhinitis, coughing, wheezing, shortness of breath) seen after exposure to gaseous formaldehyde.

Elevated serum levels of IgE, IgG or IgM antibodies were observed in several individuals exposed to formaldehyde (Thrasher *et al.*, 1987, 1988, 1990). The experimental design and methods used have been criticized, however, for lack of adequate controls, lack of a correlation between disease and immunological abnormalities, lack of information about the diseased and comparison populations and use of unproven diagnostic tests (Beavers, 1989; Greenberg & Stave, 1989).

4.2.2 Experimental systems

Formaldehyde has been shown to be toxic *in vitro* in a variety of experimental systems, including human cells. It decreased growth rate, cloning efficiency and the ability of cells to exclude trypan blue while inducing squamous differentiation of cultured human bronchial epithelial cells (Grafström, 1990). These effects occurred simultaneously with elevated levels of intracellular calcium ion, decreased levels of free low-relative-molecular-mass thiols, including glutathione, and the appearance of genotoxicity (see section 4.4).

(a) Acute effects

(i) Irritation

A quantitative measure of sensory irritation in rodents is provided by the reflex decrease in respiratory rate of mice or rats 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, eliciting a 50% decrease in respiratory frequency in B6C3F1 mice at 4.9 ppm [6.0 mg/m^3] and Fischer 344 rats at 31.7 ppm [38.7 mg/m^3] (Chang *et al.*, 1981). Swiss–Webster mice exposed to the concentration that elicits a 50% decrease in respiratory frequency (3.1 ppm [3.8 mg/m^3]) for five days (6 h/day) 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, resulting 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 of 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 four 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/F1 mice and CD rats (Jaeger & Gearhart, 1982).

Rats exposed to 28 ppm [34.1 mg/m^3] formaldehyde for four days developed tolerance to its sensory irritancy, but rats exposed to 15 ppm [18.3 mg/m^3] for one, four or 10 days did not (Chang & Barrow, 1984).

(ii) Pulmonary hyperreactivity

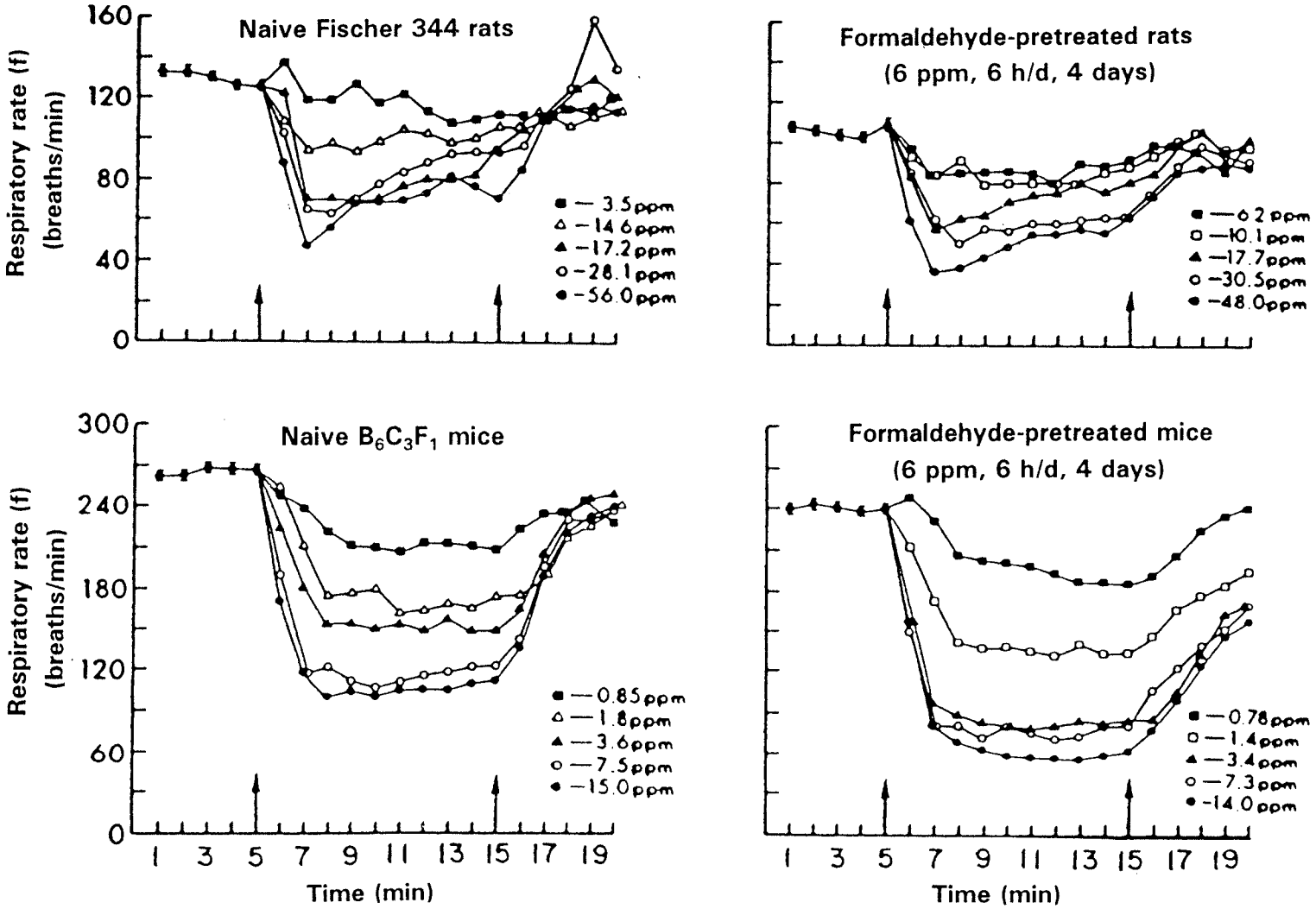
Formaldehyde induced pulmonary hyperreactivity in guinea-pigs: exposure to 0.03 ppm [0.04 mg/m^3] caused transient bronchoconstriction and hyperreactivity to infused acetylcholine when the duration of exposures was 8 h, but higher concentrations (10 ppm [12.2 mg/m^3]) were required to induce bronchoconstriction when the duration was 2 h. These effects occurred with no evidence of tracheal epithelial damage after exposure to 3.4 ppm [4.1 mg/m^3] for 8 h. The mechanism by which they occur is unknown (Swiecichowski *et al.*, 1993).

The effects of formaldehyde (vaporized formalin) on pulmonary flow were determined in cynomolgus monkeys, which were tranquilized before exposure and received an endotracheal tube transorally. Pulmonary flow resistance was increased at a concentration of 2.5 ppm [3.0 mg/m^3]. Airway narrowing 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.]

(iii) Cytotoxicity and cell proliferation in the respiratory tract

The acute and subacute effects of formaldehyde in experimental animals are summarized in Table 21. A critical issue for the mechanism of carcinogenesis is whether low concentrations of formaldehyde increase 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/day, three days) has been reported to induce a small, transient increase in nasal epithelial cell turnover in Wistar rats (Zwart *et al.*,

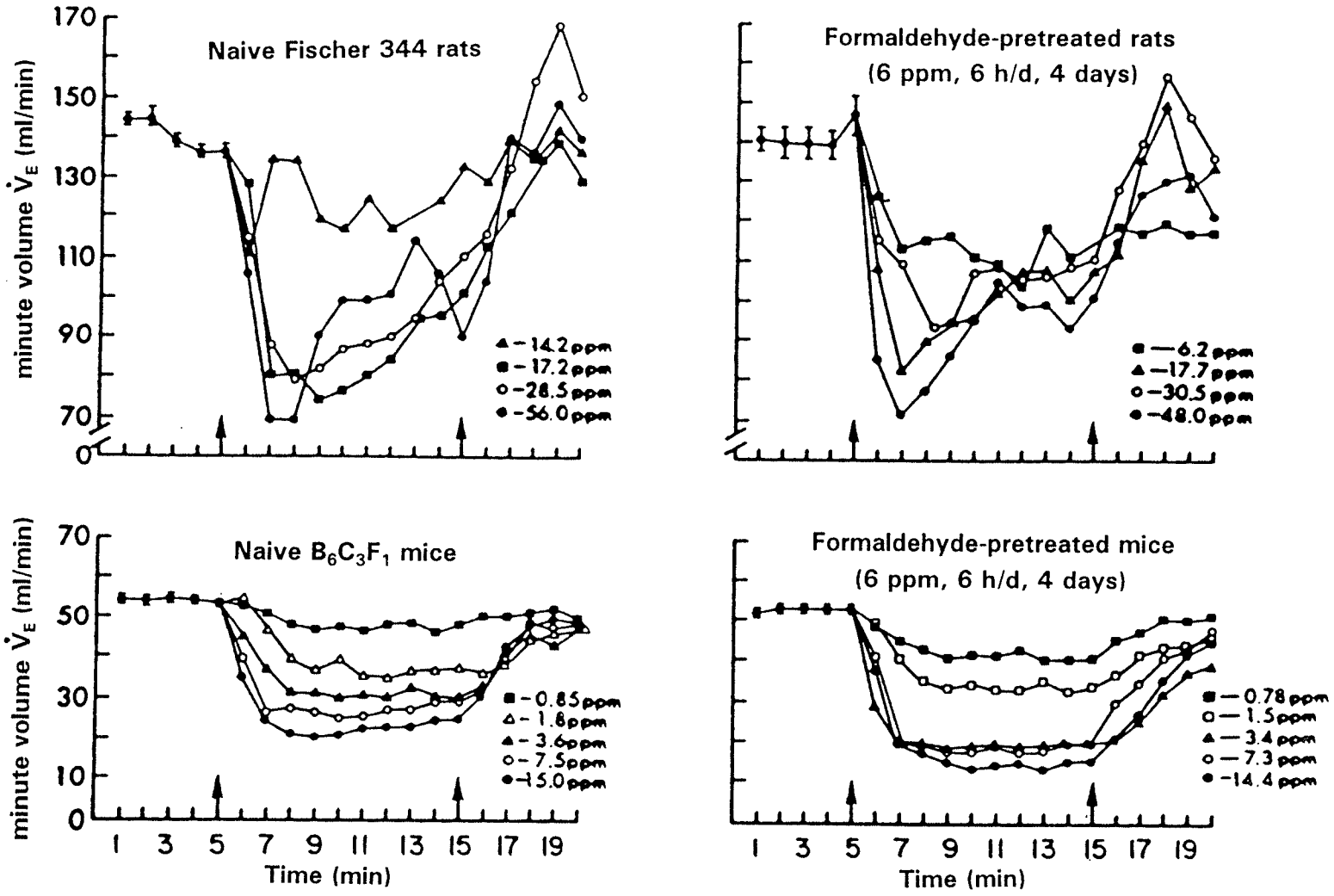
Figure 4. Representative time–response curves for the minute volume of naive and formaldehyde-treated mice and rats during 10-min exposures to various concentrations of formaldehyde



From Chang *et al.*(1981)

Data for the pre-exposure period are means ± SE of 19 or (A) 22 or (B) 28 animals, and the points for each concentration are means for four animals. Arrows indicate beginning and end of exposure.

Figure 4 (contd)



1988), but the apparent increase was not shown to be significant, and it 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/day, one, four or nine days) (Monticello *et al.*, 1991) or to 0.5 or 2 ppm [0.6 or 2.4 mg/m^3] (6 h/day, three days) (Swenberg *et al.*, 1983). Low concentrations of formaldehyde (0.5 or 2 ppm; 6 h/day, one, two, four, nine or 14 days) also did not 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/day, one or four days) (Monteiro-Riviere & Popp, 1986).

Wistar rats exposed to 3 ppm [3.7 mg/m^3] (6 h/day, three days (Zwart *et al.*, 1988) or 22 h/day, three days (Reuzel *et al.*, 1990)) had a transient increase in cell replication. Higher formaldehyde concentrations (≥ 6 ppm [7.3 mg/m^3]) induced erosion, epithelial hyperplasia, squamous metaplasia and inflammation in a site-specific manner in the nasal mucosa (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/day, one, four or nine days, or 6 h/day, five days/week, six 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 at 6 ppm (Monteiro-Riviere & Popp, 1986). Rhesus monkeys exposed to 6 ppm (6 h/day, five 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 three or 10 days (Swenberg *et al.*, 1983) or four 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).

The effects of simultaneous exposure to formaldehyde and ozone were investigated in Wistar rats exposed to 0.3, 1 or 3 ppm [0.4, 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/day, three 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 (just nonirritating) concentrations of these irritants but a

Table 21. Cytotoxicity and cell proliferation induced by acute and subacute exposure to formaldehyde

Species	Exposure	Effects	Reference
Fischer 344 rat, male; B6C3F1 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; B6C3F1 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 mouse responses	Chang *et al.* (1983)
Fischer 344 rat, male	3.7 mg/m^3 × 12 h/day, 7.4 mg/m^3 × 6 h/day, 14.4 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; proliferation less 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.* (1986c)
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.* (1986b)
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, nonkeratinized 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); 2.13 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	0.37, 1.2: Small increase in cell turnover at 1.2 ppm, but significance not shown and not confirmed in later studies (Reuzel *et al.*, 1990); 3.7: significant, transient increase in cell turnover	Zwart *et al.* (1988)

Table 21 (contd)

Species	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 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, 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)

C, concentration; *t*, time

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).

(iv) Enzyme induction

No increase in the activity of formaldehyde or aldehyde dehydrogenase was seen in the nose of Fischer 344 rats exposed to 15 ppm [18.3 mg/m^3] (6 h/day, five days/week, two weeks) (Casanova-Schmitz *et al.*, 1984a). A large increase in the activity of rat pulmonary cytochrome P450 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/day, four days) (Dallas *et al.*, 1986), although Dinsdale *et al.* (1993), using the same rat strain, could not confirm these results and found no increase in pulmonary cytochrome P450 activity after exposure to 10 ppm [12.2 mg/m^3] formaldehyde (6 h/day, four days).

(b) Chronic effects

(i) 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 22. No increases in cell turnover or DNA synthesis were found in the nasal mucosa after subchronic or chronic exposure to concentrations ≤ 2 ppm [≤ 2.4 mg/m^3] (Rusch *et al.*, 1983; Zwart *et al.*, 1988; Monticello *et al.*, 1993; Casanova *et al.*, 1995). Small, site-specific increases in the rate of cell turnover were noted at 3 ppm [3.7 mg/m^3] (6 h/day, 5 days/week, 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/day, 5 days/week, 12 weeks) in Fischer 344 rats (Casanova *et al.*, 1995). At these concentrations, however, an adaptive response occurs in rat nasal epithelium, as cell turnover rates after six weeks (Monticello *et al.*, 1991) or 13 weeks (Zwart *et al.*, 1988) are lower than those after one to four days of exposure. Monticello *et al.* (1993) 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 three months (6 h/day, 5 days/week), but, as already noted, Casanova *et al.* (1995) detected a small increase in DNA synthesis under these conditions. Large, sustained increases in cell turnover were observed at 10 and 15 ppm [12.2 and 18.3 mg/m^3] (6 h/day, 5 days/week, 3, 6, 12 or 18 months) (Monticello *et al.*, 1993). The effects of subchronic exposure to various concentrations of formaldehyde on DNA synthesis in the rat nose are illustrated in Figure 5.

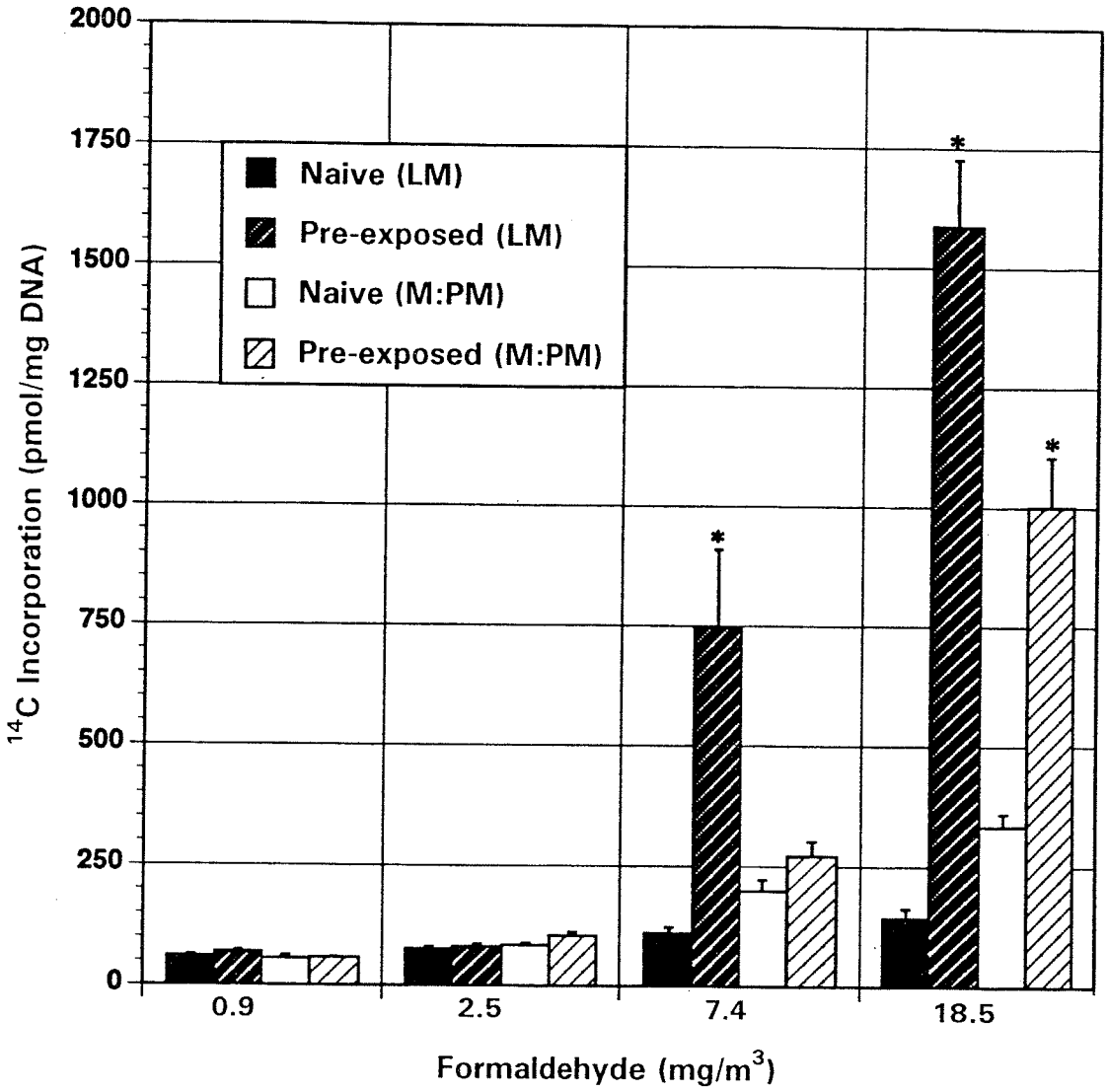
Additional studies have shown the importance of increased cell turnover in the induction of rat nasal tumours (Appelman *et al.*, 1988; Woutersen *et al.*, 1989). The investigators damaged the nasal mucosa of Wistar rats by bilateral intranasal electrocoagulation and evaluated the susceptibility of the rats to formaldehyde at concentrations of 0.1, 1 or 10 ppm [0.1, 1.2 or 12.2 mg/m^3] (6 h/day, 5 days/week, 13 or 52 weeks, 28 months, or three months of exposure followed by a 25-month observation period). 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. These noncancerous effects 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

Table 22. Cytotoxicity and cell proliferation induced by subchronic and chronic exposures to formaldehyde

Species	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	Rats: Squamous metaplasia in nasal turbinates at 3.7 mg/m^3 only Hamsters: 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, 24.7, 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	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 and degeneration in respiratory epithelium (both intact and damaged nose) and olfactory epithelium (damaged nose only)	Appelman *et al.* (1988)
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, determines 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, 3 months	0.86, 2.5, 7.4: No increase in cell replication detected 12.3, 18.5: Sustained cell proliferation	Monticello *et al.* (1993)
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.* (1995)

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 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]



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Pre-exposed rats were exposed subchronically to the same concentrations of unlabelled formaldehyde (6 h/day, 5 days/week, 11 weeks and four days), while naive rats were exposed to room air. The exposure to ^{14}C-formaldehyde occurred on the fifth day of the twelfth week. The asterisk denotes a significant difference between pre-exposed and naive rats.

mucosa was more susceptible to the cytotoxic effects of formaldehyde and that severe damage contributes to the induction of nasal tumours.

Rhesus monkeys exposed to 6 ppm [7.3 mg/m^3] formaldehyde (6 h/day, 5 days/week) had a larger percentage of the nasal mucosal surface area affected after six weeks than after five 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/day, 7 days/week, 26 weeks) developed no detectable nasal lesions (Rusch *et al.*, 1983), but Fischer 344 rats exposed to 2 ppm [2.4 mg/m^3] (6 h/day, 5 days/week, 24 months) developed mild squamous metaplasia in the nasal turbinates (Kerns *et al.*, 1983b). Although the total dose received by the former group was 2.5 times higher than that received by the latter, the incidence and severity of lesions was less, again demonstrating the greater importance of concentration than total dose (Rusch *et al.*, 1983).

(ii) Toxicity in the gastrointestinal tract after oral administration

The toxic effects of formaldehyde given by oral administration 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 91 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 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 two 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 two 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 82 mg/kg bw per day (males) or 109 mg/kg bw per day (females) (Til *et al.*, 1988, 1989).

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 two years. Estimated from the water intake, 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) receiving 0.1% formaldehyde (50 mg/kg bw per day) (Tobe *et al.*, 1989).

(c) Immunotoxicity

The possibility that formaldehyde may induce changes in the immune response was examined in B6C3F1 mice exposed to 15 ppm [18.3 mg/m^3] formaldehyde (6 h/day, 5 days/week, 3 weeks). A variety of immune function tests revealed no significant changes, except for an increase in host resistance to challenge with the bacterium, *Listeria monocytogenes*, implying an increased resistance to infection. Exposure did not alter the number or impair the function of resident peritoneal macrophages, but it increased the competence for release of hydrogen peroxide from peritoneal macrophages (Dean *et al.*, 1984; Adams *et al.*, 1987).

Sprague–Dawley rats were exposed to 12.6 ppm [15.4 mg/m^3] formaldehyde (6 h/day, 5 days/week, 22 months) and then vaccinated with pneumococcal polysaccharide antigens and tetanus toxoid. They were tested three to four 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).

In order to investigate the induction of sensitivity to formaldehyde, undiluted formalin was painted on shaven and epilated dorsal sites on guinea-pigs; a second application was administered two days later at naive sites, to give a total dose of 74 mg/animal. Other animals received diluted formalin at doses of 12–9.3 mg/animal. All animals receiving 74 mg developed skin sensitivity when tested seven days after exposure. A significant dose–response relationship was observed for degree of sensitization and for percentage of animals sensitized; however, pulmonary sensitivity was not induced when formaldehyde was administered dermally, by injection or by inhalation, and no cytophilic antibodies were detected in blood (Lee *et al.*, 1984).

4.3 Reproductive and developmental effects

4.3.1 Humans

The incidence of spontaneous abortion was studied among hospital staff in Finland who used ethylene oxide (see IARC, 1994b), glutaraldehyde and formaldehyde for sterilizing 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, and 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 smoking habits, intake of alcohol, reproductive history, including number of pregnancies and their outcome, and occupation at the time of each pregnancy. Information about exposure to chemical sterilizing agents was obtained from the supervising nurses. The crude rates of spontaneous abortions 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 learnt they were pregnant (the difference being significant) and 10.6% among controls. When adjusted for age, parity, decade of pregnancy, 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 ($p < 0.05$), and that associated with formaldehyde, with or without other agents, was 8.4%, which was comparable to the reference level of 10.5% (Hemminki *et al.*, 1982).

In a nationwide record linkage study in Finland, all nurses who had been pregnant between the years 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 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 to 5.3% matched controls, to give an odds ratio of 1.7 [95% CI, 0.39–7.7]; the latter analysis was based on eight exposed subjects (Hemminki *et al.*, 1985).

The occurrence of spontaneous abortions among women working in laboratories in Finland and congenital malformations and birth weights of the children were investigated in a matched retrospective case–control study. 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, smoking and alcohol consumption during the first trimester of the pregnancy was collected by postal questionnaire. The odds ratio for spontaneous abortion was increased among women who had been exposed to formalin for at least three days per week (odds ratio, 3.5; 95% CI, 1.1–11). 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, 1989c). The authors stated that the results for individual chemicals should be interpreted cautiously 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).

4.3.2 Experimental systems

The reproductive and developmental toxicity of formaldehyde has been reviewed (Feinman, 1988; WHO, 1989).

Whether administered by inhalation, ingestion or the skin to various rodent species, formaldehyde did not exert adverse effects on reproductive parameters or fetal development (Marks *et al.*, 1980; Feinman, 1988). Additional studies have confirmed this assessment. Groups

of 25 pregnant Sprague–Dawley rats were exposed to formaldehyde (0, 5, 10, 20 or 40 ppm [0, 6, 12, 24 or 49 mg/m^3]; 6 h/day, 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 given 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 attributable to maternal toxicity (Saillenfait *et al.*, 1989).

Groups of 25 mated female Sprague–Dawley rats were exposed to formaldehyde at 2, 5 or 10 ppm [2.5, 6 or 12 mg/m^3] (6 h/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 (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 treatment. 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 (Overman, 1985).

4.4 Genetic and related effects

The mutagenicity of formaldehyde has been reviewed (IARC, 1982, 1987d; Ma & Harris, 1988; WHO, 1989; Feron *et al.*, 1991).

4.4.1 Humans

(a) DNA–protein cross-links

No data were available to the Working Group.

(b) Mutation and allied effects

The effects of formaldehyde on the frequencies of chromosomal aberrations and sister chromatid exchange in peripheral lymphocytes of people occupationally exposed to formaldehyde were reviewed previously (IARC, 1987d). Both positive and negative results were obtained, but their interpretation was difficult because of the small number of subjects studied and inconsistencies in the findings. Since then, further data on the cytogenetic effects of formaldehyde in humans have been published.

In a study of workers exposed to formaldehyde in a factory manufacturing wood-splinter materials, short-term cultures of peripheral lymphocytes were examined from a group of 20 workers aged 27–57 (mean, 42.3 years), of whom 10 were men and 10 were women. They had been exposed to formaldehyde at 8-h time-weighted concentrations of 0.55–10.36 mg/m^3 for

periods of 5–≥ 16 years. The control group consisted of 19 people [sex and age unspecified] employed in the same plant whose habits and social status were similar to those of the exposed group but who had unknown occupational contact with chemicals. No significant difference was observed between control and exposed groups with respect to any of the chromosomal anomalies (including chromatid and chromosome gaps, breaks, exchanges, breaks per cell, percentage of cells with aberrations) scored in the study (controls: 3.6% aberrant cells, 0.08 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 the smoking habits of the subjects.]

In the study of Ballarin *et al.* (1992), described on p. 306, the frequency of micronuclei in respiratory nasal mucosa cells was also investigated. At least 6000 cells from each individual were scored for micronuclei. A significant excess of micronucleated cells was seen in the exposed group (mean percentage of micronucleated cells, 0.90 ± 0.47; range, 0.17–1.83 in exposed group; 0.25 ± 0.22; range, 0.0–0.66 in controls; Mann–Whitney U test: $p < 0.01$). The authors noted the absence of a dose–response relationship between exposure to formaldehyde and the frequency of micronuclei and that concurrent exposure to wood dust could have contributed to the excess of micronucleated cells seen in the exposed group.

In a prospective study of the effect of formaldehyde on the frequency of micronuclei in oral and nasal mucosal cells and peripheral lymphocytes from a group of 29 student morticians, samples of blood and epithelial cells were taken before the students started the course (baseline samples) and again after the first nine weeks in an embalming laboratory. During the 85-day study period, the subjects had average cumulative formaldehyde exposures of 14.8 ppm-h [17.8 mg/m^3-h], with an average air concentration of 1.4 ppm [1.7 mg/m^3]. Epithelial cells were taken with a cytopathology brush from each inner cheek and from the inferior turbinate of each nostril. Weakly positive results were found in lymphocytes, positive results in buccal epithelium and negative results in nasal epithelium (Suruda *et al.*, 1993). [The Working Group noted the inadequate reporting of the data in this study and was unable to evaluate it.]

(c) Sperm abnormalities

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 bodies. Subjects were matched for sex, 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 two- to three-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 [0.73–1.58 mg/m^3] (weekly exposure, 3–40 ppm-h [3.6–48 mg/m^3-h]). No significant difference was observed between the exposed and control groups with regard to sperm parameters (Ward *et al.*, 1984).

(d) Urinary mutagenicity

Hospital autopsy service workers in Galveston, TX (United States), consisting of 15 men and four women aged < 30–> 50, and a control group from the local medical school, consisting of 15 men and five women in the same age range and 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 two-month intervals. The time-weighted average 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 livers of Aroclor-1254-induced rats. No increase in mutagenicity was seen in the autopsy workers as compared with the control group.

4.4.2 Experimental systems

(a) DNA–protein cross-links

Formaldehyde induces DNA–protein cross-links in mammalian cells *in vitro* and *in vivo* (see Table 23). The precise nature of these cross-links is unknown. Studies of the repair of DNA–protein cross-links caused by formaldehyde *in vitro* showed that they are removed from several types of normal cells and xeroderma pigmentosum cells, with a half-time of 2–3 h. These removal rates were similar at non-toxic and toxic concentrations of formaldehyde. In formaldehyde-exposed normal cells, active removal of DNA adducts by DNA excision repair was indicated by formation of DNA single-strand breaks, which could be accumulated in the presence of DNA repair synthesis inhibitors (Grafström *et al.*, 1984).

Groups of four male Fischer 344 rats were exposed for 6 h to 0.3, 0.7, 2, 6 or 10 ppm [0.4, 0.9, 2.4, 7.3 or 12.2 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 mouth-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 concentration of cross-links increased non-linearly with the airborne concentration in both species. The concentrations of cross-links 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 they 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).

(b) Mutation and allied effects (see also Table 23 and Appendices 1 and 2*)*

Formaldehyde induced mutation and DNA damage in bacteria and mutation, gene conversion, DNA strand breaks and DNA–protein cross-links in fungi. In *Drosophila*

Table 23. Genetic and related effects of formaldehyde

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*	+	0	30	Snyder & Van Houten (1986)
PRB	Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage	+	0	0.0075	Kuykendall & Bogdanffy (1992)
PRB	Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage	+	0	20	Le Curieux *et al.* (1993)
ECB	*Escherichia coli* (or *E. coli* DNA) strand breaks, cross-links or related damage; DNA repair	+	0	600	Wilkins & MacLeod (1976)
ECB	*Escherichia coli* (or *E. coli* DNA) strand breaks, cross-links or related damage; DNA repair	+	0	60	Poverenny *et al.* (1975)
ECD	*Escherichia coli polA*/W31110-P3478, differential toxicity (spot test)	+	0	10	Leifer *et al.* (1981)
ECL	*Escherichia coli* K12 KS160-KS66 *pol*AI, differential toxicity	+	0	60	Poverenny *et al.* (1975)
ECK	*Escherichia coli* K12, forward or reverse mutation	+	0	60	Zijlstra (1989)
ECK	*Escherichia coli* K12, forward or reverse mutation	+	0	18.8	Graves *et al.* (1994)
ECK	*Escherichia coli* K12, forward or reverse mutation	+	0	120	Crosby *et al.* (1988)
SAF	*Salmonella typhimurium*, forward mutation	+	+	10	Temcharoen & Thilly (1983)
SA0	*Salmonella typhimurium* TA100, reverse mutation	(+)	0	25	Marnett *et al.* (1985)
SA0	*Salmonella typhimurium* TA100, reverse mutation	-	-	30	Gocke *et al.* (1981)
SA0	*Salmonella typhimurium* TA100, reverse mutation	-	+	16.6	Haworth *et al.* (1983)
SA0	*Salmonella typhimurium* TA100, reverse mutation	(+)	+	30 (toxic above 125 µg/plate)	Connor *et al.* (1983)
SA0	*Salmonella typhimurium* TA100, reverse mutation	+	0	7.5	Takahashi *et al.* (1985)
SA0	*Salmonella typhimurium* TA100, reverse mutation	+	+[c]	4.5	Pool *et al.* (1984)
SA0	*Salmonella typhimurium* TA100, reverse mutation	+	0	9.3	O'Donovan & Mee (1993)
SA0	*Salmonella typhimurium* TA100, reverse mutation	(+)	+	3	Schmid *et al.* (1986)
SA2	*Salmonella typhimurium* TA102, reverse mutation	+	0	10	Marnett *et al.* (1985)
SA2	*Salmonella typhimurium* TA102, reverse mutation	+	0	10	Le Curieux *et al.* (1993)
SA2	*Salmonella typhimurium* TA102, reverse mutation	+	0	35.7	O'Donovan & Mee (1993)
SA4	*Salmonella typhimurium* TA104, reverse mutation	+	0	10	Marnett *et al.* (1985)
SA5	*Salmonella typhimurium* TA1535, reverse mutation	-	-	30	Gocke *et al.* (1981)
SA5	*Salmonella typhimurium* TA1535, reverse mutation	-	-	50	Haworth *et al.* (1983)
SA5	*Salmonella typhimurium* TA1535, reverse mutation	0	-[c]	9	Pool *et al.* (1984)

Table 23 (contd)

Test system		Result[a]		Dose[b] (LED/HID)	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
SA5	*Salmonella typhimurium* TA1535, reverse mutation	-	0	143	O'Donovan & Mee (1993)
SA7	*Salmonella typhimurium* TA1537, reverse mutation	-	-	30	Gocke *et al.* (1981)
SA7	*Salmonella typhimurium* TA1537, reverse mutation	-	-	50	Haworth *et al.* (1983)
SA7	*Salmonella typhimurium* TA1537, reverse mutation	-	0	143	O'Donovan & Mee (1993)
SA8	*Salmonella typhimurium* TA1538, reverse mutation	-	-	30	Gocke *et al.* (1981)
SA8	*Salmonella typhimurium* TA1538, reverse mutation	-	0	143	O'Donovan & Mee (1993)
SA9	*Salmonella typhimurium* TA98, reverse mutation	+	0	5	Marnett *et al.* (1985)
SA9	*Salmonella typhimurium* TA98, reverse mutation	-	-	30	Gocke *et al.* (1981)
SA9	*Salmonella typhimurium* TA98, reverse mutation	-	(+)	16.6	Haworth *et al.* (1983)
SA9	*Salmonella typhimurium* TA98, reverse mutation	-	(+)	30 (toxic above 100 µg/plate)	Connor *et al.* (1983)
SA9	*Salmonella typhimurium* TA98, reverse mutation	0	(+)[c]	3	Pool *et al.* (1984)
SA9	*Salmonella typhimurium* TA98, reverse mutation	+	0	17.9	O'Donovan & Mee (1993)
SAS	*Salmonella typhimurium* TA97, reverse mutation	+	0	5	Marnett *et al.* (1985)
SAS	*Salmonella typhimurium* (other miscellaneous strains), reverse mutation	-	-	100 (toxic at 250 µg/ml)	Connor *et al.* (1983)
ECW	*Escherichia coli* WP2 *uvr*A, reverse mutation	+	0	15	Takahashi *et al.* (1985)
ECW	*Escherichia coli* WP2 *uvr*A(pKM101), reverse mutation	+	0	17.9	O'Donovan & Mee (1993)
EC2	*Escherichia coli* WP2, reverse mutation	+	0	1.2	Nishioka (1973)
EC2	*Escherichia coli* WP2(pKM101), reverse mutation	+	0	35.7	O'Donovan & Mee (1993)
EC2	*Escherichia coli* WP2, reverse mutation	+	0	60	Takahashi *et al.* (1985)
ECR	*Escherichia coli* (other miscellaneous strains), reverse mutation	+	0	900	Panfilova *et al.* (1966)
ECR	*Escherichia coli* (other miscellaneous strains), reverse mutation	+	0	80	Demerec *et al.* (1951)
ECR	*Escherichia coli* (other miscellaneous strains), reverse mutation	+	0	30	Takahashi *et al.* (1985)
SSB	*Saccharomyces* species, DNA strand breaks, cross-links or related damage	+	0	990	Magaña-Schwencke *et al.* (1978)
SSB	*Saccharomyces* species, DNA strand breaks, cross-links or related damage	+	0	500	Magaña-Schwencke & Ekert (1978)
SSB	*Saccharomyces* species, DNA strand breaks, cross-links or related damage	+	0	500	Magaña-Schwencke & Moustacchi (1980)
SCH	*Saccharomyces cerevisiae*, gene conversion	+	0	540	Chanet *et al.* (1975)
SCH	*Saccharomyces cerevisiae*, homozygosis by mitotic recombination or gene conversion	+	0	18.5	Zimmermann & Mohr (1992)

Table 23 (contd)

Test system		Result[a]		Dose[b] (LED/HID)	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
NCF	*Neurospora crassa*, forward mutation	+	0	100	de Serres *et al.* (1988)
NCR	*Neurospora crassa*, reverse mutation	-	0	732	Dickey *et al.* (1949)
NCR	*Neurospora crassa*, reverse mutation	+	0	37 500	Jensen *et al.* (1952)
NCR	*Neurospora crassa*, reverse mutation	-	0	300	Kölmark & Westergaard (1953)
PLM	Plants (other), mutation	+	0	0.0	Auerbach *et al.* (1977)
DMG	*Drosophila melanogaster*, genetic crossing over or recombination	+		2700	Ratnayake (1970)
DMG	*Drosophila melanogaster*, genetic crossing over or recombination	+		420	Alderson (1967)
DMG	*Drosophila melanogaster*, genetic crossing over or recombination	+		1260	Sobels & van Steenis (1957)
DMX	*Drosophila melanogaster*, sex-linked recessive lethal mutations	+		420	Alderson (1967)
DMX	*Drosophila melanogaster*, sex-linked recessive lethal mutations	(+)		1940	Ratnayake (1968)
DMX	*Drosophila melanogaster*, sex-linked recessive lethal mutations	+		2380	Ratnayake (1970)
DMX	*Drosophila melanogaster*, sex-linked recessive lethal mutations	+		1940	Auerbach & Moser (1953)
DMX	*Drosophila melanogaster*, sex-linked recessive lethal mutations	+		1080	Kaplan (1948)
DMX	*Drosophila melanogaster*, sex-linked recessive lethal mutations	+		420	Khan (1967)
DMX	*Drosophila melanogaster*, sex-linked recessive lethal mutations	+		270	Stumm-Tegethoff (1969)
DMX	*Drosophila melanogaster*, sex-linked recessive lethal mutations	+		1260	Sobels & van Steenis (1957
DMH	*Drosophila melanogaster*, heritable translocation	+		2700	Ratnayake (1970)
DMH	*Drosophila melanogaster*, heritable translocation	+		420	Khan(1967)
DML	*Drosophila melanogaster*, dominant lethal mutation	+		1940	Auerbach & Moser (1953)
DML	*Drosophila melanogaster*, dominant lethal mutation	+		1400	Šrám (1970)
*	*Caenorhabditis elegans*, recessive lethal mutations	+		700	Johnsen & Baillie (1988)
DIA	DNA strand breaks, cross-links or related damage, animal cells *in vitro*	+	0	6	Ross & Shipley (1980)
DIA	DNA strand breaks, cross-links or related damage, animal cells *in vitro*	+	0	3.75	Ross *et al.* (1981)
DIA	DNA strand breaks, cross-links or related damage, animal cells *in vitro*	+	0	22.5	Demkowicz-Dobrzanski & Castonguay (1992)
DIA	DNA strand breaks, cross-links or related damage, animal cells *in vitro*	+	0	7.5	O'Connor & Fox (1987)
G9H	Gene mutation, Chinese hamster V79 cells, *hprt* locus	+	0	9	Grafström *et al.* (1993)
SIC	Sister chromatid exchange, Chinese hamster cells *in vitro*	+	0	1	Obe & Beek (1979)
SIC	Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	3.2	Natarajan *et al.* (1983)
SIC	Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	1.8	Basler *et al.* (1985)
CIC	Chromosomal aberrations, Chinese hamster cells *in vitro*	+	+	6.5	Natarajan *et al.* (1983)
CIC	Chromosomal aberrations, Chinese hamster cells *in vitro*	+	0	18	Ishidate *et al.* (1981)

Table 23 (contd)

Test system		Result[a]		Dose[b] (LED/HID)	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
TCM	Cell transformation, C3H10T1/2 mouse cells	+[d]	0	0.5	Ragan & Boreiko (1981)
DIH	DNA strand breaks, cross-links or related damage, human cells *in vitro*	+	0	24	Fornace *et al.* (1982)
DIH	DNA strand breaks, cross-links or related damage, human cells *in vitro*	+	0	1.5	Craft *et al.* (1987)
DIH	DNA strand breaks, cross-links or related damage, human cells *in vitro*	+	0	3	Grafström *et al.* (1986)
DIH	DNA strand breaks, cross-links or related damage, human cells *in vitro*	+	0	3	Snyder & Van Houten (1986)
DIH	DNA strand breaks, cross-links or related damage, human cells *in vitro*	+	0	3	Saladino *et al.* (1985)
DIH	DNA strand breaks, cross-links or related damage, human cells *in vitro*	+	0	3	Grafström *et al.* (1984)
DIH	DNA strand breaks, cross-links or related damage, human cells *in vitro*	+	0	12	Grafström (1990)
UIH	Unscheduled DNA synthesis, human bronchial epithelial cells *in vitro*	-	0	3 (> 0.1 mmol/L was lethal)	Doolittle *et al.* (1985)
GIH	Gene mutation, human cells *in vitro*	+	0	3	Grafström *et al.* (1985)
GIH	Gene mutation, human cells *in vitro*	+	0	3.9	Goldmacher & Thilly (1983)
GIH	Gene mutation, human cells *in vitro*	+	0	0.9	Craft *et al.* (1987)
GIH	Gene mutation, human cells *in vitro*	+	0	4.5	Crosby *et al.* (1988)
GIH	Gene mutation, human cells *in vitro*	+	0	4.5	Liber *et al.* (1989)
GIH	Gene mutation, human cells *in vitro*	+	0	3	Grafström (1990)
RIH	DNA repair exclusive of unscheduled DNA synthesis, human cells *in vitro*	+	0	6	Grafström *et al.* (1984)
SHL	Sister chromatid exchange, human lymphocytes *in vitro*	+	0	5.4	Obe & Beek (1979)
SHL	Sister chromatid exchange, human lymphocytes *in vitro*	+	0	5	Kreiger & Garry (1983)
SHL	Sister chromatid exchange, human lymphocytes *in vitro*	+	+	3.75	Schmid *et al.* (1986)
CHF	Chromosomal aberrations, human fibroblasts *in vitro*	+	0	60	Levy *et al.* (1983)
CHL	Chromosomal aberrations, human lymphocytes *in vitro*	+	0	10	Miretskaya & Shvartsman (1982)
CHL	Chromosomal aberrations, human lymphocytes *in vitro*	+	+	7.5	Schmid *et al.* (1986)
CHL	Chromosomal aberrations, human lymphocytes *in vitro*	+	0	3.75	Dresp & Bauchinger (1988)
DVA	DNA–protein cross-links, rat cells *in vivo*	+		1.5 inhal. 6 h	Casanova-Schmitz *et al.* (1984b)
DVA	DNA–protein cross-links, rat cells *in vivo*	(+)		1.5 inhal. 6 h	Lam *et al.* (1985)
DVA	DNA–protein cross-links, rat cells *in vivo*	+		0.25 inhal. 3 h	Heck *et al.* (1986)
DVA	DNA–protein cross-links, rat cells *in vivo*	+		0.25 inhal. 3 h	Casanova & Heck (1987)
DVA	DNA–protein cross-links, rat cells *in vivo*	+		0.08 inhal. 6 h	Casanova *et al.* (1989)
DVA	DNA–protein cross-links, rhesus monkey nasal turbinate cells *in vivo*	+		0.05 inhal. 6 h	Heck *et al.* (1989)

Table 23 (contd)

Test system		Result[a]		Dose[b] (LED/HID)	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
DVA	DNA–protein cross-links, rhesus monkey nasal turbinate cells *in vivo*	+		0.05 inhal. 6 h	Casanova *et al.* (1991)
*	DNA–protein cross-links, rat tracheal implant cells *in vivo*	+		2 mg/ml instil.	Cosma *et al.* (1988)
SVA	Sister chromatid exchange, rat cells *in vivo*	-		3.9 inhal. 6 h/d × 5	Kligerman *et al.* (1984)
*	Micronucleus induction, newt (*Pleurodeles waltl*) *in vivo*	-		5 µg/ml, 12 d	Siboulet *et al.* (1984)
MVM	Micronucleus induction, mouse *in vivo*	-		25 ip × 1	Natarajan *et al.* (1983)
MVM	Micronucleus induction, mouse *in vivo*	-		30 ip × 1	Gocke *et al.* (1981)
MVR	Micronucleus induction, rat (gastrointestinal tract) *in vivo*	+		200 po × 1	Migliore *et al.* (1989)
CBA	Chromosomal aberrations, mouse bone-marrow cells *in vivo*	-		25 ip × 1	Natarajan *et al.* (1983)
CBA	Chromosomal aberrations, rat bone-marrow cells *in vivo*	+		0.07 inhal. 4 h/d, 4 months	Kitaeva *et al.* (1990)
CBA	Chromosomal aberrations, rat bone-marrow cells *in vivo*	-		3.9 inhal. 6 h/d × 5, 8 weeks	Dallas *et al.* (1992)
CLA	Chromosomal aberrations, rat leukocytes *in vivo*	-		3.9 inhal. 6 h/d × 5	Kligerman *et al.* (1984)
CCC	Chromosomal aberrations, mouse spermatocytes treated *in vivo*, spermatocytes observed	-		50 ip × 1	Fontignie-Houbrechts (1981)
CVA	Chromosomal aberrations, mouse spleen cells *in vivo*	-		25 ip × 1	Natarajan *et al.* (1983)
CVA	Chromosomal aberrations, rat pulmonary lavage cells *in vivo*	+		3.9 inhal. 6 h/d × 5	Dallas *et al.* (1992)
GVA	Gene mutation, rat cells *in vivo* (*p53* point mutations in nasal carcinomas)	+		3.9 inhal. 6 h/d, 2 years	Recio *et al.* (1992)
MST	Mouse spot test	-		3.9 inhal. 6 h/d × 3	Jensen & Cohr (1983) [Abstract]
DLM	Dominant lethal mutation, mouse	(+)		50 ip × 1	Fontignie-Houbrechts (1981)
DLM	Dominant lethal mutation, mouse	-		20 ip × 1	Epstein *et al.* (1972)
DLR	Dominant lethal mutation, rat	(+)		0.2 inhal. 4 h/d × 120	Kitaeva *et al.* (1990)
DLM	Dominant lethal mutation, mouse	-		20 ip × 1	Epstein & Shafner (1968)
MVH	Micronucleus formation, human lymphocytes *in vivo*	(+)		0.06[e] inhal. 8-h TWA	Suruda *et al.* (1993)
MVH	Micronucleus formation, human cells (buccal epithelium) *in vivo*	+		0.06[e] inhal. 8-h TWA	Suruda *et al.* (1993)
MVH	Micronucleus formation, human cells (nasal epithelium) *in vivo*	-		0.06[e] inhal. 8-h TWA	Suruda *et al.* (1993)
MVH	Micronucleus formation, human cells (nasal epithelium) *in vivo*	+		0.06[e] inhal. 8-h TWA	Ballarin *et al.* (1992)
SLH	Sister chromatid exchange, human lymphocytes *in vivo*	-		0.5 inhal. 8-h TWA	Thomson *et al.* (1984)
SLH	Sister chromatid exchange, human lymphocytes *in vivo*	-		0.5 inhal. 8-h TWA	Bauchinger & Schmid (1985)
SLH	Sister chromatid exchange, human lymphocytes *in vivo*	+		0.2 inhal. 8-h TWA	Yager *et al.* (1986)
SLH	Sister chromatid exchange, human lymphocytes *in vivo*	-		0.06[e] inhal. 8-h TWA	Suruda *et al.* (1993)
CLH	Chromosomal aberrations, human lymphocytes *in vivo*	-		0.5 inhal. 8-h TWA	Thomson *et al.* (1984)

Table 23 (contd)

Test system		Result[a]		Dose[b] (LED/HID)	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
CLH	Chromosomal aberrations, human lymphocytes *in vivo*	-		0.8 inhal. 8-h TWA	Fleig *et al.* (1982)
CLH	Chromosomal aberrations, human lymphocytes *in vivo*	+		0.5 inhal. 8-h TWA	Bauchinger & Schmid (1985)
CLH	Chromosomal aberrations, human lymphocytes *in vivo*	-		0.4 inhal.	Vargová *et al.* (1992)
SPR	Sperm morphology, rats *in vivo*	+		200 po × 1	Cassidy *et al.* (1983)
SPM	Sperm morphology, mice *in vivo*	-		100 po × 5	Ward *et al.* (1984)
SPH	Sperm morphology, humans *in vivo*	-		0.2 inhal. 8-h TWA	Ward *et al.* (1984)

*Not on profile

[a] +, positive; (+) weak positive; -, negative; 0, not tested; ?, inconclusive (variable response in several experiments within an adequate study)

[b] In-vitro tests, µg/ml; in-vivo tests, mg/kg bw

[c] Tested with S9 without co-factors

[d] Positive only in presence of 12-*O*-tetradecanoylphorbol 13-acetate (TPA)

[e] Based on a mean 8-h time-weighted average of 0.33 ppm (range, 0.1–0.96 ppm); peak exposures up to 6.6 ppm

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 in Chinese hamster V79 cells and transformation of mouse C3H10T1/2 cells *in vitro*. It 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 (Grafström, 1990; Grafström *et al.*, 1993).

While there is conflicting evidence that formaldehyde can induce chromosomal anomalies in the bone marrow of rodents exposed by inhalation *in vivo*, recent studies have shown that it induces cytogenetic damage in the cells of tissues that are more locally exposed, either by gavage or by inhalation. Thus, 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 five fold) 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 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).

In the second experiment, male Sprague–Dawley rats were exposed 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 five days per week, for one and eight weeks. There was no significant increase in chromosomal abnormalities in the bone-marrow cells of formaldehyde-exposed rats relative to controls, but there was a significant increase in the frequency of chromosomal aberrations in pulmonary lavage cells (lung alveolar macrophages) from rats that inhaled 15 ppm formaldehyde. Aberrations, which were predominantly chromatid breaks, were seen in 7.6 and 9.2% of the scored pulmonary lavage cells from treated animals and in 3.5 and 4.8% of cells from controls, after one and eight weeks, respectively (Dallas *et al.*, 1992).

Assays for dominant lethal mutations in rodents *in vivo* gave inconclusive results. In single studies, formaldehyde induced sperm-head anomalies in rats but not in mice.

(c) Mutational spectra

The spectrum of mutations induced by formaldehyde was studied in human lymphoblasts *in vitro*, in *Escherichia coli* and in naked pSV2gpt plasmid DNA (Crosby *et al.*, 1988). Thirty TK6 X-linked *hprt*$^-$ human lymphoblast colonies induced by eight treatments with 150 μmol/L 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. In *E. coli,* the mutations induced by formaldehyde were characterized in the xanthine guanine phosphoribosyl transferase (*gpr*) gene. Exposure of *E. coli* to 4 mmol/L 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 pSV2gpt plasmid DNA was exposed to 3.3 or 10 mmol/L formaldehyde and transformed into *E. coli*, most of the resulting mutations were frameshifts, again suggesting a different mechanism of mutation.

Sixteen of the 30 formaldehyde-induced human lymphoblast TK6 X-linked *hprt* mutants referred to above which 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 and three produced no detectable mRNA. Sequence analyses of cDNA prepared from *hprt* mRNA were performed on one spontaneous and seven formaldehyde-induced mutants 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.

Table 24. DNA sequence analysis of *p53* cDNA (polymerase chain reaction 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	TTC→TTA (132) phe→leu	134	II
$_{398}$G→T	TGC→TTC (133) cys→phe	135	II
$_{638}$G→T	AGC→ATC (213) ser→ile	215	
$_{812}$G→A	CGT→CAT (271) arg→his	273	V
$_{842}$G→C	CGG→CCG (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.

DNA sequence analysis of polymerase chain reaction-amplified cDNA fragments containing 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 by formaldehyde. The rats has been exposed by inhalation to 15 ppm [18.5 mg/m^3] formaldehyde for up to two years. Point mutations at GC base-pairs in the *p53* complementary DNA sequence were found in five of the tumours (Table 24). The authors pointed out that all five human counterparts of the mutated *p53* codons listed in the Table 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 occurring in many human cancers (Recio *et al.*, 1992).