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REVIEW ARTICLE

Recent trend in risk assessment of formaldehyde exposures from indoor air

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Abstract Studies about formaldehyde (FA) published since the guideline of 0.1 mg/m^3 by the World Health Organization (WHO) in 2010 have been evaluated; critical effects were eye and nasal (portal-of-entry) irritation. Also, it was considered to prevent long-term effects, including all types of cancer. The majority of the recent toxicokinetic studies showed no exposure-dependent FA–DNA adducts outside the portal-of-entry area and FA–DNA adducts at distant sites were due to endogenously generated FA. The no-observed-adverse-effect level for sensory irritation was 0.5 ppm and recently reconfirmed in hypo- and hypersensitive individuals. Investigation of the relationship between FA exposure and asthma or other airway effects in children showed no convincing association. In rats, repeated exposures showed no point mutation in the *p53* and *K-Ras* genes at $\leq$15 ppm neither increased cell proliferation, histopathological changes and changes in gene expression at 0.7 ppm. Repeated controlled exposures (0.5 ppm with peaks at 1 ppm) did not increase micronucleus formation in human buccal cells or nasal tissue (0.7 ppm) or in vivo genotoxicity in peripheral blood lymphocytes (0.7 ppm), but higher occupational exposures were associated with genotoxicity in buccal cells and cultivated peripheral blood lymphocytes. It is still valid that exposures not inducing nasal squamous cell carcinoma in rats will not induce nasopharyngeal cancer or lymphohematopoietic malignancies in humans. Reproductive and developmental toxicity are not considered relevant in the absence of sensory irritation. In conclusion, the WHO guideline has been strengthened.

Keywords Indoor air guideline · Formaldehyde · World Health Organization · Sensory irritation · Asthma · Cancer

Introduction

Formaldehyde (FA), a high-volume chemical, is ubiquitously found in the environment due to natural sources and anthropogenic activities. Major indoor air sources are building materials (e.g. wooden products as furniture, particleboard, plywood and medium-density fibreboard), consumer products and combustion processes (WHO 2010). The International Agency for Research on Cancer (IARC) has classified FA in group 1 (human carcinogen). It was based on inhalation causing squamous cell carcinoma (SCC) in rats and nasopharyngeal cancer in humans (IARC 2006). Recently, the classification has been expanded with FA causing leukaemia and limited evidence of sinonasal cancer in humans (IARC 2012).

Indoor air is the dominating contributor to FA exposure through inhalation. Therefore, the World Health Organization (WHO) developed an indoor air guideline value in 2010. The critical effects were considered the portal-of-entry effects, sensory irritation of the eyes and the upper airways, resulting in a guideline value of 0.1 mg/m^3 that should not be exceeded for any 30-min period of the day (WHO 2010). The guideline intends to prevent sensory irritation after acute and chronic exposures and cancer at the portal-of-entry as well as at remoter sites. In this review, we evaluate the literature published since the guideline was published with the purpose to evaluate whether the guideline has been compromised.

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Exposures

In Europe, Canada and the US, the general FA levels (geometric mean, median or average concentrations) in homes or dwellings were generally within 20–40 μg/m^3, the 95th percentiles were roughly two times higher. In Japan, a recent study suggests a similar mean level. A higher mean concentration has been reported in China (~240 μg/m^3). In Europa and the US, the general levels in public buildings and office buildings were lower than in homes and dwellings. Mean ambient outdoor concentrations were typically in the range 1–4 μg/m^3, but with higher levels in polluted cities (WHO 2010). Other examples from recent reports support the WHO exposure data. Exposure levels from the comprehensive review by Salthammer et al. (2010) showed that the general level in homes and dwellings in Europe, Canada and the US were overall similar to the range reported by WHO (2010), although slightly broader (10–80 μg/m^3); the 95th percentiles were mostly ≤100 μg/m^3. Another recent review reported FA levels in Europe to be typically between 10 and 50 μg/m^3. The maximum concentration was in general <100 μg/m^3. The concentration inside cars was about 15 μg/m^3 with a maximum of 27 μg/m^3 in dense traffic (Sarigiannis et al. 2011).

In France, the median FA concentration was 20 μg/m^3 (10th and 95th percentile: 9 and 47 μg/m^3) in dwellings and 14 μg/m^3 (min and max: 1.5 and 56 μg/m^3) in the day-care centres. The mean concentration was 27 μg/m^3 (min and max: 4 and 100 μg/m^3) in schools and 16 μg/m^3 (min and max: 7 and 27 μg/m^3) in cars (Mandin et al. 2012). Mean concentrations in Turkish primary school classrooms and kindergartens were in the range from 19 to 109 μg/m^3, and at outdoor playgrounds from 0.6 to 9 μg/m^3 (Sofuoglu et al. 2011). Recent measurements in Japanese dwellings aged ≤7 years showed a decrease in indoor air FA concentrations compared with previous measurements. The 25th, the median and the 75th percentile was 34, 49 and 67 μg/m^3 FA, respectively, in homes with occupants reporting sick-building syndrome (SBS) symptoms and 27, 40 and 56 μg/m^3 FA, respectively, among non-SBS reporting participants. Although the differences between the two types of homes were marginal, the differences were statistically different ($p < 0.001$) (Takigawa et al. 2010). In Houston metropolis, a highly industrial area in the US, the average ambient FA concentration was about 3–7 ppb (~4–9 μg/m^3) with peak exposures up to 37 ppb (45 μg/m^3) (Guven and Olaguer 2011); we use the conversion factor: 1 ppm is equal to 1.228 mg/m^3 at 25 °C and 1 atm throughout the studies. Low rural FA concentrations have been confirmed in a study from the northern Michigan. FA concentrations varied from 0.5 ppb (0.6 μg/m^3; night maximum) to about 4 ppb (5 μg/m^3; daytime maximum) in a mixed hardwood forest at days with a wind direction from areas with low anthropogenic pollution; FA was mainly due to oxidation of isoprene (Galloway et al. 2012).

Absorption, distribution, metabolism and elimination

The summary is based on references in IARC (2006), WHO (2010), Nielsen and Wolkoff (2010) and Wolkoff and Nielsen (2010) except where otherwise stated. Thus, due to the high water solubility and reactivity, airborne FA is absorbed mainly in the upper airways (>90 %). Deposition of reactive and water soluble gases was found to be similar in the extrathoracic area of the airways of children and adults (Ginsberg et al. 2010). In the water phase, FA forms a water addition product, FA acetal [methylene glycol, $CH_2(OH)_2$], which reacts with glutathione (GSH), forming *S*-hydroxymethylglutathione (FA glutathione thioacetal; HO–CH_2–SG). This intermediate is oxidized by the glutathione-dependent FA dehydrogenase [FDH, also termed alcohol dehydrogenase 5 (ADH5) and previously ADH3 (Just et al. 2011)] to *S*-formylglutathione and hydrolyzed to GSH and formate (Andersen et al. 2010). FA is an endogenous metabolite, for example, of serine, glycine, methionine and choline. It is essential in the one-carbon pool, which is involved in the biosynthesis of nucleic acids and certain amino acids. Formate is incorporated in metabolic products or further oxidized to carbon dioxide. Additionally, it is excreted in the urine (Hovda et al. 2005; Soleimani 2001); due to the high background formate excretion in the urine [from about 4 to ≥39 mg/day (Berode et al. 2000)] no increase is expected due to inhalation of FA. Inhalation of FA does not increase the concentration of FA in the blood (about 2–3 mg/L). Rat studies indicated that the half saturation of the nasal FA metabolizing enzyme system is about 2.6 ppm (3.2 mg/m^3). Higher exposure levels cause a disproportionate increase in nasal cellular levels of FA (Andersen et al. 2010). The half-life of FA in blood is about 1–1½ min. The mean exhaled breath FA concentrations have been reported to be about 2 ppb (Cáp et al. 2008; Fuchs et al. 2010) and the maximum about 12 ppb (Cáp et al. 2008). FA is an electrophile, which reacts with DNA, RNA and proteins forming reversible adducts or irreversible cross-links. Thus, formation of DNA–protein cross-links (DPX) in nasal tissue in rats increased disproportionate at exposures above 2–3 ppm (2.5–3.6 mg/m^3).

Several recent inhalation studies using labelled FA ($^{13}CD_2$) did not find DNA adducts outside the nasal tissue in rats and monkeys; thus, FA was not considered to reach the internal organs or the blood compartment (Lu et al. 2010, 2011; Moeller et al. 2011; Swenberg et al. 2011). This agrees with a dosimetry study that suggests

intracellular FA concentrations in the nasal cells are only marginally changed at exposures up to 2 ppm (Andersen et al. 2010). Furthermore, gene expression studies showed no change at 0.7 ppm (Andersen et al. 2008). In a 6-h inhalation study in rats exposed to 10 ppm ^{13}C-FA, no inhaled FA was detected in the blood, neither during the exposure nor after the exposure (Kleinnijenhuis 2012). This should also apply to humans (Franks 2005). Overall, these and previous studies support that FA acts as a portal-of-entry toxicant if detoxification mechanisms are not overwhelmed (WHO 2010; Nielsen and Wolkoff 2010).

However, a recent study considered biomonitoring of airborne FA exposures from the terminal N-methylenvaline residue in blood haemoglobin (Hgb). This unstable adduct was reduced to the stable N-methylvaline adduct and analysed after derivatization. Adduct formations were compared with FA exposures (8-h sampling) in pathologists, plastic laminate workers and low-exposed controls. The mean FA concentration was 189, 210 and 41 μg/m^3, respectively, and the N-methylenvaline concentration 378, 343 and 145 nmol/g Hgb, respectively. Dividing exposures into quartiles showed a significant exposure–response relationship (Bono et al. 2012). For an evaluation, we assume an 8-h working day (inhalation rate: 10 m^3/8 h; exposure concentration: 189 and 210 μg/m^3, respectively) and 16 h at the control level (inhalation rate: 20 m^3/24 h; exposure concentration: 41 μg/m^3) in the non-occupational exposure period, and 24 h exposure (inhalation rate: 20 m^3/24 h; exposure concentration: 41 μg/m^3) in the controls. Thus, the inhaled FA dose during a 24-h day would be 2,437, 2,647 and 820 μg/day, respectively. In general, absorption of airborne FA into the blood is considered low. In humans, it was estimated that 1.9 ppm increases blood FA at maximum by 15 μg/L during repeated daily 8-h exposures (Franks 2005); the normal background blood FA level is about 2,000–3,000 μg/L. In rats exposed 6 h/day for 5 days to 10 ppm FA, the exogenous FA did not result in detectable FA–DNA adducts in blood lymphocytes (Lu et al. 2010); this also indicated a low FA absorption into the blood compartment. Furthermore, an in vitro study showed that FA-exposed nasal epithelial cells did not release FA subsequently (Neuss et al. 2010b); this suggests that the nasal epithelium is an efficient barrier between inhaled FA and the blood compartment. Nevertheless, we accept, as an upper boundary, that 1 % of the inhaled dose may be delivered to the blood compartment, which would be 24, 26 and 8 μg/day, respectively. Part of the delivered dose would be metabolized in the blood compartment, which is a fast process, and part of the delivered dose would react with blood proteins, including Hgb with valine as one of the binding sites. No accumulation is considered to occur over long-term exposures due to the fast metabolism of FA in blood and the reversible nature of the reaction products (methylol derivatives) with amino, hydroxyl and thiol groups in proteins; the primary reaction product can undergo further (secondary) reactions. The secondary reaction products may also be reversible, as shown for DPX removal in human lymphocytes due to spontaneous hydrolysis (Quievry and Zhitkovich 2000). The total N-methylenvaline bound FA in Hgb can be estimated to 8,514, 7,725 and 3,266 μg/person, accepting a molecular mass of FA at 30.03, a Hgb concentration of 150 g/L blood and a blood volume of 5 L/person. Thus, the delivered dose corresponds to 0.28, 0.34 and 0.24 %, respectively, of the detected blood N-methylenvaline amount. This calculation assumes no metabolism in the blood compartment and furthermore, that the only binding site in the blood compartment is the valine sites, which clearly is an upper boundary assumption. However, even in this case, can the inhaled FA only explain a marginal amount of the measured N-methylenvaline adduct level. Thus, it is difficult to corroborate that the inhaled FA would be responsible for the observed differences in N-methylenvaline adduct in Hgb. It is noted that in the evaluation of the N-methylenvaline levels, neither high peak exposures were investigated, which might overwhelm the detoxification mechanisms, nor were potential differences in gender considered; among the pathologists, 42 % were males, 100 % were males among the plastic laminate workers and 29 % among the controls were males.

Non-carcinogenic airway effects

Odour and non-specific symptoms

A recent high-quality study determined the mean 50 % odour detection threshold of FA to 110 ppb (0.135 mg/m^3), but the individual thresholds varied from 23 to 505 ppb (0.028–0.62 mg/m^3) (Berglund et al. 2012). This further supports the WHO's (2010) conclusion that a significant fraction of the population may perceive FA at or below 0.1 mg/m^3.

A Japanese study comprised 5,709 randomly selected households in dwellings (age ≤7 years); 2,297 returned a preliminary questionnaire, and of these, 425 households with 1,479 residents participated in the study. FA and 41 other volatile organic compounds (VOCs) were measured in the dwellings (passive sampling for 24 h) and a questionnaire with sick-building syndrome (SBS) symptoms (eye irritation, nasal, throat and lung, skin and general symptoms as fatigue, heavy head, headache, nausea, dizziness and difficulties in concentrating) were obtained from the participants. FA and other aldehydes were the dominating VOCs. Aldehydes and other VOCs were often significantly higher in the SBS positive participants. The positive participants comprised more females, younger

participants, exposed to environmental tobacco smoke, participants spending more time in their homes, reporting mould growth and suffering from allergic diseases. Multiple logistic regression analysis was used to calculate adjusted odds ratios (ORs) in the quartiles of air concentrations. For the SBS positive participants, the ORs for FA exposures were significantly increased in the second (OR: 2.1), third (OR: 2.3) and fourth quartiles (OR: 2.4; maximum FA: 203 μg/m^3). In the fourth quartile, ORs were significantly increased for *n*-nonane (OR: 2.4; maximum: 160 μg/m^3), 1,2-dichloropropane (OR: 15; maximum: 3 μg/m^3) and chlorodibromomethane (OR: 6; maximum: 6 μg/m^3); the maximum concentration is the highest measured concentration in all dwellings. OR for eye irritation at FA exposure was 2.0, 4.2 and 1.7, respectively; the value in the third quartile was statistically significant. ORs for nasal irritation were 2.9, 2.6 and 1.7, respectively; the first two values were significantly increased. It was concluded that after adjustment for other possible risk factors, FA dose dependence showed to be a significant risk factor for SBS (Takigawa et al. 2010). Overall, the study was conducted on a highly selected population and highly increased ORs for SBS were observed at very low exposure levels of some VOCs; this challenges a cause–effect relationship. It is apparent that the study shows no concentration-dependent effect of FA exposure. This weakens that FA is causally related to the reported effects, except if they are odour driven as the odour threshold may have been exceeded. If FA at all is involved in the increased symptom reporting, the odour of FA is not considered adverse below 0.1 mg/m^3 (WHO 2010).

Sensory irritation

Perceived (sensory) irritation from the eyes and the upper airways is considered the critical effect of acute effects of FA. Formaldehyde activates the TRPA1 receptor (McNamara et al. 2007) on the trigeminal nerves, which triggers the sensation of sensory irritation (WHO 2010; Wolkoff and Nielsen 2010). Sensory irritation sensitivity was not related to genetic endpoints. This was observed in a study with non-smoking male volunteers where nasal irritation sensitivity to CO_2 (a model sensory irritant) was compared with FA dehydrogenase gene expression in leucocytes, with in vitro FA-induced DPX formation and DPX removal in leucocytes, and with FA-induced sister chromatide exchange (SCE) in cultured lymphocytes (Zeller et al. 2011b).

A review of the sensory irritation studies indicated that the most informative (key) study was a double-blinded 4-h controlled chamber study by Lang et al. (2008). The concentration 0.5 ppm can be considered the lowest-observed-adverse-effect level (LOAEL) where peaks of 1 ppm were added or the no-observed-adverse-effect level (NOAEL) without peaks. This was in reasonable agreement with other chamber studies and with results from predictive animal testing. Contrary, epidemiological studies have reported associations, although not always consistent, between symptoms and FA levels at more than 10 times lower concentrations. These effects were from mixed exposures where causative agents are not clear and confounders are likely present (Paustenbach et al. 1997; WHO 2010; Wolkoff and Nielsen 2010; Golden 2011); these studies were disregarded in the WHO's (2010) guideline setting. Sensory irritation induced by FA is considered concentration dependent and essentially not time dependent after few hours of exposure (Paustenbach et al. 1997; WHO 2010; Wolkoff and Nielsen 2010; Golden 2011). Neither asthmatics (Paustenbach et al. 1997; WHO 2010; Wolkoff and Nielsen 2010) nor children (WHO 2010; Wolkoff and Nielsen 2010) were found to be at increased risk. Thus, the NOAEL from the Lang et al.'s (2008) study was used as the point of departure for setting the guideline value. An assessment factor of five was used based on the steepness of the exposure–response relationship and the standard deviation of nasal pungency thresholds (WHO 2010; Wolkoff and Nielsen 2010), which after rounding led to a guideline value of 0.1 mg/m^3 (WHO 2010; Wolkoff and Nielsen 2010). A nearly identical value [0.1 ppm (0.12 mg/m^3)] was derived in a recent review (Golden 2011).

Inter individual variations in FA-induced sensory irritation were investigated in 4-h exposures in 41 males (aged 32 ± 10 years), where hypo- and hypersensitivity were identified from CO_2-induced nasal sensory irritation. The FA concentrations were 0.5 and 0.7 ppm, 0.3 ppm with four 15-min peaks at 0.6 ppm and 0.4 ppm with four 15-min peaks at 0.8 ppm. The FA exposures caused no consistent effect on conjunctival redness, eye-blinking frequency, self-reported tear film break-up time, mean nasal flow rate, eye and nasal irritation intensity symptoms. Furthermore, no difference between hypo- and hypersensitive volunteers was observed. The FA exposures were associated with increased olfactory triggered symptoms, which increased in the hypersensitive subjects compared with the hyposensitive subjects. NOAELs were 0.7 ppm at a constant exposure and 0.4 ppm with peaks at 0.8 ppm, comprising both hypo- and hypersensitive individuals (Mueller et al. 2012). Thus, this study is in overall agreement with the Lang et al.'s (2008) study used in the guideline setting.

A prerequisite for the use of an acute study in the guideline setting is that the FA-induced sensory irritation effect does not increase upon daily repeated exposures. This was addressed from repeated exposures of airborne limonene–ozone reaction products in mice. In this mixture, FA is responsible for a considerable part of the sensory

irritating effect (Wolkoff et al. 2008). One-hour exposures were conducted on 10 consecutive days. No increase was observed in sensory irritation, airflow limitation and deep lung effects at exposures from low to high sensory irritation levels (Wolkoff et al. 2012). This supports that the sensory effects of FA can be established from acute chamber studies in humans as is the case with the WHO's (2010) guideline. Also, the recent study by Mueller et al. (2012) indicates that the WHO guideline is highly protective and takes into account the sensitive subjects.

Asthma

FA-dependent exposure–response relationships have not been substantiated from lung function effects in controlled chamber studies neither in healthy adults nor in asthmatics below 1 mg/m^3. Furthermore, associations between FA exposures and exacerbation of asthma or sensitization have not been convincingly demonstrated in children or adults at exposures in homes or in schools (WHO 2010; Wolkoff and Nielsen 2010). Similarly, a recent review concluded that there was no clear association between FA concentrations in dwellings and development of childhood asthma; consistent environmental risk factors were environmental tobacco smoke, living close to busy roads and living in damp homes with visible moulds (Heinrich 2011).

The association between FA exposures and asthma in children was addressed in a recent meta-analysis (McGwin et al. 2010). The fixed-effects model showed an OR of 1.03 (95 % CI 1.02–1.04) and the random effects model an OR of 1.17 (1.01–1.36), both for an increment of 10 μg/m^3 of FA. The two most influential studies in the analyses were the study of Rumchev et al. (2002) and the study by Garrett et al. (1999).

The Rumchev et al.'s (2002) study is a case–control study comprising 88 children (mean age: 25 months), who were discharged from an emergency treatment for asthma, and 104 non-asthmatic controls (mean age: 20 months). FA was measured both in the winter and the summer period and in the living rooms and the bedrooms of the children. Children exposed to 60 μg/m^3 FA had a 39 % increased risk of asthma compared with children with <10 μg/m^3 FA in the air. The study acknowledged the difficulty of diagnosing asthma in the studied age group. Later on, an entirely similar study was published by the same group. It showed the presence of VOCs that were dominated by benzene and toluene. Additionally, house-dust mites were present in fine dust (Rumchev et al., 2004). The median benzene and toluene concentration among the cases was 25 and 12 μg/m^3, respectively, and among the controls, 12 and 6 μg/m^3, respectively. Dust mite allergen levels were significantly associated with total VOC levels. Benzene levels of ≥20 μg/m^3 were associated with an eightfold increase in emergency treatment. As benzene and toluene are markers of combustion products, it suggests that the FA exposure in the first study is one component in a complex combustion mixture. Due to the limitations, the Rumchev et al.'s (2002) study has not been considered appropriate for establishing exposure effects of FA (WHO 2010; Wolkoff and Nielsen 2010; Golden 2011; NRC 2011).

In a cross-sectional study, Garrett et al. (1999) compared 53 asthmatic and 95 non-asthmatic children, aged 7–14 years. It was stated that "there was no significant increase in adjusted risk of asthma or respiratory symptoms with FA exposure". However, there was a marginal higher geometric mean FA concentration ($p = 0.06$) in the bedroom at the atopic children (19 μg/m^3) compared with the non-atopic children (16 μg/m^3). This was significant ($p = 0.002$) if the highest recorded FA concentrations from the bedrooms, the living rooms, the kitchens and the outdoor levels were used, 38 vs. 29 μg/m^3, respectively. The OR (about 1.3–1.4) for atopy increased with an increase in bedroom FA levels by 10 μg/m^3. Results from an exactly similar group of children showed that asthma was associated with exposure to *Penicillium* and atopy with exposure to *Aspergillus* (Garrett et al. 1998). Overall, the use of mixed exposure effects and lumping together unrelated endpoints makes it difficult to interpret this meta-analysis as previously pointed out (Golden 2011; Heinrich 2011).

A study was conducted in two cross-sectional-based case-control populations with children, aged about 13 years, living in a French city (32 asthmatics and 31 controls) and its country side (24 asthmatics and 27 controls). Asthma was identified by means of the questions: "has your child ever had asthma?", "has your child had wheezing in the chest in the past 12 months?" or whether a child has taken medication against an asthma crisis. The controls answered "no" to the questions. Air pollutants, including FA, were measured in the living room. Measurements were carried out summer and winter in the urban homes, but in the rural homes only in summer. The winter-median FA concentration was 20.3 μg/m^3 and the summer-median concentration was 20.6 μg/m^3 in the urban homes and the summer-median concentration was 15.7 μg/m^3 in the rural homes. The FA exposure was marginally, but significantly increased in the asthmatics [19.8 μg/m^3 (range 5.8–75.1)] compared with the non-asthmatics [17.2 μg/m^3 (range 3.7–50.8)]. ORs were determined by logistic models. In the combined population, the association between FA and asthma was increased non-significantly [OR (95 % CI) 1.7 (0.7–4.4)]. The increased OR was driven by a significant increased OR (∼11) in the rural children, whereas the OR (∼0.2–0.6) was non-significantly decreased in the urban children (Hulin et al. 2010). As the highest risk was observed in the lowest exposed FA group (rural children), it is counterintuitive that the increase in

ORs expresses a causal relationship. Also, cross-sectional studies have a limited ability to establish causations.

In a study of 1,005 school children (aged 8–13 years) in Korea, 12.8 % had self-reported asthma and 6.8 % physician-diagnosed asthma. A case–control study was conducted with 33 asthmatic and 40 non-asthmatic children. Personal exposures to FA and VOCs were obtained during a 3-day period. No significant difference was observed between FA and VOC exposures in the two groups; the geometric mean FA concentration was 27 $\mu g/m^3$ in the cases and 29 $\mu g/m^3$ in the controls. Multiple logistic regression analysis (adjusted for confounders) did not show any significant difference between the children, suggesting other causative factors for development of asthma (Hwang et al. 2011).

A cross-sectional study was conducted among 2,453 Korean school children, aged 10 years. Respiratory symptoms were obtained by means of a questionnaire, and indoor and outdoor exposures, including FA concentrations, were measured. The mean FA concentration was 28 $\mu g/m^3$ (range 16–47) in class rooms and the outdoor level was 4.3 $\mu g/m^3$ (range 2–9). There was no association between class room FA exposures and wheeze, doctor diagnosed asthma or current asthma, but wheeze was significantly associated with reported indoor dampness and mould growth in the home environments (Kim et al. 2011).

In a French cross-sectional study comprising 6,590 children with a mean age of 10 years from 401 randomly selected classrooms, past-year rhinoconjunctivitis and past-year asthma were obtained by means of a questionnaire. Furthermore, exercise-induced asthma and skin prick test reactions to 10 common allergens were investigated. Classroom concentrations of fine particles ($PM_{2.5}$) were obtained by filter-based sampling, and NO_2, FA, acetaldehyde and acrolein were obtained by passive sampling. A significantly increased odds ratio (OR: 1.19) was observed for past-year rhinoconjunctivitis at FA exposures exceeding 28.4 $\mu g/m^3$, but not for past-year asthma (OR: 0.90). Furthermore, exercise-induced asthma was not correlated with FA exposure. The authors concluded that school air quality may affect rhinitis (FA) and asthma-related ($PM_{2.5}$, acrolein and NO_2) morbidity. However, it was realized that a cross-sectional study cannot provide causal relationships (Annesi-Maesano et al. 2012)

Overall, recent field studies are about mixed exposures where the effects of FA are indistinguishable from effects of other components. None of the studies present a convincing association between FA exposures and asthma, in agreement with previous studies (WHO 2010).

Other airway or lung effects

In an Australian study, lung effects of low-NO_x unflued combustion products from gas heaters and gas heaters with non-indoor-air emitting flue gas were compared in 4–6 grade school children ($N = 400$). The geometric mean (95 % CI) concentration of NO_2 in classroom was 31.6 ppb (7.4–135.2) and 17.5 ppb (3.5–88.4), respectively, and of FA 32.6 ppb (3.1–62.1) and 24.7 ppb (3.3–46.1), respectively. However, when the heaters were used ≥30 % of the day, the NO_2 concentration was 2.33 times higher with the unflued heater and the FA concentration was 20.1 ppb higher compared with the flued heater. The only effect of unflued heaters was minor, but statistically significant, improvement in lung function at high-exposure days that also applied to the subgroup of asthmatics. No adverse symptom effect was observed in the non-atopics. Also, the estimated effect of heater type on symptoms did not differ significantly by current asthma status. However, the use of unflued gas heaters was significantly associated with evening cough (OR = 1.16) and morning wheeze (OR = 1.38); adjustment of the use of gas heating, open fire and environmental tobacco smoke exposure at home increased the OR for morning cough to 1.60. The values were no longer significant if analyses were limited to days where heaters were used ≥30 % of the day. In the subgroup analyses with atopics, the use of unflued heaters was significantly associated with a reduction in morning cough (OR = 0.77) and an increase in morning wheeze (OR = 1.85), stomach ache (OR = 1.63) and use of bronchodilators for relief of symptoms (OR = 1.87). If the analyses in atopics were restricted to days where heaters were used ≥30 %, increase in morning wheeze was significantly increased (OR = 7.72) as was evening wheeze (OR = 3.97) (Marks et al. 2010). The study has a strong (double blind, cluster randomized, crossover) design; it indicates that gas combustion products were associated with reported wheeze and cough mainly in the subgroup of atopics. The study cannot disentangle which components of the complex flue mixture are responsible for the associations. However, if it is assumed that NO_2 or FA are causally related to the symptoms, they are most likely due to deep lung effects of NO_2 (WHO 2010) and not to FA, which is mainly deposited in the upper airways (WHO 2010).

Children in a Paris birth cohort ($N = 2{,}940$) were investigated for lower respiratory tract infections (LRIs) and LRIs with wheeze (wLRIs) during first year of life (Roda et al. 2011); the "never LRI" group was the reference group. Health data were obtained by questionnaires, and building characteristics and lifestyle factors from phone interviews. FA exposures were predicted for all children from a model, which was derived from FA measurements in about 200 randomly selected dwellings, their building characteristics and the occupant activities. The model classified 73 % of the dwellings correctly in the group below and above the median, that is, 27 % could be

misclassified and a random allocation is expected to classify 50 % correctly. The annual quartile ranges were <14.4, 14.4–19.5, 19.5–26.8 and >26.8 μg/m^3 FA and the interquartile range from 14.2 to 26.8 μg/m^3. In the interquartile range, the association between symptoms and FA exposures was significantly increased [OR (95 %): 1.32 (1.11–1.55)] for LRI and for wLRI [1.41 (1.14–1.74)] after adjustment for risk factors. No OR was given for the upper quartile range. However, the ORs were given for the upper tertile range that was 1.31 (1.10–1.57) for LRI and 1.43 (1.14–1.79) for wLRI; no concentration was given for the tertile ranges. Nevertheless, the upper tertile range includes the upper quartile range and the upper part of the interquartile range. As the ORs are identical at the interquartile range and the upper tertile range, the associations between FA exposures and LRIs and wLRIs were not exposure dependent. This and the fact that much higher exposure concentrations are not expected to reach the lower airways in biologically significant concentrations cast doubt on a potential cause–effect relationship. The authors mentioned that "the duration of window opening reported by parents was inversely associated with FA levels". A biologically plausible explanation could be that the FA concentration is a proxy for the ventilation in a dwelling where an increase in FA concentration indicates an accumulation of all types of indoor air pollutants of which some may be the causal factor(s).

Overall, the two studies do not challenge the indoor air guideline set by WHO (2010).

Genotoxicity

Different susceptibility to FA due to gene polymorphisms has been addressed in several recent publications.

As FDH is the key enzyme in the FA metabolism, the *FDH* gene polymorphisms were studied in blood cells. Two polymorphisms reported in the literature did not show polymorphic sequences in the studied population. In contrast, a third polymorphism (rs13832) showed a variable allele in a group of 105 subjects. The differences did not result in different *FDH* mRNA transcription between the heterozygous and the homozygous groups. Also, the in vitro DPX formation induced by FA was similar. Overall, no relevant polymorphism in transcription of the *FDH* gene was identified (Just et al. 2011).

FA-induced DPX formation and DPX removal were studied in vitro in blood cultures from male smokers, female non-smokers and children. The DPX formation was slightly increased in children compared with the male smokers, but not with the female non-smokers. The DPX removal was slightly decreased compared with both adult groups. However, the authors considered these small differences to be biological insignificant. Additionally, they may be explained by assay variability. FA-induced DNA lesions were determined by SCE in cultivated lymphocytes, which showed no statistical difference between the groups. The expression of *FDH* gene was not different between children and the other two groups. Furthermore, the *FDH* gene expression was neither correlated with DPX nor with SCE formation. Blood glutathione *S*-transferase (GST) M1 and GSTT1 genetic polymorphisms were not related to DPX formation in FA-treated blood cultures from all subjects, but a slightly higher induction of SCE was found in cultures from subjects with homozygous deletion of the GSTM1 (null) gene; this, however, was not consistent with other studies. The authors concluded that there was no biologically relevant difference in FA-induced genetic susceptibility between the three groups (Zeller et al. 2012).

Overall, no important gene polymorphism was identified that caused increased susceptibility to FA. This is in agreement with results from blood cell studies in FA-exposed workers (see below).

Reaction with DNA in animal studies

Formaldehyde is genotoxic in multiple in vitro models and in exposed humans and laboratory animals, including formation of DPX, micronuclei (MN) (WHO 2010; Nielsen and Wolkoff 2010), DNA monoadducts and DNA–DNA cross-links (Swenberg et al. 2011). The DNA lesions showed a nonlinear increase with increasing FA concentrations (WHO 2010; Nielsen and Wolkoff 2010; Lu et al. 2011; Swenberg et al. 2011). DNA lesions can result in mutations if DNA replication takes place before they are repaired (Swenberg et al. 2011). A major progress has shown how to distinguish FA adducts generated from the endogenous metabolism and adducts generated from external exposures. External exposure of isotope labelled FA ($^{13}CD_2$) can be distinguished from internal CH_2O-FA adducts by mass spectrometry (Lu et al. 2010, 2011, 2012; Moeller et al. 2011; Swenberg et al. 2011).

Monoadducts [N^2-hydroxymethyl-deoxyguanosine (FA-dG) and N^6-hydroxymethyl-deoxyadenosine (FA-dA)] and DNA–DNA cross-links from deoxyguanosine-FA-deoxyguanosine (dG-FA-dG) cross-links were present in the rat nasal mucosa. Both exogenous and endogenous FA caused FA-dG and dG-FA-dG formation, where the dG-FA-dG adducts were about ten times lower than the FA-dG adducts. Thus, the most sensitive adduct indicator was FA-dG formation. Exogenous exposure did not induce FA-dA formation, indicating that endogenous FA caused solely additional DNA adducts compared with exogenous FA exposure. The endogenous concentrations of FA adducts were similar across the number of exposures, whereas the

Table 1 Number of monoadducts [N^2-hydroxymethyl-deoxyguanosine (FA-dG) and N^6-hydroxymethyl-deoxyadenosine (FA-dA)] and DNA–DNA cross-links from deoxyguanosine-FA-deoxyguanosine (dG-FA-dG) cross-links per 10^7 dG, dA and dG nucleosides, respectively, were studied in the male Fischer 344 rat nasal mucosa

Number of days	Concentration (ppm)	FA-dG Exogenous	FA-dG Endogenous	FA-dA Endogenous	dG-FA-dG Exogenous	dG-FA-dG Endogenous
1	10	1.3[a]	2.6[a]	4.0[a,b]	0.14[a]	0.17[a]
5	10	2.4	2.8	3.6[b)]	0.26	0.18

FA exposures were for 6 h/day for 1 or 5 days (Lu et al. 2010)

[a] The coefficient of variation was between 7 and 43 % across all adducts and all exposures

[b] Exogenous adduct was not detected in the nasal mucosa

Table 2 The monoadducts N^2-hydroxymethyl-deoxyguanosine (FA-dG) was used as a biomarker of FA-induced DNA adducts in rat nasal mucosa (Lu et al. 2011) and in the maxilloturbinates of the non-human primates (cynomolgus macaques) (Moeller et al. 2011)

Species	Time	Concentration (ppm)	Endogenous FA-dG adducts/10^7 dG	Exogenous FA-dG adducts/10^7 dG	Exogenous/endogenous FA-dG ratio	Frequency of SCC (%) in rats
Rats	6 h	0.7	3.6[a]	0.04[b]	0.01[c]	0/90 (0)
	6 h	2	6.1	0.19	0.03	0/96 (0)
	6 h	6	5.5	1.0	0.2	1/90 (1.1)
	6 h	9/10[d]	3.4	2.0	0.6	20/90 (22)
	6 h	15	4.2	11.2	2.8	69/147 (47)
Monkeys	6 h, 2 days	2	2.5[e]	0.26[f]	0.10[g]	–
	6 h, 2 days	6	2.1	0.41	0.20[g]	–

The nasal epithelial squamous cell carcinoma (SCC) frequency in male rats was from a 2-year inhalation study (Monticello et al. 1996)

[a] The coefficient of variation was in the range 13–50 % for all exposure concentrations

[b] The coefficient of variation was in the range 21–49 % for all exposure concentrations

[c] Since the endogenous FA-dG did not vary in an exposure-dependent manner, the ratio was calculated from the endogenous and the exogenous adducts in each rat

[d] Exposures in the FA-dG group was 9 ppm and in the 2-year study 10 ppm

[e] The coefficient of variation was in the range 16–26 % for all exposure concentrations

[f] The coefficient of variation was in the range 12–15 % for all exposure concentrations

[g] Ratio from the endogenous and the exogenous columns; exposures for 2 days causes a higher exogenous accumulation than exposures for 1 day

exogenous adducts increased with the number of exposures (Table 1). Additionally, the three adducts were studied in lung, liver, spleen, bone marrow, thymus and white blood cells. The endogenous FA-dG adducts were in the range from 1.1 to 3.2 adducts/10^7 dG nucleosides, the FA-dA adducts from 1.9 to 4.0/10^7 dA nucleosides and dG-FA-dG adducts from 0.09 to 0.21/10^7 dG nucleosides. However, no exogenous adducts were detected. This shows that in rats, no FA passes beyond the nasal mucosa even at a 10-ppm FA exposure. The marked increase in cell proliferation in the nasal tissue induced by exposure to 10 and 15 ppm was considered to play a critical role in converting both endogenous and exogenous labile promutagenic adducts into mutations (Lu et al. 2010). After 6 h of exposure to 10 ppm in rats, nearly half of the adducts was lost during the first 6-h post-exposure due to cytotoxicity-induced cell death and then followed by a decrease corresponding to a half-life of about 94 h (Swenberg et al. 2011).

A single 6-h FA exposure in rats was used to investigate exposure–response relationships for the FA-dG adducts (Lu et al. 2011). The relationship was nonlinear; at low exposures, the endogenous FA adducts dominated. The exogenous adduct level followed a relationship similar to the development of squamous cell carcinoma in a 2-year rat study (Table 2). Furthermore, external FA-dG adducts were not detected in the bone marrow. Adducts after inhalation of FA in monkeys can be compared with adducts in rats (Table 2). The number of exogenous adducts after one exposure in rats and two exposures in monkeys was similar at 2 ppm, but about 2.5-fold lower in primates at 6 ppm. This suggests that the nasal cancer risk from FA exposures may be lower in primates. Also in the primates, no exogenous FA adduct was detected in the bone marrow.

As in rats, this suggests that inhaled FA does not reach the bone marrow (Moeller et al. 2011).

Overall, the recent molecular dosimetry studies seriously question the biological plausibility of the epidemiological studies, suggesting that inhaled FA causes leukaemia and Hodgkin lymphoma (Swenberg et al. 2011).

Point mutations in rat nasal tissue

It was not clear whether FA-induced gene mutations are the key events in FA-induced nasal carcinogenesis. As genetic lesions on oncogenes or tumour suppressor genes are the key events in carcinogenesis in general, the rat *p*53 codon 271 CGT to CAT and the K-*Ras* codon 12 GGT to GAT mutations were studied as hotspot cancer inducing mutations and because these mutations had been observed in nasal tumours from FA-exposed rats. Rats were exposed to 0, 0.7, 2, 6, 10 and 15 ppm FA for 6 h/day, 5 days/week for 13 weeks as epithelial changes are known to occur within 1 week of FA exposures. No exposure-dependent increase was observed in *p*53 mutations and no K-*Ras* mutation was detected at all despite a strong exposure-dependent increase occurred in cell proliferation at the higher concentrations. These point mutations have the strongest association with FA-induced nasal tumours in rats, suggesting that FA is not carcinogenic through a point mutation mode-of-action and that the observed mutations occur after key events like formation of DPX, cytotoxicity, cell proliferation, and pre-neoplastic lesions (Meng et al. 2010).

Overall, this study did not find point mutations from FA exposure in rats and thus does not indicate a linear extrapolation for risk assessment.

Gene expression in rat nasal tissue

A microarray study was conducted with FA exposures at 0, 0.7, 2 and 6 ppm for 6 h/day, 5 days/week for up to 3 weeks in rats. Additionally, a single 6-h exposure to 15 ppm was included. No increase was observed in cell proliferation, histopathology and change in gene expression in rat nasal tissue at 0.7 ppm FA. At 2 ppm, cell proliferation was non-significantly increased and histopathology showed minimal inflammatory infiltration and epithelial hyperplasia; the maximum was within the first 5 days. On day 5, 15 genes had changed expression, but no gene expression was changed significantly at day 15, indicating tissue adaptation and reduced tissue sensitivity. At 6 ppm, cell proliferation was increased significantly, histopathological changes, including squamous metaplasia, were increased and major changes occurred in gene expression. Benchmark dose (BMD) analyses were performed on the day 1 gene expression, which showed the lowest BMD (1.60 ppm) for the gene expression related to the basal plasma membrane. FA at 2 ppm primarily affected the extracellular matrix or external plasma membrane proteins of the epithelium (Andersen et al. 2008).

The above-mentioned microarray study was extended (Andersen et al. 2010). Rats were exposed to 0, 0.7, 2, 6, 10 and 15 ppm 6 h/day for 1, 4 or 13 weeks. The nasal tissue was analysed by histopathology and for cell proliferation. Nasal tissue gene expression was studied at all concentrations, but gene set enrichment analysis [groups of genes that share common biological function, chromosomal location or regulation (Subramanian et al. 2005)] was limited to the three highest FA concentrations. The developed pharmacokinetic model took the endogenous FA level into account. At low dose external FA exposures, the tissue $CH_2(OH)_2$ showed a plateau. Exposures at 0.7 ppm FA had little effect on the tissue concentration of $CH_2(OH)_2$ and GSH, and 2 ppm caused only a minimal change in cellular GSH and $CH_2(OH)_2$. Exposure to several ppm FA was needed before the predicted levels would change significantly. A steeper increase in $CH_2(OH)_2$ and a more pronounced decrease in GSH occurred above 4 ppm. Treatment-related nasal lesions were found at exposure $\geq$2 ppm FA. Cell proliferation was not increased at 0.7 and 2 ppm, but increased at higher FA concentrations. At 13 weeks of exposure at $\geq$6 ppm, the most significant enriched pathways were related to cell cycle and DNA damage. Seven genes upregulated at 2 ppm after 1 week of exposure in the previous study (Andersen et al. 2008) and upregulated at 6 ppm in this study were combined in a group comprising "sensitive response genes" and analyzed by the BMD method. For all three exposure durations, this group had the lowest BMD ($\geq$1 ppm) of upregulated genes. Presumably, they represent responses to extracellular irritancy, reduction in extracellular membrane antioxidant thiols, responses to maintain reduced extracellular thiols and export of GSH to the extracellular space. The BMDs for cell-cycle control and DNA repair were in the 3–5 ppm range. It was concluded that (1) exposure–response relationships were nonlinear, (2) a threshold exists and (3) that "FA concentrations below 1 or 2 ppm would not increase risk of cancer in the nose or any other tissue or affect FA homeostasis within epithelial cells".

Genotoxicity in human nasal and buccal mucosa cells

Several studies have been conducted on the frequency of MN in the nasal and buccal mucosa cells in relation to FA exposures in humans (Speit and Schmid 2006; Nielsen and Wolkoff 2010; Knasmueller et al. 2011). Further, 21 volunteers were exposed up to 0.5 ppm with four 15-min peaks at 1 ppm, respectively; exposures were for 4 h/day over a period of 10 working days. Buccal cells did not show a statistically significant increase in MN (Speit et al.

2007). Overall, this was in agreement with the conclusion that if FA induced such an effect, it would be limited to high mean or high peak exposures (Nielsen and Wolkoff 2010).

In a Portuguese study, 50 employees from pathology and anatomy laboratories and 30 workers from a FA and FA-based resin production factory were investigated for induction of MN in exfoliated epithelial cells from the buccal mucosa; 85 non-exposed were the controls. The buccal cell MN frequency was significantly increased in factory workers (mean ± SD: 1.3 ± 1.6) and in those from the laboratories (0.6 ± 1.7) compared with the frequency in the controls (0.1 ± 0.5). Student's *t* test was used for the analyses, but it is apparent from the SDs that the distributions are skewed. This indicates that the statistical outcome should to be taken cautiously. The MN frequency in buccal cells was significantly, but weakly correlated ($r^2 = 0.04$) with the years of exposure. Mean FA exposure in the factory was 0.21 ppm (range 0.20–0.22) and the mean ceiling concentration was 0.52 ppm (0.003–1.04); since both values were based on only two samples, the data from the factory population cannot be considered robust. However, it was also reported that ceiling values at reactor sample collection and impregnation machine operation exceeded 1 ppm FA. The mean exposure in the laboratory employees was 0.28 ppm (0.05–0.51), based on 29 samples, and the mean ceiling concentration 2.52 ppm (0.02–5.02); the highest exposure was at macroscopic examinations by pathologists (Viegas et al. 2010). It is noted that both groups had exposures exceeding 1 ppm FA. Furthermore, the apparently lowest FA-exposed factory workers had the highest frequency of MN, which suggests that the exposures among the factory workers have been greatly underestimated, if the MN formation is driven by FA exposure.

In another Portuguese report, buccal cells were studied for MN formation in 56 employees exposed to FA in pathological laboratories and 85 non-exposed controls. The mean FA concentration was 0.16 ppm (0.04–0.51) and the mean ceiling concentration was 1.14 ppm (0.18–2.93). The MN in the buccal cells was significantly higher among the exposed (0.96 ± 0.28) compared with the controls (0.16 ± 0.06) (Ladeira et al. 2011). It is noted that the control groups in the Viegas et al. (2010) and the Ladeira et al. (2011) studies have the same number of males and females, have the same age distribution and the same distribution of smokers and non-smokers; this may indicates that the two studies were not independent.

In a controlled chamber study, non-smoking males were exposed 4 h/day for 5 consecutive days to FA. The FA concentrations were 0, 0.3 (with four 15-min peaks at 0.6 ppm), 0.4 (with four 15-min peaks at 0.8 ppm), 0.5 and 0.7 ppm. Induction of MN formation was studied in nasal epithelial cells up to 3 weeks post-exposure. Full-genome DNA microarray analyses were performed on nasal biopsies. The FA concentrations did not increase MN formation or cause change of expression of genes in the mucosa cells (Zeller et al. 2011a), consistent with a study in rats (Andersen et al. 2010).

The genomic results from the rat studies were supported from in vitro studies in human nasal epithelial cells (Neuss et al. 2010a). Thus, increased gene expression was exposure dependent with a no-effect-level (NOEL). Additionally, genes involved in FA detoxification showed no change in expression. Up regulation of genes at high FA exposures were similar to the up regulations seen in an inhalation study in rats (Andersen et al. 2008).

Overall, in both studies in pathology laboratory employees, MN formation in buccal cells was associated with high FA peak exposures, which agrees with the previous evaluation (Nielsen and Wolkoff 2010). Furthermore, if a low mean exposure concentration is due to series of high peak exposures, the mean level is not a representative of a mean indoor air level of FA as the indoor air FA levels are roughly constant. Therefore, mean exposure concentrations due to series of high peak exposures cannot be used for setting an indoor air guideline.

Chromosomal effects in peripheral blood lymphocytes

Human biomonitoring studies have shown increased DPX formation, SCE and MN formation in blood cell cultures. However, the human studies showed no robust exposure–response relationship. The studies included mean and peak exposure concentrations at ≥1 ppm. It was concluded that high FA levels caused the effects and overwhelmed the epithelial detoxification mechanisms, if the effects were due to FA exposures. Therefore, it was concluded that the mean and the peak concentrations have to be below 1 ppm at a set guideline. As 1 ppm is the NOAEL for histopathological effects in rats, risk characterization based on the nasal effects in rats was considered to protect against chromosomal effects in peripheral lymphocytes in humans (Nielsen and Wolkoff 2010). The National Research Council (NRC) recently acknowledged that inhaled FA has a cytogenetic effect in peripheral blood cells, but it is not possible to conclude that FA causes cytogenetic effects at distant sites and extrapolation to typical environmental exposures is difficult. It was hypothesized that the observed effect was from an unproven mechanism in portal-of-entry tissues (NRC 2011). Newer studies are listed in Table 3.

In a Hungarian study in pathology departments, 37 women exposed for 4–34 years to FA and 37 non-exposed controls were compared; 16 of the FA exposed were also exposed to solvents, whereas 21 were mainly exposed to FA. In the FA exposed, ex vivo cultivated lymphocytes

Table 3 Cytogenetic effects in peripheral blood lymphocytes in formaldehyde (FA)-exposed individuals

Exposure	Number of participants; exposed (*E*), controls (*C*) and smokers (*S*)	Exposure in years, mean (range) or as indicated	Exposure in ppm Mean (*M*) (range) or indicated Peak (*P*) (range) or indicated	Statistically significant outcome of FA exposures
Pathology departments (Jakab et al. 2010)	*E* (FA + Solvents): 16 (*S*: 6)	22 (7–34)	Both FA-exposed groups: *M*: 0.7 (0.2–1.0) (8 h TWA)	Apoptosis: increased CA: increased Aneuploidy: decreased UDS: no effect HPRT: no effect
	E (FA mainly): 21 (*S*: 5)	18 (4–34)		Apoptosis: increased CA: increased Aneuploidy: decreased UDS: no effect HPRT: decreased
	C (unexposed): 37 (*S*: 6)	Non-exposed		
Plywood industry workers (Jiang et al. 2010)	*E*: 151 (*S*: 79)	2.51 (SD: 2.0)	*M*: 0.83 (0.08–6.3) (8 h TWA)	Comet: pos MN: increased
	C: 112 (*S*: 48)		*M*: <0.008	
Pathology/anatomy laboratories (Costa et al. 2011)	*E*: 48 (*S*:10)	14 (1–31)	*M*: 0.4 (0.04–1.6) (8 h TWA)	MN: increased Comet assay showed DNA damage
	C: 50 (*S*: 7)	Non-exposed		
Histopathology laboratories (Ladeira et al. 2011)	*E*: 56 (*S*: 11)	14.5 (1–33)	*M*: 0.16 (0.04–0.51) (8 h TWA) *P*: 1.14 (0.18–2.93)	MN: increased NPB: increased NBUD: increased
	C: 85 (*S*: 25)			
Pathology/anatomy laboratories	*E*: 50	14.5 (1–33)	*M*: 0.28 (0.05–0.51) (8 h TWA) *P*: 2.52 (0.02–5-02)	MN: increased
FA and FA-based resins production (Viegas et al. 2010)	*E*: 30 *S*: 25 in the combined groups ($N = 80$)	6.2 (1–27)	*M*: 0.21 (0.20–0.22) (8 h TWA) *P*: 0.52 (0.003–1.04) Both are from 2 samples	MN: not significantly increased
	C: 85 (*S*: 26)			
Pathology departments (Santovito et al. 2011)	*E*: 20 (*S*: 0)	13 (2–27)	*M*: 0.059 (SD: 0.010) (8 h TWA)	CA: increased
	C: 16 (*S*: 0)		*M*: 0.030 (SD: 0.005) (8 h TWA)	

CA chromosomal aberrations, *Comet* comet assay and pos positive for genotoxicity , *HPRT* HPRT mutations, *MN* micronucleus, *NBUD* nuclear buds, *NPB* nucleoplasmic bridges, *SCE* sister-chromatid exchange, *TWA* time-weighted average exposure, *UDS* UV-induced unscheduled DNA repair

showed approximately doubling of apoptosis and chromosomal aberrations (CAs), which were mostly breaks of the chromatid type; both effects were statistically significant. In the group mainly exposed to FA, the half of the employees with the longest exposure (mean employment: 26 years; $N = 10$) had a CA frequency of 1.7 %, whereas the half with the shortest exposure (mean employment: 10 years; $N = 11$) had a frequency of 4.4 % that was significantly different. SCE- and UV-induced DNA-repair synthesis was similar among exposed and controls. Aneuploidy was significantly lower (approximately halved) in both FA groups compared with the controls. HPRT gene mutations were similar in controls and the FA plus solvent group, but significantly reduced in the FA mainly group. FA exposures were measured at fixed locations and thus, personal exposures may have been higher. The 8-h time-weighted average (TWA) was 0.9 mg/m^3 (range 0.23–1.21 mg/m^3) [0.7 (0.2–1.0) ppm)]. High-exposure levels were confirmed from all employees reporting eye symptoms (Jakab et al. 2010). If exposures are part of the

working day, task-specific exposures are higher. Also, peak exposures are higher than the mean concentrations.

In a Chinese study, 151 FA-exposed plywood industry workers were compared with 112 non-exposed controls from a machine manufactory. FA was determined as the 8-h time-weighted average concentration by personal sampling. The mean FA concentration was 0.83 ppm (range 0.08–6.3) in the exposed and below 0.008 ppm in the controls. Genotoxicity was studied in isolated lymphocytes by means of the comet assay and results presented as the olive tail moment (TM). The TM increased in a concentration-dependent manner and was significantly increased at ≥0.11 ppm FA compared with the controls. However, there was no exposure-dependent effect in the range 0.11–0.39 ppm. Also, genotoxicity was studied in the cytokinesis-block micronucleus assay (CBMN) in cultivated lymphocytes. The CBMN frequency increased in a concentration-dependent manner and the frequency was significantly increased at 0.28 ppm. None of the biomarkers were affected by age, current smoking or alcohol consumption. GST polymorphisms (GSTM1, GSTT1 and GSTP1) were not significantly associated with TM or CBMN frequency, although the GSTM1 null genotype had a slightly higher TM than the GSTM1 non-null genotype (3.86 vs. 3.27, $p = 0.07$), and GSTP1 (Ile/Val + Val/Val) were borderline increased compared with the GSTP1 (Ile/Ile) genotype (6.32 vs. 5.01, $p = 0.05$) in the CBMN test (Jiang et al. 2010).

In a Portuguese study, 48 FA-exposed employees in pathology and anatomy laboratories were compared with 50 non-exposed controls. Lymphocyte cultures in the FA exposed showed increased MN formation and blood lymphocytes had increased DNA damage identified by the comet assay. The 8-h mean TWA concentration was 0.43 (range 0.04–1.6) ppm (Costa et al. 2011), also suggesting that high peak exposures were present.

In an another Portuguese study by Viegas et al. (2010), which was discussed in relation to effects in buccal cells, workers from a FA and FA-based resin production factory and employees from pathology and anatomy laboratories were also investigated for induction of MN in peripheral blood lymphocyte cultures; for further discussion and limitations of the study, see the discussion in relation to buccal cells. The MN frequency in the blood lymphocytes was non-significantly increased in the factory workers (mean ± SD: 1.8 ± 2.1) and significantly increased in the laboratory employees (3.7 ± 3.9) in comparison with the controls (1.2 ± 2.0). The MN frequency in blood lymphocytes increased significantly ($r^2 = 0.16$) with years of exposure. Mean FA exposure in the factory was 0.21 ppm (range 0.20–0.22) and the mean peak exposure was 0.52 ppm (0.003–1.04), both were based only on two samples and thus not robust for establishing exposure–response relationships. The mean exposure in the laboratory employees was 0.28 ppm (0.05–0.51), based on 29 samples, and the mean peak (ceiling) concentration was 2.52 ppm (0.02–5.02).

In a third study in Portugal, peripheral lymphocytes were studied in 56 employees exposed to FA in pathological laboratories and 85 non-exposed controls by means of the cytokinesis-block MN test, comprising MN formation (biomarker of chromosomal breakage or loss), nucleoplasmic bridges (NPB; biomarkers of chromosome rearrangement, poor repair and/or telomere fusion) and nuclear buds (NBUD; biomarker of elimination of amplified DNA). The mean exposure concentration was 0.16 ppm (0.04–0.51) and the mean peak concentration 1.14 ppm (0.18–2.93). Compared with the controls, the FA-exposed employees had significantly higher occurrence of MN (4.0 ± 0.53 vs. 0.81 ± 0.17), NPB (3.0 ± 0.52 vs. 0.18 ± 0.06) and NBUD (0.98 ± 0.27 vs. 0.07 ± 0.3) (Ladeira et al. 2011). The MN frequency from the study was re-evaluated blindly by two trained and experienced scorers (2 and 3). In the original study, all slides were scored by the first author (scorer 1). Scorer 2 evaluated 32 exposed and 36 non-exposed participants and the MN frequency from 1,000 binucleated cells per participant was 9.9 and 6.8, respectively; the difference was not significant ($p = 0.17$). Scorer 3 evaluated 20 exposed and 46 non-exposed participants and the frequency was 19 and 13, respectively, $p = 0.09$. However, the combined results from scorer 2 and 3 was 13.4 and 10.2, respectively, which was significant ($p = 0.03$) and thus supported that there was a difference between exposed and non-exposed as found by scorer 1. Nevertheless, there were conspicuous differences between the absolute frequencies from the different scorers. Furthermore, when slides were evaluated twice by each scorer, the results were highly variable. Overall, it was concluded that the reproducibility was poor and reliability of the MN frequencies was limited (Speit et al. 2012).

In an Italian study, 20 pathology workers (30 % males) were compared with 16 hospital workers (44 % males). None of the participants smoked, consumed alcohol or drugs for a period of at least 1 year prior to the study. The mean (±SD) 8-h time-weighted average FA concentration was 73 ± 57 μg/m^3 (0.059 ± 0.010 ppm) and 36 ± 6.8 μg/m^3 (0.030 ± 0.005 ppm), respectively. Blood cells were cultivated 48 h and scored for CAs in 100 metaphases per subject; CAs were defined as chromatid breaks, chromosome breaks, dicentrics and chromatid exchange. The mean CA frequency per cell was significantly increased in pathologists compared with the controls (0.03 vs. 0.01). The different GST phenotype polymorphisms (GSTT positive, GSTT null, GSTM positive and GSTM null) were not associated with CA frequencies (Santovito et al. 2011). It is noted that peak exposures were not measured and no other chemical confounders were included in the study.

Effects of FA at distant to the portal-of-entry site have been proposed; FA may be transported to internal organs

where it acts or FA may affect cells in the blood. Furthermore, effect may be on cells passing through the nasal tissue or residing in the nasal tissue (Zhang et al. 2010a). The two first mechanisms are unlikely based on toxicokinetics of FA. As lymphocytes pass through the nasal tissue, FA effects were studied in an in vitro assay. Human nasal epithelial cells were exposed in vitro to high concentrations of FA, which caused DPX formation. After change of the exposure medium, cells were co-cultivated with human lymphocytes. No DPX formation was observed in the lymphocytes and no biological relevant amount of FA was released to the new medium. It was therefore considered unlikely that inhaled FA could cause genotoxic effects in blood cells as FA was not considered to pass through the epithelial barrier and reach the blood compartment (Neuss et al. 2010b). Overall, this study did not support the last of the three hypotheses by Zhang et al. (2010a).

In highly exposed (mean: 2.1 ppm and 90 percentile: 4.1 ppm FA) Chinese workers, blood progenitor cells of the myeloid line were derived by cultivation of blood cells. The cultivated progenitor cells showed increased loss (monosomy) of chromosome 7 and increase (trisomy) of chromosome 8 compared with 12 non-exposed workers (Zhang et al. 2010b); limitations in context of risk characterization have been discussed (WHO 2010; Nielsen and Wolkoff 2010; Rhomberg et al. 2011). Furthermore, a recent study did not find aneuploid effect of FA in vitro by exposure of human progenitor cells; in contrast to vincristine, a known aneugen. The authors concluded that it is unlikely that the aneuploidy in the Zhang et al. study could be caused by FA exposure (Kuehner et al. 2012).

In a controlled chamber study with non-smoking males, FA exposures were 4 h/day for 5 consecutive days. The FA concentrations were 0, 0.3 (with 4, 15-min peaks at 0.6 ppm), 0.4 (with 4, 15-min peaks at 0.8 ppm), 0.5 and 0.7 ppm. In vivo blood genotoxicity was evaluated by the comet assay, and from induction of SCE and MN in cultured lymphocytes. FA dehydrogenase expression was studied in blood cells. Full-genome DNA microarray analyses were performed on blood samples. The FA concentrations did not cause genotoxic effects in peripheral blood cells and had no effect on FA dehydrogenase gene expression. Additionally, human blood cells were stimulated in vitro for 4 h with up to 200 μM FA (6 mg/L) and studied for gene expression. The FA stimulation increased gene expression at 200 μM, but not at ≤100 μM (3 mg/L) (Zeller et al. 2011a); the normal blood FA level is 2–3 mg/L.

Overall, the controlled chamber study showed no genotoxicity in white blood cells up to 0.7 ppm FA. In the epidemiological studies where genotoxicity was observed, mean peak FA concentrations occasionally exceeded 1 ppm and peak concentrations were considerably above this level. This is in agreement with the earlier conclusion that a guideline value has to be below 1 ppm both for the mean and peak concentrations (Nielsen and Wolkoff 2010).

Portal-of-entry carcinogenicity

Nasal cancer in rats

Nasal squamous cell carcinoma in rats was considered the critical cancer effect due to FA exposure and together with the mode-of-action and it constituted the basis for the cancer risk assessment of indoor air exposures (WHO 2010). For development of SCC, Fischer 344 and Sprague–Dawley rats were more sensitive than Wistar rats. Rats were more sensitive than mice and Syrian golden hamsters. The results from four long-term studies with sensitive rat strains are combined in Table 4, showing the frequency of SCC among exposed rats. Apparently, the concentration–response relationship is nonlinear with an NOAEL for SCC at 2 ppm (Nielsen and Wolkoff 2010).

DPX levels in nasal tissue have been used as a marker of FA-induced DNA damage. At similar FA concentrations, the DPX level was higher in rats compared with monkeys (a proxy for humans), which suggests that rats is a sensitive species (Nielsen and Wolkoff 2010). Similar results have recently been seen from FA–DNA adducts (Moeller et al. 2011; Swenberg et al. 2011). This indicates that no assessment factor is needed for extrapolation from rats to humans (Nielsen and Wolkoff 2010).

In the development of SCC, cytolethality-regenerative cell proliferation plays a key role. Increase in cell proliferation was observed at ≥2 ppm FA. No histopathological effect was observed at continuous exposure to ≤1 ppm FA in the nose (WHO 2010; Nielsen and Wolkoff 2010),

Table 4 Nasal epithelial squamous cell carcinomas (SCC) in combined groups of male and female rats from four long-term inhalation studies with formaldehyde (FA) exposures

FA (ppm)	Rats with SCC/group size (% with SCC)
0	0/453 (0)
0.3	0/32 (0)
0.7	0/90 (0)
2	0/364 (0) (Apparent NOAEL)
6	3/325 (0.9)
10	20/90 (22)
14	103/232 (44)
15	120/278 (43)

In the four studies, all exposures were for 6 h/day, 5 days/week. Kerns et al. (1983) exposed Fischer rats for 24 months which was followed by 6 months of non-exposure; FA exposures: 0, 2, 6 and 14 ppm. Groups contained from 117–119 males and 114–118 females. Sellakumar et al. (1985) exposed male Sprague–Dawley rats, 99–100/group, lifelong; FA exposures: were 0 and 15. Monticello et al. (1996) exposed male Fischer 344 rats, 90–147 per group, for 24 months; FA exposures: 0, 0.7, 2, 6, 10 and 15 ppm. Kamata et al. (1997) exposed male Fischer 344 rats, 32/exposure level, for up to 28 months; FA exposures: 0, 0.3, 2 and 15 ppm

considered to be the NOAEL for cytotoxicity. This value was used as the point of departure for evaluation of FA-induced nasal cancer (WHO 2010; Nielsen and Wolkoff 2010). Also, it should be noted that there is no indication that points to a linear exposure–response relationship (Table 4). Furthermore, a nonlinear exposure–response relationship is indicated from the recent toxicokinetic studies, which showed that the FA–DNA products are driven by endogenous formed FA at low airborne exposures.

Nasal cancer in humans

That FA may cause nasopharyngeal cancer (NPC) in humans was based on a non-significant increase in the standardized mortality ratio [SMR (exact 95 % CI): 2.10 (0.91–4.14)] in the US National Cancer Institute (NCI) cohort comprising 25,619 workers employed in 10 US FA producing or using facilities; workers were employed prior to 1 January 1966 and were followed-up through 31 December 1994 (Hauptmann et al. 2004). Four metrics (average exposure, highest peak exposure, cumulative exposure and duration of exposure) were used for the study of exposure–response relationships. Relative risks (RR) were obtained with unexposed as the reference group. The average exposure intensities were divided into groups: >0 to <0.5, 0.5 to <1 and >1 ppm where RRs were not obtainable (0/3,640 deaths), 0.38 (1/1,405) and 1.67 (6/1,450), respectively. As observed in the rat studies, the trend was nonlinear and an NOAEL was established from the exposure–response relationships; the increased risk was at ≥1 ppm. However, the trend was not significant. In the peak exposure group, all deaths in the FA groups were in the highest exposure group (≥4 ppm), and the trend was significant. An exposure-dependent trend was found in the cumulative exposure groups, which was appeared to be driven by the highest exposure level.

The study has several limitations (WHO 2010; Nielsen and Wolkoff 2010; Golden 2011); the majority of FA cases were in one of the plants [SMR (95 %CI): 10 (4–22)] and with a low risk in the other nine plants [0.65 (0.08–2.33)]. Most cases in the high-risk plant had short exposures and confounder exposures from previous occupations. Also, the study missed 1006 deaths from the cohort, which may have influenced the risk estimates (Marsh et al. 2010). The two other major occupational cohorts (Coggon et al. 2003; Pinkerton et al. 2004) evaluated by the WHO (2010) had no excess in nasopharyngeal cancer. Additionally, the NCI case–control study on lymphohematopoietic malignancies in FA exposed in the funeral industry showed no excess in NPC [OR (95 % CI): 0.1 (0.01–1.2)] (Hauptmann et al. 2009).

Ignoring the limitations of the study, it was accepted by the WHO (2010) that a mean exposure level has to be below 1 ppm and the peak exposures were below 4 ppm for guideline setting. As the rat nasal histopathological effect and the nasal cell proliferation had an NOAEL at 1 ppm FA, it was accepted that a risk assessment based on nasal histopathology in rats would protects against NPC in humans (WHO 2010). Also at present, this stands as a sound conclusion.

Other portal-of-entry cancers

A recent meta-analysis, comprising six case–control studies and five cohort studies, found no association between FA exposure and laryngeal cancer [meta relative risk (mRR (95 % CI): 1.13 (0.98–1.31)] (Paget-Bailly et al. 2012).

The association between FA exposure and the incidence of lung cancer was investigated in a case–cohort study nested within a cohort of 267,400 female textile workers in Shanghai. From the period 1989–1998, the incident lung cancer cases ($N = 628$) were compared with a randomly selected subcohort ($N = 3{,}185$) from the entire cohort. Exposures were from a job-exposure matrix for textile factories with dichotomous (exposed vs. non-exposed) classification. The relative risk [hazard ratio, HR (95 % CI)] was adjusted for age and smoking. Two cases among the 628 cases had a FA exposure of ≥10 years of exposure whereas five among the 3,185 non-cases had a FA exposure of ≥10 years, with a HR of 2.1 (0.4–11). It was noted that the non-significant result is based on a small number of cases and non-cases (Checkoway et al. 2011), but the result agrees with the view that only a small fraction of inhaled FA reaches the lungs and FA is not found to cause lung cancer (WHO 2010).

An ecological study merged emission to the environment (air, land, surface water, sewage treatment plants, off-site locations for recycling and waste disposal) in the USA from the Toxics Release Inventory Database and the lung cancer incidence and survival from the Surveillance, Epidemiology and End Results Programme. After adjustment for confounders, a statistically significant association was observed between FA release and lung cancer at the county level, which was also found for chromium and nickel. However, after stratification into metro and non-metro counties, the association was only significant for non-metro counties (Luo et al. 2011). The study has several limitations that include lack of personal exposure data on FA and confounders (e.g. smoking and occupational exposures) and lack of concordance between metro and non-metro counties; thus, it cannot be used for evaluation of FA effects.

Lymphohematopoietic malignancies

Animal studies

In three drinking-water studies with 2-year FA exposure in rats, two well-conducted studies showed no increase in

lymphatic and hematopoietic (lymphohematopoietic) malignancies, whereas one study with methodological limitations showed an effect at high concentrations. Three long-term inhalation studies (≥2 years) with FA exposures up to 15 ppm for 6 h/day, 5 days/week in mice and rats neither showed a convincing increase (WHO 2010; Nielsen and Wolkoff 2010; Golden 2011; Rhomberg et al. 2011). However, one limitation was that the increased mortality in the high-exposure groups may have masked an increase in the lymphohematopoietic malignancies. Adjusting for increased mortality suggested that if an increase had occurred, it would occur at ≥14 ppm (WHO 2010; Nielsen and Wolkoff 2010; Golden 2011; IARC 2012). For risk assessment, it was apparent that a potential exposure–response relationship in rats has to be nonlinear and lymphohematopoietic malignancies to be less sensitive endpoints than SCC (WHO 2010).

Epidemiological studies

Lymphohematopoietic cancers, including leukaemia, did not increase consistently in three meta-analyses, contrasting ever vs. never exposed (WHO 2010; Nielsen and Wolkoff 2010). Opposite, a meta-analysis by Zhang et al. (2009) found increased mRR for all types of cancer combined, for all leukaemia, myeloid leukaemia and multiple myeloma, but not for Hodgkin and non-Hodgkin lymphoma. The study used one RR from each study selected in the order: peak, average intensity, cumulative exposure and exposure duration. The RR for the highest level was used from each study. The meta-analysis was updated for leukaemia [mRR (95 % CI): 1.53 (1.11–2.11)], myeloid leukaemia [2.47 (1.42–4.27)] and lymphatic leukaemia (mRR: 0.95) (Schwilk et al. 2010); studies were selected in the same hierarchically manner as in the first study except that "earlier date of hire" was added to the list. Also, where a study provided RRs for different exposure levels, the RR for the highest level was used. The updated analysis confirmed that risk was not increased if contrasting exposed vs. non-exposed (mRR: 1.07) as found in the previous meta-analyses. Thus, the differences between the analyses depend on the selection of the studies (WHO 2010; Nielsen and Wolkoff 2010; Schwilk et al. 2010). The WHO (2010) concluded that if lymphohematopoietic malignancies are due to FA, they may appear at high FA levels, which exceed the detoxification mechanisms at the portal-of-entry. This conclusion is conservative as recent toxicokinetic studies challenge that FA can reach the bone marrow or other internal organs. Furthermore, a recent comprehensive analysis of 24 cohort and 8 case–control studies for lymphohematopoietic malignancies neither found consistency across studies nor consistent exposure-dependent effects of FA (Rhomberg et al. 2011). Another recent and extensive review of 22 cohort and 17 case–control studies concluded that the relative risk of lymphohematopoietic malignancies and FA exposures was close to null, apart from some isolated exceptions; furthermore, supporting evidence for dose–response relationships was negligible (Checkoway et al. 2012).

Four studies provided information about exposure-dependent effects of FA, three major cohort studies (Coggon et al. 2003; Pinkerton et al. 2004; Freeman et al. 2009) and the NCI embalmer case–control study (Hauptmann et al., 2009). None of the three cohort studies showed an increase in standardized mortality ratio (SMR) for the different lymphohematopoietic malignancies (WHO 2010; Nielsen and Wolkoff 2010). The NCI cohort has been updated three times and none of the updates showed an increase in SMR for any of the lymphohematopoietic malignancies (Cole et al. 2010). However, Freeman et al. (2009) and the previous update by Hauptmann et al. (2003) subdivided exposures into non-exposed and three FA exposure categories within the exposure metrics, peak exposure (estimated and not measured), average intensity and cumulative exposure. The RR was obtained within each exposure metric with the lowest exposed group as the reference group (RR = 1); evaluations looked for significantly increased RRs and exposure-dependent trends. The RRs and trends for the average intensity metric were the most relevant for setting an indoor guideline value as indoor air exposures are roughly at constant levels. There was no consistent significant increase in RR and trend for all lymphohematopoietic malignancies, for non-Hodgkin's lymphoma and for multiple myeloma (Hauptmann et al. 2003; Freeman et al. 2009); data were corrected for 1,006 missing deaths in the Hauptmann et al. (2003) study. In the highest exposure group (≥1 ppm), myeloid leukaemia was increased (RR (95 % CI): 2.19 (0.9–5.3) in the Hauptmann et al. (2003) study and the Freeman et al. (2009) study [1.61 (0.76–3.39)], but none of the updates showed an exposure-dependent trend. A significant increase across studies was only observed for Hodgkin's lymphoma, where a similar risk was observed among the controls and the lowest FA group (0.1–<0.5 ppm FA) with an abrupt increase in RR (∼2–4) to a plateau level at the two higher exposure groups; the trends were significant in both updates. As Hodgkin's lymphoma has not been associated with chemical-induced cancer (known risk factors include socioeconomic status, family size and Epstein-Barr virus infection (Herbertson and Hancock 2005; Punnett et al. 2010), it was considered precautionary, if the guideline value was set below 0.5 ppm. A similar analysis was conducted on the peak exposure metric and in this case it was concluded that the peak level had to be below 2 ppm (WHO 2010; Nielsen and Wolkoff 2010).

The NCI case–control study in embalmers estimated adjusted ORs for effects of FA and lymphohematopoietic malignancies (Hauptmann et al. 2009). The main focus was on myeloid leukaemias, which included one subject with myeloid leukaemia in the reference group, individuals who had never embalmed. A more stable analysis was obtained with 5 myeloid leukaemias in the reference group (performed <500 embalmments); this analysis was not evaluated statistically and the statistical results from the first (unstable) analysis were adhered to the results of the more stable analysis. In the more stable analysis, the ORs for myeloid leukaemia showed little difference in the FA exposure groups and thus, no exposure-dependent trend was apparent (Cole et al. 2010; Golden 2011; Nielsen and Wolkoff 2010; Rhomberg et al. 2011). Furthermore, no increase in mortality was observed for all lymphohematopoietic malignancies, for all myeloid leukaemias, for acute leukaemias and for nasal cancer (Cole et al. 2010). Overall, the limitations excluded that the study being used for hazard identification and risk assessment.

In summary, the WHO (2010) guideline was below reported the effect levels of lymphohematopoietic malignancies. Taking into account that recent toxicokinetic studies support that FA does not reach the blood compartment or internal organs, including the bone marrow; this indicates that the WHO guideline is precautionary.

Reproductive and developmental toxicity

These endpoints were not discussed in the previous evaluations (WHO 2010; Nielsen and Wolkoff 2010) as indoor air relevant FA exposures were not considered to reach internal organs; later supported by recent toxicokinetic studies. The lack of the effects was supported by a review and meta-analysis (Collins et al. 2001), which was not discussed in the evaluations. However, a recent review and meta-analyses have questioned the conclusion of the first meta-analysis (Duong et al. 2011), why we evaluate these endpoints.

Humans

The review by Collins et al. (2001) found that there was no convincing evidence of reproductive or developmental toxicity in animal studies at FA exposures by routes, which were relevant for risk assessment of workplace exposure levels. Eleven human studies, nearly all comprising exposed females, were described. Eight studies were included in an overall meta-analysis, where either the male or the female individuals were exposed. In this case, there was no overall association with spontaneous abortions; the mRR was 1.4 (95 % CI 0.9–2.1). However, combining potential effects of FA on female and male reproductive organs and expressing the outcome in females may not be biologically interpretable due to differences of the sexual organs and their functions. When the analysis was limited to the 7 studies with only exposed females, the mRR was 1.6 (0.9–2.7). The studies with exposed females were further stratified into studies where exposures were self-reported [2.0 (1.4–2.8)] and obtained from work tasks [0.7 (0.5–1.0)]; self-assessment has a low reliability compared with job-exposure matrix-based assessments (Burdorf et al. 2011). This suggests that the increased risk may be due to recall bias. Neither was birth defect nor was low birth weight associated with FA exposures, but infertility was increased. However, the few studies were considered insufficient to reach robust conclusions. A single study was evaluated where only the males were exposed and the outcome studied was spontaneous abortion (mRR: 1.0 (0.8–1.4) for high exposed men) (Lindbohm et al. 1991), see below. All included studies had at least one potential confounding exposure (Collins et al. 2001). This is expected to increase the observed RR independent of the FA exposures.

An opposite conclusion was reached in a recent systematic review, including meta-analyses (Duong et al. 2011). This review describes 18 human studies, but only one was published in the period between the two reviews. This study investigated pregnancy outcome among 1,025 female laboratory technicians and 8,037 female teachers (the reference group) from the Danish National Birth Cohort (1997–2003) using a follow-up study design (Zhu et al. 2006); a job-exposure matrix was used in the exposure assessment and an exposure index was used to classify exposures into three groups: the unexposed reference group (OR = 1), the lower exposure levels and the highest exposure level. No difference was found in preterm birth (lower exposures: 1.0 (95 % CI 0.5–2.0) and highest exposure [0.7 (0.3–1.7)], small for gestational age [1.2(0.7–2.0) and 1.2 (0.6–2.2), respectively] and "major" malformations [1.2 (0.6–2.1) and 1.5 (0.8–2.9), respectively]. In contrast, work with radioimmunoassay and radiolabelling were associated with adverse pregnancy outcome, lending support to the outcomes being plausible. Overall, this study does not suggest developmental effects of FA.

The first meta-analysis by Duong and co-workers included seven studies and addressed spontaneous abortion exclusively in FA-exposed female employees. The mRR (95 % CI) was 1.76 (1.29–2.41), indicating a significant increase. However, if the mRR was obtained from studies where exposures were assessed by other than self-reports to minimize recall bias, the risk was no longer significantly increased [1.29 (0.52–3.21)]. The recent analysis was similar to the study of Collins and co-workers, except that

the highest exposure group was included where available. Further, the study by Stücker et al. (1990) in the analysis by Collins [(OR (95 % CI): 1.0 (0.5–2.0)] was replaced by a study of Saurel-Cubizolles et al. (1994) [crude OR: 1.68 (1.01–2.82)]; the latter accounted for 37.2 % of the weight in the first meta-analysis by Duong et al. (2011).

The Stücker study investigated the RR (95 % CI) of spontaneous abortion among nurses working with cytostatics, where the control group was non-exposed nurses. The exposure to cytostatics was associated with an increased risk [1.7 (1.2–2.5)]. A similar comparison after exclusion of all FA-exposed nurses resulted in a slightly higher risk estimate [2.1 (1.3–3.4)] and thus, FA exposure did not add to the spontaneous abortion risk. In the other study, the pregnancy outcome was investigated in female operating room nurses in hospitals in Paris (Saurel-Cubizolles et al. 1994); the control group conducted no operating room work. Exposure assessment was from self-reported exposure to anaesthetics (yes/no), ionizing radiation (yes/no), FA (yes/no) and antineoplastics (yes/no). Such an assessment method has low reliability (Burdorf et al. 2011). The crude OR was significantly increased for spontaneous abortion among nurses exposed to anaesthetics, ionizing radiation and FA [OR (95 % CI): 1.7 (>1.0–2.8)], which was not adjusted for the two other exposures. This value was an overestimate as it was mentioned that "the exposure during pregnancy were closely interrelated: exposures to FA and to ionizing radiation were much more common among pregnancies exposed to anaesthetics than among non-exposed pregnancies: 84 and 64 %, respectively, compared with 20 % ($p < 0.001$) and 11 % ($p < 0.001$)." Furthermore, after adjustment for confounding factors (age, number and outcome of previous pregnancies, smoking and exposure to antineoplastic drugs), the OR increased [2.6 (1.3–5.2)], if the exposure included all three risk factors (anaesthetics, FA and ionizing radiation). The OR did not increase [0.9 (0.4–1.8)] with only one or two risk factors. Thus, the crude OR from this study and its high weighting (37.2 %) in the meta-analysis will artificially increase the mRR risk; the appropriate value should be much closer to unity and thus similar to the value in the Stücker study.

The second meta-analysis by Duong et al. (2011) comprised 12 studies and it included all reproductive and developmental outcomes combined, where a single outcome was selected from each study in the order: spontaneous abortion, birth defects or malformations and low birth weight. The risk estimate from the highest exposure group was used where available. The mRR was significantly increased [1.54 (1.27–1.88)]. The analysis has several limitations, because the combination of different endpoints may not be interpretable since the effect of different endpoints may be independent (Carmines and Rajendran 2008). Furthermore, the analysis combined occupational and environmental exposures. In the three environmental studies included, the FA exposures may exceed 5 μg/m^3, but the majority of the exposures were lower. The environmental studies had ORs from 1.24 to 2.09 and their weight in the analysis was 47 %. The ORs in the occupational studies was from 0.87 to 3.5. The similar ORs with different exposure levels violate the paradigm about exposure–response relationships. Also, the FA exposure levels in the environmental studies were similar to reported levels of FA in exhaled breath. Selection within studies is problematic. For example, in the Zhu et al. (2006) study, the non-significant ORs from the highest exposure group is available from three endpoints, preterm birth (OR: 0.7), small for gestational age (OR: 1.2) and "major" malformation (OR: 1.5). In the meta-analysis, only the highest value was taken into account and the other non-significant values were disregarded. This may cause selection bias against a higher mRR. Thus, this meta-analysis is uninterpretable and cannot be used for hazard identification and setting an indoor guideline value for FA.

Overall, the results from the meta-analysis by Collins et al. (2001) and the first meta-analysis by Duong et al. (2011) are not substantially different. No significant increase is observed in studies with low recall bias. A somewhat increased mRR, that was observed in both studies, can freely be explained by the lack of confounder control as indicated from the crude OR (1.7) and the adjusted OR (0.9) in the study of Saurel-Cubizolles et al. (1994). Thus, no convincing effect of FA was observed in pregnant women in agreement with the toxicokinetic studies indicating that FA does not reach the internal organs.

The effect of FA exposure on paternal fertility has only been studied to a limited extent. A nationwide register-based study addressed chemical risk factor for spontaneous abortion in 99186 pregnancies in Finland. Both the male and the female occupation and industry were used for exposure classification, comprising "none", "potentially low" and "moderate or high". The analysis also included socioeconomic status, age and maternal exposure to reproductive toxicants. For paternal exposure, no association was observed with low FA exposure [OR (95 % CI): 1.1 (0.9–1.4)] and moderate or high exposure [1.0 (0.8–1.4)], but increased risks were associated with paternal exposure to ethylene oxide, solvents, rubber chemicals and rubber products. The study was not able to address potential confounding by alcohol consumption and smoking (Lindbohm et al. 1991). Nevertheless, it supports that no effect on male fertility is expected at the indoor guideline level.

Paternal exposure to FA and effects on reproduction was evaluated in a case–control study, where endpoints were

time to pregnancy (TTP), spontaneous abortion, preterm birth, sex ratio and major structural birth defects (Wang et al. 2012). Effects were obtained from a questionnaire presented to the males. For inclusion in the case group, 1,035 employees in the wood processing industry were screened and 302 (29.2 %) were accepted as cases. A group of 816 males, mainly salesmen and clerks, were screened for the control group; 305 (37.4 %) were accepted. Thus, both groups were highly selected. Exclusion criteria included that a couple had genital malformations or other chronic diseases; 16.7 % were excluded for participating in the case group and 7.1 % for the control group. Exclusion due to unknown TTP and other uncompleted questionnaires were 11.3 % for inclusion in the case group and 16.8 % for the control group. This suggests different health status and different recall in the groups. In the case group, 88.1 % smoked and 85.3 % smoked in the control group, that is, both groups consisted mainly of smokers. In the case group, the odds ratio (95 % CI) was significantly increased for TTP ([2.8 (1.1–7.4)]; adjusted for body mass index and alcohol consumption) and for spontaneous abortion ([1.9 (1.1–3.3)]; adjusted for smoking). FA exposure did not increase preterm birth, low birth weight, skewed sex ratio distribution and birth defects. In general, self-assessment may have a limited reliability (Burdorf et al. 2011). Limitations of the study include the high selection of the groups, an unusual high smoking prevalence in both groups, lack of reported FA levels although measured and the lack of description of the females in the two groups. For example, TTP depends on the age, the educational level, parity (first vs. second child) and smoking among the females (Burdorf et al. 2011). Most important, no confounder control was carried out for other chemicals. Overall, the study cannot be used for hazard identification of indoor air FA exposures on male reproduction.

Although the effect of FA exposure on male reproduction has been studied only to a limited extent, there is no convincing indication that it is affected. This is further supported from toxicokinetic studies, which show that FA does not reach internal organs.

Animal studies—frame of interpretation

Evaluation of reproductive and developmental effects of FA has to consider other toxicological effects (reflex bradypnea, decreased body temperature, reduced oxygen supply and reduced nasal mucociliary clearance) as well as stress-induced reactions (NRC 2011). First, high concentrations of FA induce strong eye and upper airway irritation. Thus, the concentration decreasing the respiratory rate by 50 % (RD50) in mice due to activation of a protective trigeminal reflex is considered "intolerable in humans" (Kane et al. 1979). The RD50 in mice is about 4 ppm; the respiratory rate begins to decrease already at 0.3 ppm (Chang et al. 1981; Nielsen et al. 1999). Thus, exposures in animals from 4 ppm and above induce strong eye and airway irritation and thus, exposure-dependent pain-induced stress reactions are expected. As the tidal volume up to about 4 ppm FA in mice is virtually constant, the respiratory ventilation is halved at the RD50, resulting in decreased oxygen supply in mice and presumably hypoxia-induced stress reactions. The effect on respiratory rate and respiratory ventilation were less pronounced in rats. The decrease in respiratory ventilation at repeated exposure to 2, 6 and 15 ppm was about 20, 25 and 30 %, respectively (Chang et al. 1981, Fig. 5). A decrease in the respiratory ventilation of 50 % required about 50 ppm FA (Chang et al. 1981). The pronounced decrease in minute volume in mice compared with that in rats was confirmed in a study with 15 ppm FA exposure. This was associated with a reduced steady-state CO_2 exhalation of 42 % of that in the control mice, whereas CO_2 exhalation in rats transiently decreased to 76 % of that in the control group. Thus, the decreased ventilation was associated with a decrease in metabolism. Additionally, the body temperature decreased from 37.8 to 34.7 °C in mice and from 37.3 to 36.8 °C in rats (Jaeger and Gearhart 1982). Overall, the decreased respiratory ventilation may cause hypoxia-induced stress.

Stress-induced effects can be suggested from different stress models. Restraint is used as a standard model in rodents. In pregnant mice, restraint may cause implementation failure, cleft palate, supernumerary ribs and resorption; adrenocortical hormones have been implicated in the stress-induced induction of cleft palate, but not on supernumerary ribs, and implantation failure. In pregnant rats, there is some evidence for restraint-induced implantation failure, but not for morphological anomalies (Golub et al. 2004). Long-term hypoxaemia in sheep decreased the function of the hypothalamic–pituitary–adrenal axis at the adrenal gland level in the foetuses that had a decreased ability to respond with cortisol production. The adrenals play a key role in the initiation of labour and, therefore, the adaptation may play an important role in a prevention of preterm delivery at chronic stress. In rats and sheep, hypoxia decreased the contractile response of the myometrium to oxytocin due to a decrease in the oxytocin receptor level (Ducsay 1998). Thus, in pregnant animals several mechanisms may blunt stress-induced adverse developmental outcome.

In men, stress may suppress spermatogenesis, decrease testosterone levels and change testicular blood flow. In rats and mice, stress induced by immobilization or surgery decreased testosterone and increased corticosterone levels (McGrady 1984). In general, terrestrial animal tissue exposed to prolonged fasting, hypoxia and ischaemia/reperfusion responds with oxidative stress and tissue

damage (Liu et al. 2011; Vázquez-Medina et al. 2012), which at least partly depend on the tissue antioxidant level (Vázquez-Medina et al. 2012). Thus, hypoxia due to chronic obstructive pulmonary diseases in men decreased serum testosterone levels (Aasebø et al. 1993) and caused atrophy of the testosterone producing Leydig cells (Gosney 1987). Also, unacclimatized men exposed for 4 weeks to high altitude induced hypoxia resulted in a decrease in sperm counts and mobility, and an increase in abnormal forms (Donayre et al. 1968) Hypobaric hypoxia has been shown to cause testicular degeneration in monkeys and rats (cf. Cikutovic et al. 2009). In one study, male rats were housed 7 days at 3 km altitude followed by 7 days at sea level, a cycle repeated 6 times. The control group was housed at sea level. The hypoxic rats had decreased testicular diameter of the tubules and decreased thickness of the spermatogenic epithelium compared with that in the controls. The cauda epididymis sperm concentration in the hypoxic males was decreased to 20 % of the controls (Cikutovic et al. 2009). At least partly, these effects were due to changes in the hypophysis–gonad axis as 30 days with hypobaric hypoxia in rats decreased the plasma luteinizing hormone level, which causes a decrease in Leydig cell stimulation, and thereby a decrease in the plasma testosterone level and the height of the spermatogenic epithelium (Farias et al. 2008). In rats, treatment with ascorbic acid prevented the hypobaric hypoxia-induced decrease in epididymal sperm count and increase in testicular and epididymal lipid peroxidation; this suggests that oxidative stress plays a role in these effects (Farias et al. 2010). In contrast, hypoxia induced by 12 % oxygen in the air, 8 h/day for 4 days in rats increased the plasma testosterone level and its release forms the Leydig cells (Hwang et al. 2009). However, the short-term effects are not considered as they are not relevant for the evaluation of the longer lasting studies described below. Overall, different types of stress may have profound adverse effects on male testis.

The previously conducted long-term studies (≥2 years of FA exposure for 6 h/day, 5 days/week) in male and female rats (group size about 100 animals per sex) with up to 15 ppm FA concentrations did not mention specific effects on sexual organs, but Kerns et al. (1983) mentioned no effect outside the upper airways in males and females where "all major tissues from each organ system" were investigated. Sellakumar et al. (1985) mentioned no effect on the testes, which were included in the histopathology; the study included only male rats.

Exposure of female rats

A developmental toxicity study was conducted in rats exposed to 5, 10, 20 or 40 ppm FA for 6 h/day from gestational day 6–20, and with caesarean section on day 21; each group contained 25 rats. None of the dams died, but the body weight gain was significantly decreased (halved) in the 40 ppm group. The mean implantation sites/litter, mean total foetal loss/litter, mean resorption sites/litter, mean live foetuses/litter and foetal sex ratio were similar in all groups. However, the male foetal body weight was significantly decreased in the 20- and 40-ppm groups, whereas the female foetal body weight gain decreased only in the 40-ppm group. External, visceral and skeletal examinations of the foetuses did neither reveal major abnormalities nor were there any difference in other soft-tissue and skeletal variations, such as dilated ureter, extra fourteenth ribs, rudimentary thirteenth ribs and delayed ossification of thoracic vertebrae (Saillenfait et al. 1989). Thus, a decreased body weight gain was observed in the dams at the highest exposure level (40 ppm), but no effect was observed at 20 ppm (NOAEL). A slight foetotoxic effect (reduced weight in male foetuses) was observed with an NOAEL at 10 ppm. No teratogenic effect was observed (NOAEL: 40 ppm).

Another developmental toxicity study was conducted in rats exposed to 2, 5 or 10 ppm FA for 6 h/day from gestational day 6 to 15; each group contained 25 rats. Two control groups were included: one was air exposure and in the other was maintained in the animal room throughout the study. No maternal death occurred. The maternal food consumption was significantly decreased in the 10-ppm group. Formaldehyde exposure had no effect on numbers of corpora lutea, implantation sites, live foetuses, dead foetuses and resorptions, foetal weights, sex ratios and pre- and post-implantation losses. "The incidences of reduced ossification of pubic and ischial bones in the 5 and 10 ppm treated groups were significantly increased when compared to the air control group but not the room control group. These findings were considered to be related to slightly larger litter sizes and slightly lower foetal weights in the 5 and 10 ppm treated groups" (Martin 1990). This study showed an NOAEL for maternal toxicity (reduced food consumption) at 5 ppm and no teratogenic effect at 10 ppm. The study, although only briefly reported, is in overall agreement with Saillenfait et al. (1989), except that the NOAEL for maternal toxicity appears to be somewhat lower (5 ppm).

Also, the lack of effect of FA exposures on female rat pregnancy outcome was reported in a recent study (Carmines and Rajendran 2008). Pregnant rats were exposed nose-only for 2 h/day on gestational days 6–19 to an aldehyde mixture or to air. Caesarean sections were performed on day 20; each group contained 27 dams. The aldehyde mixture contained 1 mg/m^3 FA (0.8 ppm), 41 mg/m^3 acetaldehyde (23 ppm) and 4 mg/m^3 acrolein (1.7 ppm). The mixture and thus FA did not affect maternal

body weight gain, food consumption and mean uterine weight. Neither was viable litters, live foetuses per litter, resorptions per litter, mean foetal weight nor foetal sex ratio affected. No external malformations (micrognathia/agnathia, umbilical hernia or filamentous tail) were observed. This study also investigated interactions between compounds affecting different developmental toxicity endpoints. Inhalation of nicotine decreased the pregnant rat body weight gain, but it did not affect the foetal body weight. Opposite, carbon monoxide [~30 % carboxyhemoglobin (COHb)] had no effect on the maternal body weight gain, but decreased foetal body weight; COHb may have compromised oxygen supply to the foetus. The aldehyde mixture had no effect on the maternal weight gain or the foetal body weight. Binary and ternary mixtures of these exposures showed no interaction of the exposure effects on the weights. Thus, this study suggests that developmental toxicity endpoints have to be evaluated separately.

Embryo culture studies show that FA can induce severe embryotoxicity, including death (Hansen et al. 2005). However, as such effects were not observed in inhalation studies with exposures up to 40 ppm, this indicates that FA does not reach the embryo by inhalation.

Several Russian inhalation studies with exposures from 0.01 to 1.2 ppm in female rats showed adverse reproductive and developmental outcomes (Thrasher and Kilburn 2001), however, with unusual methods. These results are inconsistent with the above-reviewed studies, which showed no teratogenic effect up to 40 ppm in spite of potential pain-induced and hypoxia-induced stress.

Exposures of male rats

Adult male rats were exposed for 8 h/day, 5 days/week for 4 and 13 weeks to 0 (controls), 12.2 and 24.2 mg/L; the concentrations are probably in mg/m^3 and thus, equal to 10 and 20 ppm, respectively, as the US NIOSH analytical method was used for calibration. Each group contained 7 rats. In the 4-week study, the body weight gain was 30 and 13 %, respectively, and the testis weights 98 and 97 %, respectively, in the low and high FA groups compared with that of the control group. The corresponding concentrations of metals in the testis were 48 and 39 % for Zn, 66 and 52 % for Cu and 117 and 130 % for Fe. In the 13-week study, the body weight gain was 62 and 37 %, respectively, and the testis weight 92 and 90 %, respectively. The respective testis content of Zn was 42 and 35 %, of Cu 60 and 32 % and of Fe 158 and 176 %. The authors concluded that the altered metal levels may induce oxidative damage of testicular tissue (Ozen et al. 2002).

In groups of rats ($N = 6$) exposed 8 h/day, 5 days/week to 0 (controls) at 5 or 10 ppm for 13 weeks, "unsteady breathing, an increase in nose cleaning, excessive licking, frequent sneezes, and haemorrhage in the nasal mucosa" were observed. However, there was no mortality in the groups. Food and water consumption were relatively decreased in the FA groups. In the low and high FA exposure groups, the serum testosterone concentration was significantly decreased to 60 and 35 %, respectively, of that in the control group. It was proposed that the decrease was due to a dysfunction of the hypothalamic–pituitary–gonadal axis. Furthermore, the mean seminiferous tubule diameter was significantly decreased to 91 and 90 %, respectively, in the FA groups. Immunohistochemistry showed exposure-dependent increase in heat shock protein 70 in spermatogonia, spermatocytes and spermatids, but without heat shock protein in Sertoli cells (Ozen et al. 2005).

In a 2-week study, male rats were exposed to 10 mg/m^3 FA (8 ppm) for 12 h/day; the control rats were exposed orally to physiological saline. Each group contained 10 rats. In the FA group, the testicular weight was significantly decreased. Histopathology showed atrophy of the seminiferous tubules, decrease in spermatogenic cells, seminiferous epithelial cell disintegration and shedding into the lumen, interstitial tissue oedema with vascular dilatation and hyperaemia. Luminal azoospermia was observed. Epididymal sperm counts were decreased as was percentage of motile sperm. Abnormal sperm counts were increased in the FA group. The testicular superoxide dismutase, glutathione peroxidase and glutathione level were significantly decreased, and the malondialdehyde level was significantly increased. A daily oral administration of 30 mg/kg/day vitamin E in the exposure period blunted the adverse effects of FA, suggesting that the effects were due to oxidative stress (Zhou et al. 2006).

In a recent study in male rats, the effects of 0 (controls), 0.5 and 2.46 mg/m^3 FA (0, 0.4 and 2 ppm, respectively) for 8 h/day for 60 consecutive days were investigated; each group comprised 10 animals. The groups showed no clinical difference. Neither was differences observed in serum testosterone concentrations, in testicular and epididymal weights, nor in epididymal tubular diameters. However, in the high-exposure group, atrophy of the testicular seminiferous tubules, decreased spermatogenic cells and oligozoospermia were observed. Epididymal lumina were also oligozoospermic. Additionally, testicular seminiferous tubular diameter, superoxide dismutase and glutathione peroxidase and epididymal sperm count were significantly decreased. Testicular malondialdehyde and epididymal percentage of abnormal sperm were significantly increased. No difference was found between the control group and the 0.5 mg/m^3 group (Zhou et al. 2011). Thus, the NOAEL was 0.4 ppm and the LOAEL 2 ppm.

Inhalation is the relevant route of exposure for setting an indoor air guideline. None of the above-mentioned

inhalation studies interpreted the FA-induced testicular effects in context of known biological effects of FA; thus, the prominent clinical symptoms apparent at 5 ppm agree with expected occurrence of severer irritation-induced stress. Also, 5 ppm decreased food and water consumption that may reasonably explain the observed decrease in body weight gain at 10 ppm. At 10 ppm, the testicular levels of Zn and Cu were decreased that may be due to one or more of the potential indirect mechanisms causing testicular damage; these include stress from irritation, hypoxia and reduced intake of food. The latter may cause insufficient supply of the metals. The increased Fe would be in line with an increase in hyperaemia in the testes, which was observed at 10 mg/m^3 (8 ppm) FA. The LOAEL (2 ppm) was a level that causes moderate sensory irritation-induced stress and hypoxia-induced stress (20 % decrease in respiratory minute volume); higher levels caused exposure-dependent increase in testicular effects. At the LOAEL, no increase is expected in FA absorption. The NOAEL was 0.4 ppm where neither sensory irritation nor decreased respiratory minute volume was observed; no effect was observed in the absence of sensory irritation, which is the case at the indoor air guideline value. Further, recent toxicokinetic studies do not support that FA reaches the sexual organs.

Evaluation of risk from indoor air formaldehyde

The WHO (2010) approach

The WHO approach used sensory irritation of the eyes and the upper airways as the critical effect. The most comprehensive controlled chamber study was used for setting the guideline value, 0.1 mg/m^3 for each 30-min period of the day. This result agreed with previous studies and further supported by a recent chamber study. The value is also to be considered a long-term guideline value as sensory irritation was not considered to increase at repeated exposures as found in a recent mouse study. In contrast, epidemiological studies were not considered sufficiently robust for setting a guideline value on sensory irritation due to insufficient confounder control. Furthermore, the guideline value was considered to protect against nasal cancer based on the analysis of the mode-of-action, the nonlinear concentration–response relationship, the occurrence of an apparent NOAEL and two risk assessment approaches. They were based on an assessment factor approach. The results from the assessment factor approaches were strongly supported by a biologically based dose–response model, which is one of the best-developed models today for any chemical, even with its uncertainties (NRC 2011). All models suggested that there was no risk of nasal cancer at the guideline level established from sensory irritation; this is supported from recent toxicokinetic studies. These studies showed that, exogenous FA-induced DNA adduct levels in the nose are marginal in comparison with adduct levels generated from endogenously formed FA at low concentrations. Lymphohematopoietic cancer was suggested from epidemiological studies. Analyses of concentration–response relationships showed that if caused by FA, effects occurred at high-exposure levels and exposure–response relationships were nonlinear, and prevention of nasal cancer would also prevent lymphohematopoietic malignancies, including leukaemia. However, recent toxicokinetic studies have shown that FA is not absorbed beyond the respiratory tract and thus, questions that FA can reach the bone marrow and cause leukaemia. Altogether, the WHO (2010) guideline value is not contradicted, but rather supported by recent studies.

The French approach

France has two indoor air guidelines (AFSSET 2007). A short-term exposure level has been set to 50 μg/m^3 over 2-h periods; it was based on a study of Pazdrak et al. (1993). A long-term exposure level was set to 10 μg/m^3, which was based on a study of Holmström et al. (1989). The values have recently been used in a study of the risk of FA exposure in the French population (Mandin et al. 2012). Additionally, France has set long-term standards for public buildings at 30 μg/m^3 from 1 January 2015 with a further reduction to 10 μg/m^3 from 1 January 2023 (Décret 2011)

In the study of Pazdrak et al. (1993), nine patients with skin hypersensitivity to FA were compared with 11 healthy volunteers in a 2-h study with an exposure at 0.5 mg/m^3 FA; all subjects were non-smokers. The study parameters were the combined nasal symptom score from sneezing, mucosal oedema, rhinorrhea and eye itching, and the nasal lavage fluid (NALF) content of eosinophils, basophils, epithelial cells, neutrophils and mononuclear cells as well as albumin and total protein content. Pre-exposure levels were used for exposure-effect assessment, which was studied up to 16-h post-exposure. The pre-exposure parameters were similar in the groups; exposure to clean air showed no effect. In both groups, the combined symptom score increased in a similar manner after the exposure (roughly 2–10 times) and decreased in a similar manner in the post-exposure period; the increase was still significant at the end of the period. In both groups, the NALF content of eosinophil counts approximately doubled after the FA challenge. The counts decreased in the post-exposure period, but they were still significantly increased at the end of the period. The NALF content of albumin and total protein followed a similar pattern as the eosinophils. The epithelial cell fraction was significantly decreased in both groups,

whereas no effect was seen on the fraction of basophils, neutrophils and mononuclear cells and the content of tryptase. The authors stated that there was no difference in NALF parameters between the groups. Additionally, it was stated that the FA exposure caused a "transient burning sensation of the eyes and the nasal passages". Recent high-quality chamber studies do not find adverse symptoms at 0.5 mg/m^3, except for odour; they might have occurred if high peak exposures were present. However, from the comparable effects in individuals with and without skin hypersensitivity (type IV) reactions, it can be concluded that type IV skin allergic individuals are not a sensitive group at FA exposures.

In a second study by the same group, 10 workers with asthma, ascribed to FA, and 10 healthy workers were exposed to 0.5 mg/m^3 FA for 2 h and followed for up to 24 h post-exposure. Clean air exposure did not induce symptoms or influenced the nasal parameters. Symptoms (sneezing, itching and nasal congestion) were similar in the two groups and highest immediately after the inhalation of FA; the scores were roughly similar to that in the first study. In both groups, leucocytes counts in NALF were similar and roughly doubled immediately after the provocation, but only significant in the healthy workers. The eosinophil counts followed a similar pattern and was significantly increased in both groups. The protein concentrations were less than doubled; significance testing was not reported. Eosinophil cationic protein and tryptase concentrations in NALF were not affected significantly. No effect of FA was observed on forced expiratory volume in 1 s (FEV_1), peak expiratory flow (PEF) and lung function at histamine challenge. None of the asthmatics has FA specific IgE antibodies. The authors interpreted the findings as a transient and non-specific reaction that was similar in asthmatics and non-asthmatics (Krakowiak et al. 1998). Neither this study described the FA generation system. However, from a risk assessment point of view, it can be concluded that subjects with asthma do not constitute a sensitive group.

The short-term French reference exposure guideline value was derived by setting the LOAEL to 500 μg/m^3 based on the first study. An assessment factor (AF) of 3 was used because the point of departure was the LOAEL. An additional AF of 3 was included for protection of sensitive individuals; neither the reason for including this AF nor its size was substantiated.

The French long-term guideline value was based on a study of Holmström et al. (1989), which investigated nasal histopathology in two groups of workers: one group was from a chemical plant and the other group comprised wood workers. From this study, the LOAEL was considered to be 0.3 mg/m^3. An AF of 3 was used for departing from the LOAEL and an AF of 10 was used for intrahuman variability (AFSSET 2007). In the chemical plant workers, the median FA concentration was 0.3 mg/m^3 with frequent peak exposures above 1 mg/m^3. In the furniture industry, the FA concentration was in the range 0.2–0.3 mg/m^3 in general and seldom exceeded 0.5 mg/m^3; wood dust exposures were up to 4.9 mg/m^3. The mean concentration in the control group was 0.09 mg/m^3 FA. The mean nasal biopsy score was 1.56 (range 0–4) in the controls, 2.07 (range 0–6) in the furniture workers (not statistically significant) and 2.16 (range 0–4) in the chemical plant (statistically significant); the grading scores were from 0 to 8. The FA levels were divided into exposure levels for current exposure and cumulative exposure; they showed no exposure-dependent trend. The limitations, including high peak exposures, confounding by wood dust and lack of exposure-dependent effects, hinder that the study can be used for deriving a guideline value (WHO 2010). Recently, the National Research Council reached a similar conclusion based on a comprehensive evaluation (NRC 2011).

Overall, the French guideline values cannot be considered reliable alternatives to the WHO (2010) guideline value.

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