```
PROG:

2
AU  - Lucier GW ; Lee IP ; Dixon RL
AD  - Natl. Inst. Environ. Health Sci., NIH, Research Triangle Park, N. C.
TI  - Effects of environmental agents on male reproduction
SI  - CA/088/032593D
SO  - Testis; VOL 4., 1977.577-604
LA  - ENG
AB  - CBAC  COPYRIGHT: CHEM ABS  A review with many refs. of the effects
      of pesticides, food additives and contaminants, industrial chems.,
      and air pollutants on male reprodn.

SS 2 /C?
USER:
stop Y
TIME  0:03:10     NLM TIME 15:07:19
. . .

PROG:

GOOD-BYE!
301 20 DISCONNECTED 00:00:03:22 47 18

@
```

COMPLETE ARTICLE CAN BE FOUND IN TIER I BOOK

Title ---> Formaldehyde	K-2574-(100),	Purple copy pp. 317-349
Acetaldehyde	K-2575-	
Propionaldehyde	K-2355-	
Butyraldehyde	K- 692-	
Acrolein	K-2576-	
Crotonaldehyde	K- 693-	
Benzaldehyde	K- 715-	pp. 317-326, 343-349
Furfural	K -688-	
Glyoxal	K-5489-	
Malonaldehyde	DR-0167-5827-	
Glutaraldehyde	K-20301-	
Chloral Hydrate	K-2577-	
Hexamethylene Tetramine	K-3057-	
Ethyl Alcohol	K-2544-	
Vinyl Chloride	K-1711-(1977)	

Mutation Research, 39 (1977) 317--362

GENETIC AND CYTOGENETICAL EFFECTS OF FORMALDEHYDE AND RELATED COMPOUNDS

C. AUERBACH [1], M. MOUTSCHEN-DAHMEN [2] and J. MOUTSCHEN [2]

[1] *Institute of Animal Genetics, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JN (Great Britain) and* [2] *Laboratoire de Génétique, Université de Liège, Rue Forgeur, 15, B-4000 Liège (Belgique)*

(Received February 9th, 1977)
(Accepted March 2nd, 1977)

Contents

Historical outline

The cytological applications of formalin have been known since 1892 (ref. [179]). A lot of publications on its use as a fixative agent in mixtures for animal and plant cells have been written. More recently, it has been suggested to replace formalin by other, less strong aldehydes especially glyoxal and glutaraldehyde, this latter substance principally in the field of electron microscopy.

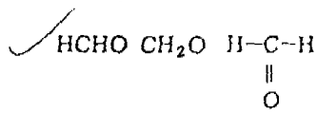
At lower concentrations than those used in Cytology, the action of formaldehyde on viral nucleoproteins has been amply exploited in the preparation of vaccines. Subsequently, it has been used as an inactivating agent of tobacco mosaic virus (TMV) [52,171] and transforming factor [188,189].

Genetical research on formaldehyde started in 1946 with Rapoport's publication on the production of sex-linked lethals in Drosophila larvae fed on medium containing formalin [135]. Until the middle fifties, this research was followed up vigorously in several laboratories (section Ca) and was extended also to a few other aldehydes (section Cb). With the advent of the Watson-Crick era, interest shifted to chemical mutagens whose action at the molecular level could be more easily understood than that of formaldehyde. Towards the end of the fifties, research on formaldehyde mutagenesis had almost completely stopped. This has changed only recently. Over the last years, there has been renewed interest in the genetical effects of formaldehyde and other aldehydes. This has various reasons. First, the widespread use of these substances and their derivatives in industry and medicine (section D) calls urgently for an assessment of their potential hazards. Second, the reactions of formaldehyde with nucleotide bases and nucleic acids are now fairly well understood [49] and find applications in studies on the molecular structure of DNA and RNA. This should facilitate the formulation and testing of hypotheses on the molecular nature of genetic damage by formaldehyde. Finally, the recent discovery that, in micro-organisms, formaldehyde-induced genetical effects are subject to excision repair (section Ca) removes this substance from its isolated position and opens the way to analysis by means that have been successful in the study of other chemical mutagens.

(A) The molecules

The following list of compounds is not exhaustive. It deals only with substances that are wide-spread in the human environment and appear important from the point of view of genetic toxicity.

Monofunctional saturated



Methanal; formaldehyde; methylaldehyde; oxomethane; methylene oxide; oxymethylene; formic aldehyde.

CH_3CHO
Ethanal; acetaldehyde; acetic aldehyde; ethylaldehyde.

CH_3CH_2CHO
Propanal; propionaldehyde; propylaldehyde; methylacetaldehyde.

$(CH_3)_2CHCHO$
2-Methylpropanal; *iso*butyraldehyde; *iso*butyric aldehyde.

$(CH_3)_3CCHO$
2,2-Dimethylpropanal; pivaldehyde; pivalaldehyde.

$HOCH_2CHOHCHO$
2,3-Dihydroxypropanal; glyceraldehyde; glyceric aldehyde; α,β-dihydroxypropionaldehyde.

$CH_3(CH_2)_2CHO$
Butanal; butyraldehyde; butylaldehyde.

$CH_3(CH_2)_3CHO$
Pentanal; valeraldehyde; valeric aldehyde; valeral.

$(CH_3)_2CHCH_2CHO$
2-Methylpentanal; isovalderaldehyde.

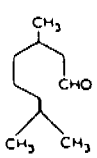
$CH_3(CH_2)_5CHO$
Heptanal; heptylaldehyde; heptaldehyde; aldehyde C-7.

$NH_2(CH_2)_4NH(CH_2)_2CHO$
Oxidized spermidine.

$CH_3CH(OC_2H_5)COCHO$
Kethoxal; β-ethoxy-α-ketobutylaldehyde.

Monofunctional unsaturated
$CH_2 = CHCHO$
2-Propenal; acrolein; acrylic aldehyde; acrylaldehyde; acraldehyde.

$CH_3CH = CHCHO$
Trans-2-butenal; crotonaldehyde; crotonic aldehyde; β-methylacrolein.

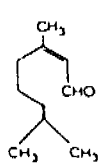


3,7-Dimethyl-6-ortenal; citronellal; d-rhodinal (a mixture of stereoisomeric aldehydes):

$CH_2 = C(CH_3)(CH_2)_3CH(CH_3)CH_2CHO$

$(CH_3)_2C = CH(CH_2)_2CH(CH_3)CH_2CHO$

$(CH_3)_2COH(CH_2)_3CH(CH_3)CH_2CHO$
Hydroxycitronellal (a saturated derivative).

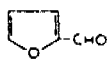


3,7-Dimethyl-2,6-octadienal; citral (*from natural sources*) = a mixture of 2 geometric isomers : geranial and neral :

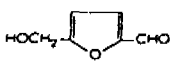
$(CH_3)_2C = CH(CH_2)_2CCH_3$ (HCCHO) — citral a = geranial

$(CH_3)_2C = CH(CH_2)_2CCH_3$ (OHCCH) — citral b = neral

Monofunctional (cyclic compounds)

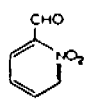


Furfural; fural; 2-furaldehyde; pyromucic aldehyde; 2-furancarboxaldehyde

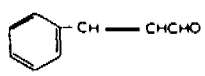


5-(hydroxymethyl)-2-furfural; 5-(hydroxymethyl)-2-furfuraldehyde; 5-(hydroxymethyl)-2-furancarbonal; 5-hydroxymethyl-2-formylfuran; HMF.

Benzenecarbonal; benzaldehyde; benzoic aldehyde; benzenecarboxaldehyde.



o-Nitrobenzaldehyde.



3-Phenylpropenal; cinnamaldehyde; cinnamic aldehyde; β-phenylacrolein; cinnamal.

Monofunctional with heterologous atoms

$ClCH_2CHO$

2-Chloro-1-ethanal; chloroacetaldehyde; monochloroacetaldehyde.

$Cl_3CCHO \cdot H_2O$

Chloral hydrate; trichloroacetaldehyde monohydrate; 2,2,2-trichloro-1,1-ethanediol.

Bifunctional

OHCCHO

Ethanedial; glyoxal; biformyl; diformyl; oxalaldehyde.

CH_3OCCHO
2-oxo-propanal; methyl glyoxal (monofunctional derivative).

$OHCCH_2CHO$
Propanedial; malonaldehyde.

$OHC(CH_2)_3CHO$
Pentanedial; glutaraldehyde; glutaric aldehyde.

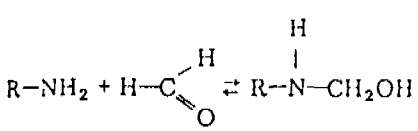
$OHC(CH_2)_3NH(CH_2)_4NH(CH_2)_3CHO$
Oxidized spermine.

The natural or man-made distribution of aldehydes in the human environment will be considered in section D.

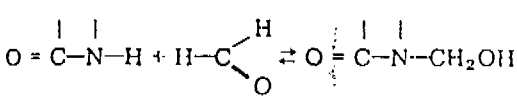
(B) Chemical reactions

The reactions of formaldehyde with various components of increased complexity and of biological importance have been exhaustively reviewed by Feldman [49] to whom we refer here. The reactions of other aldehydes have so far been much less investigated. Only those reactions likely to play a part in the production of genetical effects will be reviewed. Various hypotheses on the mechanism of formaldehyde mutagenesis have been based on these chemical findings; they will be discussed in section Ca.

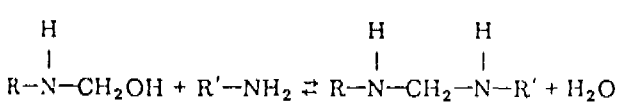
With formaldehyde, reactions with amino groups are certainly the most likely to occur in biological materials. These reactions can be summarized as follows:



(I)



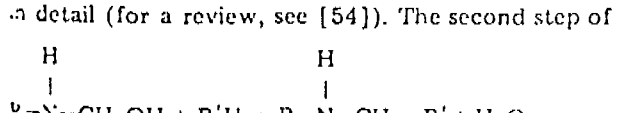
(II)



(III)

(Ba) Reactions with amino acids and proteins

Reaction (I) occurs with the amino groups of amino acids and polypeptidic chains of proteins. The first step involves the formation of unstable methylol derivatives. The kinetics of the reactions with amino acids has been investigated in detail (for a review, see [54]). The second step of the reaction is as follows:



(IV)

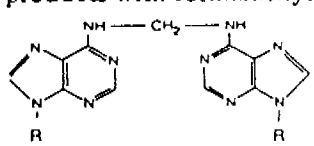
This slow step yields stable condensation products. The condensation reaction of formaldehyde with proteins is proved, among other things, by the fact that after treatment of casein with formaldehyde vapours, water is eliminated [121]. The possibility of a formation of methylene bridges with proteins has been long debated. Under conditions close to physiological ones, the reaction of formaldehyde gives intermolecular crosslinks, but the opinions of the researchers differ somewhat in regard to the groups actually involved in the secondary reaction.

In view of the important part played by enzymes in mutation and chromosome breakage, the reactions of formaldehyde with these molecules are of special significance for this review. This refers in particular to catalase which according to two investigations [172,175] is inhibited by formaldehyde while in a third [180] this could not be observed. The reaction of 2-chloroacetaldehyde with the sulfydryl group of glutathione and cysteine of rat liver has also been demonstrated [70,71].

(Bb) Reactions with nucleosides, nucleotides and nucleic acids

Reaction (II) occurs with nucleosides, nucleotides and nucleic acids. Reaction (III) takes place only with amino purines. Reaction (II) can occur with the groups —CO—NH of pyrimidine and purine heterocycles.

As regards the biological action of formaldehyde, the reaction with —NH groups in position 9 or 7 of the heterocycle ring of purines is especially significant. Monomethylol derivatives involved in reaction (II) are only intermediary labile products, which by secondary reaction give rise to stable methylene structures according to reaction (IV). In particular, the reactions of formaldehyde with compounds of the adenine series have received considerable attention. With AMP, a stable product methylene-bis-adenylic acid (V) is formed. In contrast, pyrimidine derivatives do not form stable condensation products with formaldehyde.



R = ribose phosphate

The kinetics of the reactions with purine has been carefully investigated (see [49]). The rate of the second step leading to methylene compounds is much slower than that of the first one: e.g. for adenosine, at room temperature and optimal conditions, more than a week is needed to get a negligible amount of the methylene compound, whereas the equilibrium of the first step is reached within 70 h.

The reactions of formaldehyde with DNA are less well known than those with nucleosides and nucleotides. Native double-stranded DNA does not react with formaldehyde. Treatments of DNA which destroy the hydrogen bonds allow the reaction to occur; after this reaction, DNA is not any more capable of renaturation. There is evidence that in a variety of conditions only unwound sites of DNA can react with formaldehyde and that these sites are not randomly distributed but occur in a rather regular fashion.

The primary reaction of formaldehyde with both nucleic acids, resulting

to monomethylol derivatives, occurs as it is the case for smaller molecules. The evidence is not so strong for the secondary reaction. However, from the existence of a fraction of formaldehyde firmly bound to DNA it can be inferred that methylene bridges actually occur between purines, though there are still no methods available to isolate these bridges from DNA.

Among other aldehydes, 2-chloroacetaldehyde is known to react with nucleosides in a specific way, forming fluorescent cyclic derivatives by acting on the N-1 and N^6 nitrogens of adenosine and the N-3 and N^4 nitrogens of cytidine [27,85,90]. It can also react with DNA in single stranded regions. This reaction may be involved in the mutagenic effect of chloroacetaldehyde ([100]; section Cc).

Binding of methylglyoxal to tRNA and DNA has been demonstrated [89]. Dicarbonyl compounds such as glyoxal, kethoxal and pyruvaldehyde interact with nucleic acids or their components in a different way from formaldehyde, possibly by two successive reactions with two carbonyl groups. In particular they do not form bridges between two bases (quoted in [49]). Malonaldehyde has been reported to react with guanine and cytidine and to cross-link DNA [33]. Particularities of reactions with oxidized spermine will be discussed below.

(Ec) Reactions with nucleoproteins

In reactions of aldehydes with nucleoproteins, steric factors should be taken into account, since they may accelerate these reactions. Special attention has been given to nucleohistones. It has been shown in pea buds that proteins closely associated with DNA are completely linked to it even by mild treatments with formaldehyde [34], whereas under comparable conditions DNA itself is not cross-linked [53]. All the data suggest that methylene bridges between nucleic acid and protein are responsible for formation of a complex with increased stability to heating (references in [49]).

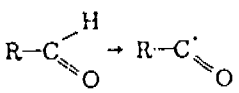
As models of the reaction with nucleoproteins, experiments in which various amino acids or lysine-rich histones were mixed with aldehydes during their reaction with nucleotides or DNA are of special interest. In the first experiments [153], the following amino acids were mixed with deoxynucleotides or DNA during treatment with formaldehyde : L-histidine, DL-valine, DL-alanine, DL-lysine and glycine. It was shown that these amino acids are involved in the reaction of formaldehyde with nucleic acid components, since the reaction products contained the amino acid residues. In addition, it was found that the rate of reaction of formaldehyde with DNA was enhanced in the presence of lysine-rich proteins [153]. The kinetics of the reaction of glycine with nucleotides, deoxynucleotides or DNA was investigated in further experiments with similar results [154]. These investigations demonstrated that methylol derivatives of the glycine or lysine-rich histones are first formed within a short time (several seconds) and interact secondarily with nucleotides or DNA. The stability of the end product depends on the nature of the amino acid and the nucleotides. The stability of products of the reactions with lysine seems greater than that of complexes with other amino acids [153]. With glycine stability is greater for GMP than for other nucleotides. The reactions with DNA when amino acid or a lysine-rich fraction of histone are added occur according to the

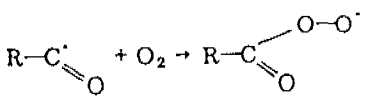
same general model. However, breaks appear at a rather high rate [153,154] in the sugar-phosphate moiety of the double helix. They produce an acid-soluble fraction of degraded molecules [154]. Some conclusions drawn from the experiments on formaldehyde interactions could be extended to other aldehydes, in particular in regard to the interactions with DNA [130].

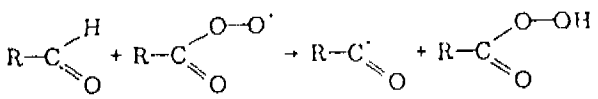
Other researchers [186] are also of the opinion that the most dramatic action of formaldehyde is to induce DNA—protein cross-links some of which bind to the two strands of DNA. It should be remembered that the in vitro experiments monitored for purely chemical effects were intended to simulate in vivo conditions. Recent experiments with bacteria actually confirmed the conclusions drawn from in vitro ones [130].

(Bd) Formation of peroxides and peroxidic radicals

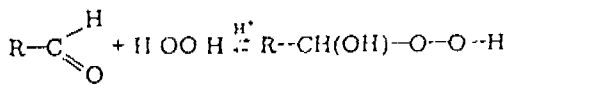
It has long been shown that under several experimental conditions when oxidizing molecules are available, aldehydes can form reactive hydroxyalkyl peroxides and/or free radicals. The general reactions are as follows:

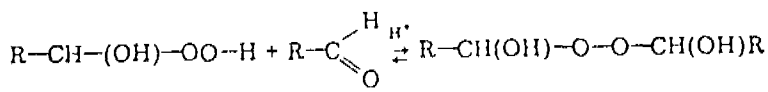

Free radical (VI)


free peroxy radical (VII)

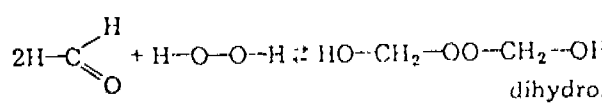

Peracid (VIII)

With H_2O_2 the reactions are:


(IX)


(X)

In the particular case of formaldehyde:


dihydroxycimethyl peroxide (XI)

In vitro, a polymerization reaction of formaldehyde can occur, but the condensation reaction is not energetic enough to explain biological effects [87].

(Be) Precursor molecules

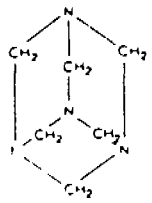
In certain circumstances, some molecules can be considered as precursors of

aldehydes. They include

Hexamethylene tetramine (urotropin)

Condensation reactions of formaldehyde with ammonia can give rise to this compound. This reaction (XII) is reversible, and at acidic pH's formaldehyde is slowly liberated.

$$6CH_2O + 4NH_3 \underset{H^+}{\overset{OH^-}{\rightleftharpoons}}$$



(XII)

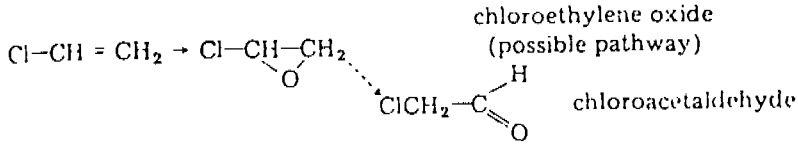
Ethyl alcohol

There has long been evidence that acetaldehyde is an intermediary product in ethyl alcohol metabolism. In vitro, it can be easily isolated as the first oxidation product (XIII), and it is found in vivo in the blood during alcohol metabolism. Acetaldehyde is very readily oxidized in vivo in acetic acid.

$$CH_3{-}CH_2OH \xrightarrow[-2H]{} CH_3{-}CHO \qquad \text{(XIII)}$$

Vinyl chloride

The mutagenic and carcinogenic effects of vinyl chloride have been attributed to reaction products formed in the liver by metabolic activation [134]. One of these products is 2-chloroacetaldehyde. The most possible route is indicated below (XIV). Other routes are considered unlikely in vitro [57].

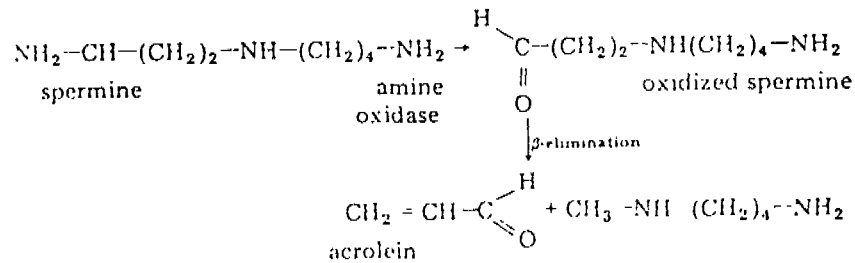


(XIV)

Both metabolites appear in urine of man and rats exposed to vinyl chloride [65].

Polyamines

Spermine and spermidine can give rise to aldehydes by oxidation, being transformed respectively into oxidized spermine (a dialdehyde) or oxidized spermidine (a monoaldehyde (Section A)). These are unstable substances which readily decompose by β-elimination giving acrolein (XV).



(XV)

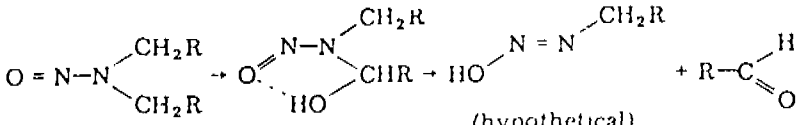
There is certainly a potential risk due to the appearance of this aldehyde (section Cb). Treatments of DNA with oxidized spermine result in covalent cross-links of complementary chains which do not occur with oxidized spermidine, suggesting that both carbonyl groups are needed for cross links [23,24]. The reaction of oxidized spermine is not specific for DNA since it can react with all bases containing amino groups and to a much lesser extent with thymine and uracil [24,44]. No firm conclusion can yet be drawn about the mechanism of reaction of these aldehydes.

Peroxidized polyunsturated fatty acids

The oxidative decomposition of polyunsaturated fatty acids produces the three-carbon dialdehyde : malonaldehyde. The mutagenic effects of this substance will be dealt with in Section Cb.

Dialkylnitrosamines

Aldehydes are generated after oxidative monodealkylation by microsomal enzymes. They are thought to be rapidly further oxidized to yield CO_2 (for a survey, see [107]).



nitrosamine (hypothetical)

Triazenes

It has been shown that 1-phenyl 3,3-dimethyltriazenes was dealkylated to formaldehyde and probably monomethyltriazene by isolated microsome fractions from rat liver or to other organs, but only in presence of a NADPH-generating system. The ultimate mutagen reacts with DNA like an alkylating agent [132].

(C) Genetical and cytological effects

(Ca) Formaldehyde K-2574

We start by discussing the findings on Drosophila that had been given formaldehyde in the food (FF), because these are by far the most numerous and most fully analysed ones. Results obtained by exposure of Drosophila and other organisms to vapour or aqueous solutions of formaldehyde, and by combination treatments between formaldehyde and other mutagens will be dealt with subsequently.

Treatment of Drosophila with formaldehyde-food (FF)

The first to test FF for mutagenic effects on Drosophila was Rapoport [135]. He transferred eggs or young larvae (up to 48 h old) to food containing a "sublethal" admixture of formaldehyde. The emerging males were tested by the CIB method and yielded about 6% lethals, in some experiments more. Kaplan repeated these experiments with similar results [73]. He transferred larvae

and strange enough, this denaturation process is prevented by some cations such as sodium. It is difficult to say to what extent these in vitro findings can be extrapolated to in vivo situations. A clear picture of the relative importance of the different mechanisms of action does not yet emerge. A possible way to tackle this question would be to look for correlations between the effects of the drug on cellular processes and its well-known anaesthetic action.

(Cc) Precursor molecules as potential mutagens

Hexamethylene tetramine (urotropin)

This substance, its salts and other substances of the same type as amphotropin, helmitol and anotropin were suggested as potential mutagens. Rapoport [135,136] states that these substances can produce mutations in Drosophila when mixed with the larval medium at much higher concentrations than those used in therapy.

Ethyl alcohol

Ethyl alcohol by itself has a chromosome breaking action in *Vicia faba* [104] and induces specific X-chromosome breakage in the grasshopper *Phloeoba antennata* but not in another species *Oxya velox* [98]. Since there is a great variety of response between organisms, possibly due to different metabolic pathways, it is not yet possible to evaluate which effect can be attributed to acetaldehyde.

Vinyl chloride

The mutagenic and carcinogenic effects of vinyl chloride have been reviewed [29]. Chloroethanol can be considered as an intermediary step leading to chloroacetaldehyde. The toxicity of chloroethanol in the rat was attributed to chloroacetaldehyde [71]. The mutagenic effects of one of its metabolites, chloroacetaldehyde, are described in section Cb.

Polyamines

As mentioned before (section Cb), the aldehydes produced by oxidation of spermine and spermidine are toxic for a certain number of organisms. Since the decomposition of these products can give rise to acrolein by β-elimination, this creates a potential hazard. However, without oxidation, polyamines are not likely to be mutagenic. In fact, spermine has been found to be an antimutagen [72].

Peroxidized polyunsaturated fatty acids

These substances have not yet been tested as such for genetical effects. The effects of one of its metabolites, malonaldehyde, are described in Section Cb.

Dialkylnitrosamines and triazenes

As compared with the strong mutagenicity of the activated metabolites obtained in the reactions with microsomes it is unlikely that the amount of aldehydes produced can induce a significant part of the mutations observed [107,132].