Original Research

Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

Journal of Environmental Biology

JEB™ p-ISSN: 0254-8704
e-ISSN: 2394-0379
CODEN: JEBIDP

DOI : http://doi.org/10.22438/jeb/41/2/MRN-1271

The role of nucleotide excision repair on 2,6- and 3,5-dimethylaniline-induced genotoxicity

M.Y. Kim*

Toxicology Laboratory, Faculty of Biotechnology (Biomaterials), College of Applied Life Science, SARI, Jeju National University, Jeju, 63243, Republic of Korea

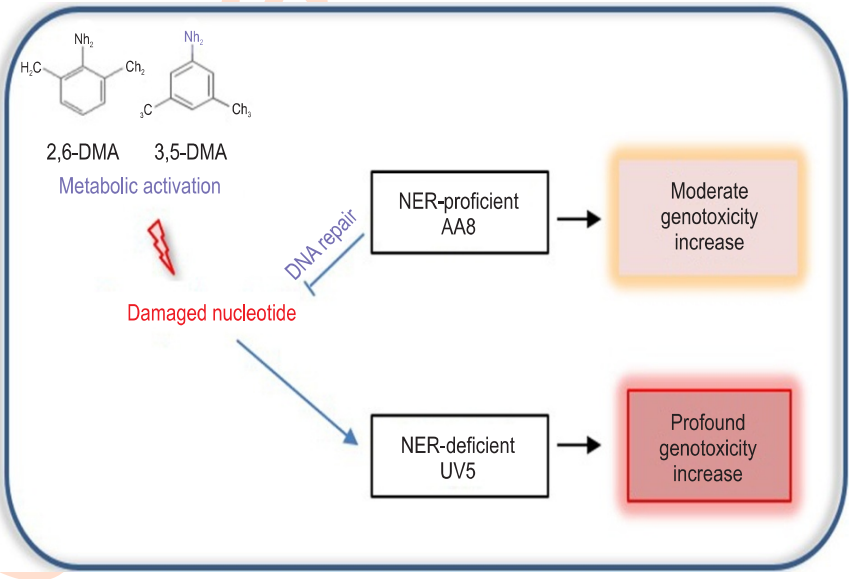
***Corresponding Author Email :** jeffmkim@jejunu.ac.kr

Paper received: 07.09.2019 *Revised received*: 11.12.2019 *Accepted*: 13.01.2020

Abstract

Aim: To examine the possible role of nucleotide excision repair (NER) in affecting the ultimate mutagenic potency of 2,6- and 3,5-dimethylaniline (DMA) and their metabolites.

Methodology: Two cell lines, nucleotide excision repair (NER)-proficient AA8 and deficient UV5 cells were treated with 50, 100, 250, 500 and 1000 µM of 2,6- and 3,5-DMA for 48 hr or their N-hydroxyl and aminophenol metabolites for 1 hr. Cell survival was determined by trypan blue exclusion assay, and 8-azaadenine-resistant mutants at adenine phosphoribosyltransferase (*aprt*) gene locus were evaluated.



Results: A dose-dependent increase in cytotoxicity and mutant fraction was observed in AA8 and UV5 cells, treated with 2,6- and 3,5-DMA and their metabolites, but showed considerable variation in potency; N-hydroxyl and aminophenol metabolites of 2,6- and 3,5-DMA in serum-free α-minimal essential medium (MEM) having the highest potency, and 2,6- and 3,5-DMA in regular MEM at least. Repair-deficient UV5 cells were more sensitive to cytotoxic and mutagenic action than repair-proficient AA8 cells.

Interpretation: These findings suggest that 2,6- and 3,5-DMA-induced DNA damage response may trigger cytotoxicity and mutagenicity when not completely repaired.

Key words: Dimethylaniline, Genotoxicity, Mutagenicity, Nucleotide excision repair

How to cite : Kim, M.Y.: The role of nucleotide excision repair on 2,6- and 3,5-dimethylaniline-induced genotoxicity. *J. Environ. Biol.*, **41**, 216-221 (2020).

Introduction

The nucleotide excision repair (NER) play a key role for recognition and removal of alkylated and highly mutagenic DNA lesions, nucleic acid adducts from oxidative damage caused by intrinsic chemical instability or environmental genotoxins (Zebian *et al.,* 2019; Friedberg *et al.,* 2001). If NER does not function, 104-105 DNA lesions per day in mammalian cell may get loaded and generate chromosomal aberrations and mutagenesis (Marteijn *et al.,* 2014; Friedberg *et al.,* 2001). They can subsequently increase the risk of developing cancer (Marteijn *et al.,* 2014; Friedberg *et al.,* 2001). Arylamines such as 2,6- and 3,5-dimethylaniline (2,6- and 3,5-DMA) are ubiquitous in the environment due to widespread use of industrial synthetic compounds. Experimental studies have shown that administration of 2,6-DMA can induce nasal cancer and neoplastic nodules in the liver of rats (U.S. National Toxicology Program, 1990; IARC, 1993). 2,6-DMA has been classified as a group 2B carcinogen (IARC, 1993). Recent studies have found that 2,6- and 3,5-DMA are associated with the risk for developing bladder cancer in non-smoker (Tao *et al.,* 2013). Arylamines require metabolic activation, usually in the liver, to be transformed into fully carcinogenic agents (Hecht, 2003). They are oxidized to N-hydroxylamines and dimethylaminophenol *in-vivo,* which may ultimately result in bladder cancer (Yu *et al.,* 2002).

Genotoxicity of 2,6- and 3,5-DMA has received limited and controversal investigation to date. Some authors have reported mutagenic action of N-hydroxylated metabolite of 2,6-DMA towards *Salmonella typhimurium* TA100 (Beland *et al.,* 1997) while others have reported that aminophenol metabolite of 3,5-DMA does not induce significant increase in the number of revertant colonies in both TA98 and TA100 *Salmonella typhimurium* and effect the hypoxanthine phosphorybosyl transferase (*HPRT*) mutation frequency in CHO cells (Erkekoglu *et al.,* 2017). *HPRT* gene is located on X chromosome of mammalian cells, and it is used as a model gene to investigate gene mutations in mammalian cell lines. The assay can detect a wide range of chemicals which can damage DNA and lead to gene mutation (Erkekoglu *et al.,* 2017; Gravells *et al.,* 2015).

We have recently reported that 2,6- and 3,5-DMA are cytotoxic and mutagenic in *gpt, HPRT* and *aprt* genes of AS52, TK6 and AA8 cells with human S9 (Moon and Kim, 2018; Chao *et al.,* 2012; Chao *et al.,* 2015). 2,6- and 3,5-DMA were mutagenic and cytotoxic in AS52, TK6 and AA8 cells when activated by P450-mediated hydroxylation and phase II conjugation, in which aminophenol metabolites were considerably more potent than the corresponding N-hydroxylamines (Moon and Kim, 2018; Chao *et al.,* 2012; Chao *et al.,* 2015). *Invivo* gene mutation study have demonstrated that 2,6- and 3,5-DMA increase *lacZ* and *cIIgene* mutations in the nasal tissues of Muta™ mice and increased the incidence of AT to GC transitions and GC to TA transversions (Kohara *et al.,* 2018). The present study focus on the role of NER in protecting mammalian cells against the genotoxic effects of 2,6- and 3,5-DMA, and their metabolites at the hemizygous adenine phosphoribosyltransferase (*aprt*) locus of Chinese hamster ovary cell line AA8 and its NER-defective derivative Uv5. The *aprt* gene was selected because the small size of aprt gene permits mutation to be easily localized and sequenced (Sodimbaku and Pujari, 2014).

Materials and Methods

Cell lines and culture conditions: The NER-competent parental CHO AA8 and NER-deficient UV5 cells, which are heterozygous at *aprt* locus, gifted from Prof. Gerald N. Wogan (Massachusetts Institute of Technology, Cambridge, MA, USA), were used to test the effect of NER on 2,6- and 3,5-DMA-induced genotoxicity. Both cells were cultured in α-minimal essential medium (MEM) with 100 units ml^{-1} penicillin, 100 µg ml^{-1} streptomycin and 10% heat-inactivated fetal bovine serum (FBS, Atlanta Biologicals, Lawrenceville, GA) at 37 ºC with 5% CO_2. Cells in a humidified atmosphere were cleansed of pre-existing *aprt* mutants by culturing in CAAT medium (10 µM cytidine, 100 µM adenine, 1 µM aminopterin and 17.5 µM thymidine) for 2 days and transferred 2-5 days to medium enriched with thymidine (17.5 µM), adenine (100 µM) and cytidine (10 µM) for. All cultures were exponentially grown at the time of treatment.

Treatment with 2,6 and 3,5-DMA, and their metabolites: Cells were plated at approximately 0.5×10^6 cells in 100 mm tissue culture dish in 10 ml of media and exposed to 50, 100, 250, 500 and 1000 µM of 2,6- (Sigma-Aldrich, MO, USA) and 3,5-DMA (Acros Organics, Geel, Belgium) dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, MO, USA) for 48 hr at 37 °C in the absence or presence of human S9. Human liver S9 fraction was obtained from BD Biosciences (San Jose, CA). Approximately, 440 µg S9 protein and 65 µl of sterile-filtered core mixture [25 mg ml^{-1} NADP (Boehringer Mannheim, Indianapolis, IN) and 45 mg ml^{-1} DL-isocitric acid (Sigma)] were added per ml of MEM medium. N-hydroxy and aminophenol metabolites of 2,6- and 3,5-DMA were synthesized as described by Chao *et al.* (2012). Cells were seeded at 1×10^6 and incubated overnight with the regular medium. The medium was changed to serum-free medium, and cells were treated in triplicate with desired concentrations of N-hydroxy or aminophenol metabolites.

After 1 hr treatment, the cultures were washed with PBS, amended with complete MEM, and incubated in complete MEM for additional 24 hr prior to determining cell survival. Concentrations and exposure time of 2,6-DMA and 3,5-DMA and their metabolites used for cytotoxicity and mutagenicity experiments were established based on MTT cytotoxicity assays (data not shown). Stock solutions of 2,6-DMA and its metabolites (N-hydroxy and aminophenol) were prepared by dissolving the accurately weighed compounds in DMSO to give a final concentration of 100 and 25 mM, respectively. The final concentration of DMSO to which cells were exposed was less than 1%.

Assessment of cell viability: To investigate the dose-dependent effects of 2,6- and 3,5-DMA, and their metabolites on cell viability, AA8 and UV5 cells were subjected to trypan blue exclusion assay. Twenty four hours after treatment, the cell suspension was mixed with 0.5% trypan blue solution at 1:1 ratio. The mixture was incubated at room temperature for 1-2 min and loaded onto one chamber of hemocytometer and squares of the chamber were observed under a light microscope. Viable/ live (clear) and non-viable/dead (blue) cells were counted and viability was calculated by the formula (number of live cells counted/ total number of cells counted) × 100.

Assay for mutation at *aprt* locus: Following treatment, AA8 and UV5 cells were rinsed twice with PBS and detached with trypsin. Thereafter, treated cells were maintained in growth medium for at least 7 days to allow full expression of *aprt* mutant phenotype. At the end of phenotypic expression, cultures were plated for cloning efficiency and mutation. Total 6×10^5 cells from each group were placed in 100 ml selective medium containing 80 µg ml^{-1} of 8-Azaadenine (8-AA, Sigma-Aldrich, MO, USA) and plated at 600000 cells 10 ml^{-1} 100 mm $dish^{-1}$ (ten replicates) for determining of mutagenicity at *aprt* locus after 14 days. In addition, each mutant was rescreened in selective media in order to confirm the stability of mutant phenotype. Cloning efficiency (CE) dishes were seeded with 200 cells 10 ml^{-1} 100 mm $dish^{-1}$ in triplicate and allowed to grow until colonies were visible for 8 days in the absence of selecting agent, 8-AA. Colonies were stained with 0.5% crystal violet in 50% methanol and counted. Mutation fraction (MF) was calculated as ratio of mean CE in selective medium to that in non-selective medium. Cells treated with 0.2 µM PhIP (Toronto Research Chemicals, Ontario, Canada) for 48 hr served as positive control for mutation induction.

Statistical analyses: All the data were expressed as mean of three replicates ± SD. Two-tailed Student's *t*-test was calculated and to compare cell survival and mutation fraction between AA8 and UV5 cells. The results were considered statistically significant at $p<0.05$ using SPSS statistical software (SPSS, ver. 12.0; SPSS Inc., Chicago, IL, US).

Results and Discussion

The aim of the present study was to investigate the role of NER in protecting rodent cells against the genotoxic effects of 2,6- and 3,5-DMA, and their metabolites. For this purpose, Chinese hamster ovary cell line AA8 and its NER-deficient derivative UV5 were used. UV5 was derived from AA8 cells mutagenized with ethyl methanesulfonate (Thompson *et al.,* 1980), indicating that both cell lines are genetically and closely identical and, therefore, are probably better suited for comparative analyses than studies

Table 1: Mutation fraction after treatment with 2,6-DMA and its metabolites in AA8 and UV5 cells

	Mutation fraction (×10^{-5})					
Dose (µM)	**0**	**50**	**100**	**250**	**500**	**1000**
2,6-DMA (AA8)	2.9±0.27	3.1±0.77	3.1±0.31	3.1±0.23	3.2±0.26	3.2±0.17
2,6-DMA (UV5)	2.9±0.30	3.1±0.09	3.4±0.20	3.6±0.15	3.6±0.30	
2,6-DMA+S9 (AA8)	2.8±0.60	3.1±0.49	3.7±0.25	3.7±0.55	4.2±0.29	5.0±0.81
2,6-DMA+S9 (UV5)	2.9±0.43	3.8±0.20	4.5±0.05	7.0±0.27*	9.4±0.25*	
N-OH-2,6-DMA (AA8)	2.9±0.27	3.0±0.64	3.2±0.58	3.2±0.25	3.3±0.35	4.9±0.63
N-OH-2,6-DMA (UV5)	2.9±0.33	5.2±0.17	6.7±0.25*			
2,6-DMAP (AA8)	2.9±0.27	3.0±0.11	3.1±0.33	3.6±0.65	4.0±0.24	
2,6-DMAP (UV5)	2.9±0.11	7.0±0.49*				

Each value is expressed as mean of three replicates ±SD; *$p< 0.05$ compared to AA8 cells by Student's *t*-test

Table 2: Mutation fraction after treatment with 3,5-DMA and its metabolites in AA8 and UV5 cells

	Mutation fraction (×10^{-5})					
Dose (µM)	**0**	**50**	**100**	**250**	**500**	**1000**
3,5-DMA (AA8)	2.9±0.51	3.3±0.52	3.4±0.51	3.3±0.58	2.9±0.70	3.5±0.40
3,5-DMA (UV5)	2.9±0.30	2.8±0.22	3.2±0.56	3.7±0.21	3.7±0.43	
3,5-DMA+S9 (AA8)	2.8±0.60	3.5±0.20	3.7±0.65	4.4±0.31	4.7±0.81	5.1±0.21
3,5-DMA+S9 (UV5)	2.9±0.43	5.1±0.40	7.8±0.60*	9.2±0.14*		
N-OH-3,5-DMA (AA8)	2.9±0.51	3.0±0.36	3.2±0.26	3.3±0.14	4.7±0.15	4.9±0.44
N-OH-3,5-DMA (UV5)	2.8±0.12	7.4±0.31*				
3,5-DMAP (AA8)	2.9±0.33	3.0±0.24	3.4±0.24	3.9±0.55	4.1±0.34	
3,5-DMAP (UV5)	2.8±0.18	9.0±0.30*				

Each value is expressed as mean of three replicates ±SD; *$p<0.05$ compared to AA8 cells by Student's *t*-test

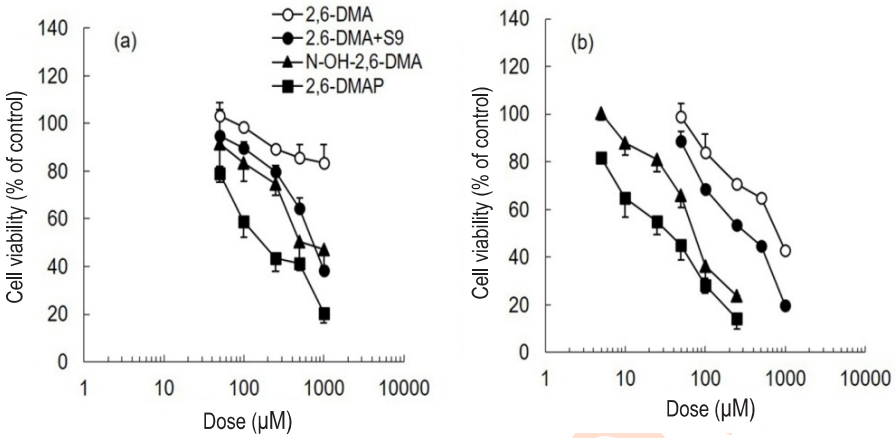


Fig. 1: Percentages of cell viability after exposure to 2,6-DMA and its metabolites. Dose-dependent of cell survival after treatment with 50, 100, 250, 500 and 1000 μM of 2,6-DMA and its metabolites in AA8 (a) and UV5 (b) cells. Survival was determined by trypan blue exclusion assay 24 hr after treatment. Results are presented as a percentage of control cells (mean of three replicates ±S.D.); **p*<0.05 compared to AA8 cells by Student's *t*-test.

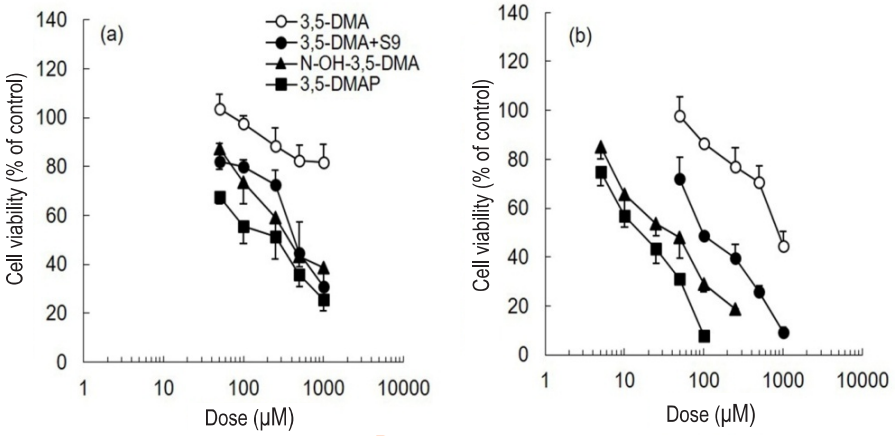


Fig. 2: Percentage of cell viability after exposure to 3,5-DMA and its metabolites. Dose-dependent of cell survival after treatment with 50, 100, 250, 500 and 1000 μM of 3,5-DMA and its metabolites in AA8 (a) and UV5 (b) cells. Survival was determined by trypan blue exclusion assay 24 hr after treatment. Results are presented as a percentage of control cells (mean of three replicates ±S.D.); **p*<0.05 compared to AA8 cells by Student's *t*-test.

using cell lines with different genetic backgrounds. Dose-dependent cytotoxic effects of 50, 100, 250, 500 and 1000 μM of 2,6- and 3,5-DMA, and their metabolites were seen in NER-proficient AA8 and NER-deficient UV5 cell lines (Fig. 1, 2).

The survival of both cell lines following 2,6- and 3,5-dimethylaminophenol (2,6- and 3,5-DMAP) treatment was lower than that observed in the corresponding N-hydroxy metabolite, parent compound with or without human S9; for instance, cell viability was 43% and 51% in AA8 cells 24 hr after a dose of 250 μM of 2,6-DMAP and 3,5-DMAP, compared to 75 and 59% after treatment with 250 μM of N-hydroxy-2,6-DMA (N-OH-2,6-DMA) and N-OH-3,5-DMA, or 250 μM of 2,6-DMA (80% or 89%) and 3,5-DMA (73% or 88%) in the presence or absence of human S9 (Fig. 1, 2). Survival of UV5 cells following 2,6- and 3,5-DMA, and their metabolites treatment was lower than that observed in the corresponding AA8 cells (Fig. 1, 2). The two dosing regimens of 1000 μM for 2,6- and 3,5-DMAP in AA8 and 100 μM for 2,6- and 3,5-DMAP in UV5 resulted in similar cell survivals of nearly 20% ($p < 0.05$) (Fig. 1, 2). Cells with functional NER were more resistant to cytotoxic effects of 2,6- and 3,5-DMA, and their metabolites than cells deficient in NER. This suggests that cells may utilize DNA damage repair mechanisms which can permit the survival or continued growth of cells.

In previous studies, it has been reported that if the relative survive rate is 30% or more after chemical exposure, there will be sufficient live cells to determine mutation responses (Li *et al.,* 2002). Thus, based on the results of cytotoxicity experiment, three cultures with concentration ranges for 2,6- and 3,5-DMA with or without human S9 (50-1000 µM and 50-500 µM), N-OH-2,6-DMA and N-OH-3,5-DMA (50-1000 µM and 50-100 µM), and 2,6-DMAP and 3,5-DMAP (50-500 µM and 50 µM) were used to determine mutagenic responses in AA8 and UV5 cells (Table 1, 2). For NER-deficient UV5 cell lines, 2,6-DMA with S9 and 2,6-DMAP treated with 50 µM produced slightly increase in *aprt* mutant factors over the average control value of 2.9×10^{-5}, with the observed *aprt* mutant factors being 3.3×10^{-5} and 4.0×10^{-5}, respectively (Table 1). 3,5-DMA with S9 and its metabolites was more toxic than its 2,6-DMA counterpart in NER-deficient UV5 cells (Table 1, 2). There was an increase in mutant factors compared with untreated controls ($2.8\text{-}2.9 \times 10^{-5}$) following exposure to 3,5-DMA in the presence of human S9 (5.1×10^{-5}) and two metabolites, N-OH-3,5-DMA (7.4×10^{-5}) and 3,5-DMAP (9.0×10^{-5}), for 50 µM investigated in UV5 cells (Table 2).

2,6- and 3,5-DMA, and their metabolites also increased the number of mutants in NER-proficient AA8 cell lines, but the magnitude of induced response was lower than that observed in the matching NER-deficient UV5 cell lines ($p< 0.05$) (Table 1, 2). For instance, in the NER-deficient cell line UV5, mutant factors in the *aprt* gene of 50 µM 3,5-DMAP induced 9.0×10^{-5}, while 500 µM induced 4.1×10^{-5} in the NER-proficient cell line AA8 (Table 2). These data suggest that 2,6- and 3,5-DMA, and their metabolites induce DNA damage, *i.e.,* a substrate for NER and that leads to cytotoxicity and gene mutations when not properly removed (Fig. 1, 2, Table 1, 2). Our results are consistent with the previous reportes on the increased mutation frequency in NER-deficient bacteria (Sidorenko *et al.,* 2015; Kivisaar, 2010; Kondo *et al.,* 1970), yeast (Gangloff and Arcangioli, 2017; Ruhland and Brendel, 1979) and CHO cells (Brooks *et al.,* 2008; Perez and Skarsgard, 1986; Op het Veld *et al.,* 1997).

Mutations can affect a wide variety of biological aberrations, including disrupted protein homeostasis and age-associated diseases (Ames, 2010). Induction of DNA damage and erroneous repair or replicative bypass of lesions can result in mutation or chromosomal aberrations affecting oncogenes and tumor suppressor genes (Torgovnick and Schumacher, 2015). Mutations caused by defective DNA repair enable a normal cell to become cancerous (Brooks *et al.,* 2008). NER is the major DNA repair pathway that repairs most of bulk DNA damage generated by some endogenous and environmental agents (Friedberg, 2001; Marteijn *et al.,* 2014). Several lines of evidence suggest that NER play a role in the development and progression of cancer (Dai *et al.,* 2013; Marteijn *et al.,* 2014; Zebian *et al.,* 2019).

In summary, the present study shows that 2,6- and 3,5-DMA investigated appear to have significant genotoxic potential. The evidence also suggests that NER plays a significant role in protecting cytotoxicity and mutagenicity via formation of hydroxyl and aminophenol metabolites of 2,6- and 3,5-DMA. Further investigations are required to examine the mutational mechanism produced by 2,6- and 3,5-DMA and to elucidate the contribution of oxidative stress plays in this process.

Acknowledgment

This research was supported by Basic Science Research Program (2017R1D1A1B03028849, 2016R1A6A1A03012862 and 2014R1A1A2056292) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology, Republic of Korea.

References

Ames, B.N.: Prevention of mutation, cancer, and other age-associated diseases by optimizing micronutrient intake. *J. Nucleic Acids,* **2010**, 11 (2010).

Beland, F.A., W.B. Jr Melchior, L.L.G. Mourato, M.A. Santos and M.M. Marques: Arylamine-DNA adduct conformation in relation to mutagenesis. *Mutation Res.,* **367**, 13-19 (1997).

Brooks, B., T.J. O'Brien, S.Ceryak, J.P. Wise, S.S. Wise, J.P. Wise, E. Defabo and S.R. Patierno: Excision repair is required for genotoxin-induced mutagenesis in mammalian cells. *Carcinogenesis,* **29**, 1064-1069 (2008).

Chao, M.W., M.Y. Kim, W. Ye, J. Ge, L.J. Trudel, C. Belanger, P.L. Skipper, B.P. Engelward, S.R. Tannenbaum and G.N. Wogan: Genotoxicity of 2,6- and 3,5-dimethylaniline in cultured mammalian cells: The role of reactive oxygen species. *Toxicol. Sci.,* **130**, 48-59 (2012).

Chao, M.W., P. Erkekoglu, C.Y. Tseng, W. Ye, W., L.J. Trudel, P.L. Skipper, S.R. Tannenbaum and G.N. Wogan: Protective effects of ascorbic acid against the genetic and epigenetic alterations induced by 3,5-dimethylaminophenol in AA8 cells. *J. Appl. Toxicol.,* **35**, 466-477 (2015).

Dai, Q.S., R.X. Hua, R. Zhang, Y.S. Huang, Z.M. Hua, C.T. Yun, R.F. Zeng and L.T. Long: Poly (AT) deletion/insertion polymorphism of the XPC gene contributes to urinary system cancer susceptibility: A meta-analysis. *Gene,* **528**, 335-342 (2013).

Erkekoglu, P., M.W. Chao, O. Kose, S. Sabuncuoglu and B. Kocer-gumusel: The mutagenic potential of 3,5-dimethylaminophenol in bacterial and mammalian cells. *FABAD J. Pharm. Sci.,* **42**, 87-93 (2017).

Friedberg, E.C.: How nucleotide excision repair protects against cancer. *Nat. Rev. Cancer,* **1**, 22-33 (2001).

Gangloff, S. and B. Arcangioli: DNA repair and mutations during quiescence in yeast. *FEMS Yeast Res.,* **17**, 1-14 (2017).

Gravells, P., S. Ahrabi, R.K. Vangala, K. Tomita, J.T. Brash, L.A. Brustle, C. Chung, J.M. Hong, A. Kaloudi, T.C. Humphrey and A.C. Porter: Use of the HPRT gene to study nuclease-induced DNA double-strand break repair. *Hum. Mol. Genet.,* **24**, 7097-7110 (2015).

Hecht, S.S.: Tobacco carcinogens, their biomarkers and tobacco induced cancer. *Nat. Rev. Cancer,* **3**, 733-744 (2003).

IARC: 2,6-dimethylaniline (2,6-xylidine). IARC monographs on the evaluation of carcinogenic risks to humans, **57**, 323-335 (1993).

Kivisaar, M.: Mechanisms of stationary-phase mutagenesis in bacteria: Mutational processes in pseudomonads. *FEMS Microbiol. Lett.,* **312**, 1-14 (2010).

Kohara, A., M. Matsumoto, A. Hirose, M. Hayashi, M.Honma and R. Suzuki: Mutagenic properties of dimethylaniline isomers in mice as

evaluated by comet, micronucleus and transgenic mutation assays. *Gene Environ,* **40**, 18 (2018).

Kondo, S., H. Ichikawa and K.K.T. Iwo: Base-change mutagenesis and prophage induction in strains of *Escherichia coli* with different DNA repair capacities. *Genetics,* **166**, 187-217 (1970).

Li, C.Q., L.J. Trudel and G.N. Wogan: Nitric oxide-induced genotoxicity, mitochondrial damage, and apoptosis in human lymphoblastoid cells expressing wild-type and mutant p53. *Proc. Natl. Acad. Sci. USA,* **99**, 10364-10369 (2002).

Marteijn, J.A., H. Lans, W. Vermeulen and J.H. Hoeijmakers: Understanding nucleotide excision repair and its roles in cancer and ageing. *Nat. Rev. Mol. Cell Biol.,* **15**, 465-481 (2014).

Moon, S.H. and M.Y. Kim: Genotoxicity of N-hydrocy and aminophenol metabolites of 2,6-and 3,5-dimethylaniline at the hypoxanthine guanine phosphoribosyltransferase locus in TK6 cells. *Asian J. Pharm. Clin Res.,* **11**, 398-401 (2018).

Op het Veld, C.W., S. van Hees-Stuivenberg, A.A. van Zeeland and J.G. Jansen: Effect of nucleotide excision repair on *hprt* gene mutations in rodent cells exposed to DNA ethylating agents. *Mutagenesis,* **12**, 417-424 (1997).

Perez, C.F. and L.D. Skarsgard: Radiation enhancement of the efficiency of DNA-mediated gene transfer in CHO UV-sensitive mutants. *Radiat. Res.,* **106**, 401-407 (1986).

Ruhland, A. and M. Brendel: Mutagenesis by cytostatic alkylating agents in yeast strains of differing repair capacities. *Genetics,* **92**, 83-97 (1979).

Sidorenko, J., K. Ukkivi and M. Kivisaar: NER enzymes maintain genome integrity and suppress homologous recombination in the absence of exogenously induced DNA damage in *Pseudomonas putida. DNA Repair,* **25**, 15-26 (2015).

Sodimbaku, V. and L. Pujari: Urolithiasis-an updated review over genetics, pathophysiogy and its clinical management. *Int. J. Pharm. Pharm. Sci.,* **6**, 23-31 (2014).

Tao, L., B.W. Day, B. Hu, Y.B. Xiang, R. Wang, M.C. Stern, M. Gago-Dominguez, V.K. Cortessis, D.V. Conti, D. Van Den Berg, M.C. Pike, Y.T. Gao, M.C. Yu and J.M. Yuan: Elevated 4-aminobiphenyl and 2,6-dimethylaniline hemoglobin adducts and increased risk of bladder cancer among lifelong nonsmokers-The Shanghai Bladder Cancer Study. *Cancer Epidemiol. Biomar. Prev.,* **22**, 937-945 (2013).

Thompson, L.H., J.S. Rubin, J.E. Cleaver, G.F. Whitmore and K. Brookman: A screening method for isolating DNA repair-deficient mutants of CHO cells. *Somatic Cell Genet.,* **6**, 391-405 (1980).

Torgovnick, A. and B. Schumacher: DNA repair mechanisms in cancer development and therapy. *Front. Genet.,* **6**, 157 (2015).

U.S. National Toxicology Program: Toxicology and carcinogenesis studies of 2,6-xylidine (2,6-dimethylaniline) (CAS No. 87-62-7) in Charles River CD rats (Feed Studies). Technical Report Series No. 278. Research Triangle Park, NC: NTP (1990).

Yu, M.C., P.L. Skipper, P.L., S.R. Tannenbaum, K.K. Chan and R.K. Ross: Arylamine exposures and bladder cancer risk. *Mutat. Res.,* **506-507,** 21-28 (2002).

Zebian, A., A. Shaito, F. Mazurier, H.R. Rezvani and K. Zibara: XPC beyond nucleotide excision repair and skin cancers. *Mutat. Res. Rev. Mutat.,* **782**, 108286 (2019).