

MUT/07/12

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)

DRAFT WORKING PAPER: FORMALDEHYDE: EVIDENCE FOR SYSTEMIC MUTAGENICITY

Introduction

Background to COM consideration of Formaldehyde

Exposure

1. Formaldehyde is produced worldwide on a large scale and is used in the production of phenolic, urea, melamine and polyacetal resins. Formaldehyde is also used as an intermediary in the manufacture of industrial chemicals and as an aqueous solution (formalin) as a disinfectant and preservative in many situations. Formaldehyde also occurs as a natural product in most living systems and in the environment. There are also a number of non occupational sources of exposure including vehicle emissions, from building and household materials, from food and cooking and from tobacco smoke. Most formaldehyde released to the environment is rapidly degraded and human exposure from environmental sources is most likely to occur when there is a continuous source present.^{1,2}

Toxicokinetics

2. Formaldehyde is a normal intermediary metabolite in humans. It is estimated that endogenous blood concentrations of formaldehyde are approximately 0.1 mM. Absorbed formaldehyde can be metabolised to formate enter the one carbon pool for incorporation in DNA, RNA and proteins. Other routes of metabolism include oxidation to carbon dioxide. Absorbed formaldehyde is rapidly excreted. A small proportion of material may be metabolised by a number of non saturable pathways at the site of contact to form cross links with proteins and a small amount to DNA. The Committee agreed that a consideration of the toxicokinetics of formaldehyde would be important with regard to consideration of potential for systemic mutagenicity.

Mutagenicity and Carcinogenicity of Formaldehyde

3. Formaldehyde has recently been considered by IARC and placed into group 1 (carcinogenic to humans).¹ There is 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 cohort and garment workers in the USA and because they conflict with the non-positive findings from the British cohort of workers.*¹ This conclusion stimulated some discussion in the published literature regarding the possible mechanism of formaldehyde induced increased risk of leukaemia.³ The IARC working group commented on the possible mechanism and ‘..noted evidence for clastogenic damage to circulatory cells, but overall since there was 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

Introduction to COM discussion

4. The COM was asked to consider the evidence for systemic mutagenicity from animal experiments and biomonitoring studies of workers exposed to formaldehyde. The objective was to consider the potential for systemic mutagenicity following inhalation exposure, the predominant route of exposure in the occupational groups for which evidence of leukaemia had been reported.
5. The COM were aware of a number of recent internationally recognised reviews which had reported on both carcinogenicity and mutagenicity and agreed there was no need to specifically review all of the mutagenicity data on formaldehyde.^{1,2,4} This included a recently published evaluation of the mode of action (MOA) for nasopharyngeal cancer.⁴ The COM acknowledged that formaldehyde is a direct acting *in vitro* mutagen in bacterial and mammalian cells (including rodent and human cell lines). Mutagenic effects reported included point mutations, chromosome aberrations, sister chromatid exchanges, DNA strand breaks and UDS in rat nasal turbinate cells.^{1,2,4} With regard to the MOA for rat nasopharyngeal tumours, most reviewers had considered the formation of formaldehyde 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 key elements in the proposed MOA. The magnitude of the formaldehyde induced local site of contact cell proliferation had been emphasised in the available reviews.^{2,4}
6. The Committee agreed to focus its initial discussion on the available peer reviewed scientific literature regarding toxicokinetics of absorbed formaldehyde and the evidence in the published literature for systemic in-vivo mutagenicity.¹⁻³⁰

The kinetics of absorbed formaldehyde

Studies in experimental animals^{3,14,15,16}

7. The COM considered information from published inhalation studies in rats and Rhesus monkeys (using either single exposure to 6 ppm for 6 h or repeated exposure to the same concentrations 5 days/week for 4 weeks with sampling after the last exposure) and agreed the majority of absorbed formaldehyde was incorporated into intermediary metabolism ($\geq 91\%$) with small amounts bound as DPX (ca $\leq 9\%$).^{3,14,15} Importantly there was no evidence for an increase in blood formaldehyde concentrations in these studies. In some relatively old published experiments the metabolic incorporation and covalent binding of formaldehyde was investigated in nasal tissue and bone marrow following inhalation exposure of rats to formaldehyde (dual labelled 3H- and 14C-) at a number of concentrations up to 15 ppm for 6 h. There was evidence for covalent binding of formaldehyde to rat nasal DNA but no evidence for binding to bone marrow.¹⁵ In a further study using a similar inhalation exposure rats were pre-treated with an intraperitoneal injection of phorone designed to deplete non protein sulphhydryl levels and hence increase the potential for DNA binding of absorbed formaldehyde. Metabolic incorporation into rat nasal tissue and bone marrow were significantly decreased, but there was no evidence for covalent binding to bone marrow DNA.¹⁶

Modelling of toxicokinetics in humans

8. The committee noted that a number of research groups had attempted to model the systemic uptake and distribution of formaldehyde in humans exposed to formaldehyde at exposure levels close to or at the U.K. occupational exposure standard of 2 ppm for 8 hours.^{2,3,5,29} These studies had concluded that the systemic blood levels of formaldehyde resulting for such exposures would be $\leq 0.1\%$ of the endogenously formed blood concentrations of formaldehyde (i.e. approximately 0.001mM compared to an endogenous level of approximately 0.01 mM).
9. The Committee concluded that systemic exposure to formaldehyde at potential cancer target organs resulting from inhalation exposure would be a negligible amount compared to endogenously formed formaldehyde. Members acknowledged that their limited information on the proportion of free and bound formaldehyde and the potential for release of adsorbed formaldehyde bound to macromolecules might occur but overall considered that the potential for redistribution of formaldehyde was very small compared to endogenously formed formaldehyde.

Potential for *in vivo* systemic mutagenicity

*Studies in Experimental animals*⁶⁻¹³

10. The COM noted that a number of test materials had been used in *in vivo* studies in experimental animals which included aqueous solutions (30-50%) stabilised with 10% methanol. Alternatively paraformaldehyde hydrolysed using sodium hydroxide might be used as a source of formaldehyde generation. Formaldehyde is intrinsically reactive and thus unstabilised solutions may be oxidised to formic acid and at low temperatures a precipitate of trioxymethylene may be formed.² The precise composition of the test material used was not clear in many of the studies reviewed but was presumed to be predominantly formaldehyde. Members agreed the presence of methanol would complicate the evaluation of *in vivo* genotoxicity studies.
11. The COM agreed that the available *in vivo* tests for micronucleus induction and chromosome aberrations in bone marrow in rodents using inhalation exposure or intraperitoneal administration were predominantly negative.^{7,8,9,10,11} A slight and apparently dose related increase in micronucleated cells/1000 PCEs was noted in mice given two intraperitoneal doses of formaldehyde (derived from paraformaldehyde at 6.25, 12.5 or 25 mg/kg bw) separated by 24 hours at two samplings (16 h and 40 h post final dose).⁸ It was noted that the study authors had not considered that a statistically significant increase had been documented in this study. The Committee considered a recently published *in vivo* comet assay in rats exposed by inhalation to up to 10 ppm 6h/day for 5days/week. An apparent dose-related increase in comet tail moment had been reported following examination of 50-100 cells/animal in peripheral blood lymphocytes and in the liver. It was noted that in principle a cross linking agent such as formaldehyde might be expected to reduce comet tail moment and it was possible that the effects might have been due to oxidative damage and possibly apoptosis. The COM noted that formaldehyde induced disturbances of protein and lipid oxidation in this study.¹³
12. The Committee considered the evidence for a dominant lethal effects and noted two positive results had been reported. Thus a single intraperitoneal dose of formaldehyde (50 mg/kg bw) administered to Q strain mice with separate matings each week for seven weeks resulted in increased embryonic death in the 1st and 3rd weeks.⁶ There was no evidence for a clastogenic effect on spermatocytes in this study. The COM concluded it was unlikely that the effects reported resulted from a systemic mutagenic effect of formaldehyde.

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13. 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 intraperitoneal doses of 0.125-0.5 mg/kg bw of formaldehyde.¹² These doses were reported to be between 1/16 and 1/4 of the intraperitoneal LD50 in this strain of rat and are very much lower than the dose levels used in other in-vivo mutagenicity studies with formaldehyde. The dominant lethal study examined matings 1-7 days, 8-14 days and 15-21 days post dosing. A significant increase in the number of dead implants was reported in the period 1-7 days post dose which was accompanied by a reduction in sperm counts and abnormal spermatozoa.¹² Members noted that the use of methanol to stabilise formaldehyde could have contributed to the observed effects on spermatozoa morphology reported in rats.
14. The COM concluded mechanism by which formaldehyde could have induced the observed effects in the dominant lethal studies was unclear but did not involve a systemic mutagenic response.

Other site of contact in-vivo mutagenicity studies

15. The Committee noted clear evidence for an increase in micronucleated cells of the basal epithelium of the stomach in a study in rats where a single oral dose of 200 mg/kg formaldehyde was administered.¹¹ In a separate study using repeated inhalation exposure of rats to formaldehyde (up to 15 ppm 6h/day for 1 or 8 weeks), lung lavage samples in addition to bone marrow samples were examined for chromosome aberrations. There was no evidence for a clastogenic effect in bone marrow samples but a statistically significant increase in chromosome aberrations was noted in lung lavage samples.¹⁰
16. The COM agreed that formaldehyde was a site of contact in-vivo mutagen. Members noted that the site of contact effects in the gastrointestinal tract might not all be due to formaldehyde reacting directly within the target cells as the effects extended down the gastrointestinal tract further than would be expected for a highly reactive chemical.

Biomonitoring studies of formaldehyde exposure¹⁷⁻²⁸

17. Biomonitoring studies of genotoxicity in workers exposed during a variety of activities including manufacture of formaldehyde and use of formaldehyde in mortuary and anatomy departments and in paper impregnation were evaluated. A further group used in biomonitoring studies were dialysis patients where the dialysis equipment was sterilised with formaldehyde. A number of these studies had reported evidence for an increase in MN or DPXs in PBLs.¹⁷⁻²⁸

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18. The COM considered these data with regard to the evaluation of data from toxicokinetic studies and in-vivo genotoxicity studies in experimental animals which suggested there would be no biological rationale for a systemic mutagenic effect in biomonitoring studies. None of the studies collected the appropriate information previously identified by COM to assess background variation in results. Thus members noted that the quality of the biomonitoring studies was poor with limited account for confounding factors, including age, and also considered that the method for determination of DNA-protein cross links (SDS separation of protein-linked DNA) in PBLs had not been adequately validated.
19. The COM considered that no definite conclusions could be reached with regard to the small increases in DNA-protein cross links reported in the studies published by Saham et al.^{22,26}
20. The Committee considered if any of the available studies was of sufficient quality to draw conclusions with regard to systemic mutagenicity of formaldehyde. Members noted the study by Ye and colleagues²⁷ clearly showed an increase in micronuclei in nasal mucosal cells in workers exposed to formaldehyde during manufacture whilst no concurrent increase in micronuclei in peripheral blood lymphocytes was noted.²⁷ The increase in SCE formation in peripheral blood lymphocytes in this study may have resulted from a secondary mechanism following oxidative DNA damage.
21. Members commented on the publication by Orsiere T et al (2006).²⁸ The apparent increase in micronuclei with centromeres was not consistent with the proposed mechanism of formaldehyde effects cross linking DNA and proteins. It was noted the protocol was not optimal for identification of aneuploidy, and that individual data were not available. Members considered that no definite conclusions could be reached on the data presented in this publication.
22. The Committee concluded that there was no convincing evidence regarding systemic mutagenic effects of formaldehyde from the available biomonitoring studies.

Additional *in vitro* study of formaldehyde induced DPX.

23. The Committee noted that recent *in vitro* studies had demonstrated that DPXs formed from formaldehyde were effectively removed at concentrations up to 100 µM.³⁰

COM conclusions

24. The COM concluded that the amount of formaldehyde systemically available following inhalation exposure at the occupational exposure standard would be negligible.

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25. The COM was aware that formaldehyde was a direct acting *in vitro* mutagen. The COM concluded that there was no convincing evidence from *in vivo* mutagenicity studies in experimental animals and from biomonitoring studies of genotoxicity in workers exposed to formaldehyde for an *in vivo* systemic mutagenic effect of inhaled formaldehyde.
26. The COM concluded that there was no reason to consider that systemic mutagenicity would be involved in the mechanism of formaldehyde induced systemic tumourigenicity.
27. The Committee concluded that it was not possible to draw a conclusion regarding whether there was a threshold for in-vivo systemic mutagenicity existed.

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