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**Thesis title:** *In vitro* chemically-induced DNA damage in cancer patients and healthy individuals

### **Keywords**

Comet assay, micronucleus assay, sister chromatid exchanges, FISH, food mutagens, nanoparticles

### **Abstract**

In the present study DNA damage was measured in peripheral blood lymphocytes from polyposis coli and colorectal cancer patients, treated with different dietary and environmental compounds and compared with lymphocytes from healthy individuals. In addition, confounding factors such as age, gender, alcohol intake and smoking habits were taken into consideration. The assays used in this study included the Comet assay, the Micronucleus assay, the Micronucleus – FISH assay and the sister chromatid exchange assay.

The food mutagens, PhIP and IQ, as well as titanium dioxide nanoparticles (TiO<sub>2</sub> NPs) induced a dose dependent increase in the DNA damage and chromosomal abnormalities in all tested groups regardless of confounding factors. Prior to experiments physicochemical characterisation of nanoparticles was conducted. In the presence of the flavonoids, quercetin and rutin that were acting in an antioxidant manner, the DNA damage resulting from the highest doses of food mutagens was significantly reduced. Thus, dietary supplementation with flavonoid-rich vegetables and fruits may prove very effective in protection against oxidative stress.

The polyposis coli and colon cancer patients were more susceptible to food mutagens, PhIP and IQ, as well as TiO<sub>2</sub> NPs, and in the majority of cases had a higher level of DNA damage in the Comet assay and higher cytogenetic damage in the Micronucleus assay.

In the final project, twelve frequently encountered (NewGeneris) chemical compounds were evaluated to establish their damaging potential in lymphocytes and spermatozoa from healthy donors. The highest damage was produced by DNA reactive aldehydes, food mutagens and benzo[a]pyrene when assessed with the neutral and alkaline Comet assay with and without metabolic activation.

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## LIST OF ABBREVIATIONS

<b>8-MeIQx</b>	2-amino-3,8-dimethylimidazo[4,5 -f]quinoxaline
<b>8-OHdG</b>	8-hydroxydeoxyguanosine
<b>AA</b>	acrylamide
<b>AJCC</b>	American Joint Committee on Cancer
<b>ALS</b>	alkali labile sites
<b>APC</b>	adenomatous polyposis coli gene
<b>ATB1</b>	aflatoxin B1
<b>Au</b>	gold
<b>BaP</b>	benzo[ <i>a</i> ]pyrene
<b>BiBuds</b>	nuclear buds in binucleated cells
<b>BiMN</b>	micronuclei in binucleated cells
<b>BiNC</b>	binucleated cells
<b>BiNPBs</b>	nucleoplasmic bridges in binucleated cells
<b>Bmpr1</b>	Bone morphogenetic protein receptor
<b>BN</b>	binucleated
<b>BPDE</b>	benzo[ <i>a</i> ]pyrene diol epoxide
<b>BRAF</b>	v-Raf murine sarcoma viral oncogene homolog B1
<b>BrdU</b>	bromodeoxyuridine
<b>BRI</b>	Bradford Royal Infirmary
<b>C+MN</b>	centromere positive micronucleus
<b>CA</b>	chromosomal aberrations
<b>Caco-2</b>	human epithelial colorectal adenocarcinoma cell line
<b>CBMN</b>	the Cytochalasin B/ Cytokinesis Block Micronucleus assay
<b>CHO</b>	Chinese hamster ovary cell line
<b>C-MN</b>	centromere negative micronucleus
<b>CRC</b>	colorectal cancer
<b>CTC</b>	CT colonography
<b>CYP1A1/2</b>	} cytochrome P450 enzymes
<b>CYP1B1</b>	

<b>Cyt-B</b>	cytochalasin B
<b>Cytb5Rd</b>	cytochrome b5 reductase
<b>DABCO</b>	1,4-Diazabicyclo[2,2,2]octane
<b>DAPI</b>	4',6-diamidino-2-phenylindole (binds to DNA)
<b>dATP</b>	2'-deoxyadenosine 5'-triphosphate
<b>DBPs</b>	disinfection by-products
<b>DCBE</b>	double contrast barium enema
<b>dCTP</b>	2'-deoxycytidine 5'-triphosphate
<b>ddH<sub>2</sub>O</b>	double distilled water
<b>dGTP</b>	2'-deoxyguanosine 5'-triphosphate
<b>DLS</b>	dynamic light-scattering
<b>DMSO</b>	dimethyl sulfoxide
<b>DNA</b>	deoxyribonucleic acid
<b>DON</b>	deoxynivalenol
<b>DSB</b>	double strand breaks
<b>DTT</b>	dithiothreitol
<b>dTTP</b>	2'-deoxythymidine 5'-triphosphate
<b>EDTA</b>	ethylenediaminetetraacetic acid
<b>ER</b>	estrogen receptor
<b>EtBr</b>	ethidium bromide
<b>EtOH</b>	ethanol
<b>FAP</b>	Familial adenomatous polyposis
<b>FBS</b>	foetal bovine serum
<b>FCS</b>	foetal calf serum
<b>FISH</b>	fluorescence in-situ hybridisation assay
<b>FIT</b>	faecal immunochemical test
<b>FITC</b>	Fluorescein isothiocyanate
<b>FKHRL1</b>	forkhead transcription factor
<b>FPG</b>	fluorescence plus Giemsa
<b>FSIG</b>	flexible sigmoidoscopy
<b>G-6-P DH</b>	glucose-6-phosphate dehydrogenase
<b>G-6-P</b>	glucose-6-phosphate
<b>GA</b>	glycidamide
<b>gFOBT</b>	guaiac faecal occult blood test

<b>Hb</b>	haemoglobin
<b>HCA</b>	heterocyclic amines
<b>Hep3B</b>	human hepatoma cell line
<b>HepG2</b>	human derived hepatoma cell line
<b>HNE</b>	4-hydroxynonenal
<b>HNPCC</b>	hereditary non-polyposis colorectal cancer
<b>HSP</b>	heat shock protein
<b>IARC</b>	the International Agency for Research on Cancer
<b>IL6</b>	Interleukin 6
<b>IQ</b>	2-amino-3-methylimidazo-[4,5- <i>f</i> ]quinoline
<b>IWGTP</b>	the International Workshop on Genotoxicity Test Procedures
<b>KCl</b>	potassium chloride
<b>KRAS</b>	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue
<b>L5178Y</b>	mouse lymphoma cell line
<b>LMPA</b>	low melting point agarose
<b>MAP</b>	MUTYH attenuated FAP
<b>MCF10A</b>	mammary epithelial cells
<b>MCF-7</b>	breast cancer cell line
<b>MDA</b>	malondialdehyde
<b>MLH1</b>	MutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)
<b>MMC</b>	Mitomycin C
<b>MN</b>	micronucleus
<b>MonoMN</b>	micronuclei in mononucleated cells
<b>MSH2/6</b>	MutS homolog 2/6, colon cancer, nonpolyposis type 1 (E. coli)
<b>MUTYH</b>	Mut Y homolog gene (MYH)
<b>NADP</b>	nicotinamide adenine dinucleotide phosphate
<b>NaOH</b>	sodium hydroxide
<b>NDMA</b>	N-nitrosodimethylamine
<b>NIH</b>	National Institute of Health
<b>NMPA</b>	normal melting point agarose
<b>NO<sub>2</sub><sup>-</sup></b>	nitrites
<b>NO<sub>3</sub><sup>-</sup></b>	nitrates
<b>NOC</b>	N-nitroso compounds
<b>NPBs</b>	nucleoplasmic bridges

<b>NSAIDs</b>	nonsteroidal anti-inflammatory drugs
<b>OTM</b>	Olive tail moment
<b>p53</b>	tumour protein 53
<b>p63</b>	tumour protein 63
<b>PCB77</b>	3,3,4,4-tetrachlorobiphenyl
<b>PCBs</b>	polychlorinated biphenyls
<b>PCR</b>	polymerase chain reaction
<b>PHA-M</b>	phytohaemagglutinin M
<b>PhIP</b>	2-amino-1-methyl-6-phenylimidazo[4,5- <i>b</i> ]pyridine
<b>PIK3CA</b>	phosphoinositide-3-kinase
<b>PK</b>	proteinase K
<b>PLP</b>	polyposis coli
<b>PMS2</b>	Mismatch repair endonuclease
<b>PTEN</b>	phosphatase and tensin homolog
<b>Q</b>	quercetin
<b>R</b>	rutin
<b>RNA</b>	ribonucleic acid
<b>ROS</b>	reactive oxygen species
<b>SCEs</b>	sister chromatid exchanges assay
<b>SCGE</b>	Single Cell Gel Electrophoresis
<b>sDNA</b>	stool DNA test
<b>SEM</b>	Scanning Electron Microscopy
<b>SMAD2/4</b>	Mothers against decapentaplegic homolog 2/4
<b>SS</b>	salmon sperm
<b>SSB</b>	single strand breaks
<b>TCDD</b>	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
<b>TiO<sub>2</sub> NPs</b>	titanium dioxide nanoparticles
<b>TNM</b>	tumour-node-metastasis classification
<b>TP53</b>	gene encoding p53
<b>V79</b>	Chinese hamster lung fibroblast cell line

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