FORMALDEHYDE

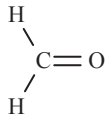
Formaldehyde was considered by previous IARC Working Groups in 1981, 1987, 1994, and 2004 (IARC, 1982, 1987, 1995, 2006). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1 Exposure Data

1.1 Identification of the agent

From (IARC, 2006).

Chem. Abstr. Serv. Reg. No.: 50-00-0
Chem. Abstr. Name: Formaldehyde
IUPAC Systematic Name: Methanal
Synonyms: Formaldehyde gas; formic aldehyde; methaldehyde; methyl aldehyde; methylene oxide; oxomethane; oxymethylene



CH_2O
Relative molecular mass: 30.03
Description: Colourless gas with a pungent odour
Conversion factor: $mg/m^3 = 1.23 \times ppm$; calculated from: $mg/m^3 = (\text{relative molecular mass}/24.45) \times ppm$, assuming standard temperature (25 °C) and pressure (103.5 kPa).

1.2 Use

Formaldehyde is produced worldwide on a large scale by catalytic, vapour-phase oxidation of methanol. Formaldehyde is used mainly in the production of various types of resin. Phenolic, urea, and melamine resins have wide uses as adhesives and binders in the wood-production, pulp-and-paper, and the synthetic vitreous-fibre industries, in the production of plastics and coatings, and in textile finishing. Polyacetal resins are widely used in the production of plastics. Formaldehyde is also used extensively as an intermediate in the manufacture of industrial chemicals, such as 1,4-butanediol, 4,4′-methylenediphenyl diisocyanate, penta-erythritol, and hexamethylenetetramine. Formaldehyde is used directly in aqueous solution (known as formalin) as a disinfectant and preservative in many applications (IARC, 2006).

1.3 Occurrence and exposure

1.3.1 Environmental occurrence and exposure

Formaldehyde is found as a natural product in most living systems and in the environment. It occurs naturally in fruits and some foods, and it is formed endogenously in mammals, including humans, as a consequence of oxidative metabolism. In addition to these natural sources, common non-occupational sources of exposure to formaldehyde include combustion processes, e.g. through emissions from motor vehicles, power plants, incinerators, refineries, wood stoves, and kerosene heaters. Formaldehyde may be released from particle boards and similar building materials, carpets, paints and varnishes, during cooking of some foods, and during its use as a disinfectant. It is also present in tobacco smoke. An indirect source of exposure to formaldehyde is its formation via photochemical oxidation of hydrocarbons, such as methane, and other precursors emitted from combustion processes (NTP, 2005; IARC, 2006). Formaldehyde has a short half-life in the environment, because it is removed from the air by photochemical processes and by precipitation and biodegradation (NTP, 2005).

Concentrations of formaldehyde in outdoor air are generally below 0.001 mg/m^3 in remote areas and below 0.02 mg/m^3 in urban settings. The levels of formaldehyde in indoor air of houses are typically 0.02–0.06 mg/m^3; indoor combustion sources can significantly increase these levels. Cigarettes may contribute as much as 10–25% of the indoor exposure. Average concentrations of 0.5 mg/m^3 or more have been measured in 'mobile homes', but these have declined since the late 1980s as a result of standards that require that building materials – e.g. particle boards – emit lower concentrations of formaldehyde. A recent study of emissions from mosquito coils found the average concentration of formaldehyde exceeded 100 µg/m^3 (IARC, 2006, 2010; Lee & Wang, 2006). Data on formaldehyde concentrations in outdoor air in residential and public settings, and information on exposure to formaldehyde associated with household use of solid fuels and high-temperature frying, have been reviewed in *IARC Monograph* Volumes 88 and 95 (IARC, 2006, 2010).

Automobile exhaust is a major source of formaldehyde in ambient air. Recent reports suggest that formaldehyde emissions may be higher from vehicles powered by compressed natural gas compared with those running on ethanol or gasohol (Corrêa & Arbilla, 2005), and that these emissions may be decreased by substitution of an ethanol-biodiesel-diesel blend for diesel fuel (Shi *et al.*, 2006). In addition, formaldehyde can be absorbed through the skin from cosmetics or via contact with other consumer products containing formaldehyde, such as unwashed permanent-press fabrics treated with formaldehyde-releasing resins (NTP, 2005).

1.3.2 Occupational exposure

Occupational exposure to formaldehyde occurs in a wide variety of occupations and industries. CAREX (CARcinogen EXposure) is an international information system on occupational exposure to known and suspected carcinogens based on data collected in the European Union (EU) from 1990 to 1993. The CAREX database provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Table 1.1 presents the results for formaldehyde in the EU by industry (CAREX, 1999).

In *IARC Monograph* Volume 88 (IARC, 2006) data were reviewed on occupational exposure to formaldehyde by type of industry. The highest continuous exposures (2–5 ppm; 2.5–6.1 mg/m^3) were measured in the past during varnishing of furniture and wooden floors, in the finishing of

Table 1.1 Estimated numbers of workers exposed to formaldehyde above background levels in the European Union

Industry, occupational activity	
Manufacture of furniture and fixtures, except primarily of metal	179000
Medical, dental, and other health and veterinary services	174000
Manufacture of wearing apparel, except footwear	94000
Manufacture of wood and wood and cork products, except furniture	70000
Personal and household services	62000
Construction	60000
Manufacture of textiles	37000
Iron and steel basic industries	29000
Manufacture of fabricated metal products, except machinery	29000
Manufacture of other non-metallic mineral products	23000
Manufacture of machinery, except electrical	20000
Manufacture of industrial chemicals	17000
Manufacture of other chemical products	17000
Manufacture of plastic products not classified elsewhere	16000
Agriculture and hunting	16000
Manufacture of paper and paper products	13000
Printing, publishing and allied industries	13000
Wholesale and retail trade and restaurants and hotels	13000
Manufacture of transport equipment	11000
Manufacture of electrical machinery, apparatus and appliances	10000
Manufacture of footwear	9000
Manufacture of glass and glass products	8000
Research and scientific institutes	7000
Non-ferrous metal basic industries	6000
Manufacture of leather and products of leather or of its substitutes	6000
Beverage industries	4000
Manufacture of instruments, photographic and optical	4000
Other manufacturing industries	3000
Food manufacturing	3000
Crude petroleum and natural gas production	2000
Manufacture of rubber products	4000
Financing, insurance, real estate and business services	3000
Education services	2000
Sanitary and similar services	2000
Services allied to transport	2000
Manufacture of miscellaneous products of petroleum and coal	1000
Other industries	2000
Total (all industries)	971000

From Kauppinen *et al.* (2000), CAREX (1999)

textiles, in the garment industry, in the treatment of fur, and in certain jobs within manufactured board mills and foundries. Short-term exposures to high levels (3 ppm and higher; ≥ 3.7 mg/m^3) have been reported for embalmers, pathologists, and paper workers. Lower concentrations have usually been encountered during the manufacture of man-made vitreous fibres, abrasives and rubber, and in formaldehyde-production industries. A very wide range of exposure levels has been observed in the production of resins and plastic products. The development of resins that release less formaldehyde, and improved ventilation have resulted in lower exposure levels in many industrial settings in recent decades (IARC, 2006).

2. Cancer in Humans

2.1 Cancer of the nasopharynx

In *IARC Monograph* Volume 88 (IARC, 2006) it was concluded that there was *sufficient evidence* for the carcinogenicity of formaldehyde, based primarily on its association with nasopharyngeal cancer. There have been relatively few new studies published on this association since that time, although there have been several re-evaluations and meta-analyses.

2.1.1 Cohort studies

In the most recent follow-up of the largest cohort study from the USA of industrial workers exposed to formaldehyde, a statistically significant excess of deaths from nasopharyngeal cancer was observed in comparison with the US national population, with statistically significant exposure–response relationships for peak exposure and cumulative exposure (Hauptmann *et al.*, 2004; see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-24-Table2.1.pdf). Based on eight cases, a significant excess mortality from nasopharyngeal cancer was observed among formaldehyde-exposed workers (SMR, 2.10; 95%CI: 1.05–4.21). A highly statistically significant (P_{trend} < 0.001) exposure–response relationship was observed between peak-exposure to formaldehyde and risk for nasopharyngeal cancer in a Poisson regression-analysis. All exposed cases were in the highest category of peak-exposure, and the relative risk was 1.83. This analysis excluded one case which, according to cancer registry data, had been misclassified as nasopharyngeal cancer. Weaker exposure–response relationships were observed between nasopharyngeal cancer and average or cumulative exposure, and duration of exposure (P_{trend} = 0.07, 0.03 and 0.15, respectively).

In the two other large cohort studies of industrial workers, cases of nasopharyngeal cancer were fewer than expected, but the power of these studies to detect an effect on nasopharyngeal cancer was low and the deficits were small. In the first study, of British chemical workers, one death was observed when 2.0 were expected (Coggon *et al.*, 2003); in the second study, no deaths were observed among US garment-manufacturers, where 0.96 were expected (Pinkerton *et al.*, 2004).

An excess of deaths from nasopharyngeal cancer was observed in a proportionate mortality analysis of the largest US cohort of embalmers (Hayes *et al.*, 1990) and in a Danish study of proportionate cancer incidence among workers at companies that used or manufactured formaldehyde (Hansen & Olsen, 1995; see Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-24-Table2.2.pdf).

Marsh *et al.* (1996) conducted a cohort study in one of the plants considered in the NCI study (where five of the nine cases of nasopharyngeal cancer occurred). The cohort included earlier year of entry and was enumerated independently. Significantly increased mortality due to nasopharyngeal cancer was observed among formaldehyde-exposed workers compared with US and regional populations (Connecticut State and

local county). In a recent follow-up through 2003, Marsh *et al.* (2007a) showed elevated SMRs when both national and local county rates were used. In addition, when conducting a case–control study nested within the cohort and including seven deaths from nasopharyngeal cancer, the authors obtained information on employment outside the formaldehyde industry and showed that five of these workers had been employed as a silversmith. However, while there was some evidence of effect modification by activities as a silversmith (based on small numbers), confounding alone did not explain the relatively high number of deaths from nasopharyngeal cancer in this plant (Marsh *et al.*, 2007a).

Two analyses have been conducted to re-analyse the data from the most recent update of the NCI cohort, with a focus on solid tumours (Hauptmann *et al.*, 2004). The first included an analysis of exposure category and SMR, as well as an analysis of Plant 1, where five of nine deaths from nasopharyngeal cancer occurred, compared with all other plants in the cohort (Marsh & Youk, 2005). Using their own cut-points of exposure, the authors concluded that their analysis lent uncertainty to the findings from the NCI cohort. In another re-analysis, the authors further controlled for the effect of plant for the peak-exposure metric and performed sensitivity analyses by imputing additional cases, which showed instability in the risk estimates (Marsh *et al.*, 2007b). The authors concluded that an interaction between plant group and exposure makes generalization beyond Plant 1 difficult.

2.1.2 Case–control studies

The relationship between nasopharyngeal cancer and exposure to formaldehyde has also been investigated in seven case–control studies, five of which found elevated risks for overall exposure to formaldehyde or in higher exposure categories, although not all were statistically significant (see Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-24-Table2.3.pdf; Vaughan *et al.*, 1986b; Roush *et al.*, 1987; West *et al.*, 1993; Vaughan *et al.*, 2000; Hildesheim *et al.*, 2001). One study found an elevation among women, but not men (Olsen *et al.*, 1984) and one found no evidence of an association (Armstrong *et al.*, 2000). Two case–control studies were considered as the most informative because of their size, their exposure assessment, and the evaluation of potential confounders. The first, a population-based case–control study in the USA, showed a significant association for the workers whose exposure duration had been the longest (OR = 2.1; 95%CI: 1.0–4.5, P_{trend} = 0.07), but not for maximum exposure (P_{trend} = 0.57) (Vaughan *et al.*, 2000). When the analysis was limited to differentiated squamous-cell and epithelial NOS, there was a significant association in the highest exposure category for both duration and cumulative exposure with significant exposure-response trends (P_{trend} = 0.014 and 0.033, respectively). In the other study, conducted in Taiwan, China, an OR of 1.6 (95%CI: 0.91–2.9, P_{trend} = 0.08) was found in the category with the longest duration of exposure (Hildesheim *et al.*, 2001). For cumulative exposure, there was a non-significant elevation in the highest exposure category and the trend test was not significant (P = 0.10). In subanalyses that were restricted to cases and controls who were seropositive for antibodies against Epstein-Barr virus, the association between exposure to formaldehyde and nasopharyngeal cancer appeared to be stronger, with an OR for ever exposure of 2.7 (95%CI: 1.2–6.2). However, no clear dose–response pattern was observed with increasing duration of exposure, or with estimated cumulative exposure.

2.1.3 Meta-analyses

A meta-analysis published in 1997 included some but not all of the above studies, and found an overall meta-relative risk for nasopharyngeal

cancer of 1.3 (95%CI: 1.2–1.5) (Collins *et al.*, 1997). From a pooled analysis including the three recently updated industrial cohorts (Coggon *et al.*, 2003; Hauptmann *et al.*, 2004; Pinkerton *et al.*, 2004), Bosetti *et al.* (2008) reported an overall SMR of 1.33 (95%CI: 0.61–2.53). A recently published meta-analysis included both case–control studies (n = 6) and cohort studies (n = 7) (Bachand *et al.*, 2010). For the case–control studies, the overall OR was 1.22 (95%CI: 1.00–1.50), with the meta-regression OR no longer significant when limited to studies that included adjustment for socioeconomic status, smoking or location. The risk estimate for cohort studies was 0.72 (95%CI: 0.40–1.29), including seven studies (Bachand *et al.*, 2010). For the cohort studies, the authors used a re-analysis of the NCI cohort study from which Plant 1 was left out (Marsh & Youk, 2005).

2.2 Leukaemia

In *IARC Monograph* Volume 88 (IARC, 2006) it was concluded that there was strong, but not sufficient evidence for the leukaemogenic effects of formaldehyde. Since that time, an update to the NCI cohort and a nested case–control study of workers in the funeral industry have been published (Beane Freeman *et al.*, 2009; Hauptmann *et al.*, 2009), as well as three meta-analyses (Bosetti *et al.*, 2008; Zhang *et al.*, 2009; Bachand *et al.*, 2010; see Table 2.1 online, and Table 2.5 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-24-Table2.5.pdf).

2.2.1 Cohort studies

Excess mortality from leukaemia has been observed relatively consistently in studies of professional workers (i.e. embalmers, funeral parlour workers, pathologists and anatomists), with six mortality studies showing positive associations (Walrath & Fraumeni, 1983, 1984; Levine *et al.*, 1984; Stroup *et al.*, 1986; Hayes *et al.*, 1990; Hall *et al.*, 1991) and one not (Logue *et al.*, 1986; see Table 2.2 online).

A weakness of the proportionate mortality studies among professionals has been the lack of exposure assessment. A recently published nested case–control study conducted among professionals in the funeral industry examined lifetime work practices and exposure in the funeral industry to develop metrics of exposure among this group, which included duration of jobs held while embalming, number of embalmings, average intensity of embalming and peak exposure (Hauptmann *et al.*, 2009). Details of work practices were obtained by interviews with next of kin and co-workers. Positive associations were seen – at many levels of exposure and for multiple exposure metrics – for deaths from lymphohaematopoietic malignancies of non-lymphoid origin (n = 48). For myeloid leukaemia (n = 34) the OR was 13.6 (95%CI: 1.6–119.7; P_{trend} = 0.020) for the longest duration of work in jobs with embalming. Because only one case was reported to have never embalmed, additional analyses were conducted in which those who reported to have embalmed ≤ 500 times were taken as the referent group, to provide a more stable estimate. Results were attenuated, but still significant (OR = 3.9; 95%CI: 1.2–12.5). [There was a considerable amount of missing data that required imputation for analyses.]

The findings for leukaemia in studies of professional workers appeared to be contradicted by the lack of such findings among industrial workers. However, some evidence for an excess of deaths from leukaemia has been reported in the recent updates of two of the three major cohort studies of industrial workers. Since the previous evaluation (IARC, 2006), the NCI cohort of industrial workers in the USA has been updated with an additional ten years of mortality data resulting in 123 deaths from leukaemia, including 48 from myeloid leukaemia (Beane Freeman *et al.*, 2009). This update extended the mortality follow-up

through 2004 and included additional deaths before 1994 that had not been previously considered. Risk estimates from follow-up through 2004 were diminished for leukaemia and myeloid leukaemia compared with the follow-up through 1994 (Hauptmann *et al.*, 2003), when both conditions had been significantly associated with increasing peak-exposure and average intensity of exposure to formaldehyde. As in the previous analysis of leukaemia, the association in the most recent update was stronger for myeloid leukaemia and peak exposure than for lymphatic leukaemia and for other metrics of exposure (Beane Freeman *et al.*, 2009). However, because the last known exposure occurred in 1980 and median follow-up was over 40 years, the authors not only examined risks at the end of follow-up in 2004, but also assessed associations over time by extending follow-up in yearly increments. Risks appeared to be highest before 1980, but only achieved statistical significance in the mid-1990s, when a sufficient number of deaths had accrued. Additional analyses with time since first exposure and time since first high peak-exposure indicated that risks were highest during the first twenty-five years. Patterns were similar, but attenuated, for average intensity of exposure; no association was observed with cumulative exposure.

Mortality from leukaemia was also found to be in excess in an update of the study of US garment workers exposed to formaldehyde (Pinkerton *et al.*, 2004). A small and statistically non-significant excess was observed for the entire cohort in comparison with rates among the general population (SMR = 1.09; 95%CI: 0.7–1.63). This excess was somewhat stronger for myeloid leukaemia (SMR = 1.44; 95%CI: 0.80–2.37), which is consistent with the findings from the study of industrial workers in the USA and several of the studies of medical professionals and embalmers. The excess was also stronger among workers with a longer duration of exposure and longer follow-up, and among those who had been employed early in the study period when exposures to formaldehyde were believed to be highest. The positive associations observed in the subgroup analyses presented in the study of US garment workers were based on a relatively small number of deaths, and were thus not statistically stable.

The updated study of British industrial workers found no excess mortality for leukaemia among all workers exposed to formaldehyde (SMR = 0.91; 95%CI: 0.62–1.29) or among those with the highest exposure (SMR = 0.71; 95%CI: 0.31–1.39) (Coggon *et al.*, 2003). The lack of positive findings in this study is difficult to reconcile with the findings from the studies of garment workers and industrial workers in the USA, and with the results of studies on professionals exposed to formaldehyde. This British study is a relatively large, high-quality study with sufficiently long follow-up to have had a reasonable chance to detect an excess of deaths from leukaemia. It did not examine specifically the risk for myeloid leukaemia, which represented the strongest finding in the studies of garment workers and industrial workers in the USA and in several of the studies of medical professionals and funeral workers.

2.2.2 Case–control studies

Three case–control studies evaluated exposure to formaldehyde and risk for leukaemia (Linos *et al.*, 1990; Partanen *et al.*, 1993; Blair *et al.*, 2001; Table 2.5 online). However, the numbers of exposed cases were few, and no significant elevations of risk were found.

2.2.3 Meta-analyses

A meta-analysis published in 2004 for 'ever exposure' to formaldehyde and leukaemia included eighteen studies and presented separate analyses by type of job: for industrial workers, the mRR was 0.9 (95%CI: 0.8–1.0); for

embalmers 1.6 (95%CI: 1.2–2.0); and for pathologists and anatomists 1.4 (95%CI: 1.0–1.9), with an overall mRR of 1.1 (95%CI: 1.0–1.2) (Collins & Lineker, 2004). In another meta-analysis, analysis was restricted to 13 cohort or proportionate mortality studies and similar results were found, with a pooled RR based on the weighted average of the SMRs for leukaemia among industrial workers of 0.9 (95%CI: 0.75–1.07), based on 122 deaths, and of 1.39 (95%CI: 1.15–1.68) among professionals, based on 106 deaths (Bosetti *et al.*, 2008). A further meta-analysis differed from these two previous ones by excluding all proportionate mortality studies and including the most recent update of the NCI cohort (Bachand *et al.*, 2010). For leukaemia overall, a risk estimate of 1.05 (95%CI: 0.93–1.20) was calculated for 'ever exposure', based on 15 studies with the use of a fixed-effects model. For myeloid leukaemia, the calculated mRR was 1.09 (95%CI: 0.84–1.40, based on three studies) and for lymphatic leukaemia the mRR was 1.11 (95%CI: 0.81–1.52, based on two studies).

Zhang *et al.* (2009) published a meta-analysis that included 15 cohort or case–control studies. The authors selected only studies where it was clear that the workers had been exposed to formaldehyde. In contrast to the other meta-analyses, this one used one exposure metric from each study and considered the highest exposure category for calculating the mRR. For leukaemia, the mRR was 1.54 (95%CI: 1.18–2.00). In addition, a separate analysis of myeloid leukaemia – for the six studies that reported it – found an mRR of 1.90 (95%CI: 1.31–2.76).

2.3 Cancer of the nasal sinuses

2.3.1 Cohort studies

An analysis of proportionate cancer incidence among industrial workers in Denmark showed an increased risk for squamous-cell carcinomas (Hansen & Olsen, 1995, 1996). No excess of mortality from sinonasal cancer was observed in the three recently updated studies of industrial and garment workers in the USA, and of chemical workers in the United Kingdom (see Table 2.1 online; Coggon *et al.*, 2003; Hauptmann *et al.*, 2004; Pinkerton *et al.*, 2004).

2.3.2 Case–control studies

The association between exposure to formaldehyde and the risk for sinonasal cancer has been evaluated in six case–control studies that primarily focused on formaldehyde (see Table 2.4 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-24-Table2.4.pdf; Olsen *et al.*, 1984; Hayes *et al.*, 1986; Olsen & Asnaes, 1986; Vaughan *et al.*, 1986a; Roush *et al.*, 1987; Luce *et al.*, 1993; Pesch *et al.*, 2008). Four of these six studies reported an increased risk (Olsen *et al.*, 1984; Hayes *et al.*, 1986; Vaughan *et al.*, 1986a; Luce *et al.*, 1993).

2.3.3 Pooled analysis

Four of the cohort studies contributed to a pooled analysis that collated occupational data from 12 case–control investigations (Luce *et al.*, 2002). After adjustment for known occupational confounders, this analysis showed an increased risk for adenocarcinoma associated with high exposure (> 1 ppm) to formaldehyde in both men (OR, 3.0; 95%CI: 1.5–5.7) and women (OR, 6.3; 95%CI: 2.0–19.7). An exposure–response trend was observed in relation to an index of cumulative exposure. There was some evidence of an association with squamous-cell carcinoma.

[Most epidemiological studies of sinonasal cancer have not distinguished between tumours that arise in the nose and those that develop in the nasal sinuses. Thus, any effect on the risk for nasal cancer specifically would tend to be diluted if there were no corresponding effect on the risk for cancer in the sinuses and could mask its detection, particularly in cohort studies that

have relatively low statistical power. However, the apparent discrepancy between the results of the case–control as compared with the cohort studies might also reflect residual confounding by wood dust in the former. Almost all of the formaldehyde-exposed cases in the case–control studies were also exposed to wood dust, which resulted in a high relative risk, particularly for adenocarcinomas.]

2.4 Other cancers

Several studies have identified statistically significant positive associations between exposure to formaldehyde and cancer at other sites, including the oral cavity, oro-and hypopharynx, larynx, lung, brain, pancreas, Hodgkin lymphoma, and multiple myeloma. However, the results are inconsistent (see Tables 2.4 and 2.5 online; Table 2.6 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-24-Table2.6.pdf, and Table 2.7 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-24-Table2.7.pdf).

2.5 Synthesis

The Working Group noted one industrial cohort study with both a strong overall association between exposure to formaldehyde and nasopharyngeal cancer, and the most elevated risks in the highest exposure category. Positive associations were also observed in many of the case–control studies, in particular those of larger size and higher-quality exposure assessment. While there was no association observed in the two other large industrial cohort studies, the expected number of cases in those studies was quite small. It is concluded that occupational exposure to formaldehyde causes nasopharyngeal cancer in humans. The Working Group noted that it was unlikely that confounding or bias could explain the observed association.

Elevated risks of leukaemia have been consistently observed in proportionate mortality studies of professionals exposed to formaldehyde (i.e. embalmers, workers in the funeral industry, pathologists and anatomists). Results from a nested case–control study of workers in the funeral industry show elevated risks for many measures of exposure, which are strongest for myeloid leukaemia. In two of the three large industrial cohort studies positive associations were observed for leukaemia, which were somewhat stronger for myeloid leukaemia. It is difficult to reconcile the lack of association observed in the third industrial cohort study with the overall positive associations in the others. However, there seems to be no strong evidence that confounding or bias explains the positive associations seen in multiple settings. On balance, the Working Group concluded that the epidemiologic evidence shows that occupational exposure to formaldehyde causes leukaemia.

Many case–control studies show positive associations for exposure to formaldehyde and sinonasal cancer, some with evidence of an exposure–response pattern. However, many of these cases were also exposed to wood dust, which was strongly associated with sinonasal cancer in these studies. The industrial cohort studies show no such association, which may be due to lack of statistical power, or could indicate that uncontrolled confounding to wood dust partially explains the observed associations in the case–control studies. The Working Group could not rule out the possibility of residual confounding in the case–control studies and noted the discordant results between the cohort and case–control studies.

3. Cancer in Experimental Animals

Carcinogenicity studies with mice, rats and hamsters exposed to formaldehyde by inhalation, via the drinking-water, or through the skin were reviewed in *IARC Monograph* Volume 88 (IARC, 2006). Results of adequately conducted carcinogenicity studies are summarized in Table 3.1. There have been no additional carcinogenicity studies in experimental animals reported since the previous review.

3.1 Inhalation

In one inhalation study in B6C3F1 mice, formaldehyde marginally increased the incidence of squamous cell carcinomas of the nasal cavity in males. The incidence of lymphoma in females exposed to 14.3 ppm (27/121) was also marginally increased ($P = 0.06$) when compared (pair-wise) with controls (19/121) (CIIT, 1981; Kerns *et al.*, 1983a, b; Gibson, 1984).

In six studies (Swenberg *et al.*, 1980; CIIT, 1981; Albert *et al.*, 1982; Kerns *et al.*, 1983a, b; Gibson, 1984; Sellakumar *et al.*, 1985; Feron, *et al.*, 1988; Woutersen *et al.*, 1989; Monticello *et al.*, 1996; Kamata *et al.*, 1997) in different strains of rats (F344, Wistar, and Sprague-Dawley), there were treatment-related increases in tumours of the nasal cavity (primarily squamous-cell carcinomas but also squamous-cell papillomas, polypoid adenomas, carcinomas, rhabdomyosarcomas, adenocarcinomas, and mixed/combined tumours). In one study (CIIT, 1981), the incidences of undifferentiated leukaemia [Fischer rat leukaemia, as indicated in the report] were 12/120 (control), 17/120 (2 ppm), 16/120 (5.6 ppm) and 7/120 (14.3 ppm) in females; there was a marked decrease in survival in the animals exposed to the high dose. Based on a survival-adjusted analysis, the incidence of leukaemia in females exposed to 14.3 ppm was increased compared with controls ($P = 0.0056$; Tarone-extension of the Cox test; level of significance, $P < 0.0167$). [The Working Group noted that this type of leukaemia is a very common, spontaneously occurring neoplasm in the F344 rat strain].

3.2 Oral administration (drinking-water)

In one drinking-water study in male Wistar rats, there was a treatment-related increase in fore-stomach squamous-cell papillomas (Takahashi *et al.*, 1986). Another study in male and female Wistar rats did not report any increase in tumours (Til *et al.*, 1989).

In a study with life-long exposure to formaldehyde, beginning *in utero* (transplacentally), there was an increased incidence of smooth-muscle tumours of the small intestine (leiomyosarcoma) in female offspring (Soffritti *et al.*, 1989). In another study in male and female rats, increased incidences of total malignant tumours, haematopoietic tumours, and interstitial-cell adenomas were observed in males (Soffritti *et al.*, 1989, 2002). [The Working Group reaffirmed the concerns of the previous Working Group (IARC, 2006) regarding the pooling of all 'leukaemias' diagnosed as lymphoblastic leukaemias and lymphosarcomas, immunoblastic lymphosarcomas, and "other types" of leukaemia and haemolymphoreticular sarcomas. Also noted were the lack of reporting of non-neoplastic lesions and historical control data, and the numerous discrepancies in tumour incidence between the first (Soffritti *et al.*, 1989) and second report (Soffritti *et al.*, 2002) of the results of this study].

3.3 Skin application

In one study in male and female hairless Oslo mice, topical application of 10% formaldehyde in water reduced the latency of 7,12-dimethylbenz[*a*]anthracene-induced skin tumours (Iversen, 1986).

Table 3.1 Carcinogenicity studies in experimental animals exposed to formaldehyde

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Inhalation studies				
C3H mouse (unspecified) 35 wk (some for 64 wk) Horton *et al.* (1963)	0, 50, 100, 200 mg/m^3 1 h/d, 3 d/wk 42–60/group	No pulmonary tumours	NS	USP grade Due to severe toxicity, exposure to 200 mg/m^3 was discontinued after the 11th exposure. Thirty-six mice exposed to 50 mg/m^3 were exposed to 150 mg/m^3 for 29 additional wk. Basal-cell hyperplasia, squamous metaplasia and atypical hyperplasia were observed in trachea and bronchi of many exposed mice. Nasal tissues were not examined. Short period of exposure and short duration of study.
B6C3F1 mouse (M) 30 mo Kerns *et al.* (1983a, b), Gibson (1984)	0, 2, 5.6, 14.3 ppm (0, 2.5, 6.9, 17.6 mg/m^3) 6 h/d, 5 d/wk for 24 mo 119–120/group	No increased tumour incidence Nasal cavity (malignant)[a]: 2/17 (14.3 ppm) vs 0/21 (controls) at 24 mo	NS	> 97.5% purity Interim sacrifices (10/group) at 6 and 12 mo; 0–1 at 18 mo; 17–21 at 24 mo. Squamous-cell hyperplasia, metaplasia and dysplasia were commonly present in nasal passages of mice exposed to 14.3 ppm.
B6C3F1 mouse (F) 30 mo Kerns *et al.* (1983a, b), Gibson (1984), CIIT (1981)	0, 2, 5.6, 14.3 ppm (0, 2.5, 6.9, 17.6 mg/m^3) 6 h/d, 5 d/wk for 24 mo 120–121/group	No increased tumour incidence Lymphoma: 27/121 (14.3 ppm) vs 19/121 (controls)	NS (P = 0.06)	> 97.5% purity Interim sacrifices (10/group) at 6 and 12 mo; 19–20 at 18 mo; 26–41 at 24 mo; 9–16 at 27 mo. Squamous-cell hyperplasia, metaplasia and dysplasia were commonly present in nasal passages of mice exposed to 14.3 ppm.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 rat (M) 30 mo Swenberg *et al.* (1980), Kerns *et al.* (1983a, b), Gibson (1984)	0, 2, 5.6, 14.3 ppm (0, 2.5, 6.9, 17.6 mg/m^3) 6 h/d, 5 d/wk for 24 mo 119–120/group	Nasal cavity (malignant)[a]: 0/118, 0/118, 1/119, 51/117*	*$P < 0.001$	> 97.5% purity Interim sacrifices (10/group) at 6 and 12 mo; 20 at 18 mo; 13–54 at 24 mo; 5–10 at 27 mo; 0–6 at 30 mo. Squamous-cell hyperplasia, metaplasia and dysplasia were commonly present in nasal passages of rats exposed to 14.3 ppm.
		Nasal cavity (malignant)[b]: 0/118, 0/118, 0/119, 4/117	NS	
		Nasal cavity (benign)[c]: 1/118, 4/118, 6/119, 4/117	NS	
F344 rat (F) 30 mo Swenberg *et al.* (1980), Kerns *et al.* (1983a, b), Gibson (1984), CIIT (1981)	0, 2, 5.6, 14.3 ppm (0, 2.5, 6.9, 17.6 mg/m^3) 6 h/d, 5 d/wk for 24 mo 120/group	Nasal cavity (malignant)[a]: 0/114, 0/118, 1/116, 52/115*	*$P < 0.001$	> 97.5% purity Interim sacrifices (10/group) at 6 and 12 mo; 19–20 at 18 mo; 14–47 at 24 mo; 0–10 at 27 mo; 0–5 at 30 mo. Squamous-cell hyperplasia, metaplasia and dysplasia were commonly present in nasal passages of rats exposed to 14.3 ppm.
		Nasal cavity (malignant)[b]: 0/114, 0/118, 0/116, 1/115	NS	
		Nasal cavity (benign)[c]: 0/114, 4/118, 0/116, 1/115	NS	
		Haematopoietic tissue (spleen, F344 rat leukaemia diagnosed as undifferentiated leukaemia): 12/120, 17/120, 16/120, 7/120	$P = 0.0056$; Tarone-extension of the Cox test (adjustment for mortality), level of significance is $P < 0.0167$	
Sprague-Dawley rat (M) Lifetime Albert *et al.* (1982); Sellakumar *et al.* (1985)	0, 14.3 ppm (0, 17.6 mg/m^3) 6 h/d, 5 d/wk 99–100/group	Nasal cavity (malignant)[a]: 0/99, 38/100	$P \leq 0.001$	A mixed carcinoma and fibrosarcoma of the nasal cavity was also present in the formaldehyde-treated group.
		Nasal cavity (benign): 0/99, 10/100	$P \leq 0.001$	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Wistar rat (M) 126 wk Feron *et al.* (1988)	0, 10, 20 ppm (0, 12.3 or 25 mg/m^3) 6 h/d, 5 d/wk for 4, 8 or 13 wk 45/group/interval	Nasal cavity: 2/134, 2/132, 10/132 (denominator combines all intervals of exposure for control and treated groups); the authors considered 6/10 tumours[d] in the high-dose group as treatment-related.	NR	Purity NR Hyperplasia and metaplasia of nasal epithelium were observed in all rats exposed to formaldehyde. Authors considered most nasal-cavity tumours in the high-dose group to be related to the treatment.
Wistar rat (M) 28 mo Woutersen *et al.* (1989)	0, 0.1, 1, 10 ppm (0, 0.123, 1.23, and 12.3 mg/m^3) 6 h/d, 5 d/wk for 3 or 28 mo 30/group (U: undamaged) or 60/group (D: damaged)	Nasal cavity (malignant)[e]: 28 mo exposure U: 0/26, 1/26, 1/26, 1/26 D: 1/54, 1/58, 0/56, 17/58* 3 mo exposure U: 0/26, 0/30, 0/29 2/26 D: 0/57, 2/57, 2/53, 2/54	*[$P < 0.001$; Fisher's exact test]	Purity NR Mucosa severely damaged by electro-coagulation during the first wk. Eight squamous-cell carcinomas from the nasolacrimal duct were excluded by the authors.
Sprague Dawley rat (F) 104 wk Holmström *et al.* (1989)	0, 12.4 ppm (0, 15.3 mg/m^3) 6 h/d, 5 d/wk 16/group	Nasal cavity: 0/16, 1/16[a]	NS	Purity NR Pronounced squamous-cell metaplasia and/or dysplasia in 10/16 rats exposed to formaldehyde vs 0/15 controls. Small group-size noted.
F344 rat (M) 24 mo Monticello *et al.* (1996)	0, 0.69, 2.05, 6.01, 9.93, 14.96 ppm (0, 0.84, 2.4, 7.2, 12, 19 mg/m^3) 6 h/d, 5 d/wk 90 or 147 (high dose group only)/group	Nasal cavity (malignant)[a]: 0/90, 0/90, 0/90, 1/90, 20/90, 69/147* Nasal cavity (benign)[c]: 0/90, 0/90, 0/90, 0/90, 5/90, 14/147[$] One nasal rhabdomyosarcoma and nasal adenocarcinoma each present in groups given 9.93 and 14.96 ppm	NR,*[$P < 0.001$] NR,[$][$P < 0.02$]	Formaldehyde vapour of paraformaldehyde Interim sacrifices at 3, 6, 12 and 18 mo (6/group).

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 rat (M) 28 mo Kamata *et al.* (1997)	0, 0, 0.3, 2.17, 14.85 ppm (0, 0, 0.36, 2.6, 17.8 mg/m^3) 6 h/d, 5 d/wk 32/group (one room-air control and one methanol-exposed control group)	Nasal cavity (malignant)[a]: 0/32, 0/32, 0/32, 0/32, 13/32* Nasal cavity (benign)[f]: 0/32, 0/32, 0/32, 0/32, 3/32	*$P < 0.01$ NR	Formaldehyde vapour of 37% aqueous formaldehyde solution with 10% methanol (4.2 ppm) Interim sacrifices at 12, 18 and 24 mo (5/group).
Rat (strain not specified) (F) Lifetime Yanysheva *et al.* (1998)	0, 0.003, 0.03, 0.3 mg/m^3, 7 h/d, 5 d/wk for 12 mo Intratracheal injection of a total dose of 0, 0.02, 0.1 or 5.0 mg B[*a*]P/animal over 20 wk 50/group	Lung tumours: 24/35 (68.6%, 5.0 mg B[a]P + 0.3 mg/m^3 formaldehyde) vs 8/28 (28.1%, 5.0 mg B[a]P)	$P < 0.01$	Purity NR Promotion effect
Hamster, Syrian golden (M) Lifetime Dalbey (1982)	0, 10 ppm (0, 12.3 mg/m^3) 5 h/d, 5 d/wk 88/group (132 controls)	No tumours	-	Purity NR Hyper- and metaplastic areas were each observed in the nasal epithelium of 5% of exposed animals
Hamster, Syrian golden (M) Lifetime Dalbey (1982)	0, 30 ppm (0, 36.9 mg/m^3) 5 h/d, 5 d/wk 50/group	No tumours	-	Purity NR
Hamster, Syrian golden (M) Lifetime Dalbey (1982)	0, 30 ppm (0, 36.9 mg/m^3) 5 h/d, 5 d/wk Both groups subcutaneously injected wkly with 0.5 mg NDEA for 10 wk 50/group	Tracheal tumours: [~2.8 tumours/tumour-bearing animal (NDEA + formaldehyde) vs ~1.7 tumours/tumour-bearing animal (NDEA)]	$P < 0.05$	Purity NR Promotion effect
Drinking-water studies				
Wistar rat (M) 40 wk Takahashi *et al.* (1986)	Experiment #1 0 or 0.5% formaldehyde 10/group	Fore-stomach squamous-cell papillomas: 8/10 vs 0/10	$P < 0.01$	Purity NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Wistar rat (M) 40 wk Takahashi *et al.* (1986)	Experiment #2 0 and 0.5% formaldehyde. MNNG administered in the drinking-water to both groups for first 8 wk with a diet containing 10% NaCl 30 (control) and 17/group	Adenocarcinoma of the pylorus: 4/17 vs 1/30	$P < 0.05$	Purity NR Weak promotion of tumour incidence in the glandular stomach. Incidence of fore-stomach papillomas was also increased vs controls, but was similar to that in the group treated with formaldehyde only (experiment #1)
Wistar rat (M) 105 wk Til *et al.* (1989)	0, 1.2, 15, 82 mg/kg bw/d 70/group	No increase in tumour incidence	NS	Formaldehyde generated from 95% pure paraformaldehyde. Interim sacrifices of 10 rats after 53 and 79 wk.
Wistar rat (F) 105 wk Til *et al.* (1989)	0, 1.8, 21, 109 mg/kg bw/d 70/group	No increase in tumour incidence	NS	Formaldehyde generated from 95% pure paraformaldehyde. Interim sacrifices of 10 rats after 53 and 79 wk.
Sprague Dawley (M, F) Lifetime Soffritti *et al.* (1989)	0 and 2 500 ppm Exposure of breeders (18–20/group/sex) and offspring (36–59/group/sex) for 104 wk. Exposure of offspring began on d 13 of gestation.	Leukaemias: Breeders (M): 2/18 (11.1%) vs 0/20 (0%) Breeders (F): 2/18 (11.1%) vs 1/20 (5%) Offspring (M): 4/36 (11.1%) vs 3/59 (5.1%) Offspring (F): 0/37 (0%) vs 3/49 (6.1%) Small intestine leiomyosarcoma: Offspring (F): 6/37 (16.2%) vs 0/49 (0%)	 NS NS NS NS $P < 0.01$	Previous and current Working Group and other authors (Feron *et al.*, 1990; IARC, 2006) noted concern about study design, significance of tumour findings and laboratory-control incidences. The Working Group also noted that leukaemias included three types, diagnosed as lymphoblastic leukaemias and lymphosarcomas, immunoblastic lymphosarcomas, and other types of leukaemias and haemolymphoreticular sarcomas. Increased incidence of a variety of benign and malignant stomach and intestinal tumours was also reported. The stomach tumours in treated groups represented single occurrences of neoplasm of different sites/cell type fore-stomach/squamous, smooth muscle and glandular stomach epithelium.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Sprague Dawley (M, F) Lifetime Soffritti *et al.* (1989, 2002)	0 (control), 0 (15 mg/L methanol control), 10, 50, 100, 500, 1 000, 1 500 mg/L for 104 wk 50/group/sex (100/control group)	Testicular interstitial-cell adenomas: 24/50 (1 000 mg/L) vs 6/50 (methanol control)	[$P < 0.01$]	Purity of 99% with 0.3% methanol as stabilizer. Concerns by the Working Group about pooling of several types of lymphohaematopoietic tumours. Concern also about interpretation of the results for lymphohaematopoietic tumours due to the numerous and extensive discrepancies in tumour incidences between the first (Soffritti *et al.*, 1989) and second report (Soffritti *et al.*, 2002) on results for this study. Increased incidence of total malignant tumours in males treated with 1 500 mg/L [$P < 0.01$].
		Lymphohaematopoietic tumours: Males 8/100, 20/50, 8/50, 20/50, 26/50, 24/50, 22/50, 46/50*	[$P < 0.01$, trend], *[$P < 0.01$, vs methanol control]	

[a] Nasal cavity tumours were squamous-cell carcinomas.
[b] Nasal cavity tumours (combined) included carcinomas (2), undifferentiated carcinomas or sarcomas (2) or carcinosarcomas (1).
[c] Nasal cavity tumours were polypoid adenomas.
[d] Three squamous-cell carcinomas, 2 polypoid adenomas, and 1 carcinoma in situ.
[e] Nasal cavity tumours were squamous-cell carcinomas except for one adenosquamous carcinoma and one adenocarcinoma at 10 ppm (D) with 28 months of exposure and one carcinoma in situ at 10 ppm (D) and one polypoid adenoma at 10 ppm (U) with 3 mo of exposure.
[f] Nasal cavity tumours were squamous-cell papillomas.
B[*a*]P, benzo[*a*]pyrene; d, day or days; h, hour or hours; F, female; M, male; mo, month or months; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; NDEA, *N*-nitrosodiethylamine; NR, not reported; NS, not significant; vs, versus; wk, week or weeks

4. Other Relevant Data

In *IARC Monograph* Volume 88 (IARC, 2006) mechanistic considerations supported a role for cytotoxicity and genotoxicity in formaldehyde-induced nasal tissue carcinogenesis. With regards to leukaemia, it was unclear to the Working Group at the time how this reactive compound could penetrate to the bone marrow, and no animal model of formaldehyde-induced leukaemia was available.

The discussion below focuses on mechanistic issues related to the potential causal association between formaldehyde inhalation and haematological cancers, and includes considerations on the mechanism underlying nasal carcinogenesis in laboratory animals and humans. A more detailed review can be found in Volume 88 (IARC, 2006).

4.1 Absorption, distribution, metabolism, and excretion

One-carbon metabolism is central to many biological processes, including the biosynthesis of purines and thymidine – essential components of nucleic acids –, the biosynthesis of certain amino acids, and the demethylation of a variety of important biological compounds that are central to cell function and survival. Formaldehyde is an intermediate in the one-carbon pool and is present in measurable concentrations in all metabolically active cells and tissues (Heck *et al.*, 1982, 1985; Casanova *et al.*, 1988). In aqueous solution, formaldehyde is rapidly converted to its diol form, methanediol (formaldehyde hydrate, $CH_2(OH)_2$, methylene glycol), which enters in a dynamic equilibrium with formaldehyde. The concentration of the diol and that of formaldehyde depend on the precise conditions (temperature, pH, formaldehyde concentration) under which the reaction occurs (Walker, 1964). Importantly, methanediol, with a molecular weight of only 48, can readily penetrate into tissue (Fox *et al.*, 1985). Thus, formaldehyde may reach the bone-marrow through the blood as methanediol, where it equilibrates again to reactive formaldehyde. Further investigation of this equilibrium in living biological systems is warranted.

The absorption of formaldehyde occurs readily in the upper respiratory tract (Casanova *et al.*, 1991; Kimbell *et al.*, 2001a, b). Once inhaled, formaldehyde can react directly with mucus or with macromolecular cellular components including proteins and nucleic acids; it can be incorporated into biological molecules through folate-dependent enzymatic processes; it can be oxidized to formic acid or to carbon dioxide through enzymatic processes dependent on formaldehyde dehydrogenase, aldehyde dehydrogenase and, in limited situations, catalase (Hedberg *et al.*, 2002), or itcan be exhaled. It has been estimated that as much as 22–42% of inhaled formaldehyde may be removed by mucus flow (Schlosser, 1999).

Formaldehyde reacts readily and reversibly with amino groups to form Schiff bases, and with sulfhydryl groups resulting in the formation of *S*-hydroxymethylglutathione, which is oxidized by alcohol dehydrogenase-3 (ADH3) to *S*-formylgluthahione. The latter is further metabolized by *S*-formylgluthione hydrolase to generate formate and gluthione. The formate can also be formed non-enzymatically (Hedberg *et al.*, 2002). Incubation of 0.1–5.0 mM formaldehyde with reduced glutathione in solution followed by addition to deoxyguanosine or to calf-thymus DNA leads to the formation of the relatively stable adduct *S*-[1-(N^2-deoxyguanosinyl) methyl]glutathione (Lu *et al.*, 2009). This adduct may form endogenously, as both formaldehyde and reduced glutathione are present in reasonably high concentrations within cells. It may also serve as a biomarker to study the penetration of inhaled radio-labelled formaldehyde, to distinguish endogenous from exogenous

formaldehyde-derived adducts. Whether this could also be a mechanism by which inhaled formaldehyde could lead to bone-marrow toxicity has not been studied.

Red blood cells have relatively high levels of enzymes that rapidly metabolize formaldehyde. The concentration of formaldehyde in the blood of six human volunteers did not change immediately after exposure to 1.9 ppm [2.34 mg/m^3] formaldehyde for 40 minute (Heck *et al.*, 1985), and no change in formic acid concentration was observed in the urine of medical students over a three-week period during which they were exposed to air concentrations < 0.5 ppm [0.62 mg/m^3] (Gottschling *et al.*, 1984). No statistically significant change in the concentration of formaldehyde in blood was found after inhalation of this substance at 1.9 ppm [2.34 mg/m^3] for 40 minute by six human volunteers; at 14.4 ppm [17.8 mg/m^3] for two hours in rats (Heck *et al.*, 1985); and at 6 ppm [7.4 mg/m^3] for six hours/day, five days per week, for four weeks in Rhesus monkeys (Casanova *et al.*, 1988). Blood was drawn approximately 7 min and 45 hours after the end of the exposure period, from monkeys whose blood levels were 1.84 ± 0.15 μg/g and 2.04 ± 0.40 μg/g, respectively. However, there are methodological concerns with these studies. In the monkey study three animals were used to determine control levels and three others were exposed to formaldehyde. The mean levels were then compared. It would have been better if the monkeys had served as their own control. A similar lack of change in formaldehyde levels was reported in rats (2.25 ± 0.07 *vs* 2.24 ± 0.07 μg/g blood) and humans (2.77 ± 0.28 *vs* 2.61 ± 0.14 μg/g blood; approximately 0.1 mM) exposed for short periods to 14.4 and 1.9 ppm, respectively (Heck *et al.*, 1985; Casanova *et al.*, 1988; IARC, 2006). [The Working Group noted that, given the short half-life for formaldehyde observed in rodents, the time from end-of-exposure to sampling in monkeys and in humans was likely too long; this could not be evaluated for rats].

Studies of the uptake of radio-labelled formaldehyde by inhalation, ingestion and through the skin do not provide information that would help to determine whether unreacted formaldehyde reaches the bone marrow, because it is rapidly taken up in the one-carbon pool and incorporated in macromolecules. There was no evidence of the formation of formaldehyde-specific DNA–protein crosslinks in the bone marrow of Rhesus monkeys exposed for six hours to 0.7, 2.0 and 6.0 ppm formaldehyde (Heck & Casanova, 2004), or in rats, including glutathione (GSH)-depleted rats, exposed to concentrations of formaldehyde up to 10 ppm (Casanova-Schmitz *et al.*, 1984; Casanova & Heck, 1987). The formation of formaldehyde-DNA adducts was demonstrated in lymphocytes of smokers (Wang *et al.*, 2009). In this study, liquid chromatography-electrospray ionization-tandem mass spectrometry was used to quantify the adduct N^6-hydroxymethyldeoxyadenosine (N^6-HOMe-dAdo) in leukocyte-DNA samples from 32 smokers (≥ 10 cigarettes per day) and 30 non-smokers. This adduct would be expected to be formed upon exposure to formaldehyde. N^6-HOMe-dAdo was detected in 29 of the 32 samples from smokers, but in only 7 of the 30 samples from non-smokers ($P < 0.001$). These findings would support a role for inhaled formaldehyde in causing the DNA adducts that may ultimately lead to smoking-associated leukaemia. The authors caution that the observed adducts may result from cigarette-smoke components other than formaldehyde – such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) or nicotine, which may be metabolized to formaldehyde within the cell – or from other sources, rather than reflecting the actual penetration of inhaled formaldehyde to lymphocytes.

Rietbrock (1965) reported that the half-life in plasma of formaldehyde injected intravenously in the rat was about one minute. [Assuming a similar half-life of formaldehyde in humans, this would be sufficient time for inhaled formaldehyde

to reach the blood and circulate to the bone marrow in humans].

4.2 Toxic effects

Formaldehyde produces irritation of the nose and pharynx in humans and laboratory animals under a variety of circumstances. There appears to be a large inter-individual variation in the human response to the irritating effects of formaldehyde. Under controlled exposure conditions, symptoms of irritation were noted by healthy individuals exposed to formaldehyde concentrations of 2–3 ppm during periods that varied between 40 minute and three hours (for details, see Table 30 in *IARC Monograph* Volume 88 (IARC, 2006)).

Formaldehyde is a known cause of allergic contact dermatitis and, somewhat more controversial, of occupational asthma. Nasal biopsies of workers chronically exposed to formaldehyde showed chronic inflammation, loss of cilia, mild dysplasia, hyperplasia and squamous metaplasia, although the latter finding has been inconsistent and may have been confounded by other exposures, such as to wood dust (IARC, 2006).

The cytotoxicity of formaldehyde has been confirmed in numerous in-vitro systems. Irritation of the nasal and upper respiratory tract is also noted in animal studies. Dose-dependent pathological findings include inflammation, hyperplasia, degenerative changes, necrosis and squamous metaplasia.

Recently, a finding relevant to the possible involvement of formaldehyde in leukaemogenesis was reported by Zhang *et al.* (2010). Their study showed that colony formation by colony-forming unit-granulocyte-macrophage (CFU-GM) progenitor cells is inhibited in cell cultures exposed to formaldehyde at toxicologically relevant concentrations. Furthermore, colony formation by the more primitive CFU-granulocyte- erythrocyte-monocyte-megakaryocyte (CFU-GEMM) progenitors, which give rise to formation of all myeloid cells, showed a linear negative dose–response when treated with formaldehyde. These effects were observed at formaldehyde concentrations of 100–200 μM [3–6 μg/mL], which are toxicologically relevant since background levels of formaldehyde in human blood have been reported to be 50–100 μM [1.5–3 μg/mL] (Heck *et al.*, 1985; Casanova *et al.*, 1988). Because the CFU-GEMM multipotent myeloid progenitor cells and the pluripotent stem cells are the target cells for leukaemogenesis and are converted to leukaemic stem cells in acute myeloid leukaemia, the finding that formaldehyde damages these cells *in vitro* adds some weight to the notion that it may be associated with myeloid leukaemia.

4.3 Genetic and related effects

The genotoxicity of formaldehyde was thoroughly reviewed in *IARC Monograph* Volume 88 (IARC, 2006). Genotoxicity has been observed *in vitro* in many systems with multiple endpoints.

4.3.1 Humans

Micronucleus formation has been repeatedly reported to occur in cells of the nasal and oral mucosa of formaldehyde-exposed humans. The outcome of studies on induction of micronuclei, sister chromatid exchange and chromosomal aberrations in the lymphocytes of exposed humans – which is pertinent to the question concerning the potential of formaldehyde to cause lympho-haematopoietic cancer – has been less consistent (see Table 4.1).

DNA–protein crosslinks in circulating white blood cells were found to be higher in 12 workers exposed to formaldehyde in an anatomy department and a pathology institute than in eight controls (P = 0.03) (Shaham *et al.*, 1996). The number of crosslinks tended to be higher in workers who had been exposed longer (exposure duration, 2–31 years). Smoking had no effect. In a

Table 4.1 Cytogenetic studies on formaldehyde–exposed humans

Reference	Description of exposed and controls	Exposure levels	Cytogenetic/genotoxic end-point	Response in exposed	Response in controls	Comments
Costa *et al.* (2008), Portugal	Workers in an anatomy-pathology laboratory (n = 30) Controls non-exposed (n = 30)	0.44 ± 0.08 ppm (range 0.04–1.58)	Micronucleus formation in lymphocytes (MN/1000 cells)	5.47 ± 0.76	3.27 ± 0.69	P = 0.003
			Sister-chromatid exchange(SCE)	6.13 ± 0.29	4.49 ± 0.16	P < 0.05
			DNA-breakage (comet tail length, µm) in lymphocytes	60.00 ± 2.31	41.85 ± 1.97	P < 0.05
Pala *et al.* (2008), Italy	Laboratory workers (n = 36) in a cancer research institute	Exposure range: 4.9–268.7 µg/m^3 Low: < 26 µg/m^3 High: ≥ 26 µg/m^3		High:	Low:	
			Micronucleus formation (MN)	MN: 0.31 ± 0.17	MN: 0.26 ± 0.24	
			Chromosomal aberrations (CA)	CA: 2.22 ± 1.27	CA: 2.95 ± 1.79	
			SCE in lymphocytes	SCE: 5.06 ± 0.76	SCE: 6.57 ± 1.38	
Iarmarcovai *et al.* (2007), France	Pathologists/anatomists (n = 18) Controls (n = 18)	2.3 ppm (range 0.4–7 ppm)	MN in lymphocytes (MN/1000 cells)	21.0 ± 12.6	14.4 ± 8.1	P < 0.01
Yu *et al.* (2005), the People's Republic of China	Workers (n = 151; low-exposed, 62; high-exposed, 89) in two plywood factories; 112 non-exposed controls.	TWA: 0.10–7.88 mg/m^3 (< 0.01 mg/m^3 in controls)	MN in lymphocytes (MN/1000 cells)	High exp. 6.5 ± 3.6 Low exp. 4.1 ± 2.5	2.7 ± 1.3	P < 0.05
			DNA-breakage (comet tail length, µm) in lymphocytes	High exp., 12.6 (95% CI: 11.8–13.4) Low exp., 11.3 (95% CI: 10.1–12.5)	6.8 (95%CI: 6.1–7.6)	P < 0.05
Ye *et al.* (2005), the People's Republic of China	Workers (n = 18; all non-smokers) in a formaldehyde factory (mean exposure duration, 8.5 yrs), and waiters (n = 16; all non-smokers) exposed to formaldehyde for 12 wk in a newly fitted ballroom. Controls were 23 students (non-smokers)	Factory: 8-h TWA, 0.99 ± 0.29 mg/m^3, with peak exposure 1.69 mg/m^3. Ballroom: 5-h TWA 0.11 ± 0.07 mg/m^3, with peak exposure 0.3 mg/m^3 Control: 0.01 ± 0.003 mg/m^3, with peak 0.015 mg/m^3	MN in nasal mucosa (MN/1000 cells) of factory workers	2.70 ± 1.50	1.25 ± 0.65	P < 0.05
			SCE in lymphocytes of factory workers	8.24 ± 0.89	6.38 ± 0.41	P < 0.05
			No effects in ballroom waiters			

Table 4.1 (continued)

Reference	Description of exposed and controls	Exposure levels	Cytogenetic/genotoxic end-point	Response in exposed	Response in controls	Comments
Shaham *et al.* (2002), Israel	Workers (n = 90) in hospital pathology departments; controls were 52 unexposed workers from the same hospitals. Average duration of exposure 15.4 yrs (range, 1–39)	Low: 0.4 ppm (0.04–1.7) High: 2.24 ppm (0.72–5.6)	SCE in lymphocytes (mean number of SCE per chromosome)	0.27 ± 0.003	0.19 ± 0.002	$P < 0.01$
Burgaz *et al.* (2002), Turkey	a) Workers (n = 22) in a shoe factory	n-hexane, toluene, methyl ethyl ketone	MN in buccal cells (MN/1000 cells)	a) 0.62 ± 0.45		$P < 0.05$
	b) Pathologists (n = 28), anatomy laboratories	2–4 ppm formaldehyde		b) 0.71 ± 0.56		$P < 0.05$
	c) Controls (n = 18): university staff	none			c) 0.33 ± 0.30	
Burgaz *et al.* (2001), Turkey	Workers (n = 23) in pathology and anatomy laboratories, and 25 non-exposed controls	Air concentration of formaldehyde in the breathing zone 2–4 ppm.	MN in nasal mucosa (MN/1000 cells; mean ± SD).	1.01 ± 0.62	0.61 ± 0.27	$P < 0.01$
Suruda *et al.* (1993), USA	Students (n = 29) taking 85-d course in embalming; average number of embalmings, 6.9; total number 144.	Average air concentration of formaldehyde: 1.4 ppm, range 0.15–4.3 mean duration of embalming 125 minute with peak exposures > 4 ppm.	MN per 1 000 cells:	MN after course	MN before course	
			- buccal cells	0.60 ± 1.27	0.046 ± 0.17	$P < 0.05$
			- nasal cavity	0.50 ± 0.67	0.41 ± 0.52	NS
			- lymphocytes	6.36 ± 2.03	4.95 ± 1.72	$P < 0.05$
			SCE in lymphocytes	7.14 ± 0.89	7.72 ± 1.26	$P = 0.01$ (decrease in SCE)
Ying *et al.* (1999), China	Students (n = 23; non-smokers) in 8-wk (3h x 3 times weekly) anatomy class.	TWA 0.51 ± 0.30 mg/m^3, with peak exposure 1.28 Background level in dormitories 0.012 ± 0.0025 mg/m^3		SCE after course	SCE before course	
			SCE in lymphocytes	6.61 ± 0.79	6.38 ± 0.41	NS

Table 4.1 (continued)

Reference	Description of exposed and controls	Exposure levels	Cytogenetic/genotoxic end-point	Response in exposed	Response in controls	Comments
He *et al.* (1998), China	Students (n = 13; non-smokers) in 12-wk (10h per wk) anatomy class. Ten other students (non-smokers) served as non-exposed controls.	Average air concentration of formaldehyde: 2.37 ppm (3.17 mg/m^3)	MN per 1 000 cells in lymphocytes	6.38 ± 2.50	3.15 ± 1.46	$P < 0.01$
			CA in lymphocytes	5.92 ± 2.40	3.40 ± 1.57	$P < 0.01$
			SCE in lymphocytes	5.91 ± 0.71	5.26 ± 0.51	$P < 0.05$
Ying *et al.* (1997), China	Students (n = 25; non-smokers) in 8-wk (3h x 3 times weekly) anatomy class.	TWA 0.51 ± 0.30 mg/m^3, with peak exposure 1.28 Background level in dormitories 0.012 ± 0.0025 mg/m^3	MN per 1 000 cells in			
			- nasal exfoliated cells	3.84 ± 1.48	1.20 ± 0.67	$P < 0.001$
			- oral mucosa cells	0.86 ± 0.56	0.57 ± 0.32	$P < 0.01$
			- lymphocytes	1.11 ± 0.54	0.91 ± 0.39	n.s.
Titenko-Holland *et al.* (1996), USA	Students (n = 28) taking a 90-d embalming class; average number of embalmings, 6.9; total number 144.	Accumulated dose over 90 d: 4.3–26.4 ppm Maximum peak exposure: 2.86 ppm	MN per 1 000 cells with centromere-specific probes (FISH) in:	MN after class	MN before class	[between parentheses: values for centromere-negative MN]
			- buccal cells	2.0 ± 2.0 (0.9 ± 1.1)	0.6 ± 0.5 (0.1 ± 0.2)	$P = 0.007$ ($P = 0.005$)
			- nasal cells	2.5 ± 1.3 (1.0 ± 0.6)	2.0 ± 1.3 (0.5 ± 0.5)	$P = 0.20$ ($P = 0.03$)

Table 4.1 (continued)

Reference	Description of exposed and controls	Exposure levels	Cytogenetic/genotoxic end-point	Response in exposed	Response in controls	Comments
Dobiás *et al.* (1988), Czechoslovakia	- Schoolchildren (n = 20 children) in school building constructed with wood-particle boards - Control group (n = 17 children) in brick school building in the same area - Control group in brick school building elsewhere	Formaldehyde concentration in air: 1984: 317.0 µg/m^3 1985: 130.0 µg/m^3 1986: 36.5 µg/m^3	CA in lymphocytes (per 100 cells) in lymphocytes	1984: 7 1985: 4.2 1986: NR	1984: 4.2 NR NR The frequency of chromosomal aberrations is stated 'normal' in 1986 but the value is not given	
		Building was cleaned in '85, '86	Percentage of aberrant cells	1984: 4.71 ± 2.09 1985: 2.82 ± 1.64 1986: 2.06 ± 1.51	1984: 1.37 ± 0.87 1985: 1.4 ± 0.79 NR	P = 0.005
Bauchinger & Schmid (1985), Germany	Workers (n = 20; 6 smokers) in a paper factory, where formaldehyde was used for impregnating the paper. Duration of exposure 2–30 yrs, average 14.5 ± 7.2 yrs. Controls were 20 non-exposed workers (13 smokers) elsewhere in the factory	Formaldehyde exposure, < 0.2 ppm Peak during repair and cleaning, 3 ppm	CA in lymphocytes (dicentrics, rings) per 100 cells SCE/cell ± SE	0.13 ± 0.05 8.87 ± 0.24	0.05 ± 0.02 9.53 ± 0.35	 NS

CA, chromosomal abberations; d, day or days; h, hour or hours; min, minute or minutes; MN, micronuclei; NR, not reported; NS, not significant; SCE, sister chromatid exchange; TWA, time-weighted average concentration

subsequent study, Shaham *et al.* (2003) reported an increase in the number of DNA–protein crosslinks in lymphocytes and in serum concentrations of the p53 protein in hospital pathology-department workers. The exposed subjects were assigned to a high-exposure subgroup (mean formaldehyde concentration in air, 2.24 ppm) and a low-exposure subgroup (mean, 0.4 ppm), based on personal sampling and field sampling for 15-minute periods on typical working days. The control group consisted of personnel of the administrative sections in the hospital. The amount of protein–cross-linked DNA was statistically significantly higher in the exposed group than in the controls (0.20 *vs* 0.14; these values are the ratios between protein-bound DNA – precipitable with sodium dodecyl sulfate – and total DNA) after controlling for age, smoking and other factors. Very little difference in DNA–protein crosslink levels was observed between the high- and low-exposure groups (0.20 *vs* 0.19); or between workers with > 16 years or < 16 years of exposure (0.20 *vs* 0.19). The percentage of formaldehyde-exposed male workers who had pantropic p53-protein (wild-type plus mutant p53) concentrations in serum higher than 150 pg/ml was statistically significantly greater than in the control group (54.8% *vs* 36.5%, $P < 0.05$; this difference was not seen in female workers). Formaldehyde-exposed workers with DNA–protein crosslink levels above the median had a significantly greater likelihood of having p53 concentrations in serum above 150 pg/ml. [The Working Group noted that the rationale for using a p53-protein level of 150 pg/ml as a cut-point was based upon previous experience with this assay; no reason is given for using 16 years as the cut-point between longer/shorter exposure. Questions have also been raised about the persistence of DNA–protein crosslinks, which are thought to be rapidly repaired within the cell (Schmid & Speit, 2007)]. In an earlier study, Casanova-Schmitz *et al.* (1984) failed to observe DNA–protein crosslinks in bone marrow of Fischer-344 rats exposed to [^{14}C]- and [^{3}H]-formaldehyde.

Compared with matched controls, pathology/anatomy workers from five hospitals, who were exposed to mean formaldehyde concentrations of 2.0 ppm (range, < 0.1 to 20.4 ppm) during 15 minute, or to 0.1 ppm (range < 0.1 to 0.7 ppm) during 8 hours, showed a statistically significant increase in bi-nucleated cells and in mono-centromeric micronucleus formation in a cytokinesis-blocked micronucleus assay combined with fluorescence in situ hybridization (FISH) (Orsière *et al.*, 2006).

A higher frequency of micronuclei was found in exfoliated nasal and oral cells of students with short-term exposure (3 hours per day; 3 days per week, for 8 weeks) to an average of 0.508 ± 0.299 mg/m^3 formaldehyde, compared with controls (Ying *et al.*, 1997, 1999). No increase in micronucleus formation or in the level of sister chromatid exchange (SCE) was observed in lymphocytes. Ye *et al.* (2005) reported a comparative analysis of 18 workers involved for various periods (mean, 8.5 years; range, 1–15 years) in a formaldehyde-manufacturing process, 16 waiters exposed for 12 weeks to an indoor source of formaldehyde during interior renovations, and a control group of 23 students; all were non-smokers. Average formaldehyde exposure-concentrations were 0.011 mg/m^3 for the student controls; 0.107 mg/m^3 for the waiters; and 0.99 mg/m^3 for the formaldehyde-plant workers. There was a statistically significantly higher frequency of micronuclei in nasal mucosal cells and of SCE in peripheral lymphocytes in the workers at the formaldehyde-manufacturing plant, but not in the waiters, although both groups were exposed to formaldehyde at comparable concentrations (Table 4.1). The result is in line with the much longer exposure duration for the plant workers. The same authors (Ye *et al.*, 2005) reported an increase in B-cells and changes in the ratios between lymphocyte subsets, similar to those reported by Madison *et al.* (1991) in an

Alaskan community subject to acute formaldehyde exposure (estimated at 2–5 ppm) for a few days. Blood analyses were done three years after the accident. Total white blood cell counts and total lymphocyte counts did not differ from those in the control community in Alaska, also measured three years later (Madison *et al.*, 1991).

An increase in SCE and other genotoxic effects were observed in the lymphocytes of workers with long-term exposure to formaldehyde (Costa *et al.*, 2008). Thirty workers from four pathology/anatomy hospital units and 30 matched controls were included in the study. Compared with the control group, statistically significant effects were seen in SCE (6.13 ± 0.29 *vs* 4.49 ± 0.16 SCE/cell, $P < 0.05$), micronucleus frequency (in 1000 bi-nucleated cells: 5.47 ± 0.76 ‰ *vs* 3.27 ± 0.69 ‰, $P = 0.003$) and tail length in the comet assay (60.0 ± 2.31 µm *vs* 41.85 ± 1.97 µm, $P < 0.05$). A statistically significant positive correlation was found between formaldehyde exposure levels and micronucleus frequency and tail length. The mean formaldehyde exposure was 0.44 ppm (range, 0.04–1.58 ppm). None of the observed effects were related to the duration of exposure.

No genotoxic effects were observed in a study of 36 laboratory workers at a cancer-research institute who were exposed to 4.9–268.7 µg/m^3 formaldehyde. There was a direct relationship between formaldehyde exposure levels and the presence of a formaldehyde human serum-albumin (FA-HSA) conjugate. The genotoxic endpoints measured were SCE, micronuclei and chromosome aberrations, but these did not show significantly elevated levels (Pala *et al.*, 2008). Although a small study, its strength is its linkage to a biological marker of formaldehyde exposure.

Hayes *et al.* (1997) evaluated O^6-Alkylguanine-DNA–alkyltransferase (AGT) activity as a measure of DNA-repair capacity in blood lymphocytes of 23 science students in a mortuary, before and after a nine-week period of classroom exposure to approximately 1.5 ppm formaldehyde. A statistically significant finding was that more students had a reduction in AGT activity than an increase. There was no clear link between the extent of exposure to formaldehyde and AGT activity.

Zhang *et al.* (2010) cultured myeloid progenitor cells from the peripheral blood of formaldehyde-exposed workers and controls and measured leukaemia-specific chromosomal changes. In a subset of ten of the most highly exposed subjects in their study, monosomy (loss) of chromosome 7 and trisomy (gain) of chromosome 8 were significantly elevated in the myeloid progenitor cells of formaldehyde-exposed workers compared with the same phenomena in 12 unexposed controls. The loss of chromosome 7 and gain of chromosome 8 were examined because they are among the most frequent cytogenetic changes observed in myeloid leukaemia and myelodysplastic syndromes; these events have been shown to be affected by exposure to the established human leukemogen, benzene. [The Working Group noted that the study is small and needs to be replicated].

4.3.2 Experimental systems

(a) In-vivo studies (laboratory animals)

Studies on a variety of genotoxic endpoints in laboratory animals inhaling formaldehyde have generally shown effects in the nasal tissues of these animals (IARC, 2006). Much less consistent have been the findings of genotoxic effects in the blood lymphocytes from exposed animals. Among the recent studies, Im *et al.* (2006) reported genotoxicity based on a positive result in the comet assay in the lymphocytes of rats inhaling 5 or 10 ppm formaldehyde for two weeks, six hours/day, five days/week. In contrast, in a review of their own work and of the literature, Speit *et al.* (2009) concluded that there was no evidence of systemic genotoxic effects in laboratory animals inhaling formaldehyde. Their own negative studies in this review focused on

lymphocyte genotoxicity, measured as micronuclei, SCE, and DNA-breakage – the latter determined with a sensitive form of the comet assay – in rats exposed for four weeks (six hours/day, five days/week) to formaldehyde concentrations of 0.5, 1, 2, 6, 10 and 15 ppm.

More recently, DNA strand-breaks were induced by formaldehyde *in vivo*, in mouse liver (maternal and fetal), and in lung cells in the rat (Wang & Liu, 2006; Sul *et al.*, 2007).

(b) In-vitro studies

The spectrum of mutations related to exposure to formaldehyde *in vitro* and *in vivo* was presented and discussed in *IARC Monograph* Volume 88 (IARC, 2006). In-vitro studies since then have expanded the wide range of potential mutagenic mechanisms, to include the hydroxymethylation of DNA and DNA-microsatellite instability (Zhong & Que Hee, 2004; Wang *et al.*, 2007).

The evidence of formaldehyde-induced mutations in various experimental systems is consistent, encompassing both clastogenic effects and direct DNA mutation. Formaldehyde showed mutagenic potential in several bacterial systems, both with and without S9 activation. Formaldehyde induced deletions, point mutations, insertions, and cell transformation in in-vitro assays with mammalian cells (IARC, 2006).

Formaldehyde-induced DNA strand-breaks (SSB) have been demonstrated in several mammalian cell systems, including hepatocytes, lymphosarcoma cells, and epithelial cells from the rat, leukaemia L1210 cells from the mouse, and lung/bronchial epithelial cells, skin fibroblasts, keratinocytes, and peripheral blood lymphocytes from humans (IARC, 2006).

Chromosomal aberrations, micronuclei and SCE were all increased *in vitro* in numerous rodent and human primary cells and cell lines treated with formaldehyde (IARC, 2006). Consistent with these findings, more recent data show increased numbers of chromosomal aberrations in Syrian hamster embryo cells, and chromosomal aberrations and SCE in Chinese hamster ovary and embryo cells (Hikiba *et al.*, 2005, Hagiwara *et al.*, 2006; Lorenti Garcia *et al.*, 2009).

Further evidence of formaldehyde-induced micronucleus formation was obtained in studies with human lymphocytes isolated from whole-blood cultures exposed *in vitro* to formaldehyde, 44 hours after the start of the culture (Schmid & Speit, 2007). Both micronuclei and SCE were induced upon in-vitro treatment of Chinese hamster V79 lung epithelial cells with formaldehyde (Speit *et al.*, 2007). In a recent study, SCE was induced in A549 human lung cells and V79 Chinese hamster cells following incubation with 0.1 mM [3 µg/mL] or higher concentrations of formaldehyde. One hour after the addition of the agent to the A549 cells, the culture medium still retained the capacity to produce SCE in non-exposed V79 cells, suggesting that genotoxicity persists despite the high reactivity of formaldehyde with macromolecules in the culture medium (Neuss & Speit, 2008). When the formaldehyde-exposed A549 cells were washed and then suspended in fresh culture medium containing the V79 cells, SCE formation was not observed in the latter. [The authors present no evidence that it is formaldehyde itself that persists, rather than a formaldehyde product that is responsible for genotoxicity. The Working Group noted that there is no reason to preclude the transfer of formaldehyde from cell to cell].

Formaldehyde has also been reported to interfere with DNA repair. A recent finding that chicken DT40 cells deficient in the FANC/BRCA (Fanconi's anaemia complementation groups/breast cancer A) pathway are hypersensitive to formaldehyde in plasma, is consistent with a role for this pathway in repairing DNA–protein cross-links caused by formaldehyde. The DT40 mutants were also more sensitive to acetaldehyde, but not to acrolein and other aldehydes (Ridpath *et al.*, 2007). Endogenous formaldehyde may be

important in producing leukaemia in patients with Fanconi's anaemia, a genetic disorder that is characterized by progressive pancytopenia. DT40 cells with deficient repair mechanisms have also been shown to be more sensitive to other cross-linking agents such as cisplatin, a myelotoxic chemotherapeutic agent that leads to pancytopenia and acute myelogenous leukaemia (AML) (Nojima *et al.*, 2005).

4.4 Mechanistic considerations

4.4.1 Cancer of the nasopharynx and nasal sinuses

Mechanistic evidence supporting a causal relation between inhalation of formaldehyde and induction of cancer of the nasopharynx and nasal sinuses is based on the chemical reactivity of formaldehyde in producing DNA–protein cross-links, and its genotoxicity *in vitro* and *in vivo*, including in the nasal cells of exposed humans. Computational fluid-dynamic models of formaldehyde in the nasal passages of rats, monkeys and humans have generally been accurate in predicting the area in the nose with the highest number of DNA–protein crosslinks (Georgieva *et al.*, 2003). Local effects in the nasal passages, genotoxicity, and cell-proliferation rate appear to be the major determinants of nasal carcinogenicity after exposure to formaldehyde.

4.4.2 Leukaemia

The findings reviewed in *IARC Monograph* Volume 88 (IARC, 2006) pertaining to a potential mechanism for formaldehyde-induced leukaemogenesis were summarized as follows: "Based on the data available at this time, it was not possible to identify a mechanism for the induction of myeloid leukaemia in humans." The Working Group further stated that "It is possible that formaldehyde itself can reach the bone marrow following inhalation, although the evidence is inconsistent." Since that time, Zhang *et al.* (2009), reviewed potential pathways by which formaldehyde could act as a leukaemogen. Three mechanisms were suggested:

- by damaging stem cells in the bone marrow directly, as most other leukaemogens do;
- by damaging haematopoietic stem/progenitor cells circulating in the peripheral blood and
- by damaging the primitive pluri-potent stem cells present within the nasal turbinates and/or olfactory mucosa.

This subject was reviewed by Heck & Casanova (2004), Pyatt *et al.* (2008), and Goldstein (2011).

(a) Studies in animals

Studies of bone marrow cells in formaldehyde-exposed animals have been inconsistent. Kitaeva *et al.* (1990) described clastogenic and cytogenetic effects in the bone marrow of rats inhaling 0.5 mg/m^3 or 1.5 mg/m^3 of formaldehyde during four hours/day for four months. In contrast, Dallas *et al.* (1992) found no evidence of cytogenetic abnormalities in the bone marrow of rats exposed to 0.5, 3 or 15 ppm [0.62, 3.7 or 18.45 mg/m^3] formaldehyde for six hours/day, five days per week, for one or eight weeks. Mice that received up to 25 mg/kg bw formaldehyde in two intra-peritoneal injections within 24 hours showed no increase in chromosomal aberrations or micronuclei in the femoral bone marrow (Natarajan *et al.*, 1983). As described in section 4.1 above, no increase in formaldehyde-specific DNA–protein cross-links was observed in the bone marrow of Rhesus monkeys or rats under various experimental conditions (Heck & Casanova, 2004).

(b) Considerations of formaldehyde as a leukaemogen in relation to other known myeloleukaemogens

Known myeloid leukaemogens in humans include benzene, ionizing radiation and a variety of chemotherapeutic anti-neoplastic agents, all of which give rise to pancytopenia. There is evidence that for each of these myeloleukaemogens, pancytopenia is caused by genotoxic damage leading to destruction of primitive progenitor cells in the bone marrow. These cells are responsible for the formation of red blood cells, white blood cells and platelets, and they are the same progenitor cells in which mutations and clonal expansion leads to myeloid leukaemia.

In view of the wide variety of genotoxic mechanisms shown by the diverse agents that have pancytopenia and myeloleukaemogenesis in common, it could be anticipated that genotoxic effects of formaldehyde on myeloid progenitor cells would also result in pancytopenia.

Pancytopenia has not been among the haematological findings in experiments with laboratory animals exposed to relatively high doses of formaldehyde, including classic long-term safety assessment studies. An increase in haemoglobin and monocytes and a decrease in lymphocytes were observed in rats receiving 0, 20, 40, or 80 mg/kg bw formaldehyde by gastric intubation on five days/week for four weeks. Lymph-node weights were increased but no change in lymph-node cellularity was observed (Vargová *et al.*, 1993). In one long-term study there was actually a statistically significant increase in bone-marrow hyperplasia in rats exposed to formaldehyde at 15 ppm (Batelle, 1981), the opposite of what would be expected for an agent that has effects similar to those of other known myeloleukaemogens.

In contrast to the findings in laboratory animals, there has been some evidence suggesting a mild pancytopenic effect in humans. A study of 50 haemodialysis nurses exposed to formaldehyde compared with 71 non-exposed ward nurses from five different hospitals comprised measurements of formaldehyde and two different blood counts recorded one a year apart. Both personal and ambient measurements of formaldehyde varied widely, from non-detectable up to 2.8 ppm. Average duration of employment was three years for both groups. Symptoms attributable to formaldehyde were reported in the exposed group. For the second blood count, but not the first, there was a statistically significant inverse correlation ($P < 0.05$) between white blood-cell count and formaldehyde concentration, as well as between white blood-cell count and symptom score. No statistically significant correlation was observed between formaldehyde concentrations or symptoms and platelet or red blood-cell counts. The exposed group had a lower white blood-cell count than the control group (Kuo *et al.*, 1997). [The Working Group noted that absolute data for blood counts were not given, nor was the statistical methodology described]

An increase in B-lymphocytes and changes in ratios of lymphocyte subsets were noted in formaldehyde-plant workers exposed to an average of 0.99 mg/m^3 formaldehyde for a mean duration of 8.5 years (Ye *et al.*, 2005). Differences in the ratios of lymphocyte subsets were also observed in an Alaskan community with an acute formaldehyde exposure (2–5 ppm for a few days), but no differences with the control community were seen in total white blood-cell counts or lymphocyte counts (Madison *et al.*, 1991). Likewise, no significant differences in blood counts were found in a comparative study of students of two schools, in one of which there were elevated concentrations of formaldehyde and toluene (Vozenílková *et al.*, 1991).

In a review of formaldehyde exposure in the People's Republic of China, Tang *et al.* (2009) mentioned eight studies on formaldehyde-exposed individuals. Lower white blood-cell counts were observed in the six studies that provided information on this point, four of which were statistically significant; platelet

counts were decreased in all three studies where this was measured, two of which were statistically significant; and haemoglobin was lower in one of the three studies for which data were reported. The one study that found lower than normal values for each blood count – consistent with a pancytopenic effect – was specifically the study with the lowest exposure to formaldehyde (0.022–0.044 mg/m^3), although the largest cohort. [From the Table in the Tang *et al.* (2009) paper, it is not clear whether these are the same individuals or separate individuals who have each of the lower counts, i.e. how many were pancytopenic. There also is no information about the usual confounders, including gender and age].

The finding of statistically significant, moderately lower blood counts in formaldehyde-exposed Chinese workers as compared to a matched control group would be consistent with formaldehyde-induced damage to either circulating haematopoietic precursor cells, or with a direct effect on such cells within the bone marrow (Zhang *et al.*, 2010). In this study the 43 exposed workers at a formaldehyde-melamine producing factory or a factory in which formaldehyde-melamine resins were used to produce utensils, were exposed to a median of 1.28 ppm formaldehyde (10–90%, range 0.63–2.51 ppm; 8-hour time-weighted average), compared with a median level, in a matched control group of 51 individuals, of 0.026 ppm (10–90%, range 0.0085–0.026 ppm). Absolute blood counts were only given for total white blood-cell counts: in controls, mean (SD) 6269 (1452) cells per µl blood; and in exposed: mean 5422 (1529) cells per µl blood, $P = 0.0016$. Data for the other blood counts are presented in a bar chart, and for red blood cells, platelets, granulocytes and lymphocytes there are small but statistically significant decreases that appear to fall within the clinical range of normal. Also of note is a statistically significant increase in the mean corpuscular volume (MCV) of red blood cells. The MCV tends to be increased in myelodysplastic conditions. The study appeared to have adequately taken into account possible confounders such as alcoholism and nutritional issues that might cause pancytopenia and an increased MCV.

(c) Leukaemogenesis on the basis of reactions with myeloid stem cells within the nose

As indicated above, Zhang *et al.* (2009) have suggested that one mechanism of formaldehyde-induced leukaemogenesis might involve reaction of formaldehyde or a reactive formaldehyde derivative with myeloid precursors present within the nose. This has been questioned on two indirect grounds (Goldstein, 2011). Nasal tissue does not seem to have been reported as a location for chloromas, which are isolated collections of myeloid leukaemia cells, despite the presence of chloromas in virtually all other tissues. Second, known nasal carcinogens, including cross-linking agents such as nickel and chromium, are not reported to cause an increase in acute myelogenous leukaemia. The one possible exception is sulfur mustard, a nasal carcinogen for which an increase in leukaemia (13 deaths observed; 8.51 expected; not statistically significant) was reported by Easton *et al.* (1988) in workers producing sulfur mustard gas during World War II. However, this agent also produces pancytopenia, an outcome that led to the development of nitrogen mustard as a chemotherapeutic compound. None of the other known human nasal carcinogens has been reported to cause pancytopenia.

(d) Formaldehyde and lymphoid cancers

Genotoxicity studies on blood lymphocytes from laboratory animals that inhaled formaldehyde have tended to be negative, although not consistently so. In comparison, somewhat more studies with the lymphocytes of humans exposed to formaldehyde have reported genotoxicity, although the findings are also inconsistent. Genotoxicity in circulating lymphocytes would be consistent with the possibility that

formaldehyde is a cause of lymphatic tumours. Particularly at risk would be mucosa-associated lymphatic tissue in the nasal area.

The evolution of our understanding of lymphohaematopoitetic cancers has led to ongoing reclassification of these tumours. There is also recognition of their inter-relatedness through a common stem cell, and the fact that there is a risk for malignant transformation during various stages of the differentiation and maturation process of the precursor cells. Recent evidence suggests that an underlying cytogenetic abnormality in an early precursor cell predisposes to subsequent mutations leading to a specific haematological cancer. The possibility of a mutagenic effect of formaldehyde on circulating lymphocytes or local lymphatic tissue cannot be excluded.

4.5 Synthesis

The current data strongly indicate that genotoxicity plays an important role in the carcinogenicity of formaldehyde in nasal tissues in humans, and that cellular replication in response to formaldehyde-induced cytotoxicity promotes the carcinogenic response. Three possible mechanisms, all focused around genotoxicity, are moderately supported as the underlying mechanism for induction of haematological malignancies in humans. Further research is needed to decide which of the mechanisms is the most important.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of formaldehyde. Formaldehyde causes cancer of the nasopharynx and leukaemia.

Also, a positive association has been observed between exposure to formaldehyde and sinonasal cancer.

There is *sufficient evidence* in experimental animals for the carcinogenicity of formaldehyde.

The Working Group was not in full agreement on the evaluation of formaldehyde causing leukaemias in humans, with a small majority viewing the evidence as sufficient of carcinogenicity and the minority viewing the evidence as limited. Particularly relevant to the discussions regarding sufficient evidence was a recent study accepted for publication which, for the first time, reported aneuploidy in blood of exposed workers characteristic of myeloid leukaemia and myelodysplastic syndromes, with supporting information suggesting a decrease in the major circulating blood-cell types and in circulating haematological precursor cells. The authors and Working Group felt that this study needed to be replicated.

Formaldehyde is *carcinogenic to humans (Group 1)*.

References

Albert RE, Sellakumar AR, Laskin S *et al.* (1982). Gaseous formaldehyde and hydrogen chloride induction of nasal cancer in the rat. *J Natl Cancer Inst*, 68: 597–603. PMID:6951075

Armstrong RW, Imrey PB, Lye MS *et al.* (2000). Nasopharyngeal carcinoma in Malaysian Chinese: occupational exposures to particles, formaldehyde and heat. *Int J Epidemiol*, 29: 991–998. doi:10.1093/ije/29.6.991 PMID:11101539

Bachand AM, Mundt KA, Mundt DJ, Montgomery RR (2010). Epidemiological studies of formaldehyde exposure and risk of leukemia and nasopharyngeal cancer: a meta-analysis. *Crit Rev Toxicol*, 40: 85–100. doi:10.3109/10408440903341696 PMID:20085478

Batelle (1981). *Final report on: A chronic inhalation toxicology study in rats and mice exposed to formaldehyde to Chemical Industry Institute of Toxicology.* Columbus, Ohio: Columbus Laboratories, No. CIIT Docket No:10922, 1:1–281

Bauchinger M & Schmid E (1985). Cytogenetic effects in lymphocytes of formaldehyde workers of a paper factory. *Mutat Res*, 158: 195–199. doi:10.1016/0165-1218(85)90085-0 PMID:4079950

Beane Freeman LE, Blair A, Lubin JH *et al.* (2009). Mortality from lymphohematopoietic malignancies

among workers in formaldehyde industries: the National Cancer Institute Cohort. *J Natl Cancer Inst*, 101: 751–761. doi:10.1093/jnci/djp096 PMID:19436030

Blair A, Zheng T, Linos A *et al.* (2001). Occupation and leukemia: a population-based case-control study in Iowa and Minnesota. *Am J Ind Med*, 40: 3–14. doi:10.1002/ajim.1066 PMID:11439392

Bosetti C, McLaughlin JK, Tarone RE *et al.* (2008). Formaldehyde and cancer risk: a quantitative review of cohort studies through 2006. *Ann Oncol*, 19: 29–43. doi:10.1093/annonc/mdm202 PMID:17897961

Burgaz S, Cakmak G, Erdem O *et al.* (2001). Micronuclei frequencies in exfoliated nasal mucosa cells from pathology and anatomy laboratory workers exposed to formaldehyde. *Neoplasma*, 48: 144–147. PMID:11478696

Burgaz S, Erdem O, Cakmak G *et al.* (2002). Cytogenetic analysis of buccal cells from shoe-workers and pathology and anatomy laboratory workers exposed to n-hexane, toluene, methyl ethyl ketone and formaldehyde. *Biomarkers*, 7: 151–161. doi:10.1080/13547500110113242 PMID:12101634

CAREX (1999). *Carex industry specific estimates – Summary.* Available at http://www.ttl.fi/en/chemical_safety/carex/Documents/5_exposures_by_agent_and_industry.pdf.

Casanova M & Heck (1987). Further studies of the metabolic incorporation and covalent binding of inhaled [3H]- and [14C]formaldehyde in Fischer-344 rats: effects of glutathione depletion. *Toxicol Appl Pharmacol*, 89: 105–121. doi:10.1016/0041-008X(87)90181-5 PMID:2438809

Casanova M, Heck HD, Everitt JI *et al.* (1988). Formaldehyde concentrations in the blood of rhesus monkeys after inhalation exposure. *Food Chem Toxicol*, 26: 715–716. doi:10.1016/0278-6915(88)90071-3 PMID:3198038

Casanova M, Morgan KT, Steinhagen WH *et al.* (1991). Covalent binding of inhaled formaldehyde to DNA in the respiratory tract of rhesus monkeys: pharmacokinetics, rat-to-monkey interspecies scaling, and extrapolation to man. *Fundam Appl Toxicol*, 17: 409–428. doi:10.1016/0272-0590(91)90230-2 PMID:1765228

Casanova-Schmitz M, Starr TB, Heck HD (1984). Differentiation between metabolic incorporation and covalent binding in the labeling of macromolecules in the rat nasal mucosa and bone marrow by inhaled [14C]- and [3H]formaldehyde. *Toxicol Appl Pharmacol*, 76: 26–44. doi:10.1016/0041-008X(84)90026-7 PMID:6207627

CIIT Chemical Industry Institute of Toxicology (1981). *Final report on a chronic inhalation toxicology study in rats and mice exposed to formaldehyde.* CIIT docket #10922. Colombus, OH: Battelle Colombus Laboratories

Coggon D, Harris EC, Poole J, Palmer KT (2003). Extended follow-up of a cohort of british chemical workers exposed to formaldehyde. *J Natl Cancer Inst*, 95: 1608–1615. PMID:14600093

Collins JJ, Acquavella JF, Esmen NA (1997). An updated meta-analysis of formaldehyde exposure and upper respiratory tract cancers. *J Occup Environ Med*, 39: 639–651. doi:10.1097/00043764-199707000-00009 PMID:9253725

Collins JJ & Lineker GA (2004). A review and meta-analysis of formaldehyde exposure and leukemia. *Regul Toxicol Pharmacol*, 40: 81–91. doi:10.1016/j.yrtph.2004.04.006 PMID:15450712

Corrêa SM & Arbilla G (2005). Formaldehyde and acetaldehyde associated with the use of natural gas as a fuel for light vehicles. *Atmos Environ*, 39: 4513–4518. doi:10.1016/j.atmosenv.2005.03.042

Costa S, Coelho P, Costa C *et al.* (2008). Genotoxic damage in pathology anatomy laboratory workers exposed to formaldehyde. *Toxicology*, 252: 40–48. doi:10.1016/j.tox.2008.07.056 PMID:18721846

Dalbey WE (1982). Formaldehyde and tumors in hamster respiratory tract. *Toxicology*, 24: 9–14. doi:10.1016/0300-483X(82)90058-0 PMID:7135407

Dallas CE, Scott MJ, Ward JB Jr, Theiss JC (1992). Cytogenetic analysis of pulmonary lavage and bone marrow cells of rats after repeated formaldehyde inhalation. *J Appl Toxicol*, 12: 199–203. doi:10.1002/jat.2550120309 PMID:1378462

Dobiás L, Janca L, Lochman I, Lochmanova A (1988). Genotoxic action of formaldehyde in exposed children. *Mutat Res*, 216: 310

Easton DF, Peto J, Doll R (1988). Cancers of the respiratory tract in mustard gas workers. *Br J Ind Med*, 45: 652–659. PMID:3196660

Feron VJ, Bruyntjes JP, Woutersen RA *et al.* (1988). Nasal tumours in rats after short-term exposure to a cytotoxic concentration of formaldehyde. *Cancer Lett*, 39: 101–111. doi:10.1016/0304-3835(88)90045-6 PMID:3345504

Feron VJ, Til HP, Woutersen RA (1990). Formaldehyde in Sprague-Dawley rats. *Toxicol Ind Health*, 6: 637–639. PMID:2097822

Fox CH, Johnson FB, Whiting J, Roller PP (1985). Formaldehyde fixation. *J Histochem Cytochem*, 33: 845–853. doi:10.1177/33.8.3894502 PMID:3894502

Georgieva AV, Kimbell JS, Schlosser PM (2003). A distributed-parameter model for formaldehyde uptake and disposition in the rat nasal lining. *Inhal Toxicol*, 15: 1435–1463. doi:10.1080/08958370390249085 PMID:14648358

Gibson JE (1984). Coordinated toxicology: An example study with formaldehyde. *Concepts Toxicol.*, 1: 276–282.

Goldstein BD (2011). Hematological and toxicological evaluation of formaldehyde as a potential cause of human leukemia. *Hum Exp Toxicol*, 30: 725–735. doi:10.1177/0960327110381682 PMID:20729258

Gottschling LM, Beaulieu HJ, Melvin WW (1984). Monitoring of formic acid in urine of humans exposed to low levels of formaldehyde. *Am Ind Hyg Assoc J*, 45: 19–23. doi:10.1080/15298668491399299 PMID:6702592

Hagiwara M, Watanabe E, Barrett JC, Tsutsui T (2006). Assessment of genotoxicity of 14 chemical agents used in dental practice: ability to induce chromosome aberrations in Syrian hamster embryo cells. *Mutat Res*, 603: 111–120. PMID:16406784

Hall A, Harrington JM, Aw TC (1991). Mortality study of British pathologists. *Am J Ind Med*, 20: 83–89. doi:10.1002/ajim.4700200108 PMID:1867220

Hansen J & Olsen JH (1995). Formaldehyde and cancer morbidity among male employees in Denmark. *Cancer Causes Control*, 6: 354–360. doi:10.1007/BF00051411 PMID:7548723

Hansen J & Olsen JH (1996). [Occupational exposure to formaldehyde and risk of cancer *Ugeskr Laeger*, 158: 4191–4194. PMID:8701536

Hauptmann M, Lubin JH, Stewart PA *et al.* (2003). Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries. *J Natl Cancer Inst*, 95: 1615–1623. PMID:14600094

Hauptmann M, Lubin JH, Stewart PA *et al.* (2004). Mortality from solid cancers among workers in formaldehyde industries. *Am J Epidemiol*, 159: 1117–1130. doi:10.1093/aje/kwh174 PMID:15191929

Hauptmann M, Stewart PA, Lubin JH *et al.* (2009). Mortality from lymphohematopoietic malignancies and brain cancer among embalmers exposed to formaldehyde. *J Natl Cancer Inst*, 101: 1696–1708. doi:10.1093/jnci/djp416 PMID:19933446

Hayes RB, Blair A, Stewart PA *et al.* (1990). Mortality of U.S. embalmers and funeral directors. *Am J Ind Med*, 18: 641–652. doi:10.1002/ajim.4700180603 PMID:2264563

Hayes RB, Klein S, Suruda A *et al.* (1997). O6-alkylguanine DNA alkyltransferase activity in student embalmers. *Am J Ind Med*, 31: 361–365. doi:10.1002/(SICI)1097-0274(199703)31:3<361::AID-AJIM13>3.0.CO;2-Z PMID:9055960

Hayes RB, Raatgever JW, de Bruyn A, Gerin M (1986). Cancer of the nasal cavity and paranasal sinuses, and formaldehyde exposure. *Int J Cancer*, 37: 487–492. doi:10.1002/ijc.2910370403 PMID:3957459

He JL, Jin LF, Jin HY (1998). Detection of cytogenetic effects in peripheral lymphocytes of students exposed to formaldehyde with cytokinesis-blocked micronucleus assay. *Biomed Environ Sci*, 11: 87–92. PMID:9559107

Heck H & Casanova M (2004). The implausibility of leukemia induction by formaldehyde: a critical review of the biological evidence on distant-site toxicity. *Regul Toxicol Pharmacol*, 40: 92–106. doi:10.1016/j.yrtph.2004.05.001 PMID:15450713

Heck HD, Casanova-Schmitz M, Dodd PB *et al.* (1985). Formaldehyde (CH2O) concentrations in the blood of humans and Fischer-344 rats exposed to CH2O under controlled conditions. *Am Ind Hyg Assoc J*, 46: 1–3. doi:10.1080/15298668591394275 PMID:4025145

Heck HD, White EL, Casanova-Schmitz M (1982). Determination of formaldehyde in biological tissues by gas chromatography/mass spectrometry. *Biomed Mass Spectrom*, 9: 347–353. doi:10.1002/bms.1200090808 PMID:7126766

Hedberg JJ, Hoog JO, Grafstrom RC (2002). *Assessment of Formaldehyde Metabolizing Enzymes in Human Oral Mucosa and Cultured Oral Keratinocytes Indicate High Capacity for Detoxification of Formaldehyde*. In: *Crucial Issues in Inhalation Research — Mechanistic, Clinical and Epidemiologic (INIS Monographs)*. Heinrich U, Mohr U, editors. Stuttgart: Fraunhofer IRB Verlag, pp. 103–115.

Hikiba H, Watanabe E, Barrett JC, Tsutsui T (2005). Ability of fourteen chemical agents used in dental practice to induce chromosome aberrations in Syrian hamster embryo cells. *J Pharmacol Sci*, 97: 146–152. doi:10.1254/jphs.FPJ04044X PMID:15665446

Hildesheim A, Dosemeci M, Chan CC *et al.* (2001). Occupational exposure to wood, formaldehyde, and solvents and risk of nasopharyngeal carcinoma. *Cancer Epidemiol Biomarkers Prev*, 10: 1145–1153. PMID:11700262

Holmström M, Wilhelmsson B, Hellquist H (1989). Histological changes in the nasal mucosa in rats after long-term exposure to formaldehyde and wood dust. *Acta Otolaryngol*, 108: 274–283. doi:10.3109/00016488909125528 PMID:2816342

Horton AW, Tye R, Stemmer KL (1963). Experimental carcinogenesis of the lung. Inhalation of gaseous formaldehyde or an aerosol of coal tar by C3H mice. *J Natl Cancer Inst*, 30: 31–43. PMID:13964040

IARC (1982). Some industrial chemicals and dyestuffs. *IARC Monogr Eval Carcinog Risk Chem Hum*, 29: 1–398. PMID:6957379

IARC (1987). Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl*, 7: 1–440. PMID:3482203

IARC (1995). Wood dust and formaldehyde. *IARC Monogr Eval Carcinog Risks Hum*, 62: 1–405.

IARC (2006). Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. *IARC Monogr Eval Carcinog Risks Hum*, 88: 1–478. PMID:17366697

IARC (2010). Household use of solid fuels and high-temperature frying. *IARC Monogr Eval Carcinog Risks Hum*, 95: 1–424. PMID:18756632

Iarmarcovai G, Bonassi S, Sari-Minodier I *et al.* (2007). Exposure to genotoxic agents, host factors, and lifestyle influence the number of centromeric signals in micronuclei: a pooled re-analysis. *Mutat Res*, 615: 18–27. doi:10.1016/j.mrfmmm.2006.09.005 PMID:17198715

Im H, Oh E, Mun J *et al.* (2006). Evaluation of toxicological monitoring markers using proteomic analysis

in rats exposed to formaldehyde. *J Proteome Res*, 5: 1354–1366. doi:10.1021/pr050437b PMID:16739987

Iversen OH (1986). Formaldehyde and skin carcinogenesis. *Environ Int*, 12: 541–544. doi:10.1016/0160-4120(86)90148-0

Kamata E, Nakadate M, Uchida O *et al.* (1997). Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fisher-344 rats. *J Toxicol Sci*, 22: 239–254. doi:10.2131/jts.22.3_239 PMID:9279826

Kauppinen T, Toikkanen J, Pedersen D *et al.* (2000). Occupational exposure to carcinogens in the European Union. *Occup Environ Med*, 57: 10–18. doi:10.1136/oem.57.1.10 PMID:10711264

Kerns WD, Donofrio DJ, Pavkov KL (1983b). *The chronic effects of formaldehyde inhalation in rats and mice: A preliminary report*. In: *Formaldehyde Toxicity*. Gibson JE, editor. Washington DC: Hemisphere, pp. 111–131

Kerns WD, Pavkov KL, Donofrio DJ *et al.* (1983a). Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. *Cancer Res*, 43: 4382–4392. PMID:6871871

Kimbell JS, Overton JH, Subramaniam RP *et al.* (2001b). Dosimetry modeling of inhaled formaldehyde: binning nasal flux predictions for quantitative risk assessment. *Toxicol Sci*, 64: 111–121. PMID:11606807

Kimbell JS, Subramaniam RP, Gross EA *et al.* (2001a). Dosimetry modeling of inhaled formaldehyde: comparisons of local flux predictions in the rat, monkey, and human nasal passages. *Toxicol Sci*, 64: 100–110. PMID:11606806

Kitaeva LV, Kitaev EM, Pimenova MN (1990). [The cytopathic and cytogenetic sequelae of chronic inhalational exposure to formaldehyde on female germ cells and bone marrow cells in rats] *Tsitologiia*, 32: 1212–1216. PMID:2103083

Kuo H, Jian G, Chen C *et al.* (1997). White blood cell count as an indicator of formaldehyde exposure. *Bull Environ Contam Toxicol*, 59: 261–267. doi:10.1007/s001289900473 PMID:9211697

Lee SC & Wang B (2006). Characteristics of emissions of air pollutants from mosquito coils and candles burning in a large environmental chamber. *Atmos Environ*, 40: 2128–2138. doi:10.1016/j.atmosenv.2005.11.047

Levine RJ, Andjelkovich DA, Shaw LK (1984). The mortality of Ontario undertakers and a review of formaldehyde-related mortality studies. *J Occup Med*, 26: 740–746. doi:10.1097/00043764-198410000-00014 PMID:6491780

Linos A, Blair A, Cantor KP *et al.* (1990). Leukemia and non-Hodgkin's lymphoma among embalmers and funeral directors. *J Natl Cancer Inst*, 82: 66 doi:10.1093/jnci/82.1.66 PMID:2293657

Logue JN, Barrick MK, Jessup GL Jr (1986). Mortality of radiologists and pathologists in the Radiation Registry of Physicians. *J Occup Med*, 28: 91–99. PMID:3950788

Lorenti Garcia C, Mechilli M, Proietti De Santis L *et al.* (2009). Relationship between DNA lesions, DNA repair and chromosomal damage induced by acetaldehyde. *Mutat Res*, 662: 3–9. doi:10.1016/j.mrfmmm.2008.11.008 PMID:19084543

Lu K, Ye W, Gold A *et al.* (2009). Formation of S-[1-(N2-deoxyguanosinyl)methyl]glutathione between glutathione and DNA induced by formaldehyde. *J Am Chem Soc*, 131: 3414–3415. doi:10.1021/ja808048c PMID:19239220

Luce D, Gérin M, Leclerc A *et al.* (1993). Sinonasal cancer and occupational exposure to formaldehyde and other substances. *Int J Cancer*, 53: 224–231. doi:10.1002/ijc.2910530209 PMID:8425759

Luce D, Leclerc A, Bégin D *et al.* (2002). Sinonasal cancer and occupational exposures: a pooled analysis of 12 case-control studies. *Cancer Causes Control*, 13: 147–157. doi:10.1023/A:1014350004255 PMID:11936821

Madison RE, Broughton A, Thrasher JD (1991). Immunologic biomarkers associated with an acute exposure to exothermic byproducts of a ureaformaldehyde spill. *Environ Health Perspect*, 94: 219–223. doi:10.2307/3431314 PMID:1683282

Marsh GM, Stone RA, Esmen NA *et al.* (1996). Mortality among chemical workers in a factory where formaldehyde was used. *Occup Environ Med*, 53: 613–627. doi:10.1136/oem.53.9.613 PMID:8882119

Marsh GM & Youk AO (2005). Reevaluation of mortality risks from nasopharyngeal cancer in the formaldehyde cohort study of the National Cancer Institute. *Regul Toxicol Pharmacol*, 42: 275–283. doi:10.1016/j.yrtph.2005.05.003 PMID:15978711

Marsh GM, Youk AO, Buchanich JM *et al.* (2007a). Work in the metal industry and nasopharyngeal cancer mortality among formaldehyde-exposed workers. *Regul Toxicol Pharmacol*, 48: 308–319. doi:10.1016/j.yrtph.2007.04.006 PMID:17544557

Marsh GM, Youk AO, Morfeld P (2007b). Mis-specified and non-robust mortality risk models for nasopharyngeal cancer in the National Cancer Institute formaldehyde worker cohort study. *Regul Toxicol Pharmacol*, 47: 59–67. doi:10.1016/j.yrtph.2006.07.007 PMID:17000042

Monticello TM, Swenberg JA, Gross EA *et al.* (1996). Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. *Cancer Res*, 56: 1012–1022. PMID:8640755

Natarajan AT, Darroudi F, Bussman CJ, van Kesteren-van Leeuwen AC (1983). Evaluation of the mutagenicity of formaldehyde in mammalian cytogenetic assays in vivo and vitro. *Mutat Res*, 122: 355–360. doi:10.1016/0165-7992(83)90019-2 PMID:6656818

National Toxicology Program (2005). Formaldehyde (Gas). NTP 11th Report on Carcinogens. *Rep Carcinog*, 11: 1–A32.

Neuss S & Speit G (2008). Further characterization of the genotoxicity of formaldehyde in vitro by the sister

chromatid exchange test and co-cultivation experiments. *Mutagenesis*, 23: 355–357. doi:10.1093/mutage/gen025 PMID:18477654

Nojima K, Hochegger H, Saberi A *et al.* (2005). Multiple repair pathways mediate tolerance to chemotherapeutic cross-linking agents in vertebrate cells. *Cancer Res*, 65: 11704–11711. doi:10.1158/0008-5472.CAN-05-1214 PMID:16357182

Olsen JH & Asnaes S (1986). Formaldehyde and the risk of squamous cell carcinoma of the sinonasal cavities. *Br J Ind Med*, 43: 769–774. PMID:3790457

Olsen JH, Jensen SP, Hink M *et al.* (1984). Occupational formaldehyde exposure and increased nasal cancer risk in man. *Int J Cancer*, 34: 639–644. doi:10.1002/ijc.2910340509 PMID:6500742

Orsière T, Sari-Minodier I, Iarmarcovai G, Botta A (2006). Genotoxic risk assessment of pathology and anatomy laboratory workers exposed to formaldehyde by use of personal air sampling and analysis of DNA damage in peripheral lymphocytes. *Mutat Res*, 605: 30–41. PMID:16584911

Pala M, Ugolini D, Ceppi M *et al.* (2008). Occupational exposure to formaldehyde and biological monitoring of Research Institute workers. *Cancer Detect Prev*, 32: 121–126. doi:10.1016/j.cdp.2008.05.003 PMID:18639989

Partanen T, Kauppinen T, Luukkonen R *et al.* (1993). Malignant lymphomas and leukemias, and exposures in the wood industry: an industry-based case-referent study. *Int Arch Occup Environ Health*, 64: 593–596. doi:10.1007/BF00517706 PMID:8314619

Pesch B, Pierl CB, Gebel M *et al.* (2008). Occupational risks for adenocarcinoma of the nasal cavity and paranasal sinuses in the German wood industry. *Occup Environ Med*, 65: 191–196. doi:10.1136/oem.2007.033886 PMID:17881467

Pinkerton LE, Hein MJ, Stayner LT (2004). Mortality among a cohort of garment workers exposed to formaldehyde: an update. *Occup Environ Med*, 61: 193–200. doi:10.1136/oem.2003.007476 PMID:14985513

Pyatt D, Natelson E, Golden R (2008). Is inhalation exposure to formaldehyde a biologically plausible cause of lymphohematopoietic malignancies? *Regul Toxicol Pharmacol*, 51: 119–133. doi:10.1016/j.yrtph.2008.03.003 PMID:18440686

Ridpath JR, Nakamura A, Tano K *et al.* (2007). Cells deficient in the FANC/BRCA pathway are hypersensitive to plasma levels of formaldehyde. *Cancer Res*, 67: 11117–11122. doi:10.1158/0008-5472.CAN-07-3028 PMID:18056434

Rietbrock N (1965). Formaldehydoxydation bei der Ratte. *Naunyn Schmiedebergs Arch Exp Pathol Pharmakol*, 251: 189–190. doi:10.1007/BF00420161

Roush GC, Walrath J, Stayner LT *et al.* (1987). Nasopharyngeal cancer, sinonasal cancer, and occupations related to formaldehyde: a case-control study. *J Natl Cancer Inst*, 79: 1221–1224. PMID:3480373

Schlosser PM (1999). Relative roles of convection and chemical reaction for the disposition of formaldehyde and ozone in nasal mucus. *Inhal Toxicol*, 11: 967–980. doi:10.1080/089583799196736 PMID:10509029

Schmid O & Speit G (2007). Genotoxic effects induced by formaldehyde in human blood and implications for the interpretation of biomonitoring studies. *Mutagenesis*, 22: 69–74. doi:10.1093/mutage/gel053 PMID:17158519

Sellakumar AR, Snyder CA, Solomon JJ, Albert RE (1985). Carcinogenicity of formaldehyde and hydrogen chloride in rats. *Toxicol Appl Pharmacol*, 81: 401–406. doi:10.1016/0041-008X(85)90411-9 PMID:4082190

Shaham J, Bomstein Y, Gurvich R *et al.* (2003). DNA-protein crosslinks and p53 protein expression in relation to occupational exposure to formaldehyde. *Occup Environ Med*, 60: 403–409. doi:10.1136/oem.60.6.403 PMID:12771391

Shaham J, Bomstein Y, Meltzer A *et al.* (1996). DNA–protein crosslinks, a biomarker of exposure to formaldehyde–in vitro and in vivo studies. *Carcinogenesis*, 17: 121–125. doi:10.1093/carcin/17.1.121 PMID:8565120

Shaham J, Gurvich R, Kaufman Z (2002). Sister chromatid exchange in pathology staff occupationally exposed to formaldehyde. *Mutat Res*, 514: 115–123. PMID:11815250

Shi X, Pang X, Mu Y *et al.* (2006). Emission reduction potential of using ethanol-biodiesel-diesel fuel blend on a heavy-duty diesel engine. *Atmos Environ*, 40: 2567–2574. doi:10.1016/j.atmosenv.2005.12.026

Soffritti M, Belpoggi F, Lambertin L *et al.* (2002). Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. *Ann N Y Acad Sci*, 982: 87–105. doi:10.1111/j.1749-6632.2002.tb04926.x PMID:12562630

Soffritti M, Maltoni C, Maffei F, Biagi R (1989). Formaldehyde: an experimental multipotential carcinogen. *Toxicol Ind Health*, 5: 699–730. PMID:2815102

Speit G, Schütz P, Högel J, Schmid O (2007). Characterization of the genotoxic potential of formaldehyde in V79 cells. *Mutagenesis*, 22: 387–394. doi:10.1093/mutage/gem031 PMID:17855734

Speit G, Zeller J, Schmid O *et al.* (2009). Inhalation of formaldehyde does not induce systemic genotoxic effects in rats. *Mutat Res*, 677: 76–85. PMID:19539779

Stroup NE, Blair A, Erikson GE (1986). Brain cancer and other causes of death in anatomists. *J Natl Cancer Inst*, 77: 1217–1224. PMID:3467114

Sul D, Kim H, Oh E *et al.* (2007). Gene expression profiling in lung tissues from rats exposed to formaldehyde. *Arch Toxicol*, 81: 589–597. doi:10.1007/s00204-007-0182-9 PMID:17285311

Suruda A, Schulte P, Boeniger M *et al.* (1993). Cytogenetic effects of formaldehyde exposure in students of mortuary science. *Cancer Epidemiol Biomarkers Prev*, 2: 453–460. PMID:8220090

Swenberg JA, Kerns WD, Mitchell RI *et al.* (1980). Induction of squamous cell carcinomas of the rat nasal

cavity by inhalation exposure to formaldehyde vapor. *Cancer Res*, 40: 3398–3402. PMID:7427950

Takahashi M, Hasegawa R, Furukawa F *et al.* (1986). Effects of ethanol, potassium metabisulfite, formaldehyde and hydrogen peroxide on gastric carcinogenesis in rats after initiation with N-methyl-N'-nitro-N-nitrosoguanidine. *Jpn J Cancer Res*, 77: 118–124. PMID:3082823

Tang X, Bai Y, Duong A *et al.* (2009). Formaldehyde in China: production, consumption, exposure levels, and health effects. *Environ Int*, 35: 1210–1224. doi:10.1016/j.envint.2009.06.002 PMID:19589601

Til HP, Woutersen RA, Feron VJ *et al.* (1989). Two-year drinking-water study of formaldehyde in rats. *Food Chem Toxicol*, 27: 77–87. doi:10.1016/0278-6915(89)90001-X PMID:2714719

Titenko-Holland N, Levine AJ, Smith MT *et al.* (1996). Quantification of epithelial cell micronuclei by fluorescence in situ hybridization (FISH) in mortuary science students exposed to formaldehyde. *Mutat Res*, 371: 237–248. doi:10.1016/S0165-1218(96)90112-3 PMID:9008725

Vargová M, Wagnerová J, Lísková A *et al.* (1993). Subacute immunotoxicity study of formaldehyde in male rats. *Drug Chem Toxicol*, 16: 255–275. doi:10.3109/01480549309081819 PMID:8404546

Vaughan TL, Stewart PA, Teschke K *et al.* (2000). Occupational exposure to formaldehyde and wood dust and nasopharyngeal carcinoma. *Occup Environ Med*, 57: 376–384. doi:10.1136/oem.57.6.376 PMID:10810126

Vaughan TL, Strader C, Davis S, Daling JR (1986a). Formaldehyde and cancers of the pharynx, sinus and nasal cavity: I. Occupational exposures. *Int J Cancer*, 38: 677–683. doi:10.1002/ijc.2910380510 PMID:3770995

Vaughan TL, Strader C, Davis S, Daling JR (1986b). Formaldehyde and cancers of the pharynx, sinus and nasal cavity: II. Residential exposures. *Int J Cancer*, 38: 685–688. doi:10.1002/ijc.2910380511 PMID:3770996

Vozenílková H, Tmějová M, Srb V *et al.* (1991). [Environmental monitoring and biological monitoring of young people exposed to nonoccupational levels of formaldehyde, toluene and other hydrocarbons] *Sb Ved Pr Lek Fak Karlovy Univerzity Hradci Kralove Suppl*, 34: 407–476. PMID:1815345

Walker FJ (1964). *Formaldehyde*. American Chemical Society Monographs 159, 3rd edition. New York: Reinhold

Walrath J & Fraumeni JF Jr (1983). Mortality patterns among embalmers. *Int J Cancer*, 31: 407–411. doi:10.1002/ijc.2910310403 PMID:6832852

Walrath J & Fraumeni JF Jr (1984). Cancer and other causes of death among embalmers. *Cancer Res*, 44: 4638–4641. PMID:6467219

Wang B & Liu DD (2006). [Detection of formaldehyde induced developmental toxicity assessed with single cell gel electrophoresis] *Fen Zi Xi Bao Sheng Wu Xue Bao*, 39: 462–466. PMID:17117557

Wang M, Cheng G, Balbo S *et al.* (2009). Clear differences in levels of a formaldehyde-DNA adduct in leukocytes of smokers and nonsmokers. *Cancer Res*, 69: 7170–7174. doi:10.1158/0008-5472.CAN-09-1571 PMID:19738046

Wang W, Xu J, Xu L *et al.* (2007). The instability of (GpT)n and (ApC)n microsatellites induced by formaldehyde in Escherichia coli. *Mutagenesis*, 22: 353–357. doi:10.1093/mutage/gem023 PMID:17630409

West S, Hildesheim A, Dosemeci M (1993). Non-viral risk factors for nasopharyngeal carcinoma in the Philippines: results from a case-control study. *Int J Cancer*, 55: 722–727. doi:10.1002/ijc.2910550504 PMID:7503957

Woutersen RA, van Garderen-Hoetmer A, Bruijntjes JP *et al.* (1989). Nasal tumours in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. *J Appl Toxicol*, 9: 39–46. doi:10.1002/jat.2550090108 PMID:2926095

Yanysheva NA, Balenko NV, Chernichenko IA *et al.* (1998). [Characteristics of modifying effects of formaldehyde on carcinogenesis] [in Russian]*Gig Sanit*, 8: 51–54.

Ye X, Yan W, Xie H *et al.* (2005). Cytogenetic analysis of nasal mucosa cells and lymphocytes from high-level long-term formaldehyde exposed workers and low-level short-term exposed waiters. *Mutat Res*, 588: 22–27. PMID:16257574

Ying CJ, Yan WS, Zhao MY *et al.* (1997). Micronuclei in nasal mucosa, oral mucosa and lymphocytes in students exposed to formaldehyde vapor in anatomy class. *Biomed Environ Sci*, 10: 451–455. PMID:9448927

Ying CJ, Ye XL, Xie H *et al.* (1999). Lymphocyte subsets and sister-chromatid exchanges in the students exposed to formaldehyde vapor. *Biomed Environ Sci*, 12: 88–94. PMID:10560533

Yu LQ, Jiang SF, Leng SG *et al.* (2005). [Early genetic effects on workers occupationally exposed to formaldehyde] *Zhonghua Yu Fang Yi Xue Za Zhi*, 39: 392–395. PMID:16329798

Zhang L, Tang X, Rothman N *et al.* (2010). Occupational exposure to formaldehyde, hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells. *Cancer Epidemiol Biomarkers Prev*, 19: 80–88. doi:10.1158/1055-9965.EPI-09-0762 PMID:20056626

Zhang LSCED, Steinmaus C, Eastmond DA *et al.* (2009). Formaldehyde exposure and leukemia: a new meta-analysis and potential mechanisms. *Mutat Res*, 681: 150–168. doi:10.1016/j.mrrev.2008.07.002 PMID:18674636

Zhong W & Que Hee SS (2004). Formaldehyde-induced DNA adducts as biomarkers of in vitro human nasal epithelial cell exposure to formaldehyde. *Mutat Res*, 563: 13–24. PMID:15324745