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CONTENTS

A. INTRODUCTION

A recent formaldehyde inhalation study sponsored by CIIT, though not yet complete, demonstrated the development of nasal cancer when rats and mice were exposed for two years to formaldehyde at concentrations above the current MAK - and TLV-limits. Since formaldehyde is a widely used chemical, found not only in the working environment but also in the domestic environment of man, it is important to assess whether the findings in animals indicate a carcinogenic risk for man and if so under what conditions.

A Formaldehyde Toxicity Working Group, (see list of members in Appendix 1) was set up by ECETOC, with the following terms of reference :

"To maintain awareness of and evaluate past, current and proposed studies of relevance to the carcinogenicity of formaldehyde; to advise the ECETOC and other industry formaldehyde groups on the significance of the results of such studies for human exposure; to advise on the necessity for any future studies related to carcinogenicity".

Some members of the Working Group were present at the Conference organised by the CIIT (Nov. 20-21,1980) which was devoted to the toxicology of formaldehyde. The proceedings of the meeting are not yet published and many of the results quoted were from studies which were then incomplete. This report summarises some of the relevant data presented at that conference.

B. MUTAGENIC EFFECTS OF FORMALDEHYDE

1. RESULTS OF MUTAGENIC TESTING

In 1946 Rapoport first reported that feeding of Drosophila larvae with a medium containing formaldehyde induced lethal mutations (1). Subsequent studies showed that this mutagenicity was specific for male larvae, in which it was confined to early spermatocytes (2). Adult Drosophila were not susceptible to the mutagenicity of formaldehyde in the food and, although mutations could be induced by injection of formaldehyde solutions into the imago (3), the mutagenic efficiency was never as good as in formaldehyde-fed larvae. In chemically defined media, adenylic acid or adenosine were essential for the expression of mutagenic effects (4). These findings suggest that products of reaction of formaldehyde with food constituents are most probably responsible for the mutagenic effects in this system. The feeding of food containing formaldehyde has also been shown to induce chromosome and chromatid breaks in the grasshopper Tristria pulvinata (5).

The fact that only early spermatocytes are susceptible to formaldehyde-induced mutagenicity suggests that DNA-replication may be required for the induction of these effects. Similar findings have been obtained with the yeast Saccharomyces cerevisiae (6-9). In synchronised cultures it has been shown that the mutagenic effect was most marked during phases G_2 and S of the cell cycle (6). This again implies that during mitosis the cell is most susceptible to formaldehyde. (The significance of this finding will be discussed later). Yeast cells seem to be able to repair the initial DNA-lesion, since the production of DNA single-strand breaks appeared to be dependent on metabolic processes within the yeast (8), and formaldehyde induced DNA-protein cross-links were removed (9) (10).

Formaldehyde mutagenicity has also been reported in viruses (11) and in bacteria (E. Coli B (12-15); E. Coli K_{12} (16-19); and Pseudomonas fluorescence (13)). Both positive and negative results have been reported in a Salmonella typhimurium mutagenicity assay (Ames Test) (20-22). In eukaryotic test systems formaldehyde was mutagenic for the fungi Neurospora crassa and Aspergillus nidulans (23), and for mouse lymphoma cells (21). It also induced sister-chromatid exchanges in Chinese hamster ovary cells (21) (24) and in cultures of human peripheral lymphocytes (24), but a test for induction of chromosome aberrations in cultured Chinese hamster ovary cells proved negative (21). Transcription termination was induced by formaldehyde in monkey kidney cells (CV-1 cells) (25), and it caused transformation of balb c 3T3 cells (21), baby hamster kidney cells (22) and C3H/10T1/2 cells (26), although in the latter case transformation occurred only if cells were also treated with the tumour-promotor, tetradecanoyl phorbol acetate (TPA).

The mutagenicity of formaldehyde in bacteria is relatively weak, but has several characteristics in common with that in yeast and Drosophila. Thus, it would appear that replicating DNA is most sensitive since mutations occur close to the replication point (19). Moreover, the initial DNA-lesion is subject to repair since E. Coli mutants which were deficient in DNA-excision repair were more susceptible to the mutagenic effects of formaldehyde than the wild type (15), and an E. Coli strain deficient in DNA-polymerase (pol A-) appeared to be more sensitive to formaldehyde-induced cytotoxicity (Rosenkranz test) (73). In the CV-1 cells the initial lesion was repaired, but this repair was not accompanied by unscheduled DNA-synthesis. This suggests that excision repair is not involved (25) in this case.

Definitive evidence that formaldehyde may induce mutations in mammals in vivo has not been found. Tests for the induction of sister-chromatid exchanges in mouse bone-marrow cells gave equivocal results (21). Dominant lethal tests in ICR - Ha Swiss mice were reported to be negative with doses up to 40 mg/kg (27) (28) whilst a more recent study in Q-strain mice showed effects except during the first week and in the third week, after treatment of males with 50 mg/kg formaldehyde (74). A chromosomal analysis at metaphase 1 failed to reveal any formaldehyde-induced chromosomal lesions (74). However, alkylation of sperm heads has been reported following treatment of mice with 1 to 150 mg/kg bw formaldehyde (29).

2. MECHANISM OF FORMALDEHYDE-INDUCED GENETIC EFFECTS

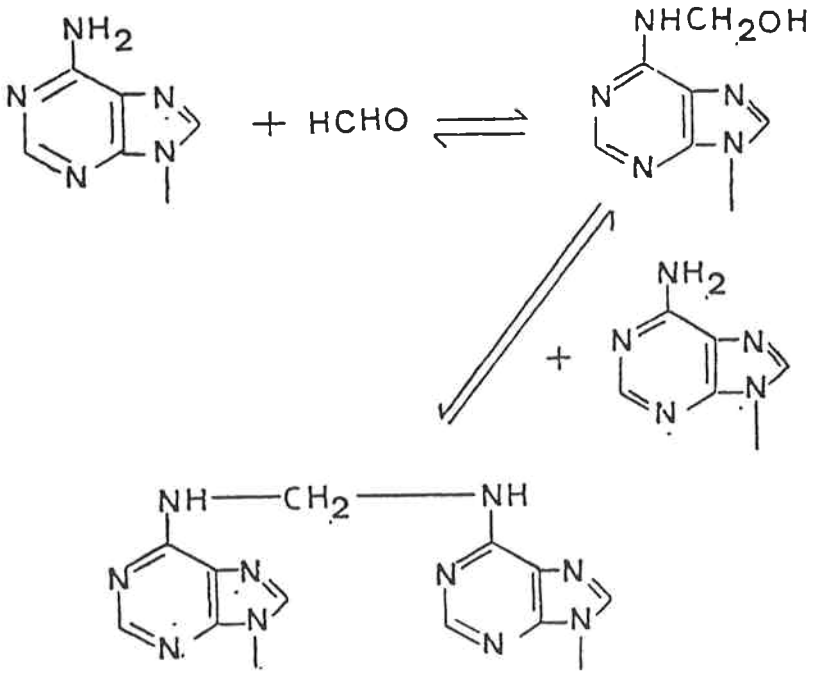
Biologically the most significant reaction of formaldehyde is that with amino groups. This results in the formation of unstable amino-methylol derivatives which may then undergo condensation reactions as shown below.

$$R\text{-}NH_2 + HCHO \rightleftharpoons R\text{-}NH.CH_2OH \quad \text{(REACTION 1)}$$

$$R\text{-}NH.CH_2OH + R^1\text{-}NH_2 \rightleftharpoons R\text{-}NH.CH_2.NH\text{-}R^1 + H_2O \quad \text{(REACTION 2)}$$

In aqueous solutions reaction 1 proceeds rapidly whilst reaction 2 , occurs relatively slowly.
Formaldehyde may react with amino groups in amino acids, proteins, nucleotides, nucleosides and nucleic acid (30). Reactions with proteins, which result in the formation of inter- and intra-molecular cross-links, are responsible for the fixative properties of formaldehyde and for its ability to inactivate bacterial toxins. They are also most probably responsible for the cytotoxicity of formaldehyde. Reaction with nucleic acids (particularly DNA) is, however, most probably responsible for the genetic effects

of formaldehyde. In this respect it should be noted that formaldehyde will react with RNA, and single-stranded DNA, but not with double-stranded DNA. The reaction occurs mainly at the amino group of purine nucleotides, pyrimidine bases being much less reactive. Formaldehyde reacts most readily with the amino group on carbon atom 6 of adenine, a reaction which can result in the formation of adenine dimers (as shown below). Although the condensation reaction occurs very slowly between the free bases in aqueous solution, it could probably occur much more rapidly in a structurally more organized nucleic acid.



As mentioned previously, adenylic acid or adenosine are obligatory for the mutagenic action of formaldehyde on Drosophila larvae fed a chemically-defined diet. This suggests that, in this case, 6-hydroxymethylamino-

adenosine (or 6-hydroxylmethylamino adenylic acid) formed in the food may be incorporated into the DNA of the larval spermatocytes, and so induce the mutations. However, as discussed previously, many findings demonstrate that the sensitivity of formaldehyde-induced mutagenesis is greatest during DNA replication, which is the only period in which large amounts of single-stranded DNA are found in the cell. Formaldehyde reacts with single-stranded DNA, but not with double-stranded DNA. Therefore, the high sensitivity of replicating cells is consistent with a direct interaction of formaldehyde with the DNA. Thus, it is possible that formaldehyde reacts with amino groups of adenine bases at the point of replication, forming hydroxymethylamino derivatives which may subsequently form dimers, resulting in the cross-linking of DNA strands or intra-strand linkage of purine base. Alternatively, it may react with nuclear proteins, forming cross-links between DNA and protein. All of these reaction products would have mutagenic potential.

C. FORMALDEHYDE METABOLISM

Biologically, formaldehyde is rapidly converted to formate. The half-life of formaldehyde in the blood has been estimated to be about 1.5 min or less in rats (43)(44), guinea pigs, rabbits and cats (44). The rapid conversion of formaldehyde to formate has been demonstrated in many tissues, including human erythrocytes, liver and brain; sheep liver; rat brain, kidney and muscle; and bovine brain and adrenals (45),(46). The reaction is catalysed by :

a) The glutathione and NAD^{+} dependent formaldehyde dehydrogenase (46-49).
b) Non-specific aldehyde dehydrogenases
c) Reaction with tetrahydrofolic acid (50)
d) The peroxidative activity of catalase.

In man, formate is the major excretion product of formaldehyde but in the rat the formate is further oxidised to CO_2.

D. THE EFFECTS OF CHRONIC EXPOSURE TO FORMALDEHYDE

1. RESULTS OF ANIMAL STUDIES

Groups of rats (Fischer 344) and mice (B6C3F1) (120 males and 120 females per group) were exposed to 2, 6 and 15 ppm formaldehyde vapour for 24 months, 6 hours/day, 5 days/week. After 6, 12 and 18 months, 10 animals per group were killed. At 24 months some animals in each group were maintained without treatment to assess the possible reversibility of the non-neoplastic changes caused by the formaldehyde exposure. The remaining animals were killed (31) (32) (33) (34). At the end of the 24-months exposure period, metaplastic and dysplastic changes of the respiratory epithelium of the nasal cavity were observed in rats at all dose levels and in mice exposed to 6 and 15 ppm formaldehyde. The severity and the extent of these lesions in the nasal cavity were related to the formaldehyde concentration and duration of exposure. Changes were first seen in the anterior region of the nasal cavity. Changes in the deeper regions of the nasal cavity were only observed after extensive exposure to high formaldehyde concentrations. Animals maintained for 3 months after the 24-month exposure period showed

some regression of the metaplasia, particularly in the mice.
The histopathological study is not yet completed. A high number of squamous cell carcinomas in the nose was observed. The following Table 1 gives the incidence of nasal tumour in rats and mice as presented during the CIIT conference (33).

Table 1

Dose (ppm in air)	Number of nasal tumours/ Number of animals at risk (rat)	(mouse)
In the control groups no nasal tumours were observed.		
2 (2.1) *	0/205	0/220
6 (5.6) *	3/204	0/220
15 (14.1)*	95/220	2/85

* Average measured concentrations.

In another life-time study (39), squamous cell carcinomas were also observed in the nasal cavities of S.D. rats exposed by inhalation 6 hours/day, 5 days/week to a mixture of formaldehyde (14.7 ppm) and hydrogen chloride (10.6 ppm). Of a group of 100 male animals, 27 developed nasal tumours of which 25 were squamous carcinomas and 2 were papillomas. Bis-(chloromethyl) ether (BCME) was detected in the inhalation chamber at a concentration of 1 ppb. It is not clear whether the formation of these low levels of BCME had any influence on the development of tumours bearing in mind that in other studies (40) different types of tumours were observed following exposure to BCME alone.

The first experiment (38) which indicated a possible tumorigenic action of formaldehyde involved sub-

cutaneous injection of aqueous formaldehyde (0.4%, 1 ml per week for 15 months). Two of the 10 treated rats developed injection site sarcomas. Sarcomas were also seen in the liver and omentum of 2 other rats. Because of the lack of controls, and inappropriateness of the route of administration, the significance of these results is questionable.

From an unpublished study cited by the National Academy of Science (53) and by the Federal Panel on Formaldehyde (72), the following information is available. No treatment-related tumours were observed in 88 Syrian hamsters exposed to 10 ppm formaldehyde for 5 hrs/day, 5 days/week for their lifetime (average 18 months) (37). A second study with 50 ppm exposure levels is also mentioned but the quoted details of the experimental design differ significantly according to the information source (72) (53).

A group of 60 C3H mice was exposed to 40 ppm formaldehyde for 35 weeks, 1 hour/day, 3 times/week (36). After this period, 36 of these animals were further exposed to 125 ppm formaldehyde vapour for an additional period up to 33 weeks. Basal cell hyperplasia, stratification of the epithelium and squamous cell metaplasia in the trachea and the major bronchi were observed, but no tumours were found.

Studies (41) with hexamethylene tetramine (HMT) are also significant because this compound can decompose to formaldehyde and ammonia. Three strains of mice were given up to 1 % HMT in the

drinking water for 60 weeks; the group size varied between 30 and 100 animals per sex. Additionally, a group of male and female Wistar rats (48 per sex) were given 1% HMT for 104 weeks in the drinking water. No carcinogenic activity was observed in any of the groups.

The studies mentioned above have shown that repeated injection (38) or prolonged inhalation (31)(33)(39)(71) of formaldehyde at doses which provoke severe irritation can result in tumour developments in the irritated region. The response at different concentrations appears to be species-dependent (33)(37)(71). Repeated brief exposure by inhalation to high concentrations showed signs of irritation, but no tumour development (36). The study with HMT (41) suggests that oral intake of compounds which may release formaldehyde without significant irritation does not result in tumour formation.

2. MECHANISM OF FORMALDEHYDE CARCINOGENICITY

Further investigations of the mode of action of formaldehyde are being performed by CIIT and the results available from these studies indicate that many different processes are involved in the tumour development. It was shown that mice were more sensitive than rats to respiratory irritation by formaldehyde. Thus, reduction of the minute volume on inhalation of formaldehyde was much more marked in mice than in rats, leading to a better physiological defense mechanism in mice. It was also shown that radiolabelled formaldehyde was mainly retained in the nasal mucosa following inhalation. This

suggests that tumour induction is a local effect. When the dose received after exposure to 15 ppm was related to the surface area of the nasal epithelium, the values calculated for mice were about half of those calculated for rats. Therefore the amounts of formaldehyde actually effective per unit area in mice exposed to 15 ppm were similar to those of rats exposed to 6 ppm, which is in accordance with the tumour incidence in these two groups. Thus, mice and rats appear to be equally susceptible to the carcinogenic effects of formaldehyde when related to the actually effective amounts. This also indicates that the action of formaldehyde depends not only on the atmospheric concentration but also on the local dose in the respiratory tract which is influenced by various factors such as mode of breathing, minute volume, defence mechanism, etc.

Although it has been demonstrated that formaldehyde has a very short half-life in blood, the half-life of the total radio-activity after intravenous administration of labelled formaldehyde was shown to be relatively long. Toxicokinetic studies showed that formate and formaldehyde behaved very similarly. This indicates that the radioactivity from the formaldehyde which remained within the animals was the result of metabolic incorporation, and most probably not of the alkylation of macromolecules which is thought to be one of the steps necessary for tumour formation.

As stressed previously, formaldehyde will react only with single-stranded DNA, thus making replicating cells much more sensitive to formaldehyde-induced mutagenicity. In this respect it is of interest that exposure of rats to 15 ppm formaldehyde for several days resulted in a marked increase in the number of cells undergoing division within the nasal mucosa. This rapid cell replication is probably due to cell replacement arising from the cytotoxicity of formaldehyde which was shown to destroy nasal epithelium during the exposure. Squamous cells were reported to be less sensitive to formaldehyde-induced cytotoxicity, and thus formaldehyde may also selectively stimulate replication of resistant cells (34). Faber has suggested that selection of cells more resistant to cytotoxicity is important in liver tumour pathogenesis (42).

The experiments reported at the CIIT meeting (34) also indicated that formaldehyde induced DNA-protein cross-linking and possibly DNA-DNA cross-linking. This DNA damage was rapidly repaired. Therefore, rapid cell division would be required to allow interaction of the formaldehyde with the DNA and to fix the resulting DNA damage as a mutation before the lesion has been repaired (41). Thus, when all these factors are considered it becomes apparent that formaldehyde carcinogenicity involves many different processes, e.g. cellular uptake, physiological compensation (breathing rate depression, increased mucous secretion) metabolic inactivation, local irritation, cell replication,

mutagenic activity, DNA repair and regression of the metaplasia (scheme 1). It should also be borne in mind that formaldehyde is a normal metabolic product.

Finally, it should be stressed that the experimental results suggest that the metaplastic change is at least in part reversible. A full evaluation of the findings presented at the CIIT Conference must await completion of the studies. However, if chronic irritation is taken as a prerequisite for tumour development, then it is possible that no tumours would develop at sub-irritant concentrations. To date, the results of limited epidemiological studies have not indicated any increased tumour incidence in exposed human populations.

3. MATHEMATICAL EXTRAPOLATION TO LOW EXPOSURE LEVELS

At the CIIT Conference (70)(71) some results of low-dose extrapolations were presented in which various mathematical models were applied to the animal data. Extrapolations based on multi-stage models indicated that concentrations in the range 1-2 ppm and 0.1 - 1 ppm could result in one additional tumour per 10^5 and 10^8 exposed, respectively.

Linear extrapolation resulted in concentrations several orders of magnitude lower for the same levels of risk. The estimates from the multi-stage model correspond approximately to the threshold for sensory irritation in man.

All data so far support the hypothesis that tumour formation results from a multistage process, and that any extrapolations must take into account every single process and not only the final event (tumour formation) as such. It is, however, the view point of ECETOC that no