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Draft

Annex 1 to MUT/04/14

## COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

### Genotoxic Carcinogens and DNA Repair at Low Doses:

#### Introduction:

This review was requested following the CoM's consideration of a position paper on genotoxic carcinogens and the impact of DNA repair at low doses (MUT/04/3). This in turn had been prompted by the CoC discussions of a horizon scanning paper in which the validity of the hormesis concept (occurrence of 'U' or 'J' shaped dose response curve) in toxicology was considered.

In the Nature paper, Calabrese and Baldwin (2003) argue that assuming dose response curves are linear or have a threshold, when assessing carcinogens or other toxicological end-points respectively, is incorrect and that the hormetic dose response curve is the 'norm' not the exception, and can be detected in 40% of toxicological studies which are performed with an appropriate protocol. The phenomenon is believed to be a consequence of low-dose stimulation/high-dose inhibition or via an overcompensation response to an initial disruption of homeostasis. Although it is stated that this is a broad-based, generally applicable principle (Calabrese and Baldwin 2002, 2003), only generalised dose response plots accompany these statements and there is very little specific data presented or indeed referenced. The quantitative features of the 'J' shaped deviation from linear in a typical hormetic response may only be 30-60% of control value and the range of doses in which stimulation occurs is generally less than a factor of 20.

Considerations for risk assessment include the possibility of the stimulation of DNA repair following low doses of mutagens and it is that possibility that is addressed herein. The current view is that, in general, thresholds do not exist for in *vivo* mutagenesis, and if there is data that suggests their existence, that this should be supported by appropriate evidence on a case-by-case basis. If hormetic responses were evident than a concept of a threshold would follow logically.

This paper aims to appraise data collected from a limited review of the literature in which the effects of low doses of mutagens on DNA adduct formation, mutation rates and the significance of different DNA repair mechanisms are assessed. The search concentrated on the low molecular weight compounds N-nitroso-N'-methyl-N-nitrosoguanidine (MNNG), ethyl methane sulphonate (EMS) and methyl methane sulphonate (MMS) and a series of papers were singled out and reviewed. These include

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evaluation of O<sup>6</sup>-methyl transferases (O<sup>6</sup>MT) in bacterial and mammalian cell systems (a DNA repair pathway known to be induced by low doses of mutagens and gives rise to response thresholds) and a limited number of other or unspecified DNA repair mechanisms. Some are papers which were published prior to a detailed understanding of the O<sup>6</sup>MT DNA repair system or as part of the pathway elucidation. Some papers are based on direct comparisons of DNA repair efficient with deficient strains/cells, from which an understanding of the importance of the repair system to the shape of the dose response curve can be assessed. However, there are only a few papers in which the premise for the research was specifically the role of the repair enzyme at low doses. Therefore for most reviews, relevant data has been extracted and evaluated in an attempt to provide the information required. Importantly, these data are most likely to indicate thresholds for mutagenic responses; the detailed low dose data needed to assess the presence or absence of a hormetic response were generally not available.

It is recognised that this literature assessment is not exhaustive by any means, that there are repair mechanisms and other appropriate genotoxins which have not been covered here, and more importantly, that *in vivo* systems have not been considered. However, it is hoped that this review will provide an appropriate springboard for further consideration of the relevance of the hormesis concept in genetic toxicology.

### **Investigations of low dose mutagens on O<sup>6</sup>-methyl transferase**

Investigations of the O<sup>6</sup>-methyl transferase (O<sup>6</sup>MT) DNA repair system supplied the vast majority of papers with data on the role of DNA repair at low doses, although this is likely to be in part due to the use of MNNG in the search strings. The repair process is inducible, commonly referred to as the 'adaptive response' (Samson and Cairns 1977). *E. Coli* has two methyltransferases repairing O<sup>6</sup>-MG and O<sup>6</sup>-MT, now known to be the products of the *ada* and *ogt* genes respectively. However there are known differences in the activity of these genes and hence proteins, in *E. Coli* and *S. Typhimurium* and again in mammalian cells. It must be noted that it is difficult to compare responses across papers due to the use of different 'wild type' strains which differ in their inherent DNA repair capacities. Therefore each paper has been assessed in isolation.

Olsson and Lindhal (1980), using a system in which labelled O<sup>6</sup>-methyl guanine residues generated by addition of <sup>3</sup>H- or <sup>14</sup>C-MNU to an *in vitro* system (*micrococcus luteus* DNA). The adaptive response induced by addition of MNNG to *E. Coli* B/r cells was identified and the resulting methyltransferase enzyme was isolated. It was concluded that the repair process has a finite capacity and thus the ability to remove only a fixed number of adducts. This in turn may give rise to an apparent threshold or plausibly a hormetic response.

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## Bacterial systems:

In a study designed to follow-up to Samson and Cairns' initial findings, Jeggo et al (1978) aimed to more closely define the nature of the inducible response. *E. coli* WP2 strains with defined mutations (eg *uvrA*, *lexA*, *rec* etc) were tested in MNNG-adapted and non-adapted states, to determine whether these gene products were involved in the induced pathway. Cultures treated with adaptive doses of 0.075-0.5 µg/ml (generally the highest dose which did not affect growth rate) were challenged with 1-100µg/ml MNNG, dependent on strain. In the wild type strain WP2, the mutation response was apparently linear (lowest dose tested 0.1µg/mL) for both the adaptive and challenge series of doses, although the absolute number of mutations was substantially reduced in the adapted cultures compared to the non-adapted cultures. Strains containing *lexA*, *polA1* and *recA56* mutations exhibited apparent thresholds for induced mutagenesis (at approximately 20, 2 and >10µgMNNG/ml respectively) as well as reduced overall responses (Figure 1). These thresholds were not apparent in a non-adapted state. In *lexA*<sup>-</sup> and *lexA*<sup>+</sup> strains, protection against killing by MNNG was afforded by prior incubation for both strains, which suggests that the SOS and MNNG adaptive pathway act independently. Mutation in *lexA*<sup>-</sup> is reduced significantly even though these strains are unable to induce the SOS functions.

Rebeck and Samson (1991) investigated the increased spontaneous mutation and alkylation sensitivity of *E. coli* strains lacking the O<sup>6</sup>-methylguanine (*ogt*) and O<sup>6</sup>-methylthymine (*ada*) DNA repair methyltransferases. Strains lacking *ogt* and both *ogt* and *ada* were more susceptible to MNNG induced mutagenesis, but all dose responses were linear to 0. In contrast wild type strains or those lacking *ada* only, exhibited a threshold for response in which resistance to MNNG- induced mutagenesis was observed at approx 0.1-0.3µg/ml (Figure 2). It may be inferred that *ada*, unlike *ogt*, is not inducible.

Todd and Schendel (1983) examined the relative roles of O<sup>6</sup> methylguanine methyltransferase induction (the adaptive response; invoked by MNNG pre-treatment of 3.3µM for 90min) and SOS repair (*uvrA* and *umuC* strains) in K-12 strains. Here Ethyl, Propyl, Butyl and Amyl NNG were looked at; no true threshold type effects were seen in any of the treated or pre-treated cultures, although varying effects on both cell killing and mutagenesis were seen in both non-adapted and adapted cultures. It is possible that the larger alkylating adducts do not respond to the MNNG-induced adaptive response.

A complex investigation described by Abril et al (1992) present data apparently in contrast to these findings. Herein, a series of *E. coli* K-12 strains with differing repair capacities were evaluated using a variety of alkylating agents with the aim of establishing the role of *ada*. A spontaneously arising culture, more susceptible to

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monofunctional alkylators, was compared to a series of further derived/manipulated strains as shown in the following table:

**TABLE I. *E. coli* Bacterial Strains**

Name	Relevant Genetic Characteristics	Construction
343/286	<i>arg56, nad113, Δ(uvrB-bio)</i>	Mohn et al. [1984]
UC376 <sup>a</sup>	as 343/286 but <i>araD81</i> , pKM101	Abril and Pueyo [1990]
UC581	as UC376 but pKM101 <sup>-</sup>	spontaneous
UC612	as UC581 but <i>umuC122::Tn5</i>	P1 ( <i>umuC::Tn5</i> ) × UC581
UC574	as UC581 but <i>uvrB</i> <sup>+</sup>	P1 (wt) × UC581
UC619	as UC581 but <i>ada10::Tn10</i>	P1 ( <i>ada10::Tn10</i> ) × UC581
UC1101	as UC376 but hypermutable to ethylating compounds	spontaneous
UC5710	as UC1101 but pKM101 <sup>-</sup>	spontaneous
UC351	as UC5710 but <i>umuC122::Tn5</i>	P1 ( <i>umuC::Tn5</i> ) × UC5710
UC575	as UC5710 but <i>uvrB</i> <sup>+</sup>	P1 (wt) × UC5710
UC2109	as UC5710 but <i>ada10::Tn10</i>	P1 ( <i>ada10::Tn10</i> ) × UC5710

<sup>a</sup>Previously named AUG101.

EMS induced a biphasic response with an apparent threshold at 100μmole/plate in a wild-type strain (UC376) in contrast to its hypermutable counterpart, UC1101 which exhibited a greatly increased mutation rate with no threshold (see attached paper [Appendix XX](#)). MMS and 4NQO were equally mutagenic to both these strains indicating the hypermutability (assumed to be *ogt*<sup>+</sup>) is specific to ethylating agents. A further set of experiments, utilising the ethylating and methylating agents EMS, MMS, DES, DMS, ENU, MNU, ENNG and MNNG4, assessed the role of the *ada* mutation, in a wild type and the hypermutable strains (all deficient in *uvrB*). Estimations of the dose response curves presented indicate the presence or absence of thresholds as follows:

- UC2109 (hypermutable *ogt*<sup>+</sup> and *ada*<sup>-</sup>) no thresholds for any compound tested and, in general exhibited significantly greater mutation frequencies
- UC5710 (hypermutable *ogt*<sup>+</sup> and *ada*<sup>+</sup>) exhibited thresholds for methylating agents only
- UC619 (wild type, pKM101<sup>-</sup> ie *ogt*<sup>-</sup>, *ada*<sup>-</sup>) produced apparent thresholds for all compounds tested
- UC581 (as UC619 but *ada*<sup>+</sup>) exhibited thresholds essentially for ethylating agents only

Although on first assessment these results appear to provide good evidence for the occurrence of thresholds, unfortunately, they are not consistent with results obtained in other studies. It is therefore difficult to ascertain with any certainty the relevance of *ogt* and *ada* mutations to the occurrence of thresholds. One conclusion drawn is that the biological significance of hypermutability capacity, associated with the *ogt* protein, is its ability to prevent mutagenesis by low levels of bulky ethylation products. However the significance of *ada* remains unclear. The paper is appended.

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Investigations of *Salmonella typhimurium* O<sup>6</sup>MT have been undertaken by Guttenplan and Milstein (1982) and Yamada and colleagues (1997). In the former study, the apparent ability of TA1535 to resist low dose MNNG-induced mutagenesis was examined by assessing O<sup>6</sup>-methylguanine and 7-methylguanine residues by HPLC analyses of hydrolysed bases. Formation of O<sup>6</sup>-MeG and measurement of mutation frequency exhibited a threshold effect at 8µM, followed by an approximate linear increase thereafter, whereas 7-MeG was linear throughout the dose range (Figure 3).

The aim of the study by Yamada et al (1997) was to produce new 'Ames' test type strains. TA1535 derivatives with a series of *ogt* and/or *ada* mutations were tested with a variety of mutagens (MNU, ENU, EMS, 4NQO, DMN, DEN). From assessment of the data presented, it appears that a threshold exists for ENU, when tested with TA1535, i.e. 'wild type'/presence of DNA *ada* and *ogt* repair (up to 100µg/plate). Concurrent evaluation of strains carrying *ada* and/or *ogt* mutations did not (Figure 4). It is possible that a threshold exists for MNU, although from the scale it is not possible to make this claim with absolute certainty. This phenomenon was not apparent for the other chemicals examined. In addition, the sensitivity of the *ogt* strains were much greater to ENU than in MNU-induced responses, suggesting that the *ogt* encoded repair mechanisms plays a role in DNA lesions induced by methylating agents in *S Typhimurium*. This suggests differing responses to those seen in *E Coli*.

The presence of both *ada* and *ogt* mutations the sensitivity to methylating agents was increased substantially, but this difference was not noticeable for ethylating, butylating etc agents.

### **Conclusion:**

In both *E coli* and *S typhimurium* thresholds are apparent in cultures which have inherent, induced or 'adapted' O<sup>6</sup>-MT capacity (*ogt*). This system appears to be more effective at repairing damage caused by ethylating agents than methylating or bulkier alkylating agents. Data from studies investigating the *ada* gene are more variable, and it is possible that the presence or absence of other repair systems impacts on it's effects. In *S. Typhimurium*, there is evidence that suggests, in the absence of the *ogt* protein, the *ada* protein can repair lesions induced by methylating agents.

### **Mammalian cell systems:**

An early study in which survival and mutagenesis were assessed in cultured primary human fibroblasts following MNNG treatment, pointed towards non-linear dose response curves, although the data therein are difficult to interpret due to varying survival rates for mutant and non-mutant colonies (Jacobs and Demars 1978).

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Unlike bacterial systems already examined, mammalian CHO cells are usually MT (*ada*) deficient. Bignami et al (1987) investigated the O<sup>6</sup>MT activity in a CHO cell line transfected with human liver DNA to produce a cell line with MT efficiency (paper attached [Appendix zz](#)). The induction of ouabane mutations showed an apparent threshold of response in MT proficient cells (0.2µg/ml), whereas a linear dose dependent increase was observed in MT deficient cells (lowest dose 0.024µg/ml). In addition, MT proficient cells were more resistant to the induction of SCEs induced by MNNG (increases only above 70ng/mL) although the threshold was not less evident; SCEs were induced in repair deficient cells at 6-70ng/ml.

A study in which the principle aim was to examine the shape of dose response curves at low doses was performed by Jenssen and Ramel (1980). Herein, dose responses generated by x-rays, UV, ethylating and methylating agents in V79 cells (6TG-resistant mutants) were examined, with view to establishing whether thresholds exist in this cell system (lowest doses tested ENU:0.007mMh, EMS:0.38mMh, MNU:0.04mMh, MMS:0.08mMh; these figures represent integrated dose/time, where the active half life of each chemical was taken into consideration). Comparisons of repair proficient vs. deficient cells were not undertaken. The methodology relied on statistical analyses providing anticipated low dose mutation frequencies following extrapolation from a high dose. Deviation from linearity was seen with methylating agents, but not for ethylating agents. Interestingly, for MMS a putative 'J' shaped dose response curve for mutation frequency was produced (see appended paper), although its magnitude was extremely small. However total alkylation for MMS was linear. The paper is attached [\(Appendix WW\)](#)

## **Conclusions:**

As for bacterial cultures the presence of O<sup>6</sup>-MT can give rise to apparent threshold effects, although this is not an inherent capacity of mammalian cells. A detailed assessment of mutations at low doses suggests 'J' shaped curves are possible.

## **Other DNA repair systems:**

If the concept of hormesis is applicable to all biological systems, then in principle invoking all DNA repair systems would produce U shaped dose response curves or a biphasic dose response curve, albeit this being apparent only at very low doses. Here a few studies in which the role of DNA repair processes other than the O<sup>6</sup>-MT systems are evaluated. It is accepted that this review is limited; it is possible that broadening the review to other chemicals would bring together other relevant papers. However search strings which included, for example *uvr A, B or C* or excision repair, revealed limited data applicable to the current discussion. This may simply reflect the lack of data on low dose effects.

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## ***Bacterial systems:***

Matijasevic and Zeiger (1998) utilised *S. typh* G46 strain and its excision repair deficient derivative TA1950 (*hisG46, DuvrB*) to evaluate the role of excision repair on EMS induced mutagenesis. EMS was tested at doses of 1-50mM for 1 or 2 hours, and induced mutants and ethylations /nucleotide were assessed. One hour exposure to 1mM yielded  $\sim 1.6 \times 10^{-5}$  alkylations/ nucleotide for both strains and when data from 2-20mM were plotted on a log-log scale linear responses were seen for both strains. However, when mutation frequency was plotted, a low dose convergence of the lines was observed, where, although *uvrB* deficient strain had a greater number of mutations at high doses, at low doses it was more resistant to mutagenic effects (at 5mM 10-fold difference) and an apparent threshold was observed. This observation receives extensive discussion. The commentary indicates the biphasic response for EMS is also seen in *S cerevisiae*, but a linear response was seen in mammalian cells. It may be construed that if less mutations were induced in the DNA repair deficient strain at low doses compared to the repair efficient strain, that induction of the repair system under study has not influenced the results. As no differences in DNA binding were observed it is surmised that the involvement of nucleotide excision repair is crucial in determining mutation frequency and that the threshold may be a consequence of another DNA repair pathway, O<sup>6</sup>-MT for example. This points towards the inducibility of the O<sup>6</sup>MT pathway in both or either of these bacterial strains.

Some information can be gleaned from an early study (Schnedel et la 1978) whose aim was to investigate the role of *lexA* product during alkylating agent induced mutagenesis. However this was based on the premise that this was involved in the inducible pathway, which was later shown to be associated with the *ada* and *ogt* genes. As the characteristic 'threshold' curves were produced for *lex A* efficient but not the deficient cultures, this may therefore indicate that the product is inducible in the same manner or as stated above, that O<sup>6</sup> MT induction was invoked.

## ***Mammalian systems;***

Humbert and colleagues (1999) investigated the long-patch mismatch repair system in a series of mouse and human cells. MNNG induced cytotoxicity is potentiated by mismatch repair of O<sup>6</sup>-me G adducts. In this study MNU was investigated and O<sup>6</sup>MT activity was blocked with a specific inhibitor. Human cells were more efficient than mouse cells at mismatch correction. However there are several human and mouse cell lines which have apparent thresholds at low doses. Cytotoxicity is thought to be reduced due to methylation tolerance, but this is believed to be an inherent attribute of the cells; induction of this at low doses is not discussed.

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## Conclusions

Thresholds not in evidence to the same degree as seen with O<sub>6</sub>-MT in bacterial systems; and those that are apparent are possibly due to its incidental induction.

## General discussion and conclusions:

Thresholds for the induction of mutations are evident, but appear to principally be a consequence of O<sub>6</sub> MT (*ogt*) induction in bacterial systems. The phenomenon appears to be more apparent for ethylating agents than methylating or other alkylating agents. The ability of *ada* repair mechanisms to produce threshold type effects remains unclear. Evidence of 'J' shaped dose response curves was scant, but this could be attributed to study designs insufficient to detect these effects.

In order to derive conclusions to the relevance of these findings to *in vivo* risk assessment it will be necessary to evaluate the biological significance of different DNA repair pathways, investigate the relevance of *in vitro* tests in bacterial and mammalian cells and the approaches to interpreting the dose responses used.

In addition there are a number of areas not covered by this review which may be of interest for future discussions; these include,

- an evaluation of similarities and differences of the responses arising from error free or error prone DNA repair mechanisms
- if the process is relevant *in vivo*, how DNA repair fidelity in different tissues would affect interpretation
- how would the variability in responses be taken into account when it is likely that the magnitude will be different for each chemical in question.

## Questions Arising:

- Do the data for O<sub>6</sub>-MT induction suggest threshold or hormesis type dose response curves could occur *in vivo*?
- Are there sufficient data to draw a conclusion with regards to the relevance of other DNA repair mechanisms in relation to the hormesis concept?
- Should any further work be undertaken before final conclusions can be drawn ?

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## References:

- Abril, N., Roldan-Arjona, T., Prieto-Alamo, M-J., van Zeeland, A.A. and Pueyo C. (1992). "Mutagenesis and DNA repair for alkyl;ation damages in Escherichia coli K-12." Environ.Mol. Mut. **19**: 288-296.
- Bignami, M., Terlizese, M., Calcagnile, A., Froshina, G., Abbondandolo, A. and Dogliotti E. (1987). "Cytotoxicity, mutations and SCE's induced by methylating agents are reduced in CHO cells expressing an active mammalian O6-methylguanine-DNA methyltransferase gene." Carcinogenesis **8**(10): 1417-1421.
- Calabrese, E. J. a. B. L. A. (2003). "Toxicology rethinks its central belief." Nature **421**: 691-692.
- Calabrese, E. J. a. B. L. A. (2002). "Applications of hormesis in toxicology, risk assessment and chemotherapeutics." TIPS **23**(7): 331-337.
- Guttenplan. J.B. and Milstein, S. (1982). "Resistnace to Salmonella typhimurim TA1535 O6-methylguanine methylation and mutagenesis induced by low doses of N-methyl-N'-nitro-N-nitrosoguanidine: an apprent constitutive repair activity." Carcinogenesis **3**(3): 327-331.
- Humbert, O., Fuimucino, S., Aquilina, G., Branch, P., Oda, S., Zijno, A., Karran, P. and Bignami, M. (1999). "Mismatch repair and differential sensitivity of mouse and human cells to methylating agents." Carcinogenesis **20**(2): 205-214.
- Jacobs. L. and Demars, R. (1978). "Quantification of chemical mutagenesis in diploid human fibroblasts: induction of azaguanine-resistant mutants by N-methyl-N'-nitrosoguanidine." Mut. res. **53**: 29-53.
- Jeggo, P., Defais M., Samson L. and Schendel P. (1977). "An adaptive response of E Coli to low levels of alkylating agent: comparison with previously characterised DNA repair pathways." Molec. Gen. Genet. **157**: 1-9.
- Jenssen, D., and Ramel, C. (1980). "Relationship between chemical damage of DNA and mutations in mammalian cells: I. Dose response curves for the induction of 6-thioguanine-resistant mutants by low doses of monofunctional alkylating agents, x-rays and uv radiation in V79 chinese hamster cells." Mut. Res. **73**: 339-347.
- Matijasevic, Z. I., and Zeiger, E. (1988). "DNA binding and mutagenicity of ethyl methanesulfonate in wild-type and uvr B cells of Salmonella typhimuirium." Mut. Res. **198**: 1-8.
- Olsson, M. and Lindhal, T. (1980). "Repair of alkylated DNA in Escherichia coli." J.Biol.Chem. **255**(22): 10569-10571.

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Rebeck G.W., and Samson. L. (1991). "Increased spontaneous mutation and alkylation sensitivity of Escherichia coli strains lacking *ogt* O6-methylguanine DNA repair methyltransferase." J.Bacteriol. **173**(6): 2068-2076.

Samson, L. and Cairns, J. (1977). "A new pathway in DNA repair in Escherichia coli." Nature **267**: 281-282.

Schendel, P. F., Defais, M., Jeggo P., Samson L., and Cairns J. (1978). "Pathways of mutagenesis and repair in Escherichia coli exposed to low levels of simple alkylating agents." J.Bacteriol. **135**(2): 366-475.

Todd, M. L. and Schendel, P.F. (1983). "Repair and Mutagenesis on escherichia coli K-12 after exposure to various alkyl-nitrosamines." J. Bacteriol. **156**(1): 6-12.

Yamada M., M. K., Sofuni T. and Nohmi T. (1997). "New tester strains of Salmonella typhimurium lacking O6-methylguanine DNA methyltransferases and highly sensitive to mutagenic alkylating agents." Mut. Res. **381**: 15-24.

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Figure 1: From Jeggo et al 1978

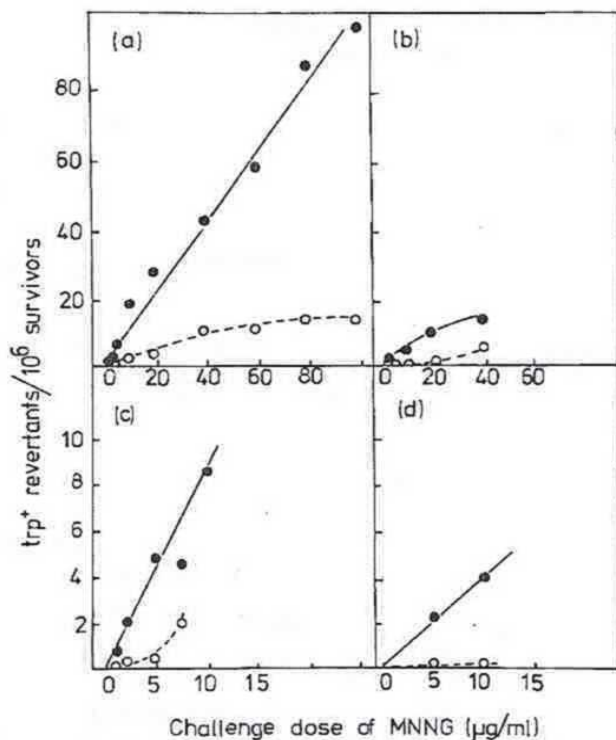


Fig. 3a-d. Mutation response of several strains after varying challenge doses of MNNG under non-adapted (●—●) and MNNG-adapted (○—○) conditions. Concentrations of MNNG used for adaptation are given in Table 2. All challenges of MNNG were for 5 mins at 37°. a Wild type, b *lexA*, c *polA1*, d *recA56*

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Figure 2: from Rebeck and Samson 1991

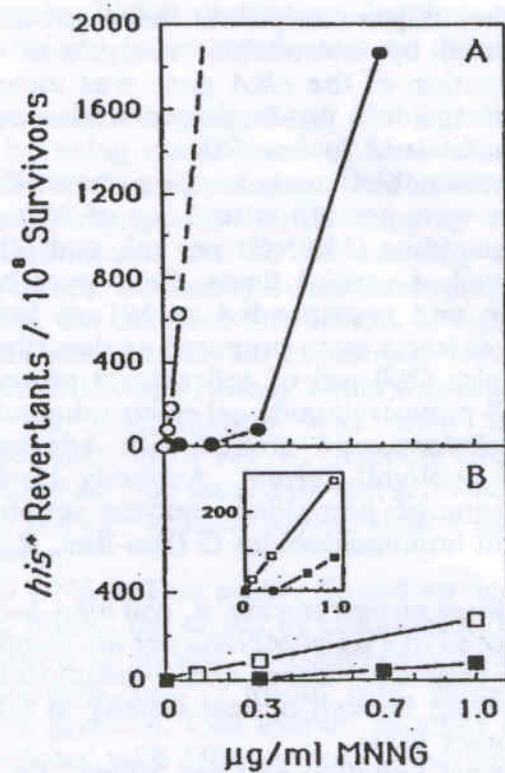


FIG. 3. MNNG-induced mutagenesis. *his* *E. coli* were grown to log phase in LB, treated with MNNG at the indicated doses for 15 min, concentrated in M9 salts, and plated on minimal plates lacking histidine (for mutants) and on minimal plates with histidine (for survivors). (A) BS23 ( $\Delta ada-alkB$ ; ●) and GWR109 ( $\Delta ada-alkB ogt$ ; ○); (B) F26 (wild type; ■) and GWR110 (*ogt*; □).

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Figure 3 : from Guttenplan and Milstein 1982

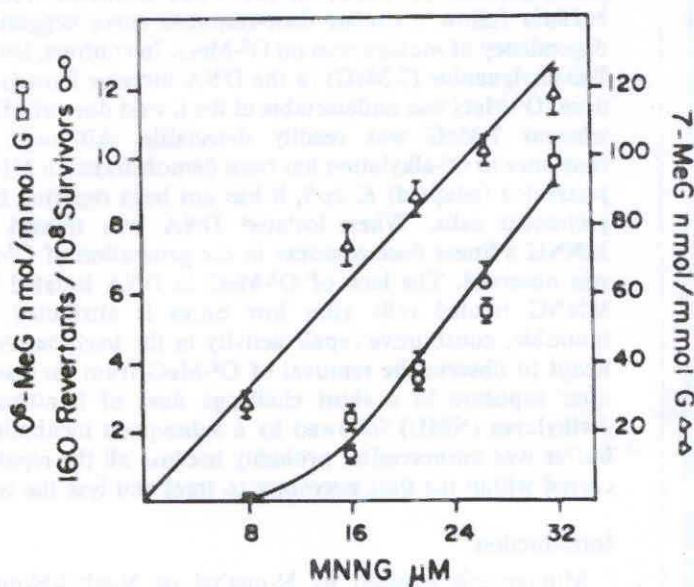


Fig. 2. Relationship between mutagenesis (given in units of 160 revertants/ $10^8$  survivors), O<sup>6</sup>-MeG formation and 7-MeG formation induced by exposure of *Salmonella typhimurium* TA 1535 to [methyl-<sup>3</sup>H]MNNG. Exposure of cells to MNNG was for 60 min at pH 6.5. Details of the treatment are described in **Materials and Methods**. For the mutagenesis assay 0.1 ml of a 1/250 dilution of each incubation mix was overlaid onto minimal agar (22) and the numbers of revertants for each dose of MNNG were 8  $\mu\text{M}$ , 5; 16  $\mu\text{M}$ , 45; 21.3  $\mu\text{M}$ , 120; 26.7  $\mu\text{M}$ , 230; 32  $\mu\text{M}$ , 347; 0  $\mu\text{M}$ , 2. There was no toxicity at any of the doses. Values for 7-MeG were obtained from the neutral thermal hydrolysates of DNA isolated from treated cells, and values for O<sup>6</sup>-MeG were obtained from the acid treated precipitate from the neutral hydrolysate.

nearly coincident with that for O<sup>6</sup>-MeG formation when normalized to the same scale. In order to rule out the possibility of complex decomposition behavior of MNNG accounting

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Figure 4: From Yamada et al 1997

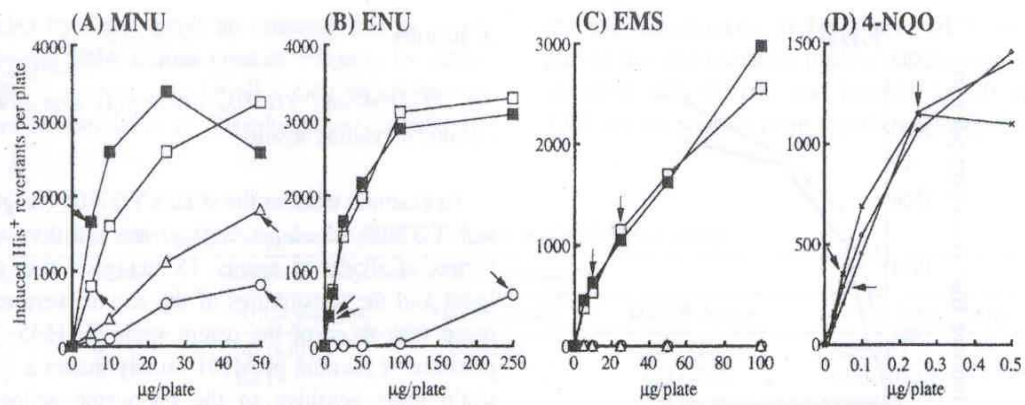


Fig. 1. Typical representation of the mutagenic response of *S. typhimurium* tester strains lacking different MTs. The chemicals used are MNU (A); ENU (B); EMS (C) and 4-NQO (D). The arrow indicates the dose which was used for the calculation of induced His<sup>-</sup> revertants per nmol or µmol in Table 2. The strains used are: ○, TA1535; △, YG7100; □, YG7104; ■, YG7108 for A, B and C. ×, TA100; ◇, YG7112; and ■, YG7113 for D.

*Δada Δogt Δada ogt*

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