DNA damage in Atlantic cod (*Gadus morhua*) tissues

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MSc thesis in Ecotoxicology
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Abstract

Aquatic organisms are exposed to more or less continuous inputs of a wide range of potentially hazardous substances. The sources of the substances could be natural and/ or anthropogenic. This exposure may have deleterious effects on the health of organisms. The intensity of such effects may vary between different tissues. The main aim of this study was therefore to clarify if cells from different tissues differed in their sensitivity to DNA damage and to quantify DNA repair following oxidative stress. This was done using different tissues from the Atlantic cod (*Gadus morhua*). Cells from blood, gills, liver and intestinal mucosa were exposed to two concentrations of peroxide (5 µM and 300 µM). Exposure was followed by the enzyme amended comet assay, using lesion-specific enzymes (fpg and ogg1).

The results indicated high background damage in all tissues, with intestinal mucosa being the most sensitive and liver the least. Causes for the high baseline damage were not clear although it may be speculated that the sampling method for the cells was too aggressive. However, some significant increases were observed in gill and liver cells that were exposed to 5 µM H_2O_2 when compared to the unexposed cells while those exposed to 300 µM H_2O_2 did not cause any significant difference from unexposed cells. This could have been due to high variability, which may have masked effects at high doses. Digestion with enzymes indicated significant increases in oxidative stress for most cells. All these increases were mostly observed at 300 µM H_2O_2, except for liver cells that indicated differences at 0 µM H_2O_2. Earlier studies have also indicated increases in oxidative stress with the use of fpg and ogg1.

The enzyme amendment of the comet assay did not produce clear results in this study, possibly because the high baseline DNA damage masked the effects of repair endonucleases.

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Abbreviations

BSA	Bovine Serum Albumin
Fpg	Formamidopyrimidine DNA glycosylase
Ogg1	8-Oxoguanine DNA glycosylase
EDTA	Ethylenediaminetetraacetic acid
DMSO	Dimethyl sulfoxide
Na_2HPO4	Sodium hydrogen sulphate
NaH_2PO4	Sodium dihydrogen phosphate
LMP	Low melting point
NaCl	Sodium chloride
NaOH	Sodium hydroxide
KCl	Potassium chloride
HCl	Hydrochloric acid
Ca	Calcium
Mg	Magnesium
PBS	Phosphate buffered saline
dH_2O	Distilled water
G	Relative centrifugal force
min	Minute
hr	Hour
Conc.	Concentration
DNA	Deoxyribonucleic acid
DNAase	Deoxyribonuclease
SCGE	Single cell gel electrophoresis
$^{\cdot}OH$	Hydroxyl radical
$^{\cdot}O_2$	Superoxide anion

H_2O_2	Hydrogen peroxide
ROS	Reactive oxygen species
PAH	Polycyclic Aromatic Hydrocarbons
OCP	Organochlorine Pesticides
PCB	Polychlorinated Biphenyls
PAC	Polycyclic Aromatic Compounds
MFO	Mixed function oxidase
OSPAR	Oslo-Paris Commission
JAMP	Joint Assessment and Monitoring Programme
CEMP	Co-ordinated Environmental Monitoring Programme
ICES	International Council for the Exploration of the Sea
ESCODD	European Standard Committee on Oxidative DNA Damage
HPLC	High Performance Liquid Chromatography
TE	Tris EDTA

1 INTRODUCTION

1.1 Marine pollution

The marine environment, which covers about 70% of the earth`s surface, serves as habitat for most animal phyla. Unfortunately, this environment is also a major sink for many potentially hazardous chemicals. Some of these chemicals include organic trace pollutants such as polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) polychlorinated biphenyls (PCBs) (Van der Oost et al., 2003), as well as metals (Mance, 1987). Although PAHs occur naturally at low levels in the marine environment (Hylland, 2006), the increase in their concentrations as well as that of other pollutants may either be due to direct discharges or hydrologic and atmospheric processes (Stegeman et al., 1994.). The sources of PAHs to the marine environment are both natural and/or anthropogenic with the main ones being natural oil leaks from the ocean floor, runoff from roads (Durand et al., 2004), direct disposal into the water (Macdonald et al., 1996) and offshore oil and gas exploration. Once in the environment, most of these pollutants tend to exist as complex mixtures that may interact with other contaminants (additivity, potentiation, synergism or antagonism) (Pape-Lindstrom et al., 1997; Sharma et al., 1999) and understanding the consequences of such mixtures is challenging (Calabrese, 1995). The Oslo-Paris commission (OSPAR) has introduced techniques aimed at monitoring the biological effects of chemicals at national and international levels, through some of their programs (JAMP/CEMP). This monitoring also measure the quality of the marine environment (Thain et al., 2008). Despite attempts to reduce inputs, most of these substances are still present in marine ecosystems. This affects the health of the oceans (Anonymous, 2000), thereby creating potentially life threatening conditions for most aquatic organisms especially fish, which may have a high sensitivity to these pollutants (Schnurstein et al., 2001).

1.2 Atlantic cod

The Atlantic cod (*Gadus morhua*) has a wide distribution and relatively high abundance along the Norwegian coast. Cod has been widely used in environmental monitoring, and is one of three marine fish species recommended by the International Council for the Exploration of the Sea (ICES) for marine monitoring in the north Atlantic. Cod is considered to be stationary (Hop et al., 1992) and is predominantly a demersal feeder. Its diet composition varies with

body size and season. For example, cod with length between 15 – 60 cm feed mainly on decapods and bottom-dwelling caridean shrimp during winter, and on bivalves, amphipods, polychaetes and fish during spring (Hop et al., 1992). Most organisms used by cod as food are highly exposed to environmental pollutants, some of which are known to be persistent and bioaccumulate. This feeding habit could lead to biomagnification of some environmental pollutants in cod. The spawning period of cod differs geographically. Along the Norwegian Skagerrak coast, cod usually spawns in early March (Fromentin et al., 2000), although some variations may occur (Brander, 1994; Wieland et al., 2000). Ocean currents help in drifting the cod larvae and this leads to high genetic similarity among cod populations (Knutsen et al., 2004; Stenseth et al., 2006). However, recent studies show cod populations to be quite genetically local (Halvor et al., 2007).

1.3 DNA damage

The exposure of marine fish species to environmental pollutants may have an impact at the molecular and/or cellular level as well as on the physiology of e.g. fish (Lawrence et al., 2007). Response to pollution may be detected in individuals or the population of the species in question (Elliott et al., 2007).

Damage to DNA may have serious consequences at cell (Cajaraville et al., 2007) and tissue levels (Reichert et al., 1998) as well as on the health of the individual (Hylland et al., 2007). At the cellular level, exposure of fish to pollutants may lead to the formation of reactive oxygen species (ROS) including superoxide anion radical (O^{-}_{2}), hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2). ROS may be genotoxic and can cause severe oxyradical damage to cellular macromolecules such as DNA strand breaks, lipid peroxidation or alteration in protein oxidation (Cajaraville et al., 2007; Winzer et al., 2000). Consequently, DNA damage by oxyradicals can lead to tumor formation. Ultraviolet radiation as well as mutagenic chemicals such as polycyclic aromatic compounds (PACs) may also cause DNA damage, eventually leading to neoplasia (Baumann et al., 1987, 1995; Myers et al., 1987, 1990, 1991, 1992, 1994). Other structural changes that could be incurred by genotoxins are covalent binding to bases and alteration of bases (Devaux et al., 1997).

1.4 Measurement of DNA damage

DNA damage has been investigated over the years using different methods amongst which we have: measurement of DNA adducts (Akcha et al., 2000) using for example, ^{32}P - postlabeling analysis method and high - performance liquid chromatography (HPLC) (Dunn et al., 1987; Ericson et al., 1999, 2000; Holth et al., 2009; Rojas et al., 1994), DNA precipitation assays (Olive et al., 1988) and measurement of DNA breaks using alkali unwinding and the comet assay (Gedik et al., 2005; Singh et al., 1988). The latter, also known as single cell gel electrophoresis (SCGE) was first established by Ostling and Johanson (, 1984), and has been used since then as one of the methods for detecting DNA damage in single cells (Wilson et al., 1998). The alkali version has been used to detect single strand breaks, double strand breaks , and alkali labile sites (Affentranger et al., 1992; Miyamae et al., 1997). This assay has been widely applied not only in DNA damage and DNA repair studies (Speit et al., 2005), but also in genotoxicity testing (Moller, 2005), as well as human biomonitoring (Kassie et al., 2000; Moller, 2006; Sipinen et al., 2010; Valverde et al., 2009). It is used for genotoxic studies of fish and other aquatic organisms which range from freshwater (Devaux et al., 1997; Pandrangi et al., 1995; Russo et al., 2004) to marine (Bombail et al., 2001; Brown et al., 2004; Lee et al., 2003). Apart from its limitation in the measurement of a wide range of break frequencies (Collins et al., 2008), the comet assay remains an important tool for quantifying DNA damage due to its relatively low cost, simplicity, sensitivity and short period of time needed to complete an experiment. The specificity and sensitivity of the comet assay has been improved through the introduction of lesion specific enzymes as suggested by the European Standards Committee on Oxidative DNA Damage (ESCODD) (Gedik et al., 2005). The comet assay is unique from other assays in that it requires cell viability and not cell growth and also documents effects at the single cell level (Ross et al., 1995).

1.5 Different tissues

For a pollutant to produce any biological effects, it must be able to interact with molecules in the target organ (Hylland et al., 2009). Each organ carries out specific functions, all of which contribute to the survival of the organism in its environment. The liver is the main site for metabolism of xenobiotics (Au et al., 1999). It contains mixed function oxidase enzymes

(MFO) which play a major role in detoxification or toxification of e.g. PAHs in fish (Kleinow et al., 1987), through phase I reactions (oxidation reactions). This helps in converting relatively insoluble organic substances to more soluble ones (Buhler et al., 1988), which are either excreted or further transformed into more water soluble substances by phase II enzymes (conjugation reactions). Although most studies on biotransformation in fish have focused on liver (Lemaire et al., 1990), phase I and/or phase II enzymes have been measured in gill (Goddard et al., 1987), kidney (Lindström-Seppä et al., 1981; Payne et al., 1982), heart (Payne Jerry et al., 1979), and intestinal tissues (Lindström-Seppä et al., 1981; Van Veld et al., 1987, 1988; Vetter et al., 1985). Gills play a major role in absorption, ion regulation and gas exchange. Besides their direct involvement in metal uptake (Mieiro et al., 2011), gills are useful in environmental assessment because they are considered as an important route of entry of contaminants as well as a reflection of the environmental status (Pereira et al., 2010). When compared to liver and kidneys, gills could be more susceptible to pollutant-induced oxidative stress (Ahmad et al., 2004). The intestine serves as an interphase between animals and their environment in relation to exchange of material (Gaucher et al., 2012). It plays a role in the digestion and absorption of nutrients (Murray et al., 1996). The length of the intestine gives an idea on the diet of the fish (Albrecht et al., 2001; Xiong et al., 2011) although some studies have shown that the intestinal length sensibly responds to changes in feeding rates and environmental conditions (Kapoor et al., 1976). Blood is the organ responsible for transportation of nutrients and gases. It transports oxygenated blood rich in nutrients such as iron from the gills to the heart, and deoxygenated blood rich in toxins and metabolites such as urea from the heart back to the gills. Based on the knowledge of their roles in toxicokinetics of the test organism, gill, intestinal mucosa, liver and blood tissues were chosen for this study. Also, the possibility for a quick extraction of a sufficient number of single cells or nuclei in suspension was a prerequisite for the selection of these tissues (Hartmann et al., 2003).

1.6 Aim and hypotheses

The main aim of this study was to clarify whether cells from different tissues of Atlantic cod (red blood cells, gills, liver, intestinal mucosa) differed in their sensitivity to DNA damage, and to quantify DNA repair following oxidative stress. All these were done using the enzyme-amended comet assay. To accomplish this, a protocol had to be developed for the separation and subsequent preparation and analysis of "new tissues" (gills, liver and intestinal mucosa). The tissues are referred to as "new" because they have not been very much used in the comet

assay to quantify DNA damage in fish. These aims could further be divided into the following hypotheses:

H_0: There were no increases in DNA damage, measured using enzyme-amended comet, in selected tissues of Atlantic cod following exposure to two concentrations of peroxide.

H_0: There were no differences in DNA damage of different tissues from Atlantic cod.

2 MATERIALS AND METHODS

Details on the preparation of solutions and other media are given in appendix 3.

2.1 Collection

The collecting and sampling of the cod was carried out on the 23-24 March 2011 using FF Trygve Braarud. The sampling area was “Travbanen” in the outer Oslo fjord (Fig.1), which is considered to be relatively unpolluted. A total of 10 individuals of around 1 kg were collected by bottom trawling. The aim of using fish with almost the same size was to avoid variability and standardize the selection. The sampling depth was 110-120 m. The water temperature was 6°C at the bottom and 1°C at the surface. The individuals were immediately transferred into tanks containing flow-through seawater to keep the fish in good condition until sampling.

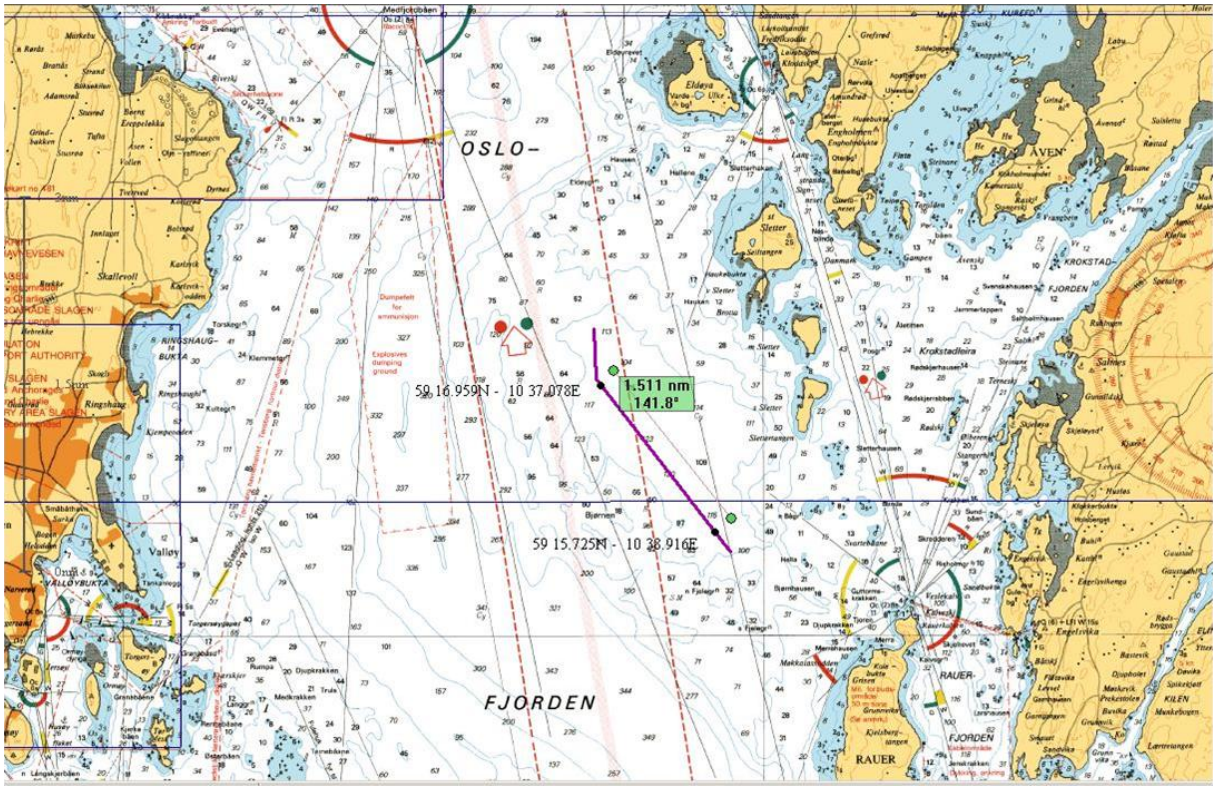

Figure.1: The outer Oslo fjord with track lines indicated in purple.

2.2 Tissue sampling

The following tissues were sampled: blood from the caudal vein; the third gill arc; 0.5 g of liver from the central, front part and the mucosa from a 5 cm section of the intestine, immediately posterior to the blind sacs. All tissues and samples were kept on ice throughout.

A pilot study was carried out to test cell isolation procedures and also to determine the dilution factors for the different cells. The pilot study was carried out on day one of the sampling.

Fish were sacrificed by a knock on the head and several parameters noted (table 2.1). A total of 10 fish were sampled and 7 out of the 10 were female. The mean weight for the fish was 1.105 kg and the mean length, 47.9 cm. The average time spent on sampling each fish was 15 min.

Table 2.1 Details on the weight, length and sex of each fish.

Fish	Weight (kg)	Length (cm)	Sex
1	0.833	45	F
2	1.365	53.5	F
3	1.443	52	M
4	1.324	49.5	F
5	1.074	47	F
6	1.017	47	M
7	1.089	48,5	F
8	0.920	45	F
9	0.883	44	M
10	1.097	47	F

2.2.1 Blood

One hundred µl of blood was collected from the caudal vein of each fish with a heparinated syringe, transferred into an eppendorf tube containing buffer that was composed of 900 µl phosphate buffered saline (PBS) and 10 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.4) and kept on ice for later processing.

2.2.2 Gills

Gill cell isolation was according to the protocol by Kelly et al. (2000). After placing the fish on a bench with paper towel, the operculum was cut open with a pair of scissors that has been cleaned with 70% ethanol and the third gill arc isolated. Its filaments were cut parallel to the axis of the cartilaginous arc, into a small Petri dish containing buffer (PBS + 10 mM EDTA) and kept on ice for about 10 min, with buffer covering the filaments. During this time, filaments were washed manually by shaking gently from time to time to rinse out blood cells. After the first wash, the filaments were removed from the buffer and transferred into 900 µl fresh buffer (PBS + 10 mM EDTA) in an eppendorf tube, held on ice for about 10 min, and later centrifuged for 1 min at 54.5 G. Excess buffer was aspirated and separation of gills cells followed by the addition of 500 µl of trypsin (0.25%) + EDTA solution to the filaments. The cells were shaken manually and incubated for 6 min at room temperature. Centrifugation followed for 4 min at 54.5 G. Excess trypsin + EDTA was then aspirated. Six hundred µl buffer was added to cells and the mixture gently mixed and kept on ice for further processing.

2.2.3 Liver

After cutting open the fish from the anus up to the pectoral fin with the aid of clean dissecting material, about 0.5 g of the liver sample was collected from the center of large blood vessels into buffer and kept on ice. Thereafter, it was chopped into smaller pieces, which were aspirated with a Pasteur's pipette into an eppendorf tube containing 900 µl buffer (PBS + 10 mM EDTA) and kept on ice for about 10 min. The mixture was later centrifuged for 1min at 54.5 G and excess buffer aspirated. The liver pieces were digested in 500 µl trypsin + EDTA and incubated for 10 min at room temperature, followed by centrifugation for 4 min at 54.5 G. Upon removal of excess trypsin, 300 µl buffer was added and the mixture mixed gently, followed by centrifugation for 4 min at 54.5 G. Excess buffer was aspirated and the liver pieces put into tissue mincers, from where they were gently squeezed into an eppendorf tube containing 900 µl fresh buffer (PBS + 10 mM EDTA). The cell suspension was manually and gently shaken and kept on ice for further processing.

2.2.4 Intestinal mucosa

About 5 cm of a piece of intestines was cut directly behind the blind sacs. After cutting open the intestines, the internal medium was gently rinsed with buffer (PBS + 10 mM EDTA), and scraped off into an eppendorf tube on ice, containing 900 µl buffer. Centrifugation followed for 1min at 66 G and excess buffer aspirated. Due to the slimy nature of the tissues of the intestinal mucosa, removal of excess buffer was carefully done in order to avoid sucking up some of the tissues. Trypsination followed with the addition of 500 µl of Trypsin + EDTA. The mixture was incubated for 10 min at room temperature and later centrifuged for 4 min at 66 G. Excess trypsin was removed and 250 µl PBS + EDTA added. The mixture was manually and mildly shaken and kept on ice for further processing.

2.2.5 Cell viability test and quantification

The viability of the newly obtained single cells was determined by the trypan blue exclusion method (Pappenheimer, 1917). Dead cells or cells with damaged membranes will be trypan blue positive (Collins, 2004) while the live ones will not. This can be explained by the fact that live cells do not take up the dye because of the integrity of their membranes. Trypan blue and the cell suspension were mixed in the ratio 2:1 respectively (20 µL + 10 µL) in an eppendorf vial. A drop of the mixture was placed in a Bürker counting chamber and the cells located using a microscope with a 40x magnification lens. The cell concentration of each aliquot was also noted and this gave an idea on the extent to which the samples should be diluted.

Dilution was used in order to avoid cell overlap and facilitate counting. The aim of dilution was to have 100000 cells/mL. Blood was diluted 10 times, gill and liver cells 20 times and the intestinal mucosa in equal volumes.

2.2.6 Embedding cells in agarose gel

Ten µl of the cell suspension were carefully mixed with 90 µl low melting point (LMP) agarose gel at 37^{o}C in an eppendorf tube. Ten µl of the mixture were carefully pipetted from the respective tubes to the corresponding spots on the hydrophilic surface of a gel bond® film. Care was taken during mixing and pipetting to avoid air bubbles. The gel was allowed to set for about 5 min on an aluminium plate. Three sets of replicates were included for each

sample. The purpose of embedding was to immobilize the cells thereby creating a stable environment throughout the protocol.

2.2.7 Exposure to H_2O_2 and lysis

Films were exposed to H_2O_2 that has been diluted in distilled water in different concentrations; 300 µM were used for high concentration and 5 µM for low concentration. Films were immersed each into 120 mL of the corresponding solutions in flat bottom recipients and incubated for 10 min. The control (unexposed) films were immersed in buffer with no H_2O_2. After incubation, each film was rinsed by immersion in buffer for 5 min, and later immersed in 120 mL chilled lysis solution (lysis buffer).

Prior chilling of the lysis solution was mainly to stabilize the agarose gel (Tice et al., 2000). While still in lysis solution, films were stored (for about 2 weeks) in the dark at 4^{o}C until enzyme treatment and subsequent electrophoresis.

The purpose of lysis was to remove cellular and nuclear membranes as well as the majority of proteins (Collins et al., 1995, 2008), thereby liberating DNA (nucleoids) into the surroundings (Cook et al., 1976).

The comet assay protocol used in this analysis was updated by Azqueta et al. (2011). This updated version of the protocol was based on that of Singh et al. (1988).

2.2.8 Enzyme treatment

Enzymes help to improve the specificity and sensitivity of the comet assay through their role in the specific recognition of base damage (Collins et al., 1993, 1996, 1997) and creation of breaks (Collins, 2004) at these points of damage.

Upon removal from lysis solution, each film was washed twice, by immersion in 200 mL enzyme reaction buffer (40 mM Hepes + 0.1 M KCl + 0.5 mM EDTA) at 4^{o}C. The first immersion lasted for 10 min, after which the enzyme reaction buffer was discarded and replaced with a fresh one, in which the films were immersed for another 50 min. This was to completely get rid of any detergents and salts from the lysis solution. The respective thawed

enzyme extracts (1 µg/mL for fpg and 2 units/mL for ogg1) were each added to 40 mL of the corresponding heated enzyme reaction buffer containing 200 µl of bovine serum albumin (BSA). The control films remained untreated. After homogenizing, respective mixtures were poured on the corresponding films, and all of them (including the control), incubated at 37^{o}C for 1 hr.

2.2.9 Alkali treatment and electrophoresis

Electrophoresis permits the migration of the strand breaks towards the anode (Olive et al., 2006), leading to the formation of a tail, whose length and intensity gives an idea on the degree of damage.

Films were immersed in electrophoresis buffer (0.3 M NaOH + 1.0 mM Na_2EDTA, pH 13.2) for 5 min. After rinsing, they were placed on a platform in an electrophoresis tank containing electrophoresis solution that has been cooled at 4^{o}C for at least 1 hr. Unwinding followed for 15 min and electrophoresis run for 20 min at 10^{o}C with voltage level set at 0.8 V/cm across the platform.

2.2.10 Neutralization

After rinsing in distilled water for 1min, films were neutralized twice for 5 min each in 400 mL neutralizing buffer (0.4 M trizma base + dH_2O, pH 7.5) at room temperature (RT). The neutralizing buffer was discarded after the first 5 min and replaced by a fresh one in which the films remained immersed for the next 5 min.

2.2.11 Fixation

After rinsing quickly in distilled water, the films were fixed in 70% ethanol for 5 min, and later immersed in 96% ethanol for 1.5 hrs at RT. They were dried for about 14 hrs and stored in between layers of papers at RT in the dark.

2.2.12 Rehydration and staining

Visualization of the comets is made possible with the use of fluorescent gel stains. SybrGold® is a nucleic acid gel stain, well known for its sensitivity (Tuma et al., 1999), as

well as signal quenching properties (Tebbs et al., 1999) and whose binding to DNA emits fluorescence that permits the visualization of comets.

A mixture of 40 µL SybrGold® solution (10x dilution of SybrGold® stock in DMSO) and 50 mL Tris EDTA (TE) buffer (pH 8) was poured on each film in a staining recipient at RT. Staining lasted for 20 min in the dark with mild shaking.

The films were later removed and rinsed properly in water to remove excess dye. A drop of distilled water was added to the films before covering them with large cover slides.

2.3 Scoring

Comets analysis was done using an Olympus BX51 (Japan) microscope with Olympus Burner (Osram Mercury Short-Arc HBO® 100 W/2 lamp), an A312 camera from Basler Vision Technologies (Germany) and a computerized image analysis system from perspective instrument (UK). A total of 50 comets were targeted per gel although some gels did not have the required number. Consequently, the total score for some gels was less than 50 (details of scores are presented in raw data in appendix 1). Scoring of the comets was done visually and each comet was given a value of 0, 1, 2, 3 or 4, where 0 represented no damage and 4, complete damage (Collins et al., 1995). The choice of visual scoring was due to the high concentration of cells in some gels, leading to overlapping of cells. This made scoring using the comet assay software impossible. While scoring, certain rules were followed with respect to the selection of comets; scoring was done in a regular pattern (to avoid double scoring of each comet) while ensuring that the selected comets represented the whole gel (Collins, 2004), comets with unusual characteristics (wrong tail orientation, unusual form of the cell), overlapping comets as well as those around foreign objects and air bubbles were avoided (the damage in these areas may not be a good representative of the gel). The above rules aimed at preventing favoritism in this process where selection of comets was done manually.

Percentage of DNA in tail was used to present results because it has been described as the most useful parameter in visual scoring (Collins, 2004) and also as a good indicator for DNA damage (Kumaravel et al., 2006). Scores were calculated by summing the products of category and frequency. This gave the weighted sum which was then multiplied by a factor 25 to obtain results on a scale of 0 to 100%. Median DNA damage is given for cells scored and is expressed relative to the maximum visual tail size (all cells in class 4 = 100%).

2.4 Statistical analysis

All data was analyzed using JMP® 9.0.2 (SAS Institute Inc.) and GraphPad Prism 5 (GraphPad Software Inc.). Levene's test was used to check for homogeneity of variances between the different tissues and their treatments (Levene, 1960.). In cases of variance equality, tissues were compared using one way analysis of variance (ANOVA) (Zar, 2010.). If the ANOVA was significant, Dunnett's post hoc test was used to compare treated tissues against respective controls (Dunnett, 1955). Tukey-Kramer's post hoc test was used to compare more than two groups. In cases of variance inequality, the data was $\log_{10}$ – transformed and Levene's test run again. If the variance still remained unequal, a non-parametric Kruskal-Wallis test was used to compare more than two groups. In cases where the Kruskal-Wallis test was significant, Dunn's post hoc test was used to compare the differences between treated tissues and their controls. A probability of $p < 0.05$ was considered significant (reject H_0: no difference between treatments or tissues) for all statistical analyses (Zar, 2010.).

Data from all H_2O_2 exposed tissues was $\log_{10}$ – transformed because it revealed variance inequality according to Levene's test. For tests on enzyme treatment, only data from blood and intestinal mucosa required $\log_{10}$ – transformation.

3 RESULTS

3.1 DNA damage in tissues

A separate analysis was made for tissues that were not exposed to H_2O_2 to clarify any differences in baseline DNA damage (table.3.1). There were no significant differences in tail DNA between unexposed samples of blood, gills, intestinal mucosa and liver tissues (ANOVA; DF=3, F=0.32, p=0.8).

Table 3.1 Tail DNA in comet presented as median and 10–90 percentiles.

Tissue	**% Tail DNA**	**Number of individuals**
Blood	85; 60 - 100	10
Gill	81; 42.5 - 92.5	10
Intestinal mucosa	87.5; 37.5 - 95	10
Liver	79.3; 62.5 - 92.5	8

3.2 Sensitivity of different tissues to peroxide

Variation in the concentration of tail DNA was not homogenous between tissues upon exposure to 5 and 300 µM H_2O_2.

3.2.1 Blood

There were no significant differences in tail DNA of blood cells exposed to the different concentrations of H_2O_2 (Kruskal-Wallis; DF=2, p=0.07) (Figure 3.1). The median values for tail DNA were 85 for unexposed, 97.5 for 5 µM and 89.3 for 300 µM H_2O_2.

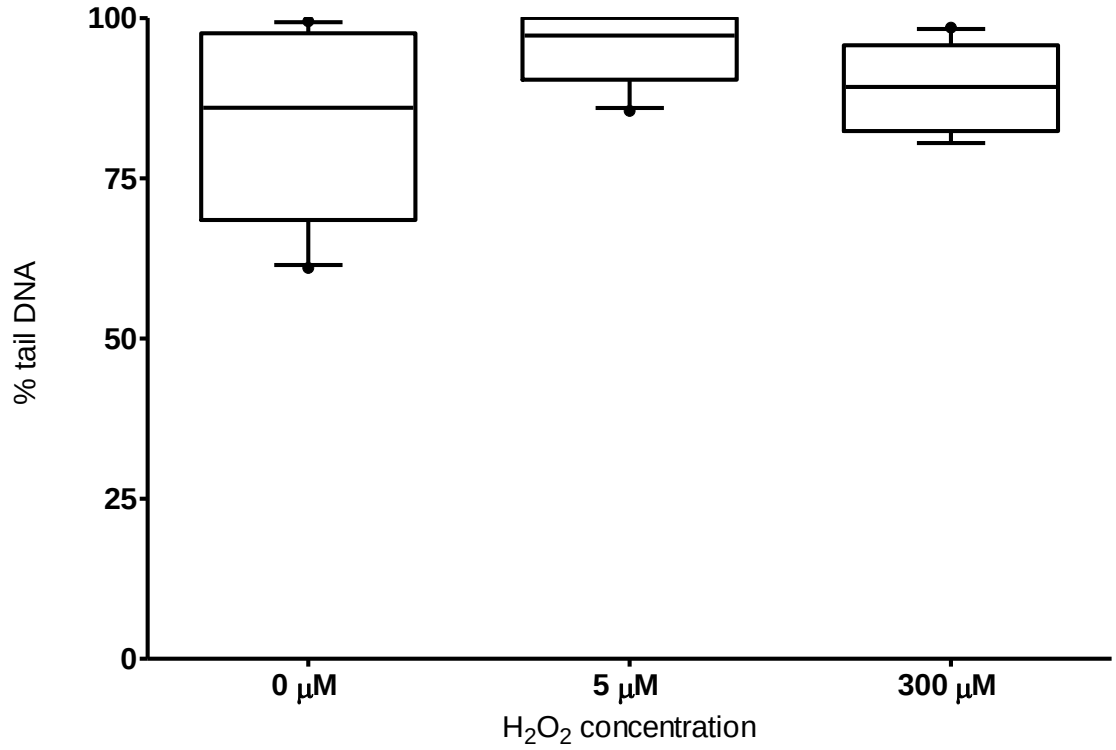

Figure 3.1 Tail concentration of DNA in blood presented as median, quartiles and 10-90 percentile.

3.2.2 Gills

There were significant differences in tail DNA of gill cells exposed to different concentrations of H_2O_2 (Kruskal-Wallis; DF=2, p=0.002) (Figure 3.2). Tail DNA was significantly higher for gill cells exposed to 5 µM H_2O_2 concentrations when compared to unexposed cells (Dunn; n=10, Z=3.51 p=0.001). There were no significant differences in tail DNA of gill cells that have been exposed to 300 µM H_2O_2 concentrations when compared to unexposed cells (Dunn; n=10, Z=1.51, p=0.26). The median values for tail DNA were 81.3 for unexposed, 95.3 for 5 µM and 88.5 for 300 µM H_2O_2.

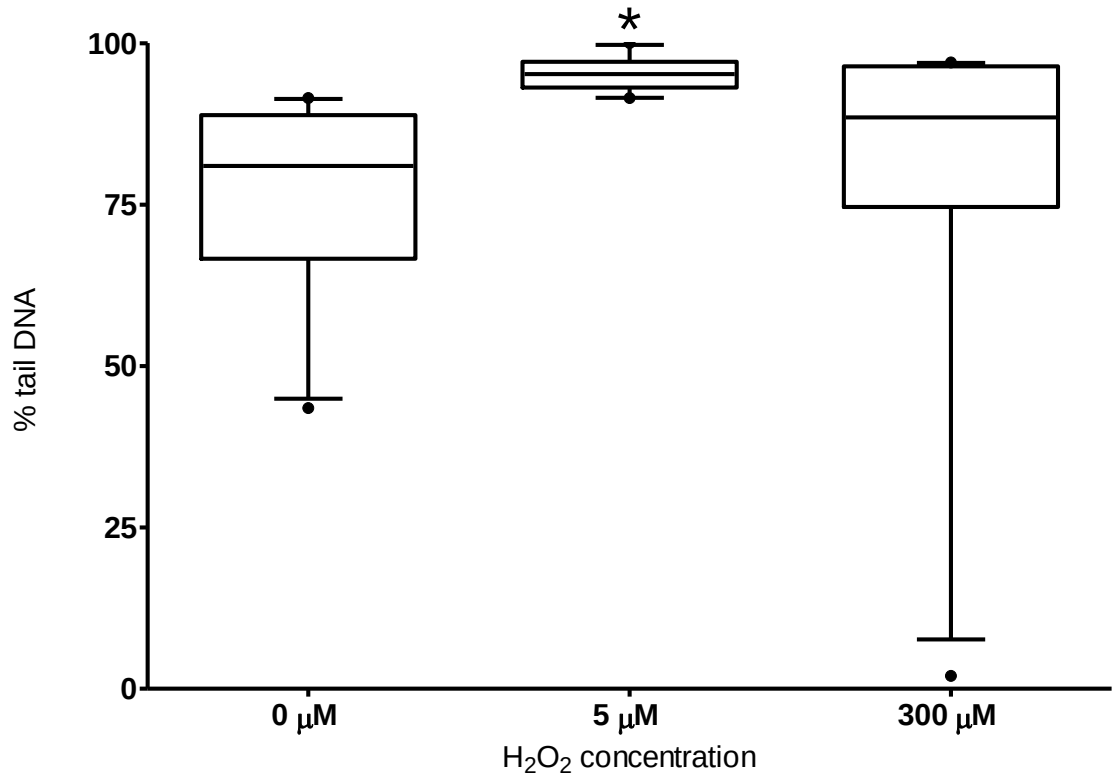

Figure 3.2 Tail concentration of DNA in cells isolated from gills presented as median, quartiles and 10-90 percentile. *significantly different from unexposed (0 µM H_2O_2) (Dunn; $p<0.05$).

3.2.3 Liver

There were significant differences in tail DNA of liver cells between the different concentrations of H_2O_2 (Kruskal-Wallis; DF=2, p=0.0002) (Figure 3.3). The tail DNA of liver cells treated with 5 µM H_2O_2 was significantly higher when compared to unexposed cells (Dunn; n=9, Z=2.99, p=0.006). There were no significant differences in tail DNA of cells exposed to 300 µM H_2O_2, when compared to unexposed cells (Dunn; n=9, Z=-0.70, p=0.96). The median values were 79.3 for unexposed, 95 for 5 µM and 75 for 300 µM H_2O_2

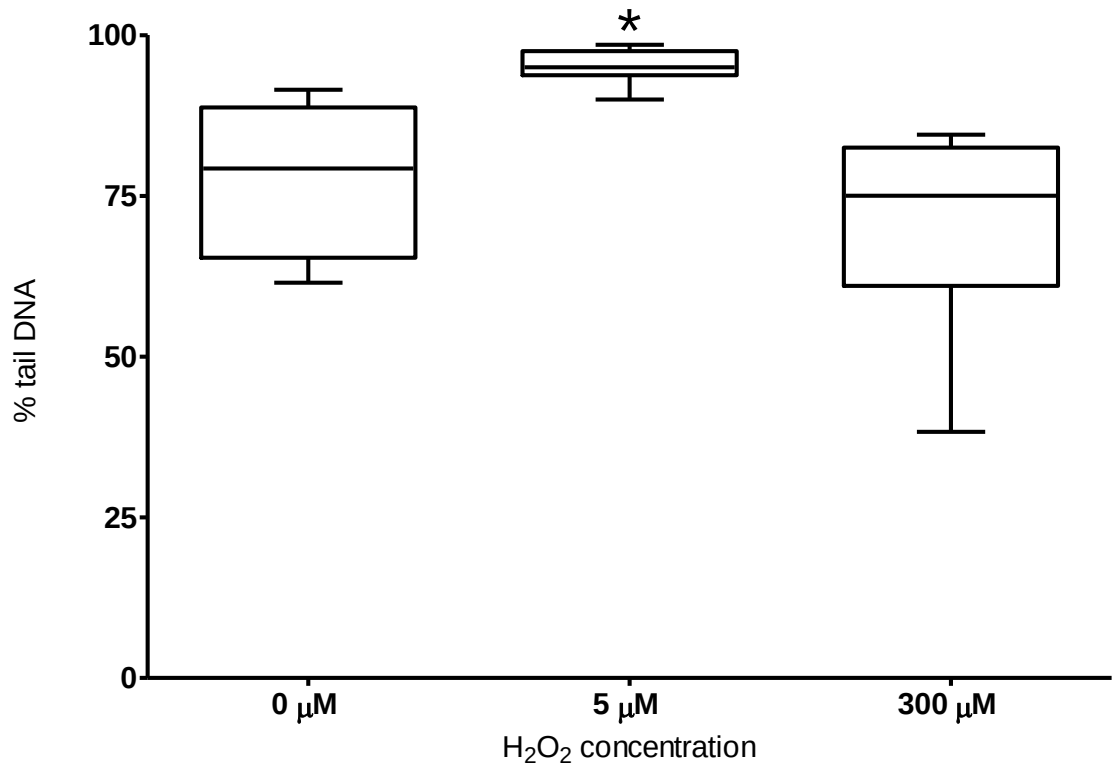

Figure 3.3 Tail concentration of DNA in cells isolated from liver presented as median, quartiles and 10-90 percentile. *significantly different from the unexposed (Dunn; $p<0.05$).

3.2.4 Intestinal mucosa

There were significant differences in tail DNA of cells of the intestinal mucosa between the different concentrations of H_2O_2 (Kruskal-Wallis; DF=2, p=0.01) (Figure 3.4). However, there were no significant differences in tail DNA of cells of the intestinal mucosa that have been exposed to 5 µM H_2O_2 (Dunn; n=10: Z=1.93, p=0.11) and 300 µM (Dunn; n=10, Z=-0.86, p=0.78) when compared to unexposed cells. The median values were 87.8 for unexposed, 94.8 for 5 µM and 79.3 for 300 µM H_2O_2.

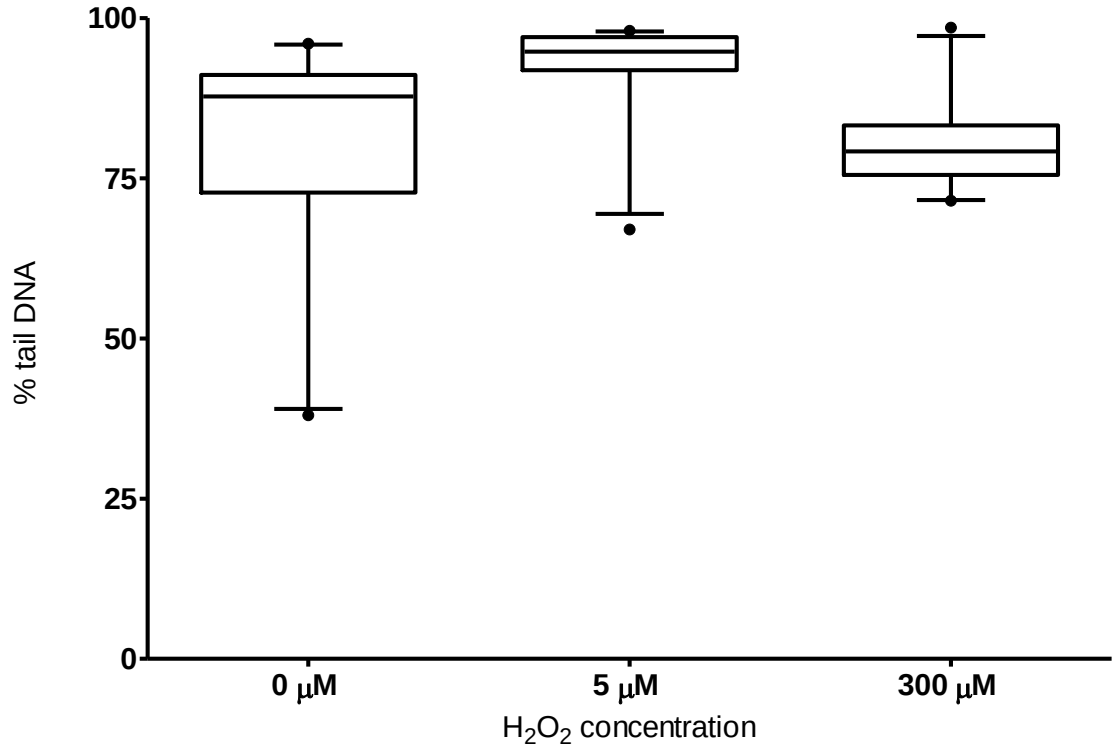

Figure 3.4 Tail concentration of DNA in cells isolated from intestinal mucosa presented as median, quartiles and 10-90 percentile.

3.3 Enzyme treatment

3.3.1 Blood

Variance in tail DNA was homogenous for blood cells exposed to 5 µM H_2O_2 (Figure 3.5). There were no significant differences in oxidative stress between the different treatments measured by specific enzymes (Kruskal-Wallis, DF=2, p=0.7). A one - way ANOVA indicated no significant differences between enzyme treated cells that were not exposed to H_2O_2 (ANOVA, DF=2, F=0.74, p=0.5), as well as those exposed to 5 µM H_2O_2 (ANOVA, DF=2, F=0.9, p=0.4). Median values for the different treatments were as follows: For 0 µM H_2O_2; 85 for no enzyme, 90 for fpg and 87.5 for ogg1; For 5 µM H_2O_2; 97.5 each for no enzyme and ogg1, and 87.5 for fpg; For 300 µM H_2O_2; 90 each for no enzyme and fpg and 70 for ogg1.

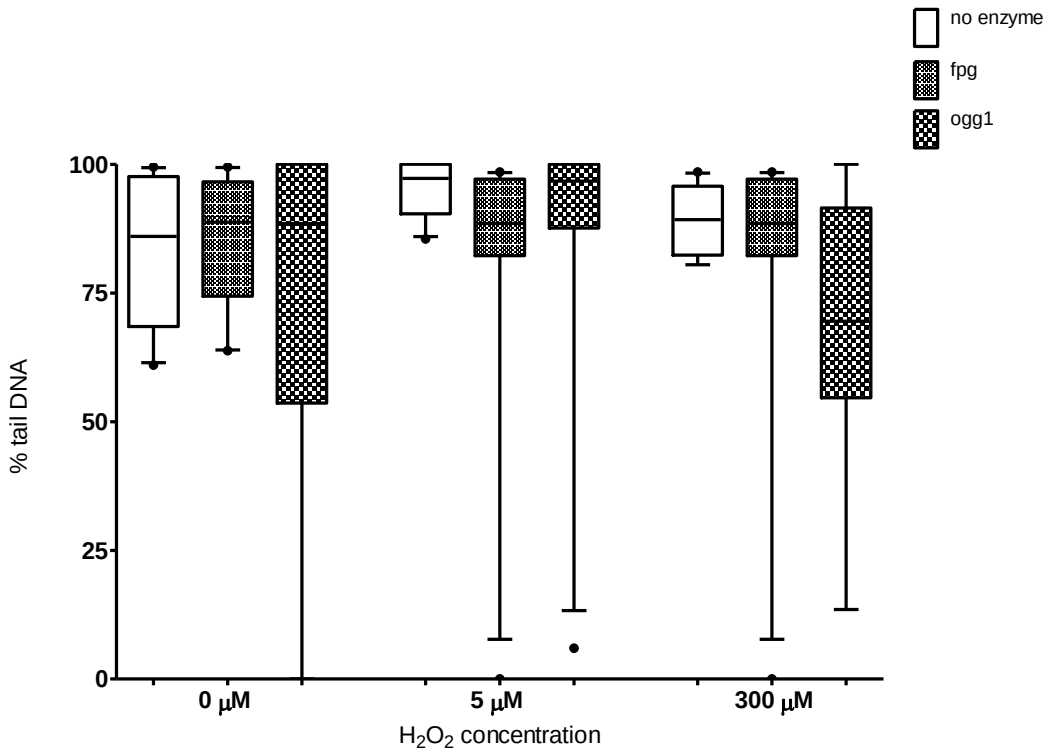

Figure 3.5 Tail concentration of DNA indicating no differences in oxidative stress between different treatments of blood cells presented as median, quartiles and 10-90 percentile.

3.3.2 Gills

Variation in tail DNA of gill cells was homogenous for most treatments (Figure 3.6). There were significant differences in oxidative stress between the different enzyme treatments that were exposed to 300 µM H_2O_2 (Kruskal-Wallis, DF=2, p=0.03). At 300 µM H_2O_2, gill cells treated with fpg had significant increases in oxidative stress (Dunn, n=10, Z=2.6, p=0.02), while those treated with ogg1 indicated no significant difference (Dunn, n=10, Z=0.8, p=0.8) when compared to non treated cells. A one - way ANOVA indicated no significant differences in oxidative stress, between unexposed enzyme treated cells, (ANOVA, DF=2, F=0.07, p=0.93), as well as those exposed to 5 µM H_2O_2 (ANOVA, DF=2, F=1, p=0.4). Median values for the different treatments were as follows: For 0 µM H_2O_2; 81.3 for no enzyme, 92.8 for fpg and 89.5 for ogg1; For 5 µM H_2O_2; 95 each for no enzyme and ogg1 and 90 for fpg; For 300 µM H_2O_2; 87 for no enzyme, 97.5 for fpg and 90 for ogg1.

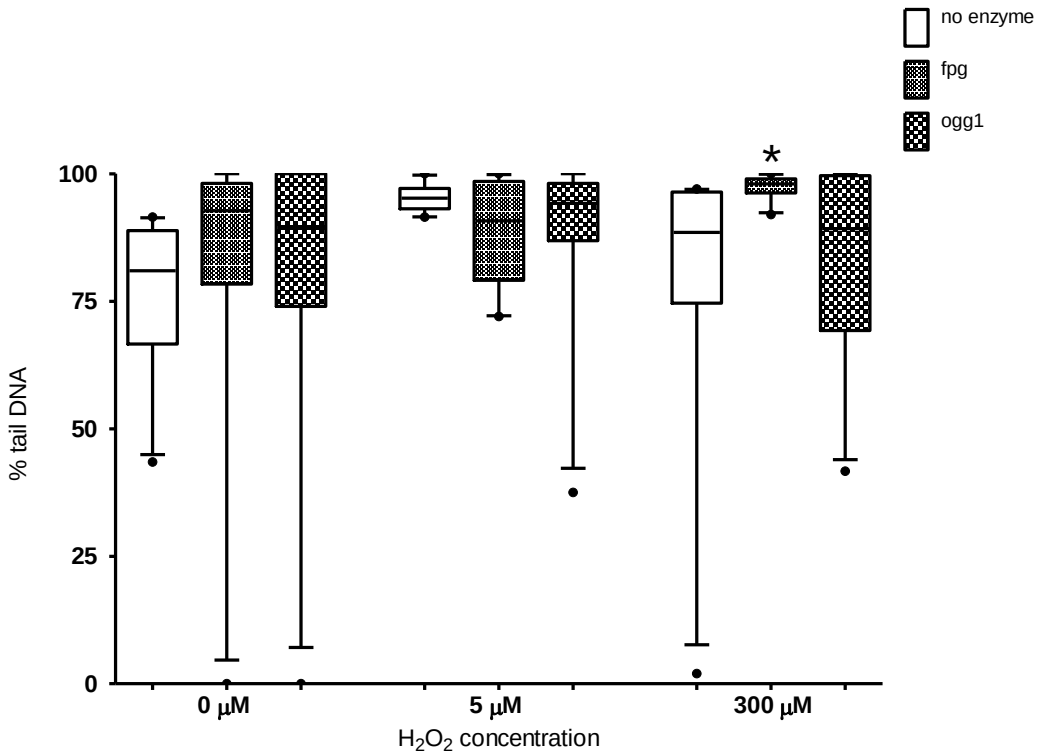

Figure 3.6 Tail concentration of DNA for gill tissues presented as median, quartiles and 10-90 percentile.*significantly different from no enzyme treatment (Dunn, $p<0.05$).

3.3.3 Liver

Variation in tail DNA between the different liver exposures was not homogenous for all treatments (Figure 3.7). There were significant differences in oxidative stress between enzyme treated liver cells that were exposed to 300 µM H_2O_2 (Kruskal-Wallis, DF=2, p=0.001). On the contrary, enzyme treated liver cells exposed to 5 µM H_2O_2 indicated no significant differences in oxidative stress (Kruskal-Wallis, DF=2, p=0.6). A one-way ANOVA indicated significant differences in oxidative stress between unexposed enzyme treated cells (ANOVA, DF=2, F=7.36, p=0.003). There were significant increases in oxidative stress for unexposed liver cells that were treated with either fpg or ogg1 when compared to non treated cells (Dunnett, $n\geq8$; fpg, p=0.002: ogg1, p=0.02). Similar observations were made for liver tissues treated with 300 µM H_2O_2 (Dunn, $n\geq8$. fpg, Z=3.6, p=0.001: ogg1, Z=3, p=0.01). The oxidative stress in unexposed cells was significantly different for the pairs ogg1-no enzyme (Tukey-Kramer, p=0.03) and no enzyme-fpg (Tukey-Kramer, p=0.003) while no significant differences were observed for the pair fpg-ogg1 (Tukey-Kramer, p=0.6). Median values for the different treatments were as follows: For 0 µM H_2O_2; 80 for no enzyme, 97.5 for fpg and

95 for ogg1; For 5 µM H_2O_2; 95 each for no enzyme and ogg1 and 92.5 for fpg; For 300 µM H_2O_2; 75 for no enzyme, and 97.5 each for fpg and ogg1.

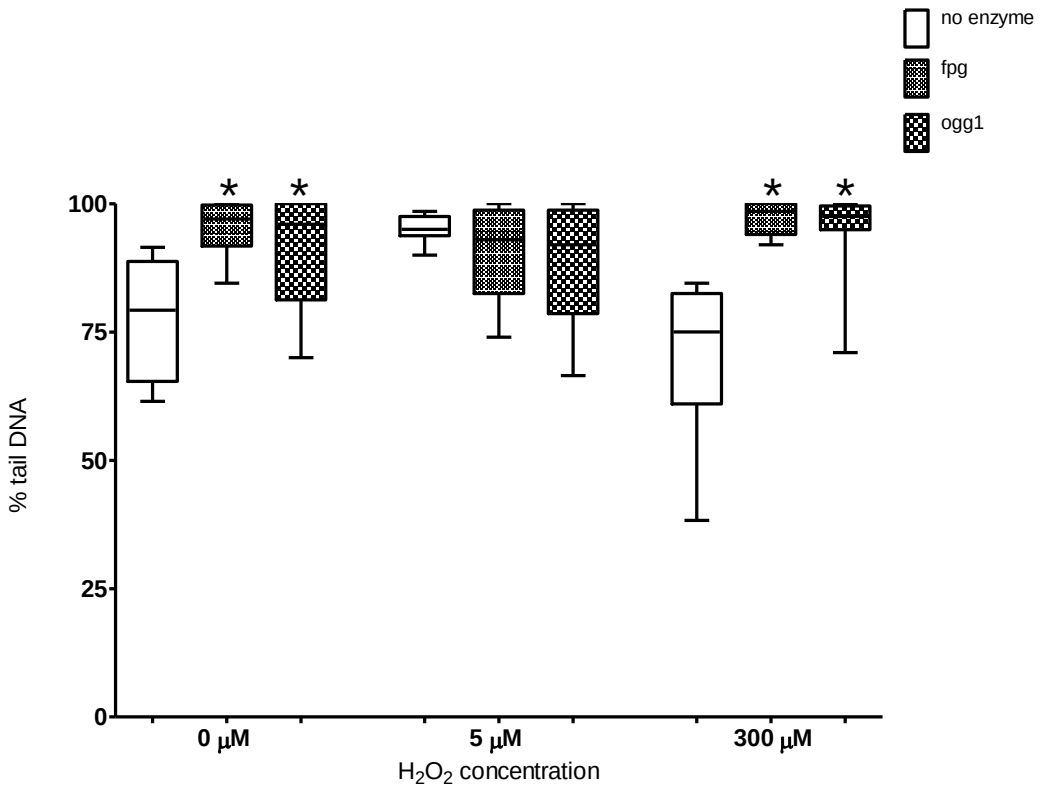

Figure 3.7 Tail concentration of DNA of liver cells presented as median, quartiles and 10-90 percentile. *significantly different from respective no enzyme treatment (Dunn, $p<0.05$; Dunnett, $p<0.05$).

3.3.4 Intestinal mucosa

Variation in tail DNA between the different cells of the intestinal mucosa was not homogenous for most treatments. (Figure 3.8).There were no significant differences in oxidative stress between unexposed enzyme treated cells (Kruskal-Wallis, DF=2, p=0.05) and also between those exposed to 5 µM H_2O_2 (Kruskal-Wallis, DF=2, p=0.06). A one-way ANOVA indicated significant differences between tissues exposed to 300 µM H_2O_2 (ANOVA, DF=2, F=4.7, p=0.02). At 300 µM H_2O_2 concentrations, cells treated with fpg indicated significant increases in oxidative stress when compared to the untreated (Dunnett, $n\geq9$, fpg, p=0.01), while those treated with ogg1 indicated no significant differences (Dunnett, n=10, p=0.1) when compared to untreated cells. Oxidative stress in cells exposed to 300 µM H_2O_2 was significantly different for the pair fpg-no enzyme (Tukey-Kramer, p=0.02), while

no significant differences were indicated for the pairs ogg1-no enzyme (Tukey-Kramer, p=0.1) and fpg-ogg1 (Tukey-Kramer, P=0.6). Median values for the different treatments were as follows: For 0 µM H_2O_2; 87.5 for no enzyme, 85 for fpg and 90 for ogg1; For 5 µM H_2O_2; 95 for no enzyme, 97.5 for fpg and 92.5 for ogg1; For 300 µM H_2O_2; 80 for no enzyme, 97.5 for fpg and 90 for ogg1.

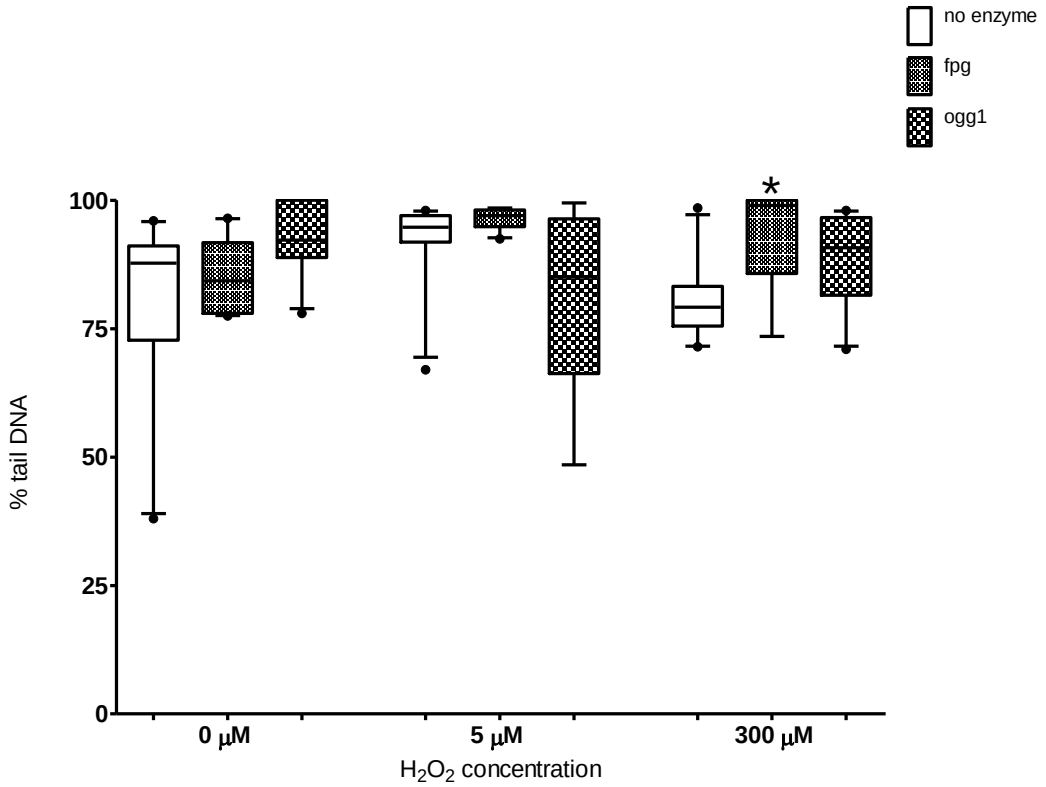

Figure 3.8 Tail concentration of DNA of tissues of the intestinal mucosa presented as median, quartiles and 10-90 percentile. *significantly different from no enzyme treatment (Dunnett, p<0.05).

4 DISCUSSION

4.1 Differences between tissues

The study indicated some differences in sensitivity to DNA damage between fish cells used i.e. blood, gills, liver and intestinal mucosa. The baseline DNA damage was surprisingly high in unexposed cells from all tissues. Cells from the intestinal mucosa were the most sensitive (87.5), followed by blood (85) then the gills (81) and finally the liver (79.3).

Earlier studies have shown that gills are more sensitive than the liver (Ahmad et al., 2004). This could be related to its (gills) role as an important route for contaminant uptake. Increase in the sensitivity of damage of intestinal mucosa could be related to earlier studies which indicate that monooxygenase activities in most fish are relatively low in the intestinal mucosa compared to the liver (Hänninen et al., 1987). Other studies by Ericson et al. (1998) to measure the effects of contaminants on fish, revealed that DNA adducts were higher in gill than blood cells. This also was an indication that gills are more sensitive to DNA damage than blood which is the opposite of what was detected in this study.

Some studies have been carried out on DNA damage in fish cells, not only with respect to contaminants exposure, but also on the methods of sample preservation prior to analysis (Skei, 2010). Skei in this study was able to show that baseline DNA could still be observed after prolonged storage of samples in lysis buffer, but not when frozen.

4.2 Sensitivity of different tissues to peroxide

High background levels in DNA damage made it challenging to compare tissues. When compared with the unexposed cells, median values of DNA damage were higher for cells of blood, gills, liver and intestinal mucosa that were exposed to 5 µM H_2O_2 concentrations, although significant differences were only apparent for hepatocytes and gill cells. These significant effects relate with previous findings in which the DNA damage in cells treated with 5 µM H_2O_2 was found to be about 1.5 to 4 times higher than in control cells (Nacci et al., 1996).

Results from exposure of cells to 300 µM H_2O_2 concentrations were not clear. When compared with unexposed cells, blood and gill cells that were exposed to 300 µM H_2O_2 had

higher median levels of DNA damage, but differences were not significant. The liver and intestinal mucosa appeared to have lower levels of DNA damage under the same conditions (300 µM H_2O_2) when compared with unexposed cells. This finding was contrary to that of Nacci et al (1996), in which exposure of cells to higher H_2O_2 concentrations led to DNA damage of about 11-17 times higher than the control. The sensitivity of tissues exposed to 5 µM H_2O_2 could be as a result of low variability in this group. High variability may have masked effects at high doses. Selective damage at low concentrations could be another possibility for the high sensitivity at 5 µM H_2O_2. The selectivity could be related to previous studies which have shown that cell damage occur at lower H_2O_2, concentrations (Imlay et al., 1988). These same studies explain that "actively growing cells are killed by lower, more physiological doses of H_2O_2, particularly if they lack enzymes required for recombinational or base excision DNA repair pathways."

4.3 Enzyme treatment

The high background damage again made it difficult to interpret the results from enzyme treatments. The two enzymes would both be expected to increase DNA damage as measured using the comet assay. Following exposure to 300 µM H_2O_2, there was a significant increase in oxidative stress for at least one of the enzyme-treated groups of cells from gills, liver and intestinal mucosa. Significant increases in oxidative stress were also detected in unexposed liver cells (0 µM H_2O_2) that were treated with Formamidopyrimidine DNA glycosylase (fpg) and 8-oxoguanine DNA glycosylase (ogg1). These increases in oxidative stress were clearer for fpg than ogg1 treatments.

Fpg and ogg1 are repair endonucleases that specifically recognize damaged bases and create breaks at these points of damage. The higher levels of damage in fpg treatments conform with previous findings which say that fpg detects altered purines especially 8-oxoguanine (Albertini et al., 2000; Dušinská et al., 1996; Tice et al., 2000), and also identifies AP sites and open ringed N-7 guanine adducts (Akcha et al., 2003; Epe et al., 1993; Li et al., 1997; Tchou et al., 1994; Tudek et al., 1998). Ogg1 is more specific than fpg and detects 8-oxoguanine and methyl-fapy-guanine (Boiteux et al., 1998; Smith et al., 2006) during the comet assay. The sensitivity of the assay may be affected by high intra-individual variability (Akcha et al., 2003).

4.4 Comet methods for cod tissues

The results for unexposed cells show that there was high baseline DNA damage in all isolated tissues. The comet classes that represented baseline damage in this study were 3 and 4. This contradicts with results from previous studies, which indicate that DNA damage in unexposed samples range mostly from classes 0 to 1 (Collins et al., 1997, 2004). Causes for the high background in DNA damage for the tissues in the present study are unknown. However, some speculations can be made as to what might have possibly been the cause for this high background damage. DNA of cells could be affected by their duration in lysis solution, i.e. the increase in background could be caused by oxidative damage during storage in lysis solution. Although Nacci et al. (1996) reported that it was possible to store samples in lysis buffer for several months, other studies have shown that storing of samples for a prolonged period in lysis buffer can produce changes in the comet patterns (Belpaeme et al., 1998). Following standard protocols for embedding of isolated cells in low melting agarose gel prior to the comet assay, an aluminum plate was used as a cooling surface for the films. Formation of ice crystals in the cells could occur in a situation where cooling was rapid, leading to DNA damage (Mazur, 1984). This could be possible if the temperature of the cooling surface was too low. The use of chemical and mechanical methods in cell isolations may also lead to DNA damage, although this is usually to a lesser extent when compared to other methods (Kosmehl et al., 2006). It could be that sampling was carried out on fish that might have been physically stressed by trawling. Stress can lead to the elevation of steroid hormones like cortisol (Barton, 2002; Espelid et al., 1996), which might have detrimental effects on the fish (Cajaraville et al., 2007). Also the fact that different tissues were extracted from the same fish could contribute to cell stress as a result of time lapse between tissue extraction and putting on ice for subsequent processing. Following comet assay protocols, EDTA is used as one of the components of the buffer during tissue processing. It is an anticoagulant that prevents the activity of several enzymes like DNase from DNA degradation (Yagi et al., 1996), by chelating divalent metal ions (Kumar et al., 2000), which are cofactors for many active enzymes inside the cell. That notwithstanding, damage to DNA could still be possible through enzymes that do not depend on these cofactors (Skei, 2010).

High background damage is not common in control cells although sampling of red blood cells from cod has previously also yielded to high background DNA damage with unknown cause (Brunborg, pers. comm.).

5 CONCLUSION

Variability and selective damage could be related to factors that could not be identified and therefore not be controlled. There could also be a possibility of coincidence where most of the apparently healthy individuals might have actually been having health problems that could not be physically detected at the time of catch.

The enzyme amendment of the comet assay did not produce clear results in this study, presumably because the high baseline DNA damage masked the effects of repair endonucleases.

6 Future Work

Improvement on the knowledge for methods of separation and preparation of "new tissues" such as gills, liver and intestinal mucosa should be considered.

An increase in knowledge of factors affecting the different steps of the comet assay protocol for fish tissues, and how these factors should be controlled need to be considered.

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Appendices

Appendix 1: Raw data

					Frequency for each comet class						
Tissue	**Sample**	**H_2O_2 conc.**	**Enzyme**	**Cells scored**	**0**	**1**	**2**	**3**	**4**	**weighted sum**	**% tail DNA**
Blood	1	control	None	50	.	.	0.42	0.54	0.04	2.62	65.5
Blood	2	control	None	50	0.12	0.06	0.26	0.38	0.18	2.44	61
Blood	3	control	None	50	0.02	0.12	0.24	0.3	0.32	2.78	69.5
Blood	4	control	None	50	0.04	0.1	0.22	0.28	0.36	2.82	70.5
Blood	5	control	None	50	0.02	0.04	0.18	0.28	0.48	3.16	79
Blood	6	control	None	50	.	.	.	0.18	0.82	3.82	95.5
Blood	7	control	None	50	.	.	0.02	0.24	0.74	3.72	93
Blood	8	control	None	50	.	.	.	0.08	0.92	3.92	98
Blood	9	control	None	50	.	.	.	0.02	0.98	3.98	99.5
Blood	10	control	None	50	.	.	.	0.1	0.9	3.9	97.5
Blood	1	control	fpg	50	.	.	0.22	0.18	0.6	3.38	84.5
Blood	2	control	fpg	50	.	.	0.28	0.34	0.38	3.1	77.5
Blood	3	control	fpg	50	0.04	0.2	0.2	0.24	0.32	2.6	65
Blood	4	control	fpg	40	0.08	.	0.45	0.25	0.225	2.55	63.8
Blood	5	control	fpg	50	.	.	0.08	0.14	0.78	3.7	92.5
Blood	6	control	fpg	50	.	.	0.08	0.3	0.62	3.54	88.5
Blood	7	control	fpg	50	.	.	0.08	0.28	0.64	3.56	89
Blood	8	control	fpg	50	.	.	.	0.02	0.98	3.98	99.5
Blood	9	control	fpg	46	.	.	.	0.07	0.93	3.93	98.4
Blood	10	control	fpg	50	.	.	.	0.16	0.84	3.84	96
Blood	1	control	ogg1	50	1	.	.	.	.	0	0
Blood	2	control	ogg1	50	.	0.12	0.34	0.1	0.44	2.86	71.5
Blood	3	control	ogg1	50	.	0.22	0.16	.	0.62	3.02	75.5
Blood	4	control	ogg1	50	.	.	0.12	0.1	0.78	3.66	91.5
Blood	5	control	ogg1	50		0.08	0.12	0.1	0.7	3.42	85.5
Blood	6	control	ogg1	50	.	.	.	.	1	4	100
Blood	7	control	ogg1	50	.	.	.	.	1	4	100
Blood	8	control	ogg1	50	1	.	.	.	.	0	0
Blood	9	control	ogg1	50	.	.	.	.	1	4	100
Blood	10	control	ogg1	50	.	.	.	.	1	4	100
Blood	1	low	none	50	.	.	.	0.58	0.42	3.42	85.5
Blood	2	low	none	50	.	.	.	0.12	0.88	3.88	97
Blood	3	low	none	50	.	.	.	.	1	4	100
Blood	4	low	none	50	.	.	0.06	0.26	0.68	3.62	90.5
Blood	5	low	none	50	.	.	.	.	1	4	100
Blood	6	low	none	50	.	.	.	0.04	0.96	3.96	99
Blood	7	low	none	50	.	.	.	0.28	0.72	3.72	93

Blood	8	low	none	50	.	.	.	.	1	4	100
Blood	9	low	none	50	.	.	.	0.4	0.6	3.6	90
Blood	10	low	none	50			.	0.1	0.9	3.9	97.5
Blood	1	low	fpg	50	1	.	.	.	.	0	0
Blood	2	low	fpg	50	.	.	0.14	0.36	0.5	3.36	84
Blood	3	low	fpg	50	.	.	0.1	0.34	0.56	3.46	86.5
Blood	4	low	fpg	50	.	.	0.04	0.36	0.6	3.56	89
Blood	5	low	fpg	50	.	.	0.3	0.32	0.38	3.08	77
Blood	6	low	fpg	50	.	.	.	0.48	0.52	3.52	88
Blood	7	low	fpg	50	.	.	.	0.16	0.84	3.84	96
Blood	8	low	fpg	50	.	.	.	0.06	0.94	3.94	98.5
Blood	9	low	fpg	50	.	.	.	0.12	0.88	3.88	97
Blood	10	low	fpg	50	.	.	.	0.1	0.9	3.9	97.5
Blood	1	low	ogg1	50	0.8	0.16	0.04	.	.	0.24	6
Blood	2	low	ogg1	50	.	.	0.18	0.48	0.34	3.16	79
Blood	3	low	ogg1	50	.	.	.	0.38	0.62	3.62	90.5
Blood	4	low	ogg1	50	.	.	.	0.36	0.64	3.64	91
Blood	5	low	ogg1	50	.	.	.	0.2	0.8	3.8	95
Blood	6	low	ogg1	50	.	.	.	.	1	4	100
Blood	7	low	ogg1	50	.	.	.	0.04	0.96	3.96	99
Blood	8	low	ogg1	50	.	.	.	.	1	4	100
Blood	9	low	ogg1	50	.	.	.	0.06	0.94	3.94	98.5
Blood	10	low	ogg1	50	.	.	.	.	1	4	100
Blood	1	high	none	50	.	0.02	0.2	0.32	0.46	3.22	80.5
Blood	2	high	none	50	.	0.08	0.08	0.28	0.56	3.32	83
Blood	3	high	none	50	.	0.02	0.08	0.26	0.64	3.52	88
Blood	4	high	none	50	.	0.02	0.08	0.28	0.62	3.5	87.5
Blood	5	high	none	50	.	.	0.3	0.18	0.52	3.22	80.5
Blood	6	high	none	50	.	0.02	.	0.12	0.86	3.82	95.5
Blood	7	high	none	50	.	.	.	0.06	0.94	3.94	98.5
Blood	8	high	none	50	.	.	.	0.38	0.62	3.62	90.5
Blood	9	high	none	50	.	.	.	0.26	0.74	3.74	93.5
Blood	10	high	none	50	.	.	.	0.14	0.86	3.86	96.5
Blood	1	high	fpg	50	1	.	.	.	.	.	0
Blood	2	high	fpg	50	.	.	0.14	0.36	0.5	3.36	84
Blood	3	high	fpg	50	.	.	0.1	0.34	0.56	3.46	86.5
Blood	4	high	fpg	50	.	.	0.04	0.36	0.6	3.56	89
Blood	5	high	fpg	50	.	.	0.3	0.32	0.38	3.08	77
Blood	6	high	fpg	50	.	.	.	0.48	0.52	3.52	88
Blood	7	high	fpg	50	.	.	.	0.16	0.84	3.84	96
Blood	8	high	fpg	50	.	.	.	0.06	0.94	3.94	98.5
Blood	9	high	fpg	50	.	.	.	0.12	0.88	3.88	97
Blood	10	high	fpg	50	.	.	.	0.1	0.9	3.9	97.5
Blood	1	high	ogg1	50	0.16	.	0.18	0.22	0.44	2.78	69.5
Blood	2	high	ogg1	50	.	.	.	0.54	0.46	3.46	86.5

Blood	3	high	ogg1	50	.	.	0.24	0.64	0.12	2.88	72
Blood	4	high	ogg1	50	0.22	.	0.18	0.14	0.46	2.62	65.5
Blood	5	high	ogg1	50	0.28	0.02	.	0.12	0.58	2.7	67.5
Blood	6	high	ogg1	24	0.79	0.08	.	0.04	0.08	0.54	13.5
Blood	7	high	ogg1	50	.	.	.	.	1	4	100
Blood	8	high	ogg1	28	0.32	0.11	0.29	0.07	0.21	1.75	43.8
Blood	9	high	ogg1	50	.	.	.	0.14	0.86	3.86	96.5
Blood	10	high	ogg1	.	.	.	.	.	.	.	.
Gill	1	control	None	50	0.36	.	0.28	0.26	0.1	1.74	43.5
Gill	2	control	None	50	0.02	0.16	0.38	0.36	0.08	2.32	58
Gill	3	control	None	50	0.02	0.06	0.34	0.28	0.3	2.78	69.5
Gill	4	control	None	50	.	.	0.34	0.44	0.22	2.88	72
Gill	5	control	None	50	.	0.02	0.28	0.28	0.42	3.1	77.5
Gill	6	control	None	50	.	.	.	0.46	0.54	3.54	88.5
Gill	7	control	None	50	.	.	.	0.34	0.66	3.66	91.5
Gill	8	control	None	50	.	.	.	0.54	0.46	3.46	86.5
Gill	9	control	None	50	.	.	.	0.62	0.38	3.38	84.5
Gill	10	control	None	50	.	.	.	0.4	0.6	3.6	90
Gill	1	control	fpg	50	.	.	.	.	1	4	100
Gill	2	control	fpg	50	.	.	.	.	1	4	100
Gill	3	control	fpg	50	1	.	.	.	.	0	0
Gill	4	control	fpg	50	0.08	0.42	0.2	0.16	0.14	1.86	46.5
Gill	5	control	fpg	50	.	.	.	0.1	0.9	3.9	97.5
Gill	6	control	fpg	50	.	.	.	0.44	0.56	3.56	89
Gill	7	control	fpg	50	.	.	0.06	0.14	0.8	3.74	93.5
Gill	8	control	fpg	50	.	.	.	0.44	0.56	3.56	89
Gill	9	control	fpg	50	.	.	.	0.32	0.68	3.68	92
Gill	10	control	fpg	50	.	.	.	0.24	0.76	3.76	94
Gill	1	control	ogg1	50	0.12	0.02	0.1	0.26	0.5	3	75
Gill	2	control	ogg1	50	.	.	0.06	0.14	0.8	3.74	93.5
Gill	3	control	ogg1	50	0.04	0.22	0.12	0.1	0.52	2.84	71
Gill	4	control	ogg1	50	.	0.02	0.36	0.14	0.48	3.08	77
Gill	5	control	ogg1	50	.	0.06	0.16	0.08	0.7	3.42	85.5
Gill	6	control	ogg1	50	.	.	.	.	1	4	100
Gill	7	control	ogg1	50	.	.	.	.	1	4	100
Gill	8	control	ogg1	50	1	.	.	.	.	0	0
Gill	9	control	ogg1	50	.	.	.	.	1	4	100
Gill	10	control	ogg1	50	.	.	.	.	1	4	100
Gill	1	low	none	50	.	.	.	.	1	4	100
Gill	2	low	none	50	.	.	0.02	0.1	0.88	3.86	96.5
Gill	3	low	none	50	.	.	.	0.1	0.9	3.9	97.5
Gill	4	low	none	49	.	.	0.04	0.14	0.82	3.78	94.4
Gill	5	low	none	49	.	.	0.02	0.14	0.84	3.82	95.4
Gill	6	low	none	50	.	.	.	0.32	0.68	3.68	92
Gill	7	low	none	50	.	.	.	0.12	0.88	3.88	97

Gill	8	low	none	50	.	.	.	0.34	0.66	3.66	91.5
Gill	9	low	none	50	.	.	.	0.26	0.74	3.74	93.5
Gill	10	low	none	50	.	.	.	0.2	0.8	3.8	95
Gill	1	low	fpg	50	.	0.12	0.28	0.2	0.4	2.88	72
Gill	2	low	fpg	50	.	.	.	0.48	0.52	3.52	88
Gill	3	low	fpg	50	.	.	0.2	0.36	0.44	3.24	81
Gill	4	low	fpg	50	.	.	0.38	0.3	0.32	2.94	73.5
Gill	5	low	fpg	50	.	.	0.14	0.22	0.64	3.5	87.5
Gill	6	low	fpg	50	.	.	.	0.06	0.94	3.94	98.5
Gill	7	low	fpg	50	.	.	.	0.06	0.94	3.94	98.5
Gill	8	low	fpg	50	.	.	.	.	1	4	100
Gill	9	low	fpg	50	.	.	.	0.1	0.9	3.9	97.5
Gill	10	low	fpg	50	.	.	.	0.26	0.74	3.74	93.5
Gill	1	low	ogg1	50	0.12	0.3	0.54	0.04	.	1.5	37.5
Gill	2	low	ogg1	50	.	.	0.04	0.42	0.54	3.5	87.5
Gill	3	low	ogg1	49	.	.	.	0.27	0.73	3.73	93.4
Gill	4	low	ogg1	50	.	.	.	0.42	0.58	3.58	89.5
Gill	5	low	ogg1	50	.	.	0.12	0.36	0.52	3.4	85
Gill	6	low	ogg1	50	.	.	.	.	1	4	100
Gill	7	low	ogg1	50	.	.	.	0.1	0.9	3.9	97.5
Gill	8	low	ogg1	50	.	.	.	0.2	0.8	3.8	95
Gill	9	low	ogg1	50	.	.	.	0.14	0.86	3.86	96.5
Gill	10	low	ogg1	50	.	.	.	.	1	4	100
Gill	1	high	none	50	0.92	0.08	.	.	.	0.08	2
Gill	2	high	none	50	.	0.12	0.46	0.38	0.04	2.34	58.5
Gill	3	high	none	50	.	.	.	0.22	0.78	3.78	94.5
Gill	4	high	none	49	.	.	0.02	0.1	0.88	3.86	96.4
Gill	5	high	none	50	.	.	.	0.12	0.88	3.88	97
Gill	6	high	none	50	.	.	0.04	0.06	0.9	3.86	96.5
Gill	7	high	none	50	.	.	.	0.26	0.74	3.74	93.5
Gill	8	high	none	50	.	.	.	0.76	0.24	3.24	81
Gill	9	high	none	50	.	.	.	0.66	0.34	3.34	83.5
Gill	10	high	none	50	.	.	0.06	0.68	0.26	3.2	80
Gill	1	high	fpg	50	.	.	.	.	1	4	100
Gill	2	high	fpg	50	.	.	.	0.32	0.68	3.68	92
Gill	3	high	fpg	50	.	.	.	0.14	0.86	3.86	96.5
Gill	4	high	fpg	50	.	.	.	0.18	0.82	3.82	95.5
Gill	5	high	fpg	50	.	.	.	0.06	0.94	3.94	98.5
Gill	6	high	fpg	50	.	.	.	0.12	0.88	3.88	97
Gill	7	high	fpg	50	.	.	.	0.04	0.96	3.96	99
Gill	8	high	fpg	50	.	.	.	0.1	0.9	3.9	97.5
Gill	9	high	fpg	50	.	.	.	0.04	0.96	3.96	99
Gill	10	high	fpg	50	.	.	.	0.04	0.96	3.96	99
Gill	1	high	ogg1	50	.	.	.	0.5	0.5	3.5	87.5
Gill	2	high	ogg1	50	.	.	0.28	0.6	0.12	2.84	71

Gill	3	high	ogg1	50	.	.	.	0.02	0.98	3.98	99.5
Gill	4	high	ogg1	50	.	.	.	0.36	0.64	3.64	91
Gill	5	high	ogg1	50	.	0.1	0.48	0.18	0.24	2.56	64
Gill	6	high	ogg1	42	.	0.07	0.21	0.24	0.48	3.12	78
Gill	7	high	ogg1	50	.	.	.	.	1	4	100
Gill	8	high	ogg1	50	.	.	.	.	1	4	100
Gill	9	high	ogg1	50	.	.	.	0.12	0.88	3.88	97
Gill	10	high	ogg1	39	0.38	0.21	0.03	0.13	0.26	1.67	41.7
Liver	1	control	None	50	0.08	0.04	0.34	0.4	0.14	2.48	62
Liver	2	control	None	50	0.08	.	0.2	0.26	0.46	3.02	75.5
Liver	3	control	None	50	.	0.08	0.16	0.16	0.6	3.28	82
Liver	4	control	None	50	.	0.02	0.58	0.32	0.08	2.46	61.5
Liver	5	control	None	50	.	.	0.14	0.66	0.2	3.06	76.5
Liver	6	control	None	.	.	.	.	.	.	.	.
Liver	7	control	None	50	.	.	.	0.38	0.62	3.62	90.5
Liver	8	control	None	.	.	.	.	.	.	.	.
Liver	9	control	None	50	.	.	.	0.34	0.66	3.66	91.5
Liver	10	control	None	50	.	.	.	0.66	0.34	3.34	83.5
Liver	1	control	fpg	50	.	.	.	0.02	0.98	3.98	99.5
Liver	2	control	fpg	50	.	.	0.04	.	0.96	3.92	98
Liver	3	control	fpg	50	.	.	.	.	1	4	100
Liver	4	control	fpg	50	.	.	0.02	0.08	0.9	3.88	97
Liver	5	control	fpg	50	.	.	.	.	1	4	100
Liver	6	control	fpg	.	.	.	.	.	.	.	.
Liver	7	control	fpg	50	.	.	.	0.62	0.38	3.38	84.5
Liver	8	control	fpg	50	.	.	.	0.26	0.74	3.74	93.5
Liver	9	control	fpg	50	.	.	.	0.4	0.6	3.6	90
Liver	10	control	fpg	50	.	.	.	0.22	0.78	3.78	94.5
Liver	L1	control	ogg1	50	.	0.04	0.02	.	0.94	3.84	96
Liver	L2	control	ogg1	50	.	.	0.14	0.12	0.74	3.6	90
Liver	L3	control	ogg1	50	.	0.06	0.18	0.14	0.62	3.32	83
Liver	L4	control	ogg1	50	.	0.08	0.22	0.52	0.18	2.8	70
Liver	L5	control	ogg1	50	.	0.06	0.24	0.16	0.54	3.18	79.5
Liver	L6	control	ogg1	.	.	.	.	.	.	.	.
Liver	L7	control	ogg1	50	.	.	.	.	1	4	100
Liver	L8	control	ogg1	50	.	.	.	.	1	4	100
Liver	L9	control	ogg1	50	.	.	.	.	1	4	100
Liver	L10	control	ogg1	50	.	.	.	.	1	4	100
Liver	1	low	none	50	.	0.02	0.16	0.02	0.8	3.6	90
Liver	2	low	none	50	.	.	.	0.06	0.94	3.94	98.5
Liver	3	low	none	50	.	.	.	0.06	0.94	3.94	98.5
Liver	4	low	none	50	.	.	0.02	0.1	0.88	3.86	96.5
Liver	5	low	none	50	.	.	0.04	0.16	0.8	3.76	94
Liver	6	low	none	.	.	.	.	.	.	.	.
Liver	7	low	none	50	.	.	.	0.2	0.8	3.8	95

Liver	8	low	none	50	.	.	.	0.2	0.8	3.8	95
Liver	9	low	none	50	.	.	.	0.26	0.74	3.74	93.5
Liver	10	low	none	50	.	.	.	0.18	0.82	3.82	95.5
Liver	1	low	fpg	50	.	.	0.04	0.2	0.76	3.72	93
Liver	2	low	fpg	50	.	.	0.12	0.36	0.52	3.4	85
Liver	3	low	fpg	50	.	.	0.06	0.3	0.64	3.58	89.5
Liver	4	low	fpg	50	.	.	0.3	0.44	0.26	2.96	74
Liver	5	low	fpg	50	.	.	0.2	0.4	0.4	3.2	80
Liver	6	low	fpg	.	.	.	.	.	.	.	.
Liver	7	low	fpg	50	.	.	.	0.04	0.96	3.96	99
Liver	8	low	fpg	50	.	.	.	0.14	0.86	3.86	96.5
Liver	9	low	fpg	50	.	.	.	0.06	0.94	3.94	98.5
Liver	10	low	fpg	50	.	.	.	.	1	4	100
Liver	1	low	ogg1	50	.	0.02	0.36	0.56	0.06	2.66	66.5
Liver	2	low	ogg1	33	0.03	0.06	0.21	0.55	0.15	2.73	68.2
Liver	3	low	ogg1	44	.	.	.	0.27	0.73	3.73	93.2
Liver	4	low	ogg1	50	.	.	0.02	0.4	0.58	3.56	89
Liver	5	low	ogg1	50	.	.	.	0.34	0.66	3.66	91.5
Liver	6	low	ogg1	.	.	.	.	.	.	.	.
Liver	7	low	ogg1	50	.	.	.	0.06	0.94	3.94	98.5
Liver	8	low	ogg1	50	.	.	.	.	1	4	100
Liver	9	low	ogg1	50	.	.	.	0.04	0.96	3.96	99
Liver	10	low	ogg1	50	.	.	.	0.32	0.68	3.68	92
Liver	1	high	none	50	0.06	0.14	0.3	0.36	0.14	2.38	59.5
Liver	2	high	none	32	0.25	0.16	0.41	0.19	.	1.53	38.3
Liver	3	high	none	30	0.03	0.13	0.3	0.37	0.17	2.5	62.5
Liver	4	high	none	50	.	.	0.1	0.8	0.1	3	75
Liver	5	high	none	50	0.04	.	0.42	0.42	0.12	2.58	64.5
Liver	6	high	none	.	.	.	.	.	.	.	.
Liver	7	high	none	50	.	.	.	0.72	0.28	3.28	82
Liver	8	high	none	50	.	.	.	0.62	0.38	3.38	84.5
Liver	9	high	none	50	.	.	.	0.74	0.22	3.18	79.5
Liver	10	high	none	50	.	.	.	0.68	0.32	3.32	83
Liver	1	high	fpg	50	.	.	.	0.08	0.92	3.92	98
Liver	2	high	fpg	50	.	0.1	.	0.02	0.88	3.68	92
Liver	3	high	fpg	50	.	0.08	.	0.06	0.86	3.7	92.5
Liver	4	high	fpg	50	.	.	.	0.18	0.82	3.82	95.5
Liver	5	high	fpg	50	.	.	.	.	1	4	100
Liver	6	high	fpg	.	.	.	.	.	.	.	.
Liver	7	high	fpg	50	.	.	.	0.06	0.94	3.94	98.5
Liver	8	high	fpg	50	.	.	.	.	1	4	100
Liver	9	high	fpg	50	.	.	.	.	1	4	100
Liver	10	high	fpg	50	.	.	.	.	1	4	100
Liver	1	high	ogg1	50	.	.	0.4	0.36	0.24	2.84	71
Liver	2	high	ogg1	50	.	.	.	0.14	0.86	3.86	96.5

Liver	3	high	ogg1	50	.	.	.	.	1	4	100
Liver	4	high	ogg1	50	.	.	.	0.12	0.88	3.88	97
Liver	5	high	ogg1	40	.	.	0.03	0.18	0.8	3.78	94.4
Liver	6	high	ogg1	.	.	.	.	.	.	.	.
Liver	7	high	ogg1	29	.	.	.	0.07	0.93	3.93	98.3
Liver	8	high	ogg1	50	.	.	.	.	1	4	100
Liver	9	high	ogg1	50	.	.	.	0.08	0.92	3.92	98
Liver	10	high	ogg1	.	.	.	.	.	.	.	.
Intestinal M.	1	control	None	50	0.5	0.08	0.06	0.12	0.24	1.52	38
Intestinal M.	2	control	None	50	.	.	.	0.16	0.84	3.84	96
Intestinal M.	3	control	None	50	.	.	0.18	0.4	0.42	3.24	81
Intestinal M.	4	control	None	50	.	.	0.18	0.32	0.5	3.32	83
Intestinal M.	5	control	None	50	0.2	0.08	0.44	0.16	0.12	1.92	48
Intestinal M.	6	control	None	50	.	.	.	0.54	0.46	3.46	86.5
Intestinal M.	7	control	None	50	.	.	.	0.44	0.56	3.56	89
Intestinal M.	8	control	None	50	.	.	.	0.4	0.6	3.6	90
Intestinal M.	9	control	None	50	.	.	.	0.22	0.78	3.78	94.5
Intestinal M.	10	control	None	50	.	.	.	0.4	0.6	3.6	90
Intestinal M.	1	control	fpg	49	.	.	0.29	0.1	0.61	3.33	83.2
Intestinal M.	2	control	fpg	50	.	0.06	0.12	0.4	0.42	3.18	79.5
Intestinal M.	3	control	fpg	50	0.02	0.2	0.06	0.1	0.62	3.1	77.5
Intestinal M.	4	control	fpg	50	.	.	0.02	0.1	0.88	3.86	96.5
Intestinal M.	5	control	fpg	50	.	.	0.04	0.1	0.86	3.82	95.5
Intestinal M.	6	control	fpg	50	.	.	.	0.58	0.42	3.42	85.5
Intestinal M.	7	control	fpg	50	.	.	.	0.88	0.12	3.12	78
Intestinal M.	8	control	fpg	50	.	.	.	0.88	0.12	3.12	78
Intestinal M.	9	control	fpg	50	.	.	.	0.38	0.62	3.62	90.5
Intestinal M.	10	control	fpg	47	.	.	.	0.47	0.53	3.53	88.3
Intestinal M.	1	control	ogg1	50	.	.	0.08	0.08	0.84	3.76	94
Intestinal M.	2	control	ogg1	50	0.04	.	0.2	0.32	0.44	3.12	78
Intestinal M.	3	control	ogg1	50	.	.	0.06	0.3	0.64	3.58	89.5
Intestinal M.	4	control	ogg1	50	0.02	.	0.1	0.1	0.78	3.62	90.5
Intestinal M.	5	control	ogg1	50	.	0.08	0.08	0.12	0.72	3.48	87
Intestinal M.	6	control	ogg1	50	.	.	.	0.38	0.62	3.62	90.5
Intestinal M.	7	control	ogg1	50	.	.	.	.	1	4	100
Intestinal M.	8	control	ogg1	50	.	.	.	.	1	4	100
Intestinal M.	9	control	ogg1	50	.	.	.	.	1	4	100
Intestinal M.	10	control	ogg1	50	.	.	.	.	1	4	100
Intestinal M.	1	low	none	50	.	.	0.06	0.12	0.82	3.76	94
Intestinal M.	2	low	none	50	.	.	.	0.08	0.92	3.92	98
Intestinal M.	3	low	none	50	.	0.08	0.36	0.36	0.2	2.68	67
Intestinal M.	4	low	none	50	.	.	.	0.18	0.82	3.82	95.5
Intestinal M.	5	low	none	50	.	.	0.04	0.24	0.72	3.68	92
Intestinal M.	6	low	none	50	.	.	.	0.32	0.68	3.68	92
Intestinal M.	7	low	none	50	.	.	.	0.18	0.82	3.82	95.5

Intestinal M.	8	low	none	50	.	.	.	0.34	0.66	3.66	91.5
Intestinal M.	9	low	none	50	.	.	.	0.12	0.88	3.88	97
Intestinal M.	10	low	none	50	.	.	0.02	0.08	0.9	3.88	97
Intestinal M.	1	low	fpg	50	.	.	.	0.22	0.78	3.78	94.5
Intestinal M.	2	low	fpg	50	.	.	.	0.08	0.92	3.92	98
Intestinal M.	3	low	fpg	50	.	.	.	0.06	0.94	3.94	98.5
Intestinal M.	4	low	fpg	50	.	.	.	0.1	0.9	3.9	97.5
Intestinal M.	5	low	fpg	50	.	.	.	0.3	0.7	3.7	92.5
Intestinal M.	6	low	fpg	50	.	.	.	0.16	0.84	3.84	96
Intestinal M.	7	low	fpg	50	.	.	.	0.2	0.8	3.8	95
Intestinal M.	8	low	fpg	50	.	.	.	0.06	0.94	3.94	98.5
Intestinal M.	9	low	fpg	50	.	.	.	0.14	0.86	3.86	96.5
Intestinal M.	10	low	fpg	50	.	.	.	0.1	0.9	3.9	97.5
Intestinal M.	1	low	ogg1	50	0.06	0.22	0.46	0.24	0.02	1.94	48.5
Intestinal M.	2	low	ogg1	50	.	0.02	0.34	0.58	0.06	2.68	67
Intestinal M.	3	low	ogg1	50	.	0.06	0.42	0.34	0.18	2.64	66
Intestinal M.	4	low	ogg1	50	.	.	0.08	0.32	0.6	3.52	88
Intestinal M.	5	low	ogg1	50	.	.	.	0.46	0.54	3.54	88.5
Intestinal M.	6	low	ogg1	.	.	.	.	.	.	.	.
Intestinal M.	7	low	ogg1	.	.	.	.	.	.	.	.
Intestinal M.	8	low	ogg1	21	.	.	0.05	0.62	0.33	3.29	82.1
Intestinal M.	9	low	ogg1	50	.	.	.	0.02	0.98	3.98	99.5
Intestinal M.	10	low	ogg1	50	.	.	.	0.04	0.96	3.96	99
Intestinal M.	1	high	none	50	.	0.06	0.3	0.16	0.48	3.06	76.5
Intestinal M.	2	high	none	50	.	0.08	0.26	0.38	0.28	2.86	71.5
Intestinal M.	3	high	none	50	.	.	0.4	0.3	0.3	2.9	72.5
Intestinal M.	4	high	none	50	.	.	0.2	0.46	0.34	3.14	78.5
Intestinal M.	5	high	none	40	.	.	0.3	0.1	0.6	3.3	82.5
Intestinal M.	6	high	none	50	.	.	.	0.06	0.94	3.94	98.5
Intestinal M.	7	high	none	50	.	.	.	0.58	0.42	3.42	85.5
Intestinal M.	8	high	none	50	.	.	.	0.7	0.3	3.3	82.5
Intestinal M.	9	high	none	50	.	.	0.06	0.76	0.18	3.12	78
Intestinal M.	10	high	none	46	.	.	0.04	0.72	0.24	3.2	79.9
Intestinal M.	1	high	fpg	50	.	.	.	0.36	0.64	3.64	91
Intestinal M.	2	high	fpg	50	.	0.04	0.02	0.22	0.72	3.62	90.5
Intestinal M.	3	high	fpg	50	.	.	0.3	0.16	0.54	3.24	81
Intestinal M.	4	high	fpg	50	.	.	0.4	0.26	0.34	2.94	73.5
Intestinal M.	5	high	fpg	50	.	.	.	0.04	0.96	3.96	99
Intestinal M.	6	high	fpg	50	.	.	.	.	1	4	100
Intestinal M.	7	high	fpg	50	.	.	.	.	1	4	100
Intestinal M.	8	high	fpg	50	.	.	.	.	1	4	100
Intestinal M.	9	high	fpg	50	.	.	.	.	1	4	100
Intestinal M.	10	high	fpg	.	.	.	.	.	.	.	.
Intestinal M.	1	high	ogg1	50	.	0.04	0.22	0.36	0.38	3.08	77
Intestinal M.	2	high	ogg1	50	.	.	0.26	0.64	0.1	2.84	71

Intestinal M.	3	high	ogg1	50	.	.	.	0.48	0.52	3.52	88
Intestinal M.	4	high	ogg1	50	.	.	.	0.46	0.54	3.54	88.5
Intestinal M.	5	high	ogg1	50	.	.	.	0.22	0.78	3.78	94.5
Intestinal M.	6	high	ogg1	50	.	0.06	0.12	0.26	0.56	3.32	83
Intestinal M.	7	high	ogg1	50	.	.	.	0.14	0.86	3.86	96.5
Intestinal M.	8	high	ogg1	50	.	.	.	0.08	0.92	3.92	98
Intestinal M.	9	high	ogg1	50	.	.	.	0.12	0.88	3.88	97
Intestinal M.	10	high	ogg1	50	.	.	.	0.28	0.72	3.72	93

. indicates that no data was registered; M=Mucosa

Appendix 2: Chemicals

Name	**Supplier**	**Country**
LMP	SIGMA	USA
EDTA	SIGMA	USA
NaH_2PO4	SIGMA ALDRICH	USA
Na_2HPO4	MERCK	Germany
NaCl	MERCK	Germany
NaOH	MERCK	Germany
Na_2EDTA	MERCK	Germany
Trizma base	SIGMA	USA
Triton X-100	SIGMA ALDRICH	USA
Distilled water	Locally produced	Norway
DMSO	MERCK	Germany
SYBR® Gold	Invitrogen	USA

GelBond® Film	Cambrex	USA
Ethanol (70% and 96%)	Arcus	Norway
Fpg	Locally made	Norway
Ogg1	New England BioLabs	USA
Hepes	SIGMA	USA
H_2O_2	MERK	Germany
KCl	MERK	Germany
BSA	SIGMA	USA
HCl (37 %)	AnalaR NORMAPUR	France
Trypan blue	SIGMA ALDRICH	USA

Appendix 3: Solutions and media

Phosphate buffered saline (PBS)

Chemical	Quantity	End concentration
Na_2HPO4	1.44 g	0.9%
NaH_2PO4	0.24 g	
NaCl	8 g	
dH2O	Adjust pH to 7.4 with conc.HCl and add water to 1 L	

Tris EDTA (TE)-Buffer

Chemicals	**1 L**	**End concentration**
0.5 M Tris-HCl (acid =8.88 mL and base=5.3 g/mol), pH 8	20 mL	10 mM
0.5 M EDTA, pH 8	2 mL	1 mM
dH_2O	Adjust to pH 8, and volume to 1 L	

PBS without Ca and Mg + EDTA (10 mM)

Chemical	**Quantity**	**End concentration**
EDTA	1.86 g	10 mM
PBS without Ca and Mg	500 mL	
Adjust pH to 7.4 with NaOH		

Trypsin + EDTA solution pH 7.7

Trypsin was diluted with PBS containing 10 mM EDTA and this dilution was done in the ratio 1:4 (trypsin:EDTA)

H2O2 stock solution is 8.8 M
H2O2 working solution

Stock	**dH2O**	**End concentration**
100 mL	900 mL	0.1 M

Lysis stock solution pH 10

Chemicals	Quantity required (2670 mL)	Concentration
Distilled water	2100 mL	
NaCl (58.44 g/mol)	438.30 g	2.8 M
NaOH (40 g/mol)	24.00 g	0.224 M
Na_2EDTA (372.2 g/mol)	111.66 g	0.0112 M
Trizma base (121.2 g/mol)	3.66 g	0.0113 M

Lysis working solution. 60mins, +4^{o}C

Chemicals	Quantity(600mL)	End Concentration
Lysis stock solution	534 mL	
Distilled water	60 mL	
Triton X-100	6 mL	1%

Enzyme treatment

Enzyme	Stock	Aliquot stock	Collins' Buffer	BSA
FPG	19.14 µg/mL	1 µg/mL	40 mL/film	200 µl
OOG1	1600 units/mL	2 units/mL	40 mL/film	200 µl

Enzyme reaction buffer (Collins' buffer)

Chemical	**4 L**	**End concentration**
Hepes	47.65 g	40 mM
KCl	37.25 g	0.1 M
EDTA	0.90 g	0.5 mM
dH_2O	Adjust to pH 7.6 with KOH(7M) Add dH_2O to volume of 5L	

Unwinding/electrophoresis buffer stock solution (x10), +4^oC

Chemicals	**4 L**	**End Concentration**
NaOH (Mw:40.0)	600 g	3 M
Na_2EDTA(372.2 g/mol)	18.61 g	10 mM
dH2O	Adjust volume to 5Lwhen dissolved	

Unwinding/electrophoresis buffer working solution(x10). +4^oC

Stock	**dH_2O**	**End concentration**
300 mL	2700 mL	0.3 M (NaOH) 1.0 M (Na_2EDTA)

pH was adjusted to 13.2 with 18 mL conc. HCl

Neutralizing buffer. RT

Chemicals	**2 L**	**End concentration**
Trizma base (121.2g/mol)	96.96 g	0.4 M
dH2O	Adjust to pH 7.5 with conc. HCl, and volume to 2 L	

75% LMP Agarose

Medium or solution	**Quantity**
LMP	75 mg
PBS + 10 mM EDTA	10 mL
Adjust pH to 7.5	

75 mg LMP dissolved in 10 mL PBS and 10 mM EDTA, Heated to boiling point until dissolving of agarose and kept at 37^{o}C on a heat block.

SYybrGold®-aliquoting. It was diluted in the ratio 1:10 in DMSO (100 µl of SybrGold with 900 µl of DMSO)

Chemicals	**1 L**
SybrGold	100 µl
DMSO	900 µl

SybrGold®-staining

40 µL of diluted SybrGold® was mixed with 50 mL TE-buffer for each film