

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT. (COM)**Formaldehyde: Evidence for systemic mutagenicity.****Introduction**

1. Formaldehyde has recently been considered by IARC and placed into group 1 (carcinogenic to humans).¹ There was sufficient evidence that formaldehyde causes nasopharyngeal cancer in humans. There was limited evidence that formaldehyde causes sinonasal cancer in humans. The working group concluded that there ‘*is strong but not sufficient evidence for a causal association between leukaemia and occupational exposure to formaldehyde. Increased risk for leukaemia has consistently been observed in studies of professional workers and in two of three of the most relevant studies of industrial workers. These findings fall slightly short of being fully persuasive because of some limitations in the findings from cohorts of industrial and garment workers in the USA and because they conflict with the non-positive findings from the British cohort of industrial workers.*’ This conclusion has stimulated some discussion in the published literature regarding the possible mechanism by which formaldehyde could induce leukaemia and some critical comments that inhaled formaldehyde does not result in any systemic delivery of the compound and hence no potential for systemic mutagenicity.² (See appended references at Annex 1 IARC review and Heck review reference 2) *The IARC working group commented on the possible mechanism and noted evidence for clastogenic damage to circulatory cells, but overall since there were no good animal models for acute myeloid leukaemia, did not reach a conclusion with regard to the mechanism of acute myeloid leukaemia reported in epidemiological studies.*¹

2 The objective of this discussion paper is to present and evaluate the evidence for systemic mutagenicity in animal experiments and biomonitoring studies of workers exposed to formaldehyde. Information on mutagenicity post dating the IARC working group will be considered. A key part of the discussion will refer to kinetics and metabolism of adsorbed formaldehyde. A very brief overview of the mode of action and in particular the involvement of mutagenicity in nasopharyngeal cancer is presented as background information, taken predominantly from the WHO CICAD review (Concise International Chemical Assessment Document 40³) and a draft (in –confidence) discussion paper on formaldehyde induced nasal tumours using the IPCS proposed approach for human relevancy assessment of mode of action for humans (subsequently published in full in Critical Reviews in Toxicology Nov-Dec, 36 (10) 803-819, 2006).⁴ A second objective is to consider the genotoxicity biomonitoring data on formaldehyde in the light of the COM evaluation of risk factors for background variance in MN and CAs in peripheral blood lymphocytes in biomonitoring studies.⁵ The final topic discussed in this paper concerns whether the default approach of COM, to assume no threshold would apply to systemic formaldehyde resulting from occupational or environmental exposure. The closest analogous chemical previously reviewed by the COM is acetaldehyde formed from consumption of ethanol. The conclusions reached by COM in 2000 are given below for information.

Mutagenicity of acetaldehyde

The committee agreed that the most recent experiments using human lymphoma

cells had confirmed earlier studies that acetaldehyde induces protein-DNA cross links, but only at concentrations which resulted in cell death. In addition acetaldehyde induced HPRT mutations in human T cells. Members agreed that no conclusions could be drawn from the finding of acetaldehyde DNA adducts in peripheral white blood cells of alcoholics in view of lack of control for the effects of smoking by alcoholics in the study group and the well known abnormalities in metabolism in alcoholics.

The Committee reaffirmed its previous conclusions with regard to acetaldehyde. The available data show that acetaldehyde includes chromosome aberrations in mammalian cells in the absence of an exogenous metabolising fraction. There is some evidence to show that covalent binding (DNA-protein cross links) in the nasal mucosa of rats exposed to high levels of acetaldehyde by inhalation.

The mutagenic profile of acetaldehyde is very similar to that of formaldehyde. The compound has direct acting mutagenic potential *in-vitro*, but would only be expected to have the potential of *in vivo* activity at sites where it is not rapidly metabolised to acetic acid. The COC has concluded that the observation of tumours in animals exposed to high inhalation doses of acetaldehyde is not relevant to drinking alcohol.

Background

Exposure

4. In brief as summarised predominantly from the CICAD review, formaldehyde is a normal product of intermediary metabolism. Endogenous concentrations in blood are estimated to be approximately 0.1 mM.² It is also widely dispersed in the environment following release through natural combustion processes and through human activity (e.g. automotive and in environmental tobacco smoke). Formaldehyde has a wide number of industrial uses (e.g. production of resins, glues and in consumer products). Most formaldehyde released to the environment is rapidly degraded and human exposure is most likely to occur when there is a continuous source of exposure. The following reported levels of exposure are for member's guidance and background information and are not meant as definitive data as there is considerable variance in exposure levels. In general mean outdoor exposure levels are around 3 µg/m³, indoor air levels around 30 µg/m³. Occupational exposure levels can be higher with most data reporting 8h-TWA levels of around 1.2 mg/m³ (ca 1 ppm). Some higher levels have been reported for a number of activities, e.g. Disinfection of brooding houses and occasional transient peaks (in anatomy and mortuary laboratories). There are comparatively few data reported in the CICAD document with regard to water and food borne sources.

Mutagenicity and MOA of rat nasal carcinogenicity

5. With regard to *in-vitro* mutagenicity testing, formaldehyde is a direct acting *in-vitro* mutagen in bacteria and mammalian cells (including rodents and human cell lines). Mutagenic effects reported include point mutations, CAs, SCEs, DNA strand breaks and UDS in rat nasoturbinate cells.¹⁻⁴

6. An increased incidence of squamous cell carcinoma of rat nasal turbinate epithelium has been documented in a number of long term inhalation carcinogenicity bioassays. A non linear dose response has been identified with a LOAEL of around 7.2 mg/m³. No systemic target organ tumours were identified in these studies up to 17-18 mg/m³. No increases in tumours were seen in a long term inhalation study in mice reviewed by the IARC but it is noted that other reviewers have cited positive

results in inhalation studies in mice (this aspect is not considered further in this discussion paper). It is noted that there is one oral drinking water study where an increase in haematopoietic tumours in Sprague-Dawley rats was reported, but this wasn't documented in a separate oral carcinogenicity bioassay in Wistar rats.

7. Regarding the proposed MOA for rat nasal tumours, most reviewers have considered the occurrence of formaldehyde induced DNA-protein cross links (DPX) with a similar dose-response to the formation of nasal tumours in rats, with consequent marked local effects on cytotoxicity, cell proliferation and local site of contact mutagenic effects as a key part of the MOA. Reviewers emphasise the magnitude of formaldehyde induced local site cell proliferation.^{3,4}

The kinetics of absorbed formaldehyde.

8. All of the available published data cited in this discussion paper refer to inhalation exposure. Heck H d'A and Casanova M (2004) reviewed the available toxicokinetic and metabolism studies (appended in Annex 1).² Briefly, studies in rats and Rhesus monkeys show the majority of inhaled material (>90%) is incorporated into intermediary metabolism at the site of contact (FA metabolism includes saturable conversion of formaldehyde to formate and into the one carbon pool for incorporation into DNA, RNA and proteins. Other routes include oxidation to carbon dioxide and water and rapid excretion via urine and exhalation). A number of non saturable pathways result in the remaining material being bound predominantly to proteins and a small amount to DNA. Studies in rats using inhalation of 6ppm for 6h resulted in 91% metabolic incorporation and 9% bound as DPX. In the Rhesus monkey, exposure to 6 ppm for 6 h resulted in 96% metabolic incorporation and 4% covalently bound as DPX. Inhalation studies in rats and Rhesus monkeys (6 ppm for 6h/day, for 5days/week for 4 weeks, with blood drawn 7 mins and 45 h post last exposure¹⁴) showed no increase in blood formaldehyde concentrations. In a study using human volunteers, inhalation of 1.9 ppm for 40 min resulted in an increase in blood levels for 3 volunteers and a decrease for the remaining three volunteers. The authors noted that there had been no control for other potential external sources of formaldehyde and these data might reflect background variance.

9. Casanova and colleagues undertook some studies to investigate the metabolic incorporation and covalent binding of formaldehyde in male F344 rat nasal mucosa and bone marrow using a mixture of ³H- or ¹⁴C- formaldehyde at up to 15 ppm total formaldehyde for 6 h, 1 day following a pre-exposure to up to 15 ppm unlabelled formaldehyde (nose only exposure).¹⁵ An increase in the ratio of ³H compared to ¹⁴C formaldehyde indicated an increase in covalent binding compared to metabolic incorporation. There was a non linear increase (of 10.5x) in covalent binding to rat nasal DNA from an exposure of 2 ppm to and exposure to 6 ppm. There was no evidence of covalent binding to olfactory mucosa or to bone marrow.¹⁵ In a subsequent experiment, the same research group exposed male F344 rats to a mixture of ³H- or ¹⁴C- formaldehyde at up to 10 ppm for 3h, 1 day after a pre-exposure to unlabelled formaldehyde (nose only exposure).¹⁶ Two hours prior to the exposure to labelled formaldehyde, the animals were given an i.p injection of phorone (17% in corn oil at 300 mg/kg bw) to deplete nonprotein sulphhydryl levels. This latter treatment reduced nonprotein sulphhydryls in nasal respiratory mucosa to 10% of control animals. This resulted in a significant increase in DNA-protein cross linking in rat nasal tissue at all exposure concentrations. Metabolic incorporation into rat nasal, olfactory tissue and bone marrow were significantly decreased. Covalent binding to macromolecules was not detected in the bone marrow.¹⁶

10. Thus there is no evidence from radiolabel studies of DNA adduction in the bone marrow of rats exposed by inhalation to relatively high concentrations of formaldehyde. There is evidence for local site DNA adduction from such studies.

11. Overton and colleagues published a dosimetry model of inhalation of formaldehyde in humans and reported that 95% of inhaled formaldehyde would be retained by the respiratory tract and thus presumably the maximum amount available for the systemic circulation assuming there was no site of contact binding to proteins etc would be 5%.⁵ Heck and colleagues calculated that the maximum potential blood formaldehyde concentration in an adult man exposed to 2 ppm for 8 h would be 0.001 mM compared to an endogenous blood concentration of 0.1 mM.² The calculation assumed 7% of inhaled material would be available to the systemic circulation but did not take into account potential reductions in the amount potentially available systemically due to loss via non-saturable mechanisms such as protein binding. More recently Franks has published a model of formaldehyde absorption and metabolism. Using an exposure concentration of 1.9 ppm (just below the Occupational Exposure Limit) it was calculated that maximum blood concentrations of free formaldehyde would be rapidly attained in a few seconds and were predicted to be approximately 0.00044 mg/l. (This equates to just below 0.0004 ppm and is a little lower than the estimated blood levels reported by Heck and colleagues for exposure to 2 ppm formaldehyde²)

12. Overall these data are consistent with the view that inhalation exposure (e.g. occupational and environmental) at levels around the OEL of 2ppm results in systemic blood levels of formaldehyde which are about or most likely to be below 0.1% of the endogenously formed blood concentrations of formaldehyde. This evaluation could be modified to some extent by taking full account of the free to bound ratio of formaldehyde arising from different sources of exposure.

13. The question for COM is whether such a small transfer of exposed formaldehyde to systemically available as free formaldehyde could have any significant biological implications for systemic mutagenicity.

Evaluation of mutagenicity and biomonitoring data

14. The IARC summary evaluation did not reach a conclusion regarding the mechanism for the reported increase in incidence of leukaemia among occupational cohorts but did note the observation of clastogenicity damage to circulatory stem cells. The COM are asked for their views on the available published literature and for comments on the mechanism regarding any positive mutagenicity data.

In-vivo FA studies in experimental animals (Summaries of all studies Annex 2, Appended selected studies Annex 4)

Test materials

15. The available studies are summarised in Annex 2. One generic problem in assessing these studies is that many authors have not commented on the stability of the dosing solution. Formaldehyde is intrinsically reactive and is commercially available as aqueous solutions of 30-50% where methanol has been added (ca 10%) to reduce polymerisation. Alternatively paraformaldehyde may be hydrolysed with sodium hydroxide but information on the dosing solution is often not provided. Formaldehyde solutions left to stand may be oxidised to formic acid and on standing at a low temperature a precipitate of trioxymethylene may be formed.

Bone-marrow studies

16. A number of published *in-vivo* mutagenicity studies of bone-marrow using mice and rats with *i.p.* and inhalation dosing are available. Gocke E et al (1981) reported that *i.p.* dosing of male and female NMRI mice with 10, 20 or 30 mg/kg FA did not result in an increase in BM MN PCEs 30 h post dosing.⁷ No increase in BM MN PCEs was reported in CBA mice given two *i.p.* doses of 6.25, 12.5 or 25 mg/kg bw FA separated by 24 hours with sampling at 16 and 40 hours post dose. The authors also undertook metaphase analysis of the bone marrow and spleen cells examining 100 cells/animal. No evidence for a clastogenic effect of FA was reported.

17. No increase in CAs in PBLs from whole blood cultures of blood taken by cardiac puncture from male and female F-344 rats exposed to up to 15 ppm FA for 6h/day, for 5 days was reported. Metaphase analysis was undertaken on 50 cells/animal.⁹ Negative results were also reported in an inhalation study of BM CAs in Sprague-Dawley (50 cells/animal) following exposure to up to 15 ppm FA for 6 hours/day, for 5 days/week for 1 or 8 weeks. However a clear clastogenic effect was reported in lung lavage cells from the same animals.

Germ cell studies

18. In contrast to the studies of bone-marrow in rats and mice, evidence for dominant lethal effects were reported in one study in mice and one study in rats. Thus a single *i.p.* dose of FA (50 mg/kg bw) of male Q strain mice with separate matings each week for seven weeks resulted in increased embryonic death in the 1st and 3rd weeks. There was no evidence for a clastogenic effect in spermatocytes in this study. In a separate investigation an increase in the number of abnormal spermatozoa and evidence for a dominant lethal effect was reported in a study using isogenic University of Lagos rats given *i.p.* doses of 0.125-0.5 mg/kg bw of FA. These doses were reported to be 1/16 to 1/4 of the *i.p.* LD50 and are very much lower than the dose levels used in other mutagenicity studies with FA. The Dominant lethal study examined matings 1-7, 8-14 and 15-21 post dosing. A significant increase in the number of dead implants was reported in period 1-7 post dose which was accompanied by a reduction in sperm counts and abnormal spermatozoa.¹²

Comet assay of PBLs and liver cells.

19. An *in-vivo* Comet assay was undertaken using PBLs and isolated hepatocytes from rats exposed to 5 ppm or 10 ppm for 6 h/day for 5 days/wk for 2 weeks. An increase in tail moment was reported following examination of 50-100 cells/animal. The authors also reported that dose levels resulted in disturbances of protein and lipid oxidation (through evaluation of malondialdehyde and proteomic analysis) in PBLs and liver cells.¹³

Other site of contact in-vivo mutagenicity studies

20. Clear evidence for an increase in micronucleated cells of the basal epithelium of the stomach and duodenum was documented in a study where an oral dose of 200 mg/kg bw FA was given to Sprague-Dawley rats. There was no concurrent blood sampling in this study. A positive response was documented in the duodenum using MNNG as a positive control chemical.¹¹

Discussion: In-vivo studies with FA

21. Formaldehyde is an in-vivo site of contact mutagen. There are several negative bone-marrow mutagenicity studies available in rats and mice where i.p. dose levels used have been sufficient to induce signs of toxicity but there are no blood measurements for absorbed FA. Most of these studies did not include a positive control chemical. It can be argued that Gocke et al did report positive results with some other chemicals tested using the same protocol at the same laboratory at probably around the same time, e.g hydroquinone and pyrogallol. Dallas et al (1992)¹⁰ didn't include a positive control chemical but did undertake lung lavage to confirm the site of contact mutagenic activity of FA. Natarajan et al (1983) included sampling of two systemic tissues (bone-marrow and spleen) using multiple i.p. dose levels and two sampling times. Members are asked whether this is sufficient evidence for a negative in-vivo systemic tissue evaluation without additional kinetic studies given the likely rapid absorption and elimination of FA. A number of key in-vivo studies in experimental animals are appended as Annex 5.

22. Positive results were reported in the germ cell assays and in one Comet assay using two systemic tissues. Members are asked for their views on the significance of these findings?

Biomonitoring studies of FA exposure (Summaries of all studies Annex 3, selected studies Annex 5)

23. The relevant studies have been summarised in Annex 3 in order of publication. The review has focused on studies of CAs and MN formation, with little weight placed on reporting studies of SCE formation. A short overview of each study is given in the table below along with a proposed conclusion regarding the outcome of each study. The primary aim is to consider whether the occupational biomonitoring studies provide convincing evidence for a systemic mutagenic effects in PBLs. Data on site of contact effects have been reported where these provide a useful comparison. Non of the studies included some of the essential conclusions reached by COM on biomonitoring studies (e.g. the need to evaluate individual scorer performance). A number of the studies have included information on potential risk factors for CA and MN formation in PBLs, but none specifically measured vitamin B₁₂ status. It is known that these factors may account for the small 1-2 fold increases in MN in PBLs seen in biomonitoring studies

24. Thus since the amount of formaldehyde reaching the systemic circulation following occupational exposure is likely to be only a small fraction of that continuously formed endogenously, the most convincing evidence is likely to come from biomonitoring studies of genotoxicity where there is good assessment of cumulative exposure to formaldehyde with sufficient numbers of individuals evaluated at each exposure level to discern a potential dose response.

Biomonitoring Studies reporting positive results in Bone marrow or PBLs

Study	Main findings	Proposed conclusion
Goh K et al ¹⁷ Bone marrow smears from Dialysis patients with estimated exposure to FA following sterilisation of equipment. FA exposure estimated.	Variation in diploid number in dialysis patients with potential exposure to FA. Uncertainties include difficulties in consistently preparing bone marrow smears and influence of a range of disease conditions on metaphase analysis. No data on FA exposures documented	No conclusions relating to FA can be drawn.

<p>Bauchinger M and Schmid E²⁰ G-banding study of PBLs from paper workers. Exposure for up to 90 mins/shift. 8h TWA data not reported. Peak levels up to 3 ppm reported.</p>	<p>Increase in dicentrics and ring chromosomes in exposed paper workers. No exposure related effects documented. Noted that supervisors in exposure group were older than controls. Relatively little data on potential confounding reported</p>	<p>Inconclusive data from a small study.</p>
<p>Suruda et al²¹ Mortuary science students examined prior to and at end of 9 week course. Buccal, nasal epithelial and PBLs evaluated for MN formation. Average exposure during embalmings was 1.4 ppm (8 h TWA was 0.33 ppm).</p>	<p>Small statistically significant increase in MN formation in PBLs (28%). A cumulative exposure-MN formation association was not documented. A 22 fold increase in buccal MN formation which was related to cumulative exposure in males was documented.</p>	<p>No convincing evidence for a FA induced genotoxic effect on PBLs.</p>
<p>Shaham J et al²² Study of DPX in PBLs from pathology workers. Personal sampling showed FFA levels of up to 3 ppm during work activities. 8TWA data not reported.</p>	<p>A small but reported statistically significant increase in DPX in PBLs in a small study. Relatively little data on factors affecting the identification and measurement of DPX reported.</p>	<p>Inconclusive data from a small study reporting a very small change in DPX with exposure. Insufficient information to ascertain accumulation of DPX with cumulative exposure.</p>
<p>Shaham J et al²⁶ Study of DPX in PBLs from pathology workers (including lab techs, physicians and hospital orderlies). Static sampling reported FA levels during work activities to vary between 0.4 ppm for lab assistants up to 2.2 ppm for hospital orderlies. 8 h TWA not reported.</p>	<p>A small but statistically significant increase in DPX in PBLs from a much larger study of pathology workers. The increase did not reach statistical significance with regard to level and duration of FA exposure. Relatively little data on factors affecting the identification and measurement of DPX reported.</p>	<p>Inconclusive data from study reporting increase in DPX in PBLs in workers exposed to FA but no exposure or cumulative exposure relationship could be established.</p>
<p>Orsiere T et al²⁸ Study of pathology and anatomy lab workers. DNA damage and CBMN in PBLs assessed. The 8h TWA was 0.1 ppm (<0.1-0.7ppm).</p>	<p>Statistically significant increases in CBMN and in particular in centromere positive BNMNs. Authors report. Correlation with exposure not statistically significant after adjustment for age. CBMN data did not correlate with data for DNA damage.</p>	<p>Inconclusive data from study investigating CBMN and centromere positive CBMN in PBLs as no clear exposure and duration of exposure relationship was demonstrated. Noted 8 h TWA exposure was relatively low.</p>

25. The table above summarises those studies where positives in PBLs have been documented. There are other biomonitoring studies which have documented negative results in PBLs for genotoxicity. Many of these (as summarised in Annex 3) are relatively small and do not adequately investigate potential risk factors for CAs or MN formation in PBLs and in some instances used 72h cultures of PBLs. (e.g references 18,18,23). The investigation by Cheng-Jing and colleagues are of interest in that the authors studied MN formation both buccal/nasal tissue as well as in PBLs in anatomy students with samples take before the commencement of a course and at the end of a course. 8h-TWA exposures in this study were below 1 ppm. The investigations of relatively small numbers of individuals by these authors have the same limitations identified above for other negative PBLs biomonitoring studies of genotoxicity in formaldehyde exposed workers but do report clear statistically significant increases in site of contact MN formation in nasal tissue.²⁴

26. Recently Schmid and Speit²⁹ investigated the LOEL for DPX formation in PBLs in culture and the LOELs for subsequent conversion of DPX to SCEs and MNs. They found that DPX could be identified at exposure concentrations of 25µM and above, but that up to 100 µM, such DPXs are completely removed. The LOELs for SCE and MN induction were >100 µM and >250 µM respectively. These authors consider that systemic cytogenetic effects of FA are very unlikely to occur in FA exposed subjects.

27. A number of the relatively recent biomonitoring and associated studies are appended as Annex 5. Some recent studies on dosimetry and stability of DPXs from FA are appended as Annex 6.

COM discussion and conclusions

28. The COM is asked to advice on the potential for formaldehyde to be a systemic in-vivo mutagen. The in-vitro mutagenic activity of formaldehyde (direct acting through DPX) is generally accepted.

29. Do members agree that the available toxicokinetic and metabolism data from animals and trials using human volunteers suggest that at exposures up to the maximum attained under occupational circumstances there is exceedingly low levels of free systemic formaldehyde following inhalation exposure, equating to a maximum of 0.1% of endogenously formed formaldehyde?

30. The available toxicokinetic and metabolism data suggest that the risk of any systemic toxic effects including mutagenic effects of absorbed formaldehyde would be very low.

31. The available in-vivo animal studies using high parenteral doses of formaldehyde do not suggest any mutagenic effects in the bone marrow.

32. Do members agree that the available positive studies in male rodent dominant lethal studies and a comet assay using PBLs and liver cells from rats do not raise any concerns or should these data suggest further work?. Do members consider that the weight of evidence from kinetic studies and biomonitoring studies suggest that there is no need for further evaluation of the positive animal data?

33. Do members consider that the available biomonitoring studies of genotoxicity in PBLs do not give rise to concerns for systemic mutagenicity of formaldehyde and that any documented positive findings do not require further

evaluation in view of the weight of evidence indicating a lack of systemic exposure and subsequent mutagenicity of formaldehyde.?

34. Do members agree that the evidence is consistent with a threshold for systemic mutagenicity following inhalation exposure?

**Secretariat
January 2007**

**Annex 1 (IARC summary and review by Heck on ADME of FA.
Annex 2 Summaries of in-vivo mutagenicity studies in animals.
Annex 3 Summaries of Biomonitoring studies
Annex 4 Selected in-vivo studies in animals.
Annex 5 selected biomonitoring studies
Annex 6 Studies and commentaries on FA interaction with DNA and removal of DPX.**

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Annex 2 to MUT/07/01

**In-vivo mutagenicity studies in experimental animals with formaldehyde
(Summarised in publication date order.)**

Reference	Results	Comment
<p>6. Fontignie-Houbrechts N (1981) Mutation Research, 88, 109-114.</p> <p>Groups of 2 male mice (3 month, Q strain) were given an i.p. dose of 50 mg/kg FA (35% solution in acetate buffer pH 4.8). Mice were killed at 8-15 days post treatment and 200 diakinesis-Metaphase 1 spermatocytes/animal examined for translocations, fragments, XY or autosomal univalents and for polyploidy. 10 males were caged with 2 virgin females each for 1 week for up to 7 weeks post treatment. Females were examined for presence of vaginal plug. 14 days after detection of the plug, the females were killed and the number of corpora lutea, and of living dead embryos scored.</p> <p>No concurrent positive control.</p>	<p>No significant increase in chromosomal lesions was reported. For controls the percentage of metaphases with XY univalents was 25.96%, autosomal univalents (2.10%), polyploidy (1.04%) and fragments (0.36%). For treated animals these data varied over days 8-15 as follows; XY (10.0-23.9%), autosomal (1.00-7.38%), Polyploidy (0.74%-3.25%) and fragments (0%-0.75%).</p> <p>The authors did not report an effect on the number of pregnant females in the dominant lethal test. An increase in embryonic mortality in the first week was due to both pre and post implantation deaths. (cf post implantation deaths 14 (0.8/female) compared to 12 (0.2/female in controls), pre implantation 33 (1.8/female) compared to 51 (0.9/female). In week 3 preimplantation deaths were 17 (1.7/female).</p>	<p>Authors considered that a clear positive had not been demonstrated as the dose used was very high (although not lethal). Authors noted previous studies (Shafner 1968, Epstein 1972) using FA in Swiss CD-1 or ICR/HA Swiss mice using doses of 16-40 mg/kg/bw (LD5-LD25) did not increase early fetal deaths or pre implantation losses.</p>
<p>7. Gocke E et al. (1981). Mutation Research, 90, 91-109. Groups of 4 NMRI mice (2 of each sex) were given an intraperitoneal dose of 0, 10, 20, 30 mg/KG bw FA in Hanks balanced salt solution. Bone marrow smears were made 30 hours post dose and 1000 polychromatic erythrocytes scored for MN/animal. positive</p>	<p>One animal died in each dose group at 10 and 20 mg/kg bw.</p> <p>There was no increase in MN PEs.</p> <p>Cf 1.0‰ in controls, 2.3 at 10, 1.7 at 20 and 2.3 at 30. All values within concurrent control tests with other chemicals reported in the publication.</p>	<p>Clear effect of hydroquinone, and pyrogallol reported in the publication.</p>

No concurrent control.		
<p>8. Natarajan AT et al (1983). Mutation Research, 122, 355-360. Paraformaldehyde was dissolved in distilled water, NaOH added and the pH adjusted to 7.2. Male and female CBA mice (1-12 weeks old) were given i.p injections of FA 6.25, 12.5 or 25.0 mg/kg at 0 and 24 h. Cells were sampled 16 and 40 hour after the second dose. Colcemid was injected 2hours before sampling. Bone marrow cells were processed for MN (1000 PCEs) and CA analysis (100 metaphases/animal). Spleen cells were obtained for CA analysis (100 metaphases/animal). No concurrent positive control.</p>	<p>There was no increase in PCE with MN or in CAs in bone marrow or spleen. Data are appended at the end of this discussion paper.</p>	<p>Authors recommended that repeat dose studies were required to draw a definite negative conclusion with regard to the potential for in-vivo mutagenicity of FA.</p>
<p>9. Kligerman AD et al (1984). Toxicology Letters, 21, 241-246. Groups of 6 (3 of each sex) CDF (F-344)/CrIBR rats were exposed to FA for 6h/day for 5 days to 0.5, 6 or 15 ppm FA. Blood was removed within 1 h after the termination of exposures by cardiac puncture. Whole blood cultures were undertaken for 54 (4 uM BrdU was added at 20 h). 1000 nuclei scored for mitotic activity, 100 metaphases scored for cell cycle kinetics. 50 metaphases from the high dose were scored for CAs. No concurrent positive control.</p>	<p>No effects on CAs (% of cells with one or more chromosome aberration) in peripheral blood lymphocytes was reported ca 2.0±1.3% in controls, 2.0 ±2.3 (n=4) in treated. No effects on MI reported.</p> <p>(In controls 1 dicentric, 3 chromatid deletions, 1 paired fragment, 1 isochromatid break. In 15 ppm 2 chromatid deletions, 2 paired fragments.)</p>	<p>(the authors noted that 15 ppm was reported to be carcinogenic in inhalation studies in rats) and that a repeat exposure for 5 days to 15 ppm resulted in significant local site effects (rhinitis, mucostasis, ciliastasis)</p>
<p>10. Dallas CE et al (1992). J of Applied Toxicology, 12, 199-203. Groups of 4 or 5 male Sprague-Dawley rats were exposed to 0,</p>	<p>There was no overall increase in CAs reported in FA exposed animals (combined chromatid breaks, chromosome</p>	<p>A significant increase in CAs was reported in lung lavage fluid from high dose animals after 1 or 8 weeks exposure.</p>

<p>0.5, 3 or 15 ppm for 6 h/day, 5 days per week for 1 or 8 weeks. Colchicine was dosed i.p (1 mg/kg bw) 2 h prior to sacrifice. Cells were obtained by lung lavage and bone marrow cells from excised tibia. Cells were treated hypotonically (0.075 M KCL), fixed and stained with Giemsa. 50 cells were scored per animal. Kruskal-Wallis statistic used to evaluate 3 or more groups and Mann-Whitney for pair wise comparisons. No concurrent positive control. Authors refer to previous studies with benzene.</p>	<p>breaks and centric fusions were used to calculate total number of cells with CAs). After one week of exposure 5 chromatid breaks, 1 chromosome break and 5 centric fusions in the 15 ppm group (250 cells examined) compared to 5 chromatid breaks, 1 chromosome break and no centric fusions in controls.</p> <p>There were no statistically significant differences between dose levels within the 1 or 8 week exposure groups or between the 1 and 8 week exposure groups.</p> <p>Data shown at end of this annex.</p>	<p>(predominantly chromosome breaks and centric fusions).</p> <p>The authors noted that the increase in CAs in lung lavage in this study was in the region of 1.9-2.2x whilst in a similar 6 week experiment with benzene the increase was approximately 4.4-5.4x the background frequency.</p> <p>Authors concluded no measurable effect on bone marrow mutagenesis in this study and also noted no measurable effect on distribution of bone marrow DNA/RNA in a similar experiment in rats exposed to FA 6h/d, 5d/wk for 24 wks.</p>
<p>11. Migliore L et al(1989). Mutagenesis, 4, 327-334, 1989. Groups of 5 male Sprague-Dawley rats were dosed with 200 mg/kg bw FA. Tissues were obtained 16, 24 and 30 h post dosing. Stomach, duodenum, ileum and colon were rinsed in saline fixed in formalin, embedded in paraffin and stained with feuglen (counter stain Fast-green). Slides were coded. 3000 cells (basal layer)/tissue were scored for MN and other damager. Nuclear anomalies (e.g karyorrhesis and pyknosis scored). Positive control MNNG 20 mg/kg bw studied.</p>	<p>FA significantly increased MN in stomach at each time point considered, in duodenum at 24 h, and at 30 h in all tissues studied.</p> <p>In contrast MNNG gave a positive in duodenal cells at 24 h only.</p> <p>FA increased NA in stomach at each time point with some evidence for NAs in other tissues.</p> <p>For MNNG increases in NA were reported in the duodenum with some evidence in other tissues examined.</p>	<p>FA is a site of contact in-vivo mutagen.</p> <p>Some data from this study presented at the end of this annex.</p> <p>The authors report a positive dominant lethal result particularly for the first week after dosing.</p> <p>The authors could not draw conclusions on the mechanism for the positive findings reported.</p>
<p>12. Odeigah PGC (1997). Mutation research, 389, 141-148. FA (reagent grade 37%, from Sigma (stabilized with 10% methanol). The mean lethal dose to isogenic rats (University Lagos colony,</p>	<p>1000 spermatozoa were scored from each of 6 rats in each treatment group 3 weeks post dosing. The frequency of abnormal sperm head abnormalities was 1.5% in controls. In treated the rates were</p>	

<p>aged 12-14 weeks) was 2 mg/kg bw. Doses of ¼ to 1/16 i.p. LD50 were used in this study. Groups of 5 male rats received 0.125, 0.250 or 0.5 mg/kg bw FA, i.p, for 5 daily doses. Rats were sacrificed 3 weeks after the last dose. Sperm counts/mg of epididymis determined by haemacytometry. For the dominant lethal test, groups of 12 males (5 controls) at the above dose levels were caged with virgin untreated females for a week for 3 consecutive weeks, The total number of mated females for control was 30. All females were examined for vaginal plug or discharge of the plug and sacrificed 13 days after the mid week of their caging. Total implants, live implants, and early fetal deaths determined. The mutation index was determined as 1- ratio of live implants treated/control x 100.</p>	<p>3.07, 7.27 and 8.57% at the low , mid and high dose levels. There were apparent dose related increases in pin head, short hook, long hook, hook at wrong angle, unusual head spermatozoas. The sperm count determined by haematocytometer showed a dose related decrease.</p> <p>In the dominant lethal study, a reduced percentage of fertile matings was noted at the high dose level at days 1-7 post dose (ca 25%). The frequency of fertile mating at days 15-21 post dose did not significantly differ between treated and controls. There was no difference in the number of implants/female between control and treated at days 15-21.</p> <p>There was a significant increase in the number of dead implants in females mated 1-7 days after treatment which appeared to be dose related.</p>	
<p>13. Im H et al(2006). J of Proteome Research, 5, 1354-1366. Groups of male Sprague-Dawley rats were exposed to 0, 5 or 10 ppm FA (Sigma) for 2 week at 6h/day for 5 day/wk. Blood samples were obtained by cardiac puncture into heparin and the Comet assay undertaken within 3 h. Lymphocytes were isolated by centrifugation and washed in PBS. Liver tissue was also processed fro the Comet assay (according to Farris Toxicology 118, 137-148,</p>	<p>In lymphocytes the control tail moment was 1.24 ± 0.04, in 5 ppm group 1.72±0.11 (p=0.0019) and 2.16 ±0.14 in the 10 ppm group (p=0.0001).</p> <p>In livers the mean tail moment in controls was 1.19±0.08, and 1.73 ±0.10 (p=0.0001) at 5 ppm and 2.49 ± 0.20 (p=0.0001)</p>	<p>Using a variety of biochemical and proteomic approaches, the authors established systemic protein oxidation and lipid peroxidation in lymphocytes and rat liver at the levels of FA exposure.</p> <p>Would such effects explain the positive Comet assay findings in this study?</p>

<p>1997.) Livers were minced and a cell suspension prepared. Comet assay was performed according to Singh (Exp Cell Res, 175, 184-191, 1988). Details are appended at the end of this Annex. For each treatment 2 slides were prepared and 50-100 cells randomly chosen for scoring. The Olive tail moment was determined.</p>		
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Annex 3 to MUT/07/01

Biomonitoring studies with formaldehyde (Summarised in publication date order.)

Reference	Results	Comment
<p>Goh K and Cestero RVM (1979). Chromosomal abnormalities in haemodialysis patients. <i>J of Medicine</i>, 10, 167-174.</p> <p>Forty stable haemodialysis patients were studied (24 men, 16 women). 16-68 y (mean 43y) with a range of diagnoses including nephrosclerosis, glomerulonephritis, diabetes, cystic disease, inactive lupus erythematosus, chronic pyelonephritis, obstructive diseases, Goodpasture's syndrome and cortical necrosis. All received maintenance haemodialysis thrice weekly (lasting for 4-6 h) Dialysers were reused on average 2.8x and were sterilised with 2% formaldehyde before use. All patients received antacids, folic acid, ascorbic acid and multi vitamins. Bone marrow aspirate was placed in culture medium and treated with colcemid (0.16 ug) for 1h and treated hypotonically, fixed and stained with Giemsa. Where possible 10 or more metaphases/ specimen were photographed and karyotyped.</p>	<p>All patients had hypocellular bone marrows. 20 showed disassociated maturation of the nucleus and cytoplasm. There were 1187 metaphases examined (average of 30/patient) with little variation among the various patient groups examined. (35 patients had between 11-59 metaphases, and five had less than 10 metaphases)</p> <p>Among 1187 metaphases, only 58% were diploid (44-67% for the various patient groups). 37% had hypoploid metaphases (27-48%) and 4.7% (1-8.6%) were hyperdiploidies. Pseudodiploidy was reported in 5.8% and acentric fragments in 3.6% of metaphases.</p> <p>The authors report these values to be significantly higher than controls (except for obstructive nephropathy and lupus nephritis patients) 88% diploid (75-95%) pseudodiploidies 3% (0-6%), breaks 2% (0-5%). Very little information on the control population was given.</p> <p>22 (1.9%) translocated chromosomes and one dicentric chromosome were found in patients bone marrow specimens.</p>	<p>The authors comment on the possibility that the range of kidney diseases could be responsible for some of the observed findings.</p> <p>They also report that the patients would have been exposed to approximately 126.75±50.84 mg of formaldehyde during each dialysis. It is not clear how this level of exposure was derived.</p>
<p>Fleig I et al (1982). Cytogenetic analysis of</p>	<p>Aberrations scored included all excluding and</p>	<p>Individual exposures were monitored by personal</p>

<p>blood lymphocytes of workers exposed to formaldehyde in formaldehyde manufacturing and processing. Journal of Occupational Medicine, 24, 1009-1012.</p> <p>A study of 15 workers employed in various aspects of formaldehyde manufacture and processing into resins. Average age 50 y (41-61y). Length of occupational exposure to formaldehyde ranged from 23-35 y with an average of 28y. 15 controls were matched by age and sex (50 y, 41-59y) were selected from office work/administrative work and had never been exposed to formaldehyde. The mean formaldehyde did not exceed 5 ppm before 1971 and 1 ppm after that date. (MAK 1 ppm).</p> <p>Whole blood cultures were established for 70-72h at 37°C in TC Chromosome medium test with colcemid (0.25 ug/ml) added during the last 2 h. After hypotonic treatment, cells were fixed in methanol: acetic acid (3:1), air dried and stained with Giemsa. 100 metaphases from each subject were read.</p>	<p>including gaps. The mean percentage of cells with abnormalities in exposed and control (excluding gaps) was 1.67% compared to 1.07%. (not statistically significant (Fisher-yates). There was no correlation between the level of formaldehyde exposure and number of aberrant metaphases. There was no evidence of increased aberrations among smokers.</p>	<p>monitoring and combined with job activity data, individual exposure was categorised in terms of 25%, 60% or 100% of the MAK before and after 1971. prior to 1971, 12/15 of the individuals were in exposure category 3 (i.e up to the MAK value). After 1971, 11/15 were in category 1 (up to 255 of the MAK), with none in category 3.</p>
<p>Thomson EJ et al (1984) Mutation research, 141, 89-91. CAs and SCEs were examined in 6 pathology workers exposed to intermittent peaks of up to 9.8-11.0 mg/m³ FA (from 26 personal air samples taken 1-3 months prior to blood sampling). Individual</p>	<p>There were no significant differences between exposed and controls with regard to CAs.</p> <p>Authors recognised small scale of study, mean age exposed 33.5, control 27.8 y. Aneuploid cells exposed 36/600, control 15/500. C cells 1 exposed, Acentric</p>	<p>TWA exposure 1.14-40.3 (mean 2.26) mg/m³ during trimming. Disposal of specimens, TWA 2.6-6.93 (mean 4.73) mg/m³</p>

<p>generally worked with FA for 2-3h/day, for 2-3 days/week. Whole blood cultures were established in RPMI 1640 with 1% PHA and 25 μM BrdU and incubated for 48 at 37°C for CA (72 h for SCE). Air dried preparations were stained with FPG. Information on smoking history was taken. 100 first division metaphases were examined.</p>	<p>fragments 1 exposed/1 control. Dicentric 1 control. Chromatid aberrations 8 exposed/6 control.</p> <p>Individuals with highest exposure showed no discernable differences to controls.</p>	
<p>Bauchinger M and Schmid E (1985). Mutation Research, 158, 195-199. A study of 20 male paper makers (mean age 40.8\pm11.2y) exposed to FA during impregnation of paper surfaces. Occupational exposure ranged from 2-30y with an average of 14.5 \pm7.2y. There were no exposures to other industrial chemicals. There were 6 smokers and 14 non-smokers.</p> <p>A group of 20 male workers from the same factory without FA exposure (13 smokers, 7 non-smokers, mean age 37.5 \pm13.4y with similar social environment employed in paper finishing and as office personnel served as controls.</p> <p>Whole blood cultures in F10 medium (44h) with colcemid added (0.1 μg/ml) for final 3 h. BrdU used to identify 1st division metaphases. 500 cells with complete chromosomes were scored for each individual using coded slides using FPG. Mann-Whitney rank U tests was applied.</p>	<p>A significantly higher incidence of dicentrics or dicentrics and ring chromosomes was reported compared to controls.</p> <p>Dicentrics exposed 0.0013\pm0.0003 (reported in 11 individuals), control 0.0005\pm0.0002 (reported in 5 individuals) . Centric rings, exposed 0.0003\pm0.0001 (reported in 3 individuals), control 0.0001\pm0.0001 (reported in one individual).</p> <p>When the data were examined for supervisors and operators, the statistical significance held for supervisors. FA exposure was reported to be approximately 2.5x longer for supervisors. It was also noted that that the mean age of the supervisors was 47.6\pm7.5y was higher than the control group. The authors considered the influence of age on aberration frequency could be excluded (P>0.05). No significant differences in the number of cells with structural chromosome changes, acentric fragments and chromatid aberrations (breaks and exchanges) were reported.</p>	<p>Workers had to enter the paper machine for periods of about 45 min (supervisors) and 90 mins (workers)/8 h shift. Exposures could be up to 3 ppm. Rarely PPE and breathing masks were worn when exposures of 20-50 ppm were encountered for 1-5 mins. Alcohol consumption reported to be inconspicuous, all were healthy and no on had received radiation or cytostatic therapy.</p> <p>The authors noted that there were no significant differences between exposed and control if 100 or 200 cells were scored. Significant differences were reported if \geq300 cells were scored.</p>

	No changes in the number of gaps was reported.	
<p>Suruda A et al (1993). Cancer Epidemiology, Biomarkers & Prevention, 2, 453-460.</p> <p>A group of mortuary science students were enrolled 3 weeks prior to their first embalming course. Details of previous embalming work, smoking, diet, medication, and X-ray exposure were documented. Swabs of nasal and oral tissue and blood samples were taken prior to embalming and after the first 9 weeks in the embalming laboratory. An interim questionnaire was administered.</p> <p>Buccal or Nasal epithelial cells were brushed and immersed in hanks salt solution, centrifuged onto a glass microscope slide, stained with Feulgen and counterstained with fast green.</p> <p>MN determination in PBLs was reported to be according to Fenech 1985. (Mut Res, 147, 29-36.)</p> <p>PBL data were analysed by a multivariate analysis. Nasal were analysed by Poisson regression and buccal data were analysed by Grubb's statistic. Differences pre and post exposure were analysed by Wilcoxon sign-rank test. Spearman's rank correlation was used to assess influence of cumulative FA exposure.</p>	<p>Personal exposure and peak air concentrations were monitored.</p> <p>During period of study, 29 students undertook 144 embalmings with autopsies 36%, normal intact bodies 21%, donated 36%, other 7%. The average exposure during 121 of these embalmings was 1.4 ppm formaldehyde (range 0.15-4.3 ppm). The man length of an embalm was 125 min. peak exposures of up to 3-9x the TWA were detected, often associated with leaks of fluid during injection or when applying embalming fluid to hands, feet, or leaks from tubing and other appliances. The highest mean exposure (1.5 ppm occurred during autopsies.) Cumulative exposure (ppm x h) was 14.8 ppm-h (range 4.3-33.6). The 8-h TWA was 0.33 ppm (0.1-0.96 ppm)</p> <p>Epithelial cells from the buccal areas showed a 12 fold increase in Mn frequency from 0.046±0.17/1000 cells pre exposure to 0.60±1.27/1000 cells post exposure. (P,0.05) Nasal MN increased 22% from 0.41±0.52/1000 cells to 0.50±0.67/1000 cells (P=0.26). The MN frequency in PBLs increased by 28% from 4.95±1.72/1000 cells to 6.36±2.03/1000 cells (P<0.05). A dose response relationship was reported between cumulative exposure to FA and buccal MN in 22</p>	<p>The authors noted potential concurrent exposure to gluteraldehyde, methanol, isopropyl alcohol and phenol No detectable gluteraldehyde, phenol and methanol were reported. Isopropyl alcohol ranged from detection limit to 12 ppm The methods of analysis were NIOSH compliant, but relatively small numbers of samples were analysed (5-16)</p> <p>Baseline data were obtained from 34 students. Post course specimens from 31. One subject with previous embalming experience 90 days prior to study and one subject who chewed tobacco were excluded, thus data on 29 students was used. 8 had no embalming experience, six had assisted (1-4 embalmings), and 15 with five or more embalmings. Average age was 23.9y, 7 were women, and 5 were current smokers (2 women smoked). 14/29 had hepatitis B vaccine in 12 months prior to study..</p> <p>The authors argued that the change in MN in PBLs over time in this study were unlikely to be artifacts.</p> <p>The authors noted that SCEs in PBLs decreased during the study.</p>

	<p>males but not 7 females. There was no correlation between cumulative exposure and MN in PBLs. A dose-response between FA and increased MN in PBLs was seen only in the 22 males and only if current smoking status and coffee were included in the model.</p>	
<p>Shaham J et al (1996) Carcinogenesis, 17, 121-125. DPXs were measured in isolated WBCs from pathology workers (which had been stored at -20°C for up to 3 weeks). 12 workers were examined with 8 controls. DNA-protein was measured by binding with SDS when the cation was changed from Na to K. Analysis used Hoechst reagent dye (200 ng/ml) at pH 7.5. Static sampling (15 mins) was used to estimate air concentrations in the pathology laboratory. The range was 1.6-1.38ppm and 6.9 ppm in the laminar flow. Personal samplers showed a range of 3.1-2.8 ppm FA at the time when most work was in progress and about 1.46 ppm at midday.</p> <p>DPX data were assessed using <i>t</i>-test. ANOVA was used to assess the influence of smoking. Multiple linear regression was used to examine levels of DPX as a function of years of exposure to FA.</p>	<p>The authors reported a significant difference (P=0.03) between the levels of DPX in PBLs (mean 28 ±6%, 21-38%) compared to control workers (mean 22%±6%, range 16-32%)Of the four workers four showed DPX above 33%.</p> <p>Subdivision into physicians and technicians as questionnaire data indicated a longer period of exposure/day for technicians. The mean DPX in technicians was 26% ± 4% range 26%-38% compared to controls mean 26%± 4%, range 21-34%.</p> <p>The authors also found a linear relationship between years exposure and DPX (Data presented at end of this Annex).</p> <p>The authors also reported differences between exposed and unexposed sub divided into smokers and non-smokers.</p>	<p>There was an exchange of correspondence between the investigators and Casanova M, Heck and Janzen from the US CIIT. Casanova noted defects in the study design, which included the report by the developers of the DPX assay of a within subject CV of 33%, lack of matching of study groups, lack of statistically significant correlation between years exposure and DPX, inconsistency of the data with evidence against distant site toxicity of FA, implausibility of the interpretation.</p> <p>The authors countered that the groups had been matched and the study was of sufficient size to determine a significant difference. Part of the counter argument regarding distant site toxicity was that humans use both oro-nasal breathing compared to nasal only for rats. The failure to find HCHO at distant sites related to sensitivity of assays and biological plausibility was possible if low concentrations of FA were present in systemic</p>

		circulation.
<p>Vasudeva N and Anand C (1996) Clinical and program Notes, 177-179. A group of 30 female medical students from lady Hardinge Medical College, New Delhi with almost 15 months exposure to FA (ca 7h/week during histology/anatomy classes) and an age matched control group of non –medial students (offsite) were used.</p> <p>Whole blood cultures using fresh blood samples were set up in RPMI 1640 with 1% PHA. Colchicine was added 2h before cell harvest at 72h. Cells were hypotonically treated, fixed in methanol: acetic acid (3:1), air dried on slides and stained with Giemsa. G-banding was undertaken following trypsin denaturing. 100 cells/student were examined.</p>	<p>The mean FA in the laboratory did not exceed 1 ppm.</p> <p>The mean frequency of aberrant cells in exposed was 1.25 compared to 0.9% in controls. There was no statistically significant difference. No other details of these results were presented.</p>	
<p>Chen-Jing Y et al (1997). Biomedical and Environmental Sciences, 10, 451-455. A group of 25 non smoking anatomy students (13 male and 12 female average age 18.8±1.0y) were included in the study. All lived in student dormitories, no drug history for 3 weeks prior to survey and no X-ray for at least 6 months (smoking history not reported). Blood samples were taken prior to and at completion of the anatomy course (8 weeks, 3x/per week, for 3h/session). Two blood samples haemolysed and were not included. Whole</p>	<p>The mean TWA exposure in the Anatomy lab was $0.508 \pm 0.299 \text{ mg/m}^3$ (range 0.071-1.284 mg/m^3)</p> <p>The authors report that bio-or-multi-micronuclei were reported in nasal tissues from 14/25 students after exposure and 3/25 before exposure. Bio-micronuclei were observed in the oral tissues of one student after exposure. No bio-or-multi-micronuclei were documented in lymphocytes.</p> <p>The difference between individual nasal and oral</p>	<p>The authors considered that the negative results in the lymphocytes in this study might be due to limited exposure.</p> <p>Nasal cells before 1.20 (SEM 0.676), after 3.85 (SEM 1.48). Oral cells before 0.568 (SEM 0.317), after 0.857 (SEM 0.558). Lymphocytes before 0.913 (0.389), after 1.11 (SEM 0.543).</p>

<p>blood cultures for the other 23 in RPMI 1640 was undertaken for 72 h. Details of culture induction and cessation were not provided. Slides were stained with Giemsa. MN were counted and confirmed by re-examination by a second trained technician.</p> <p>Control MN frequency was determined from cultures from 5 unexposed individuals. No details of the control individuals were given (eg ages, sex, smoking habits etc).</p> <p>MN were also measured in nasal and oral mucosal cells.</p>	<p>micronucleated cell frequencies before and after exposure was statistically significant (paired t test $P < 0.001$ and $P < 0.01$ respectively).</p> <p>No differences in micronucleated cell frequencies was found in the lymphocytes.</p>	
<p>Chen-Jing Y et al (1999). Biomedical and Environmental Sciences, 12, 88-94.</p> <p>Additional studies of SCEs using same group of students. No effects reported.</p>		<p>Authors reported an increase in B cells compared to T cells. An increase in the ratio of T-helper to T-cytotoxic suppressor cells was reported.</p>
<p>Shaham J et al (2003). Occupational and Environmental Medicine, 60, 403-409.</p> <p>Mononuclear cells were isolated from blood samples and stored at -20°C for up to 3 weeks. A K_SDS assay was used to measure DPX. The authors reported that DPX could be measured in-vitro at concentrations of down to 0.001mM FA. Wild-type and mutant p53 proteins were determined in serum using a quantitative ELISA kit.</p> <p>Static exposure measurements (15 mins). From this two exposure</p>	<p>The amount of DPX (DPX/total DNA) was significantly higher in exposed males (0.21 (SE 0.011) and females (0.20 (SE 0.008) and combined sexes (0.21 (SE 0.006) compared to controls males (0.15 (SE 0.008) and females (0.12 (SE 0.008) and combined sexes (0.14 (SE 0.006). Data no affected by sex, age, education, or origin.</p> <p>The authors reported that the mean DPX increased in relation to level of exposure and duration of exposure, but the analyses did not reach statistical significance.</p>	<p>Questionnaire used for demographic data, occupational history, exposures, medical history, and smoking habits.</p> <p>The exposed group consisted of 186 workers ($45.8 \pm 9.9\text{y}$, 59 men, 127 (68.3%) women) from 14 hospital pathology departments. 68 (36.6%) were smokers and 118 (63.4%) were non-smokers. The mean exposure to FA was 15.9 y (1-51y) Job descriptions included physicians, laboratory assistants, technicians, hospital orderlies. The control group consisted of 213</p>

<p>groups were identified. Low (lab assistants, technicians) mean 0.4 ppm (0.04-0.7ppm). High (physicians, hospital orderlies). Mean 2.24 ppm (0.72-5.6 ppm).</p> <p>Chi² was used to assess sex, smoking habits, origin, education. Age was assessed by Mann-Whitney U test.</p> <p>Comparison of adjusted means was by ANOVA. Comparison of DPX between low/high and ≤16y and ≥16y was estimated by Mann-Whitney. Comparison of the prevalence of high levels of p53 between exposed and unexposed was assessed by Chi². Adjusted ORs were assessed by logistic regression analysis. The association between p53 and mutant p53 was assessed using Spearman rank correlation coefficient. Mann-Whitney was used to evaluate a cut off of <150>150 pg/ml p53. The prevalence of high/low p53 and DPX was undertaken using Chi². Adjusted ORs were assessed using logistic regression using sex, age, and smoking)The authors reported that they used median DPX rather than mean as there was no established cut off point in the literature and the distribution of DPX was asymmetrical.</p>	<p>High levels of p53 were more prevalent in the exposed group 44.1% cf 36.3% in controls. The exposed males had a significantly higher p53 .150 pg/ml than unexposed males (54.8%, cf 36.5%, P<0.05). In females the prevalence of high p53 was 38.8% in exposed and 35.35 in unexposed.</p> <p>The authors reported an elevated OR for FA associated with elevated p53. (1.6 (95% 0.8-3.1)) and in males of 2.0 (95% 0.9-4.4) P<0.1</p> <p>A significant positive correlation was found between pantropic p53 and mutant p53 (rs=0.75; p<0.01) and well as pantropic p53<150 pg/ml and mutant p53 (rs=0.6; p<0.05). the eman level of mutant p53 was significantly higher.</p>	<p>workers (mean age 42.1 ±10.1y, 127 (59.6%) men, 86 (40.4%) women from the admin sections of the same hospitals. 114 (53.5%) were non smokers, 95 (44.6%) were non-smokers.</p> <p>The age distribution, sex, origin and education years differed between the two groups. There were more European/Americans in the exposed, they were older, more educated and there were more females.</p>
<p>Ye X, et al (2005). Cytogenetic analysis of nasal mucosa cells and lymphocytes from high level long-term formaldehyde exposed workers and low-level</p>	<p>The TWA for FA for workers was 0.985±0.286 ppm. For waiters 0.107±0.067ppm and in controls 0.011±0.0025ppm.</p>	<p>Demographic features, exposure, health histories, information on smoking, alcohol, drug histories, recent immunizations, infections and possible exposure to other</p>

<p>short-term exposed waiters. Mutation Research, 588, 22-27.</p> <p>This is a report of a study conducted in 1992. 18 workers (1 male and 7 female) from a FA factory were exposed to FA for periods of 1-15y (mean 8.5 y). There were 12 waitresses and 4 waiters working in a newly fitted ballroom exposed to FA from building materials, tobacco smoking and furniture. 23 undergraduates (12 males, 11 females) were chosen as a control group. There were differences in male/female ratios between these groups. The average age of controls was 19±2.3y (18-23y)</p>	<p>Nasal mucosal MN were significantly increased in the workers group. The frequency of bi and tri-micronucleated cells was also increased. No MN induction was found in the waiters.</p> <p>An increase in SCEs was documented in lymphocytes in the workers, but not in the waiters.</p> <p>The authors also reported a significant increase in the percentage of B lymphocytes with decreased total T cells, and T-cytotoxic-suppressor cells. A higher ratio of T-helper to T-cytotoxic-suppressor cells was reported.</p>	<p>chemicals were assessed by questionnaire. Those included in the study had no previous smoking, medicine use for 3 weeks prior to the survey, and no X-ray for at least 6 months prior to the survey. Subjects who routinely took drugs were excluded.</p> <p>TWA 8-h or 5h levels of FA were measured. Max levels were also reported.</p> <p>This study group also published studies of anatomy workers (see Chen-Yong 1997, 1999 above). Similar results were reported. It is noted that the study of formaldehyde workers preceded the anatomy workers and did not include an assessment of MN formation in PBLs.</p>
<p>Orsiere T et al, (2006). Genotoxic risk assessment of pathology and anatomy laboratory workers exposed to formaldehyde by use of personal air sampling and analysis of DNA damage in peripheral lymphocytes. Mutation Research, 605, 30-41.</p> <p>59 pathology/anatomy lab workers recruited from 5 hospitals in France 24% male). Control group of 37 (19% male) selected from working hospitals with no history of occupational exposure to genotoxins. Controls did not differ from exposed for age, sex or smoking. Chemiluminescence microplate assay undertaken for DNA damage. Isolated lymphocytes (from blood</p>	<p>The mean age of the exposed group was 44.7±7.9y (25-58) compared to 44.0±8.7y (23-60y) smoking 20% in exposed, 24% in control. Mean cigarettes/d in exposed 13.6±9.2y compared to 20.7±15.1 in controls.</p> <p>The mean 15 min exposure level was 2 ppm (range <0.1-20.4ppm) and the 8h TWA was 0.1 ppm (<0.1-0.7ppm). 31/59 short term exposures exceeded the French limit of 1ppm. 17/59 were higher than the STEL from USA OSHA. The highest levels were observed during macroscopic examination of formaldehyde preserved specimens. Only one personal sample for long term exposure exceeded the french mean value of</p>	<p>Data on smoking, alcohol, drug use, X-ray diagnosis. Subjects with history exposure radiotherapy or chemotherapy or use of drugs with potential mutagenic/reproductive effects excluded. FA measured by passive badges using 15 min and 8h sampling.</p> <p>The authors chose chemiluminescence as a method for identification of DNA-protein cross link DNA damage.</p> <p>The authors comment that these data were compatible with those reported by Suruda et al (see above.)</p> <p>The authors discuss the potential impact of vitamin status on MN formation but considered the study</p>

<p>samples stored in the dark at 4°C). DNA from lysed cells incubated with deoxybase triphosphates and also digeoxy- dUMP. Analysis by absorption onto digeoxy-antibody with bound alkaline-phosphatase, using Lumi-Phos as a substrate. The luminescence was proportional to repair of DNA damage. Negative control included plasma DNA, positive controls included plasma DNA exposed to UVR and als from lymphocytes treated with MMS.</p> <p>Whole blood cultures (stored for <6h at room temperature) in 199 medium, 1%PHA for 72 h. At 44 h, cytochalasin B (5ug/ml) was added. Cells were subject to mild hypotonic treatment, fixed in methanol/acetic acid and stained with Giemsa. Cell pellets were kept at -20°C for FISH analysis.</p> <p>FISH analysis was undertaken using human pancentromeric probe with FITC. Denatured DNA from treated fixed cells was hybridised overnight at 37°C and counterstained with propidium iodide. Slides were read blind by one operator for presence (C+MN) or absence (C-MN) of centromeres in MN cells.</p> <p>Differences between donor groups was examined by nonparametric Mann-Whitney test, and Chi squared for quantitative and qualitative variables.</p>	<p>0.5ppm but was below the permissible limit of 0.75 ppm.</p> <p>Chemiluminescence DNA damage was studied in 57 pathologists/anatomists at the beginning and at the end of the shift. No significant difference was reported (cf 3.9± 0.6 RLU/ng DNA before and 3.6± 0.5 RLU/ng DNA after. DNA damage was no correlated with work practices or personal air sampling data.</p> <p>The rate of BNMN cells/1000 BN cells was significantly higher in the exposed group, 16.9%±9.3 compared to 11.1%±6.0 P=0.001). The rate of BNMN cells was positively correlated with donor age, the duration of formaldehyde exposure (p=0.295, P=0.028, Spearman's test). However after correction for age, the correlation with duration of exposure was not significant.. the authors noted that gender affected BNMN cell rate. (females 16.4±8.7 compared to 8.2±3.9 for males P<0.001 for total population) Smoking and drinking had no effect. BNMN cell rate was no correlated with personal air sampling or with DNA damage data.</p> <p>FISH data for 18 exposed and 18 controls was presented. The subgroups did not differ in age and smoking habits. MN, C+MN, C-MN) were not correlated ; with donor age within controls, the exposed group or the total</p>	<p>sample size was too small to evaluate these aspects.</p> <p>The authors speculate that DNA-protein cross links can directly form MN and that aneugenic events could occur via effects of FA on tubulin proteins.</p> <p>The authors considered that the lack of exposure-response from the personal sampling data reflected accumulated DNA damage in exposed individuals.</p> <p>The authors quote an in-vitro study of effects of FA on pig tubulin in support of this argument (Pfuhrer S et al Mut Res, 514, 133-146, 2002).</p> <p>In addition cell lines derived from FA tumours have been shown to be aneuploid. Bremudez E et al Mol Carcinogen, 9, 193-199, 1994)</p> <p>The authors advocate additional studies with chromosome specific probes for specific aneuploid event.</p>
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<p>Chemiluminescence data was assessed by Wilcoxon rank. Multiple regression was used to assess the influence of variables on CBMN data and quantitative variables were assessed by the Spearman's rank test.</p>	<p>population. The BNMN rate was higher in exposed (19.1 ± 10.1) compared to 11.9 ± 5.6 ($P=0.021$) the MN frequency was higher in exposed, but the difference was not significant. The C=MN frequency was higher but the differences were not significant. The frequency of MN containing one centromere (C1+MN) was higher in pathologists and anatomists compared to controls ($11.0\% \pm 6.2$ cf $3.1\% \pm 2.4$ ($P < 0.001$), whereas the frequency of micronuclei containing more than one centromere was similar. Multiple linear regression for age, gender, smoking and drinking habits showed a clear effect of occupation on C1+MN. The ratio of C1+MN/MN and C1+MN/C=MN were significantly higher in exposed. 785 of MN were centromere positive in exposed (68% in control), 505 of the MN contained only one centromere (20% controls) 66% of C+MN were C1+MN (compared to 255 in controls). The frequency of C-MN did not differ between exposed and controls.</p>	
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