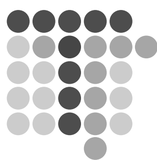

Available online at www.tox.or.kr

Measurement of DNA Damage with Fpg/Endo III FLARE Assay and Real Time RT-PCR in SD Rats Exposed to Cumene

Soo-Jin Kim[1], **Kyung-Taek Rim**[1], **Seong-Bae Lee**[1] & **Hyeon-Yeong Kim**[1]

[1]Laboratory of Occupational Toxicology, Chemical Safety & Health Research Center, Occupational Safety & Health Research Institute, KOSHA, 104-8, Moonji-Dong, Yuseong-Gu, Daejeon 305-380, Korea
Correspondence and requests for materials should be addressed to K. T. Rim (ktrim@kosha.net)

Accepted 9 July 2008

Abstract

To clarify the DNA damage from reactive oxygen species, we measured the DNA damage through Fpg/Endo III FLARE (Fragment Length Analysis with Repair Enzyme) assay and real time RT-PCR. The 80 SD rats assigned to 4 dose groups exposed to cumene vapor for 90 days. With Fpg/Endo III FLARE assay in hepatocytes, we found the OTM (Olive Tail Moment) and TL (Tail Length) significantly increased in no-enzyme treated and Fpg-treated control and 8 ppm groups with 28 days exposure. In Endo III-treated 8 ppm group, significantly increased the values with 90 days exposure. With lymphocytes, it was founded the values significantly increased in no-enzyme treated 800 ppm group in 28 and 90 days. It was significantly increased in Endo III-treated 80 ppm for 28 days and 800 ppm for 90 days. From the above findings, FLARE assay was suggested as being available as a biological marker for DNA damage induced by cumene exposure in SD rats. And we used real time RT-PCR for the OGG1 mRNA expression, it had dose-dependent biologic effects in 1 day exposure, but decrease the levels of rOGG1 mRNA. Our findings provide evidence that cumene exposure may cause suppression of rOGG1 in the rat hepatocytes or lymphocytes.

Keywords: Cumene, Fpg/Endo III FLARE assay, Real time RT-PCR, OGG1

Cumene (CAS No. 98-82-8) is an insoluble petrochemical use to manufacture a lot of chemicals such as phenol, acetone, etc. It observed with the effect to increase organ weight, low latent toxicity of reproduction, a negative result of genetic toxic test[1]. It could not classify as a carcinogen because of its inappropriate animal test, in spite of some dead animals, mild positive in micronucleus test[2]. It was reported high destructive ratio to cell membrane of human lung fibroblasts cultured with 25 mM cumene[3]. Moreover, it causes dizziness, sleepiness, ataxia, unconsciousness with short term exposure[4], inhibition of central nerve[5], toxicity by ingestion, inhalation, skin absorption[6], irritation to skin and eye[7], acts just like an general anesthetics by acute exposure[8]. The most possible routes of cumene exposure to workers are contaminated air by evaporation of petrochemicals[9], it actually measured with monitoring samples in cumene manufacturing and processing, especially 0.0001-3.35 ppm at distillation, 0.34-0.44 ppm at laboratories, 0.078-0.620 ppm at cumene unit[10]. Gasoline delivered drivers are exposed to under 0.01-0.04 ppm[11], 60-250 $\mu g/m^3$ in the air of shoe manufacturing places, 2-200 $\mu g/m^3$ at vulcanizing process, about 10 $\mu g/m^3$ at injection molding process in tire recapping places[12]. It was presumed statistically that 14,268 workers (2,760 female) would be latently exposed to cumene in the U.S.[13]. Moreover, the occupational exposure to cumene may occur by inhalation or skin contact to chemicals in workplaces with cumene manufacturing or using. It was reported the exposure by inhalation of environmental air[14], food ingestion and inhalation of gasoline vapor including cumene[15] could be occurred in the general public.

In low concentration, the ROS acts as a intra- or inter-cellular secondary messenger to regulate a variety of cell function which including initiation of gene expression, promotion and inhibition of cell proliferation, cell death[16]. However, it was known that the ROS can induce oxidative DNA damage, DNA strand breakage, chromosomal aberration, and related with variety of diseases like cancer, rheumatoid arthritis, cardiovascular and Alzheimer disease when it is overproduced[17].

An organism is being evolved many defense system for the elimination of DNA damages, and it was discovered the major repair mechanisms of oxidative

DNA damage including 8-oxodG and DNA strand breakage are base excision repair in both bacterial and mammalian cells[18]. It is the main tool of oxidative DNA repair biomarkers that the analysis of 8-hydroxyguanine formed in DNA double helix[19], and reported that the 8-OHGua is introduced by radiation, reactive oxygen induced chemicals either in vivo or in vitro, and induce G : C → T : A transversion in DNA polymerase reaction[20,21]. The GC-TA transversion is the results of cell malignant transformation resulted from the codon 12 of *ras* gene, glycine to GTT[22]. Comet assay is a method to detect DNA damage from DNA chain excision and alkali labile sites, not chromosomal aberration, so it is possible to detect DNA damage from undividable tissues[23]. It was introduced by Ostling and Johanson[24] to detect directly DNA damage in each cellular level, made more sensitive by Singh[25]. In alkali condition, it can detect base modification, alkali-labile abasic site formed when the damage site was inactively excised with DNA repair enzymes, and it can measured the DNA damage in specific sites with FLARE assay, the improved method of comet assay by treatment of endonuclease III or Fpg[26]. In last study, it was performed the alkaline (pH > 13) SCGE assay to measure the degree of DNA damage such as DNA strand breakages. It had a dose dependency for 1 day and 15 days exposure, but the DNA damages in low dose group was higher than high dose for 30 days welding fume exposure[27,28].

In this study, FLARE assay was used to investigate DNA damage and the real time RT-PCR to measure the expression of DNA damage repair gene (OGG1).

Table 1. Olive tail moment values of DNA isolated from hepatocytes.

Value / Group	Mean ± S.D			
	1 day	14 day	28 day	90 day
Control-buf	17.84 ± 23.40	44.54 ± 42.06	88.77 ± 61.12	51.37 ± 64.58
Control-Fpg	21.68 ± 28.26	88.50 ± 82.08	58.84 ± 0.00	40.06 ± 45.57
Control-Endo	4.75 ± 6.27	62.95 ± 66.47	79.17 ± 60.75	29.99 ± 39.69
Low-buf	12.68 ± 14.60	67.10 ± 80.67	79.76 ± 78.39	29.80 ± 23.75
Low-Fpg	26.49 ± 39.53	57.29 ± 55.59	31.62 ± 44.31	40.42 ± 41.65
Low-Endo	39.55 ± 48.34*	36.06 ± 38.50	59.57 ± 63.85	164.59 ± 123.75*
Medium-buf	27.06 ± 32.81	28.54 ± 29.07	32.93 ± 57.61	27.55 ± 26.15
Medium-Fpg	19.28 ± 30.68	49.34 ± 47.33	35.76 ± 60.25	25.98 ± 12.54
Medium-Endo	21.98 ± 26.50	31.93 ± 33.11	32.78 ± 53.98	33.25 ± 41.53**
High-buf	50.69 ± 67.28	21.13 ± 34.54	57.61 ± 84.55	8.90 ± 9.36**
High-Fpg	10.96 ± 19.57	37.85 ± 50.36	47.32 ± 59.08	23.73 ± 46.38**
High-Endo	16.25 ± 37.29**	49.99 ± 31.99	29.50 ± 24.96	19.07 ± 17.13**

Unit: a.u. (arbitrary unit)

All values are expressed as mean ± S.D.

*: compare with 0 ppm $P < 0.05$; **: compare with 8 ppm $P < 0.05$

Table 2. Tail length values of DNA isolated from hepatocytes.

Value / Group	Mean ± S.D			
	1 day	14 day	28 day	90 day
Control-buf	70.11 ± 81.70	162.93 ± 157.26	256.94 ± 149.79	140.67 ± 207.24
Control-Fpg	58.45 ± 79.54	249.01 ± 207.38	157.89 ± 0.00	78.66 ± 148.30
Control-Endo	15.79 ± 37.27	168.54 ± 180.80	318.42 ± 215.35	78.70 ± 120.08
Low-buf	50.25 ± 74.70	169.747 ± 193.59	246.35 ± 203.28	24.86 ± 46.03
Low-Fpg	89.05 ± 120.52	159.38 ± 147.72	67.45 ± 137.39	82.83 ± 121.52
Low-Endo	113.33 ± 143.10	115.90 ± 156.49	137.25 ± 181.45	276.20 ± 234.47
Medium-buf	81.25 ± 108.48	83.95 ± 123.77	87.78 ± 173.14	53.83 ± 89.95
Medium-Fpg	65.37 ± 122.44	152.08 ± 164.88	71.56 ± 146.44	23.69 ± 21.81
Medium-Endo	72.74 ± 98.65	109.58 ± 118.54	76.23 ± 130.75	72.81 ± 135.42
High-buf	145.01 ± 172.04	51.32 ± 95.98*	147.37 ± 235.28	2.11 ± 6.66
High-Fpg	49.67 ± 85.43	122.96 ± 150.12	121.21 ± 181.14	65.56 ± 131.46
High-Endo	50.16 ± 99.71	176.32 ± 117.28	88.37 ± 117.30	35.83 ± 85.73**

Unit: μm

All values are expressed as mean ± S.D.

*: compare with 0 ppm $P < 0.05$; **: compare with 8 ppm $P < 0.05$

DNA Damages of Hepatocytes with FLARE Assay

Table 1 and 2 refer the DNA damage of hepatocytes (mean ± S.D.) including oxidative damage in Fpg sensitive sites such as altered purines by Fpg FLARE assay, and Endo III sensitive sites, oxidized pyrimidine, by Endo III FLARE assay in control and exposed groups.

The mean values of measured Olive tail moment and tail length with no enzyme treatment had not significantly different, but had a general trend of increasing in 28 days but decreasing again in 90 days.

Olive tail moment and tail length with Fpg enzyme had not significantly different among groups, the control was higher than the exposed group from 14 to 28 days, decreased again in 90 days. These reflect a trend that the altered purines were formed in 28 days and repaired in 90 days.

In those with Endo III enzyme, the control was also higher from 14 to 28 days but statistically insignificant. However the result that low dose was significantly higher than others in 90 days reflects the oxidative DNA damage like forming the oxidized pyrimidine. Moreover these reflect the oxidative DNA damage level in specific pyrimidine base, such as recovery of DNA adducts formation.

DNA Damages of Lymphocytes with FLARE Assay

Table 3 and 4 refer the DNA damage of lympho-

Table 3. Olive tail moment values of DNA isolated from lymphocytes.

Value / Group	Mean ± S.D			
	1 day	14 day	28 day	90 day
Control-buf	0.73 ± 0.66	84.51 ± 59.73	63.33 ± 53.53	59.15 ± 65.68
Control-Fpg	24.87 ± 39.95	122.55 ± 76.98	65.82 ± 45.38	63.86 ± 52.73
Control-Endo	80.97 ± 65.17	77.41 ± 64.56	44.69 ± 34.50	94.03 ± 78.06
Low-buf	36.47 ± 49.03*	92.46 ± 73.16	70.80 ± 57.90	47.72 ± 49.22
Low-Fpg	94.28 ± 88.39*	72.00 ± 56.11*	59.75 ± 40.70	72.89 ± 78.30
Low-Endo	58.65 ± 73.21	105.88 ± 76.41*	70.66 ± 53.67*	95.32 ± 90.96
Medium-buf	9.90 ± 20.17	70.55 ± 70.92**	50.17 ± 44.24	57.22 ± 36.86
Medium-Fpg	13.18 ± 19.81**	103.80 ± 95.32	29.43 ± 20.58**	93.75 ± 57.50
Medium-Endo	71.42 ± 48.36	71.85 ± 56.16**	61.20 ± 41.26	33.81 ± 21.96**
High-buf	68.95 ± 52.03 †	75.08 ± 53.31	36.43 ± 32.56**	124.72 ± 0.00
High-Fpg	73.66 ± 48.87***	51.44 ± 45.38 †	36.74 ± 25.19**	38.87 ± 31.16
High-Endo	75.80 ± 64.23	70.69 ± 48.62**	53.91 ± 36.07	176.21 ± 89.78 †

Unit: a.u. (arbitrary unit)

All values are expressed as mean ± S.D.

*: compare with 0 ppm $P < 0.05$; **: compare with 8 ppm $P < 0.05$; ***: compare with 80 ppm $P < 0.05$; †: compare with all the 0, 8, 80 ppm $P < 0.05$

Table 4. Tail length values of DNA isolated from lymphocytes.

Value / Group	Mean ± S.D			
	1 day	14 day	28 day	90 day
Control-buf	10.53 ± 4.71	286.63 ± 148.88	275.26 ± 163.13	221.80 ± 152.12
Control-Fpg	71.34 ± 112.69	347.22 ± 156.85	273.76 ± 128.40	261.32 ± 113.07
Control-Endo	294.87 ± 199.54	237.69 ± 167.07	215.16 ± 130.28	268.72 ± 159.53
Low-buf	121.43 ± 159.12	300.21 ± 166.40	270.94 ± 140.76	205.79 ± 139.92
Low-Fpg	332.30 ± 250.39*	244.09 ± 152.65*	252.68 ± 128.38	234.58 ± 135.13
Low-Endo	179.62 ± 204.11*	302.82 ± 161.40*	290.05 ± 123.09*	285.75 ± 182.75
Medium-buf	28.95 ± 41.61	261.32 ± 214.01	232.78 ± 156.08	220.00 ± 92.28
Medium-Fpg	60.13 ± 75.08**	353.72 ± 222.25**	180.04 ± 89.65**	289.61 ± 130.62
Medium-Endo	246.14 ± 119.07	265.70 ± 159.39	303.44 ± 132.45*	111.40 ± 68.46**
High-buf	261.87 ± 153.38 †	322.82 ± 165.72***	189.27 ± 97.03**	310.53 ± 0.00
High-Fpg	254.44 ± 119.99***	218.11 ± 140.57***	186.30 ± 104.17**	221.93 ± 117.66
High-Endo	283.12 ± 181.10**	296.12 ± 127.17*	279.72 ± 133.24*	423.52 ± 113.05 †

Unit: μm

All values are expressed as mean ± S.D.

*: compare with 0 ppm $P < 0.05$; **: compare with 8 ppm $P < 0.05$; ***: compare with 80 ppm $P < 0.05$; †: compare with all the 0, 8, 80 ppm $P < 0.05$

Table 5. Fold ratio of rOGG1 mRNA expression isolated from hepatocytes with real time RT-PCR.

Fold ratio / Group	Mean ± S.D			
	1 day	14 day	28 day	90 day
Control-r*OGG1*	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Low-r*OGG1*	4.15 ± 1.00*	1.03 ± 0.06	5.58 ± 8.24*	0.42 ± 0.26
Medium-r*OGG1*	6.87 ± 7.05	0.52 ± 0.52*	2.38 ± 2.51	0.94 ± 0.48
High-r*OGG1*	11.78 ± 10.45*	0.77 ± 0.36	6.41 ± 6.93*	0.89 ± 0.76†

All values are expressed as mean ± S.D.
*: compare with 0 ppm $P < 0.05$; †: compare with all the 0, 8, 80 ppm $P < 0.05$

cytes. In no enzyme test, high dose group was significantly higher than others in 90 days, it had no dose or duration dependency in Fpg-treated lymphocytes.

The mean values of measured Olive tail moment and tail length with Fpg enzyme had no dose or duration dependency, the Endo III FLARE assay in high dose group was significantly higher than others in 90 days reflects high level of oxidative damage in specific site.

These are also reflect the oxidative DNA damage in Endo III sensitive sites such as altered pyrimidine in high dose group of 90 days.

mRNA Expression of DNA Damage Repair Enzymes with Real Time RT-PCR

Table 5 refers the changes (fold ratio) of rOGG1 mRNA expression isolated from hepatocytes with real time RT-PCR (Mean ± S.D.).

The mRNA expression of OGG1 gene was increased with dose dependency in 1 day exposure, decreased to control level in 14 days.

The expression of OGG1 mRNA of low and high dose group was higher than control group in 28 days, but those in 90 days were decreased to as similar in 14 days.

In Table 5, it reflects that the OGG1 gene expression to repair the oxidative DNA damage in liver was inhibited with dose and duration of cumene exposure.

These represent the degree of oxidative DNA damage that formamidopyrimidine, 8-oxoguanine etc. were formed in 14 and 28 days, repaired in 90 days from Fpg FLARE assay and real time RT-PCR.

Discussion

It is well known that chemicals, radiation, metal compounds can make reactive oxygen species, and the ROS induce protein or DNA damage[29]. Consequently, it have been regarded that DNA base damage may induce 8-oxodG, a type of oxidative DNA damages, is the important biomarker of carcinogenesis[26,30].

Comet assay applies widely that not only molecular DNA damage but also epidemiological method in human populations[31]. It was performed[27,28] that genetic toxicity measured with alkaline SCGE analysis with rats exposed the welding fume stainless steel manual metal arc welding.

DNA segments in alkaline SCGE assay is also temporarily present when a cell repaired DNA damage by base excision, nucleotide excision, so the high level of segments in SCGE assay is represent either high DNA damage level or some repair activities[31].

It is applied that the study with cellular repair activity with DNA segments for reveal or amplify the genotoxic effects, and that measuring the repair activity with DNA repair enzymes or inhibition of DNA damage by antioxidants[32], and quantitative measurements of specific oxidative base[26,33].

Phoa and Epe[34] was measured oxidative DNA damages with nitric oxide generated to cultured mammalian fibroblasts endogenously or exogenously, investigated the effects of DNA damages by H_2O_2 to repair enzymes, and reported that NO induced cellular DNA damages inefficiently and protected the DNA damages by H_2O_2, but flexibly inhibited the repair of oxidative DNA base modification. It supposed that the activity of NO to DNA damaged induced by welding fume is very little but helps the repair enzyme inhibition.

According to Abalea. *et al.*[35], it could determine 7 DNA base oxidation products with analysis of induced oxidative DNA damage and repair of DNA base lesion with addition of ferric nitrilotriacetate to *in vitro* primary cultured hepatocytes. In these, the oxidized-purines such as 8-oxo-guanine, xanthine, fapyadenine, 2-oxo-adenine, and DNA base repair activity have dose response with time.

It is less complicated to estimate the oxidative DNA damages directly than observe the changes with chromosomal level, it can offer information about molecular effects of the oxidative stress[36].

Moreover it was validated the FLARE assay with Fpg and Endo III, especially it can measure reducing of the oxidized pyrimidine in study of anti-oxidation

effect of food with Endo III/FLARE assay[37].

However it was analyzed that the DNA damages of workers exposed to radioactive rays had significant results in the SCGE assay with strand break, a measurement of the oxidative DNA damage with DNA repair enzyme are less effective, 8-OHGua is a better biomarker of oxidative damage[38].

Also, it can be considered as adaptive response, a protective effect in occurred to exposure higher concentration of it or chemicals with similar action mechanisms after exposure to low dose radiation or genetic toxicant, it was reported[39] the formation of adaptation related protein such as DNA methyltransferase in animal and plant cells after reported this phenomenon with E. coli in 1977[40].

Ikushima[41] reported that Chinese hamster V79 cell adapted with radioactive thymidine has adaptive effect to high dose X-ray exposure since then, the cell adapted with γ-ray has resistance to mitomycin C and UV and induced sister chromatid exchange and reduced micronucleus formation. Pant *et al*.[42] explained the inhibition of neoplastic transformation related adaptation to low dose with two mechanisms, up-regulation of antioxidant glutathione and extremely increase DNA repair capabilities (super repair).

Therefore, it is considered that the measured values of welding fume exposed *Cynomolgus* monkey are lower than the control group are caused the adaptive response or stochastic effect[43], with being studied and reported generally interested in radioactive rays.

It could be explained more easily to link the existing research results[44,45] of DNA damage repair enzyme reduction with exposure to the metal in low concentration. And then, it could be assumed that the adaptive response of 8-oxodG in welding fume exposed *Cynomolgus* monkey is rather caused up-regulation of antioxidant glutathione reduction than increase of repair enzyme with exposure of low concentration.

Also, Potts. *et al*.[46] reported that oxidative DNA damage in alveolar epithelial cell adapted with cadmium was inhibited, the results of increase thiol-containing antioxidants such as metallothionein, glutathione adapted in low concentration. But the repairing ratio of entirely oxidative damage in cells adapted with cadmium was lower than not adapted cells. They were reported that the cadmium adaptation was significantly injured to the repairing of Fpg and Endo III specific sites by comet assay with lesion specific enzyme. And these results fell in with this study results.

With LightCycler 2.0 (Roche, Germany) using *Universal Probe Library* (https://www.universalprobelibrary.com) to measure the OGG1 gene expression of rat's hepatocytes, it was not known the inhibition mechanism of this gene, but gene is regulated by several transcription factors with complex networks of protein/DNA, protein/protein interaction.

It was identified a potential transcription binding site in human OGG1 gene promoter[47], but it is scarcely known about which transcription factor actually participate to regulate the OGG1 expression.

Chemicals can decrease the ability of 8-oxoG damage repair in DNA with changing the structures of OGG1 enzyme as well as inhibit the gene expression[46].

From all of the results, we suggest that the further investigations will need to perform in rat because of its lack of correlation between the oxidative DNA damage and repair with endonuclease III enzyme.

Moreover it would be more useful as a biomarker for chemical risk assessment to perform these tests with many other hepato-toxic solvents.

Materials and Methods

Test Chemicals

Cumene (Sigma Aldrich, USA, Lot # 06211DE) was used as test chemical and Ficoll-paque plus (Amersham Biosciences, WI, USA) was used to extract lymphocytes. FLARE analysis kit (Trevigen, MD, USA) with Fpg and Endo III were used in FLARE assay. RNAqueous®-4PCR (Ambion Inc., TX, USA) and LightCycler® TaqMan® Master, Universal ProbeLibrary probe (Roche Applied Science, Germany), and primer (BIONEER, Daejeon, Korea) were used for real time RT-PCR.

Animals and Cumene Exposure

From seven-week-old male Sprague Dawley rats (230-250 g, SLC, Japan) were exposed with concentration of 8.05 ± 0.17 ppm, 80.13 ± 1.48 ppm, and 800.85 ± 10.77 ppm for 6 h/day in 1 m^3 inhalation capacity chamber (whole body type, SIBATA Co., Japan) for 13 weeks.

FLARE (Fragment Length Analysis with Repair Enzyme) Assay

FLARE assay was performed according to the method of Tice *et al*.[48]. Electrophoresis was performed at 25 V and 100 mA for 20 min in the dark. The ethidium bromide-stained electropherograms were examined with image analysis software Komet 5.0 (Kinetic Imaging, Ltd., Liverpool, UK). The Olive tail moment[49] and tail length were used to quantitatively measure the extent of DNA damage.

All results of FLARE assay are expressed as the average and standard deviation (mean $\pm$ S.D.). One way ANOVA test and Tukey test ($a = 0.05$) were used with SigmaStat 3.11.

Real Time RT-PCR (Reverse Transcription-Polymerase Chain Reaction)

cDNA was synthesized with transcriptor first strand cDNA synthesis kit (Roche Applied Science, IN, USA). The primer was designed with “ProbeFinder (version 2.35)” online primer analysis software available in *Universal Probe Library*. It was performed real time RT-PCR with LightCycler 2.0 (Mannheim, Germany), LightCycler® Software version 4 for total RNA extracted from hepatocytes to observe the change of OGG1 gene expression. The results were expressed average (mean ± S.D.) used two-way ANOVA ($P < 0.05$) and linear regression.

References

1. World Health Organization (WHO)/International Programme on Chemical Safety (IPCS). Concise international chemical assessment document No. 18. Cumene p. 4, Geneva, Switzerland (1999).
2. U.S. Environmental Protection Agency’s Integrated Risk Information System (IRIS) for Cumene (98-82-8). Available from: http://www.epa.gov/ngispgm3/iris on the Substance File list (2000).
3. Thelestam, M., Curvall, M. & Enzell, C. R. Effect of tobacco smoke compounds on the plasma membrane of cultured human lung fibroblates. *Toxicology* **15**:203-217 (1980).
4. Mackison, F. W., Stricoff, R. S. & Partridge, L. J. Jr. Occupational Health Guidelines for Chemical Hazards. *DHHS* (*NIOSH*) **3**:81-123 (1981).
5. Lewis, R. J. Sax’s Dangerous Properties of Industrial Materials. (9th ed.) *New York: Van Nostrand reinhold* **1-3**:938 (1996).
6. Lewis, R. J., Sr. Hawley’s Condensed Chemical Dictionary. (12th ed.) *New York: Van Nostrand rheinhold Co*. p. 329 (1993).
7. Documentation of the Threshold Limit Values and Biological Exposure Indices, Cincinnati, OH (5th ed.). American Conference of Governmental Industrial Hygienists (ACGIH). p. 151 (1986).
8. Doull, J., Klassen, C. D. & Amdur, M. D. Casarett and Doull’s Toxicology. 3rd ed. *New York: Macmillan Co. Inc.* p. 644 (1986).
9. Jackson, J. *et al*. Test Rule Support Document Cumene *Syracuse Res Corp* p 170 (1985).
10. Chemical Manufacturers Association (CMA). Cumene Program Panel: Industrial Hygiene survey. (1985).
11. American Petroleum Institute (API). Letter to TSCA Interagency Testing Committee USEPA (1984).
12. Cocheo, V. *et al*. Rubber manufacture: Sampling and identification of volatile pollutants. *Amer Ind Hyg Assoc J* **44**:521-527 (1983).
13. National Institute of Occupational Safety & Health (NIOSH). National Occupational Exposure Survey (NOES) (1983).
14. Shah, J. J. & Singh, H. B. Distribution of volatile organic chemicals in outdoor and indoor air. *Environ Sci Technol* **22**:1381-1388 (1988).
15. Schulz, R. C. *et al*. Kirk-Othmer Encycl Chem Technol. 4th ed. NY, *NY: John Wiley and Sons* **7**:735 (1993).
16. Droge, W. Free radicals in the physiological control of cell function. *Physiol Rev* **82**:47-95 (2002).
17. Marnett, L. J. Oxiradicals and DNA damage. *Carcinogenesis* **21**:361-370 (2000).
18. Hazra, T. K., Izumi, T., Maidt, L., Floyd, R. A. & Mitra, S. The presence of two distinct 8-oxoguanine repair enzymes in human cells: their potential complementary roles in preventing mutation. *Nucleic Acids Res* **26**:5116-5122 (1999).
19. Kasai, H. & Nishimura, S. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Res* **12**:2137-2145 (1984).
20. Kasai, H. *et al*. Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis* **7**:1849-1851 (1986).
21. Shibutani, S., Takeshita, M. & Grollan, A. P. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxo dG. *Nature* **349**:431-434 (1991).
22. Kamiya, H. *et al*. c-H-ras containing 8-hydroxyguanine at codon 12 induces point mutations at the modified and adjacent position. *Cancer Res* **52**:3483-3485 (1992).
23. Fairbairn, D. W., Olive, P. L. & O’Neill, K. L. The comet assay: A comprehensive review. *Mutat Res* **339**:37-59 (1995).
24. Ostling, O. & Johanson, K. J. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. *Biochem Biophys Res Commun* **123**:291-298 (1984).
25. Singh, N. P., McCoy, M. T., Tice, R. R. & Schneider, E. L. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res* **175**:184-191 (1988).
26. Collins, A. R., Duthie, S. J. & Dobson, V. L. Direct enzymatic detection of endogenous oxidative base damage in human lymphocyte DNA. *Carcinogenesis* **14**:1733-1735 (1993).
27. Maeng, S. H. *et al*. Changes of 8-OH-dG levels in DNA and its base excision repair activity in rat lungs after inhalation exposure to hexavalent chromium. *Mutat Res* **539**:109-116 (2003).
28. Yu, I. J. *et al*. Inflammatory and genotoxic responses during 30-day welding-fume exposure period. *Toxicol Lett* **154**:105-115 (2004).
29. Asami, S. *et al*. Effects of forced and spontaneous exercise on 8-hydroxydeoxyguanosine levels in rat organs. *Biochem Biophys Res Commun* **243**:678-682 (1998).
30. Kasai, H. Analysis of a form of oxidative DNA dam-

age, 8-hydroxy-2′-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutat Res* **387**:147-163 (1997).
31. Collins, A. R., Dobson, V., Dusinska, M., Kennedy, G. & Stetina, R. The comet assay: what can it really tell us? *Mutat Res* **375**:183-193 (1997).
32. Jekinson, A. M., Collins, A. R., Duthie, S. J., Wahle, K. W. J. & Duthie, G. G. The effect of increased intakes of polyunsaturated fatty acids and vitamin E on DNA damage in human lymphocytes. *FASEB J* **13**: 2138-2142 (1999).
33. Covallo, O. *et al*. Evaluation of oxidative damage and inhibition of DNA repair in an in vitro study of nickel exposure. *Toxicology In Vitro* **17**:603-607 (2003).
34. Phoa, N. & Epe, B. Influence of nitric oxide on the generation and repair of oxidative DNA damage in mammalian cells. *Carcinogenesis* **23**:469-475 (2002).
35. Abalea *et al*. Iron-induced oxidative DNA damage and its repair in primary rat hepatocyte culture. *Carcinogenesis* **19**:1053-1059 (1998).
36. Collins, A. R., Dusinska, M., Gedik, C. M. & Stetina, R. Oxidative damage to DNA: do we have a reliable biomarker? *Environ Health Perspect* **104**:465-469 (1996).
37. Duthie, S. J., Ma, A., Ross, M. A. & Collins, A. R. Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Res* **56**: 1291-1295 (1996).
38. Kruszewski, M., Wojewodzka, M., Iwanenko, T., Collins, A. R. & Szumiel, I. Application of the comet assay for monitoring DNA damage in workers exposed to chronic low-dose irradiation. II. Base damage. *Mutat Res* **416**:37-57 (1998).
39. Yarosh, D. B., Rice, M., Day, R. S. 3rd, Foote, R. S. & Mitra, S. O6-Methylguanine-DNA methyltransferase in human cells. *Mutation Research* **131**:27-36 (1984).
40. Samson, L. & Cairns, J. A new pathway for DNA repair in *Escherichia coli. Nature* **267**:281-283 (1977).
41. Ikushima, T. Radioadaptive response: Characterization of a cytogenetic repair induced by low-level ionizing radiation in cultured Chinese hamster cells. *Mutation Research Letters* **227**:241-246 (1989).
42. Pant, M. C. *et al*. Mechanisms of suppression of neoplastic transformation in vitro by low doses of low LET radiation. *Carcinogenesis* **24**:1961-1965 (2003).
43. Scott, B. R. A biological-based model that links genomic instability, bystander effects, and adaptive response. *Mutat Res* **568**:129-143 (2004).
44. Asmuss, M., Mullenders, L. H., Eker, A. & Hartwig, A. Differential effects of toxic metal compounds on the activities of Fpg and XPA, two zinc finger proteins involved in DNA repair. *Carcinogenesis* **21**:2097 -2104 (2000).
45. Hartwig, A. Carcinogenicity of metal compounds: possible role of DNA repair inhibition. *Toxicol Lett* **102-103**:235-239 (1998).
46. Potts, R. J. Watkin, R. D. & Hart, B. A. Cadmium exposure down-regulates 8-oxoguanine DNA glycosylase expression in rat lung and alveolar epithelial cells. *Toxicology* **184**:189-202 (2003).
47. Dhenaut, A., Boiteux, S. & Radicella, J. P. Characterization of the hOGG1 promoter and its expression during the cell cycle. *Mutat Res* **461**:109-118 (2000).
48. Tice, R. R. *et al*. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol Mutagen* **35**:206-21 (2000).
49. Olive, P. L. & Banath, J. P. Detection of DNA double -strand breaks through the cell cycle after exposure to X-rays, bleomycin, etoposide and 125IdUrd. *Int J Radiat Biol* **64**:349-358 (1993).