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TITLE

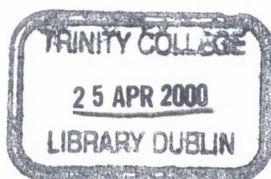
**DELAYED CELL DEATH IN HUMAN AND FISH SKIN
CELL LINES AFTER EXPOSURE TO UVA AND UVB.**

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**A thesis submitted to the University of Dublin, Trinity College,
For the award of the degree of Ph.D.**

**Department of Biochemistry, Trinity College, Dublin,
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Thesis
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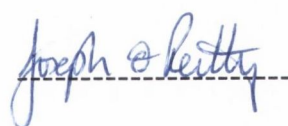
Thanks to my family and friends at home and abroad for their moral support.

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DECLARATION

- This thesis has not been submitted by me to any other University as an exercise for a degree.
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A handwritten signature in blue ink, reading "Joseph O'Reilly", written over a horizontal dashed line.

Joseph O' Reilly.

Glossary

(6-4) Photoproducts	type of photoproduct formed between two adjacent pyrimidine bases in DNA
action spectrum	a means of comparing effectiveness of different wavelengths for a given process; the effectiveness at a series of different wavelengths is expressed relative to the most effective wavelength
adenine	one of the purine bases present in DNA and RNA; pairs with thymine in DNA and uracil in RNA
apoptosis	a mode of programmed cell death. Characteristic features include nuclear and cytoplasmic condensation, blebbing of the plasma membrane followed by fragmentation of the cell into individual membrane-bound apoptotic bodies.
BCC	Basal-cell carcinoma

cyclobutane pyrimidine dimers (CPD)	type of photoproduct formed between two adjacent pyrimidine bases in DNA
cytokine	a factor that induces cell growth and division
cytosine	one of the pyrimidine bases present in RNA and DNA; pairs with guanine
D_{37}	The dose of radiation required to reduce the surviving fraction to 37%.
eukaryote	an organism consisting of cells in which the genetic material is contained within a distinct nucleus. All organisms except bacteria are eukaryotes.
gene	specific DNA sequence encoding polypeptide or protein; the basic unit of transmission of hereditary characteristics

genotype	the genetic constitution of an individual or a cell
growth factor	a factor which stimulates cells to grow and divide
guanine	one of the purine bases present in RNA and DNA; pairs with cytosine
homologous	corresponding in structure, derived from a different organism of the same type
hydroxyl radical	the univalent radical OH; a highly reactive chemical species
immunocytochemistry	detection of specific components in tissue by antibody staining
J/m^2	Joules per metre squared.
keratin	simple protein with structural and protective functions; present in skin, hair and nails

keratinocytes	epidermal cell that synthesises keratin
lymphocyte	type of immune cell divided into two subsets, T- and B- cells, which kill foreign cells and produce antibodies respectively
melanin	group of black or dark-brown pigments present in the skin to absorb UVR, produced in melanocytes and stored in melanosomes
metastasis	a process where cells break away from a tumour and spread around the body
mutation	a stable heritable change in the DNA sequence at a specific site in the genome of an organism; this constitutes a change in the genotype and, if expressed, may alter the phenotype
nucleotide	the smallest unit in the DNA or RNA coding

sequence; combination of a purine or pyrimidine base with a sugar and phosphoric acid; basic structural unit of nucleic acid

oncogene a mutated or foreign gene which contributes to cancer in a dominant fashion; the normal cellular counterpart is called a proto-oncogene

overexpression the abnormally high production of protein from a gene

proto-oncogene a normal gene which has the potential to become an oncogene if it is mutated.

psoralen one of a group of related chemical compounds which absorb UVR and photosensitise DNA damage

pyrimidine class of bases present in DNA and RNA; includes cytosine, thymine and uracil

SCC	Squamous-cell carcinoma
thymine	one of the pyrimidine bases of DNA; pairs with adenine
transcription factors	proteins which control the rate and fidelity of transcription
transformation	the genetic alteration of a cell so that it will form a tumour if injected into a suitable host animal; transformed cells exhibit characteristic growth changes in culture
tumour suppressor gene	a normal cellular gene involved in regulating cell behaviour; mutations can contribute to cancer in a recessive fashion
W/m^2	Watts per metre squared.

xeroderma

skin pigmentosum (XP)

rare inherited skin disease in humans; causes
to become abnormally sensitive to UVR or
sunlight. Patients with this disease are
unusually highly prone to cancer.

Publications and Presentations

Mothersill, C., Crean, M., Lyons, M., Mc Sweeney, J., Mooney, R., O' Reilly, J. P. and Seymour, C. B., 1998 Expression of delayed toxicity and lethal mutations in the progeny of human cells surviving exposure to radiation and other environmental mutagens. *International Journal of Radiation Biology*, 74 (6), 673-680.

O' Reilly, J. P. and Mothersill, C., 1997, Comparative effects of UVA and UVB on clonogenic survival and delayed cell death in skin cell lines from humans and fish. *International Journal of Radiation Biology*, 72 (2), 111-119.

O' Reilly, J. P. and Mothersill, C., 1997, Delayed cell death in cell lines from humans and fish exposed to UVA and UVB. In *Fundamental and Molecular Mechanisms of Mutagenesis, Proceedings of the 7th International Conference on Environmental Mutagens, Toulouse, France, 7-12 September*. S 186.

O' Reilly, J. P., Mc Entee, M., O' Reilly, S., Cantwell, T. and Mothersill, C., 1995, Comparative effects of UVA and UVB on clonogenic survival of an immortalised human keratinocyte cell line and a fish epithelial cell line.

Abstracts of 'Rad Res 95', the Association for radiation Research Meeting, University of St. Andrews, St. Andrews, UK, 5-8 April 1995. International Journal of Radiation Biology, 68 (3), 341-356.

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O' Reilly, J. P. and Mothersill, C., 1996, Comparative effects of UVA and UVB on clonogenic survival and delayed cell death in skin cell lines from humans and fish. *Proceedings of the 7th Gray workshop on research Strategies for the prevention of early events in human radiation carcinogenesis. Dublin, Ireland, 5-8 December 1996.*

O' Reilly, J. P. and Mothersill, C., 1996, Comparative effects of UVA and UVB on clonogenic survival and delayed cell death human and fish skin cell lines. *Proceedings of the International Conference on environmental UV-radiation, Risk of skin cancer and primary prevention, Hamburg, Germany, 3-*

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O' Reilly, J., O' Reilly, S. and Mothersill, C., 1994, Comparative effects of UVA, UVB and UVC on clonogenic survival of an immortalised human keratinocyte cell line. *Proceedings of the Joint meeting of The Association for Radiation Research and The Irish Radiation Research Society, University College Dublin, Ireland, 23-26 June 1994.*

Summary

The effects of ultraviolet radiation on humans and animals are receiving increasing attention and much interest has recently been focused on the environmental effects of UVA and UVB. This study compares the effects of UVA and UVB on two human cell lines, HaCaT and HaCaT-ras, and two fish cell lines, CHSE and EPC.

The HaCaT cell line was derived from human skin keratinocytes spontaneously transformed in vitro during long-term incubation of a primary culture under selected culture conditions. The HaCaT-ras cell line was derived from HaCaT cells transfected with the cellular Harvey-ras oncogene which subsequently resulted in malignant HaCaT variants. The EPC cell line originated from carp epidermal herpes virus-induced hyperplastic lesions. The CHSE-214 cell line was derived from pooled embryos of the chinook salmon. The aim of this work was to see if similar initial and delayed cell death occurred in each cell line after exposure to UVA or UVB. While there is no doubt that delayed expression of lethal or non-lethal mutations occurs post-exposure to ionizing radiation, little is known of the UV response in humans and fish. There is experimental evidence that suggests that DNA double-strand breaks (DSBs) act as the main initiator of this instability. UVA does cause

DSBs, but UVB does not and, therefore, might not be expected to cause delayed death if DSBs are important initiators. Recent publications from our laboratory suggest that the delayed reproductive death observed post-exposure to radiation may in fact be delayed apoptosis as the progeny of irradiated cells contain apoptotic bodies. Two genes *bax* and *bcl-2* are known to promote and inhibit apoptosis, respectively, and therefore expression of these gene products may indicate whether or not the cell is undergoing apoptosis. Results showed that delayed cell death occurred in the non-transformed HaCaT and CHSE cell lines but did not occur in the transformed HaCaT-ras and EPC cell lines post exposure to UVA and UVB. This indicates that DNA DSBs are not the only initiator of delayed reproductive death. The induction of repair by split-doses of radiation and also photoreactivation was investigated in the HaCaT and CHSE cell lines to see if induced repair was possible in these cells and if this repair had an effect on the appearance of delayed reproductive death. Results showed an induction of repair in both cell lines post exposure to a split-dose of UVA or UVB and that this repair ablated the appearance of delayed reproductive death. Photoreactivation was not possible in the HaCaT cells but was possible in the CHSE cells which also lead to a reduction in the appearance of delayed reproductive death post exposure to UVB. Inhibition of oxidative stress- induced damage post exposure to UVA was investigated in

the HaCaT and CHSE cells and again the effect on the appearance of delayed reproductive death was investigated. Inhibition of oxidative stress lead to a reduction in the appearance of delayed reproductive death in both the HaCaT and CHSE cell lines.

1. Introduction.

1.1 Ultraviolet Radiation.

Ultraviolet Radiation (UVR) wavelengths range between 100 and 400 nm and are the most energetic region of the optical radiation spectrum. UVR is able to induce photochemical changes in the cells and biomolecules contained in the superficial tissues of animals which may lead to acute and chronic effects (NRPB 1994). Acute effects include erythema or sunburn which, if severe enough, may result in blistering and destruction of the surface of the skin and inflammatory responses of the cornea (photokeratitis) and conjunctiva (conjunctivitis) respectively. Chronic effects include premature skin ageing, malignant and benign skin tumours. There is also clinical and experimental evidence to suggest that immune factors contribute to the pathogenesis of UV induced cancer (Streilein 1996).

The solar ultraviolet spectrum is divided into three sections: UVA (315-400 nm), UVB (280-315 nm) and UVC (200-280 nm) (Arlett *et al.*, 1993). UVC is strongly absorbed by the earth's atmosphere, and because of this UVA and UVB are the only wavelength regions of environmental significance. UVB is strongly absorbed by DNA and other biomolecules, resulting in

significant biological effects and increases in ozone depletion will lead to greater amounts of UVB reaching the Earth's surface (Kerr 1993). Solar UVB reaches the Earth in relatively small quantities but is highly effective at causing erythema (Madronich *et al.*, 1994), and because of this it is often referred to as the sunburn spectrum or the erythematous band. Action spectra for DNA damage (Rothman and Setlow, 1979, Rosenstein and Mitchell 1987), animal carcinogenicity (Sternberg and Van Der Leun, 1987) and mutation and transformation assays (Jones *et al.*, 1987, Doniger *et al.*, 1981) all indicate that UVB is very effective at inducing skin cancer. UVA is very much less damaging than UVB in damaging biological molecules such as DNA, but it comprises the main component of solar radiation because it is not absorbed by ozone.

UVA is both melanogenic and erythemogenic (NRPB 1994) but the amount of energy required to produce an effect are orders of magnitude higher than for UVB or UVC. In the past, the lack of high intensity artificial light sources strengthened the idea that UVA was innocuous. UVB was the part of the ultraviolet spectrum that was seen to cause skin cancer (Fears *et al.*, 1977, Urbach 1978). UVA is now known to be carcinogenic also, although approximately 1,000-10,000 times less than UVB in terms of Joules per metre squared (J/m^2), (De Gruijl *et al.*, 1993, Forbes *et al.*, 1982, Van Weelden *et*

al., 1988, Sterenbourg *et al.*, 1990, Klingman *et al.*, 1990). With this understanding and because the amount of solar UVA reaching the Earth's surface is enormously greater than UVB, more attention has been given to UVA now than ever before.

1.1.1. Ultraviolet Radiation and Cancer.

Skin cancer has become the most common cancer after bowel cancer. In Ireland skin cancer accounts for 35 % of male cancer and 31 % of female cancer and at present rates of occurrence, an Irish person has a one in eight chance of developing skin cancer before the age of 75. There were 6408 new cases of skin cancer in Ireland in 1994 with 36 deaths from this type of cancer (NCRR 1994).

Basal-cell carcinoma (BCC) is the most common skin cancer that occurs in Caucasians. Extensive epidemiological studies indicate that these tumours are found primarily on the head and neck (NCRR 1994). They are exceedingly rare in deeply pigmented races. There is a statistically significant tendency for patients with BCC to have light eyes, light hair, and light complexions, to sunburn easily and to spend more time outdoors than a matched group of people that do not have such tumours (NCRR 1994). These

findings add further support to the importance of sunlight in BCC formation. The distribution of this type of cancer on the head and neck does not entirely correspond with sites of greatest exposure, that is they are concentrated around the eye behind the ear and in other relatively protected sites (Elwood, 1992, Mackie, 1993).

Squamous-cell carcinoma (SCC) is the second most common skin cancer in Caucasians and is found more often than BCC in the pigmented races (NRPB 1994). The evidence for the role of sunlight in producing SCC in Caucasians is even more convincing than it is for BCC. These growths are distributed primarily over the head and neck, and, to a lesser extent, the exposed areas of the upper extremities. As noted for BCCs, SCCs are found most abundantly in people who work outdoors in geographic areas of high sun exposure (NCRR 1994).

Malignant melanoma is a serious life-threatening tumour because of its potential for biological aggressiveness and metastases. The influence of latitude of residence on the incidence of mortality from melanoma in Caucasians is the original and strongest evidence of the importance of exposure to sunlight as a cause of malignant melanoma. Malignant melanoma is less common than the other types of skin carcinoma but is associated with a much greater risk of fatality in people with a tumour. In spite of improvements

in treatment in the last generation the incidence and death rate from malignant melanoma is rising rapidly in many societies (Elwood 1992, Mackie 1993). The role of sunlight in the development of malignant melanoma is ambiguous since these tumours are most commonly found on the back and other less exposed sites (Russell Jones 1989).

1.1.2. Use of the Fish Skin Model.

The skin of man is not an appropriate experimental model for the study of skin cancer, because of the obvious moral, sociological objections, and because of the long tumour induction time in humans. There is good evidence that UVB is tumourigenic in animals and can cause transformation *in vitro*, the discovery of UV-induced squamous cell tumours in genetically hairless mice (Winkelman *et al.*, 1960, Winkelman *et al.*, 1963) was a pivotal laboratory finding (Forbes 1996).

Fish melanomas closely resemble the tumours that arise in human skin (Sobel *et al.*, 1975). It has long been established that certain hybrids of the platyfish *Xiphophorus maculatus* and the sword-tail *Xiphophorus helleri* have a predisposition to melanoma. Recent analysis of melanoma susceptibility in the *Xiphophorus* have indicated that tumour formation depends on a single

oncogene, designated *Tu*, which is regulated by one or more regulatory genes, acting as tumour suppressor genes (Schartl and Adam, 1992). Setlow *et al.*, (1989) showed that irradiation of 5-day old fish for up to 20 days with UVA or UVB broadband sources, produced tumours in around 25 % of fish within four months. Cyclobutane pyrimidine dimers (CPDs) were implicated as the principal UVR-induced lesion, since photoreactivation with white light reduced the tumour incidence to around 12 %. From these data it is possible to hypothesise that UVR may induce lesions, probably CPDs, which subsequently become fixed as mutations, inactivating regulatory tumour suppressor genes and thereby deregulating the *Tu* oncogene.

Photoreactivation which involves the enzymatic splitting of CPDs to regenerate the initial pyrimidine nucleotides, occurs in fish cells (Setlow *et al.*, 1989, Uchida *et al.*, 1995, 1997, Yasuhira and Yasui, 1992, Yasuhira *et al.*, 1992). This is another reason for using the fish skin model. If delayed cell death was found to occur in the fish skin cells then the contribution of CPDs to delayed cell death could be assessed by photoreactivating the damage.

The use of fish skin to examine the effects of UVR on melanoma induction in animals has been investigated in this thesis by using the EPC and CHSE cell lines available in the laboratory. The EPC (Epithelioma papulosum cyprini) cell line originated from carp epidermal hyperplastic lesions induced

with the herpes virus (Fijan *et al.*, 1983). The CHSE cell line (Lannan *et al.*, 1984) used was derived from pooled embryos of the chinook salmon, *Oncorhynchus tshawytscha*.

1.1.3. Direct DNA Damage

Absorption of UVR by DNA is maximal at wavelengths around 260 nm and results in photochemical damage to the pyrimidine and purine bases which absorb it (Cadet and Vigny 1990). Although this is within the UVC band UVR is also absorbed at wavelengths greater than 260 nm which is of relevance to the work presented in this thesis. The most common photoproducts involve reactions between adjacent pyrimidine bases with the formation of some dipyrimidine products. The three major photoproducts of this type are cyclobutane pyrimidine dimers (CPDs), (6-4) photoproducts and the dewar isomers (Mitchell 1988, Sage 1993, Mitchell *et al.*, 1992, Taylor and Cohrs, 1987, Matsunaga *et al.*, 1991, Clingen *et al.*, 1995).

The most common form of direct DNA damage photoproducts are the cyclobutane pyrimidine photoproducts with particular reference to the thymine-thymine dimer (Beukers and Berends, 1960, Wang 1960). Other dimers are also formed, the cytosine- cytosine and cytosine - thymine dimers,

(Setlow and Carrier, 1966). CPDs have been implicated in mutations found in mouse skin tumours and skin tumour cell lines induced by exposure to UVR, and also in human basal and squamous cell carcinomas. The malignant melanoma lesion found in the *Xiphophorus*, suggests that CPDs are significant lesions since the carcinogenic effects of UVR were reversed post exposure to photoreactivating light (Setlow *et al.*, 1989). The action spectra for the formation of CPDs has been investigated in human cells in culture (Enninga *et al.*, 1986, Rosenstein and Mitchell, 1987, Matsunaga *et al.*, 1991). CPD formation is maximal at the shorter wavelengths of the ultraviolet spectrum and much lower as the UVR wavelengths increase. Action spectra for the formation of CPDs have also been obtained in human skin (Freeman *et al.*, 1989) and it is evident from these findings that there is a high peak in dimer formation at around 300 nm.

A different photoproduct of DNA is the (6-4) photoproduct. Similar to CPDs, (6-4) photoproducts form between adjacent pyrimidines and thus may be thymine-thymine, cytosine-cytosine or mixed. The biological importance of (6-4) photoproducts has been shown in experiments using a xeroderma pigmentosum (XP) revertant cell line which is capable of repairing these lesions but not CPDs (Cleaver *et al.*, 1988). Similar experiments with Chinese hamster ovary (CHO) cells defective in the repair of CPDs or CPDs and (6-4)

photoproducts have also indicated that (6-4) photoproducts are important in mutation (Zdzienicka *et al.*, 1992). The action spectra for the formation of (6-4) photoproducts has been determined in cultured cells (Matsunaga *et al.*, 1991, Rosenstein and Mitchell, 1984) and appears to be similar to those for CPDs. At wavelengths longer than 300 nm the amount of (6-4) photoproducts falls more rapidly than that of CPDs and this results from further photodermal reaction of the (6-4) photoproducts which has an absorption maximum at 310 nm (Moan and Peak, 1989, Peak *et al.*, 1984).

The principal photochemical reaction occurring at these longer wavelengths appears to be a molecular rearrangement to form the Dewar valence isomer, the action spectrum for which is identical to the absorption spectrum of the (6-4) photoproduct (Taylor and Cohrs, 1987). Unlike pyrimidines, purines are not generally regarded as being a major source of photoproducts following DNA absorption of UVR. Studies of these products remain at an early stage and the extent to which they are of any biological significance is not clear (NRPB 1994). Action spectra for DNA damage and the absorption spectrum of DNA strongly suggests that although DNA is the principal chromophore at short wavelengths, endogenous and exogenous sensitisers play a more significant role at longer wavelengths (Eisenstark 1989).

1.1.4 Indirect DNA damage

Sensitisation via an indirect mechanism is probably a more important mechanism than direct energy transfer under most conditions and is certainly the predominant mechanism at longer wavelengths (Peak and Peak, 1987, Tyrell and Pidoux, 1989, Foote 1991, Cadet *et al.*, 1986). This involves the generation of chemically reactive species via a triplet excited sensitizer. It has been observed that this requires oxygen (Eisenstark 1989). Photosensitisation processes involving oxygen are termed photodynamic reactions and are believed to be of two types.

The first type of reaction involves electron or hydrogen abstraction from the DNA, to the triplet sensitizer. The radical thus formed reacts with molecular oxygen. The second type of reaction involves direct reaction of the triplet excited sensitizer with molecular oxygen to form either singlet oxygen ($^1\text{O}_2$) or the superoxide anion (O_2^-). In the cell $^1\text{O}_2$ may be readily converted to $^1\text{O}_2^-$ which in turn may be converted into highly reactive hydroxyl radicals. The reactive oxygen species $^1\text{O}_2$ has been implicated as a major species generated by photosensitized reactions *in vivo*.

A target of reactive oxygen species is the purine base guanine, and one of the most important products of these reactions may be 8-hydroxyguanine

(Epe 1991, Piette 1991), which appears to be mispaired with adenine leading to the formation of a T:A base-pair from an original G:C base-pair (Shibutani *et al.* 1991). The exposure of mouse keratinocytes to broadband source emitted in the range 270-380 nm with a maximum output at 320 nm resulted in a doubling of the background rate of 8-hydroxyguanine formation.

Breaks in one or both of the DNA strands can be produced by both long and short UV wavelengths. It is generally recognised that single strand breaks (SSBs) formed at short wavelengths are minor photoproducts as they are produced approximately 10^2 - 10^3 times less frequently than CPDs (Moan and Peak, 1989). At 365 nm the numbers of the two types of strand breaks are approximately equal while at visible wavelengths SSBs clearly represent the major type of photodamage. The relative increase in the frequency of SSBs observed following irradiation of normal human fibroblasts and repair deficient cells at 365 nm appeared to make little contribution to the observed cell lethality (Smith and Paterson, 1982). SSBs have generally been considered to be of little biological significance in comparison with other types of UVR induced damage (Moan and Peak, 1989, Tyrell and Keyse, 1990). Recent evidence however, indicates that a subset of SSBs induced in normal and repair deficient CHO cells (Churchill *et al.*, 1991) and XP cells (Peak and Peak, 1991) may be refractory to repair and may be indicative of a

class of SSBs that could be of biological importance.

The action spectrum DSBs is similar to that for SSBs and it has been calculated that the incidence of DSBs was too high to have been resulted from the random production of SSBs in close proximity (Peak and Peak, 1990). The formation of DSBs by UVR has been less intensively studied than that of single strand breaks principally because of the absence of an assay that is both sensitive enough and widely acceptable. It has been shown however that the induction of DSBs in cultured human P3 cells was proportional to the square of the radiant exposure over a range of wavelengths between 254 and 405nm (Peak and Peak, 1990).

Another lesion which is produced by indirect sensitisation is the DNA-Protein Cross link (DPC). Cellular DNA is intimately associated with protein to form chromatin and it is therefore to be expected that reactions may occur between excited bases and susceptible protein components in close proximity. These reactions have been established for some time and result in the formation of DPCs (Smith 1976, Varghese 1976). DPCs have been detected following exposure to 365 and 405nm radiation (Han *et al.*, 1984) which suggest that these may be good candidates for biologically important lesions at longer wavelengths.

1.1.4. DNA Repair

The development of cancer is a multistep process which is initiated when the products of ultraviolet-induced genomic DNA damage, such as cyclobutane pyrimidine dimers (CPD) and 6-4 photoproducts are not properly repaired. In response to the induction of ultraviolet-induced DNA damage, several cellular response processes are initiated, such as DNA repair, transient immune suppression, accumulation of the p53 protein and overexpression of certain p53-regulated genes that lead to either cell cycle arrest or apoptosis (Kraemer 1997, Kripke 1990, Ljungman and Zhang 1996, Mukhar and Elmetts 1996). The role of UV-induced DNA damage in skin cancer and the importance of adequate DNA repair systems to repair the damage is clearly illustrated in patients with the rare heritable disease, xeroderma pigmentosum (XP) (Bootsma et al. 1998, Freidberg *et al.* 1995).

XP patients develop skin tumours at an extremely high frequency (> 1000 fold increase as compared to normal individuals) because of their inability to repair UV-induced DNA lesions. As in the general population, these tumours comprise BCCs and SCCs on sun-exposed areas of the skin and melanomas that are more uniformly distributed (Bootsma et al. 1998, Freidberg *et al.* 1995).

Approximately 80% of XP patients who have been classified to date have a defect in the nucleotide excision repair (NER) pathway. These patients are said to have classical XP, in contrast to the remaining 20% of patients who are designated as XP-variants and most likely have a defect in postreplication repair (6). In XP-V patients, DNA replication stops or is interrupted at UV-damaged sites, and de novo DNA synthesis opposite CPD lesions is prone to errors, leading to the fixation of multiple DNA lesions and ultimately to cancer.

DNA damage occurs spontaneously as a result of natural processes and cells have consequently developed DNA repair mechanisms to remove damage and restore the genetic code, although they may not always be effective (Friedberg *et al.*, 1995). At its most extreme the burden of DNA damage may be too great for the cell either because of its magnitude or because it blocks a vital process, and the cell will die. Alternatively the cell may be able to recognise and eliminate the sites of damage, thus restoring the original DNA sequence. Such repair mechanisms are said to be error-free, in order to distinguish them from those that allow the cell to eliminate the lesion, but that do not necessarily restore the original DNA sequence. These error-prone mechanisms probably serve a useful purpose in allowing the cell to overcome an otherwise lethal burden of DNA damage, although at the cost of

introducing possible mutations into its genetic code. The differences in the fidelity with which cells repair DNA damage arise from involvement of a number of different DNA repair pathways, these can be broadly be divided into three main types, excision repair, post-replication repair and photoreactivation (Freidberg *et al.*, 1995).

Excision repair (Sancar 1995) is a general term that covers a range of different repair pathways and these may be grouped into three main categories: nucleotide excision repair, base excision repair and mismatch repair. Nucleotide excision repair (Hoeijmakers 1994, Sancar and Tang 1993, Aboussekhra 1994, Shivji *et al.*, 1995) is the most extensively studied repair mechanism. The best characterised nucleotide excision repair pathways are those found in prokaryote cell types such as bacteria (van Houten 1990). Prokaryotic nucleotide excision repair involves a complex of two types of protein binding to DNA and translocating along the DNA in search of a damaged site. Once a lesion has been identified, the complex acts as a damage recognition signal for a third protein which makes two incisions in the damaged strand, on either side of the lesion. The short oligonucleotide containing the damaged base or bases is then released by a fourth protein and a DNA polymerase. Using the other undamaged strand as a template, the polymerase fills in the resulting gap and finally the newly synthesised DNA is

joined into the existing strand by a DNA ligase. Many of the basic steps in eukaryotes appear to be similar to prokaryotes with some form of damage recognition followed by incision, removal of the damage containing oligonucleotide, and resynthesis of the strand.

Base excision repair also involves the identification and excision of damaged bases. In this case the enzyme DNA glycosylase excises them as free bases (Freidberg *et al.*, 1995).

Mismatch repair was initially identified in prokaryotes and is now known to occur in eukaryotes including humans. Mismatched bases are those which do not obey the normal base pairing rules, and may arise in a number of ways including recombination, replicative infidelity during DNA synthesis and spontaneous base changes such as deamination of cytosine to uracil. Although relatively poorly understood at present, mismatch repair appears to involve a mechanism for mismatch recognition coupled with helicase and nuclease activities to effect excision of an oligonucleotide, which includes the mismatched base, and subsequent resynthesis of the missing strand (Freidberg 1988, Lehmann *et al.*, 1992).

Post-replication repair pathways may be induced in response to DNA damage and allow cells to survive large damage burdens, although this may be at the expense of increased mutation. In these situations damage may still be

present in DNA which is being replicated, and such sites could block the progress of the DNA polymerase which is synthesising the daughter strand, thus halting replication with potentially lethal consequences. In order to avoid this, the cell activates one or more alternative pathways that allow the DNA polymerase to bypass lesions in the parent or template strand. The lesion is presumably removed by the normal repair process subsequent to replication. The process by which this bypass occurs is not well understood in eukaryotic systems, but at least two mechanisms have been suggested, discontinuous synthesis with gap formation and trans-lesion synthesis. In discontinuous synthesis it is suggested that DNA polymerase is able to skip the damage, leaving a gap which is subsequently filled in or corrected by recombination with an undamaged sister chromatid. However while the presence of gaps in the daughter strands of ultraviolet irradiated mammalian cells has been reported, it is not clear that these occur opposite lesions (Meneghini *et al.*, 1981, Painter 1985). Another study found no evidence of UVR-induced gaps, which suggests either that such gaps do not occur or that they are filled very rapidly (Van Zeeland and Filon, 1982). Trans-lesion synthesis involving the suspension of the normal base-pairing rules is currently believed to be an important mechanism for error-prone repair leading to mutation (Strauss 1991). There is evidence that DNA polymerases are able to synthesise past

unrepaired CPDs produced by low doses of UVR (Spivak and Hanawalt 1992) and that DNA polymerases encountering a non-instructional lesion during DNA synthesis default to insertion of an adenine under some circumstances (Strauss 1991).

Photoreactivation involves the enzymatic splitting of CPDs to regenerate the initial pyrimidine nucleotides. The (6-4) photoproduct is removed from DNA by nucleotide excision repair (NER) very efficiently (Van Stegg and Kramer, 1999). In contrast, CPD are a poor substrate for enzymes of NER and are repaired less efficiently by NER in both prokaryotes and eukaryotes. CPDs are most efficiently repaired by an enzyme called CPD photolyase. The CPD photolyase splits CPD with energy from visible light, and this process is called photorepair or photoreactivation. As to the photorepair of (6-4) photoproducts, a possibility for the presence of photolyase specific to (6-4) photoproducts has been discussed (Mitchell *et al.*, 1986, 1990). Recently, in *Drosophila* (Todo *et al.*, 1993) and in *Xenopus* and *Crotalus* (Kim *et al.*, 1996) the presence of (6-4) photolyase was reported.

When RBCF-1 cells derived from the caudal fin of a goldfish were illuminated with photoreactivating light (PRL), the expression of the CPD photolyase gene was induced (Yasuhira and Yasui, 1992). In addition, PRL enhanced excision repair of (6-4) photoproducts and UV resistance in the

dark. Furthermore after exposure to PRL the photorepair ability for (6-4) photoproducts as revealed by enzyme-linked immunosorbent assay (ELISA) increased (Uchida *et al.*, 1995). Thus, PRL has multiple effects on repair of DNA damage in cultured goldfish cells.

PREs have been identified in a wide range of species and cell types, including those not normally exposed to light (Eker, 1983). A human PRE has been identified in cultured leukocytes and fibroblasts (Sutherland 1974, Sutherland and Sutherland, 1975) and has been reported in human skin. The existence of a human PRE is questioned by some however (Ley 1993). Where the human enzyme has been detected, it exhibits an action spectrum that spans 300-600nm with a peak at 405nm. The enzyme is active at much longer wavelengths than PREs from most other species (Sutherland 1974, Sutherland and Sutherland, 1975). In general the cellular concentration of PRE appears to be low, although there is some evidence that it may be induced by photoreactivating light (Yashira *et al.*, 1992). Furthermore, it has been demonstrated that in the yeast *Saccharomyces cerevisiae*, the PRE gene, PHR1, is regulated by a DNA damage-responsive transcriptional repressors (Sebastian and Sancar 1991) such that its activity is increased following DNA damage.

1.2. Genomic Instability

There is a long history linking exposure to ultraviolet radiation (Setlow *et al.*, 1993, Berg *et al.*, 1993, Strickland *et al.*, 1985) and ionizing radiation (Hall 1994, Sankaranaryanan and Chakraborty, 1995) to the elevated incidence of cancer. Substantial interest has been focused on the role of genomic instability in ionizing radiation induced carcinogenesis. Cells react rapidly to ultraviolet and ionizing radiation invoking a mammalian stress response that includes many biological responses such as the initiation of signal transduction pathways (Weichselbaum *et al.*, 1991), the activation of gene transcription (Boothman *et al.*, 1993), the repair of damaged DNA (Ward 1985, 1990, Ward *et al.*, 1991), and cell-cycle specific growth arrest (Murnane, 1995, Maity *et al.*, 1994). These early events are likely to be preconditions and determinants of the later fate of irradiated cells.

If a cell survives the initial biological response to the radiation-induced insult, it may well participate in normal differentiation or acquire via genomic instability those characteristics associated with the neoplastic transformation of a normal cell to a cancerous cell. Alternatively radiation may induce a subset of lesions that are not repaired, or are fixed as mutations for generations after exposure. Several delayed effects in the progeny of cells

surviving exposure to radiation have been described, a persistent reduction in plating efficiency (Seymour *et al.*, 1986, Chang and Little, 1991, 1992), giant cell formation (Fitzek and Trott, 1993), cell fusion (Andrushkevich *et al.*, 1993), delayed mutation (Seymour *et al.*, 1986, Mothersill and Seymour, 1987, Chang and Little, 1992), transformation (Mendonca *et al.*, 1993, 1995), and delayed chromosomal instability (Kadhim *et al.*, 1992, 1994, Holmberg *et al.*, 1993, Marder and Morgan 1993, Martins *et al.*, 1993). These chromosomal changes can occur many generations after exposure to radiation (Holmberg *et al.*, 1993, Marder and Morgan, 1993).

Genomic instability is characterised by the increased acquisition of alterations of the genome. The loss of stability of the genome is becoming widely accepted as one of the most important aspects of cancer (Cheng and Loeb, 1993, Tlsty *et al.*, 1993). It has been suggested that genomic instability provides the requirements to drive the stepwise progression of genetic change required for the neoplastic phenotype (Nowell, 1993).

There is accumulating evidence that human cancers contain multiple genetic mutations (Fearon and Vogelstein, 1990). Genomic instability in cancer cells is most visible cytogenetically (Rabbits 1994), although it extends to molecular, biochemical and morphological characteristics and multiple chromosomal abnormalities have been described in a variety of human

cancers (Mitelman 1991). Patients with a range of leukaemias and lymphomas show karyotypic abnormalities which include chromosomal translocations (Mitelman 1991). Additionally the chromosome breakage syndromes, ataxia telangiectasia, Bloom's syndrome and Fanconi's anemia are all characterised by the increased incidence of cancers (Mitelman 1991). This relationship between chromosomal rearrangements and cancer would suggest that chromosomal instability may lead in some part, to cancer.

1.2.1 Delayed Reproductive Death

The end point used when investigating genomic instability in this thesis was delayed reproductive death which is described in detail below. Since the work of Puck and Marcus (1956) on the action of X-rays on mammalian cells the interpretation of radiation effects on cells, tissues, tumours and organisms has been based on the observation that the inactivation of clonogenic cells is a stochastic process. Thus, some cells lose their reproductive potential even if they can still perform some abortive divisions, whereas other cells could only go on to proliferate for approximately five divisions after irradiation. This can lead to a wide spectrum of colony sizes. This concept has been validated by studies on post irradiation growth rate (Elkind *et al.*, 1963) and colony size

distribution (Nias *et al.* 1965).

This well established concept was challenged (Seymour *et al.*, 1986, Mothersill and Seymour, 1987) and it was shown that lethal mutations occurred in clonogenic cells which had survived radiation doses of 2-10 Gy and had passed through a number of divisions. Other investigators have similarly demonstrated that the surviving progeny of irradiated cells show a persistently reduced cloning efficiency for up to 15-30 population doublings post-irradiation (Mendonca *et al.*, 1989, Gorgojo and Little, 1989, Born and Trott, 1988, O' Reilly *et al.*, 1994, Chang and Little, 1991, 1992). This phenomenon of reduced cloning efficiency in the progeny of cells surviving irradiation has been termed 'Lethal Mutations' (Seymour *et al.*, 1986) on the basis that heritable defects, including mutations with lethal effects, could occur in some of the surviving progeny and appear to be responsible for the late expression of reproductive failure in the descendants.

O' Reilly *et al.*, 1994, showed that the change in the slope of the growth curve in HPV-G cells post irradiation with γ -rays is due entirely to reduced numbers of reproductively viable cells in the population. This agrees with earlier conclusions using CHO-K1 cells (Seymour and Mothersill, 1992) where the correction of the curve for the non-viable cells in the population led to the production of a curve parallel to the control curve. This means that the

longer cell cycle time hypothesis (Nias *et al.*, 1965, Grote *et al.*, 1981) used to explain the reduced growth rate of cells post-irradiation is apparent rather than real, being due to a failure of a proportion of the cells to divide to give two viable progeny rather than to an overall elongated cell cycle time. The main evidence for parallel control and post-irradiation growth curves in the literature comes from haematological cells in suspension cultures. Non-viable progeny are probably less likely to persist in these cultures than in substrate-attached cultures, where the cells can stay in a non-viable state for many days.

1.2.2 Chromosomal Instability

Interest has been focused recently on other signs of delayed radiation-induced injuries as evidence to support the hypothesis that radiation may also induce a transmissible persistent destabilisation of the cellular genome, detectable after many cell cycles in the progeny of irradiated cells (Kadhim *et al.*, 1994, Marder and Morgan, 1993). It was inferred that radiation induced a type of genetic instability among survivors that would result in a heritable mutator phenotype. Since a correlation between the higher spontaneous Hprt mutation frequency in mutator clones and their persistently lower cloning efficiency was observed, it was also hypothesised that such an instability may be

involved in the phenomenon of delayed reproductive death. Recent studies have demonstrated the occurrence of various chromosomal changes in the descendants of irradiated cells such as aneuploidy (Brown and Trott, 1994), micronuclei or dicentric chromosomes (Jamali and Trott, 1996). It has been also found that delayed chromosomal instability, as measured by chromosome-type aberrations, also correlated with reduced plating efficiency (Marder and Morgan, 1993). Similar instability was shown after exposure to X-rays (Holmberg *et al.*, 1993) and after exposure to heavy ions (Martins *et al.*, 1993).

1.2.3 Gene Amplification

A third aspect of genomic instability characterised particularly well in cancer cells is gene amplification. Gene amplification is a dynamic process by which specific gene regions are altered at the molecular and cytogenetic levels during cell propagation and drug selection. Amplified sequences are often detected in one of two types of chromosomal anomalies. These are paired acentric circular structures called double-minute chromosomes and expanded chromosomal regions (Schimke 1982). The initial molecular event or events that lead to gene amplification are not known, but the prevailing hypotheses

for the molecular and cytogenetic chronology for gene amplification are unequal sister chromatid exchange, deletion and reintegration of genomic sequences, and/or chromosome breakage followed by fusion and bridge breakage-fusion cycles to precipitate a cascade of chromosomal instability (Windle *et al.*, 1991, Smith *et al.*, 1992, Ma *et al.*, 1993, Hahn *et al.*, 1990, Ruiz *et al.*, 1990, Muller, 1927). It has also been demonstrated that ionizing radiation could significantly enhance the frequency of both methotrexate resistance and gene amplification in CHO cells in culture (Hahn *et al.*, 1990).

1.2.4 Delayed Mutations

A fourth aspect of genomic instability is that of delayed mutations. It has been recently been demonstrated that the spectrum of structural changes differs significantly between directly induced and late arising mutations (Little 1994). More than 70 % of the directly- induced mutations involve total or partial deletions, whereas the great majority of delayed mutations appear to involve point mutations (Colucci 1997, Little 1994). Delayed mutation has also been described in mice after whole body irradiation (Selvanayagam *et al.*, 1995). When mammary tissue was isolated from these irradiated mice it became neoplastic with time *in vitro* and *in vivo*. p53 mutations arose in the progeny

of irradiated cells several generations post-irradiation (Selvanayagam *et al.*, 1995).

The conversion of a DNA lesion into a stable genetic change after mutagen treatment of bacteria usually occurs within the first or second cell generation. This process has been generally assumed to be equally rapid in mammalian cells, and most methods for measuring mutation frequency using cultured cells are based on this assumption.

It has been found that treatment of CHO cells with ethyl methane sulfonate (EMS) produced colonies containing both glucose-6-phosphate dehydrogenase (G6PD) enzyme-deficient mutant and wild type cells when assayed by histochemical staining and replating (Stamato *et al.*, 1982). This same group examined the delayed mutation phenomenon after ultraviolet exposure to determine if UVR produced mutations for up to 11 cell generations post-irradiation (Stamato *et al.*, 1995). It was found that the relative number of G6PD⁻ mutant to wild type cells in single colonies occurred in the ratios expected for delayed mutational events occurring at the fourth, sixth-ninth and eleventh cell division after ultraviolet light exposure.

1.2.5 DSBs as Initiators of Delayed Cell Death

There is evidence that DNA DSBs, as well as the endogenous repair processes that recognise and rejoin DSBs are associated with the phenotype of delayed reproductive death. Chang and Little (1992) demonstrated that restriction endonuclease-induced DSBs led to reduced plating efficiency in the progeny of treated cells. This was not observed in *xrs5* cells deficient in DSB repair (Jeggo and Kemp, 1983), suggesting that the inherent DSB repair capacity of the cell can modulate the integrity of the genome. Results by Mothersill and Seymour (1993) showed no induction of delayed reproductive death in the *xrs 5* cell line post exposure to γ -radiation, supporting previous work by Alper *et al.*, (1988). The explanation postulated for this was that since the XRS gene defect in *xrs 5* cells is known to involve DSB repair (Jeggo and Kemp, 1983), it may also be deduced that “lethal mutation” type damage must be DSB related.

While the hypothesis that DNA DSBs can initiate genomic instability is attractive, recent experimental evidence argues against DSBs being the only initiator of instability. The frequency of chromosomal aberrations in the first, second and third mitoses after treatment of one-cell mouse embryos with the restriction enzyme Alu 1 was studied recently (Wojcik *et al.*, 1996). Their

results indicate that the induction of DSBs does not lead to novel aberrations in the third post-treatment mitosis. Also recent work by Limoli *et al.*, (1997) has shown that blunt-end-cutting restriction enzymes failed to produce chromosomally unstable clones.

1.2.6. Perpetuation of Genomic Instability and Consequences.

Once genomic instability is initiated within a cell it may be perpetuated by different processes. If chromosomal instability results in a dicentric chromosome, successive cycles of dicentric bridge breakage-refusion (McClintock 1941) may generate the new karyotypic rearrangements seen in many unstable clones (Marder and Morgan, 1993). These dicentric bridge breakage-refusion cycles may also be facilitated by a recombination-mediated process involving interstitial repeat sequences.

Enhanced and persistent oxidative stress in the progeny of irradiated cells may provide the cue that initiates signalling cascades, which in turn may contribute to the perpetuation of delayed chromosomal instability (Clutton *et al.*, 1997). Although initiation of genomic instability may be a transient process, perpetuation can be a dynamic process persisting within cells for many generations (Watson *et al.*, 1996). Evidence indicates that genomic

instability contributes to tumourigenesis. It could also have an effect on how tumour cells respond to treatment involving radiotherapy or chemotherapy. Morgan and Murnane (1995) have suggested that genomic instability, particularly chromosomal destabilisation, may increase the likelihood of radioresistance in tumour cells. The majority of unirradiated cells show a tendency towards aneuploidy and numerous complex chromosomal rearrangements (Mitelman 1991). The cells composing these tumours clearly have proliferative capabilities and can adapt to new environments which may suggest that destabilised genomes confer an adaptive advantage.

Radio-resistance occurring at the site of radiation exposure in tumours may also be enhanced by the prolonged period of DNA rearrangement that follows radiotherapy. Other factors, such as newly induced proteins (Boothman *et al.*, 1989, 1991, Singh and Lavin, 1990), altered gene expression (Boothman *et al.*, 1993) and secretion of growth factors (Sherman *et al.*, 1990, Hallahan *et al.*, 1989, 1991), could also play a role by directly or indirectly modulating the sequence of events leading to chromosomal instability.

Many of the delayed cytogenetic rearrangements induced by radiation may lead to chromosome aberrations not compatible with cell survival, and may result in mitotic or delayed reproductive death (Marder and Morgan,

1993). The implications of delayed reproductive cell death for the interpretation of tissue responses has been discussed recently (Hendry and West, 1995). In tumours previously exposed to radiation, some surviving cells exhibiting delayed genomic instability may be favoured to develop changes that provide a growth advantage upon subsequent challenge to noxious agents, as in fractionated and protracted radiotherapy and chemotherapy protocols.

1.3. Mode of Delayed Cell Death and the Expression of Apoptotic and Anti-apoptotic Associated Genes.

Multicellular organisms must balance the rates of cell death and generation if they are to maintain both a constant size and a continuing ability to function normally. Organisms must also remove senescent, damaged or otherwise abnormal cells that could interfere with organ function or develop into a tumour. Cellular differentiation, specialisation, growth and organogenesis would be impossible without a mechanism to remove cells that are no longer needed. Such physiological cell death in the absence of inflammation is called apoptosis (Kerr *et al.*, 1972).

The apoptotic cell death process is associated with pronounced morphological changes in the cell, such as cell shrinkage, membrane blebbing

as well as intranucleosomal DNA fragmentation. Apoptosis usually affects scattered individual cells and proceeds quickly once initiated requiring as little as a few minutes to a few hours. Examples of apoptosis in adult somatic cells include shedding of the intestinal lining, involution of the lactating breast after weaning and death of mature neutrophils.

Apoptosis is an efficient method of preventing malignant transformation because it removes cells with genetic lesions. Failure of such cell death can promote cancer development by allowing accumulation of dividing cells and obstructing the removal of genetic variants with enhanced malignant potential (Carson and Ribeiro, 1993).

Ultraviolet radiation has been shown to induce apoptosis in a variety of cell types (Cotton and Spandau, 1997, Goderr *et al.*, 1994, Schwartz *et al.*, 1995, Kane and Maytin, 1995) and this induction of apoptosis has been shown to be mediated in part by the p53 gene (Reinke and Guillermina, 1997). This increased expression of the p53 protein is cell-type specific (Forrester *et al.*, 1995), and activation is due to an alteration in transcription (Hudson *et al.*, 1995), translation (Mosner *et al.*, 1995) or conformation (Cox and Lane, 1995). It has been shown that irradiation of cultured human cells with UVA, UVB or UVC resulted in immunocytochemically detected p53 protein (Campbell *et al.*, 1993). Furthermore, irradiation of human skin *in situ* has

been shown to result in a rapid accumulation of p53 protein that persisted for at least several days (Hall *et al.*, 1993). The transcriptional activator p53 has long been identified as a tumour suppressor. The loss or mutation of the p53 gene is a critical event in the etiology of many tumours of diverse origin, and it is indeed more commonly mutated in tumourigenesis than any other single gene (Hainaut *et al.*, 1997).

Even though the p53 status of the cell lines used in this study is compromised it is an important gene to discuss because the p53 status of the cell is important in the repair response to UVR (Kastan *et al.*, 1991). Upon damage to DNA, the levels of p53 protein increase (Kastan *et al.*, 1991, Lu and Lane, 1993, Maltzman and Czyzyk, 1984). In most cells this results in one of two fates, either an arrest in the G1 phase of the cell cycle (Lane 1992, Sanchez and Elledge, 1995, Cox and Lane, 1995, Canman, 1995) or death by apoptosis (Lowe *et al.*, 1993, Sanchez and Elledge, 1995, Cox and Lane, 1995, Chen *et al.*, 1996). It is presumed that halting the cell cycle provides an opportunity for damaged DNA to be repaired before the damage is propagated during replication. After repair has occurred the cell progresses through the cycle. In this way growth arrest would prevent the cell from becoming transformed. The decision to undergo apoptosis rather than divide also allows for suppression of transformation. p53 dependent apoptosis has been

correlated *in vivo* with tumour suppression (Symonds *et al.*, 1994).

The known functions of the genes responsive to p53 appear to strongly indicate that p53 acts directly through their induction to promote either growth arrest or apoptosis. One of the genes activated by p53 is *bax*, the protein product of the *bax* gene is known to promote apoptosis through competitive dimerisation between itself and Bcl-2 (Oltvai *et al.*, 1993). The relative levels of these two proteins determines the fate of the cell. The identification of *bax* as a down stream transcriptional target of p53 delineates a potential pathway by which p53 mediates apoptosis (Miyashita and Reed 1995).

It has been shown in many, but not all, experimental systems that *bcl-2* is able to extend cellular viability by blocking programmed cell death (Korsmeyer 1992). Identification of a number of *bcl-2* homologs, some of which bind to *bcl-2*, suggests that *bcl-2* functions at least in part, through protein-protein interactions. The first of these homologues, Bax was identified by co-immunoprecipitation with the Bcl-2 protein. Bax, a heterodimerizing partner of Bcl-2, is a death promoter (Oltvai *et al.*, 1993). Site-directed mutagenesis of BH1 and BH2 in Bcl-2 showed that two domains BH1 and BH2 were important for binding to Bax. When binding was disrupted, Bcl-2's protective function was also eliminated, suggesting that Bcl-2 must bind to Bax to exert its effect. When Bax is overexpressed in cells, apoptotic death in

response to a death signal was accelerated, earning its designation as a death promoter. When Bcl-2 was overexpressed, it heterodimerised with Bax and death was repressed (Oltvai *et al.*, 1993). Thus the ratio of Bcl-2 to Bax is important in determining susceptibility to apoptosis. Bax is widely expressed in tissues, including a number of sites in which cells die during normal maturation (Oltvai *et al.*, 1993, Krajewski *et al.*, 1994). The Bcl-2 to Bax ratio varies during the developmental history of a given lineage, such as T lymphocytes. The lack of *bcl-2* may enable thymocytes to die if they fail to receive an appropriate signal. Publications from our laboratory suggest that the delayed reproductive death observed post-exposure to radiation may in fact be delayed apoptosis as the progeny of irradiated cells contain apoptotic bodies (Lyng *et al.*, 1996). Because the two genes *bax* and *bcl-2* are known to promote and inhibit apoptosis respectively, the protein expression of these gene products was used as an indication of whether or not the cell is undergoing apoptosis.

1.4 Split-dose Recovery from Cell Death.

1.4.1 Split-dose Effects and Recovery from Initial Cell Death.

Generally there are two descriptions of the shape of the survival curve. Firstly, the multi-target model describes the curve in terms of the initial slope D_1 , final slope D_0 , due to single and multiple event killing respectively and the size or width of the shoulder D_q . The values D_1 and D_0 are the reciprocals of the initial and final slopes, in each case it is the dose needed to reduce the % surviving cells to 37 % of its previous value. D_q is the quasi threshold dose, it is defined as 'the dose at which the straight portion of the curve extrapolated backwards cuts the dose axis drawn through a surviving fraction of unity'. There is no threshold dose below which there is no cell killing so the quasi threshold dose is the closest parameter. The three parameters are related by the expression $\log e N = D_q/D_0$.

The second model used to describe the cell survival curve is the linear-quadratic model. This model assumes that there are two parts to cell killing by radiation, one is proportional to the dose and the other is proportional to the square of the dose. The idea that cell killing is proportional to the square of the dose involves the idea of dual radiation action, this follows from

chromosome abnormalities that are the result of two separate breaks in the DNA. This model is described by the equation $S = e^{-(\alpha D + \beta D^2)}$, Where S is the % of cells that survive a certain dose D and α and β are constants. The components of cell killing that are proportional to the dose and the square of the dose are equal when $\alpha D = \beta D^2$ or $D = \alpha/\beta$. This shows us that the linear and quadratic components of cell killing are equal at a dose that is equal to the ratio $\alpha:\beta$. An important point to note is that this model assumes the cell survival curve to be continuously bending, however this is not the case experimentally. When survival curves are determined to several decades of cell killing, at these levels of cell killing the survival curve represents a straight line. The linear quadratic model is useful however to analyse cell killing over the first few decades of cell killing and it has the advantage that it has only two adjustable parameters. A diagram of both types of survival curve is shown in Figure 1.

Two conflicting but not necessarily exclusive ideas have prevailed about the production and elimination of the survival-curve shoulder. One idea relates to the ability of the cell population to accumulate damage during low dose radiation exposure. As the dose increases the damage becomes lethal to progressively more cells in the population and the number of lesions per cell reaches a critical level in increasing numbers of the population. The other idea

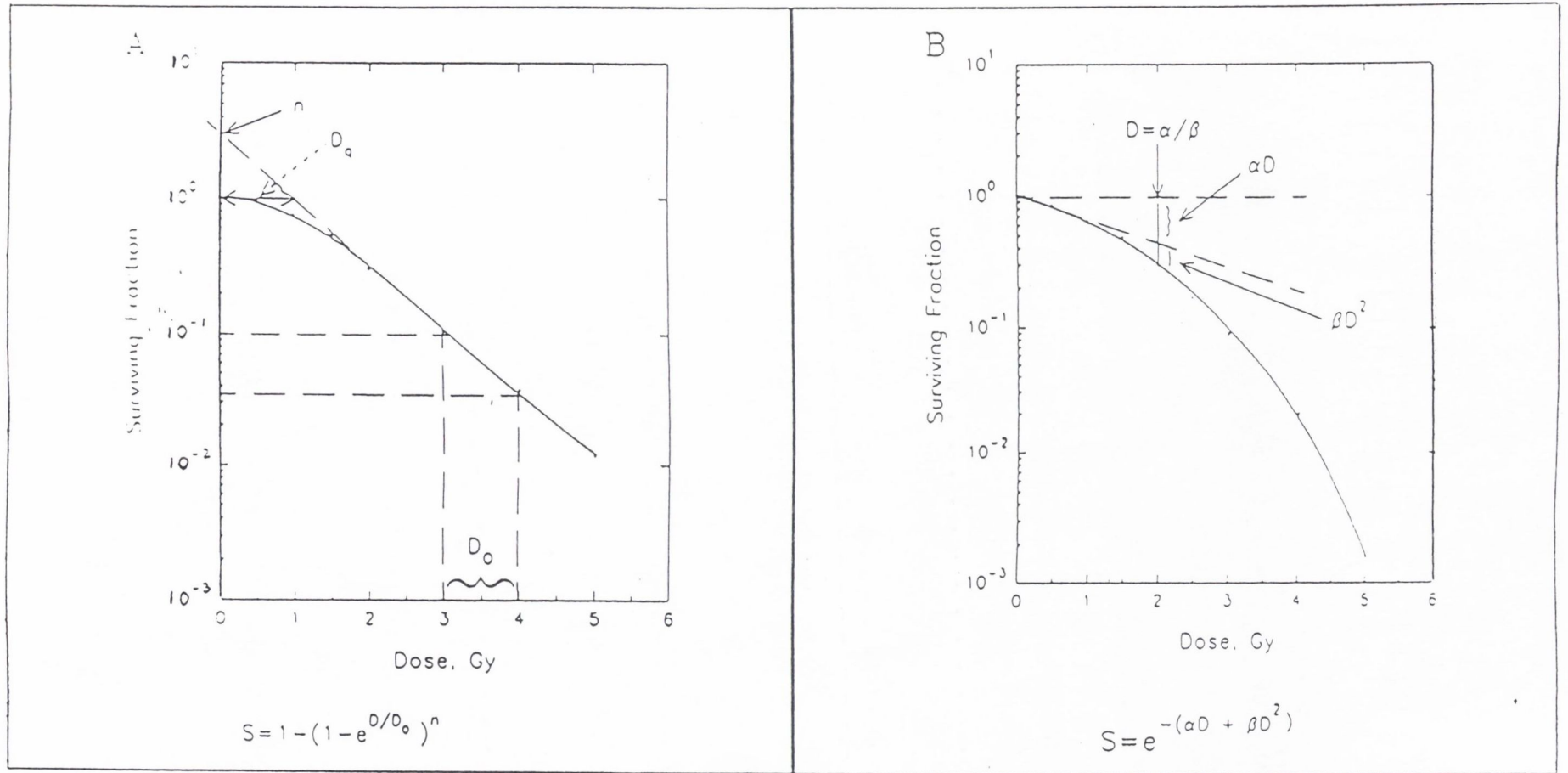


Figure 1. :- Schematic representation of simple multi-target model (A) and the linear-quadratic model (B) used to define cell survival curves following irradiation.

involves repair of damage by saturable or exhaustible reserves and that, in the absence of repair, the survival curve would be exponential.

Repair does occur in the first group of models but only influences split-dose recovery and only involves sublethally damaged cells. These cells are considered to be fully repaired and undamaged by the time the second dose of radiation is delivered. It is important that the first and second survival-curve shoulders are the same size because they are caused by the same factors, repaired in the same way and both populations are the same. In the repair model analysis, the limiting factor is the amount of repair and therefore it is possible to modulate the sizes of the first and second survival-curve shoulders separately by using certain chemicals or treatments (Alper *et al.* 1988). In these models it is assumed that if a long enough radiation-free interval is allowed between exposures to permit the restoration of energy reserves and repair substances, the shoulder width will always be restored.

Neither of these groups of models considers the possibility that repair can be induced by the first dose of radiation. There is evidence to support induction of repair (Shadley and Wolff, 1987, Olivieri *et al.*, 1991) and the idea is considered to be beyond doubt by DNA-repair biochemists working with chemical or heat damage to DNA (Samson and Cairns, 1977, Lundquist 1986, Walker 1985).

The major biological evidence against induced repair is that the radiation-survival curves do tend to show equal sized shoulders on successive survival curves when exposed using a variety of regimes. If repair was inducible then at least the very first survival curve should be different. The major evidence concerning this situation is that the single dose survival-curve shoulder can be smaller than the split-dose curve shoulder (Seymour *et al.*, 1986, Seymour and Mothersill, 1987a, Mothersill and Seymour, 1987). The findings from these experiments show that the primary survival-curve shoulder arises due to the existence of large numbers of sterile cells in so called survivor colonies, which means that these colonies are counted as survivors, having met the 50 cell criterion of Puck and Marcus (1956). But in split-dose experiments where 2-3 hours separates the dose (Seymour and Mothersill, 1989) the shoulder on the split-dose curve is unaffected and no residual damage is visible in colonies from split-dose points.

1.4.2 Split-dose and Reduction of Lethal Mutations

The induction of lethal mutations by fractionated irradiation of CHO-K1 cells has been reported to occur to a lesser extent than with single dose irradiation (Seymour and Mothersill, 1989). It was suggested that splitting the dose by

more than two hours allowed for effective repair of lethal mutations, while a recovery interval of less than two hours allowed their expression at a level comparable to that seen after single dose irradiation. When substances such as glycolysis inhibitors are used in conventional survival curve experiments (Seymour *et al.*, 1986, Seymour and Mothersill, 1987b), the results show a near exponential single dose survival curve and a shouldered split-dose curve, and no lethal mutations can be detected.

1.4.3 Protective mechanisms of N-acetyl-L-cysteine against cell death.

Irradiation of the skin with UV causes a wide range of damage to DNA and other biomacromolecules. This damage can result from direct excitation of the DNA, but also from reactions with UV-induced reactive species, such as reactive oxygen species (ROS) and lipid peroxides (Black 1987). The ROS are considered to be an important cause of skin cancer and cutaneous ageing and are thought to be involved in UV-induced immunosuppression (Black 1987, Clement-Lacroix *et al.*, 1996, Miyachi 1995). In the skin, ROS and other reactive species can be induced by UV radiation. Again, this can occur directly, but also through (endogenous) photosensitization reactions (Black 1987).

Thiol compounds are known to be direct scavengers of radicals and ROS (Van den Broeke *et al.*, 1993, Biaglow *et al.*, 1983). The -SH group can scavenge radicals by donating a hydrogen atom. The resulting thiyl radical is much less reactive and will decay by reacting with another thiyl radical, resulting in a disulfide. In addition these disulphides can quench radicals through electron donation by short-lived radical anions (Prutz 1989).

One of the most important endogenous defence mechanisms against UV-induced reactive species is the tripeptide glutathione (GSH). It can directly scavenge radicals by hydrogen donation but also acts as the unique cofactor for the enzyme GSH-peroxidase, which scavenges hydrogen peroxide. Furthermore, it acts as a reservoir of antioxidant activity and is involved in the regeneration of vitamins E and C (Meister 1991), other important endogenous photoprotectors.

Several investigators have shown cutaneous GSH levels to be reduced after UV irradiation (Connor and Wheeler, 1987, Shindo *et al.*, 1994). Low GSH levels have been shown to lead to increased UV sensitivity (Tyrell and Pidoux, 1988). Thus, maintaining or increasing skin GSH could be very beneficial.

Because of its zwitterionic structure at physiological pH, GSH has problems penetrating lipophilic barriers, such as the *stratum corneum* and the

cell membrane. Treatment of cells with exogenous GSH is not effective in raising intracellular GSH levels because it is not absorbed (Meister 1991), and it was not useful in photoprotection in mice (Kobayashi *et al.*, 1996). Presumably, it acts only as a source of cysteine after it has been broken down into amino acids (Meister 1991).

Another way to induce higher GSH levels in the cell is to provide a source of cysteine, which is usually the limiting substrate in GSH synthesis. However because it also has a zwitterionic structure, cysteine has difficulty penetrating the cell. The use of more lipophilic cysteine derivatives can overcome this uptake problem. Once inside the cell, the cysteine precursors are converted to cysteine and ultimately to GSH (Karg *et al.*, 1990, Butterworth *et al.*, 1993, Steenvoorden and Beijersbergen van Henegouwen, 1996).

In addition many thiols and therefore most cysteine derivatives can scavenge UV-induced reactive intermediates directly. The thiol group can repair radicals by donating a hydrogen atom, while the resulting much less reactive group, such as cysteine derivatives, have a protective effect independent of GSH metabolism.

Previous studies have shown that cysteine derivatives are promising candidates for use as UV radiation protectors. N-acetyl-L-cysteine (NAC) is

effective against UV-induced expression of protooncogenes (Garmyn and Degreef 1997), p53 (Renzing *et al.*, 1996) and immunosuppression (Van den Broeke and VaBeijersbergen Van Henegouwen 1995). When applied to the skin of rats, NAC and several other cysteine derivatives were found to protect biomacromolecules in viable epidermal cells from damage by a variety of reactive intermediates resulting from photoexcitation (Van den Broeke and VaBeijersbergen Van Henegouwen 1994). These compounds penetrated the skin and were able to induce radical scavenging activity. In this thesis, the effect of NAC was studied on both human and fish cells to determine if there was any protective effect on the initial survival or delayed cell death post exposure to UVA.

1.5 Aims and Strategy for achieving these Aims

It is now generally accepted that cells which survive irradiation are not equally capable of long-term reproductive survival, this has been termed lethal mutations/delayed reproductive death. While there is no doubt that delayed expression of lethal or non-lethal mutations occurs post-exposure to ionizing radiation, little is known of the UV response in humans and fish. There is experimental evidence that suggests that DNA double-strand breaks (DSBs) act as the main initiator of this instability. UVA does cause DSBs, but UVB does not and, therefore, might not be expected to cause delayed death if DSBs are important initiators. Recent publications from our laboratory suggest that the delayed reproductive death observed post-exposure to radiation may in fact be delayed apoptosis as the progeny of irradiated cells contain apoptotic bodies. Two genes *bax* and *bcl-2* are known to promote and inhibit apoptosis, respectively, and therefore expression of these gene products may indicate whether or not the cell is undergoing apoptosis. This project aims to investigate whether or not UVA and UVB cause delayed reproductive death in human and fish cell lines and whether or not this is due to genetic instability induced by the radiation or a form of delayed apoptosis controlled by the two genes *bax* and *bcl-2*. Initially I will investigate the short term effects on

clonogenic survival on both the human and fish skin cell lines to see if there is any difference in the cytotoxicity between either species post exposure to Uva and UVB. When this is done and I am aware of which dose range that I will have to work within I will perform experiments to see whether or not delayed reproductive death is apparent post exposure to UVA and UVB in the skin cell lines used. If there is a delayed effect I will investigate whether or not oxidative oxidative stress or CPDs play a role in any delayed effect by looking at the effect of NAC and photoreactivation on the cells post exposure to UVA and UVB respectively. Finally I will see if apoptosis plays a role in any delayed effect by scoring the cells for the expression of the two genes involved in apoptosis, Bax and bcl-2.

2. Materials.

2.1 General Apparatus.

Nuaire Laminar Flow cabinet

Forma Scientific Incubators	CO ₂	5 %
	Temp	37 °C, 30 °C and 21 °C.
	Humidity	99 %

Coulter Counter, Model Dn

Nikon microscope, model TMS

Nikon microscope, model OPTIPHOT

Standard Laboratory equipment and glassware were used.

2.2 Culture Flasks.

Cells were cultured in the following culture vessels:-

- 1) Nunclon 25 cm²/ 40 ml flasks (Nunclon, Denmark).
- 2) Nunclon 80 cm²/ 260 ml flasks (Nunclon, Denmark).

2.3 Ultraviolet Radiometer and Lamps used:-

UVGL-58 lamp - (UVA)

UVLM-57 lamp - (UVB)

UVGL-58 lamp - Lamp used for Photoreactivating light (PRL)

UVX Digital Radiometer

(All obtained from UVP LTD, Cambridge, UK)

2.4 Cell lines used.

The HaCaT cell line used was derived from human skin keratinocytes spontaneously transformed in vitro during long-term incubation of a primary culture under selected culture conditions (Boukamp *et al.*, 1988). This cell line was a gift from Dr. P. Boukamp, (Heidelberg, Germany).

Similar to most of the skin carcinomas these HaCaT cells show UV-specific mutations in both alleles of the p53 tumour suppressor gene. In addition they exhibit loss of one copy of chromosomes 3p, 4p, and 9p. Following transfer of the different chromosomes, only chromosome 3 could restore senescence in the HaCaT cells (Boukamp *et al.* 1996).

The HaCaT-ras cell line (Boukamp *et al.*, 1990) was derived from

HaCaT cells transfected with the cellular Harvey-ras oncogene which subsequently resulted in benign and malignant HaCaT variants. The HaCaT - ras cell line used in this thesis is the malignant variant. This cell line was also a gift from Dr. P. Boukamp, (Heidelberg, Germany). Under standard conditions both the HaCaT and HaCaT-ras cell lines have a mean plating efficiency of approximately 25 % that was stable over a wide range of cell numbers plated. Both cell lines were grown at 37 °C.

The EPC (Epithelioma papulosum cyprini) cell line originated from carp epidermal herpes virus-induced hyperplastic lesions (Fijan *et al.*, 1983). The EPC cell line was grown at 30 °C. Under standard conditions this cell line has a mean plating efficiency of approximately 20 % which was stable over a wide range of cell numbers plated. This cell line was obtained from the European Tissue Culture Collection.

The CHSE-214 cell line (Lannan *et al.*, 1984) used was derived from pooled embryos of the chinook salmon, *Oncorhynchus tshawytscha*. It grows within a narrow range of temperatures 21-24 °C and under standard conditions this cell line has a mean plating efficiency of approximately 25 % which was stable over a wide range of cell numbers plated. The CHSE cell line was grown at 21°C. This cell line was obtained from the European Tissue Culture Collection.

2.5 Cell culture media.

Medium used to grow the HaCaT and HaCaT -ras cells:-

Dulbecco's Modified Eagle Medium / Nut Mix F12 (GIBCO)

Supplemented with the following:-

L-Glutamine 200 mM (GIBCO)	1 mM
Penicillin/streptomycin (GIBCO)	
(5000 IU/ml Penicillin)	50 IU/ml
(5000 µg/ml Streptomycin)	50 µg/ml
Hepes buffer 1M (GIBCO)	12 ml
Hydrocortisone (SIGMA)	1 µg/ml
Foetal Calf Serum (GIBCO)	7.5%

Medium used to grow the CHSE cells:-

Dulbecco's Modified Eagle Medium / Nut Mix F12 (GIBCO)

Supplemented with the following:-

L-Glutamine 200 mM (GIBCO)	1 mM
Penicillin/streptomycin (GIBCO)	
(5000 IU/ml Penicillin)	50 IU/ml
(5000 µg/ml Streptomycin)	50 µg/ml

Hepes buffer 1M (GIBCO)	12 ml
Foetal Calf Serum (GIBCO)	10 %

Medium used to grow the EPC cells:-

MEM Glasgow Modified Medium (GIBCO)

Supplemented with the following:-

L-Glutamine 200 mM (GIBCO)	1mM
Penicillin/streptomycin (GIBCO)	

(5000 IU/ml Penicillin)	50 IU/ml
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(5000 µg/ml Streptomycin)	50 µg/ml
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Hepes buffer 1 M (GIBCO)	12 ml
Foetal Calf Serum (GIBCO)	10 %

2.6 Disaggregation agents.

ENZYMATIC AGENT.

Weak trypsin solution

Earle’s balanced salt solution

(w/o calcium and magnesium) (GIBCO)	500 ml
2.5 % stock trypsin (GIBCO)	50 ml

Chelating Agent.

Versene (Gibco); Ethylenediaminetetraacetic Acid (EDTA)

This agent acts as a calcium and magnesium chelator and is used in combination with weak trypsin solution to remove cells from the substratum in preparation for subculture.

2.7 Buffer.

Phosphate Buffered Saline:-

Sodium Chloride (BDH)	8.00 g
Potassium Chloride (BDH)	0.20 g
Disodium Hydrogen Phosphate	1.44 g
Potassium Dihydrogen Phosphate (BDH)	0.24 g

Adjust volume to one litre with distilled water

Adjust the pH to 7.2

2.8 Fixatives.

10% Neutral Buffered Formalin

40% Formaldehyde Solution (BDH)	100 ml
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Distilled water	900 ml
Sodium Dihydrogen Phosphate Monohydrate (BDH)	4.00 g
Disodium Hydrogen Phosphate Anhydrous (BDH)	6.50 g

2.9 N-Acetyl-L-cysteine.

N-Acetyl-L-cysteine (obtained from Calbiochem-Novabiochem Corporation, La Jolla, CA, 92039, U.S.A), was made up in a 50×10^{-3} M solution i.e. 0.408g in 50mls H_2O , so that when added to 5000 μ l of medium in a flask would give a 1X concentration of the solution. Various dilutions were carried out to give 10^{-4} M, 10^{-5} M and 10^{-6} M of the solution.

2.10 Immunocytochemical reagents.

Monoclonal Antibodies.

Bcl-2- Clone 124.

Bcl-2 oncoprotein was detected using a mouse anti-human monoclonal 124 antibody (Dako Ltd, Bucks, UK).

Bax Clone I-19.

Bax oncoprotein was detected using the rabbit polyclonal Bax (I-19) antibody (Santa Cruz Biotechnology Inc., CA, USA) which is specific for the carboxy-terminus of human Bax and is non-cross reactive with Bcl-2, Bak, or other proteins involved with apoptosis. Positive controls were sections of thyroid tissue.

Labelling of the bax and bcl-2 all antibodies was developed using peroxidase-conjugated anti-mouse IgG (Vectastain, ABC kit).

Chromogen for Peroxidase Enzyme.

Diaminobenzaldehyde (DAB) Isopac 100mg in sealed bottle, add 16 ml phosphate buffered saline (PBS) to bottle. Mix well and aliquot into 500 ml or 1 ml amounts. Store in glass tubes at -20 °C.

3% Hydrogen Peroxide Solution (BDH)

This solution is used to block endogenous peroxidase activity.

One in ten dilution of a 30 % solution of hydrogen peroxide (stored at 4 °C) in distilled water.

Glycergel Mounting Medium (DAKO C563).

An aqueous histological mounting medium for use when a permanent water-solution mounting medium is required. In plastic culture flasks an organic mounting medium cannot be used due to corrosion of the plastic.

2.11 Other Reagents

10 % Carbol Fuchsin (Clin-tech)

Harris's Haematoxylin (BDH).

3. Methods

3.1 Cell Culture and Maintenance of Cell Lines

The stock cells were grown in 260 ml Nunc flasks in the appropriate growth medium and at the appropriate temperature for the particular cell line involved. When the cells reached confluence they were passaged in the following way. Cells were rinsed for approximately one minute with 0.25 % w/v trypsin solution and 1mM versene solution in a 1:1 mixture. This solution was then poured off and a fresh trypsin/versene solution was used to trypsinise the cells for approximately 10-15 minutes at 37 °C for the human cells and 30 °C for the fish cells. 10 ml of cell growth medium was then placed in the flask to stop the action of trypsin on the cells. This stock cell suspension was poured into a universal container and aspirated to ensure a single cell suspension. The cells were counted using a Coulter counter model Dn. Three background readings were taken using a cuvette containing 20 ml of isoton only. Three readings were then taken using a cuvette containing 20 ml of isoton and 1 ml of stock cell suspension, and the number of cells per ml was thus determined using the calculation:-

$(\text{Mean cell count} - \text{Mean background cell count}) \times 42 = \text{Number of cells/ml.}$

It was then possible to carry out various dilutions and plate known numbers of cells into the flasks.

3.2 Plating Efficiencies and Cell Survival Tests

Known numbers of cells were plated into three replicate flasks per dose point. Having been allowed to attach to the surface of the flask (after approximately 4 hours unless otherwise stated) the cells were irradiated at room temperature. The number of cells per flask that were plated varied with the dose so that the number of colonies surviving would be in a range that approximately one hundred colonies could be counted conveniently.

After irradiation the cells were allowed to grow at the appropriate temperature for the particular cell line involved until the surviving cells produced macroscopic colonies that were visible with the naked eye. The cells were then stained with carbol fuchsin (Zeel Niehlson 1:15) for approximately 15 minutes and the carbol fuchsin was then rinsed off with tap water. The colonies were then counted and the plating efficiency (P.E.) and surviving fraction (S.F) was determined using the following equations:-

$$\% \text{ P.E.} = \frac{\text{no. of colonies counted per flask}}{\text{no. of cells plated per flask}} \times 100$$

$$\% \text{ S.F.} = \frac{\text{no. of colonies counted per flask}}{\text{no. of cells plated per flask}} \times \frac{100}{\text{Control P.E.}}$$

3.3 Growth Curves: Determination of Population Doubling Time.

When cells were seeded into a flask they enter a lag period, followed by a period of exponential growth and then finally enter a period of reduced or zero growth after they become confluent. These phases were characteristic for each cell line and give rise to reproducible measurements. The determination of the growth cycle was important in designing routine subculture and experimental protocols.

The population-doubling time of the cells used was determined by plating 5000 cells per 25 cm² flask. Cells were trypsinised from three flasks and counted everyday for the duration of the experiment and the total number of cells per flask was obtained. By plotting the total number of cells counted per day versus the number of days on a semi-logarithmic plot it was possible to determine the population doubling time from the straight-line exponential region of the growth curve.

3.4 Irradiation Technique

The spectral output of any ultraviolet radiation source is usually measured (from source to sensor) in W/m^2 and is called the “Irradiance” or “Radiant flux”. There is an implied dependence of irradiance on the angle of the area being irradiated relative to the beam. In a collimated, uniform beam, the irradiance on a planar surface varies directly with $\cos \theta$, where θ = angle of incidence from a normal to the surface. A diagram of the projected area of the sensor is shown in Figure 2. The “exposure dose” or “dose” is usually measured in J/m^2 and is calculated by multiplying the irradiance in W/m^2 by the exposure time in seconds.

A photograph of the apparatus used for to irradiate the cells is shown in Plate 3.1. Having been allowed to attach to the surface of the flask (after at least 4 hours) the cells were irradiated at room temperature using 6 watt ultraviolet hand lamps at a distance of 15 cm from source to flask. The lamps were held by a stand during irradiation. UVA irradiation was delivered at a dose rate of typically $3.15 \text{ Jm}^{-2} \text{ s}^{-1}$ using a UVGL-58 lamp. This lamp has a broad spectrum output of 340-400 nm and emits maximally at 365 nm. UVB irradiation was delivered at a dose rate of typically $2.15 \text{ Jm}^{-2} \text{ s}^{-1}$ using a UVLM-57 lamp. This lamp has a broad spectrum output of 280-380 nm and

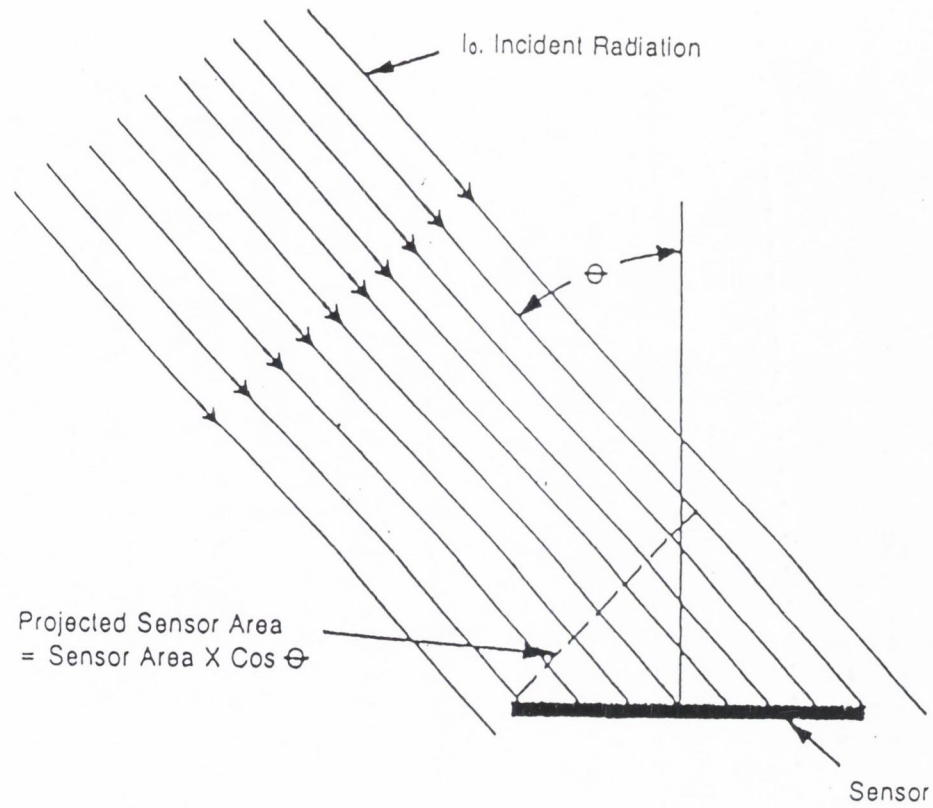


Figure 2 :- Showing the projected area of the sensor on the UVX Digital Radiometer.

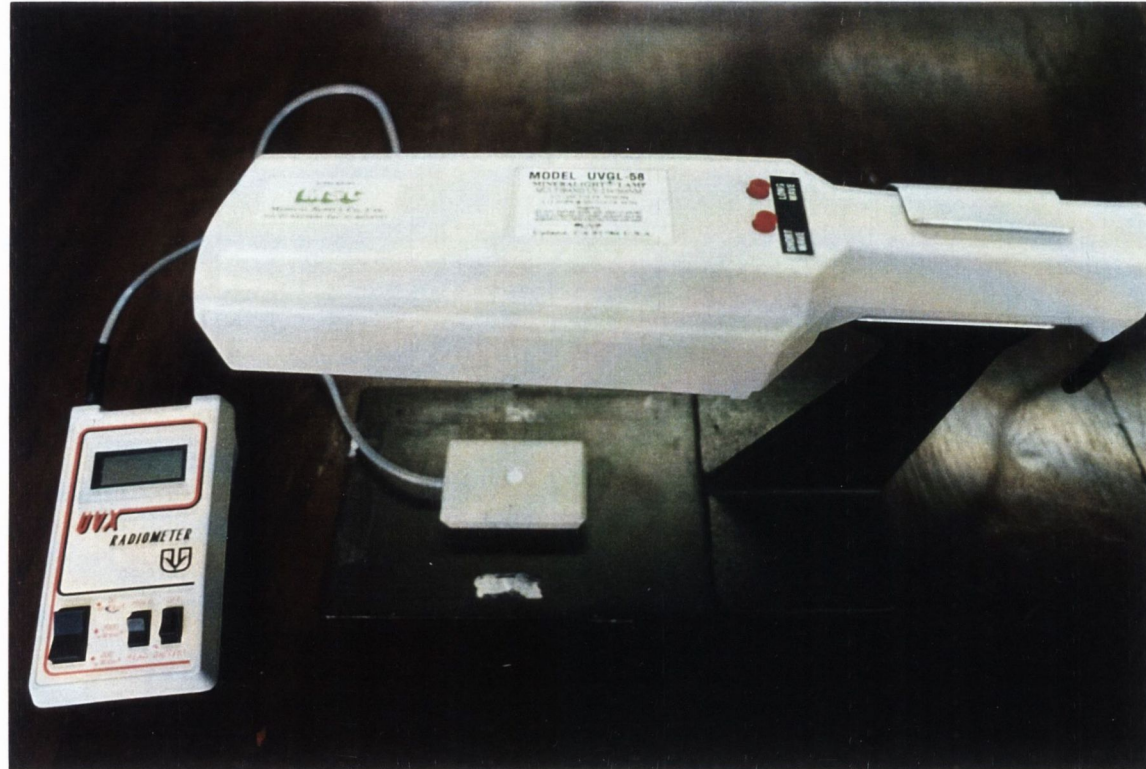


Plate 3.1:- Showing the UVGL-58 lamp used to irradiate cells with UVA and photoreactivating light.

emits maximally at 302 nm. Photoreactivating light (PRL) was delivered using the UVGL-58 (UVA) lamp. This lamp has a broad spectrum output of 340-400 nm and emits maximally at 365 nm. The spectral output from the lamps (obtained from UVP LTD, Cambridge, UK) is shown in Appendix II. A UVX digital radiometer was used for measuring the irradiance at the flask from these lamps. The radiometer has interchangeable probes sensitive in the 300 nm (UVB) and 365 nm (UVA) regions of the ultraviolet spectrum.

No more than three flasks were irradiated together at any one time. The flasks were placed as close to the centre of the field of UVR as possible. The radiometer was also placed as close to the centre of the field of UVR as possible before the cells were irradiated to measure the irradiance. The area of irradiation was approximately 10 cm^2 , and the irradiance varied by approximately $10 \mu\text{W}/\text{cm}^2$ within this area. The cells were irradiated through the top of the flask to cut out wavelengths less than 279 nm.

Before irradiation the medium was removed from the flask to prevent absorption of the UVR by the phenol red in the medium. The exposure times for UVA were quite long and so before the cells were irradiated with UVA, the medium was removed and 2 ml of PBS was left in the flask, a similar technique is used by Goderr *et al.*, 1994. This was essential to prevent the cells from drying up when left under the lamp for any length of time. When

exposing the cells to UVB the medium was removed but no PBS was needed as the exposure times were relatively short compared with UVA exposure times. The effect of leaving the cells in PBS for up to two hours when irradiating with UVA and without medium for up to two minutes when irradiating with UVB was found to alter the plating efficiency by less than 1 %. By using PBS the irradiance was increased by approximately $2 \mu\text{W}/\text{cm}^2$. This was possibly due to total internal reflection of the light within the liquid and all irradiance values have been corrected for this.

3.5 Lethal Mutation Assay

Appropriate cell numbers were seeded into six flasks per dose point. These cells were allowed to attach to the surface of the flask and were then irradiated. When the cells produced macroscopic colonies, three of the original six flasks were stained with carbol fuchsin, and the colonies counted. The medium was then removed from the remaining three flasks and a fresh 5 ml of culture medium was placed into each flask. Upon reaching confluence the cells were trypsinised and the cell suspension was counted to determine the number of cells per ml. A plating efficiency experiment was then set up for each flask and the cells in these flasks were grown to macroscopic colonies

and were then stained with carbol fuchsin. By taking into account the surviving fraction of the progeny of the initially irradiated cells and that of the initial surviving fraction it was possible to determine the residual surviving fraction (R.S.F.) from the equation:-

$$\% \text{ R.S.F.} = \frac{\text{Initial \% S.F.} \times \text{Progeny \% S.F.}}{100}$$

The residual percentage surviving fraction was a measure of the 'true' level of survival at times distant from the initially irradiated cells. When the initial % S.F and the % R.S.F were plotted versus dose on a semi-log scale the reduction in the initial surviving fraction to the residual surviving fraction would indicate the presence of lethal mutations.

3.6 Split Dose Recovery.

Three sets of flasks were set up. (1) A control set of 3 flasks, (2) a set of 3 flasks exposed to a single dose of UVR at time zero and (3) 12 flasks (four groups of three) were exposed to a similar dose except that this dose was delivered in two equal fractions. These equal fractions were separated by a time interval of 1, 2, 3, and 4 hours after time zero. Following irradiation the

flasks of cells were left undisturbed and allowed to form colonies. The surviving fraction was determined for each dose point. A comparison of the level of survival of a single dose of UVR to the level of survival when the dose was given in two equal fractions separated by a time interval, allowed the determination of the time it takes for the cells to recover from sublethal damage. This allowed the time interval that should elapse between successive fractions of radiation to be determined.

Knowing the time that was needed to elapse between successive fractions it was possible to set up split-dose survival curves to determine if delivering a split-dose to the cells could induce repair and ablate the delayed cell death present post irradiation in both the HaCaT and CHSE-214 cell lines. Two sets of flasks (6 flasks per dose point) were set up, one set was to be given a single dose UVR (with a range of doses) at time zero, while the second set was to be given an initial dose of UVR at time zero and a second similar dose 2 hours after time zero. The cells were then grown to form colonies, at this time one set (three flasks per dose point) was stained with carbol fuchsin to determine the surviving fraction of the initially irradiated cells and the other set was grown to confluence and passaged as explained in Section 3.6 to determine the plating efficiency of the progeny of the initially irradiated cells.

3.7 Photoreactivation Assay

The repair induced by illuminating cells with UVA after the cells were exposed to various doses of UVB was studied by comparing the effect of a single dose of UVB to a set of flasks and also the effect of a single dose of UVB followed with a dose of UVA to a second set of flasks. In order to determine what dose of UVA induced repair, two sets of flasks were set up. The first set of flasks (3 per dose point) were given a dose of UVB, and second set of flasks were then given the same dose of UVB followed with a single dose of UVA immediately after irradiation with UVB. From this it was possible to determine at which dose of UVA the cells were exhibiting photoreactive repair i.e. in this case an increase in survival.

Knowing the dose that was needed to photoreactivate the damage caused by UVB it was possible to set up a photoreactivation survival curve to determine if delivering a dose of UVA to the cells could induce repair and ablate the delayed cell death present post irradiation in both the HaCaT and CHSE-214 cell lines. This involved setting up three sets of flasks (6 flasks per dose point), (1) a control set of flasks, (2) a set of flasks exposed to various doses of UVB, and (3) a set of flasks exposed to a similar dose of UVB as (2) followed by a dose of UVA to photoreactivate the UVB induced damage. The

cells were then grown to form colonies, at this time one set (three flasks per dose point) was stained with carbol fuchsin to determine the surviving fraction of the initially irradiated cells and the other set was grown to confluence and passaged as explained in Section 3.5 to determine the plating efficiency of the progeny of the initially irradiated cells.

3.8 Reduction of Oxidative Stress Assay

N-acetyl-L-cysteine (NAC) is known to reduce oxidative stress in cells post exposure to an oxidant such as UVA. The reduction of oxidative stress caused by exposing cells to various doses of UVA in the presence of NAC was studied by comparing the effect of a single dose of UVA to a set of flasks and also a similar dose of UVA to a second set of flasks containing various concentrations of NAC. In order to determine at what concentration of NAC the cells were protected from oxidative stress, two sets of flasks were set up. The first set of flasks (3 per dose point) were given a dose of UVA, the second set of flasks were then given the same dose of UVA as the first set except this set of flasks contained various concentrations of NAC, 10^{-3} M, 10^{-4} M, 10^{-5} M and 10^{-6} M. 100 μ l of the various concentrations of NAC was placed in the flasks (giving a final concentration of 0.0081g/ml) for 24 hours before

irradiation. After irradiation 100µl of the various concentrations of NAC was placed in the flasks. From this it was possible to determine which concentration of NAC protected the cells from the oxidative stress induced by UVA.

Knowing the concentration of NAC that was needed to reduce the oxidative stress caused by UVA it was possible to set up a survival curve to determine if delivering a dose of UVA to the cells with NAC could inhibit the oxidative stress and ablate the delayed cell death present post irradiation in both the HaCaT and CHSE-214 cell lines. This involved setting up a control set of flasks, a set of flasks exposed to various doses of UVA, and a set of flasks exposed to a similar dose of UVA with NAC present in the medium (6 flasks per dose point). The cells were then grown to form colonies, at this time one set (three flasks per dose point) was stained with carbol fuchsin to determine the surviving fraction of the initially irradiated cells and the other set was grown to confluence and passaged as explained in Section 3.6 to determine the plating efficiency of the progeny of the initially irradiated cells.

3.9 Immunocytochemical assay for *bax* and *bcl-2*.

The cultures were washed in PBS and the endogenous peroxidase activity was

blocked with 3 % H_2O_2 in PBS for 5 minutes. The cultures were then washed in buffer for 20 minutes. The cultures were then incubated with normal diluted serum for 20 minutes. All excess serum was washed from the cultures and they were incubated with primary antibody diluted (1:20) in PBS for 60 minutes. The cultures were again washed in buffer for 10 minutes and incubated for 30 minutes with diluted biotinylated antibody solution. The cultures were again washed in buffer for 10 minutes and then incubated for 45 minutes with vectastain ABC reagent and washed in buffer for a further 10 minutes. Cultures were incubated in Diaminobenzidine (DAB) prepared as follows:- 1000 μl DAB, 9000 μl PBS and 10 μl H_2O_2 until staining became apparent. The cultures were then rinsed in tap water and counterstained with haematoxylin for 1 minute. The cultures were then rinsed in hot water and mounted in glycerol. A positive reaction was indicated by a brown staining. For negative controls the primary antibody was omitted.

Immunopositive cells were quantified under light-microscopy by performing counts on three randomly selected colonies within each flask. The total number of positive and negative cells along a marked line in each colony was estimated and expressed as percentages. Immunopositive expression was indicated as brown staining in the cell. Negative-stained cells were blue.

4. Results on the Comparative Survival of Each Cell Line Post Exposure to UVA and UVB.

Figures 4.1-4.2 show the response of HaCaT and HaCaT-ras cells to UVA and UVB. The best fit regression lines are shown for each survival curve. These regression lines are not constrained through the 100% survival point and are merely an indication of the best fit line for that survival curve. The D_{37} values are shown in Table 4.1. Both cell lines were more sensitive to UVB than UVA and show a similar response to UVB. The HaCaT cells however show a more sensitive response to UVA than the HaCaT-ras cells. Figures 4.3-4.4 show the response of CHSE and EPC cells to UVA and UVB. The D_{37} values are also shown in Table 4.1. Both cell lines were more sensitive to UVB than UVA. The EPC cells were more sensitive to UVA than the CHSE cells and the CHSE cells were more sensitive to UVB than the EPC cells. From these results it is evident that the fish cells were more sensitive than the human cells to both UVA and UVB.

Cell Line		HaCaT	HaCaT-ras	CHSE	EPC
UVA	$D_{37} / (\text{Jm}^{-2})$	19000	23600	12700	10900
UVB	$D_{37} / (\text{Jm}^{-2})$	550	550	275	320

Table 4.1:- Showing the D_{37} values for each cell line post exposure to UVA and UVB. D_{37} is the dose required to reduce the surviving fraction to 37%. This data is taken from Figures 4.1-4.4

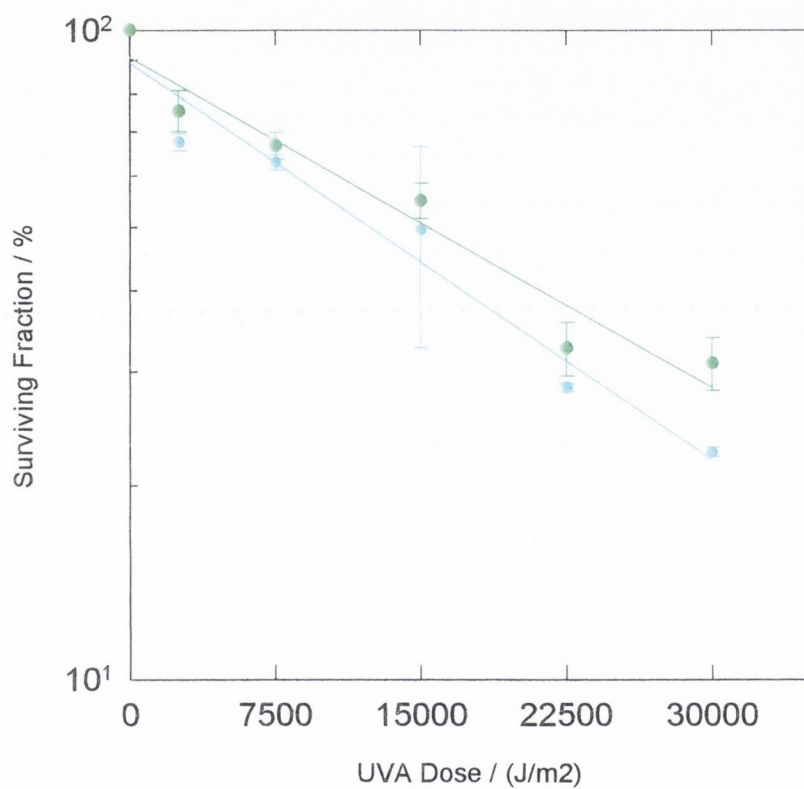


Figure 4.1:- Survival curves for the HaCaT (•) and HaCaT-ras (•) cells post exposure to UVA. Also shown are the best fit regression lines. See Appendix 1 page 237 for full data.

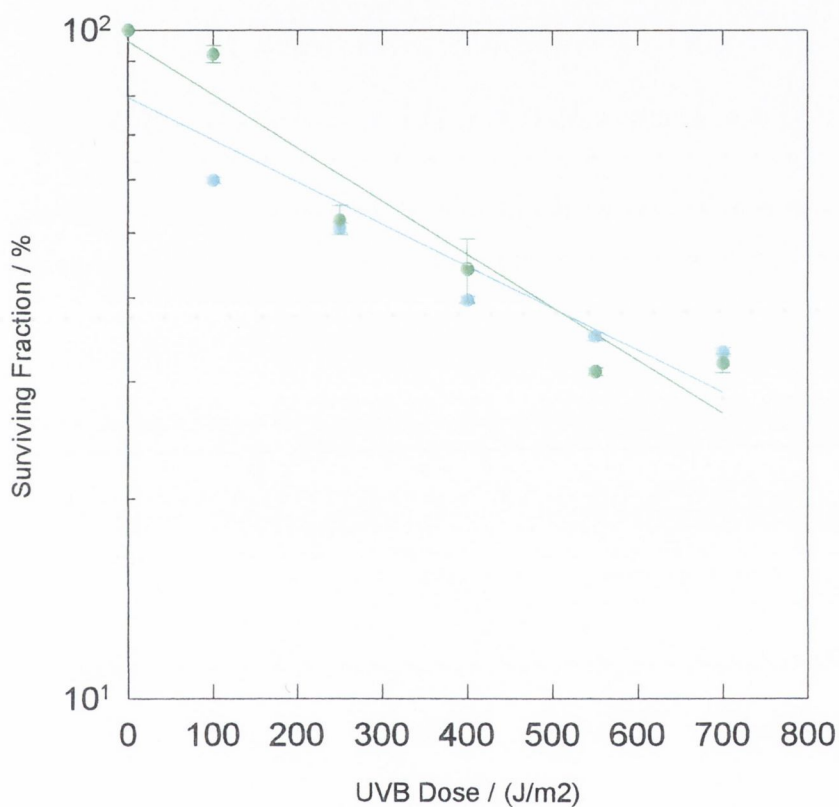


Figure 4.2:- Survival curves for the HaCaT (•) and HaCaT-ras cells (•) post exposure to UVB. Also shown are the best fit regression lines. See Appendix 2 page 240 for full data.

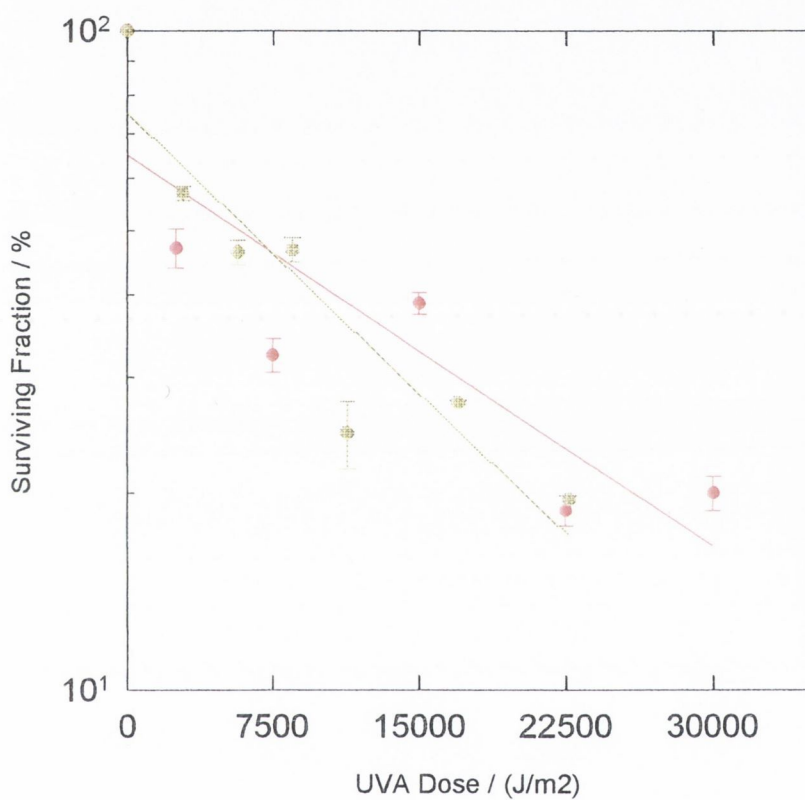


Figure 4.3:- Survival curves for the EPC (■) and CHSE (●) cells post exposure to UVA. Also shown are the best fit regression lines. See Appendix 3 page 243 for full data.

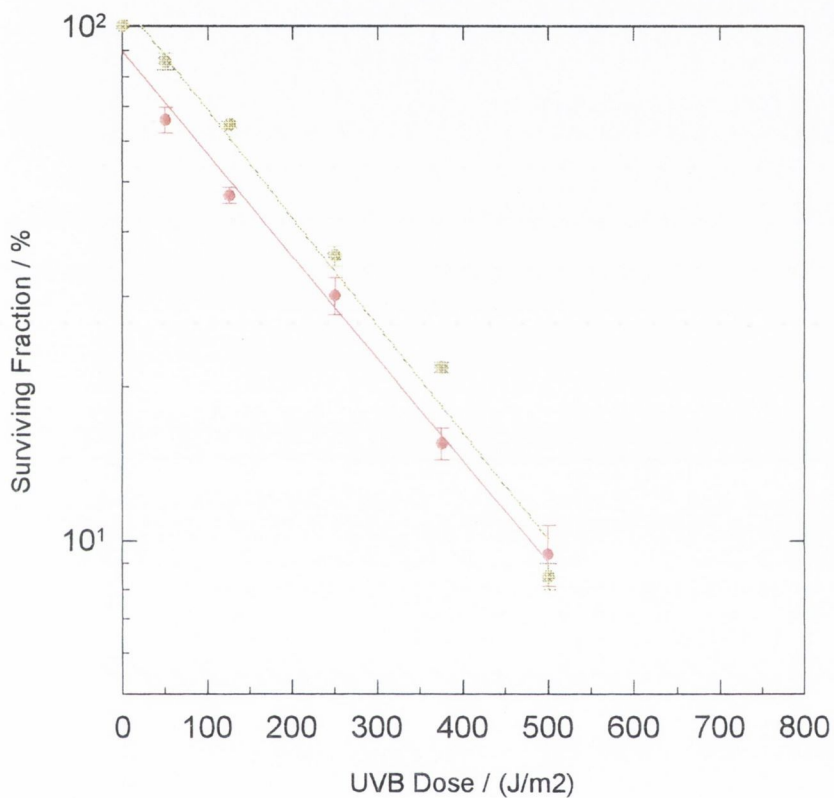


Figure 4.4:- Survival curves for the EPC (*) and CHSE (•) cells post exposure to UVB. Also shown are the best fit regression lines. See Appendix 4 page 246 for full data.

5. Results on Delayed Cell Death.

The incidence of delayed cell death was measured in the four cell lines used post exposure to UVA and UVB. The plating efficiency at each dose point was calculated and expressed as a percentage of its own control. From this a percentage surviving fraction was calculated for each sample at each dose point for a particular passage. Probability levels shown in the following tables indicate whether the difference between the initial and the residual survival curves is either not significant ($P > 0.05$), significant ($P < 0.05$) or highly significant ($P < 0.01$) at each dose point.

5.1 Delayed Cell Death in the HaCaT and CHSE cell lines.

Figures 5.1-5.5 show the initial percentage surviving fraction plotted with the residual percentage surviving fraction of the HaCaT cells post exposure to UVA. The best fit regression lines are shown for each survival curve. These regression lines are not constrained through the 100% survival point and are merely an indication of the best fit line for that survival curve. Also shown for the initial survival regression line in each case are the 95% confidence limits which indicate whether or not a point outside of these points is significantly

different or not to the regression line drawn. Table 5.1 shows the probability levels given when comparing the initial and residual survival curves in Figure 5.1.

Figures 5.7-5.11 show the initial percentage surviving fraction plotted with the residual percentage surviving fraction of the HaCaT cells post exposure to UVB. The best fit regression lines are shown for each survival curve. These regression lines are not constrained through the 100% survival point and are merely an indication of the best fit line for that survival curve. Also shown for the initial survival regression line in each case are the 95% confidence limits which indicate whether or not a point outside of these points is significantly different or not to the regression line drawn. Table 5.3 shows the probability levels given when comparing the initial and residual survival curves in Figure 5.7. The data are plotted after 10-50 population doublings with approximately 10 population doublings between each passage. In the case of the HaCaT cells, exposure to UVA and UVB resulted in considerable variation in the surviving fraction values. The clonogenic ability of the surviving progeny of the exposed HaCaT cells was reduced compared with that of the unirradiated cells. The possibility of this delayed cell death being ablated after further passaging was investigated in the HaCaT cell line. It can be seen that the effect exists for up to 50 population doublings post exposure.

Figures 5.13 and 5.15 show the initial percentage surviving fraction plotted with the residual percentage surviving fraction of the CHSE cells post exposure to UVA, and UVB respectively. The best fit regression lines are shown for each survival curve. These regression lines are not constrained through the 100% survival point and are merely an indication of the best fit line for that survival curve. Also shown for the initial survival regression line in each case are the 95% confidence limits which indicate whether or not a point outside of these points is significantly different or not to the regression line drawn. Tables 5.5 and 5.7 show the probability levels given when comparing the initial and residual survival curves in Figures 5.13 and 5.15 respectively. The data are plotted after approximately 10 population doublings. In the case of the CHSE cells, exposure to UVA and UVB did not result in considerable variation in the surviving fraction values however, it can be seen that the cloning efficiency of the surviving progeny of the exposed CHSE cells was reduced compared with that of the unirradiated cells.

5.2 Delayed Cell Death in the transformed HaCaT-ras and EPC cell lines.

Figures 5.6, 5.12, 5.14, and 5.16 show the residual percentage surviving

fraction plotted with the initial percentage surviving fraction for the HaCaT-ras and EPC cells post exposure to UVA and UVB. The best fit regression lines are shown for each survival curve. These regression lines are not constrained through the 100% survival point and are merely an indication of the best fit line for that survival curve. Also shown for the initial survival regression line in each case are the 95% confidence limits which indicate whether or not a point outside of these points is significantly different or not to the regression line drawn. Tables 5.2, 5.4, 5.6 and 5.8 show the probability levels given when comparing the initial and residual survival curves in each of these figures. The data plotted are after approximately 10 population doublings for both cells lines. The cloning efficiency of the surviving progeny of the exposed HaCaT-ras and EPC cells was not significantly reduced overall compared with that of the unirradiated cells. This indicates that there was no detectable delayed death present in these cell lines. As no delayed cell death was evident in the HaCaT-ras or EPC cell lines no further passages were performed with this cell line.

5.3 Delayed Apoptosis.

An increase in Bax protein expression is seen as an indication the cell is

undergoing apoptosis because this gene is actively involved in the promotion of apoptosis. Tables 5.9 and 5.10 show the percentage positive *bax* cells post exposure to UVA and UVB respectively in the initially irradiated cells and the progeny of those cells. In the HaCaT cells there was an increase in *bax* expression from no expression in the control up to 100 percent both in the initially irradiated cells and in the progeny. The expression in the CHSE cells also increases from no expression in the control up to 30-40 percentage at low doses of UVA and up to 100 percent at low and high doses of UVB. For the HaCaT-ras cells there was also an increase in *bax* expression but not to the same extent as with the HaCaT cells, this increase was also seen in the initially irradiated cells and the progeny. Plate 5.3 shows HaCaT-ras cells immunocytochemically stained for *bax* in the absence of exposure to UVA. The plate shows that there are no positive cells. Plate 5.4 shows HaCaT-ras cells immunocytochemically stained for *bax* post exposure to 2500 J/m² of UVA. The plate shows 75 percentage positive cells. EPC cells are tumourigenic cells and because of this should express low levels of *bax*, however, after exposure to UVA or UVB the levels of *bax* in these cells rose to 100 percent both at high and low doses in the initially irradiated and the progeny cells. Plate 5.7 shows EPC cells immunocytochemically stained for *bax* post exposure to 700 J/m² of UVB. The plate shows 100 percent positive

cells. Plate 5.8 shows EPC cells immunocytochemically stained for *bax* in the absence of exposure to UVB. The plate shows that there are no positive cells.

An increase in BCL-2 protein expression is seen as an indication the cell is not undergoing apoptosis because this gene is an anti-apoptotic gene and is actively involved in preventing apoptosis. Tables 5.11 and 5.12 show the percentage positive *bcl-2* cells post exposure to UVA and UVB respectively in the initially irradiated cells and their progeny. In the HaCaT cells there was approximately 50 percent or less expression of *bcl-2* in the control cells this level was not significantly changed following the initial irradiation and fell to zero in the progeny. At no dose point did the expression of *bcl-2* rise to 100 percent. Plate 5.1 shows the control HaCaT cells immunocytochemically stained for *bcl-2* in the absence of exposure to UVB. The plate shows that there are no positive cells. Plate 5.2 shows HaCaT cells immunocytochemically stained for *bcl-2* post exposure to 100 J/m² of UVB. The plate shows 54 percent positive cells. In both the HaCaT-ras and EPC cells there are 100 percent positive cells in the control, the initially irradiated cells and their progeny. These cells lines should have a high level of *bcl-2* because they are tumourigenic and therefore are resistant to apoptosis. CHSE cells are non-tumourigenic and because of this should express low levels of *bcl-2*. However, post exposure to UVA or UVB the levels of *bcl-2* in these

cells rose to 100 percent in the initially irradiated cells and their progeny. Plate 5.5 shows CHSE cells immunocytochemically stained for *bcl-2* post exposure to 30000 J/m² of UVA. The plate shows 100 percent positive cells. Plate 5.6 shows the control CHSE cells immunocytochemically stained for *bcl-2* post exposure to 0 J/m² of UVA. The plate shows 23 percent positive cells.

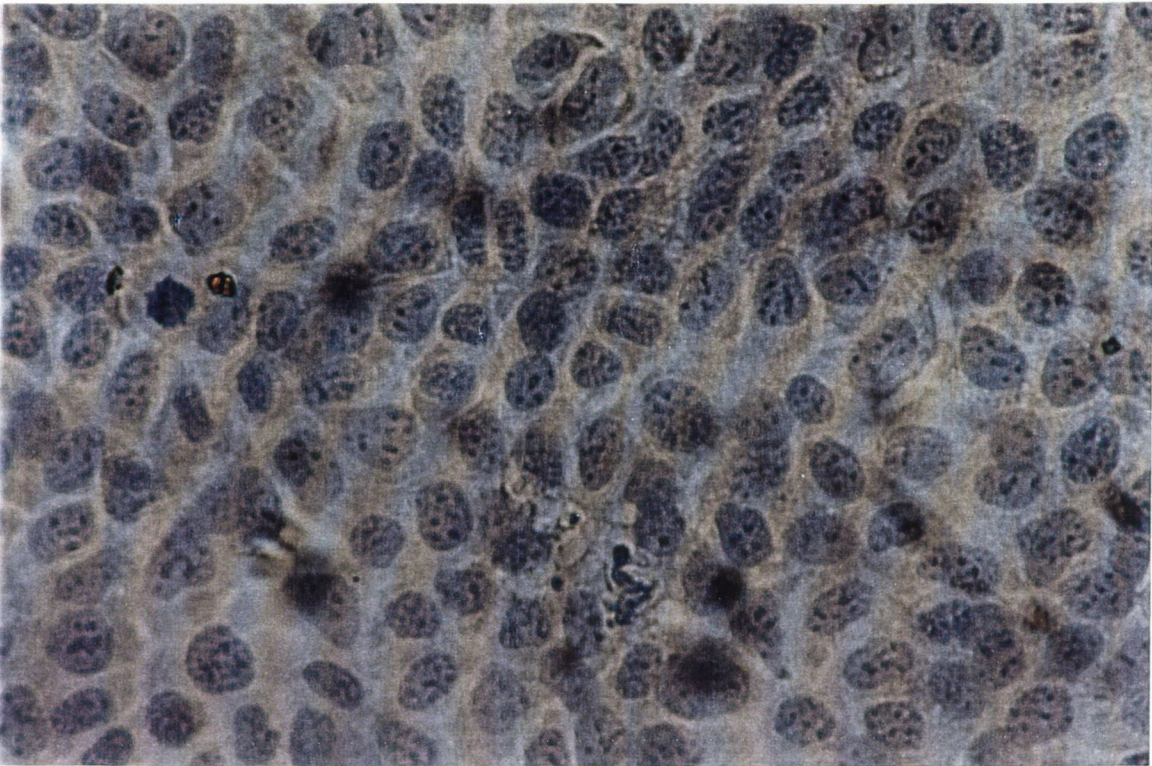


Plate 5.1:- HaCaT cells immunocytochemically stained for *bcl-2* post exposure to 0 J/m^2 of UVB. The above photograph shows 0 % positive cells.

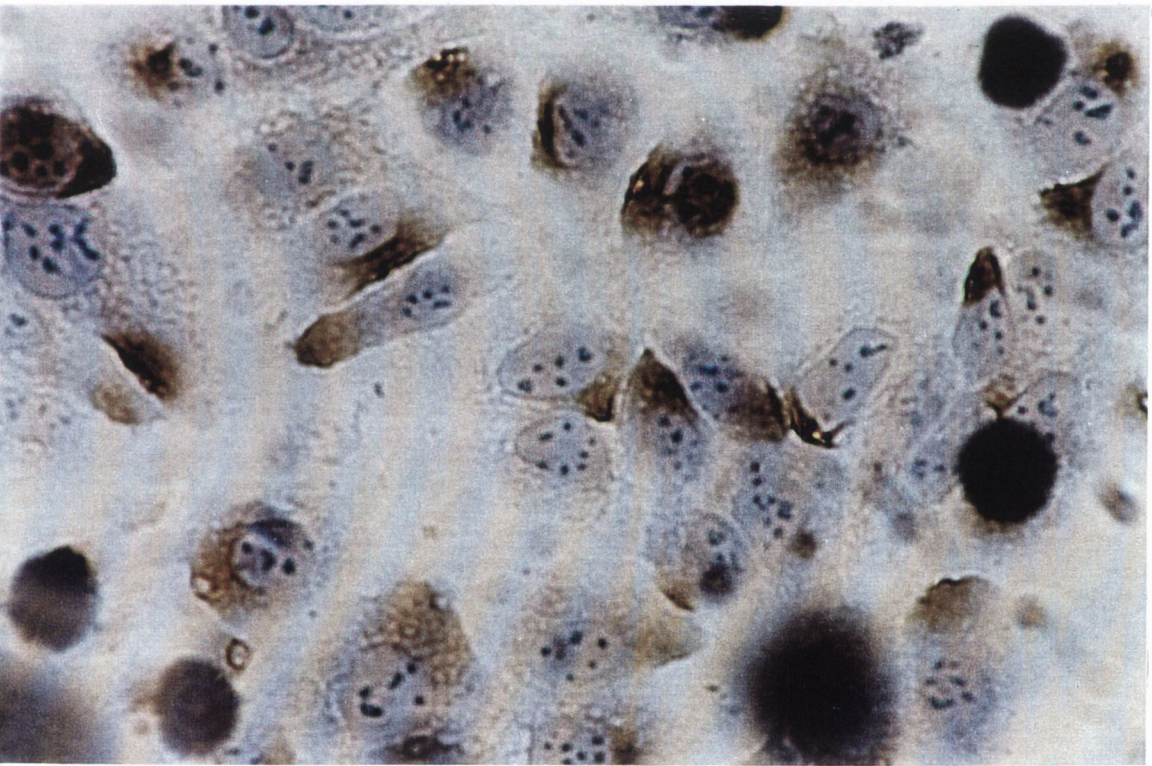


Plate 5.2:- HaCaT cells immunocytochemically stained for *bcl-2* post exposure to 100 J/m^2 of UVB. The above photograph shows 54 % positive cells.

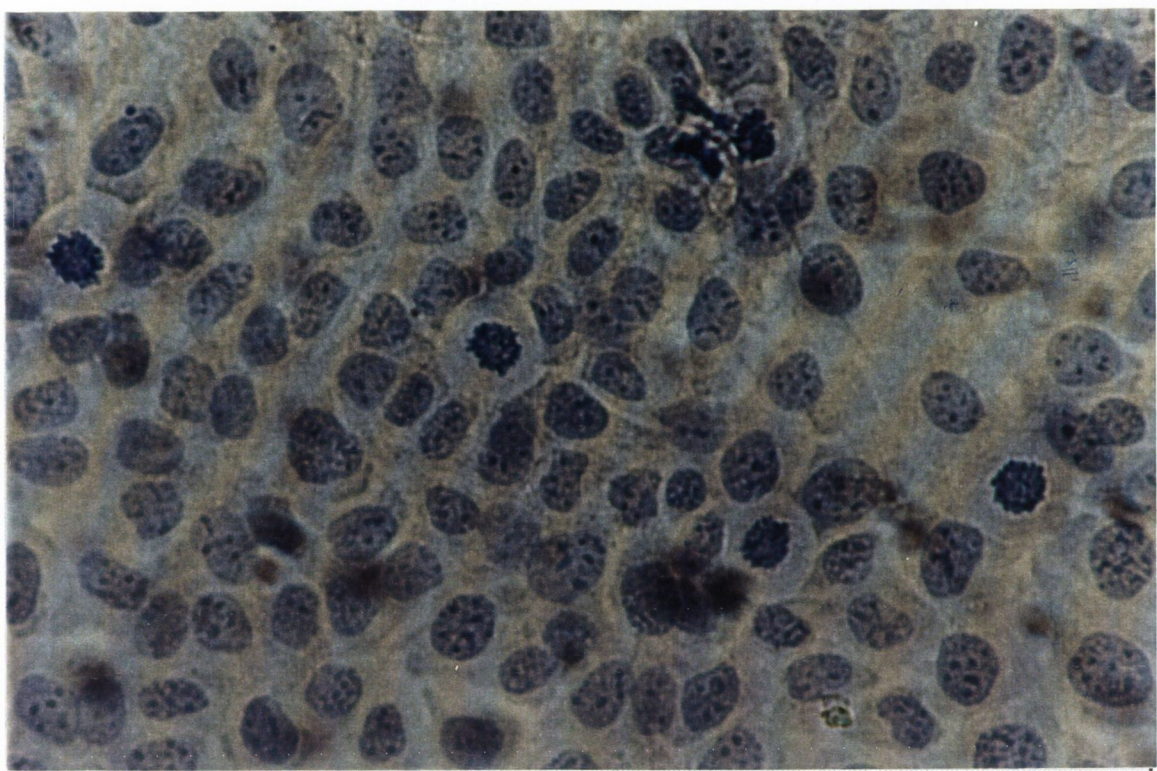


Plate 5.3:- HaCaT-ras cells immunocytochemically stained for *bax* post exposure to 0 J/m² of UVA. The above photograph shows 0 % positive cells.

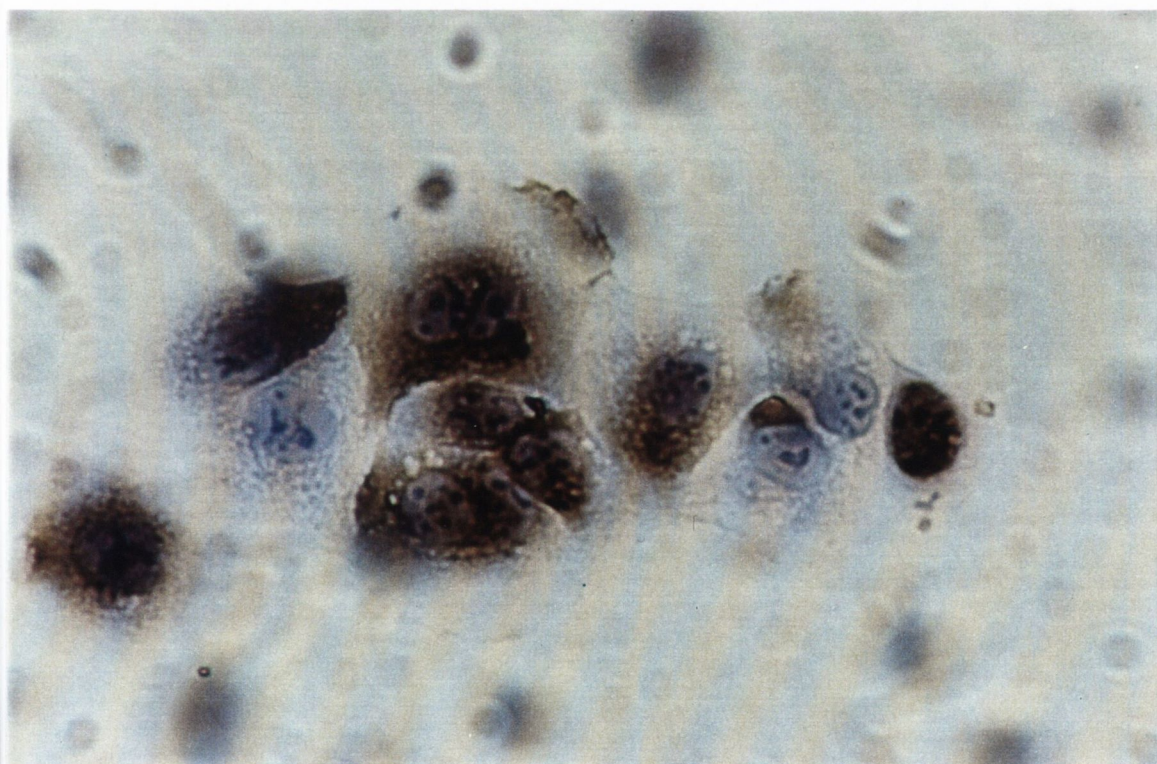


Plate 5.4:- HaCaT-ras cells immunocytochemically stained for *bax* post exposure to 2500 J/m² of UVA. The above photograph shows 75 % positive cells.

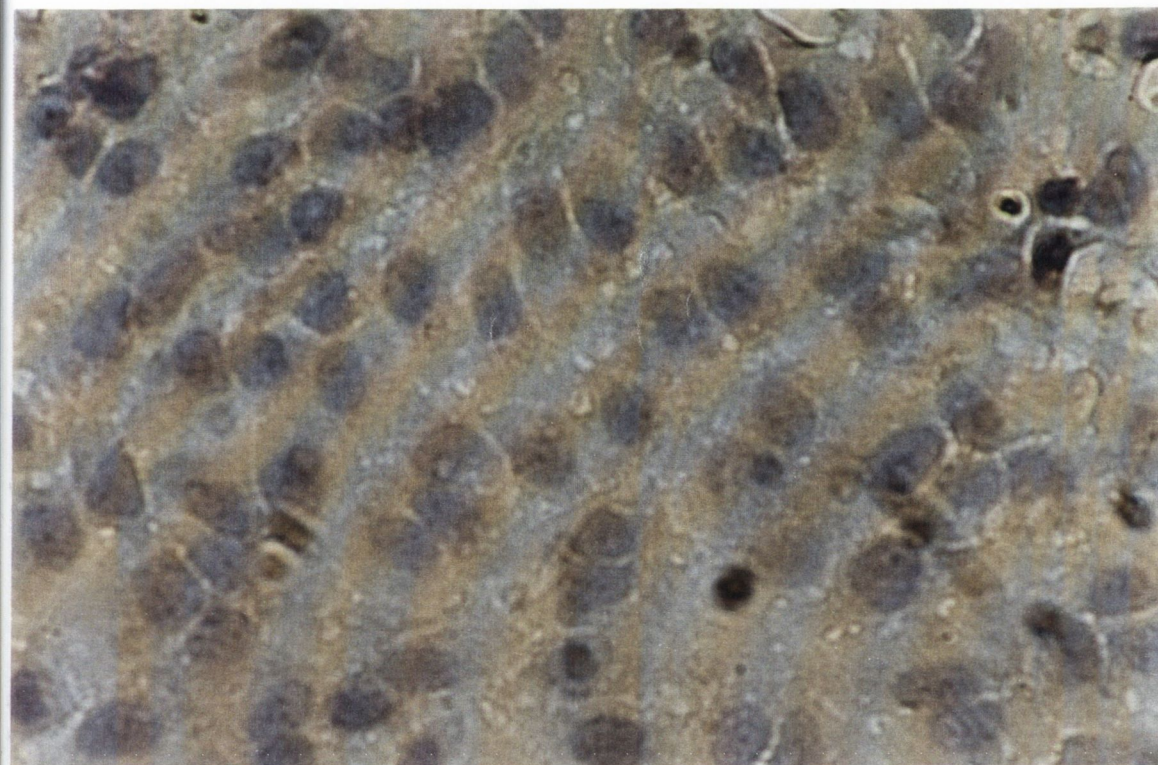


Plate 5.5:- CHSE cells immunocytochemically stained for *bcl-2* post exposure to 30000 J/m² of UVA. The above photograph shows 100 % positive cells.

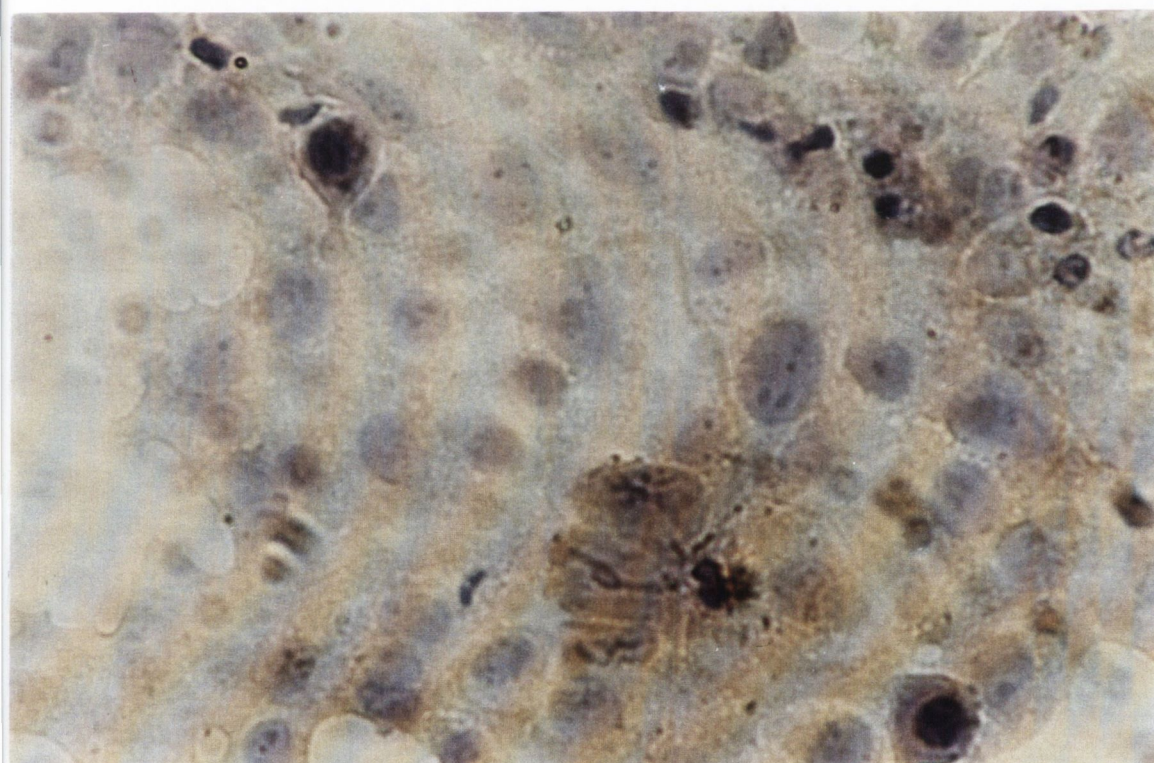


Plate 5.6:- CHSE cells immunocytochemically stained for *bcl-2* post exposure to 0 J/m² of UVA. The above photograph shows 23 % positive cells.

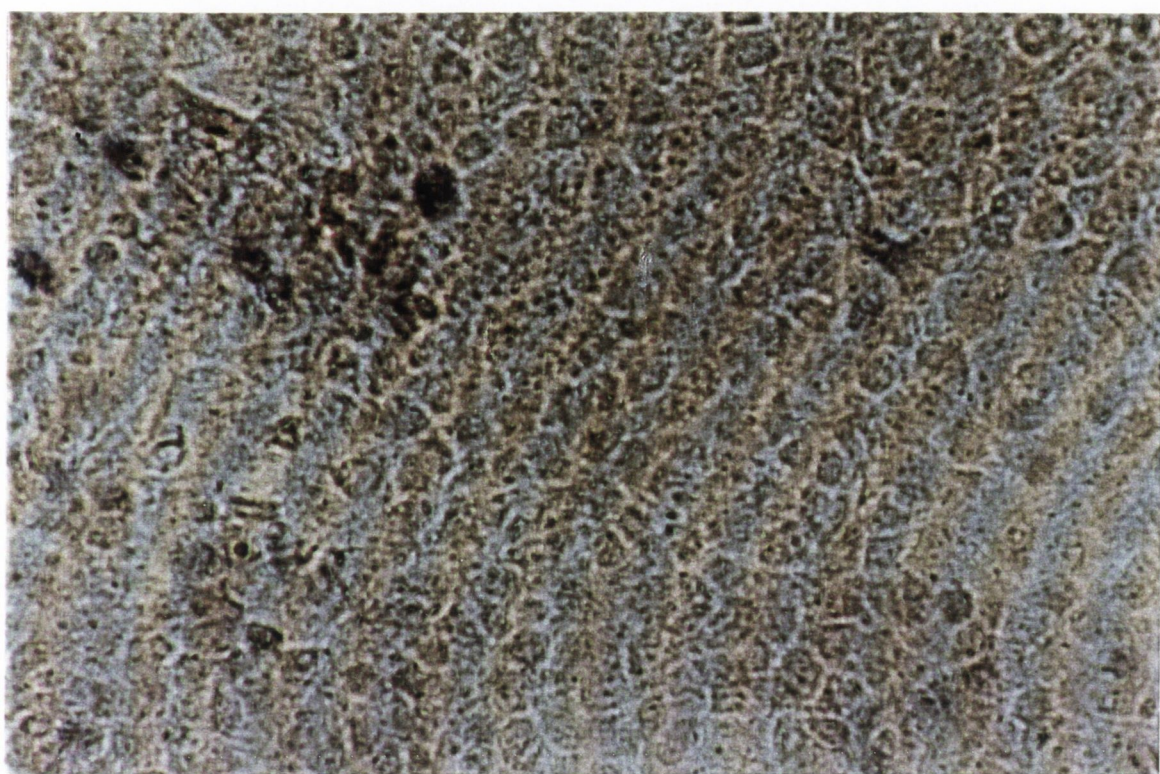


Plate 5.7:- EPC cells immunocytochemically stained for *bax* post exposure to 700 J/m² of UVB. The above photograph shows 100 % positive cells.

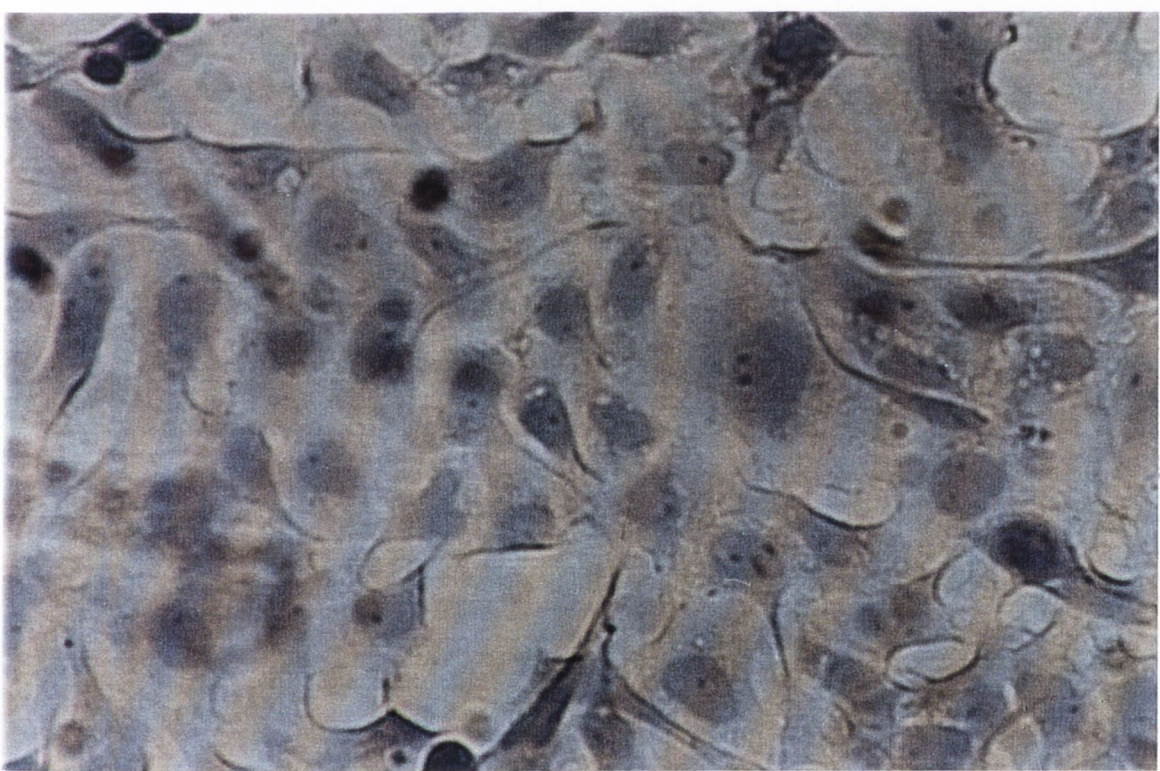


Plate 5.8:- EPC cells immunocytochemically stained for *bax* post exposure to 0 J/m² of UVB. The above photograph shows 0 % positive cells.

UVA Dose / (J/m ²)	Probability level (P)
2826	HS
5652	HS
8478	S
11304	HS
16956	S
22608	S
28260	S

Table 5.1:- Showing the probability levels given when comparing the initial and residual survival curves in Figure 5.1. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVA Dose / (J/m ²)	Probability level (P)
2500	NS
7500	NS
15000	NS
22500	NS
30000	NS

Table 5.2:- Showing the probability levels given when comparing the initial and residual survival curves in Figure 5.6. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVB Dose / (J/m ²)	Probability level (P)
63	HS
127	S
190	HS
254	S
318	HS
381	S
445	HS

Table 5.3:- Showing the probability levels given when comparing the initial and residual survival curves in Figure 5.7. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVB Dose / (J/m^2)	Probability level (P)
0	0
125	NS
250	NS
400	NS
550	NS
700	NS

Table 5.4:- Showing the probability levels given when comparing the initial and residual survival curves in Figure 5.12. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVA Dose / (J/m ²)	Probability level (P)
2500	S
7500	S
15000	HS
22500	S
30000	S

Table 5.5:- Showing the probability levels given when comparing the initial and residual survival curves in Figure 5.13. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVA Dose / (J/m ²)	Probability level (P)
2826	NS
5652	NS
8478	NS
11304	NS
16956	NS
22608	NS
28260	NS

Table 5.6:- Showing the probability levels given when comparing the initial and residual survival curves in Figure 5.14. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVB Dose / (J/m ²)	Probability level (P)
50	S
125	S
250	S
375	HS
500	S

Table 5.7:- Showing the probability levels given when comparing the initial and residual survival curves in Figure 5.15. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVB Dose / (J/m^2)	Probability level (P)
63	NS
127	NS
190	NS
254	NS
318	NS
381	NS
445	NS

Table 5.8:- Showing the probability levels given when comparing the initial and residual survival curves in Figure 5.16. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVA Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	CHSE % positive cells	EPC % positive cells
0	0	0	0	0
2500	75 ± 20	22 ± 3	42 ± 12	100
30000	100	45 ± 12	100	100
UVA Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	5 ± 0	0	0
2500	29 ± 6	29 ± 2	36 ± 10	100
30000	100	49 ± 26	100	100

Table 5.9:- Showing the percentage positive *bax* cells post exposure to UVA, initially and after 10 population doublings (passage 1). Errors are expressed as the standard error of the mean for n=3.

UVB Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	UVB Dose / (J/m ²)	CHSE % positive cells	EPC % positive cells
0	0	0	0	0	0
100	32 ± 5	13 ± 5	50	100	100
700	100 ± 0.0	0	500	100	100
UVB Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	UVB Dose / (J/m ²)	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	0	0	0	0
100	100	0	50	100	100
700	100	0	500	100	100

Table 5.10:- Showing the percentage positive *bax* cells post exposure to UVB, initially and after 10 population doublings (passage 1). Errors are expressed as the standard error of the mean for n=3.

UVA Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	CHSE % positive cells	EPC % positive cells
0	33 ± 5	100	13 ± 3	100
2500	57 ± 8	100	100	100
30000	18 ± 7	100	100	100
UVA Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	100	18 ± 5	100
2500	0	100	100	100
30000	0	100	100	100

Table 5.11:- Showing the percentage positive *bcl-2* cells post exposure to UVA, initially and after 10 population doublings (passage 1). Errors are expressed as the standard error of the mean for n=3.

UVB Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	UVB Dose / (J/m ²)	CHSE % positive cells	EPC % positive cells
0	0	100	0	22 ± 6	100
100	15 ± 7	100	50	100	100
700	0	100	500	100	100
UVB Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	UVB Dose / (J/m ²)	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	100	0	10 ± 4	100
100	0	100	50	100	100
700	0	100	500	100	100

Table 5.12:- Showing the percentage positive *bcl-2* cells post exposure to UVB, initially and after 10 population doublings (passage 1). Errors are expressed as the standard error of the mean for n=3.

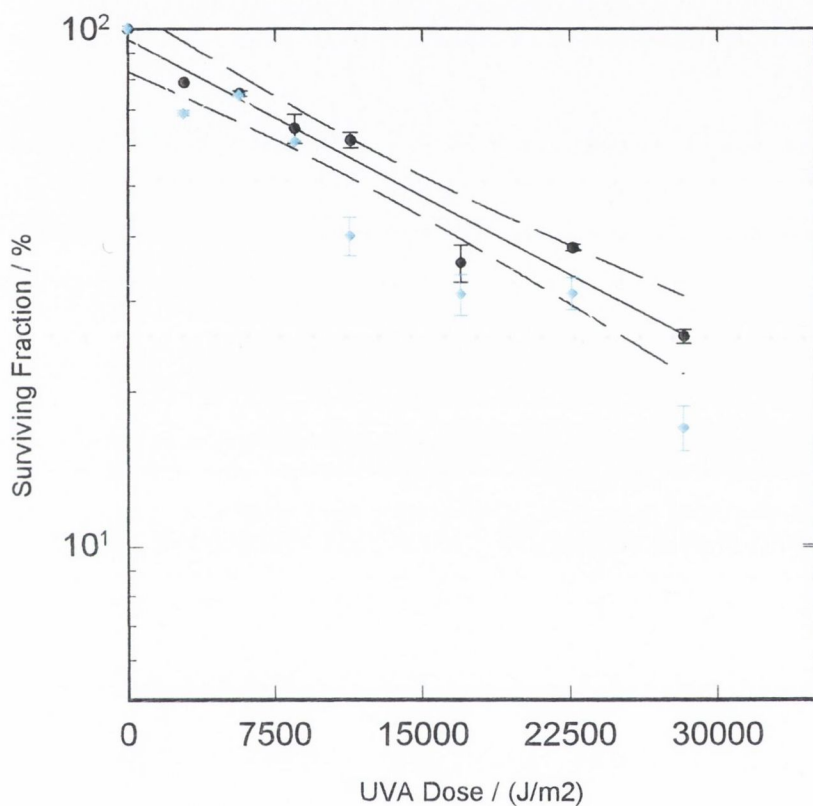


Figure 5.1:- Survival curves for the initially irradiated HaCaT cells (●) and the residual survival of the progeny of these cells (◆) after 10 population doublings post exposure to UVA. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 9 page 261 for full data.

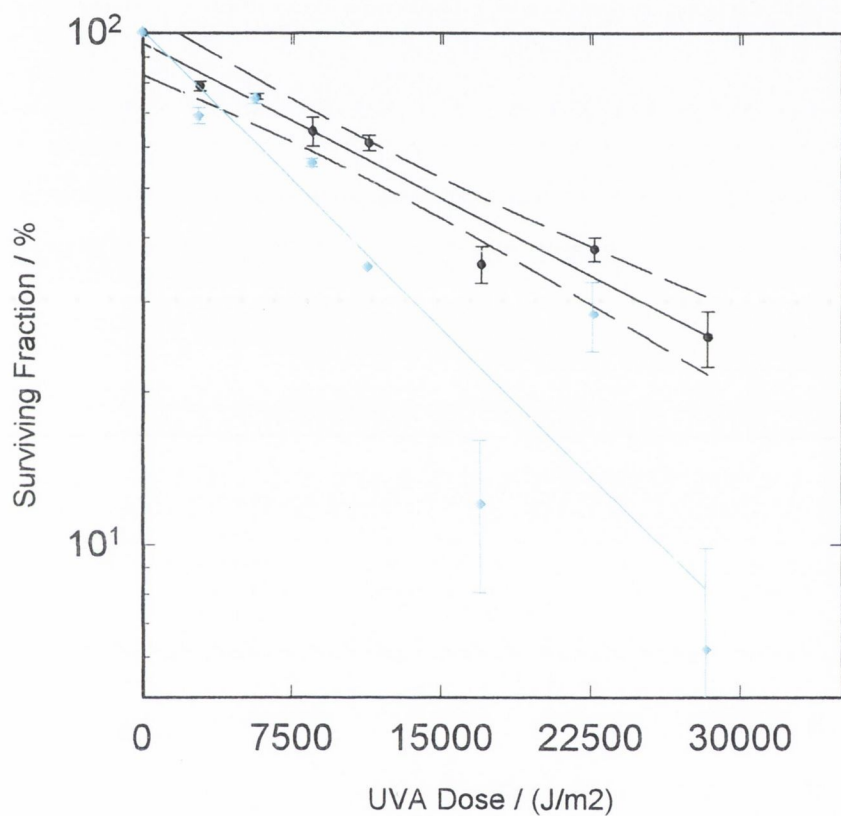


Figure 5.2:- Survival curves for the initially irradiated HaCaT cells (●) and the residual survival of the progeny of these cells (◆) after 20 population doublings post exposure to UVA. Also shown are the best fit regression lines with 95% confidence limits for the initial survival regression line. See Appendix 10 page 262 for full data.

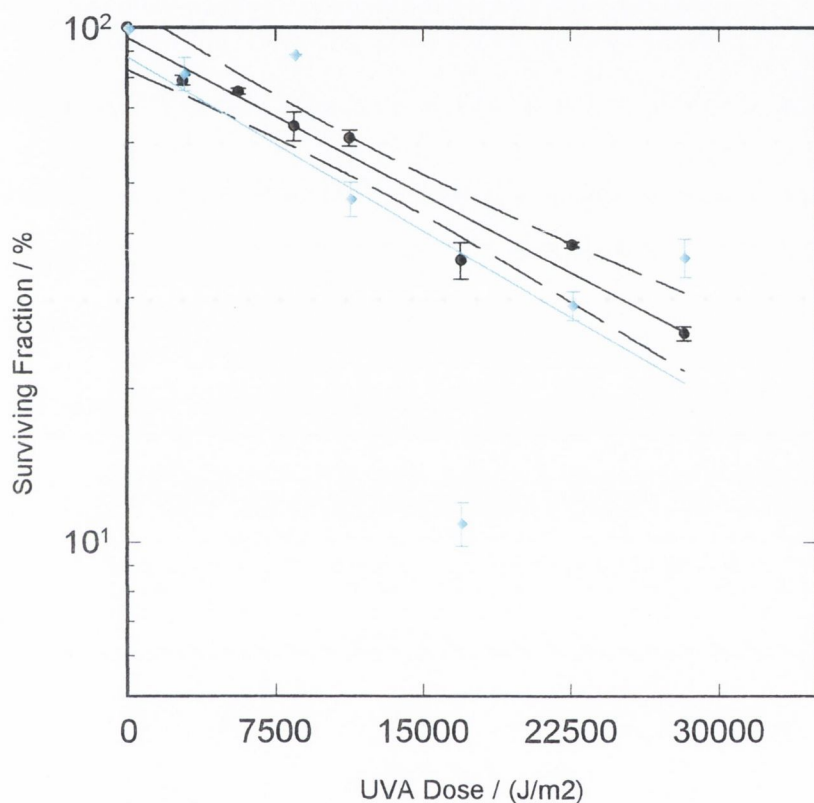


Figure 5.3:- Survival curves for the initially irradiated HaCaT cells (●) and the residual survival of the progeny of these cells (◆) after 30 population doublings post exposure to UVA. Also shown are the best fit regression lines with 95% confidence limits for the initial survival regression line. See Appendix 11 page 263 for full data.

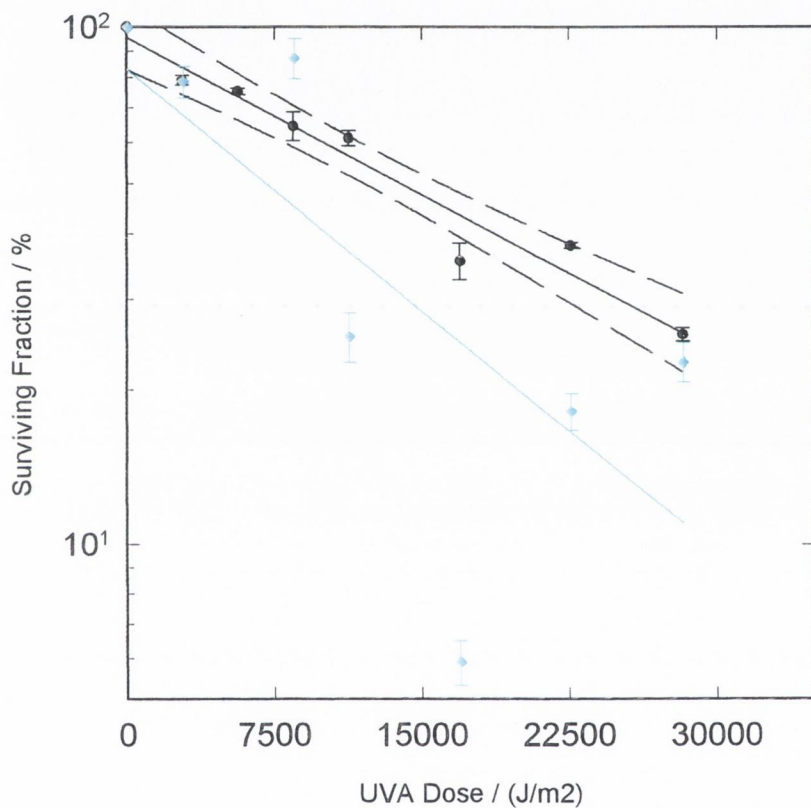


Figure 5.4:- Survival curves for the initially irradiated HaCaT cell (●) and the residual survival of the progeny of these cells (◆) after 40 population doublings post exposure to UVA. Also shown are the best fit regression lines with 95% confidence limits for the initial survival regression line. See Appendix 12 page 264 for full data.

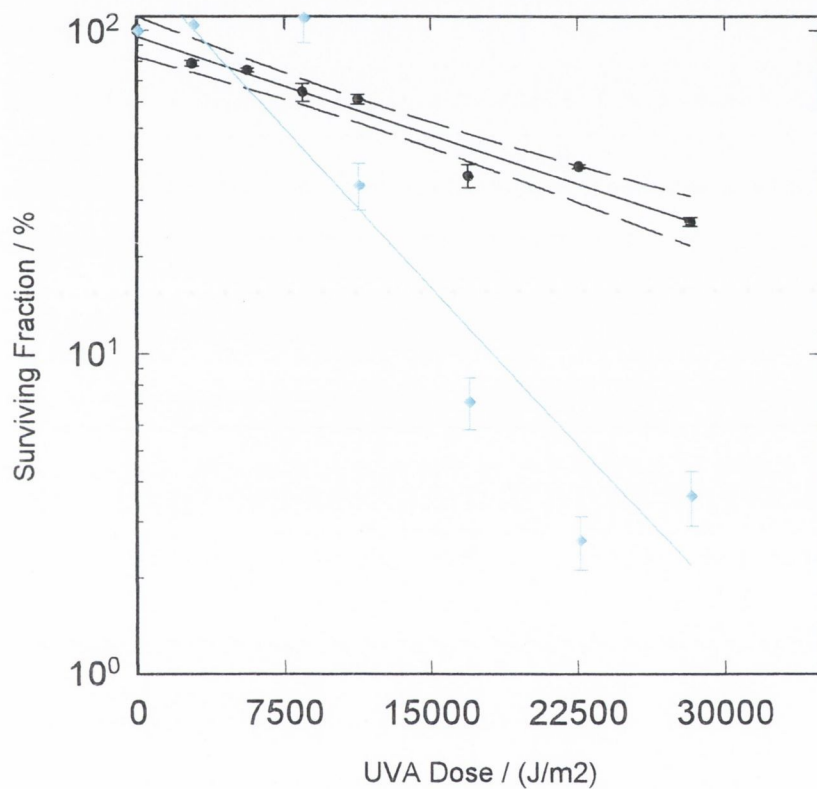


Figure 5.5:- Survival curves for the initially irradiated HaCaT cells (●) and the residual survival of the progeny of these cells (◆) after 50 population doublings post exposure to UVA. Also shown are the best fit regression lines with 95% confidence limits for the initial survival regression line. See Appendix 13 page 265 for full data.

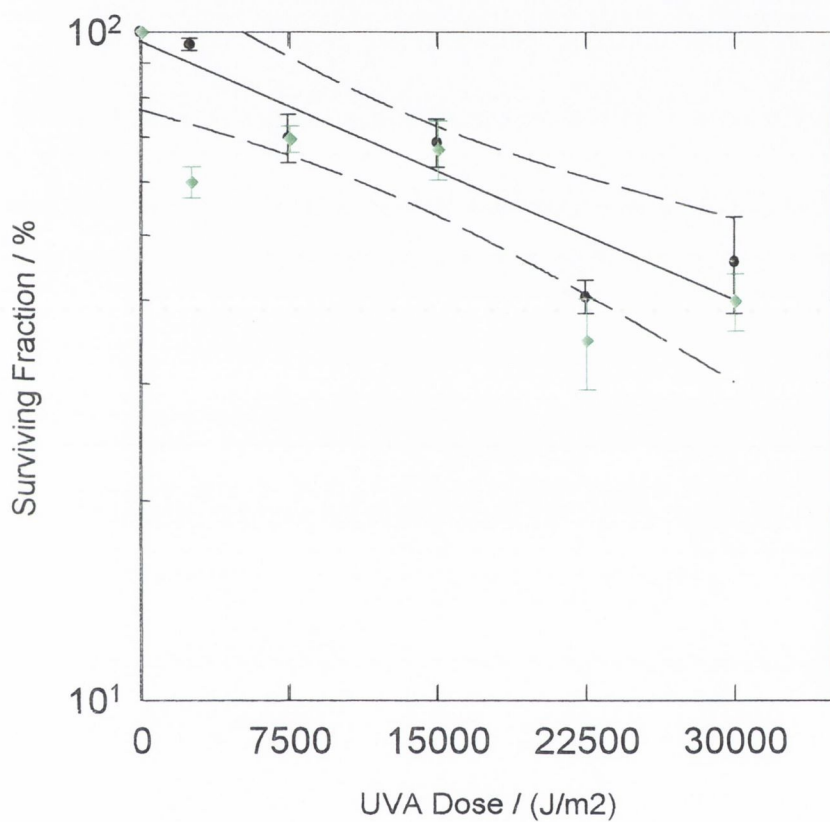


Figure 5.6:- Survival curves for the initially irradiated HaCaT-ras cells (●) and the residual survival of the progeny of these cells (◆) after 10 population doublings post exposure to UVA. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 14 page 266 for full data.

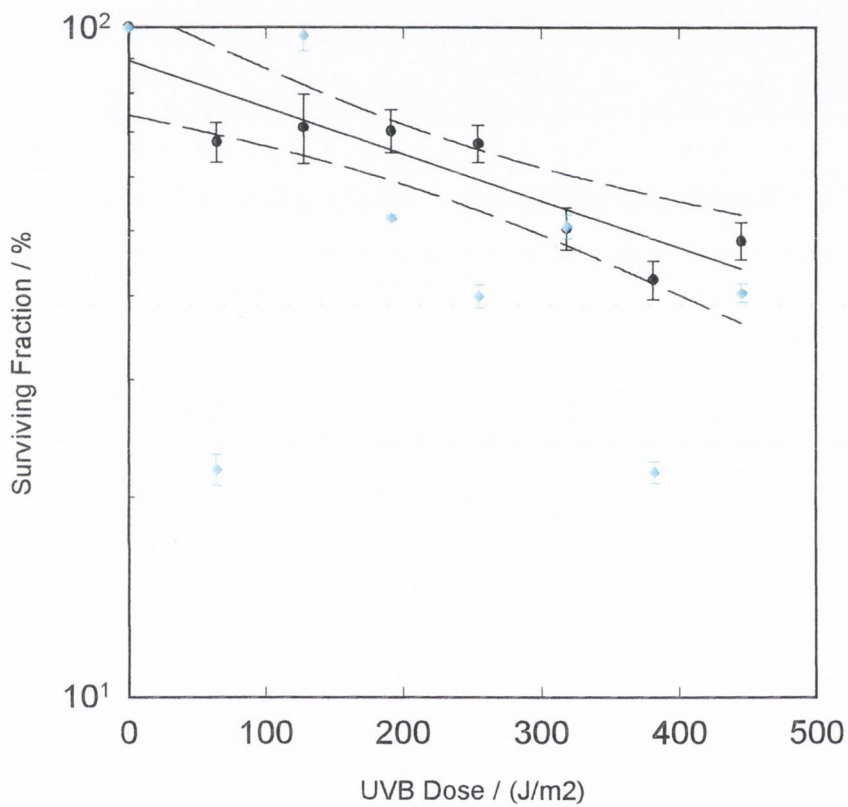


Figure 5.7:-Survival curves for the initially irradiated HaCaT cells (●) and the residual survival of the progeny of these cells (◆) after 10 population doublings post exposure to UVB. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 15 page 272 for full data.

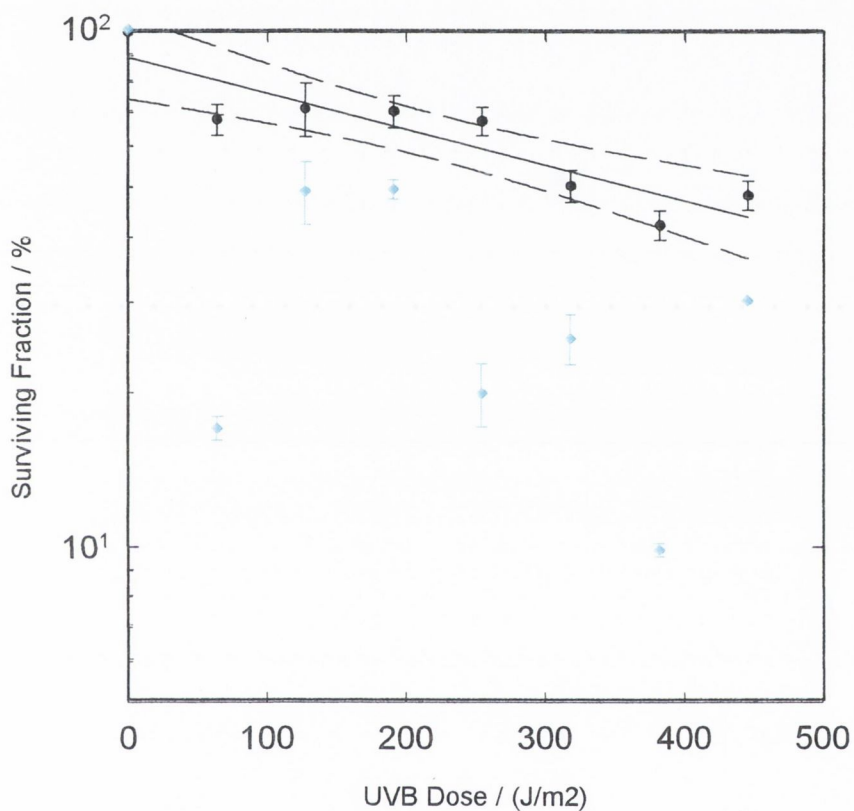


Figure 5.8:-Survival curves for the initially irradiated HaCaT cells (●) and the residual survival of the progeny of these cells (◆) after 20 population doublings post exposure to UVB. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 16 page 273 for full data.

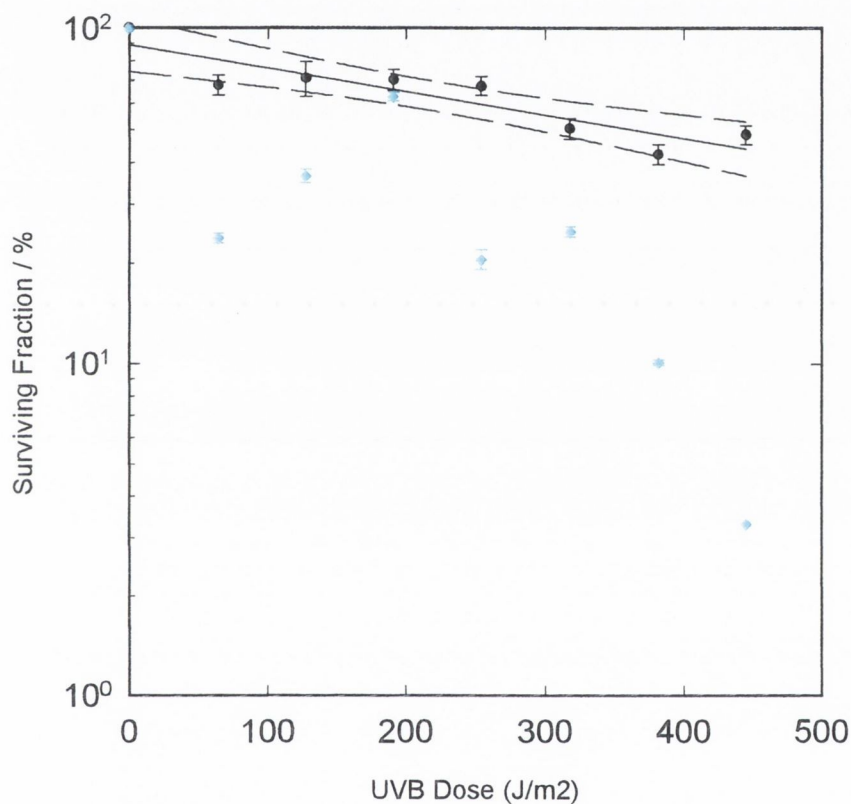


Figure 5.9:- Survival curves for the initially irradiated HaCaT cells (●) and the residual survival of the progeny of these cells (◆) after 30 population doublings post exposure to UVB. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 17 page 274 for full data.

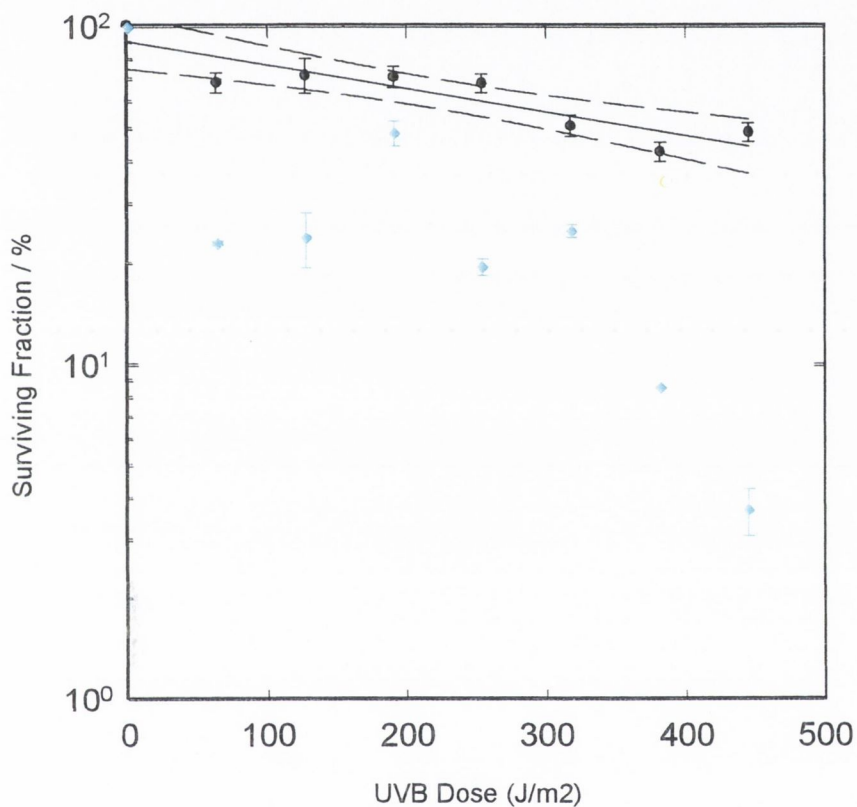


Figure 5.10:- Survival curves for the initially irradiated HaCaT cells (●) and the residual survival of the progeny of these cells (◆) after 40 population doublings post exposure to UVB. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 18 page 275 for full data.

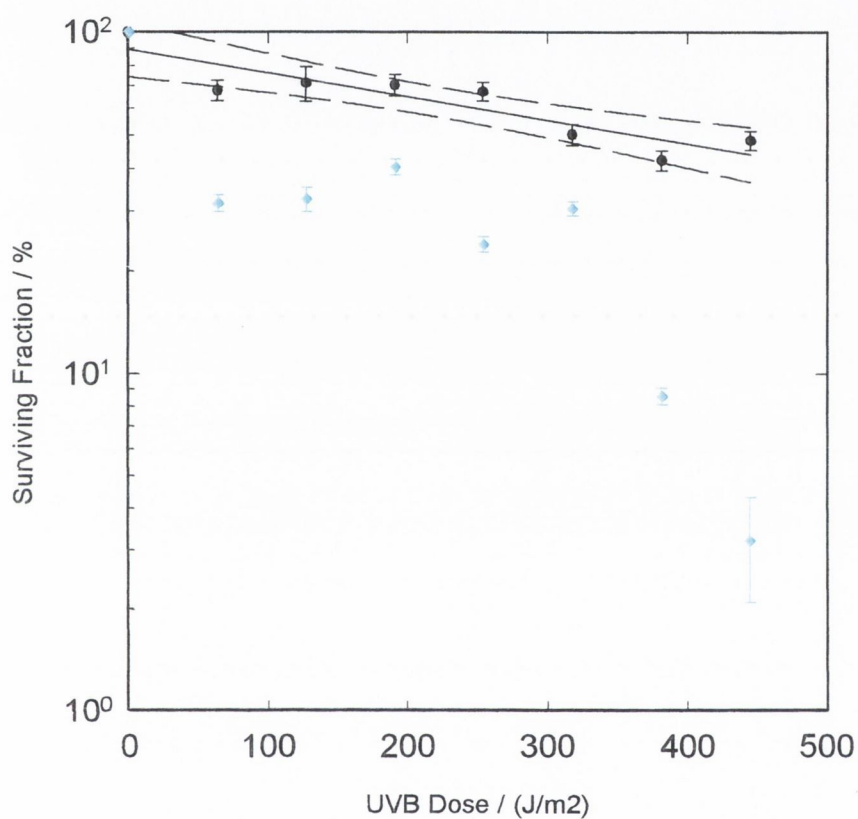


Figure 5.11:- Survival curves for the initially irradiated HaCaT cells (●) and the residual survival of the progeny of these cells (◆) after 50 population doublings post exposure to UVB. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 19 page 277 for full data.

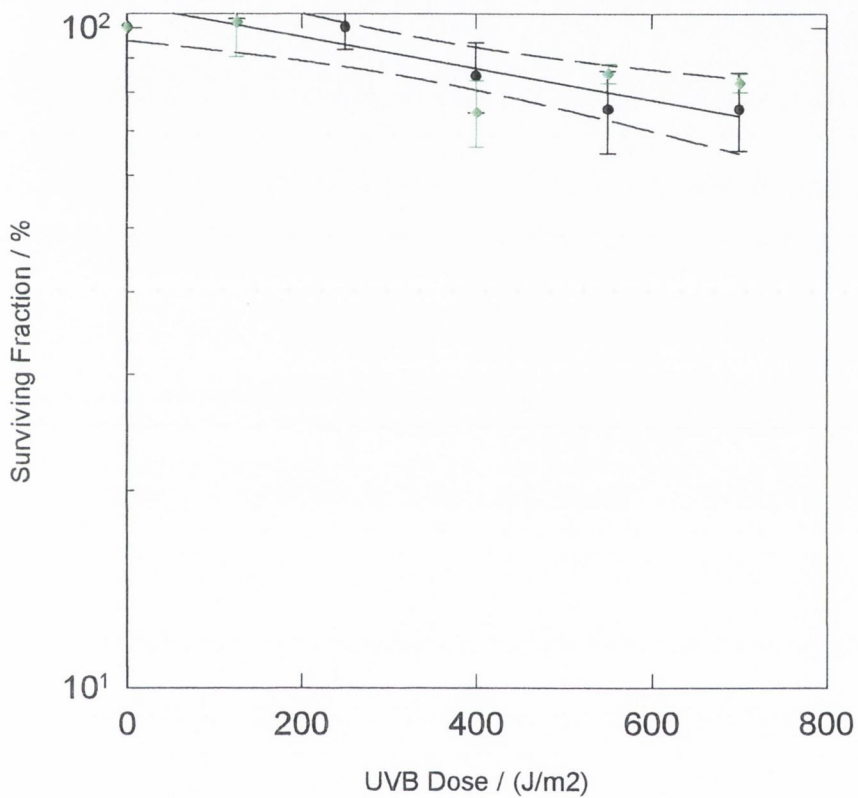


Figure 5.12:- Survival curves for the initially irradiated HaCaT-ras cells (●) and the residual survival of the progeny of these cells (◆) after 10 population doublings post exposure to UVB. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 20 page 277 for full data.

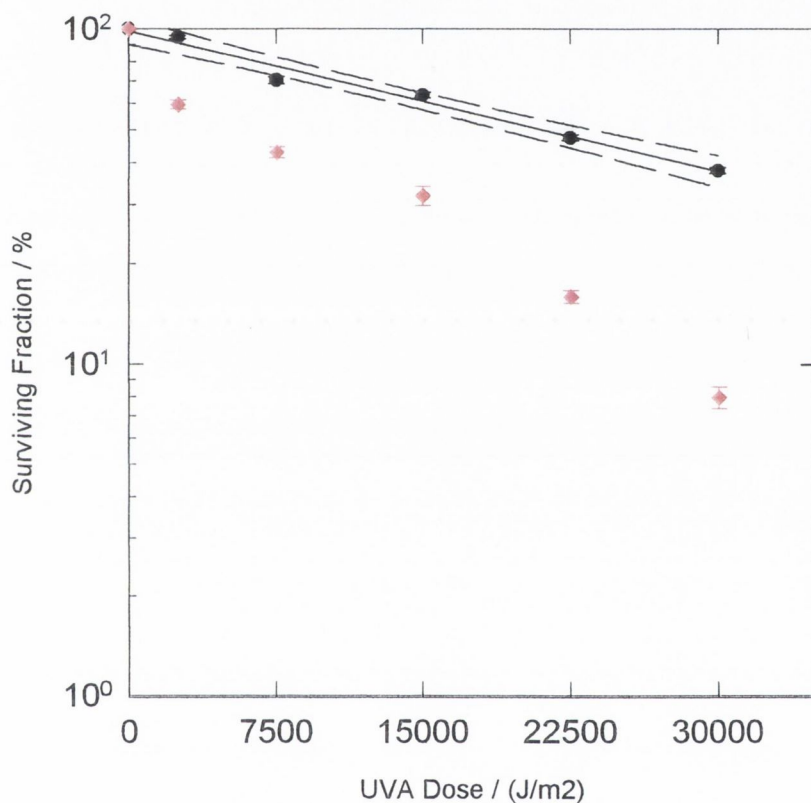


Figure 5.13:- Survival curve for the initially irradiated CHSE cells (●) and the residual survival of the progeny of these cells (◆) after 10 population doublings post exposure to UVA. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 21 page 281 for full data.

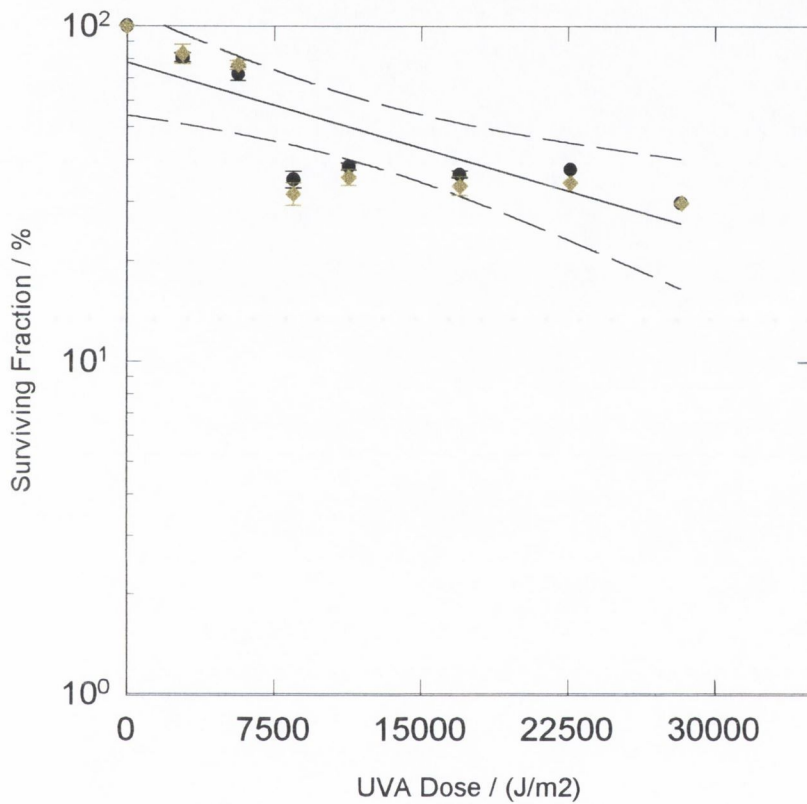


Figure 5.14:- Survival curve for the initially irradiated EPC cells (●) and the residual survival of the progeny of these cells (◆) after 10 population doublings post exposure to UVA. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 22 page 287 for full data.

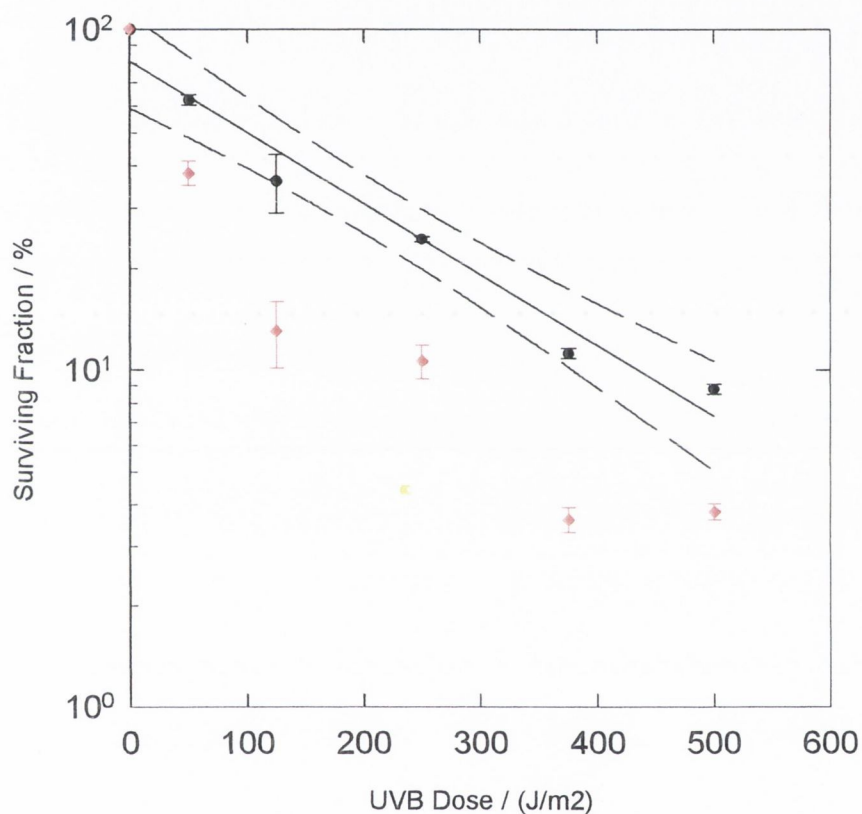


Figure 5.15:- Survival curves for the initially irradiated CHSE cells (●) and the residual survival of the progeny of these cells (◆) after 10 population doublings post exposure to UVB. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 23 page 287 for full data.

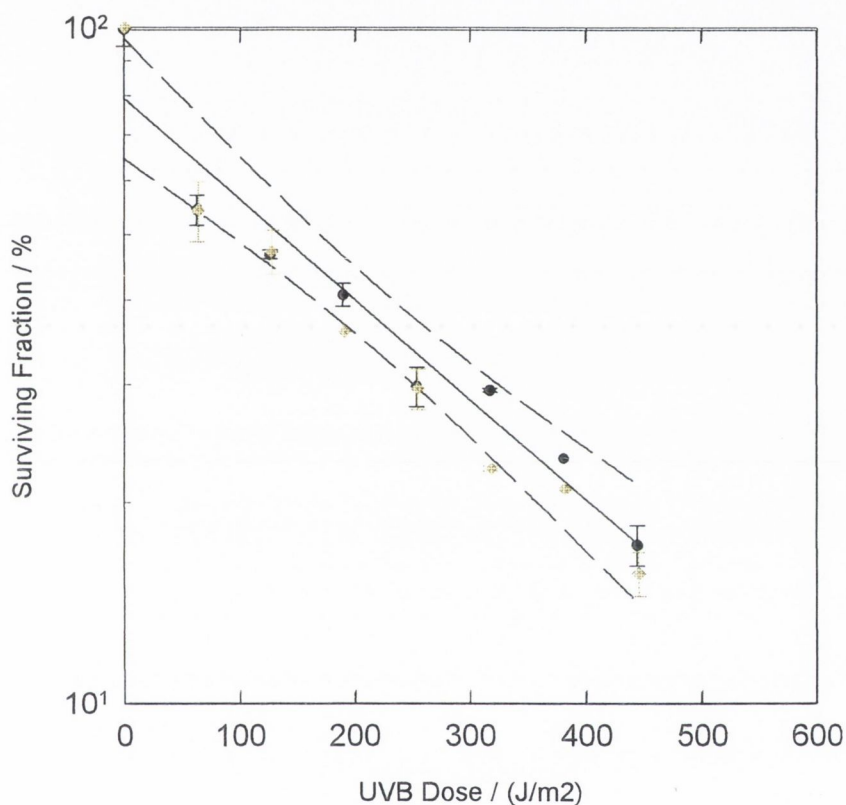


Figure 5.16- Survival of the initially irradiated EPC cells (●) and the residual survival of the progeny of these cells (◆) after 10 population doublings post exposure to UVB. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 24 page 292 for full data.

6. Results on Recovery from Initial and Delayed Cell Death

6.1 Split-dose Effects and Recovery from Initial Cell Death

Tables 6.8- 6.11 show the response of the HaCaT and CHSE cells post exposure to a split dose of UVA and a split dose of UVB delivered with various time intervals between to two irradiations. It is evident from these Tables that the HaCaT cells showed full recovery from sub-lethal damage after a time interval of 1 hour after exposure to UVB, there was no apparent recovery after exposure to UVA.

Because the HaCaT cell line recovers from sub-lethal damage 1 hour after the initial dose of UVB, 1 hour was allowed between irradiations in further experiments using split-doses with the HaCaT cell line. The response of the CHSE cells was different when a split dose was delivered with time intervals of 1, 2, 3, or 4 hours.

The CHSE cells showed full recovery from sub-lethal damage only after a time interval of 2 hours. If a time interval of only 1 hour was allowed between the two irradiations the CHSE cells showed a considerable reduction in survival to 5 percent post exposure to UVA and 2.1 percent post exposure to UVB. This would indicate that the CHSE cells are hypersensitive to split

doses of UVA and UVB if a time interval of only 1 hour is allowed between irradiations. Therefore, 2 hours was allowed between irradiations in further experiments using split-doses of UVA or UVB with the CHSE cell line.

6.2 Split-dose Effects and Recovery from Delayed Cell Death

Having determined the time that was needed to elapse between successive fractions, it was possible to set up split-dose survival curves to determine if delivering a split-dose to the cells could induce repair. The possibility that such a dose of radiation might ablate the delayed cell death present post irradiation could then be examined.

Figures 6.1 and 6.2 show the initial and residual percentage surviving fractions plotted for the HaCaT and CHSE cells after exposure to split doses of UVA. Figures 6.3 and 6.4 show the initial and residual percentage surviving fractions for the HaCaT and CHSE cells after exposure to split doses of UVB. The data were plotted after 10 population doublings. The best fit regression lines are shown for each survival curve. These regression lines are not constrained through the 100% survival point and are merely an indication of the best fit line for that survival curve. Also shown for the initial survival regression line in each case are the 95% confidence limits which indicate whether or not a point outside of these points is significantly different or not to the regression line drawn.

The probability levels when comparing the initial and residual survival curves are shown for these Figures in Tables 6.1-6.4. The trend was for the

clonogenic ability of the surviving progeny of the exposed cells not to be significantly reduced compared with the unirradiated cells suggesting that repair has been induced and that the delayed cell death has been reduced after exposure to a split-dose of either UVA or UVB, as compared to the cells exposed to a single dose of radiation.

A recovery factor is a value indicating the amount of recovery or repair allowed by splitting the dose of radiation. The value was calculated by dividing the split-dose surviving fraction, by the single-dose surviving fraction at a certain dose point. Thus the recovery factor indicates the amount of recovery induced by delivering a split dose of radiation compared with that of a single dose of radiation. If the value is equal to one there is no recovery from the split dose, however if the value is greater than one it is an indication that recovery has been induced by the split dose. The recovery factors are shown for the HaCaT and CHSE cells post exposure to UVA and UVB on Tables 6.12 -6.15.

6.3 Effect of N-acetyl-L-cysteine (NAC) on Initial Cell Death

The reduction of oxidative stress caused by exposing cells to various doses of UVA in the presence of NAC was studied by comparing the effect of a single dose of UVA to a set of flasks and also a similar dose of UVA to a second set of flasks containing a certain concentration of NAC. From this it was possible to determine which concentration of NAC protected the cells from the oxidative stress induced by UVA. Table 6.16 shows the survival data for the HaCaT and CHSE cells in the presence of various concentrations of NAC, and also post exposure to UVA in the presence of NAC at various concentrations. The percentage survival of both cell lines was taken to be 100 percent post exposure to 15000 J/m^2 in order to see the effect the NAC has on the survival of the cells at various concentrations post exposure. It can be seen that at concentrations of NAC from 10^{-3} - 10^{-5} both the HaCaT and CHSE cells showed a reduced survival because at high concentrations this compound proved to be toxic, however at low concentrations (10^{-6} M) it showed no apparent toxicity and significantly increased survival. When the cells were exposed to UVA in the presence of NAC it was only at a concentration of 10^{-6} M that the oxidative stress was reduced in both cell lines and the cells exhibited an increase in survival. Therefore in further experiments involving oxidative stress, 10^{-6} M of NAC was used.

6.4 Effect of NAC on Delayed Cell Death

Having determined the concentration of NAC that was needed to reduce the oxidative stress caused by UVA, it was possible to set up a survival curve to determine if delivering a dose of UVA to the cells in the presence of NAC might inhibit the oxidative stress and ablate the delayed cell death present post irradiation in both the HaCaT and CHSE cell lines. Figures 6.5 and 6.6 show the residual percentage surviving fraction plotted with the initial percentage surviving fraction of the HaCaT and CHSE cells post exposure to UVA. The data are plotted after approximately 10 population doublings for both cell lines. The best fit regression lines are shown for each survival curve. These regression lines are not constrained through the 100% survival point and are merely an indication of the best fit line for that survival curve. Also shown for the initial survival regression line in each case are the 95% confidence limits which indicate whether or not a point outside of these points is significantly different or not to the regression line drawn. The clonogenic ability of the surviving progeny of the exposed HaCaT and CHSE cells was still reduced compared with the unirradiated cells but not significantly. The probability levels when comparing the initial and residual survival curves are shown for Figures 6.5-6.6 in Tables 6.5-6.6. This indicates that NAC does have a

protective effect on the survival of the progeny of the initially irradiated cells that was not seen when the cells were initially irradiated without NAC present. The recovery factors are shown for the HaCaT and CHSE cells post exposure to UVA with and without NAC in Table 6.17 and 6.18.

6.5 Photoreactivation in the HaCaT and CHSE cells.

Tables 6.19 and 6.20 show the survival of the HaCaT and CHSE cells post exposure to UVB/UVA. The damage caused by UVB to the HaCaT cells was not photoreactivated by UVA because there is no difference in the survival of the cells exposed only to UVB and those illuminated with UVA post exposure to UVB. The situation with the CHSE cells was different, when exposed to UVB at a dose of 500 (J/m^2) the cells have a survival of approximately 6 percent, if these cells were then illuminated with UVA there was an increase in survival up to approximately 20 percent indicating that the damage caused by UVB has been photoreactivated. At a dose of 9000 (J/m^2) of UVA the effect is maximal because at higher doses of UVA the survival was reduced to approximately 13 percent.

6.6 The Effect of Photoreactivation on Delayed Cell Death in the CHSE cells.

Having determined the dose of UVA needed to photoreactivate the UVB damage, it was possible to set up a survival curve to determine if delivering a dose of UVA to the cells post exposure to UVB could ablate the delayed cell death present post irradiation in the CHSE cell line. Figure 6.7 shows the residual percentage surviving fraction plotted with the initial percentage surviving fraction of the CHSE cell line post exposure to UVB/UVA. The data are plotted after approximately 10 population doublings. The best fit regression lines are shown for each survival curve. These regression lines are not constrained through the 100% survival point and are merely an indication of the best fit line for that survival curve. Also shown for the initial survival regression line in each case are the 95% confidence limits which indicate whether or not a point outside of these points is significantly different or not to the regression line drawn. The clonogenic ability of the surviving progeny of the exposed CHSE cells was not reduced compared with the unirradiated cells. The probability levels when comparing the initial and residual survival curves are shown for Figure 6.7 in Table 6.7. This indicates that irradiating these cells with UVA post exposure to UVB does have a protective effect on the survival of the progeny of the initially irradiated cells that was not seen when the cells

are irradiated only with UVB. The recovery factors for the CHSE cells post exposure to UVB/UVA are shown in Table 6.21.

UVA Dose / (J/m ²)	Probability level (P)
2500	NS
7500	NS
15000	NS
22500	NS
30000	NS

Table 6.1:- The probability levels given when comparing the initial and residual survival curves in Figure 6.1. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVA Dose / (J/m ²)	Probability level (P)
2500	NS
7500	NS
15000	NS
22500	NS
30000	NS

Table 6.2:- The probability levels given when comparing the initial and residual survival curves in Figure 6.2. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVB Dose / (J/m ²)	Probability level (P)
100	NS
250	NS
400	NS
550	NS
700	NS

Table 6.3:- The probability levels given when comparing the initial and residual survival curves in Figure 6.3. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVB Dose / (J/m ²)	Probability level (P)
50	NS
125	NS
250	NS
375	NS
500	NS

Table 6.4:- The probability levels given when comparing the initial and residual survival curves in Figure 6.4. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVA Dose / (J/m ²)	Probability level (P)
2500	NS
7500	NS
15000	NS
22500	NS
30000	NS

Table 6.5:- The probability levels given when comparing the initial and residual survival curves in Figure 6.5. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVA Dose / (J/m ²)	Probability level (P)
2500	NS
7500	NS
15000	NS
22500	NS
30000	NS

Table 6.6:- The probability levels given when comparing the initial and residual survival curves in Figure 6.6. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVB Dose / (J/m ²)	Probability level (P)
50	NS
125	NS
250	NS
375	NS
500	NS

Table 6.7:- The probability levels given when comparing the initial and residual survival curves in Figure 6.7. Probability levels are shown as being not significant (NS), significant (S) and highly significant (HS).

UVA Dose / (J/m ²)	Time between Irradiations /(Hours)	% S.F $\pm$ S.E
0	0	100
7500	0	50.2 $\pm$ 1.3
3750 + 3750	1	56.8 $\pm$ 1.1
3750 + 3750	2	54.5 $\pm$ 1.5
3750 + 3750	3	55.9 $\pm$ 5.3
3750 + 3750	4	54.7 $\pm$ 2.2

Table 6.8:-Split-dose data for the HaCaT cells irradiated with a split dose of UVA.

UVB Dose / (J/m^2)	Time between Irradiations / (Hours)	% S.F $\pm$ S.E
0	0	100
700	0	38.9 ± 0.6
350 + 350	1	53.0 ± 1.4
350 + 350	2	51.3 ± 0.6
350 + 350	3	56.5 ± 1.1
350 + 350	4	56.9 ± 0.9

Table 6.9:- Survival data for the HaCaT cells irradiated with a split dose of UVB.

UVA Dose / (J/m ²)	Time between Irradiations / (Hours)	% S.F $\pm$ S.E
0	0	100
15000	0	24.1 $\pm$ 0.8
7500 + 7500	1	5.0 $\pm$ 0.9
7500 + 7500	2	29.9 $\pm$ 1.3
7500 + 7500	3	35.2 $\pm$ 1.6
7500 + 7500	4	26.4 $\pm$ 0.7

Table 6.10:- Survival data for the CHSE cells irradiated with a split dose of UVA.

UVB Dose / (J/m^2)	Time between Irradiations / (Hours)	% S.F $\pm$ S.E
0	0	100
700	0	8.6 ± 0.6
350 + 350	1	2.2 ± 0.4
350 + 350	2	7.4 ± 0.5
350 + 350	3	9.5 ± 1.3
350 + 350	4	8.2 ± 0.3

Table 6.11:- Survival data for the CHSE cells irradiated with a split dose of UVB.

UVA Dose (J/m ²)	Initial S.F ± S.E	Residual S.F ± S.E
0	100	100
2500	74.4 ± 5.7	71.8 ± 8.7
7500	67.4 ± 13.1	65.3 ± 12.9
15000	38.1 ± 8.2	15.5 ± 3.3
22500	35.1 ± 2.0	27.5 ± 2.7
30000	27.6 ± 0.2	10.8 ± 0.2
UVA Dose (J/m ²)	Initial Survival Recovery Factor	Residual Survival Recovery Factor
1250 + 1250	0.98 ± 0.1	0.84 ± 0.1
3750 + 3750	0.77 ± 0.1	0.75 ± 0.1
7500 + 7500	1.37 ± 0.3	3.66 ± 0.8
11250 + 11250	1.01 ± 0.0	1.29 ± 0.1
15000 + 15000	1.25 ± 0.2	4.21 ± 0.4

Table 6.12:- Initial and residual surviving fractions obtained when the HaCaT cells were irradiated with a single dose, and also the recovery factors for the HaCaT cells post exposure to a split-dose of UVA. Errors are expressed as the standard error of a ratio.

UVA Dose (J/m ²)	Initial S.F $\pm$ S.E	Residual S.F $\pm$ S.E
0	100	100
2500	92.4 $\pm$ 2.3	63.4 $\pm$ 13.1
7500	64.1 $\pm$ 9.0	54.7 $\pm$ 7.0
15000	36.1 $\pm$ 0.6	24.4 $\pm$ 5.2
22500	31.8 $\pm$ 0.6	6.8 $\pm$ 2.1
30000	40.8 $\pm$ 0.8	6.9 $\pm$ 2.1
UVA Dose (J/m ²)	Initial Survival Recovery Factor	Residual Survival Recovery Factor
1250 + 1250	1.07 $\pm$ 0.0	1.18 $\pm$ 0.2
3750 + 3750	0.83 $\pm$ 0.1	0.97 $\pm$ 0.1
7500 + 7500	1.34 $\pm$ 0.0	1.86 $\pm$ 0.1
11250 + 11250	1.42 $\pm$ 0.1	6.45 $\pm$ 2.2
15000 + 15000	0.71 $\pm$ 0.0	3.30 $\pm$ 1.0

Table 6.13:- Initial and residual surviving fractions obtained when the CHSE cells were irradiated with a single dose, and also the recovery factors for the CHSE cells post exposure to a split-dose of UVA. Errors are expressed as the standard error of a ratio.

UVB Dose (J/m ²)	Initial S.F $\pm$ S.E	Residual S.F $\pm$ S.E
0	100	100
100	72.7 $\pm$ 5.9	37.2 $\pm$ 1.8
250	64.7 $\pm$ 1.4	21.4 $\pm$ 1.2
400	45.7 $\pm$ 1.6	12.9 $\pm$ 2.2
550	38.5 $\pm$ 1.3	5.8 $\pm$ 0.4
700	29.2 $\pm$ 2.8	5.2 $\pm$ 0.9
UVB Dose (J/m ²)	Initial Survival Recovery Factor	Residual Survival Recovery Factor
50 + 50	1.26 $\pm$ 0.1	2.02 $\pm$ 0.1
125 + 125	0.92 $\pm$ 0.0	2.69 $\pm$ 0.1
200 + 200	1.22 $\pm$ 0.0	3.82 $\pm$ 0.6
275 + 275	0.98 $\pm$ 0.0	6.72 $\pm$ 0.5
350 + 350	1.31 $\pm$ 0.1	7.13 $\pm$ 1.2

Table 6.14:- Initial and residual surviving fractions obtained when the HaCaT cells were irradiated with a single dose, and also the recovery factors for the HaCaT cells post exposure to a split-dose of UVB. Errors are expressed as the standard error of a ratio.

UVB Dose (J/m ²)	Initial S.F ± S.E	Residual S.F ± S.E
0	100	100
100	66.6 ± 5.7	45.5 ± 2.8
250	36.4 ± 7.1	17.1 ± 1.4
400	23.7 ± 3.2	13.7 ± 1.3
550	10.9 ± 1.6	4.8 ± 0.3
700	8.6 ± 1.9	1.9 ± 0.2
UVB Dose (J/m ²)	Initial Survival Recovery Factor	Residual Survival Recovery Factor
50 + 50	1.02 ± 0.1	1.27 ± 0.1
125 + 125	1.23 ± 0.2	2.00 ± 0.2
200 + 200	0.85 ± 0.1	1.16 ± 0.1
275 + 275	1.84 ± 0.3	3.22 ± 0.4
350 + 350	0.50 ± 0.1	2.00 ± 0.2

Table 6.15:- Initial and residual surviving fractions obtained when the CHSE cells were irradiated with a single dose, and also the recovery factors for the CHSE cells post exposure to a split-dose of UVB. Errors are expressed as the standard error of a ratio.

N-acetyl-L-cysteine / (M)	HaCaT % S.F $\pm$ S.E	CHSE % S.F $\pm$ S.E
0	100	100
10^{-3}	53.1 ± 1.8	58.6 ± 2.4
10^{-4}	51.7 ± 1.1	78.9 ± 2.5
10^{-5}	62.3 ± 3.1	75.7 ± 3.7
10^{-6}	54.0 ± 3.6	89.5 ± 2.6
UVA Dose / (J/m^2) + N- acetyl-L-cysteine / (M)	HaCaT % S.F $\pm$ S.E	CHSE % S.F $\pm$ S.E
15000	100	100
$15000 + 10^{-3}$	98.5 ± 2.4	67.0 ± 2.0
$15000 + 10^{-4}$	101.4 ± 0.4	101.9 ± 1.0
$15000 + 10^{-5}$	101.8 ± 2.7	94.8 ± 2.5
$15000 + 10^{-6}$	123.5 ± 5.4	125.5 ± 2.4

Table 6.16:- Survival data for the HaCaT and CHSE cells in the presence of various concentrations of N-acetyl-L-cysteine, and also post exposure to UVA in the presence of N-acetyl-L-cysteine at various concentrations. The percentage survival of both cell lines is taken to be 100 percent post exposure to 15000 J/m^2 .

UVA Dose (J/m ²)	Initial S.F ± S.E	Residual S.F ± S.E
0	100	100
2500	85.0 ± 2.4	62.1 ± 10.3
7500	65.3 ± 2.3	46.8 ± 2.9
15000	42.3 ± 1.9	35.9 ± 2.5
22500	30.1 ± 4.2	22.6 ± 1.4
30000	37.0 ± 1.9	13.7 ± 1.1
UVA Dose (J/m ²)	Initial Survival Recovery Factor	Residual Survival Recovery Factor
2500	1.02 ± 0.0	1.30 ± 0.2
7500	1.07 ± 0.0	1.50 ± 0.1
15000	1.09 ± 0.0	1.09 ± 0.0
22500	1.46 ± 0.2	1.78 ± 0.0
30000	1.12 ± 0.1	3.11 ± 0.4

Table 6.17:- The initial and residual surviving fractions obtained when the HaCaT cells were irradiated with UVA, and also the recovery factors for the HaCaT cells post exposure to UVA in the presence of N-acetyl-L-cysteine. Errors are expressed as the standard error of a ratio.

UVA Dose (J/m ²)	Initial S.F $\pm$ S.E	Residual S.F $\pm$ S.E
0	100	100
2500	45.6 $\pm$ 2.3	35.1 $\pm$ 3.3
7500	33.3 $\pm$ 1.1	30.3 $\pm$ 3.2
15000	38.2 $\pm$ 1.9	10.6 $\pm$ 0.9
22500	20.1 $\pm$ 1.2	8.9 $\pm$ 0.6
30000	16.9 $\pm$ 2.1	3.5 $\pm$ 0.2
UVA Dose (J/m ²)	Initial Survival Recovery Factor	Residual Survival Recovery Factor
2500	1.05 $\pm$ 0.0	1.29 $\pm$ 0.2
7500	1.12 $\pm$ 0.5	1.01 $\pm$ 0.1
15000	0.80 $\pm$ 0.0	2.42 $\pm$ 0.2
22500	0.72 $\pm$ 0.0	2.43 $\pm$ 0.3
30000	0.90 $\pm$ 0.1	2.70 $\pm$ 0.1

Table 6.18:- The initial and residual surviving fractions obtained when the CHSE cells were irradiated with UVA, and also the recovery factors for the CHSE cells post exposure to UVA in the presence of N-acetyl-L-cysteine. Errors are expressed as the standard error of a ratio.

UVB Dose / (J/m^2)	UVA Dose / (J/m^2)	% S.F $\pm$ S.E
0	0	100
700	0	39.5 ± 1.6
700	3000	33.9 ± 1.9
700	6000	32.9 ± 1.9
700	9000	35.6 ± 2.2
700	12000	27.3 ± 1.6

Table 6.19:- Survival data for the HaCaT cells irradiated with UVB and then illuminated with UVA to photoreactivate the damage. Three flasks were exposed to a total dose of 700 J/m^2 of UVB and twelve flasks (four groups of three flasks) were exposed to 3000, 6000, 9000 and 12000 J/m^2 of photoreactivating light immediately post exposure to UVB.

UVB Dose / (J/m^2)	UVA Dose / (J/m^2)	% S.F $\pm$ S.E
0	0	100
500	0	5.8 ± 0.7
500	3000	8.9 ± 1.1
500	6000	14.9 ± 2.5
500	9000	20.3 ± 2.9
500	12000	13.2 ± 0.5

Table 6.20:- Survival data for the CHSE cells irradiated with UVB and then illuminated with UVA to photoreactivate the damage. Three flasks were exposed to a total dose of 700 J/m^2 of UVB and twelve flasks (four groups of three flasks) were exposed to 3000, 6000, 9000 and 12000 J/m^2 of photoreactivating light immediately post exposure to UVB.

UVB Dose (J/m ²)	Initial S.F $\pm$ S.E	Residual S.F $\pm$ S.E
0	100	100
50	83.3 $\pm$ 1.9	64.0 $\pm$ 3.1
125	38.0 $\pm$ 3.3	26.5 $\pm$ 1.2
250	38.4 $\pm$ 8.2	24.5 $\pm$ 0.9
375	13.0 $\pm$ 0.2	5.1 $\pm$ 0.2
500	5.8 $\pm$ 0.4	5.3 $\pm$ 0.5
UVB Dose (J/m ²)	Initial Survival Recovery Factor	Residual Survival Recovery Factor
50	1.02 $\pm$ 0.0	0.73 $\pm$ 0.0
125	0.97 $\pm$ 0.0	1.13 $\pm$ 0.1
250	0.76 $\pm$ 0.1	0.97 $\pm$ 0.0
375	1.83 $\pm$ 0.0	4.76 $\pm$ 0.2
500	2.75 $\pm$ 0.1	2.52 $\pm$ 0.2

Table 6.21:- The initial and residual surviving fractions obtained when the CHSE cells were irradiated with UVB, and also the recovery factors for the CHSE cells post exposure to UVB and then illuminated with 9,000 (J/m²) of UVA. Errors are expressed as the standard error of a ratio.

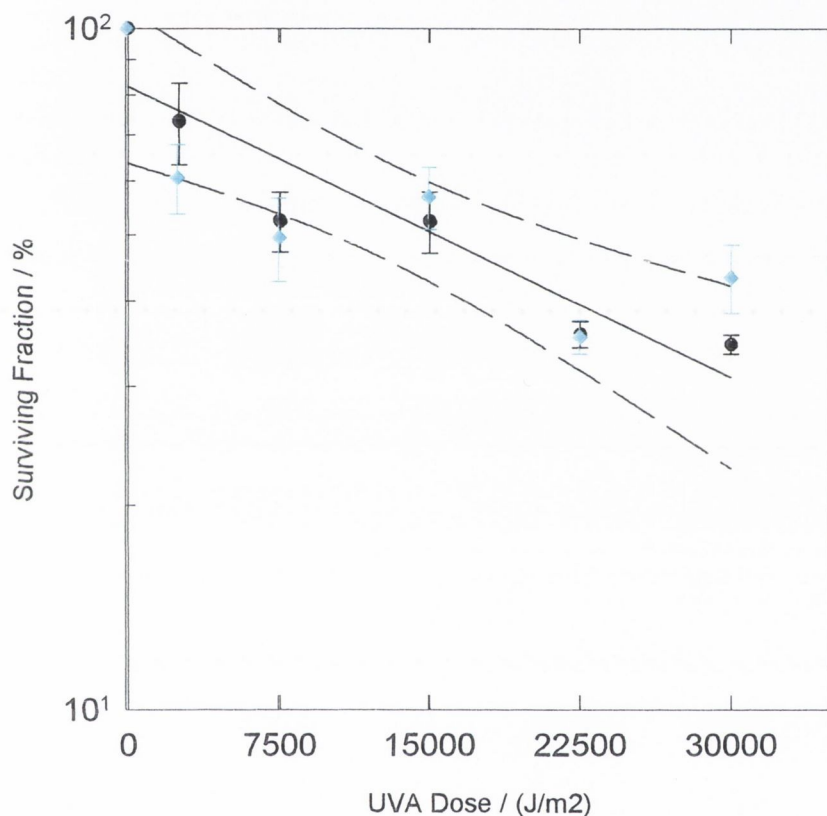


Figure 6.1:- Survival curves for the initially irradiated HaCaT cells (●) and the residual survival of the progeny of these cells (◆) after 10 population doublings post exposure to a split dose of UVA. Also shown is the best fit regression line with 95% confidence limits for the initial regression line. See Appendix 33 page 317 for full data.

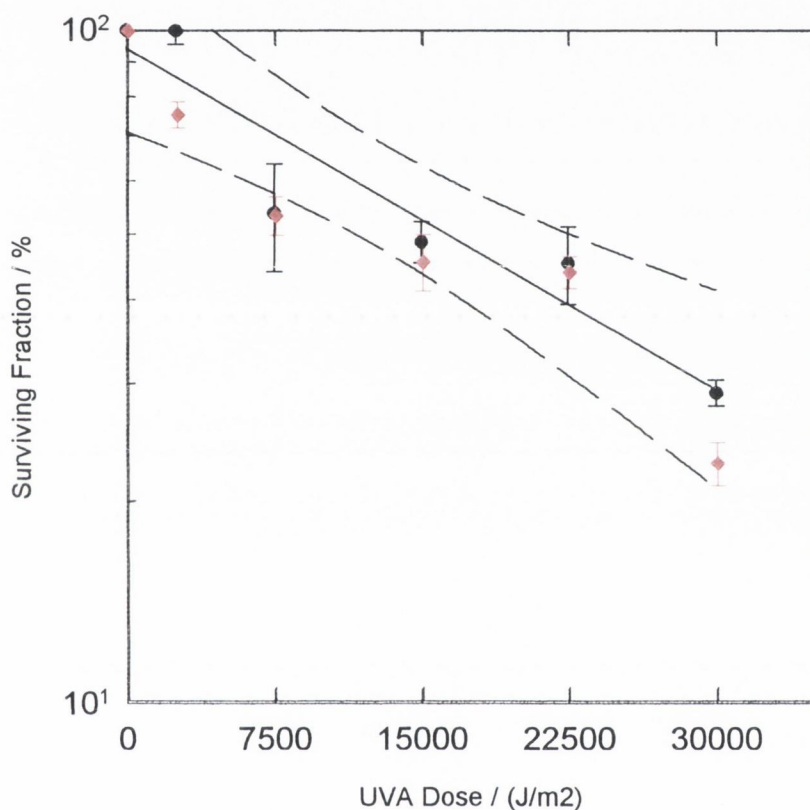


Figure 6.2:- Survival curves for the initially irradiated CHSE cells (●) and the residual survival of the progeny of these cells (◆) after 10 population doublings post exposure to a split dose of UVA. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 34 page 327 for full data.

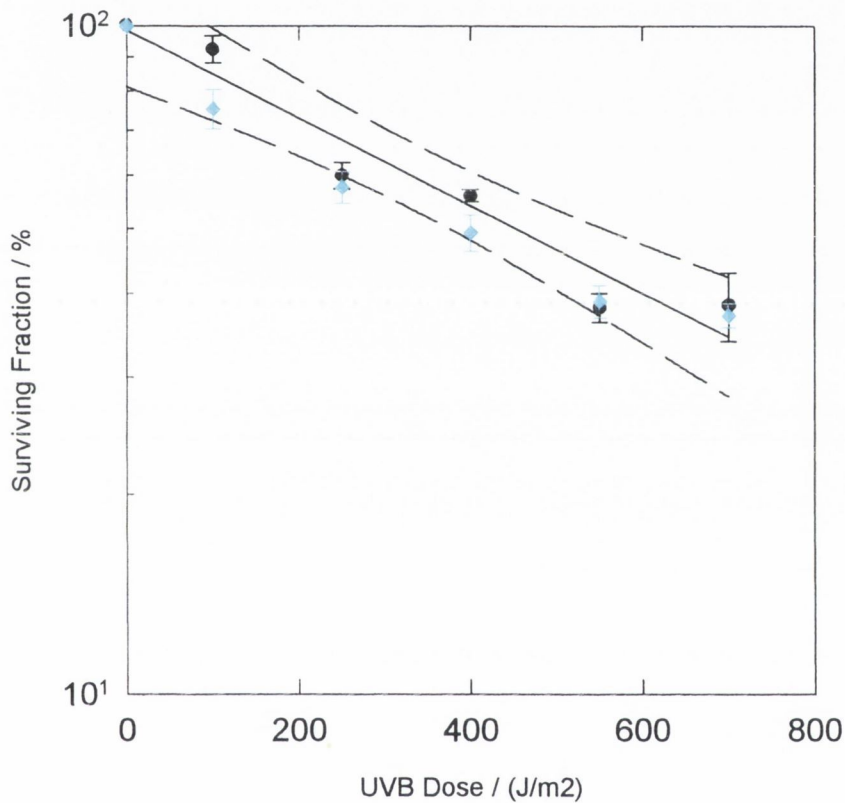


Figure 6.3:- Survival curves for the initially irradiated HaCaT cells (●) and the residual survival of the progeny of these cells (◆) after 10 population doublings post exposure to a split dose of UVB. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 35 page 337 for full data.

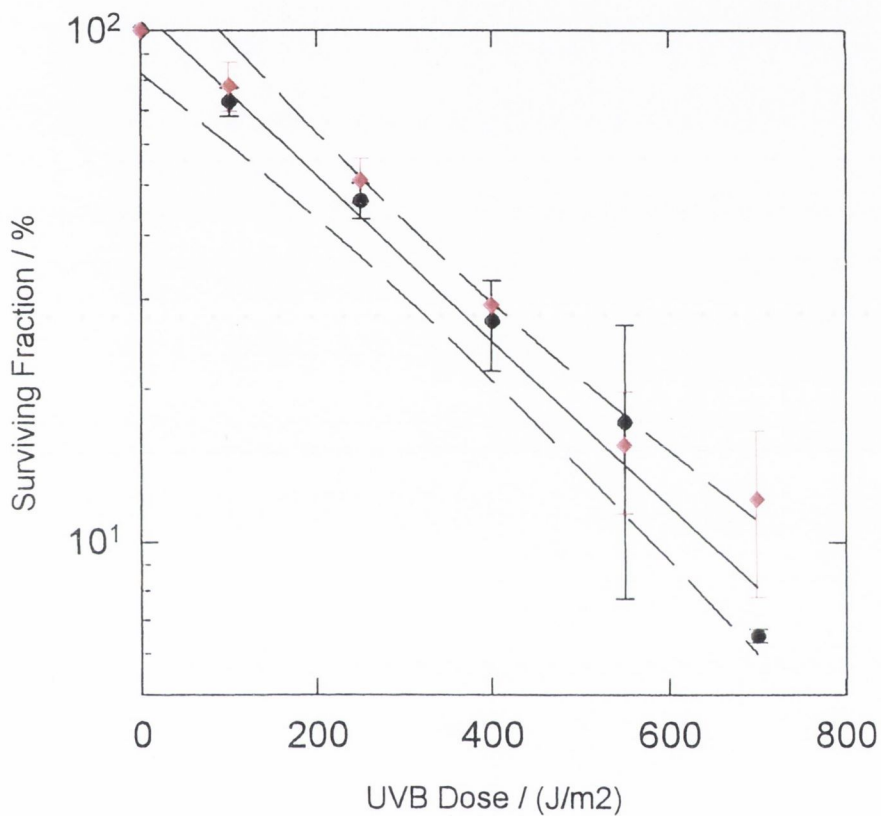


Figure 6.4:-Survival curves for the initially irradiated CHSE cells (●) and the residual survival of the progeny of these cells (♦) after 10 population doublings post exposure to a split dose of UVB. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 36 page 347 for full data.

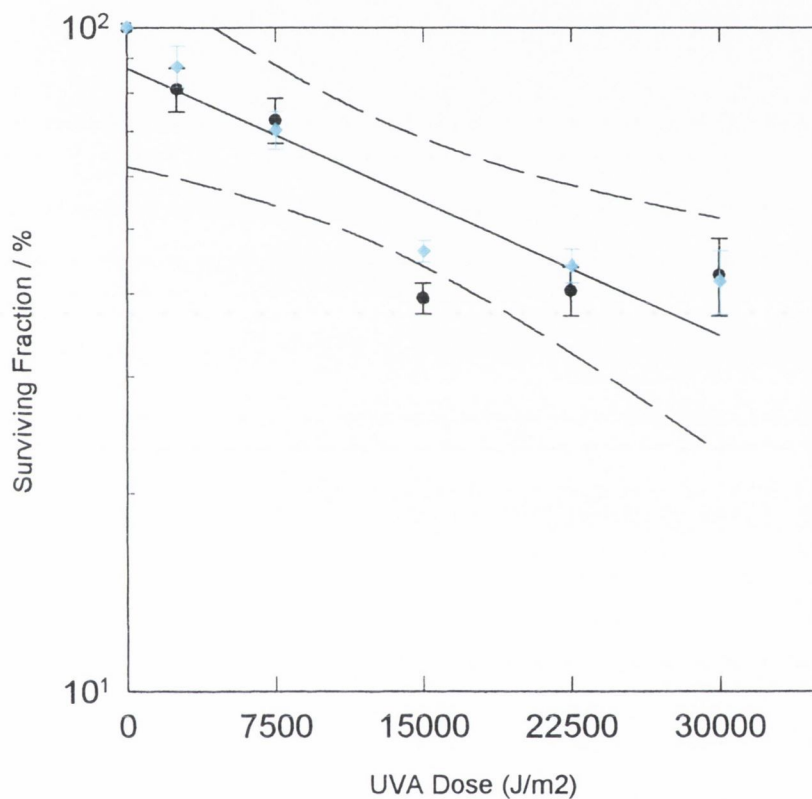


Figure 6.5:- survival curves for the initially irradiated HaCaT cells (●) and the residual survival of the progeny of these cells (◆) after 10 population doublings post exposure to UVA in the presence of (10⁻⁶m) of N-acetyl-L-cysteine. Also shown is the best fit regression line with 95% confidence limits for the initial regression line. See Appendix 37 page 357 for full data.

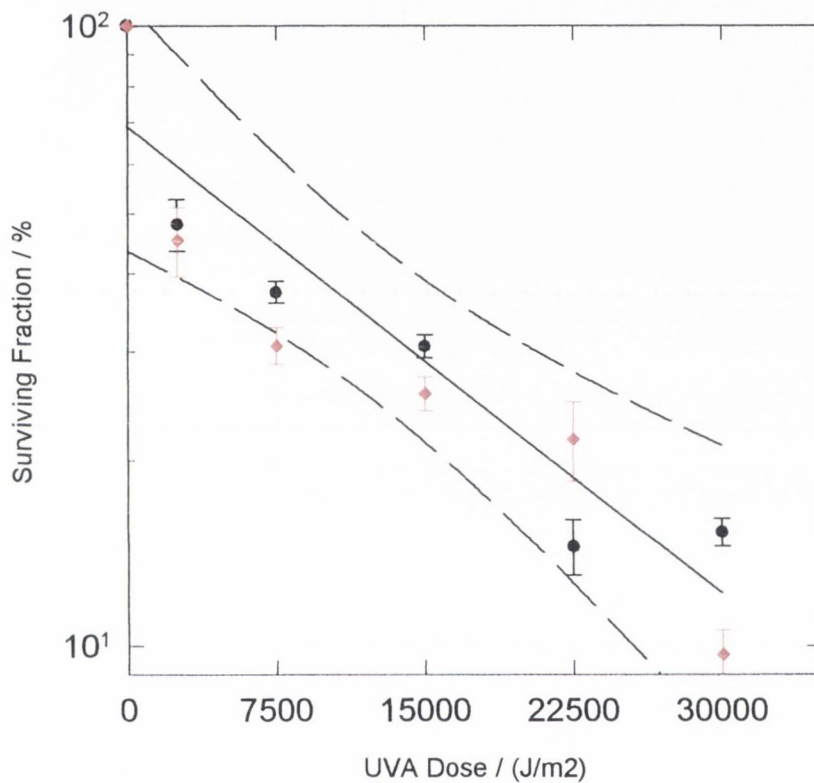


Figure 6.6:- Survival curves for the initially irradiated CHSE cells (●) and the residual survival of the progeny of these cells (◆) after 10 population doublings post exposure to UVA in the presence of (10-6m) of N-acetyl-L-cysteine. Also shown is the best fit regression line with 95% confidence limits for the initial regression line. See Appendix 39 page 367 for full data.

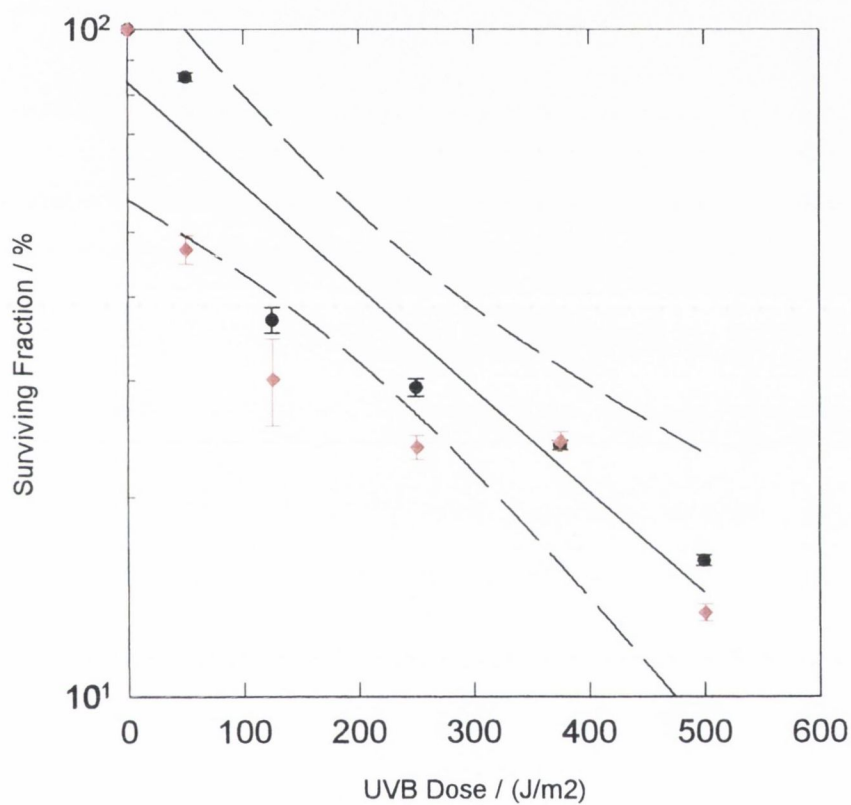


Figure 6.7:-Survival curves for the initially irradiated CHSE cells (●) and the residual survival of the progeny of these cells (◆) after 10 population doublings post exposure to UVB/UVA. Also shown is the best fit regression line with 95% confidence limits for the initial survival regression line. See Appendix 39 page 377 for full data.

7. Discussion

7.1 Initial Survival Curves

The response of the HaCaT cells to UVA and UVB was similar to that of the HaCaT-ras cells. The CHSE and EPC cells also show a similar response to UVA and UVB. When comparing the response of the human cells to that of the fish cells it is evident that the fish cells show an overall more sensitive response to both UVA and UVB.

Work on the response of human cells to ultraviolet radiation has been done using fibroblasts, lymphocytes and keratinocytes (Arlett *et al.* 1992, 1993, de Leeuw *et al.* 1995, Scharffelter *et al.* 1991). Results from the present work agree with the literature data which indicate that there is very little difference in UV sensitivity between keratinocytes, fibroblasts and lymphocytes. Further studies will need to compare all types of cells but these would also need to consider the p53 (Lane, 1992) status of the cells which is important in the repair response to UVR (Kastan *et al.* 1991). DNA damage and photorepair in both fish and human fibroblasts cell lines post exposure to UVR have been investigated by Ahmed *et al.* (1993). They showed that the fish cells have an efficient photoreactivation system at wavelengths of UVB >

304 and this photoreactivation reverses cytotoxicity and dimer formation after exposure to a shorter wavelength. They have also shown that shorter wavelengths of UVB > 304 nm are more effective in photoreversal than longer wavelengths. 50-80 % of dimers produced by wavelengths > 304 nm appear to be photoreactivated as they are being formed. Cultured human fibroblasts did not appear to possess such a repair mechanism (Ahmed *et al.*, 1993). Comparing the results presented here for the human and fish keratinocytes, the human cells appear to possess a better repair mechanism than the fish cells post exposure to either UVA or UVB.

Useful animal models for determining melanoma induction as a function of wavelength/action spectrum are the hybrid offspring from intra- and interspecific crosses between pigmented and nonpigmented fishes of the genus *xiphophorus* (Setlow and Woodhead, 1989). The F1 hybrids show atypical, extended pigmentation, while backcross hybrids, BC₁, between these F1 fish and the nonpigmented parent show appropriate Mendelian segregation of the colour pattern; 50 % have no pigment, 25 % are freckled, and 25 % are heavily pigmented. The last group is susceptible to melanoma induction by a single, relatively small exposure to UV. Classical linkage analysis have suggested that this susceptibility reflects the loss of a tumour suppressor gene and the enhanced expression of a dominant oncogene. A candidate oncogene

was isolated and sequenced (Wittbrodt *et al.*, 1989). Setlow *et al.*, 1989 irradiated fish from these backcrosses with narrow wavelength bands from 302 to 436 nm; the data showed that the wavelengths tested induced melanomas. The absolute sensitivity of the hybrid fish cells to melanoma induction at all wavelengths studied was significantly greater than for erythema induction in fair-skinned fish. For example, the average minimal erythemal doses at 302 and 313 nm for the stationary person are 400 and 8000 J/m² and the values for moving fish are 200 and 1200 J/m² respectively. At 302 and 313 nm, where the major effect in fish seems to arise from the direct absorption of radiation by DNA, these exposures result in only approximately 1.5 cyclobutane dimers per Mb of the DNA of exposed fish whereas at 302 nm a minimal erythemal dose in man corresponds to 20-50 dimers per Mb. Setlow *et al.* (1993) interpreted the high sensitivity as indicating that the tumours arise from inactivation of a suppressor gene in a pigment cell containing a single suppressor gene. This may also be the case with the CHSE and EPC cell lines used in this project. The high sensitivity of the fish cells compared with the human cells used may arise from inactivation of a similar suppressor gene making the cells more sensitive to both UVA and UVB.

7.2 Delayed Cell Death.

7.2.1 Delayed Cell Death in the HaCaT and CHSE cell lines.

The most interesting data to emerge from this study were the demonstration that lethal mutations (delayed cell death) can be induced by UVR in the human HaCaT and fish CHSE cell lines. Previous studies by Chang and Little (1992) showed no expression of delayed death following exposure to CHO-K1 cells by UVC. They suggested this was due to no DSBs being formed. The principal pathway to DNA damage post exposure to UVC is the formation of (6-4) photoproducts and cyclobutane pyrimidine dimers.

Recent evidence (Wojcik *et al.*, 1996) however indicates that DSBs are not the only initiator of genomic instability. The frequency of chromosomal aberrations in the first, second and third mitoses after treatment of one cell mouse embryos with the restriction enzyme AluI indicates that the induction of DSB does not lead to aberration in the third mitosis post treatment.

The results presented here show that UVA which is known to cause DNA damage by the formation of single and double stranded breaks, protein-DNA crosslinks and oxy-radicals does cause expression of delayed cell death at significant levels for approximately 30 population doublings in the HaCaT

cell line and at least 10 population doublings in the CHSE cell line. This supports the work of others that oxy-radical generation leading to oxidative stress induces a delayed response (Clutton *et al.* 1997, Mendonca *et al.* 1993, Mothersill and Seymour, 1997, Limoli *et al.* 1997). This would indicate further that DSBs are not the only initiator of genomic instability. Similarly UVB which principally causes DNA damage by the formation of (6-4) and cyclobutane pyrimidine photoproducts also causes delayed cell death in the HaCaT and CHSE cells.

In Figures 5.1-5.5 the HaCaT cells there appears to be a threshold dose of approximately 7,500 J/m² of UVA below which there is an induction of repair that increases the survival of the progeny of the irradiated cells to a level above that of the control cells. This phenomenon does not occur post exposure to UVB in the HaCaT cell line or the CHSE cell line post exposure to UVA or UVB. It may be possible that cells which have received either sub-lethal or a small amount of damage can repair or recover from the damage post exposure. The progeny of these cells are then selected from a population of cells that have survived an insult, do not carry lethal mutations and prove to have a better cloning efficiency than cells that have not received this insult. Because this only occurs with the human cell line post exposure to UVA it could be postulated that DNA damage in the form of double stranded breaks,

protein-DNA crosslinks and oxy-radicals is needed to create this type of phenotype.

The temporal relationship of the expression of delayed cell death in the HaCaT cell line is interesting. Evidence from the literature suggests that the cytotoxic effects of ionizing radiation include giant cell formation and the induction of a reduced plating efficiency which persists for 12-23 generations after irradiation (Seymour *et al.*, 1986). From the data on the response of the HaCaT cells it can be seen that the delayed cell death effect is maximal after approximately 20 population doublings and passage 3 progeny (about 30 population doublings post exposure) have plating efficiencies which in general approach or even exceed those of the controls. The plating efficiency returns to normal after 50 population doublings. This was similar to the results obtained by Seymour *et al.* (1986) for CHO-K1 cells exposed to ionizing radiation but disagrees with data produced by O' Reilly *et al.* (1994) for HPV-G transfected skin cells where the effect remained stable for at least 70 population doublings. O' Reilly *et al.* (1994) showed this effect persisted for over 400 cell doublings with the HPV-G transfected cell line.

Until now a persistent decrease in colony-forming ability has been observed among the progeny of the irradiated cells as compared with the controls in different species but has never been reported outside the

mammalian species. A persistent reduction in the cloning efficiency of the progeny of cells surviving environmental mutagenic exposure has been demonstrated in yeast treated with ethyl methanesulfonate (EMS) (Klein *et al.*, 1989), sheep cells exposed to γ -rays (Seymour *et al.*, 1986) and CHOK-1 cells exposed to X-rays (Gorgojo and Little, 1989, Chang and Little, 1991). This phenomenon has also been observed in the HeLa hybrid cell line (Mendonca *et al.*, 1993) and the murine CH3 10 T1/2 cell line (Mothersill and Seymour, 1987). Delayed reproductive death has also been shown to be associated with a delayed expression of mutations at the hypoxanthine-guanine phosphoribosyl transferase (*hprt*) locus in the mass population for at least 6-7 population doublings post X-irradiation (Little *et al.*, 1990). Colucci *et al.* (1997) also showed delayed mutations in p53 exons in bladder cultures exposed to γ -rays. A delayed mutational process has also been described for glucose-6-phosphate dehydrogenase (G6PD) mutations induced by EMS: an increased frequency of mutations among progeny of EMS treated cells persisted for at least 10-14 cell generations (Stamato *et al.*, 1987).

7.2.2 Delayed Cell Death in the HaCaT-ras and EPC cell lines.

No expression of lethal mutations was seen in the malignant HaCaT-ras or EPC cell lines. It is not known whether this was due to the fact that these cell lines are transformed. Cell transformation was shown by our group to suppress lethal mutations (Mothersill *et al.* 1996a), with the demonstration that transformed lines of C3H 10T1/2 cells whether spontaneous or radiation-induced have no detectable lethal mutations. They have survival curves with very resistant low dose responses and wide shoulders, and the residual curves showed an increased cloning efficiency in the irradiated survivors as compared to the control line. The use of *ras* transfected cell lines or treatment of cells with nitrosamines also suppressed lethal mutations (Mothersill *et al.*, 1996a). The results from all these experiments suggest that, using several different approaches, it can be shown that agents or situations that are associated with increased malignant potential lead to the induction of fewer post irradiation lethal mutations. Such results may support a conclusion that, in circumstances where carcinogenesis is progressing, the yield of lethal mutations is reduced and this reduction allows the persistence of damage in the genome. This supports an emerging hypothesis that lethal mutations represent some sort of cleansing mechanism which removes damage from the

genome which was not repaired or prevented by early cell cycle arrest responses to radiation.

7.2.3 Delayed Apoptosis.

From Tables 5.9 and 5.10 it is evident that the percentage of *bax* positive cells in the non-transformed HaCaT and CHSE cells rises to 100 percent from the control in the initially irradiated cells, and the progeny of those cells, post exposure to UVA or UVB. This was not the case for the transformed HaCaT-ras cells where the percentage of *bax* positive cells does not rise to 100 percentage in the initially irradiated cells, and the progeny of those cells, post exposure to UVA or UVB. The EPC cells are also tumourigenic cells and because of this should express low levels of *bax*, however, post exposure to UVA or UVB the levels of *bax* in these cells rises to 100 percent both at high and low doses in the initially irradiated and the progeny cells.

Tables 5.11 and 5.12 show that the percentage *bcl-2* positivity in the transformed EPC and HaCaT-ras cells was 100 percent in the control, the initially irradiated cells, and the progeny of those cells, post exposure to UVA or UVB. The non-transformed HaCaT cells express a very low percentage of positive cells in the controls, the initially irradiated cells, and the progeny of

those cells, post exposure to UVA or UVB. The situation with the CHSE cells was different, CHSE cells are non-tumourigenic and because of this might be expected to express low levels of *bcl-2*, however, post exposure to UVA or UVB the levels of *bcl-2* in these cells rises from a low level in the control to 100 percent both at high and low doses in the initially irradiated and the progeny cells.

Bcl-2 and Bax are homologous proteins that have opposing effects on cell life and death, with *bcl-2* serving to prolong cell survival and Bax acting as an accelerator of apoptosis (Reed, 1994). Bax, a heterodimerizing partner of Bcl-2, is a death promoter. Identification of a number of Bcl-2 homologues, some of which bind to Bcl-2, suggests that Bcl-2 functions at least in part, through protein-protein interactions. The first of these homologues, Bax was identified by co-immunoprecipitation with the Bcl-2 protein. Bax is a 21-KD protein that shares homology with Bcl-2 principally clustered in two conserved regions, BH1 and BH2. In addition an exon juncture in BH2 is conserved. Bax heterodimerises with Bcl-2 and homodimerises with itself (Oltavi *et al.* 1993). Site-directed mutagenesis of BH1 and BH2 in Bcl-2 showed that these two domains were important for binding to Bax. When binding was disrupted, Bcl-2's protective function was also eliminated, suggesting that Bcl-2 must bind to Bax to exert its effect. When Bax is

overexpressed in cells, apoptotic death in response to a death signal was accelerated, resulting in its designation as a death promoter. When Bcl-2 is overexpressed, it heterodimerised with Bax and death was repressed (Oltavi *et al.*, 1993). Thus the ratio of Bcl-2 to Bax determines the amount of Bcl-2/Bax heterodimers versus Bax/Bax homodimers and is important in determining susceptibility to apoptosis.

Delayed cell death occurs predominantly, but not exclusively, from apoptosis. All post irradiation survivor colonies contain apoptotic bodies and cells with small pycnotic nuclei but there is also evidence of increased failure to divide and of senescence or terminal differentiation (Lyng *et al.*, 1996). These observations suggested that perhaps there was programmed cell death involved in the mechanism. The conclusion was that active prevention of immediate death mediated by elevation of *bcl-2* occurs. This appears to act as a counter apoptotic factor, but obviously cannot prevent death due to division failure. These observations suggested that perhaps there might be an active or programmed cell death involved in the mechanism of genomic instability. Mothersill *et al.*, 1996a, showed that when transformed C3H 10T1/2 cells were irradiated with cobalt-60, the progeny of these cells did not express lethal mutations or delayed cell death. This observation was explained by the fact that delayed cell death was prevented by *bcl-2* and only occurred as the

level of *bcl-2* decreased within the cell. Thus the conclusion that can be drawn from these findings is that active prevention of immediate cell death mediated by *bcl-2* is occurring. This appears to act as a counter apoptotic survival factor. *Bcl-2* is induced post irradiation in several cell lines (Sentman *et al.*, 1991, Strasser *et al.*, 1991) and in primary normal human epithelial cells (Mothersill *et al.*, 1995, Harney *et al.* 1995 a, 1995 b, Mothersill *et al.*, 1996b). If induced by radiation it persists at a high level for 10-15 population doublings, after which time it falls gradually to approximately 50 % of the immediate post-irradiation levels.

Irradiation with UVB has been shown to induce apoptosis in human skin (Cotton and Spandau, 1997) and also in HaCaT cells (Schwarz *et al.* 1995). From the results presented here it is evident that UVA and UVB also induce delayed apoptosis in HaCaT cells through the elevation of *Bax* or the reduced expression of *bcl-2*. *Bcl-2* was elevated in the HaCaT-ras and EPC cells post exposure to UVA or UVB. This elevated expression continues through to the progeny of the irradiated cells and indicates why the cells are resistant to delayed cell death mediated by apoptosis. The case with the HaCaT cells was different in that these cells express a low level of *bcl-2* in the initially irradiated cells and the progeny of these cells. *Bcl-2* does not have a protective function in these cells and therefore they are prone to delayed cell

death. The expression of *bcl-2* was high in the non-transformed fish cells, this does not fit in with the model for the HaCaT-ras, EPC and HaCaT cells as the CHSE cell should exhibit a low level of *bcl-2*, they do however express high levels of *Bax* indicating that they were prone to apoptosis mediated by *Bax* in the initially irradiated cells and the progeny of these cells.

Delayed cell death occurs in cells independent of p53 status. p53 mutant human keratinocytes or CHO-K1 cells show the effect at a similar level to that found in p53 null HPV transfected cells or normal wild-type expressing epithelial cells (O'Reilly *et al.* 1994, Mothersill *et al.* 1995). A similar p53 independent mechanism has been shown for delayed chromosomal aberrations by the MRC group, (Kadhim *et al.* 1995).

7.3 Recovery from Initial and Delayed Cell Death

7.3.1 Split-dose Effects and Recovery from Initial Cell Death.

The data in Tables 6.8 and 6.9 show the response of the HaCaT cells to a split-dose of UVA or UVB, respectively. It is evident from these Tables that the HaCaT cells showed full recovery from sub-lethal damage after a time interval of 1 hour. The situation with the CHSE cell line was different. The

response of the CHSE cells showed that when a split dose was delivered with time intervals of 1, 2, 3, or 4 hours the CHSE cells showed full recovery from sub-lethal damage only after a time interval of 2 hours this is similar to CHO and other mammalian cell lines (Seymour and Mothersill, 1989). If a time interval of only 1 hour was allowed between the two irradiation's the CHSE cells showed a reduced survival showing that the cells had not recovered from sub-lethal damage at this time.

7.3.2 Split-dose Effects and Recovery from Delayed Cell Death

Figures 6.1 and 6.3 show the residual percentage surviving fraction plotted with the initial percentage surviving fraction for the HaCaT cells post exposure to a split-dose of UVA and UVB respectively. Figures 6.2 and 6.4 show the residual percentage surviving fraction plotted with the initial % surviving fraction for the CHSE cells post exposure to a split-dose of UVA and UVB respectively. The data plotted were after approximately 10 population doublings for both of the cell lines. The clonogenic ability of the surviving progeny of the exposed HaCaT cells was not reduced post exposure to a split-dose of either UVA or UVB. This result would imply that repair has been induced and that the delayed cell death has been ablated post exposure to

a split-dose of either UVA or UVB. A similar result was seen when the CHSE cells are exposed to a split-dose of UVB but not UVA where the delayed cell death was reduced but not fully ablated. Results by Mothersill and Seymour (1989) also showed that fractionating the dose prevented delayed cell death. Furthermore results by Seymour *et al.* (1986), Mothersill and Seymour (1987), and Alper *et al.* (1988) predicted that recovery or repair leading to the production of the survival-curve shoulder may be error prone and lead ultimately to death of some or all of the progeny of the repaired cell. Elkind and Sutton (1959, 1960) have demonstrated a direct relationship between the size of the primary survival-curve shoulder and the extent of split-dose recovery. The correspondence was so good that it is generally accepted that the size of the shoulder on a survival curve generated after a conditioning dose of radiation followed by an adequate radiation-free interval, was exactly the same size as the original shoulder. That is, following recovery the surviving cells respond to a second dose of radiation as though they had not been previously exposed.

Elkind and Sutton (1960) cited this as evidence that cells which had received sub-lethal damage could repair or recover from the damage during the radiation-free interval and that it was the accumulation of sub-lethal damage in the cells which resulted in the production of the shoulder. Other

authors regarded these results as evidence of the induction of a particular type of lesion (Q lesion) repaired by a particular process-Q repair (Sinclair, 1972), discussed by Alper *et al.* 1988.

There was evidence for the existence of inducible repair mechanisms in cells exposed to UVR, oxidative stress or to chemical toxins (Little and Mount 1982). However many radiobiologists do not favour such a concept although Wolff *et al.* (1988) have shown that lymphocytes exposed to low doses or radiation become refractory to high doses as well as to chemical mutagens whose effect was to cause DNA DSBs.

Results presented by Seymour and Mothersill (1989) suggest that (a) some form of repair of radiation damage was inducible in repair-deficient XRS-5 cells and in wild-type CHO-K1 cells following a dose of radiation; (b) the damage induced by this repairable system was different to damage repaired by systems available for repair of single-dose damage and (c) the repair carried out by this induced system was efficient and does not allow transmission of heritable lethal defects to progeny.

7.3.3 Effect of N-acetyl-L-cysteine (NAC) on Initial Cell Death.

The possible reduction of oxidative stress caused by exposing cells to various doses of UVA in the presence of NAC was studied by comparing the effect of a single dose of UVA, with a similar dose of UVA when the cells were in the presence of NAC. The data on Table 6.16 shows the survival data for the HaCaT and CHSE cells in the presence of various concentrations of N-acetyl-L-cysteine, and also post exposure to UVA in the presence of N-acetyl-L-cysteine at various concentrations. When the cells were exposed to UVA in the presence of N-acetyl-L-cysteine it was only at a concentration of 10^{-6} M that the oxidative stress was reduced and cell lines exhibited an increase in survival. Cysteine is known to be toxic at higher concentrations and the results presented in Table 6.16 support this fact since only at low concentrations of cysteine was there any protective effect against oxidative stress.

7.3.4 Effect of N-acetyl-L-cysteine on Delayed Cell Death

Figures 6.5 and 6.6 show the residual percentage surviving fraction plotted with the initial percentage surviving fraction of the HaCaT and CHSE cells post exposure to UVA in the presence of NAC. The data plotted are after

approximately 10 population doublings for both cell lines. In a cellular environment, $^1\text{O}_2$ may be readily converted to O_2^- , which in turn may be converted into highly reactive hydroxyl radicals. There is evidence from the literature that the lethal effects of long wavelengths result from the formation of $^1\text{O}_2$ (Tyrell and Pidoux 1989). By irradiating the cells in the presence of NAC there has been a reduction in the appearance of delayed cell death, indicating that the radiation insult was reduced in the initially irradiated cells and that this was not passed onto the progeny of these initially irradiated cells. This result was interesting in that it showed that oxidative stress may be involved in the induction of delayed cell death post exposure to UVA, this idea has been investigated by Clutton *et al.* (1997) and it was demonstrated that oxidative stress is linked to instability post exposure to α -particles.

7.3.5 Effect of Photoreactivating Light on Initial Cell Death.

The repair induced by illuminating cells with UVA after the cells were exposed to various doses of UVB was studied by comparing the effect of a single dose of UVB with a single dose of UVB followed with a dose of UVA. From this it was possible to determine at which dose of UVA the cells were exhibiting photoreactive repair resulting to a relative increase in survival.

From Table 6.19 it is evident that post exposure to a dose of UVB of 700 (J/m^2) the survival of the HaCaT cells was 39.5 % and when a similar dose is delivered to three other sets of flasks of cells followed by a dose of 3000, 6000 or 9000 (J/m^2) of UVA respectively there was little difference in the survival of the irradiated cells. Two points can be made here, it is possible that the damage caused by UVB can not be photoreactivated by UVA and also that the UVA dose of 9000 (J/m^2) should further reduce the survival to approximately 15-20 %.

Photoreactivation is a DNA repair pathway that requires the presence of a photoreactivating enzyme, DNA photolyase. DNA photolyase recognises and binds specifically to ultraviolet radiation-induced cyclobutane pyrimidine dimers in DNA. Exposure of the photolyase-dimer complex to wavelengths in the range of 300-500nm results, upon absorption of a photon, in the return of the dimerised pyrimidines to their monomeric form. Clear evidence for photoreactivation has been observed in numerous prokaryotes and certain eukaryotes. Evidence for photoreactivation in placental mammals was less convincing (Ly *et al.*, 1993). Sutherland (1974) reported that DNA photolyase was present in human leukocytes. Since that time, photolyase has been reported in human and murine cells in culture. The occurrence of photoreactivation in these studies was dependant upon the medium in which

the cells were grown, the tissue from which the extracts were prepared and the manner in which the photoreactivation treatments were administered-i.e. no photoreactivation was observed following a single exposure to UVR and subsequent visible light, but 40 percent removal of dimers was measured following three cycles of UVR/visible light given at 2.5-hr intervals. Studies by Li *et al.* (1993), indicated that photoreactivation is either missing or of no practical significance in human cells. This observation is of considerable importance as regards the significance, or potential significance, of photoreactivation to human health. The results presented here indicate that photoreactivation was not evident in the human HaCaT skin cell line and agrees with the arguments given above by several authors that photoreactivation may be missing in human skin cells post exposure to a single UVB/UVA exposure.

From Table 6.20 it is evident that post exposure to a dose of UVB of 500 (J/m^2) the survival of the CHSE cells was approximately 5 % and when a similar dose was delivered to three other sets of flasks of cells followed by a dose of 3000, 6000 or 9000 (J/m^2) of UVA respectively there was an increase in the survival of up to approximately 20 % post exposure to 9000 (J/m^2) of UVA. This would indicate that the damage caused by UVB can be photoreactivated by UVA. Ultraviolet radiation-induced DNA damage and its

photorepair in fish skin has been reported by Ahmed *et al.* 1993. Illumination with UVA after exposure to wavelengths >290nm resulted in reduction in dimers. In other experiments UVA resulted in 39 % and 49 % reduction in dimers in the dermal DNA of grown white fish exposed to wavelengths > 302nm. Photorepair in fish has also been reported by Uchida *et al.* (1997), indicating that (6-4) photolyase is present in cultured goldfish cells. These results and the results from other work done in this area (Uchida *et al.* 1995, Yasuhira *et al.* 1992) indicate that photoreactivation is possible in fish and results presented in this thesis are consistent with this observation.

7.3.6 Effect of Photoreactivating Light on Delayed Cell Death.

Figure 6.7 shows the residual percentage surviving fraction plotted with the initial percentage surviving fraction of the CHSE cells post exposure to UVB/UVA. The data were plotted after approximately 10 population doublings. Following irradiation of the cells with UVB and then illuminating them with a dose of 9000 (J/m²) of UVA there was a reduction in the appearance of delayed cell death, indicating that the radiation insult had been reduced in the initially irradiated cells and that this insult was not passed onto the progeny of these initially irradiated cells. This result suggests that

pyrimidine dimers, caused by the exposure to UVB may be involved in the induction of delayed cell death post exposure to UVB.

Final Conclusions

Primarily this project set out to compare the survival of human and fish skin cells in culture. Our group has been involved in a series of studies of delayed cell death post exposure to radiation, therefore a natural progression for this project was to investigate the possibility of delayed death occurring post exposure to UVA or UVB. Previous work in this area showed that UVC induced delayed mutations in CHO cells (Stamato *et al.*, 1995). However Chang and Little (1992a) showed that UVC did not cause delayed cell death in CHO cells. They suggested this was due to no DSBs being formed. No work had been done on delayed cell death post exposure to UVA or UVB.

The most interesting data to emerge from this study was the demonstration that lethal mutations (delayed cell death) can be induced by UVA and UVB in the human HaCaT and fish CHSE cell lines. The results presented here show that UVA which is known to cause DNA damage by the formation of single and double stranded breaks, protein-DNA crosslinks and oxy-radicals does cause expression of delayed cell death in the HaCaT and CHSE cell lines. This would indicate that DSBs are not the only initiator of delayed reproductive death. Similarly UVB which principally causes DNA damage by the formation of (6-4) and cyclobutane pyrimidine photoproducts

also causes delayed cell death in the HaCaT and CHSE-cells. Until now a persistent decrease in colony-forming ability had been observed among the progeny of the irradiated cells as compared with the controls in different species but has never been reported outside the mammalian species.

It has been shown by our group that delayed cell death occurs predominately, but not exclusively, from apoptosis. All post irradiation survivor colonies have been shown to contain apoptotic bodies (Lyng *et al.* 1996). These observations suggested that perhaps there was active or programmed cell death involved in the mechanism. It was not known how delayed cell death occurred in the cell lines studied in this project. To investigate the possibility of delayed apoptosis occurring post exposure to UVA or UVB the expression of two genes that are involved in the promotion and negative regulation of apoptosis was studied.

The percentage of *BAX* positive cells in the non-transformed HaCaT and CHSE cells rises to 100 percent from the control levels of zero in the initially- irradiated cells and in the progeny of those cells, post exposure to UVA or UVB. The percentage of *bcl-2* positive cells in the transformed EPC and HaCaT-ras cells was 100 percent in the control cells, the initially irradiated cells, and the progeny of those cells, post exposure to UVA or UVB. The non-transformed HaCaT cells express a very low percentage of

positive cells in the control, the initially irradiated cells, and the progeny of those cells post exposure to UVA or UVB.

The conclusion was that active prevention of immediate death mediated by elevation of *bcl-2* occurs. From the results presented here it is evident that *bcl-2* was elevated in the HaCaT-ras and EPC cells post exposure to UVA or UVB. This elevated expression continues through to the progeny of the irradiated cells and indicates why the cells are resistant to delayed cell death mediated by apoptosis. The situation with the HaCaT cells was different in that these cells express a low level of *bcl-2* in the initially irradiated cells and the progeny of these cells. *Bcl-2* expression does not appear to have a protective function in these cells which are therefore prone to delayed cell death. The expression of *bcl-2* was high in the CHSE non-transformed cells. This does not appear to be consistent with the model for the HaCaT-ras, EPC and HaCaT cells as the CHSE cell should exhibit a low level of *bcl-2*, they do however express high levels of *BAX* indicating that they were prone to apoptosis mediated by *BAX* in the initially irradiated cells and the progeny of these cells.

Up to this point in the project the results had established that delayed cell death could occur post exposure to UVA and UVB and that the form of death appeared to be delayed apoptosis. Previous studies by our group using γ -

radiation have shown that by delivering a dose in two equal fractions repair could be induced and that delayed death could be ablated. However it was not known whether this was the case with ultraviolet radiation. Experiments were performed to investigate this possibility. The results showed that the clonogenic ability of the surviving progeny of the exposed HaCaT and CHSE cells was not reduced post exposure to a split-dose of either UVA or UVB. This would imply that repair has been induced and that the delayed cell death has been ablated post exposure to a split-dose of either UVA or UVB.

As stated above, UVA is known to cause DNA damage by the formation of, among other things, oxy-radicals and UVB principally causes DNA damage by the formation of (6-4) and cyclobutane pyrimidine photoproducts. What extent oxy-radicals or CPDs are involved in the induction of delayed death remains to be established.

NAC is known to scavenge oxy-radicals and reduce oxidative stress. By irradiating the cells in the presence of NAC there was a reduction in the appearance of delayed cell death, indicating that the radiation insult had been reduced in the initially irradiated cells and that the effect of this insult was not been passed onto the progeny of these initially irradiated cells. These results suggested that oxidative stress may be involved in the induction of delayed cell death post exposure to UVA.

Photoreactivation is known to repair (6-4) photoproducts in cells post exposure to UVB. The results presented here indicate that photoreactivation was possible in the fish CHSE cell line, but, was not detectable in the human HaCaT skin cell line. The latter result agrees with the arguments by several authors that photoreactivation may be missing in human skin cells post exposure to a single UVB/UVA exposure. By irradiating the CHSE cells with UVB and then illuminating them with UVA there was a reduction in the appearance of delayed cell death, indicating that the radiation insult has been reduced in the initially irradiated cells and that this insult has not been passed onto the progeny of these initially irradiated cells. This result suggests that it shows that pyrimidine dimers, caused by the exposure to UVB may be involved in the induction of delayed cell death post exposure to UVB.

Suggestions for Future Work

Delayed expression of cell death is now considered by many scientists to be one manifestation of genomic instability. Division failure in clonal progeny, chromosomal instability, leading to the production of nonclonal chromosomal mutations in clonal progeny, microsatellite instability and gene amplification. These all provide evidence that surviving cells that appear normal and which can divide, have latent damage which can appear at any time in near or distant progeny to produce an abnormal event. While these phenomena are grouped together as genomic instability there is a fundamental difference between the first two and the last two which must be addressed in any future work. Microsatellite instability and gene amplification are possibly radiation induced immediate gene mutations which lead to the production of unstable clones which may have or develop a selective advantage leading to proliferation success or disadvantage leading eventually to extinction. The other two lines of investigation show clear evidence of a very frequent non-clonal events. The frequency of delayed death in distant clonal progeny is too high to have resulted from a mutation or mutator phenotype. The induction of bcl-2 could provide one with a possible mechanism. This analysis raises the interesting and yet unresolved question of whether lethal mutations are

actively dealing with this damage through a programmed cell death pathway or are the lethal end of the instability spectrum. Further studies in this area need to investigate the role of *bcl-2* in more detail.

The CHSE cell line was shown to be hypersensitive to split-doses of UVA and UVB when the time interval is less than 2 hours. It would therefore be interesting to see what the effect would be if the time intervals between irradiation's were further shortened.

The exact percentage of apoptotic cells in the progeny of the irradiated cells should also be investigated and correlated to the expression of the two genes that are involved in apoptosis i.e. *BAX* and *bcl-2*. This should be done with cultures stained with giemsa and scored under a light microscope. This result would test suggestions in this thesis that the transformed cells used are in fact apoptotic resistant and that this is the reason that they are resistant to apoptosis mediated delayed death.

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Appendix I

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Table 1:- Survival data for the HaCaT cells post exposure to UVA.

Table 2:- Survival data for the HaCaT cells post exposure to UVA.

Table 3:- Mean survival data from Figure 4.1 for the HaCaT cells post exposure to UVA.

Appendix 2

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Table 1:- Survival data for the HaCaT-ras cells post exposure to UVA.

Table 2:- Survival data for the HaCaT-ras cells post exposure to UVA.

Table 3:- Mean survival data from Figure 4.2 for the HaCaT-ras cells post exposure to UVA.

Table 1:- Survival data for the HaCaT cells post exposure to UVB.

Table 2:- Survival data for the HaCaT cells post exposure to UVB.

Table 3:- Mean survival data from Figure 4.3 for the HaCaT cells post exposure to UVB.

Table 1:- Survival data for the HaCaT-ras cells post exposure to UVB.

Table 2:- Survival data for the HaCaT-ras cells post exposure to UVB.

Table 3:- Mean survival data from Figure 4.4 for the HaCaT-ras cells post exposure to UVB.

Table 1:- Survival data for the CHSE cells post exposure to UVA.

Table 2:- Survival data for the CHSE cells post exposure to UVA.

Table 3:- Mean survival data from Figure 4.5 for the CHSE cells

post exposure to UVA.

Appendix 6

252

Table 1:- Survival data for the EPC cells post exposure to UVA.

Table 2:- Survival data for the EPC cells post exposure to UVA.

Table 3:- Mean survival data from Figure 4.6 for the EPC cells post exposure to UVA.

Appendix 7

255

Table 1:- Survival data for the CHSE cells post exposure to UVB.

Table 2:- Survival data for the CHSE cells post exposure to UVB.

Table 3:- Mean survival data from Figure 4.7 for the CHSE cells post exposure to UVB.

Table 1:- Survival data for the EPC cells post exposure to UVB.

Table 2:- Survival data for the EPC cells post exposure to UVB.

Table 3:- Mean survival data from Figure 4.8 for the EPC cells post exposure to UVB.

Table 1:- Survival data from Figure 5.1 for the initially irradiated HaCaT cells and the residual survival after 10 population doublings post exposure to UVA.

Table 1:- Survival data from Figure 5.2 for the initially irradiated HaCaT cells and the residual survival after 20 population doublings post exposure to UVA.

Appendix 11

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Table 1:- Survival data from Figure 5.3 for the initially irradiated HaCaT cells and the residual survival after 30 population doublings post exposure to UVA.

Appendix 12

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Table 1:- Survival data from Figure 5.4 for the initially irradiated HaCaT cells and the residual survival after 40 population doublings post exposure to UVA.

Appendix 13

265

Table 13:- Survival data from Figure 5.5 for the initially irradiated HaCaT cells and the residual survival after 50 population doublings post exposure to UVA.

Appendix 14

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Table 1:- Survival data initially irradiated HaCaT-ras cells post exposure to UVA.

Table 2:- Residual survival data for the irradiated HaCaT-Ras

cells in Table 1 after 10 population doublings post exposure to UVA.

Table 3:- Survival data for the initially irradiated HaCaT-Ras cells post exposure to UVA.

Table 4:- Residual survival data for the irradiated HaCaT-Ras cells in Table 3 after 10 population doublings post exposure to UVA.

Table 5:- Mean survival data from Tables 1-4 above and shown in Figure 5.6 for the initially irradiated HaCaT-Ras cells and the residual survival after 10 population doublings post exposure to UVA.

Appendix 15

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Table 1:- Survival data from Figure 5.7 for the initially irradiated HaCaT cells and the residual survival after 10 population doublings post exposure to UVB.

Appendix 16

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Table 1:- Survival data from Figure 5.8 for the initially irradiated HaCaT cells and the residual survival after 20 population

doublings post exposure to UVB.

Appendix 17 274

Table 1:- Survival data from Figure 5.9 for the initially irradiated HaCaT cells and the residual survival after 30 population doublings post exposure to UVB.

Appendix 18 275

Table 1:- Survival data from Figure 5.10 for the initially irradiated HaCaT cells and the residual survival after 40 population doublings post exposure to UVB.

Appendix 19 276

Table 1:- Survival data from Figure 5.11 for the initially irradiated HaCaT cells and the residual survival after 50 population doublings post exposure to UVB.

Table 1:- Survival data initially irradiated HaCaT-ras cells post exposure to UVB.

Table 2:- Residual survival data for the irradiated HaCaT-Ras cells in Table 1 after 10 population doublings post exposure to UVB.

Table 3:- Survival data for the initially irradiated HaCaT-Ras cells post exposure to UVB.

Table 4:- Residual survival data for the irradiated HaCaT-Ras cells in Table 3 after 10 population doublings post exposure to UVB.

Table 5:- Mean survival data from Tables 1-4 above and shown in Figure 5.12 for the initially irradiated HaCaT-Ras cells and the residual survival after 10 population doublings post exposure to UVB.

Table 1:- Survival data initially irradiated CHSE cells post exposure to UVA.

Table 2:- Residual survival data for the irradiated CHSE cells in Table 1 after 10 population doublings post exposure to UVA.

Table 3:- Survival data for the initially irradiated CHSE cells post exposure to UVA.

Table 4:- Residual survival data for the irradiated CHSE cells in Table 3 after 10 population doublings post exposure to UVA.

Table 5:- Mean survival data from Tables 1-4 above and shown in Figure 5.13 for the initially irradiated CHSE cells and the residual survival after 10 population doublings post exposure to UVA.

Appendix 22

286

Table 1:- Survival data from Figure 5.14 for the initially irradiated EPC cells and the residual survival after 10 population doublings post exposure to UVA.

Appendix 23

287

Table 1:- Survival data initially irradiated CHSE cells post exposure to UVB.

Table 2:- Residual survival data for the irradiated CHSE cells in Table 1 after 10 population doublings post exposure to UVB.

Table 3:- Survival data for the initially irradiated CHSE cells post exposure to UVB.

Table 4:- Residual survival data for the irradiated CHSE cells in Table 3 after 10 population doublings post exposure to UVB.

Table 5:- Mean survival data from Tables 1-4 above and shown in Figure 5.15 for the initially irradiated CHSE cells and the residual survival after 10 population doublings post exposure to UVB.

Appendix 24

292

Table 1:- Survival data from Figure 5.16 for the initially irradiated EPC cells and the residual survival after 10 population doublings post exposure to UVA.

Appendix 25

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Table 1:- Split-dose data for the HaCaT cells irradiated with UVA.

Table 2:- Split-dose data for the HaCaT cells irradiated with UVA.

Table 3:- Mean split-dose data for the HaCaT cells irradiated with UVA.

Appendix 26 296

Table 1:- Split-dose data for the CHSE cells irradiated with UVA.

Table 2:- Split-dose data for the CHSE cells irradiated with UVA.

Table 3:- Mean split-dose data for the CHSE cells irradiated with UVA.

Appendix 27 299

Table 1:- Split-dose data for the HaCaT cells irradiated with UVB.

Table 2:- Split-dose data for the HaCaT cells irradiated with UVB.

Table 3:- Mean split-dose data for the HaCaT cells irradiated with UVB.

Appendix 28 302

Table 1:- Split-dose data for the CHSE cells irradiated with UVB.

Table 2:- Split-dose data for the CHSE cells irradiated with UVB.

Table 3:- Mean split-dose data for the CHSE cells irradiated with UVB.

Appendix 29

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Table 1:- Survival data for the HaCaT cells in the presence of varying concentrations of N-acetyl-L-cysteine, and also post exposure to UVA in the presence of N-acetyl-L-cysteine at varying concentrations.

Table 2:- Survival data for the HaCaT cells in the presence of varying concentrations of N-acetyl-L-cysteine, and also post exposure to UVA in the presence of N-acetyl-L-cysteine at varying concentrations.

Table 3:- Mean survival data for the HaCaT cells in the presence of varying concentrations of N-acetyl-L-cysteine, and also post exposure to UVA in the presence of N-acetyl-L-cysteine at varying concentrations.

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Table 1:- Survival data for the CHSE cells in the presence of varying concentrations of N-acetyl-L-cysteine, and also post exposure to UVA in the presence of N-acetyl-L-cysteine at

varying concentrations.

Table 2:- Survival data for the CHSE cells in the presence of varying concentrations of N-acetyl-L-cysteine, and also post exposure to UVA in the presence of N-acetyl-L-cysteine at varying concentrations.

Table 3:- Mean survival data for the CHSE cells in the presence of varying concentrations of N-acetyl-L-cysteine, and also post exposure to UVA in the presence of N-acetyl-L-cysteine at varying concentrations.

Appendix 31 311

Table 1:- Survival data for the HaCaT cells irradiated with UVB and then illuminated with UVA to photoreactivate the damage.

Table 2:- Survival data for the HaCaT cells irradiated with UVB and then illuminated with UVA to photoreactivate the damage.

Table 3:- Mean survival data for the HaCaT cells irradiated with UVB and then illuminated with UVA to photoreactivate the damage.

Appendix 32 314

Table 2:- Survival data for the CHSE cells irradiated with UVB and then illuminated with UVA to photoreactivate the damage.

Table 2:- Survival data for the CHSE cells irradiated with UVB and then illuminated with UVA to photoreactivate the damage.

Table 3:- Mean survival data for the CHSE cells irradiated with UVB and then illuminated with UVA to photoreactivate the damage.

Appendix 33

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Table 1:- Survival data for the irradiated HaCaT cells post exposure to UVA.

Errors are expressed as the standard error on the mean for $n=3$.

Table 2:- Residual survival data for the HaCaT cells in Table 1 after 10 population doublings post exposure to UVA.

Table 3:- Survival data for the HaCaT cells post exposure to UVA.

Table 4:- Residual survival data for the HaCaT cells in Table 3 after 10 population doublings post exposure to UVA.

Table 5:- Mean initial and residual survival data from Tables 1-4 for the HaCaT cells irradiated with UVA.

Table 6:- Split-dose survival data for the irradiated HaCaT cells post exposure to UVA.

Table 7:- Residual split-dose survival data for the HaCaT cells in

Table 6 after 10 population doublings post exposure to UVA. .

Table 8:- Split-dose survival data for the HaCaT cells post exposure to UVA.

Table 9:- Residual split-dose survival data for the HaCaT cells after 10 population doublings post exposure to UVA.

Table 10:- Mean split-dose survival data from Figure 6.1 for the HaCaT cells irradiated with UVA.

Appendix 34327

Table 1:- Survival data for the irradiated CHSE cells post exposure to UVA.

Table 2:- Residual survival data for the CHSE cells in Table 1 after 10 population doublings post exposure to UVA.

Table 3:- Survival data for the CHSE cells post exposure to UVA.

Table 4:- Residual survival data for the CHSE cells in Table 3 after 10 population doublings post exposure to UVA.

Table 5:- Mean initial and residual survival data from Tables 1-4 for the CHSE cells irradiated with UVA.

Table 6:- Split-dose survival data for the irradiated CHSE cells post exposure to UVA.

Table 7:- Residual split-dose survival data for the CHSE cells in Table 6 after 10 population doublings post exposure to UVA.

Table 8:- Split-dose survival data for the CHSE cells post exposure to UVA.

Table 9:- Residual split-dose survival data for the CHSE cells after 10 population doublings post exposure to UVA.

Table 10:- Mean split-dose survival data from Figure 6.2 for the CHSE cells irradiated with UVA.

Appendix 35

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Table 1:- Survival data for the irradiated HaCaT cells post exposure to UVB.

Table 2:- Residual survival data for the HaCaT cells in Table 1 after 10 population doublings post exposure to UVB.

Table 3:- Survival data for the HaCaT cells post exposure to UVB.

Table 4:- Residual survival data for the HaCaT cells in Table 3

after 10 population doublings post exposure to UVB.

Table 5:- Mean initial and residual survival data for the HaCaT cells irradiated with UVB.

Table 6:- Split-dose survival data for the irradiated HaCaT cells post exposure to UVB.

Table 7:- Residual split-dose survival data for the HaCaT cells in Table 6 after 10 population doublings post exposure to UVB.

Table 8:- Split-dose survival data for the HaCaT cells post exposure to UVB.

Table 9:- Residual split-dose survival data for the HaCaT cells in Table 8 after 10 population doublings post exposure to UVB.

Table 10:- Mean split-dose survival data from Figure 6.3 for the HaCaT cells irradiated with UVB.

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Table 1:- Survival data for the irradiated CHSE cells post exposure to UVB.

Table 2:- Residual survival data for the CHSE cells in Table 1 -
after 10 population doublings post exposure to UVB.

Table 3:- Survival data for the CHSE cells post exposure to UVB.

Table 4:- Residual survival data for the CHSE cells after 10
population doublings post exposure to UVB.

Table 5:- Mean initial and residual survival data for the CHSE cells
irradiated with UVB.

Table 6:- Split-dose survival data for the irradiated CHSE cells post
exposure to UVB.

Table 7:- Residual split-dose survival data for the CHSE cells
after 10 population doublings post exposure to UVB. (/) indicates
data not obtained.

Table 8:- Split-dose survival data for the CHSE cells post exposure
to UVB.

Table 9:- Residual split-dose survival data for the CHSE cells
after 10 population doublings post exposure to UVB.

Table 10:- Mean split-dose survival data from Figure 6.4 for the CHSE
cells irradiated with UVB.

Table 1:- Survival data for the HaCaT cells irradiated with UVA.

Table 2:- Residual Survival data for the HaCaT cells in Table 1 after 10 population doublings post exposure to UVA.

Table 3:- Survival data for the HaCaT cells post exposure to UVA.

Table 4:- Residual survival data for the HaCaT cells in Table 3 after 10 population doublings post exposure to UVA.

Table 5:- Mean initial and residual survival data from Tables 1-4 for the HaCaT cells irradiated with UVA.

Table 6:- Survival data for the HaCaT cells irradiated with varying doses of UVA in the presence of 10^{-6} M of N-acetyl-L-cysteine.

Table 7:- Residual survival data for the HaCaT cells irradiated in Table 6 after 10 population doublings post exposure to UVA.

Table 8:- Survival data for the HaCaT cells post exposure to UVA in the presence of 10^{-6} M of N-acetyl-L-cysteine. Errors are expressed as the standard error on the mean for n=3.

Table 9:- Residual survival data for the HaCaT cells irradiated in

Table 8 after 10 population doublings post exposure to UVA. .

Table 10:- Mean survival data from Figure 6.5 for the HaCaT cells irradiated with UVA in the presence of 10^{-6} M of N-acetyl-L-cysteine.

Appendix 38

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Table 1:- Survival data for the CHSE cells irradiated with UVA.

Table 2:- Residual Survival data for the CHSE cells in Table 1 after 10 population doublings post exposure to UVA.

Table 3:- Survival data for the CHSE cells post exposure to UVA.

Table 4:- Residual survival data for the CHSE cells in Table 3 after 10 population doublings post exposure to UVA.

Table 5:- Mean initial and residual survival data from Tables 1-4 for the CHSE cells irradiated with UVA.

Table 6:- Survival data for the CHSE cells irradiated with varying doses of UVA in the presence of 10^{-6} M of N-acetyl-L-cysteine

Table 7:- Residual survival data for the CHSE cells irradiated in Table 6 after 10 population doublings post exposure to UVA.

Table 8:- Survival data for the CHSE cells post exposure to UVA in the presence of 10^{-6} M of N-acetyl-L-cysteine.

Table 9:- Residual survival data for the CHSE cells irradiated in Table 8 after 10 population doublings post exposure to UVA.

Table 10:- Mean survival data from Figure 6.6 for the CHSE cells irradiated with UVA.

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Table 1:- Survival data for the CHSE cells irradiated with UVB.

Table 2:- Residual Survival data for the CHSE cells in Table 1 after 10 population doublings post exposure to UVB.

Table 3:- Survival data for the CHSE cells post exposure to UVB.

Table 4:- Residual survival data for the CHSE cells in Table 3 after 10 population doublings post exposure to UVB.

Table 5:- Mean initial and residual survival data from Tables 1-4 for the CHSE cells irradiated with UVB.

Table 6:- Survival data for the CHSE cells irradiated with UVB and then illuminated with 9,000 (J/m²) UVA to photoreactivate the damage.

Table 7:- Residual survival data for the CHSE cells irradiated in Table 6 after 10 population doublings post exposure to UVB and then illuminated with 9,000 (J/m²) UVA to photoreactivate the damage.

Table 8:- Survival data for the CHSE cells post exposure to UVB and then illuminated with 9,000 (J/m²) UVA to photoreactivate the damage.

Table 9:- Residual survival data for the CHSE cells irradiated in Table 8 after 10 population doublings post exposure to UVB and then illuminated with 9,000 (J/m²) UVA to photoreactivate the damage.

Table 10:- Mean survival data from Figure 6.7 for the CHSE cells irradiated with UVB and then illuminated with 9,000 (J/m²) UVA to photoreactivate the damage.

Appendix 40

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Table 1:- Showing the % positive *bax* cells post exposure to UVA, initially and after 10 population doublings (passage 1).

Table 2:- Showing the % positive *bax* cells post exposure to UVA, initially and after 10 population doublings (passage 1).

Table 3:- Showing the mean % positive *bax* cells from Tables 1 and 2 post exposure to UVA, initially and after 10 population doublings (passage 1). This data is shown in Table 5.9 in the results

section.

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Table 1:- Showing the % positive *bax* cells post exposure to UVB, initially and after 10 population doublings (passage 1).

Table 2:- Showing the % positive *bax* cells post exposure to UVB, initially and after 10 population doublings (passage 1).

Table 3:- Showing the mean % positive *bax* cells from Tables 1 and 2 post exposure to UVB, initially and after 10 population doublings (passage 1). This data is shown in table 5.10 in the results section.

Appendix 42393

Table 1:- Showing the % positive *bcl-2* cells post exposure to UVA, initially and after 10 population doublings (passage 1).

Table 2:- Showing the % positive *bcl-2* cells post exposure to UVA, initially and after 10 population doublings (passage 1).

Table 3:- Showing the mean % positive *bcl-2* cells post exposure to UVA, initially and after 10 population doublings (passage 1). This data is shown in Table 5.11 in the results section.

Table 1:- Showing the % positive *bcl-2* cells post exposure to UVB, initially and after 10 population doublings (passage 1).

Table 2:- Showing the % positive *bcl-2* cells post exposure to UVB, initially and after 10 population doublings (passage 1).

Table 3:- Showing the mean % positive *bcl-2* cells post exposure to UVB, initially and after 10 population doublings (passage 1). This data is presented in Table 5.12 in the results section.

Figure 1:- Spectral output of the UVG-L 58 lamp used to irradiate cells with UVA and photoreactivating light.

Figure 2:- Spectral output of the UVLM-57 lamp used to irradiate cells with UVB.

Figure 3:- Spectral response of the UVX-36 long range radiometer used to measure the irradiance of the UVG-L 58 lamp.

Figure 4:- Spectral response of the UVX-31 mid-range radiometer

used to measure the irradiance of the UVLM-57 lamp.

Appendix I

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	504	156, 153, 146	100
2500	1004	203, 177, 215	65.7 $\pm$ 3.0
7500	1004	187, 192, 175	61.2 $\pm$ 1.3
15000	2007	220, 119	28.1 $\pm$ 6.0
22500	2997	261, 220	26.7 $\pm$ 1.6
30000	2997	196, 205	22.2 $\pm$ 0.3

Table 1:- Survival data for the HaCaT cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	503	164, 136, 147	100
2500	1006	206, 193, 221	69.2 ± 4.4
7500	1006	201, 204, 172	64.5 ± 2.8
15000	2012	/	/
22500	3018	269, 261, 264	29.6 ± 0.2
30000	3018	200, 221	22.9 ± 0.7

Table 2:- Survival data for the HaCaT cells post exposure to UVA.
(/) Indicate data not obtained.

UVA Dose / (J/m ²)	Mean % S.F $\pm$ S.E
0	100
2500	67.5 $\pm$ 2.0
7500	62.9 $\pm$ 1.7
15000	49.6 $\pm$ 16.9
22500	28.4 $\pm$ 0.4
30000	22.6 $\pm$ 0.4

Table 3:- Mean survival data from Figure 4.1 for the HaCaT cells post exposure to UVA.

Appendix 2

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	397	39, 49, 42	100
2500	397	39, 26, 26	70.0 ± 8.2
7500	762	63, 61, 43	66.9 ± 6.2
15000	762	56	67.4
22500	1491	74, 52, 64	38.9 ± 3.2
30000	1491	66, 64, 51	37.0 ± 2.3

Table 1:- Survival data for the HaCaT-ras cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	402	125, 130, 123	100
2500	402	109, 86, 110	80.7 $\pm$ 5.0
7500	747	157, 149, 160	66.4 $\pm$ 1.1
15000	747	108, 115, 134	50.8 $\pm$ 2.7
22500	1524	110, 139, 130	26.4 $\pm$ 1.5
30000	1524	103, 122, 130	24.7 $\pm$ 1.5

Table 2:- Survival data for the HaCaT-ras cells post exposure to UVA.

UVA Dose / (J/m ²)	Mean % S.F $\pm$ S.E
0	100
2500	75.3 $\pm$ 5.4
7500	66.6 $\pm$ 3.2
15000	54.9 $\pm$ 3.4
22500	32.6 $\pm$ 3.1
30000	30.9 $\pm$ 2.9

Table 3:- Mean survival data from Figure 4.2 for the HaCaT-ras cells post exposure to UVA.

Appendix 3

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	506	163, 157, 165	100
125	1012	185, 192, 200	59.5 ± 1.0
250	1012	160, 161, 170	50.6 ± 0.8
400	2024	251, 260, 249	39.1 ± 0.4
550	2024	229, 236, 235	36.0 ± 0.2
700	2024	210, 218, 206	32.7 ± 0.4

Table 1:- Survival data for the HaCaT cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	512	158, 169, 159	100
125	1005	186, 197, 190	60.1 ± 0.8
250	1005	150, 163, 174	51.0 ± 1.7
400	2010	243, 266, 262	40.4 ± 0.9
550	2010	220, 218, 210	33.9 ± 0.4
700	2010	215, 205, 226	33.8 ± 0.7

Table 2:- Survival data for the HaCaT cells post exposure to UVB.

UVB Dose / (J/m ²)	Mean % S.F $\pm$ S.E
0	100
125	59.8 $\pm$ 0.7
250	50.8 $\pm$ 0.9
400	39.7 $\pm$ 0.5
550	35.0 $\pm$ 0.5
700	33.2 $\pm$ 0.5

Table 3:- Mean survival data from Figure 4.3 for the HaCaT cells post exposure to UVB.

Appendix 4

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	407	90, 85, 82	100
125	407	83, 72, 75	89.6 $\pm$ 3.1
250	801	91, 96, 86	47.1 $\pm$ 2.8
400	801	86, 69, 77	45.9 $\pm$ 2.3
550	1607	99, 111, 100	30.5 $\pm$ 0.9
700	1607	104, 92, 109	30.0 $\pm$ 1.2

Table 1:- Survival data for the HaCaT-ras cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	407	112, 110, 106	100
125	407	76, 89, 77	94.8 $\pm$ 4.0
250	801	88, 94, 90	57.5 $\pm$ 0.9
400	801	71, 66, 64	42.5 $\pm$ 1.0
550	1607	106, 110, 100	31.7 $\pm$ 4.7
700	1607	102, 112, 108	34.0 $\pm$ 0.7

Table 2:- Survival data for the HaCaT-ras cells post exposure to UVB.

UVB Dose / (J/m ²)	Mean % S.F $\pm$ S.E
0	100
125	92.2 $\pm$ 2.8
250	52.3 $\pm$ 2.6
400	44.2 $\pm$ 4.8
550	31.1 $\pm$ 0.4
700	32.0 $\pm$ 1.0

Table 3:- Mean survival data from Figure 4.4 for the HaCaT-ras cells post exposure to UVB.

Appendix 5

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	497	107, 141, 130	100
2500	497	59, 59, 58	47.3 ± 0.2
7500	994	93, 93	37.5
15000	994	111, 100	42.6 ± 1.5
22500	1989	110, 106, 100	21.2 ± 0.5
30000	1989	108, 125, 104	22.6 ± 1.0

Table 1:- Survival data for the CHSE cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	497	151, 168, 143	100
2500	497	96, 58, 62	46.8 ± 6.5
7500	994	110, 83, 90	30.7 ± 2.1
15000	994	111, 109, 115	36.3 ± 0.4
22500	1989	108, 100, 98	16.5 ± 0.4
30000	1989	112, 107, 103	17.4 ± 0.3

Table 2:- Survival data for the CHSE cells post exposure to UVA.

UVA Dose / (J/m ²)	Mean % S.F $\pm$ S.E
0	100
2500	47.0 $\pm$ 3.2
7500	32.4 $\pm$ 1.9
15000	38.8 $\pm$ 1.5
22500	18.8 $\pm$ 1.0
30000	20.0 $\pm$ 1.2

Table 3:- Mean survival data from Figure 4.5 for the CHSE cells post exposure to UVA.

Appendix 6

UVA Dose / (J/m ²)	Cells Plated	Average Colonies Counted $\pm$ S.E	% S.F $\pm$ S.E
0	502	102.3 $\pm$ 8.7	100
2826	502	55.6 $\pm$ 1.1	54.1 $\pm$ 0.9
5652	1004	103.3 $\pm$ 5.3	50.2 $\pm$ 2.4
8478	1004	99.5 $\pm$ 4.4	48.7 $\pm$ 1.9
11304	1506	103.6 $\pm$ 4.9	33.4 $\pm$ 1.4
16956	1506	111.0 $\pm$ 0.5	35.9
22608	1506	74.6 $\pm$ 1.1	24.1

Table 1:- Survival data for the EPC cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	500	116, 110, 109	100
2826	500	68, 62, 71	60.0 ± 1.9
5652	1000	102, 98, 86	42.7 ± 1.7
8478	1000	90, 108, 102	44.8 ± 1.9
11304	1500	46, 28, 92	16.5 ± 4.6
16956	1500	79, 47, 70	15.4 ± 0.4
22608	1500	54, 53, 48	15.4 ± 0.4

Table 2:- Survival data for the EPC cells post exposure to UVA.

UVA Dose / (J/m ²)	Mean % S.F $\pm$ S.E
0	100
2826	57.0 $\pm$ 1.4
5652	46.4 $\pm$ 2.0
8478	46.8 $\pm$ 1.9
11304	24.7 $\pm$ 2.9
16956	27.5 $\pm$ 0.2
22608	19.6 $\pm$ 0.2

Table 3:- Mean survival data from Figure 4.6 for the EPC cells post exposure to UVA.

Appendix 7

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	509	119, 131, 135	100
50	509	100, 92, 86	72.2 ± 2.5
125	996	121, 107, 136	48.3 ± 2.7
250	996	76, 82, 65	29.5 ± 1.6
375	2016	88, 61, 57	13.5 ± 1.5
500	2016	76, 60, 43	11.7 ± 1.5

Table 1:- Survival data for the CHSE cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	509	124, 114, 142	100
50	509	90, 72, 65	59.6 ± 4.8
125	996	126, 108, 108	45.9 ± 1.9
250	996	106, 58, 72	31.6 ± 4.7
375	2016	89, 90, 87	17.6 ± 0.1
500	2016	27, 50, 32	7.1 ± 1.1

Table 2:- Survival data for the CHSE cells post exposure to UVB.

UVB Dose / (J/m ²)	Mean % S.F ± S.E
0	100
50	65.9 ± 3.8
125	47.1 ± 1.7
250	30.1 ± 2.5
375	15.5 ± 1.1
500	9.4 ± 1.3

Table 3:- Mean survival data from Figure 4.7 for the CHSE cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	503	135, 134, 149	100
50	705	113, 126, 120	85.9 $\pm$ 2.1
125	1002	141, 130, 133	64.4 $\pm$ 1.2
250	1002	84, 83, 69	37.6 $\pm$ 1.8
375	2001	95, 88, 101	21.8 $\pm$ 0.7
500	2001	40, 45, 43	9.8 $\pm$ 3.7

Table 1:- Survival data for the EPC cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	500	179, 163, 138	100
50	701	128, 125, 145	84.5 ± 2.4
125	1014	146, 136, 128	64.3 ± 11.1
250	1014	86, 80, 101	34.2 ± 2.1
375	2007	118, 123, 125	20.6 ± 0.8
500	2007	44, 30, 42	7.1 ± 0.3

Table 2:- Survival data for the EPC cells post exposure to UVB.

UVB Dose / (J/m ²)	Mean % S.F $\pm$ S.E
0	100
50	85.2 $\pm$ 3.2
125	64.4 $\pm$ 0.9
250	35.9 $\pm$ 1.6
375	21.8 $\pm$ 0.5
500	8.5 $\pm$ 0.5

Table 3:- Mean survival data from Figure 4.8 for the EPC cells post exposure to UVB.

Appendix 9

UVA Dose / (J/m ²)	Exp 1 Initial % S.F $\pm$ S.E	Exp 2 Initial % S.F $\pm$ S.E	Mean Initial % S.F $\pm$ S.E	Exp 1 Residual % S.F $\pm$ S.E	Exp 2 Residual % S.F $\pm$ S.E	Mean Residual % S.F $\pm$ S.E
0	100	100	100	100	100	100
2826	79.6 $\pm$ 1.5	78.2 $\pm$ 1.9	78.9 $\pm$ 1.0	69.5 $\pm$ 0.9	67.9 $\pm$ 0.8	68.7 $\pm$ 0.8
5652	77.3 $\pm$ 1.5	70.1 $\pm$ 0.5	75.2 $\pm$ 4.0	76.1 $\pm$ 0.6	72.5 $\pm$ 0.8	74.3 $\pm$ 0.7
8478	58.9 $\pm$ 5.3	69.9 $\pm$ 2.6	64.4 $\pm$ 4.1	53.7 $\pm$ 0.5	62.9 $\pm$ 1.5	60.6 $\pm$ 0.1
11304	57.2 $\pm$ 2.0	65.0 $\pm$ 2.2	61.1 $\pm$ 2.1	36.2 $\pm$ 3.2	43.4 $\pm$ 3.6	39.8 $\pm$ 3.4
16596	38.7 $\pm$ 3.1	32.3 $\pm$ 2.7	35.5 $\pm$ 2.9	29.8 $\pm$ 2.4	25.6 $\pm$ 3.2	30.7 $\pm$ 2.8
22608	36.7 $\pm$ 2.4	39.3 $\pm$ 0.4	38.0 $\pm$ 0.5	28.1 $\pm$ 2.4	33.5 $\pm$ 3.0	30.8 $\pm$ 2.2
28260	24.0 $\pm$ 0.9	27.2 $\pm$ 2.8	25.6 $\pm$ 0.8	16.4 $\pm$ 2.0	15.4 $\pm$ 1.4	16.9 $\pm$ 1.7

Table1:- Survival data from Figure 5.1 for the initially irradiated HaCaT cells and the residual survival after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Exp 1 Initial % S.F ± S.E	Exp 2 Initial % S.F ± S.E	Mean Initial % S.F ± S.E	Exp 1 Residual % S.F ± S.E	Exp 2 Residual % S.F ± S.E	Mean Residual % S.F ± S.E
0	100	100	100	100	100	100
2826	79.6 ± 1.5	78.2 ± 1.9	78.9 ± 1.0	65.4 ± 2.1	72.0 ± 2.7	68.7 ± 2.4
5652	77.3 ± 1.5	70.1 ± 0.5	75.2 ± 4.0	113.3 ± 1.4	127.3 ± 1.2	119.8 ± 1.8
8478	58.9 ± 5.3	69.9 ± 2.6	64.4 ± 4.1	51.3 ± 0.9	60.1 ± 1.3	55.7 ± 1.1
11304	57.2 ± 2.0	65.0 ± 2.2	61.1 ± 2.1	42.0	28.0	35.0
16596	38.7 ± 3.1	32.3 ± 2.7	35.5 ± 2.9	136.0 ± 0.6	10.4 ± 1.4	12.0 ± 1.0
22608	36.7 ± 2.4	39.3 ± 0.4	38.0 ± 0.5	32.4 ± 1.9	24.2 ± 0.7	28.3 ± 1.3
28260	24.0 ± 0.9	27.2 ± 2.8	25.6 ± 0.8	6.4 ± 0.8	6.0 ± 4.0	6.2 ± 0.9

Table 1:- Survival data from Figure 5.2 for the initially irradiated HaCaT cells and the residual survival after 20 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Exp 1 Initial % S.F $\pm$ S.E	Exp 2 Initial % S.F $\pm$ S.E	Mean Initial % S.F $\pm$ S.E	Exp 1 Residual % S.F $\pm$ S.E	Exp 2 Residual % S.F $\pm$ S.E	Mean Residual % S.F $\pm$ S.E
0	100	100	100	100	100	100
2826	79.6 $\pm$ 1.5	78.2 $\pm$ 1.9	78.9 $\pm$ 1.0	78.5 $\pm$ 5.6	85.3 $\pm$ 6.6	81.9 $\pm$ 6.1
5652	77.3 $\pm$ 1.5	70.1 $\pm$ 0.5	75.2 $\pm$ 4.0	87.3 $\pm$ 0.7	93.3 $\pm$ 0.9	90.3 $\pm$ 0.8
8478	58.9 $\pm$ 5.3	69.9 $\pm$ 2.6	64.4 $\pm$ 4.1	87.0 $\pm$ 0.1	91.0 $\pm$ 0.7	89.0 $\pm$ 0.4
11304	57.2 $\pm$ 2.0	65.0 $\pm$ 2.2	61.1 $\pm$ 2.1	44.6 $\pm$ 3.2	49.0 $\pm$ 4.0	46.8 $\pm$ 3.6
16596	38.7 $\pm$ 3.1	32.3 $\pm$ 2.7	35.5 $\pm$ 2.9	10.0 $\pm$ 1.7	11.8 $\pm$ 0.5	10.9 $\pm$ 1.1
22608	36.7 $\pm$ 2.4	39.3 $\pm$ 0.4	38.0 $\pm$ 0.5	27.9 $\pm$ 2.6	30.3 $\pm$ 1.2	29.1 $\pm$ 1.9
28260	24.0 $\pm$ 0.9	27.2 $\pm$ 2.8	25.6 $\pm$ 0.8	35.1 $\pm$ 4.0	37.1 $\pm$ 2.2	36.1 $\pm$ 3.1

Table 1:- Survival data from Figure 5.3 for the initially irradiated HaCaT cells and the residual survival after 30 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Exp 1 Initial % S.F $\pm$ S.E	Exp 2 Initial % S.F $\pm$ S.E	Mean Initial % S.F $\pm$ S.E	Exp 1 Residual % S.F $\pm$ S.E	Exp 2 Residual % S.F $\pm$ S.E	Mean Residual % S.F $\pm$ S.E
0	100	100	100	100	100	100
2826	79.6 $\pm$ 1.5	78.2 $\pm$ 1.9	78.9 $\pm$ 1.0	81.5 $\pm$ 6.2	75.9 $\pm$ 4.8	78.7 $\pm$ 5.5
5652	77.3 $\pm$ 1.5	70.1 $\pm$ 0.5	75.2 $\pm$ 4.0	187.0 $\pm$ 16.0	205.0 $\pm$ 12.8	196.0 $\pm$ 14.4
8478	58.9 $\pm$ 5.3	69.9 $\pm$ 2.6	64.4 $\pm$ 4.1	89.9 $\pm$ 8.6	84.9 $\pm$ 6.8	87.4 $\pm$ 7.7
11304	57.2 $\pm$ 2.0	65.0 $\pm$ 2.2	61.1 $\pm$ 2.1	27.3 $\pm$ 2.9	23.5 $\pm$ 2.7	25.4 $\pm$ 2.8
16596	38.7 $\pm$ 3.1	32.3 $\pm$ 2.7	35.5 $\pm$ 2.9	7.0 $\pm$ 0.8	4.8 $\pm$ 0.4	5.9 $\pm$ 0.6
22608	36.7 $\pm$ 2.4	39.3 $\pm$ 0.4	38.0 $\pm$ 0.5	19.8 $\pm$ 1.8	16.6 $\pm$ 1.2	18.2 $\pm$ 1.5
28260	24.0 $\pm$ 0.9	27.2 $\pm$ 2.8	25.6 $\pm$ 0.8	23.6 $\pm$ 3.6	21.8 $\pm$ 0.4	22.7 $\pm$ 2.0

Table 1:- Survival data from Figure 5.4 for the initially irradiated HaCaT cells and the residual survival after 40 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Exp 1 Initial % S.F ± S.E	Exp 2 Initial % S.F ± S.E	Mean Initial % S.F ± S.E	Exp 1 Residual % S.F ± S.E	Exp 2 Residual % S.F ± S.E	Mean Residual % S.F ± S.E
0	100	100	100	100	100	100
2826	79.6 ± 1.5	78.2 ± 1.9	78.9 ± 1.0	98.7 ± 3.7	109.7 ± 1.3	104.2 ± 2.5
5652	77.3 ± 1.5	70.1 ± 0.5	75.2 ± 4.0	233.4 ± 34.9	261.4 ± 45.3	247.4 ± 40.1
8478	58.9 ± 5.3	69.9 ± 2.6	64.4 ± 4.1	111.8 ± 20.5	107.2 ± 14.9	109.5 ± 17.7
11304	57.2 ± 2.0	65.0 ± 2.2	61.1 ± 2.1	28.6 ± 4.8	38.2 ± 6.2	33.4 ± 5.5
16596	38.7 ± 3.1	32.3 ± 2.7	35.5 ± 2.9	8.1 ± 1.3	6.1 ± 0.3	7.1 ± 1.3
22608	36.7 ± 2.4	39.3 ± 0.4	38.0 ± 0.5	1.9 ± 0.3	3.3 ± 0.7	2.6 ± 0.5
28260	24.0 ± 0.9	27.2 ± 2.8	25.6 ± 0.8	3.8 ± 1.1	3.0 ± 0.3	3.4 ± 0.7

Table 1:- Survival data from Figure 5.5 for the initially irradiated HaCaT cells and the residual survival after 50 population doublings post exposure to UVA.

Appendix 14

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	Initial % S.F $\pm$ S.E
0	400	40, 35, 34	100
2500	400	35, 37, 29	98.1 $\pm$ 1.9
7500	750	60	88.8
15000	750	64, 53, 42	78.5 $\pm$ 7.6
22500	1500	66, 62, 58	45.8 $\pm$ 1.3
30000	1500	90, 93, 77	64.1 $\pm$ 2.9

Table 1:- Survival data initially irradiated HaCaT-ras cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	Residual % S.F ± S.E
0	509	104, 95, 97	100	100
2500	502	38, 31, 43	38.4 ± 2.9	37.6 ± 2.9
7500	497	99, 88, 81	93.1 ± 4.4	82.6 ± 4.2
15000	499	102, 74, 101	95.8 ± 7.7	75.2 ± 9.4
22500	500	90, 79, 85	87.2 ± 8.6	40.7 ± 4.0
30000	506	60, 93, 94	84.2 ± 9.3	53.9 ± 6.4

Table 2:- Residual survival data for the irradiated HaCaT-Ras cells in Table1 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	402	111, 106, 99	100
2500	402	104, 101, 90	93.4 $\pm$ 3.3
7500	747	117, 134, 120	64.2 $\pm$ 2.1
15000	747	111, 108, 125	58.7 $\pm$ 2.2
22500	1524	140, 139, 140	35.0 $\pm$ 7.8
30000	1524	100, 107, 118	27.2 $\pm$ 1.0

Table 3:- Survival data for the initially irradiated HaCaT-Ras cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	525	114, 106, 120	100	100
2500	499	91, 102, 92	88.5 $\pm$ 2.6	82.8 $\pm$ 3.7
7500	510	104, 89, 100	89.0 $\pm$ 3.3	56.4 $\pm$ 2.0
15000	498	117, 92, 115	100.8 $\pm$ 6.1	59.1 $\pm$ 4.2
22500	499	78, 99, 98	85.4 $\pm$ 5.1	29.8 $\pm$ 6.8
30000	502	114, 93, 103	95.7 $\pm$ 4.6	26.0 $\pm$ 1.5

Table 4:- Residual survival data for the irradiated HaCaT-Ras cells in Table 3 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Initial % S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	100	100
2500	95.8 $\pm$ 2.1	60.0 $\pm$ 3.3
7500	69.7 $\pm$ 5.7	69.5 $\pm$ 3.1
15000	68.6 $\pm$ 5.7	67.1 $\pm$ 6.8
22500	40.4 $\pm$ 2.3	34.8 $\pm$ 5.4
30000	45.6 $\pm$ 7.5	39.9 $\pm$ 3.9

Table 5:- Mean survival data from Tables 1-4 above and shown in Figure 5.6 for the initially irradiated HaCaT-Ras cells and the residual survival after 10 population doublings post exposure to UVA.

Appendix 15

UVB Dose / (J/m ²)	Exp 1 Initial % S.F $\pm$ S.E	Exp 2 Initial % S.F $\pm$ S.E	Mean Initial % S.F $\pm$ S.E	Exp 1 Residual % S.F $\pm$ S.E	Exp 2 Residual % S.F $\pm$ S.E	Mean Residual % S.F $\pm$ S.E
0	100	100	100	100	100	100
63	66.2 $\pm$ 4.1	69.2 $\pm$ 5.6	67.7 $\pm$ 4.6	18.7 $\pm$ 0.8	25.3 $\pm$ 1.6	22.0 $\pm$ 1.2
127	74.2 $\pm$ 9.2	68.0 $\pm$ 7.6	71.1 $\pm$ 8.4	95.3 $\pm$ 4.9	98.9 $\pm$ 5.2	97.4 $\pm$ 5.1
190	68.9 $\pm$ 5.6	71.7 $\pm$ 4.6	70.3 $\pm$ 8.4	48.9 $\pm$ 0.6	55.7 $\pm$ 0.2	52.3 $\pm$ 0.4
254	69.3 $\pm$ 3.8	65.3 $\pm$ 4.8	68.3 $\pm$ 4.3	39.4 $\pm$ 1.5	40.6 $\pm$ 1.7	40.0 $\pm$ 1.6
318	52.2 $\pm$ 3.4	48.6 $\pm$ 3.8	50.4 $\pm$ 3.6	45.0 $\pm$ 3.0	56.6 $\pm$ 1.2	50.8 $\pm$ 2.1
381	40.1 $\pm$ 2.7	44.5 $\pm$ 2.9	42.3 $\pm$ 2.8	22.3 $\pm$ 0.9	21.3 $\pm$ 0.8	21.8 $\pm$ 0.8
445	51.3 $\pm$ 3.4	45.3 $\pm$ 2.8	48.3 $\pm$ 3.1	35.6 $\pm$ 0.9	35.8 $\pm$ 1.7	40.5 $\pm$ 1.3

Table1:- Survival data from Figure 5.7 for the initially irradiated HaCaT cells and the residual survival after 10 population doublings post exposure to UVB.

Appendix 16

UVB Dose / (J/m ²)	Exp 1 Initial % S.F ± S.E	Exp 2 Initial % S.F ± S.E	Mean Initial % S.F ± S.E	Exp 1 Residual % S.F ± S.E	Exp 2 Residual % S.F ± S.E	Mean Residual % S.F ± S.E
0	100	100	100	100	100	100
63	66.2 ± 4.1	69.2 ± 5.6	67.7 ± 4.6	19.6 ± 1.1	14.6 ± 0.7	17.1 ± 0.9
127	74.2 ± 9.2	68.0 ± 7.6	71.1 ± 8.4	52.0 ± 6.4	46.0 ± 7.2	49.0 ± 6.8
190	68.9 ± 5.6	71.7 ± 4.6	70.3 ± 8.4	56.8 ± 2.8	42.0 ± 1.4	49.4 ± 2.1
254	69.3 ± 3.8	65.3 ± 4.8	68.3 ± 4.3	22.0 ± 3.2	18.0 ± 2.4	20.0 ± 2.8
318	52.2 ± 3.4	48.6 ± 3.8	50.4 ± 3.6	27.6 ± 1.9	23.4 ± 3.7	25.5 ± 2.8
381	40.1 ± 2.7	44.5 ± 2.9	42.3 ± 2.8	11.3 ± 0.2	8.8 ± 0.4	9.9 ± 0.3
445	51.3 ± 3.4	45.3 ± 2.8	48.3 ± 3.1	28.1 ± 0.8	32.3	30.2 ± 0.1

Table1:- Survival data from Figure 5.8 for the initially irradiated HaCaT cells and the residual survival after 20 population doublings post exposure to UVB.

Appendix 17

UVB Dose / (J/m ²)	Exp 1 Initial % S.F ± S.E	Exp 2 Initial % S.F ± S.E	Mean Initial % S.F ± S.E	Exp 1 Residual % S.F ± S.E	Exp 2 Residual % S.F ± S.E	Mean Residual % S.F ± S.E
0	100	100	100	100	100	100
63	66.2 ± 4.1	69.2 ± 5.6	67.7 ± 4.6	22.9 ± 0.9	24.7 ± 0.7	23.8 ± 0.8
127	74.2 ± 9.2	68.0 ± 7.6	71.1 ± 8.4	33.5 ± 1.5	39.5 ± 1.9	36.5 ± 1.7
190	68.9 ± 5.6	71.7 ± 4.6	70.3 ± 8.4	66.4 ± 1.6	59.4 ± 2.0	62.9 ± 1.8
254	69.3 ± 3.8	65.3 ± 4.8	68.3 ± 4.3	18.5 ± 1.2	22.7 ± 3.6	20.6 ± 1.4
318	52.2 ± 3.4	48.6 ± 3.8	50.4 ± 3.6	22.1 ± 1.1	27.7 ± 0.7	24.9 ± 0.9
381	40.1 ± 2.7	44.5 ± 2.9	42.3 ± 2.8	9.8 ± 0.3	10.4 ± 0.1	10.1 ± 0.2
445	51.3 ± 3.4	45.3 ± 2.8	48.3 ± 3.1	2.2	4.4 ± 0.2	3.3 ± 0.1

Table1:- Survival data from Figure 5.9 for the initially irradiated HaCaT cells and the residual survival after 30 population doublings post exposure to UVB.

Appendix 18

UVB Dose / (J/m ²)	Exp 1 Initial % S.F $\pm$ S.E	Exp 2 Initial % S.F $\pm$ S.E	Mean Initial % S.F $\pm$ S.E	Exp 1 Residual % S.F $\pm$ S.E	Exp 2 Residual % S.F $\pm$ S.E	Mean Residual % S.F $\pm$ S.E
0	100	100	100	100	100	100
63	66.2 $\pm$ 4.1	69.2 $\pm$ 5.6	67.7 $\pm$ 4.6	25.3 $\pm$ 0.4	20.7 $\pm$ 0.2	23.0 $\pm$ 0.3
127	74.2 $\pm$ 9.2	68.0 $\pm$ 7.6	71.1 $\pm$ 8.4	20.8 $\pm$ 5.3	27.0 $\pm$ 3.5	23.9 $\pm$ 4.4
190	68.9 $\pm$ 5.6	71.7 $\pm$ 4.6	70.3 $\pm$ 8.4	46.2 $\pm$ 3.9	51.4 $\pm$ 4.3	48.8 $\pm$ 4.1
254	69.3 $\pm$ 3.8	65.3 $\pm$ 4.8	68.3 $\pm$ 4.3	14.9 $\pm$ 0.6	24.3 $\pm$ 1.6	19.6 $\pm$ 1.1
318	52.2 $\pm$ 3.4	48.6 $\pm$ 3.8	50.4 $\pm$ 3.6	21.4 $\pm$ 1.7	28.6 $\pm$ 0.5	25.0 $\pm$ 1.1
381	40.1 $\pm$ 2.7	44.5 $\pm$ 2.9	42.3 $\pm$ 2.8	7.8	9.4 $\pm$ 0.1	8.6 $\pm$
445	51.3 $\pm$ 3.4	45.3 $\pm$ 2.8	48.3 $\pm$ 3.1	4.0 $\pm$ 0.7	3.4 $\pm$ 0.5	3.7 $\pm$ 0.6

Table1:- Survival data from Figure 5.10 for the initially irradiated HaCaT cells and the residual survival after 40 population doublings post exposure to UVB.

Appendix 19

UVB Dose / (J/m ²)	Exp 1 Initial % S.F ± S.E	Exp 2 Initial % S.F ± S.E	Mean Initial % S.F ± S.E	Exp 1 Residual % S.F ± S.E	Exp 2 Residual % S.F ± S.E	Mean Residual % S.F ± S.E
0	100	100	100	100	100	100
63	66.2 ± 4.1	69.2 ± 5.6	67.7 ± 4.6	30.6 ± 3.0	32.8 ± 0.6	31.7 ± 1.8
127	74.2 ± 9.2	68.0 ± 7.6	71.1 ± 8.4	33.8 ± 2.2	31.4 ± 3.2	32.6 ± 2.7
190	68.9 ± 5.6	71.7 ± 4.6	70.3 ± 8.4	37.4 ± 2.9	43.6 ± 1.5	40.5 ± 2.2
254	69.3 ± 3.8	65.3 ± 4.8	68.3 ± 4.3	22.6 ± 1.3	25.4 ± 1.1	24.0 ± 1.2
318	52.2 ± 3.4	48.6 ± 3.8	50.4 ± 3.6	28.5 ± 1.7	32.7 ± 1.3	30.6 ± 1.5
381	40.1 ± 2.7	44.5 ± 2.9	42.3 ± 2.8	9.1 ± 0.5	8.1 ± 0.5	8.6 ± 0.5
445	51.3 ± 3.4	45.3 ± 2.8	48.3 ± 3.1	4.7 ± 1.3	1.7 ± 2.9	3.2 ± 2.1

Table1:- Survival data from Figure 5.11 for the initially irradiated HaCaT cells and the residual survival after 50 population doublings post exposure to UVB.

Appendix 20

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	Initial % S.F $\pm$ S.E
0	403	123, 107, 129	100
125	403	131, 103, 120	98.6 $\pm$ 5.5
250	807	203, 200, 193	83.2 $\pm$ 1.0
375	807	174, 156, 181	71.2 $\pm$ 2.5
550	1614	250, 266, 252	53.5 $\pm$ 0.8
700	1614	233, 255, 247	51.3 $\pm$ 1.1

Table 1:- Survival data initially irradiated HaCaT-ras cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	402	97, 94, 108	100	100
125	404	105, 128, 112	100.3 $\pm$ 3.5	98.8 $\pm$ 9.2
250	402	87, 92, 91	115.2 $\pm$ 5.5	95.2 $\pm$ 6.8
375	406	97, 82, 83	90.6 $\pm$ 1.2	64.5 $\pm$ 2.4
550	400	85, 103, 94	91.1 $\pm$ 3.8	48.5 $\pm$ 2.1
700	398	103, 90, 108	102.0 $\pm$ 4.4	52.0 $\pm$ 2.5

Table 2:- Residual survival data for the irradiated HaCaT-Ras cells in Table1 after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	509	120, 115, 125	100
125	1002	210, 206, 223	90.0 $\pm$ 16.9
250	1002	180, 185, 214	85.8 $\pm$ 3.6
375	1002	182, 177, 163	73.5 $\pm$ 1.9
550	1002	118, 112, 124	59.7 $\pm$ 8.1
700	1002	100, 87, 92	39.2 $\pm$ 1.3

Table 3:- Survival data for the initially irradiated HaCaT-Ras cells post exposure to UVB.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	419	105, 112, 103	100	100
125	417	90, 83, 86,	81.4 $\pm$ 1.5	73.2 $\pm$ 13.8
250	408	89, 78, 81	79.7 $\pm$ 2.5	68.3 $\pm$ 3.5
375	405	86, 91, 80	83.2 $\pm$ 2.5	61.1 $\pm$ 2.6
550	399	79, 105, 76	85.4 $\pm$ 7.4	50.9 $\pm$ 8.1
700	403	101, 93, 87	91.4 $\pm$ 3.2	35.8 $\pm$ 1.7

Table 4:- Residual survival data for the irradiated HaCaT-Ras cells in Table 3 after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Initial % S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	100	100
125	108.7 $\pm$ 5.6	86.0 $\pm$ 11.2
250	100.3 $\pm$ 7.6	81.7 $\pm$ 5.1
375	84.7 $\pm$ 10.2	62.8 $\pm$ 2.5
550	79.2 $\pm$ 10.7	49.7 $\pm$ 5.5
700	75.5 $\pm$ 10.1	43.9 $\pm$ 2.5

Table 5:- Mean survival data from Tables 1-4 above and shown in Figure 5.12 for the initially irradiated HaCaT-Ras cells and the residual survival after 10 population doublings post exposure to UVB.

Appendix 21

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	Initial % S.F $\pm$ S.E
0	423	100, 115, 102	100
2500	423	105, 102, 103	97.6 $\pm$ 0.6
7500	805	140, 154, 136	71.1 $\pm$ 2.2
15000	805	135, 123, 134	64.8 $\pm$ 1.5
22500	1625	207, 213, 203	51.0 $\pm$ 0.5
30000	1625	171, 162, 177	41.7 $\pm$ 0.8

Table 1:- Survival data initially irradiated CHSE cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	515	96, 82, 84	100	100
2500	499	59, 56, 50	65.1 $\pm$ 2.5	63.5 $\pm$ 2.4
7500	513	60, 53, 51	63.0 $\pm$ 2.5	44.7 $\pm$ 2.2
15000	500	51, 43, 39	52.4 $\pm$ 3.4	33.9 $\pm$ 2.3
22500	500	29, 33, 30	36.2 $\pm$ 1.1	18.4 $\pm$ 0.5
30000	505	23, 18, 16	22.2 $\pm$ 1.9	9.2 $\pm$ 0.8

Table 2:- Residual survival data for the irradiated CHSE cells in Table1 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	400	103, 108, 101	100
2500	400	97, 93, 96	91.5 ± 1.6
7500	800	141, 148, 146	69.6 ± 1.3
15000	800	132, 128, 131	62.3 ± 1.0
22500	1600	173, 194, 181	43.8 ± 1.3
30000	1600	154, 138, 136	34.2 ± 0.9

Table 3:- Survival data for the initially irradiated CHSE cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	410	103, 100, 106	100	100
2500	400	59, 65, 60	60.9 $\pm$ 1.3	55.7 $\pm$ 1.5
7500	403	58, 60, 64	59.7 $\pm$ 1.3	41.3 $\pm$ 1.1
15000	405	46, 58, 45	48.6 $\pm$ 3.2	30.2 $\pm$ 2.0
22500	405	26, 34, 36	31.4 $\pm$ 2.0	13.7 $\pm$ 0.9
30000	413	18, 24, 21	19.9 $\pm$ 1.2	6.8 $\pm$ 0.4

Table 4:- Residual survival data for the irradiated CHSE cells in Table 3 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Initial % S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	100	100
2500	94.5 $\pm$ 1.1	59.6 $\pm$ 1.9
7500	70.3 $\pm$ 1.7	43.0 $\pm$ 1.6
15000	63.5 $\pm$ 1.2	32.0 $\pm$ 2.1
22500	47.4 $\pm$ 0.9	16.0 $\pm$ 0.7
30000	37.9 $\pm$ 0.8	8.0 $\pm$ 0.6

Table 5:- Mean survival data from Tables 1-4 above and shown in Figure 5.13 for the initially irradiated CHSE cells and the residual survival after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Exp 1 Initial % S.F ± S.E	Exp 2 Initial % S.F ± S.E	Mean Initial % S.F ± S.E	Exp 1 Residual % S.F ± S.E	Exp 2 Residual % S.F ± S.E	Mean Residual % S.F ± S.E
0	100	100	100	100	100	100
2826	78.1 ± 6.4	82.3 ± 12.8	80.6 ± 9.6	96.2 ± 18.8	109.4 ± 25.2	102.8 ± 22.0
5652	79.0 ± 5.6	65.2 ± 21.6	72.1 ± 13.6	100.3 ± 7.6	112.7 ± 11.6	106.5 ± 9.6
8478	38.6 ± 3.2	31.4 ± 2.0	35.0 ± 8.0	88.6 ± 11.2	93.4 ± 8.0	91.0 ± 9.6
11304	38.6 ± 5.2	33.0 ± 4.4	38.2 ± 3.6	87.3 ± 4.8	96.7 ± 7.2	92.0 ± 6.0
16956	30.2 ± 2.8	42.2 ±	36.2 ± 3.6	95.7 ± 9.6	90.1 ± 7.2	92.9 ± 8.4
22608	32.9 ± 2.0	44.1	38.5 ± 1.0	95.0 ± 2.0	88.0 ± 0.4	91.5 ± 1.2
28260	32.2 ± 0.8	27.4	29.8 ± 0.4	105.3	95.5	100.4

Table 1:- Survival data from Figure 5.14 for the initially irradiated EPC cells and the residual survival after 10 population doublings post exposure to UVA.

Appendix 23

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	Initial % S.F $\pm$ S.E
0	406	106, 112, 105	100
50	406	58, 67, 63	58.2 $\pm$ 1.9
125	812	60, 52, 66	38.1 $\pm$ 9.4
250	812	50, 48, 53	23.9 $\pm$ 0.5
375	1500	45, 42, 46	10.5 $\pm$ 0.2
500	1500	37, 44, 40	9.5 $\pm$ 0.3

Table 1:- Survival data initially irradiated CHSE cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	400	93, 96, 98	100	100
50	400	59, 57, 53	60.5 $\pm$ 1.3	35.2 $\pm$ 1.2
125	400	32, 33, 35	33.9 $\pm$ 0.7	12.9 $\pm$ 3.1
250	400	42, 45, 40	44.4 $\pm$ 1.2	10.6 $\pm$ 0.4
375	400	28, 26, 28	28.7 $\pm$ 0.5	3.0 $\pm$ 0.1
500	401	43, 46, 41	45.9 $\pm$ 1.2	4.3 $\pm$ 0.1

Table 2:- Residual survival data for the irradiated CHSE cells in Table1 after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	503	134, 140, 116	100
50	503	89, 90, 80	66.4 $\pm$ 2.9
125	1021	112, 72, 79	33.9 $\pm$ 3.8
250	1021	66, 61, 65	24.2 $\pm$ 0.4
375	2002	68, 67, 52	12.0 $\pm$ 0.8
500	2002	43, 40, 43	8.1 $\pm$ 0.1

Table 3:- Survival data for the initially irradiated CHSE cells post exposure to UVB.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	Residual % S.F ± S.E
0	402	38, 25, 26	100	100
50	400	21, 19, 14	61.4 ± 5.9	40.7 ± 4.0
125	409	14, 14, 7	39.0 ± 6.3	13.2 ± 2.7
250	400	18, 8, 12	43.3 ± 8.1	10.6 ± 1.9
375	406	14, 10, 8	35.9 ± 4.8	4.3 ± 0.6
500	403	14, 14, 9	41.8 ± 4.6	3.3 ± 0.3

Table 4:- Residual survival data for the irradiated CHSE cells in Table 3 after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Initial % S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	100	100
50	62.3 $\pm$ 2.2	37.9 $\pm$ 3.1
125	36.0 $\pm$ 7.0	13.0 $\pm$ 2.9
250	24.3 $\pm$ 0.4	10.6 $\pm$ 1.2
375	11.2 $\pm$ 0.4	3.6 $\pm$ 0.3
500	8.8 $\pm$ 0.3	3.8 $\pm$ 0.2

Table 5:- Mean survival data from Tables 1-4 above and shown in Figure 5.15 for the initially irradiated CHSE cells and the residual survival after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Exp 1 Initial % S.F $\pm$ S.E	Exp 2 Initial % S.F $\pm$ S.E	Mean Initial % S.F $\pm$ S.E	Exp 1 Residual % S.F $\pm$ S.E	Exp 2 Residual % S.F $\pm$ S.E	Mean Residual % S.F $\pm$ S.E
0	100	100	100	100	100	100
63	49.6 $\pm$ 25.2	59.0 $\pm$ 19.6	54.3 $\pm$ 22.4	52.0 $\pm$ 20.4	54.8 $\pm$ 22.0	53.5 $\pm$ 21.2
127	48.3 $\pm$ 14.4	45.1 $\pm$ 8.0	46.7 $\pm$ 11.2	44.7 $\pm$ 5.6	51.1 $\pm$ 22.4	47.9 $\pm$ 14.0
190	39.1 $\pm$ 3.6	42.3 $\pm$ 2.0	40.7 $\pm$ 2.8	48.5 $\pm$ 7.6	47.3 $\pm$ 9.2	47.9 $\pm$ 8.4
254	31.5 $\pm$ 8.4	28.1 $\pm$ 4.4	29.8 $\pm$ 6.4	18.4 $\pm$ 13.2	21.6 $\pm$ 9.2	20.0 $\pm$ 11.2
318	27.0 $\pm$ 10.0	31.8 $\pm$ 6.0	29.4 $\pm$ 8.0	23.0 $\pm$ 24.8	29.8 $\pm$ 20.0	26.4 $\pm$ 22.4
381	23.0	19.6 $\pm$ 0.4	23.3 $\pm$ 8.0	7.8 $\pm$ 3.2	12.0 $\pm$ 1.6	9.9 $\pm$ 2.4
445	18.7 $\pm$ 6.0	15.9 $\pm$ 3.6	17.3 $\pm$ 4.8	4.8	7.6 $\pm$ 0.4	6.2

Table 1:- Survival data from Figure 5.16 for the initially irradiated EPC cells and the residual survival after 10 population doublings post exposure to UVB.

UVA Dose / (J/m ²)	Time between Irradiation's /(Hours)	Cells Plated	Colonies Counted	% S.F ± S.E
0	0	402	132, 116, 123	100
7500	0	996	153, 149, 146	48.8 ± 0.5
3750 + 3750	1	996	180, 168, 172	56.6 ± 0.9
3750 + 3750	2	996	164, 151, 156	51.3 ± 1.0
3750 + 3750	3	996	156, 168, 163	53.0 ± 0.9
3750 + 3750	4	996	148, 151, 145	49.3 ± 0.4

Table 1:- Split-dose data for the HaCaT cells irradiated with UVA. Three flasks were exposed to a total dose of 7500 J/m² at time zero and 12 flasks (four groups of three flasks) that were exposed to 3750 J/m² at time zero and then given a similar dose of 3750 J/m² at 1, 2, 3, and 4 hours after time zero.

UVA Dose / (J/m^2)	Time between Irradiation's /(Hours)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	0	402	133, 118, 113	100
7500	0	996	169, 141, 156	51.7 ± 2.1
3750 + 3750	1	996	180, 176, 157	57.0 ± 1.9
3750 + 3750	2	996	167, 173, 180	57.8 ± 1.0
3750 + 3750	3	996	184, 168, 178	58.8 ± 1.2
3750 + 3750	4	996	182, 179, 180	60.1 ± 0.2

Table 2:- Split-dose data for the HaCaT cells irradiated with UVA. Three flasks were exposed to a total dose of 7500 J/m^2 at time zero and 12 flasks (four groups of three flasks) that were exposed to 3750 J/m^2 at time zero and then given a similar dose of 3750 J/m^2 at 1, 2, 3, and 4 hours after time zero.

UVA Dose / (J/m^2)	Time between Irradiation's /(Hours)	Initial % S.F $\pm$ S.E
0	0	100
7500	0	50.2 ± 1.3
3750 + 3750	1	56.8 ± 1.1
3750 + 3750	2	54.5 ± 1.5
3750 + 3750	3	55.9 ± 5.3
3750 + 3750	4	54.7 ± 2.2

Table 3:- Mean split-dose data for the HaCaT cells irradiated with UVA. Three flasks were exposed to a total dose of 7500 J/m^2 at time zero and 12 flasks (four groups of three flasks) that were exposed to 3750 J/m^2 at time zero and then given a similar dose of 3750 J/m^2 at 1, 2, 3, and 4 hours after time zero.

Appendix 26

UVA Dose / (J/m^2)	Time between Irradiation's /(Hours)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	0	407	159, 142, 146	100
15000	0	814	75, 83, 70	25.4 ± 1.0
7500 + 7500	1	814	14, 18, 26	6.4 ± 0.9
7500 + 7500	2	814	101, 81, 93	30.7 ± 2.0
7500 + 7500	3	814	100, 121, 97	35.5 ± 2.0
7500 + 7500	4	814	76, 88, 72	26.3 ± 1.3

Table 1:- Split-dose data for the CHSE cells irradiated with UVA. Three flasks were exposed to a total dose of 15000 J/m^2 at time zero and 12 flasks (four groups of three flasks) that were exposed to 7500 J/m^2 at time zero and then given a similar dose of 7500 J/m^2 at 1, 2, 3, and 4 hours after time zero.

UVA Dose / (J/m ²)	UVA Dose / (J/m ²)	Time between Irradiation's /(Hours)	Cells Plated	Colonies Counted	% S.F ± S.E
0	0	0	407	162, 170, 158	100
15000	2500	0	814	71, 73, 80	22.8 ± 0.6
7500 + 7500	7500	1	814	10, 9	2.8 ± 0.1
7500 + 7500	15000	2	814	109, 97, 81	29.2 ± 2.0
7500 + 7500	22500	3	814	101, 108, 135	35.0 ± 2.5
7500 + 7500	30000	4	814	83, 91, 87	26.6 ± 0.5

Table 2:- Split-dose data for the CHSE cells irradiated with UVA. Three flasks were exposed to a total dose of 15000 J/m² at time zero and 12 flasks (four groups of three flasks) that were exposed to 7500 J/m² at time zero and then given a similar dose of 7500 J/m² at 1, 2, 3, and 4 hours after time zero.

UVA Dose / (J/m^2)	UVA Dose / (J/m^2)	Time between Irradiation's /(Hours)	Initial % S.F $\pm$ S.E
0	0	0	100
15000	2500	0	24.1 ± 0.8
7500 + 7500	7500	1	5.0 ± 0.9
7500 + 7500	15000	2	29.9 ± 0.3
7500 + 7500	22500	3	35.2 ± 1.6
7500 + 7500	30000	4	26.4 ± 0.7

Table 3:- Mean split-dose data for the CHSE cells irradiated with UVA. Three flasks were exposed to a total dose of 15000 J/m^2 at time zero and 12 flasks (four groups of three flasks) that were exposed to 7500 J/m^2 at time zero and then given a similar dose of 7500 J/m^2 at 1, 2, 3, and 4 hours after time zero.

Appendix 27

UVB Dose / (J/m^2)	Time between Irradiation's /(Hours)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	0	409	98, 113, 105	100
700	0	1600	163, 152, 160	38.4 ± 0.6
350 + 350	1	1600	229, 231, 200	53.4 ± 1.9
350 + 350	2	1600	222, 206, 219	52.3 ± 0.9
350 + 350	3	1600	236, 241, 250	58.8 ± 0.8
350 + 350	4	1600	218, 238, 246	56.8 ± 1.6

Table 1:- Split-dose data for the HaCaT cells irradiated with UVB. Three flasks were exposed to a total dose of 700 J/m^2 at time zero and 12 flasks (four groups of three flasks) that were exposed to 350 J/m^2 at time zero and then given a similar dose of 700 J/m^2 at 1, 2, 3, and 4 hours after time zero.

UVB Dose / (J/m^2)	Time between Irradiation's /(Hours)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	0	402	116, 120, 101	100
700	0	1608	172, 156, 161	39.4 ± 0.9
350 + 350	1	1608	220, 215, 218	52.6 ± 0.2
350 + 350	2	1608	205, 211, 208	50.3 ± 0.3
350 + 350	3	1608	231, 218, 225	54.3 ± 0.7
350 + 350	4	1608	246, 232, 230	57.0 ± 1.0

Table 2:- Split-dose data for the HaCaT cells irradiated with UVB. Three flasks were exposed to a total dose of 700 J/m^2 at time zero and 12 flasks (four groups of three flasks) that were exposed to 350 J/m^2 at time zero and then given a similar dose of 700 J/m^2 at 1, 2, 3, and 4 hours after time zero.

UVB Dose / (J/m ²)	Time between Irradiation's /(Hours)	Initial % S.F ± S.E
0	0	100
700	0	38.9 ± 0.6
350 + 350	1	53.0 ± 1.4
350 + 350	2	51.3 ± 0.6
350 + 350	3	56.5 ± 1.1
350 + 350	4	56.9 ± 0.9

Table 3:- Mean split-dose data for the HaCaT cells irradiated with UVB. Three flasks were exposed to a total dose of 700 J/m² at time zero and 12 flasks (four groups of three flasks) that were exposed to 350 J/m² at time zero and then given a similar dose of 700 J/m² at 1, 2, 3, and 4 hours after time zero.

Appendix 28

UVB Dose / (J/m^2)	Time between Irradiation's /(Hours)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	0	400	103, 97, 111	100
700	0	800	16, 18, 12	7.3 ± 0.7
350 + 350	1	800	3, 3, 2,	1.2 ± 0.1
350 + 350	2	800	20, 15, 14	7.8 ± 0.7
350 + 350	3	800	29, 25, 24	12.6 ± 0.5
350 + 350	4	800	17, 16, 17	8.0 ± 0.1

Table 1:- Split-dose data for the CHSE cells irradiated with UVB. Three flasks were exposed to a total dose of 700 J/m^2 at time zero and 12 flasks (four groups of three flasks) that were exposed to 350 J/m^2 at time zero and then given a similar dose of 350 J/m^2 at 1, 2, 3, and 4 hours after time zero.

UVB Dose / (J/m^2)	Time between Irradiation's /(Hours)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	0	400	109, 120, 115	100
700	0	800	20, 23, 25	9.8 ± 0.5
350 + 350	1	800	7, 6, 9	3.1 ± 0.3
350 + 350	2	800	13, 17, 19	7.1 ± 0.6
350 + 350	3	800	19, 15, 11	6.5 ± 0.8
350 + 350	4	800	16, 22, 20	8.4 ± 0.6

Table 2:- Split-dose data for the CHSE cells irradiated with UVB. Three flasks were exposed to a total dose of 700 J/m^2 at time zero and 12 flasks (four groups of three flasks) that were exposed to 350 J/m^2 at time zero and then given a similar dose of 350 J/m^2 at 1, 2, 3, and 4 hours after time zero.

UVB Dose / (J/m^2)	Time between Irradiation's /(Hours)	Initial % S.F $\pm$ S.E
0	0	100
700	0	8.6 ± 0.6
350 + 350	1	2.2 ± 0.4
350 + 350	2	7.4 ± 0.5
350 + 350	3	9.5 ± 1.3
350 + 350	4	8.2 ± 0.3

Table 3:- Mean split-dose data for the CHSE cells irradiated with UVB. Three flasks were exposed to a total dose of 700 J/m^2 at time zero and 12 flasks (four groups of three flasks) that were exposed to 350 J/m^2 at time zero and then given a similar dose of 350 J/m^2 at 1, 2, 3, and 4 hours after time zero.

Appendix 29

N-acetyl-L-cysteine / M	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	391	116, 109, 117	100
10^{-3}	1466	263, 226, 237	56.6 ± 2.1
10^{-4}	1271	180, 185, 181	49.0 ± 0.3
10^{-5}	997	155, 170, 154	54.9 ± 1.4
10^{-6}	997	158, 179, 199	61.5 ± 3.3
UVA Dose / (J/m ²) + N-acetyl-L-cysteine / M			
15000	1466	196, 220, 220	100
15000 + 10^{-3}	1466	217, 212, 196	98.6 ± 2.4
15000 + 10^{-4}	1271	207, 200, 201	110.6 ± 0.9
15000 + 10^{-5}	997	145, 153, 147	103.2 ± 1.3
15000 + 10^{-6}	997	196, 195, 185	133.7 ± 2.0

Table 1:- Survival data for the HaCaT cells in the presence of varying concentrations of N-acetyl-L-cysteine, and also post exposure to UVA in the presence of N-acetyl-L-cysteine at varying concentrations.

N-acetyl-L-cysteine / M	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	391	110, 135, 132	100
10^{-3}	1466	225, 241, 236	49.7 ± 0.8
10^{-4}	1271	216, 220, 229	54.3 ± 0.7
10^{-5}	997	221, 220, 229	69.7 ± 0.7
10^{-6}	997	145, 159, 143	46.5 ± 1.2
UVA Dose / (J/m^2) + N-acetyl-L-cysteine / M			
15000	1466	195, 204, 198	100
$15000 + 10^{-3}$	1466	212, 195, 178	98.5 ± 4.0
$15000 + 10^{-4}$	1271	182, 131, 162	92.2 ± 7.0
$15000 + 10^{-5}$	997	146, 123	99.8 ± 6.0
$15000 + 10^{-6}$	997	154, 170, 134	113.4 ± 6.3

Table 2:- Survival data for the HaCaT cells in the presence of varying concentrations of N-acetyl-L-cysteine, and also post exposure to UVA in the presence of N-acetyl-L-cysteine at varying concentrations.

N-acetyl-L-cysteine / M	% S.F $\pm$ S.E
0	100
10^{-3}	53.1 ± 1.8
10^{-4}	51.7 ± 1.1
10^{-5}	62.3 ± 3.1
10^{-6}	54.0 ± 3.6
UVA Dose / (J/m^2) + N-acetyl-L-cysteine / M	
15000	100
$15000 + 10^{-3}$	98.5 ± 2.4
$15000 + 10^{-4}$	101.4 ± 0.4
$15000 + 10^{-5}$	101.8 ± 2.7
$15000 + 10^{-6}$	123.5 ± 5.4

Table 3:- Mean survival data for the HaCaT cells in the presence of varying concentrations of N-acetyl-L-cysteine, and also post exposure to UVA in the presence of N-acetyl-L-cysteine at varying concentrations.

N-acetyl-L-cysteine / M	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	400	69, 86, 51	100
10^{-3}	1507	184, 147, 153	62.5 ± 3.6
10^{-4}	1310	195, 194, 178	84.1 ± 1.9
10^{-5}	1015	155, 132, 148	83.5 ± 3.2
10^{-6}	1015	175, 166, 148	93.6 ± 3.9
UVA Dose / (J/m^2) + N-acetyl-L-cysteine / M			
15000	1507	181, 197	100
$15000 + 10^{-3}$	1507	117, 133	66.3 ± 2.9
$15000 + 10^{-4}$	1310	172, 168	103.7 ± 0.8
$15000 + 10^{-5}$	1015	115, 121	92.9 ± 1.6
$15000 + 10^{-6}$	1015	164, 150	123.7 ± 3.9

Table 1:- Survival data for the CHSE cells in the presence of varying concentrations of N-acetyl-L-cysteine, and also post exposure to UVA in the presence of N-acetyl-L-cysteine at varying concentrations.

N-acetyl-L-cysteine / M	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	400	78, 69, 93	100
10^{-3}	1505	167, 159, 169	54.4 ± 0.9
10^{-4}	1300	178, 199, 199	73.8 ± 2.2
10^{-5}	1007	132, 149, 130	67.9 ± 2.4
10^{-6}	1007	167, 175, 174	85.3 ± 1.0
UVA Dose / (J/m^2) + N-acetyl-L-cysteine / M			
15000	1505	186, 194	100
$15000 + 10^{-3}$	1505	130	68.5
$15000 + 10^{-4}$	1300	162, 171, 162	100.7 ± 1.4
$15000 + 10^{-5}$	1007	117, 115, 134	96.1 ± 3.8
$15000 + 10^{-6}$	1007	166, 152, 165	126.8 ± 2.9

Table 2:- Survival data for the CHSE cells in the presence of varying concentrations of N-acetyl-L-cysteine, and also post exposure to UVA in the presence of N-acetyl-L-cysteine at varying concentrations.

N-acetyl-L-cysteine / M	% S.F $\pm$ S.E
0	100
10^{-3}	58.6 ± 2.4
10^{-4}	78.9 ± 2.5
10^{-5}	75.7 ± 3.7
10^{-6}	89.5 ± 2.6
UVA Dose / (J/m^2) + N-acetyl-L-cysteine / M	
15000	100
$15000 + 10^{-3}$	67.0 ± 2.0
$15000 + 10^{-4}$	101.9 ± 1.0
$15000 + 10^{-5}$	94.8 ± 2.5
$15000 + 10^{-6}$	125.5 ± 2.4

Table 3:- Mean survival data for the CHSE cells in the presence of varying concentrations of N-acetyl-L-cysteine, and also post exposure to UVA in the presence of N-acetyl-L-cysteine at varying concentrations.

Appendix 31

UVB Dose / (J/m ²)	UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	0	398	147, 147, 126	100
700	0	796	109, 92, 107	36.4 ± 1.8
700	3000	796	77, 110, 80	31.8 ± 3.0
700	6000	796	97, 95, 93	33.9 ± 0.3
700	9000	796	85, 91, 104	33.3 ± 1.6
700	12000	796	84, 82, 86	30.0 ± 0.3

Table 1:- Survival data for the HaCaT cells irradiated with UVB and then illuminated with UVA to photoreactivate the damage. Three flasks were exposed to a total dose of 700 J/m² of UVB and 9 flasks (three groups of three flasks) were exposed to 3000, 6000 and 9000 J/m² of photoreactivating light immediately post exposure to UVB.

UVB Dose / (J/m ²)	UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	0	398	125, 106, 119	100
700	0	750	96, 95, 90	42.7 ± 0.7
700	3000	750	86, 73, 79	36.1 ± 1.3
700	6000	750	70, 57, 83	31.9 ± 2.7
700	9000	750	68, 101, 80	37.8 ± 3.6
700	12000	750	58, 62, 42	24.6 ± 6.3

Table 2:- Survival data for the HaCaT cells irradiated with UVB and then illuminated with UVA to photoreactivate the damage. Three flasks were exposed to a total dose of 700 J/m² of UVB and 9 flasks (three groups of three flasks) were exposed to 3000, 6000 and 9000 J/m² of photoreactivating light immediately post exposure to UVB.

UVB Dose / (J/m ²)	UVA Dose / (J/m ²)	% S.F ± S.E
0	0	100
700	0	39.5 ± 1.6
700	3000	33.9 ± 1.9
700	6000	32.9 ± 1.9
700	9000	35.6 ± 2.2
700	12000	27.3 ± 1.6

Table 3:- Mean survival data for the HaCaT cells irradiated with UVB and then illuminated with UVA to photoreactivate the damage. Three flasks were exposed to a total dose of 700 J/m² of UVB and 9 flasks (three groups of three flasks) were exposed to 3000, 6000 and 9000 J/m² of photoreactivating light immediately post exposure to UVB.

UVB Dose / (J/m ²)	UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	0	416	98, 112, 90	100
700	0	810	7, 12, 13	5.4 ± 0.7
700	3000	810	8, 22, 18	8.2 ± 1.7
700	6000	810	31, 45, 41	20.0 ± 1.7
700	9000	810	19, 32, 44	16.2 ± 3.0
700	12000	810	24, 31, 25	13.6 ± 0.9

Table 2:- Survival data for the CHSE cells irradiated with UVB and then illuminated with UVA to photoreactivate the damage. Three flasks were exposed to a total dose of 700 J/m² of UVB and 9 flasks (three groups of three flasks) were exposed to 3000, 6000 and 9000 J/m² of photoreactivating light immediately post exposure to UVB.

UVB Dose / (J/m ²)	UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	0	409	132, 120, 110	100
700	0	816	8, 16, 21	6.2 ± 1.2
700	3000	816	28, 27, 15	9.6 ± 1.4
700	6000	816	26, 34, 12	9.9 ± 2.1
700	9000	816	74, 39, 64	24.5 ± 3.5
700	12000	816	33, 31, 29	12.8 ± 0.4

Table 2:- Survival data for the CHSE cells irradiated with UVB and then illuminated with UVA to photoreactivate the damage. Three flasks were exposed to a total dose of 700 J/m² of UVB and 9 flasks (three groups of three flasks) were exposed to 3000, 6000 and 9000 J/m² of photoreactivating light immediately post exposure to UVB.

UVB Dose / (J/m ²)	UVA Dose / (J/m ²)	% S.F ± S.E
0	0	100
700	0	5.8 ± 0.7
700	3000	8.9 ± 1.1
700	6000	14.9 ± 2.5
700	9000	20.3 ± 2.9
700	12000	13.2 ± 0.5

Table 3:- Mean survival data for the CHSE cells irradiated with UVB and then illuminated with UVA to photoreactivate the damage. Three flasks were exposed to a total dose of 700 J/m² of UVB and 9 flasks (three groups of three flasks) were exposed to 3000, 6000 and 9000 J/m² of photoreactivating light immediately post exposure to UVB.

Appendix 33

UVA Dose / (J/m ²)	Cells Plated	Average Colony Count $\pm$ S.E	% S.F $\pm$ S.E
0	400	90.0 $\pm$ 0.7	100
2500	400	63.2 $\pm$ 4.8	70.6 $\pm$ 5.3
7500	801	106.5 $\pm$ 24.8	59.2 $\pm$ 13.7
15000	801	76.0 $\pm$ 19.2	42.5 $\pm$ 10.6
22500	1510	135.9 $\pm$ 4.5	40.0 $\pm$ 1.3
30000	1510	75.5 $\pm$ 1.5	22.4 $\pm$ 0.4

Table 1:- Survival data for the irradiated HaCaT cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Average Colony Count ± S.E	% R.S.F ± S.E
0	400	97.2 ± 3.6	100
2500	400	75.6 ± 12.8	70.1 ± 10.6
7500	408	59.5 ± 8.5	47.1 ± 12.0
15000	410	13.1	16.7 ± 4.1
22500	413	21.4 ± 4.9	28.7 ± 2.1
30000	400	10.8 ± 0.4	7.7 ± 0.1

Table 2:- Residual survival data for the HaCaT cells in Table 1 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Average Colony Count $\pm$ S.E	% S.F $\pm$ S.E
0	401	102.2 $\pm$ 4.0	100
2500	401	79.7 $\pm$ 6.4	78.2 $\pm$ 6.2
7500	760	145.9 $\pm$ 24.3	75.6 $\pm$ 12.5
15000	760	64.6 $\pm$ 11.4	33.7 $\pm$ 5.8
22500	1513	116.5 $\pm$ 10.5	30.2 $\pm$ 2.7
30000	1513	125.5	32.8

Table 3:- Survival data for the HaCaT cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	412	80, 76, 86	100	100
2500	401	82, 67, 79	94.2 ± 4.6	73.6 ± 6.8
7500	398	87, 90, 91	110.6 ± 1.0	83.6 ± 13.8
15000	398	36, 37, 30	42.9 ± 2.2	14.4 ± 2.5
22500	400	63, 86, 62	87.4 ± 7.9	26.3 ± 3.3
30000	400	35, 31, 30	39.7 ± 1.5	13.0 ± 0.4

Table 4:- Residual survival data for the HaCaT cells in Table 3 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Initial % S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	100	100
2500	74.4 $\pm$ 5.7	71.8 $\pm$ 8.7
7500	67.4 $\pm$ 13.1	65.3 $\pm$ 12.9
15000	38.1 $\pm$ 8.2	15.5 $\pm$ 3.3
22500	35.1 $\pm$ 2.0	27.5 $\pm$ 2.7
30000	27.6 $\pm$ 0.2	10.8 $\pm$ 0.2

Table 5:- Mean initial and residual survival data from Tables 1-4 for the HaCaT cells irradiated with UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	405	128, 134, 115	100
1250 + 1250	405	127, 116, 115	94.9 $\pm$ 2.5
3750 + 3750	1014	216, 202, 186	64.0 $\pm$ 2.2
7500 + 7500	1014	200, 202, 205	64.3 $\pm$ 0.3
11250 + 11250	2030	242, 236, 234	37.6 $\pm$ 0.3
15000 + 15000	2030	212, 208, 230	34.3 $\pm$ 0.8

Table 6:- Split-dose survival data for the irradiated HaCaT cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Average Colony Count ± S.E	% S.F ± S.E	% R.S.F ± S.E
0	398	91.9 ± 0.3	100	100
1250 + 1250	405	53.4 ± 1.2	57.3 ± 1.2	54.3 ± 1.8
3750 + 3750	403	65.6 ± 12.8	70.6 ± 13.8	45.1 ± 8.9
7500 + 7500	404	76.3 ± 16.1	81.9 ± 17.3	52.6 ± 11.1
11250 + 11250	402	91.6 ± 14.0	99.1 ± 15.1	37.2 ± 5.6
15000 + 15000	412	126.8 ± 0.4	133.4	44.2 ± 1.0

Table 7:- Residual split-dose survival data for the HaCaT cells in Table 6 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	Average $\pm$ S.E	% S.F $\pm$ S.E
0	399	70, 102, 67	79.6 $\pm$ 11.1	100
1250 + 1250	399	34, 32, 57	41.0 $\pm$ 8.0	51.6 $\pm$ 8.2
3750 + 3750	992	88, 67, 66	73.6 $\pm$ 14.1	40.8 $\pm$ 3.0
7500 + 7500	992	91, 85, 63	79.6 $\pm$ 8.5	40.3 $\pm$ 3.5
11250 + 11250	992	80, 65, 57	67.3 $\pm$ 6.8	34.0 $\pm$ 2.8
15000 + 15000	992	77, 60, 70	65.6 $\pm$ 5.6	34.9 $\pm$ 2.0

Table 8:- Split-dose survival data for the HaCaT cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	Average ± S.E	% P.E ± S.E	% S.F ± S.E	% R.S.F ± S.E
0	400	31, 30, 32	31.0 ± 0.5	7.7 ± 0.1	100	100
1250 + 1250	400	31, 44, 45	40.0 ± 4.5	10.0 ± 4.1	129.8 ± 11.9	66.9 ± 12.2
3750 + 3750	400	41, 46, 36	41.0 ± 4.5	10.2 ± 0.7	133.0 ± 7.6	54.2 ± 5.0
7500 + 7500	400	43, 53, 44	46.6 ± 3.1	116. ± 0.7	151.4 ± 8.4	61.0 ± 6.2
11250 + 11250	400	32, 32, 28	30.6 ± 1.3	7.6 ± 0.3	99.5 ± 3.5	33.8 ± 3.0
15000 + 15000	400	19, 44, 50	37.6 ± 9.5	9.4 ± 2.3	122.2 ± 25.2	42.6 ± 9.1

Table 9:- Residual split-dose survival data for the HaCaT cells after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Initial % S.F ± S.E	Residual % S.F ± S.E
0	100	100
1250 + 1250	73.2 ± 10.0	60.6 ± 7.0
3750 + 3750	52.4 ± 5.2	49.6 ± 6.9
7500 + 7500	52.3 ± 5.3	56.8 ± 5.9
11250 + 11250	35.8 ± 1.6	35.5 ± 2.0
15000 + 15000	34.6 ± 1.1	43.4 ± 5.0

Table 10:- Mean split-dose survival data from Figure 6.1 for the HaCaT cells irradiated with UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	Average ± S.E	% P.E ± S.E	% S.F ± S.E
0	400	86, 79, 70	78.4 ± 3.7	19.6 ± 0.9	100
2500	400	66, 74	70.0 ± 2.3	17.5 ± 0.5	89.2 ± 3.6
7500	756	130, 125, 126	127.0 ± 1.2	16.9 ± 0.1	85.6 ± 0.8
15000	756	79, 54, 46	59.6 ± 0.1	7.9	40.2 ± 5.4
22500	1512	98, 96, 96	96.6 ± 0.5	6.4	32.5 ± 0.1
30000	1512	130, 112, 121	121.0 ± 4.2	8.0 ± 0.2	40.7 ± 1.4

Table 1:- Survival data for the irradiated CHSE cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	410	83, 88	100	100
2500	401	70, 66, 53	72.6 ± 4.8	64.7 ± 5.0
7500	400	66, 68, 80	82.5 ± 4.1	70.6 ± 3.5
15000	397	71	82.7	33.2 ± 4.4
22500	401	22, 20, 19	23.4 ± 0.8	7.6 ± 0.2
30000	400	16, 20, 19	21.1 ± 1.1	8.5 ± 0.5

Table 2:- Residual survival data for the CHSE cells in Table 1 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	Average $\pm$ S.E	% P.E $\pm$ S.E	% S.F $\pm$ S.E
0	413	100, 92, 100	97.3 $\pm$ 2.2	23.6 $\pm$ 0.5	100
2500	413	97, 88, 92	92.3 $\pm$ 2.1	22.4 $\pm$ 0.5	94.6 $\pm$ 2.1
7500	700	62, 73, 76	70.3 $\pm$ 3.4	17.0 $\pm$ 0.4	42.5 $\pm$ 2.1
15000	700	59, 52, 48	53.0 $\pm$ 2.6	7.6 $\pm$ 0.3	32.0 $\pm$ 1.6
22500	1500	105	105.0	7.0	29.0
30000	1500	144, 147	145.0 $\pm$ 0.8	9.7	41.0 $\pm$ 3.1

Table 3:- Survival data for the CHSE cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Average Colony Count ± S.E	% S.F ± S.E	% R.S.F ± S.E
0	402	100.0 ± 8.1	100	100
2500	400	61.2 ± 5.6	61.6 ± 22.4	58.2 ± 21.2
7500	401	91.0 ± 6.1	91.5 ± 24.4	38.8 ± 10.5
15000	400	75.2 ± 12.3	75.6 ± 9.2	15.7 ± 6.1
22500	400	31.2 ± 5.2	31.6 ± 20.8	6.1 ± 4.0
30000	400	18.4 ± 3.3	18.7 ± 13.2	5.4 ± 3.8

Table 4:- Residual survival data for the CHSE cells in Table 3 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Initial % S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	100	100
2500	92.4 $\pm$ 2.3	63.4 $\pm$ 13.1
7500	64.1 $\pm$ 9.0	54.7 $\pm$ 7.0
15000	36.1 $\pm$ 0.6	24.4 $\pm$ 5.2
22500	31.8 $\pm$ 0.6	6.8 $\pm$ 2.1
30000	40.8 $\pm$ 0.8	6.9 $\pm$ 2.1

Table 5:- Mean initial and residual survival data from Tables 1-4 for the CHSE cells irradiated with UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	400	99, 119, 88	100
1250 + 1250	400	106, 96, 84	93.4 ± 5.1
3750 + 3750	750	76, 91, 78	42.6 ± 2.0
7500 + 7500	750	118, 85, 78	49.9 ± 5.0
11250 + 11250	1500	178, 173, 169	45.2 ± 0.5
15000 + 15000	1500	110, 130, 123	31.5 ± 1.2

Table 6:- Split-dose survival data for the irradiated CHSE cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	499	101, 88, 90	100	100
1250 + 1250	506	80, 71, 79	81.4 ± 2.4	76.0 ± 2.3
3750 + 3750	501	82, 77, 92	89.7 ± 3.8	38.2 ± 2.4
7500 + 7500	504	82, 91, 93	94.5 ± 2.9	47.1 ± 4.9
11250 + 11250	504	84, 71, 68	82.2 ± 6.7	37.1 ± 3.0
15000 + 15000	501	42, 55, 47	51.4 ± 3.3	16.1 ± 1.2

Table 7:- Residual split-dose survival data for the CHSE cells in Table 6 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	400	87, 98, 80	100
1250 + 1250	400	104, 93, 83	106.0 $\pm$ 5.6
3750 + 3750	750	144	87.2
7500 + 7500	750	90, 88, 63	48.6 $\pm$ 4.3
11250 + 11250	1500	152, 142, 156	45.4 $\pm$ 1.0
15000 + 15000	1500	80, 95, 90	26.7 $\pm$ 1.0

Table 8:- Split-dose survival data for the CHSE cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E	% R.S.F $\pm$ S.E
0	503	80, 108, 86	100	100
1250 + 1250	508	69, 60, 65	70.2 $\pm$ 2.3	74.4 $\pm$ 4.6
3750 + 3750	506	62, 83, 71	78.6 $\pm$ 5.4	68.5 $\pm$ 4.7
7500 + 7500	514	82, 89, 82	90.6 $\pm$ 2.0	44.0 $\pm$ 4.0
11250 + 11250	506	102, 96, 11	112.0 $\pm$ 3.6	50.8 $\pm$ 1.9
15000 + 15000	508	96, 91, 120	111.0 $\pm$ 7.8	29.6 $\pm$ 2.3

Table 9:- Residual split-dose survival data for the CHSE cells after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Initial % S.F ± S.E	Residual % S.F ± S.E
0	100	100
1250 + 1250	99.7 ± 4.6	75.2 ± 3.4
3750 + 3750	53.7 ± 9.7	53.3 ± 3.5
7500 + 7500	48.7 ± 3.4	45.5 ± 4.4
11250 + 11250	45.3 ± 5.9	43.9 ± 2.4
15000 + 15000	29.1 ± 1.3	22.8 ± 1.7

Table 10:- Mean split-dose survival data from Figure 6.2 for the CHSE cells irradiated with UVA.

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UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	413	132, 148, 122	100
100	413	90, 98, 94	70.0 ± 1.4
250	756	156, 170, 166	66.6 ± 1.3
400	756	108, 111, 96	42.6 ± 1.5
550	1514	188, 175, 173	36.2 ± 0.7
700	1514	109, 109, 118	22.7 ± 6.0

Table 1:- Survival data for the irradiated HaCaT cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	400	98, 109, 93	100	100
100	403	51, 49, 51	50.9 ± 0.5	35.6 ± 0.7
250	403	25, 29, 30	28.3 ± 1.2	18.8 ± 0.8
400	400	24, 16, 21	20.7 ± 1.9	8.8 ± 0.8
550	398	11, 9, 10	10.2 ± 0.4	3.6 ± 0.1
700	401	28, 26, 12	22.3 ± 4.1	5.0 ± 1.6

Table 2:- Residual survival data for the HaCaT cells in Table 1 after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	400	84, 94, 92	100
100	400	67, 65, 72	75.5 $\pm$ 1.8
250	751	109, 112, 98	62.8 $\pm$ 2.0
400	751	78, 83, 87	56.7 $\pm$ 10.2
550	1502	140, 125, 149	40.7 $\pm$ 1.6
700	1502	110, 123, 131	35.8 $\pm$ 1.4

Table 3:- Survival data for the HaCaT cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	411	115, 124, 128	100	100
100	400	69, 65, 51	51.6 ± 3.7	38.9 ± 2.9
250	401	52, 46, 40	38.4 ± 2.3	24.1 ± 1.6
400	400	29, 46, 33	30.1 ± 3.5	17.0 ± 3.6
550	400	22, 21, 30	20.3 ± 1.9	8.1 ± 0.8
700	399	16, 20, 20	15.6 ± 0.9	5.5 ± 0.3

Table 4:- Residual survival data for the HaCaT cells in Table 3 after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Initial % S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	100	100
100	72.7 $\pm$ 5.9	37.2 $\pm$ 1.8
250	64.7 $\pm$ 1.4	21.4 $\pm$ 1.2
400	45.7 $\pm$ 1.6	12.9 $\pm$ 2.2
550	38.5 $\pm$ 1.3	5.8 $\pm$ 0.4
700	29.2 $\pm$ 2.8	5.2 $\pm$ 0.9

Table 5:- Mean initial and residual survival data for the HaCaT cells irradiated with UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	406	165,156, 153	100
50 + 50	406	138, 125, 128	82.4 $\pm$ 2.0
125 + 125	1003	209, 206, 213	53.5 $\pm$ 0.4
200 + 200	1003	211, 215, 202	53.6 $\pm$ 0.8
275 + 275	2030	260, 270, 269	33.6 $\pm$ 0.3
350 + 350	2030	230, 217, 207	27.5 $\pm$ 0.6

Table 6:- Split-dose survival data for the irradiated HaCaT cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	298	74, 46, 61	100	100
50 + 50	298	55, 57, 48	88.5 ± 3.6	72.9 ± 3.4
125 + 125	300	63, 53, 64	98.9 ± 4.7	52.9 ± 2.5
200 + 200	296	69, 46, 60	97.5 ± 9.1	52.2 ± 4.9
275 + 275	310	61, 62, 84	110.1 ± 9.7	36.9 ± 3.2
350 + 350	300	86, 77, 89	138.5 ± 4.8	38.0 ± 1.5

Table 7:- Residual split-dose survival data for the HaCaT cells in Table 6 after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	417	152, 149, 149	100
50 + 50	417	156, 150, 152	101.9 $\pm$ 0.9
125 + 125	1017	250, 236, 241	66.3 $\pm$ 0.9
200 + 200	1017	200, 218, 220	58.2 $\pm$ 1.4
275 + 275	2001	302, 314, 305	42.7 $\pm$ 0.4
350 + 350	2001	360, 350, 354	49.3 $\pm$ 0.3

Table 8:- Split-dose survival data for the HaCaT cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E	% R.S.F $\pm$ S.E
0	306	100, 101, 88	100	100
50 + 50	327	91, 98, 68	76.7 $\pm$ 6.6	78.1 $\pm$ 6.7
125 + 125	302	93, 111, 88	94.4 $\pm$ 5.5	62.5 $\pm$ 3.7
200 + 200	301	83, 86, 79	80.4 $\pm$ 1.6	46.7 $\pm$ 1.4
275 + 275	304	94, 103, 104	96.7 $\pm$ 2.5	41.2 $\pm$ 1.1
350 + 350	304	84, 70, 75	73.6 $\pm$ 3.2	36.2 $\pm$ 1.5

Table 9:- Residual split-dose survival data for the HaCaT cells in Table 8 after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Initial % S.F ± S.E	Residual % S.F ± S.E
0	100	100
50 + 50	92.2 ± 4.2	75.5 ± 5.0
125 + 125	59.9 ± 2.7	57.7 ± 2.1
200 + 200	55.9 ± 1.2	49.4 ± 3.1
275 + 275	38.1 ± 1.9	39.0 ± 2.2
350 + 350	38.4 ± 4.5	37.1 ± 1.5

Table 10:- Mean split-dose survival data from Figure 6.3 for the HaCaT cells irradiated with UVB.

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UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	413	109, 96, 121	100
50	413	76, 90, 92	79.4 ± 3.8
100	827	125, 117, 105	53.3 ± 2.1
250	827	68, 58, 72	30.5 ± 1.6
375	1654	54, 64, 73	14.6 ± 1.0
500	1654	50, 34, 46	9.9 ± 0.9

Table 1:- Survival data for the irradiated CHSE cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	500	127, 118, 129	100	100
50	514	92, 87, 83	68.1 ± 1.6	54.0 ± 2.8
100	503	52, 65, 48	43.8 ± 3.3	23.3 ± 1.9
250	508	69, 79, 65	56.0 ± 2.6	17.0 ± 1.1
375	500	65, 52, 42	45.3 ± 2.8	6.6 ± 0.6
500	500	32, 26, 25	22.1 ± 1.4	2.1 ± 0.2

Table 2:- Residual survival data for the CHSE cells in Table 1 after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	427	99, 84, 116	100
50	427	58, 49, 5	53.8 $\pm$ 2.1
100	855	40, 46, 32	19.6 $\pm$ 1.6
250	855	35, 44, 23	17.0 $\pm$ 2.4
375	1710	30, 28, 29	7.2 $\pm$ 0.1
500	1710	36, 24, 27	7.2 $\pm$ 0.7

Table 3:- Survival data for the CHSE cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	504	138, 142, 136	100	100
50	506	87, 89, 112	68.9 ± 4.6	37.0 ± 2.8
100	514	86, 73, 79	56.0 ± 2.1	10.9 ± 0.9
250	509	92, 74, 93	61.6 ± 3.6	10.4 ± 1.5
375	500	62, 55, 63	43.6 ± 1.4	3.1 ± 0.1
500	505	40, 24, 42	25.1 ± 3.2	1.8 ± 0.2

Table 4:- Residual survival data for the CHSE cells after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Initial % S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	100	100
50	66.6 $\pm$ 5.7	45.5 $\pm$ 2.8
100	36.4 $\pm$ 7.1	17.1 $\pm$ 1.4
250	23.7 $\pm$ 3.2	13.7 $\pm$ 1.3
375	10.9 $\pm$ 1.6	4.8 $\pm$ 0.3
500	8.6 $\pm$ 1.9	1.9 $\pm$ 0.2

Table 5:- Mean initial and residual survival data for the CHSE cells irradiated with UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	413	103, 96, 97	100
25 + 25	413	64, 71, 50	62.7 $\pm$ 5.1
62.5 + 62.5	827	95, 89, 81	44.8 $\pm$ 1.6
125 + 125	827	36, 29, 23	14.8 $\pm$ 1.5
187.5 + 187.5	1654	89, 103, 108	25.3 $\pm$ 1.1
250 + 250	1654	22, 18, 16	4.6 $\pm$ 0.3

Table 6:- Split-dose survival data for the irradiated CHSE cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E	% R.S.F $\pm$ S.E
0	500	147, 161, 143	100	100
25 + 25	500	121, 125, 136	84.8 $\pm$ 2.4	53.1 $\pm$ 4.5
62.5 + 62.5	513	112, 132, 125	79.8 $\pm$ 3.1	35.7 $\pm$ 1.8
125 + 125	505	110, 116, 115	75.0 $\pm$ 0.9	11.1 $\pm$ 1.1
187.5 + 187.5	500	88, 98, 106	64.8 $\pm$ 2.8	16.3 $\pm$ 0.9
250 + 250	500	115, 124, 121	79.9 $\pm$ 1.4	3.6 $\pm$ 0.2

Table 7:- Residual split-dose survival data for the CHSE cells after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	427	124, 106, 136	100
25 + 25	427	76, 92, 101	73.6 ± 4.8
62.5 + 62.5	855	110, 105, 113	39.0 ± 4.1
125 + 125	855	53, 66, 71	25.9 ± 1.7
187.5 + 187.5	1710	72, 69, 78	14.9 ± 0.4
250 + 250	1710	28, 19, 26	4.9 ± 0.4

Table 8:- Split-dose survival data for the CHSE cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	502	120, 132, 124	100	100
25 + 25	500	108, 111, 103	86.1 ± 1.5	63.3 ± 4.2
62.5 + 62.5	505	116, 104, 98	84.2 ± 3.4	32.8 ± 3.6
125 + 125	512	106, 106, 95	80.2 ± 2.3	20.7 ± 1.4
187.5 + 187.5	506	88, 95, 90	72.2 ± 1.3	10.7 ± 0.3
250 + 250	531	109, 117, 108	84.1 ± 1.7	4.1 ± 0.3

Table 9:- Residual split-dose survival data for the CHSE cells after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Initial % S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	100	100
25 + 25	68.1 $\pm$ 4.2	58.2 $\pm$ 4.3
62.5 + 62.5	44.8 $\pm$ 0.9	34.2 $\pm$ 2.7
125 + 125	20.3 $\pm$ 2.6	15.9 $\pm$ 0.7
187.5 + 187.5	20.1 $\pm$ 2.2	15.5 $\pm$ 0.6
250 + 250	4.8 $\pm$ 0.3	3.8 $\pm$ 0.2

Table 10:- Mean split-dose survival data from Figure 6.4 for the CHSE cells irradiated with UVB.

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UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	400	82, 102, 74	100
2500	400	80, 65, 68	82.5 ± 4.3
7500	750	100, 112, 110	66.5 ± 1.8
15000	750	64, 75, 79	44.8 ± 2.2
22500	1502	125, 132, 135	40.4 ± 0.7
30000	1502	102, 98, 124	33.3 ± 2.0

Table 1:- Survival data for the HaCaT cells irradiated with UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	403	98, 90, 103	100	100
2500	412	69, 73, 94	78.8 ± 12.4	65.0 ± 10.7
7500	400	57, 70, 68	67.4 ± 3.4	44.8 ± 2.5
15000	398	80, 84, 76	83.3 ± 1.9	37.3 ± 2.0
22500	400	66, 59, 83	71.8 ± 6.0	29.0 ± 2.4
30000	401	46, 50, 61	54.1 ± 3.8	18.0 ± 1.6

Table 2:- Residual Survival data for the HaCaT cells in Table 1 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	Average $\pm$ S.E	% P.E $\pm$ S.E	% S.F $\pm$ S.E
0	500	87, 100, 91	92.6 $\pm$ 3.8	18.5 $\pm$ 0.7	100
2500	500	81, 81, 81	81.0	16.2	87.5
7500	750	94, 98, 75	89.0 $\pm$ 7.1	11.8 $\pm$ 0.9	64.1 $\pm$ 4.1
15000	750	63, 52, 51	55.3 $\pm$ 3.1	7.4 $\pm$ 0.4	39.8 $\pm$ 2.2
22500	1500	54, 58, 54	55.3 $\pm$ 1.0	7.4 $\pm$ 0.4	19.9 $\pm$ 0.4
30000	1500	119, 108, 113	113.2 $\pm$ 2.5	5.5 $\pm$ 0.1	40.8 $\pm$ 0.9

Table 3:- Survival data for the HaCaT cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	523	137, 126, 129	100	100
2500	497	84, 81, 88	67.8 ± 11.4	59.3 ± 9.9
7500	502	92, 101, 95	76.4 ± 1.7	48.9 ± 3.3
15000	503	98, 121	87.0 ± 6.4	34.6 ± 3.1
22500	502	108, 99	82.4 ± 2.5	16.3 ± 0.5
30000	504	25, 32, 32	23.4 ± 1.4	9.5 ± 0.6

Table 4:- Residual survival data for the HaCaT cells in Table 3 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Initial % S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	100	100
2500	85.0 $\pm$ 2.4	62.1 $\pm$ 10.3
7500	65.3 $\pm$ 2.3	46.8 $\pm$ 2.9
15000	42.3 $\pm$ 1.9	35.9 $\pm$ 2.5
22500	30.1 $\pm$ 4.2	22.6 $\pm$ 1.4
30000	37.0 $\pm$ 1.9	13.7 $\pm$ 1.1

Table 5:- Mean initial and residual survival data from Tables 1-4 for the HaCaT cells irradiated with UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	500	86, 98	100
2500	500	72, 80, 85	86.8 ± 3.3
7500	750	116, 91, 99	73.8 ± 4.3
15000	750	67, 64	47.4 ± 0.7
22500	1500	120, 112, 146	45.5 ± 3.0
30000	1500	118, 110, 114	41.2 ± 8.5

Table 6:- Survival data for the HaCaT cells irradiated with varying doses of UVA in the presence of 10⁻⁶ M of N-acetyl-L-cysteine.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E	% R.S.F $\pm$ S.E
0	521	128, 128, 135	100	100
2500	495	115, 111, 118	92.6 $\pm$ 1.3	80.4 $\pm$ 3.2
7500	504	125, 128	100.3 $\pm$ 0.8	74.0 $\pm$ 4.3
15000	527	96, 103	75.4 $\pm$ 1.8	35.7 $\pm$ 1.0
22500	501	117, 115, 129	96.0 $\pm$ 2.8	43.6 $\pm$ 3.1
30000	503	136, 140, 132	108.1 $\pm$ 1.5	44.5 $\pm$ 9.2

Table 7:- Residual survival data for the HaCaT cells irradiated in Table 6 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Average Colonies Counted $\pm$ S.E	% P.E $\pm$ S.E	% S.F $\pm$ S.E
0	500	128.0 $\pm$ 12.3	25.6 $\pm$ 2.4	100
2500	500	113.6 $\pm$ 8.6	22.7 $\pm$ 2.4	89.0 $\pm$ 9.3
7500	751	127.3 $\pm$ 10.2	17.0 $\pm$ 1.3	66.6 $\pm$ 5.0
15000	751	86.5 $\pm$ 5.4	11.5 $\pm$ 0.7	45.0 $\pm$ 2.7
22500	1503	163.7 $\pm$ 9.4	10.9 $\pm$ 0.6	42.6 $\pm$ 2.3
30000	1503	162.0 $\pm$ 3.6	10.8 $\pm$ 0.2	42.3 $\pm$ 0.7

Table 8:- Survival data for the HaCaT cells post exposure to UVA in the presence of 10^{-6} M of N-acetyl-L-cysteine.

UVA Dose / (J/m ²)	Cells Plated	Average Colonies Counted $\pm$ S.E	% S.F $\pm$ S.E	% R.S.F $\pm$ S.E
0	512	119.8 $\pm$ 4.5	100	100
2500	502	108.4 $\pm$ 4.1	92.6 $\pm$ 3.3	82.4 $\pm$ 9.0
7500	503	126.7 $\pm$ 7.8	107.8 $\pm$ 6.3	71.7 $\pm$ 6.8
15000	515	115.8 $\pm$ 5.4	96.2 $\pm$ 4.2	43.2 $\pm$ 3.2
22500	500	102.5 $\pm$ 9.1	87.7 $\pm$ 7.5	37.3 $\pm$ 3.7
30000	501	113.2 $\pm$ 6.2	96.9 $\pm$ 5.0	40.9 $\pm$ 2.2

Table 9:- Residual survival data for the HaCaT cells irradiated in Table 8 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Initial % S.F ± S.E	Residual % S.F ± S.E
0	100	100
2500	87.4 ± 6.3	80.9 ± 6.1
7500	70.2 ± 4.6	72.8 ± 5.6
15000	46.2 ± 1.7	39.4 ± 2.1
22500	44.0 ± 2.6	40.4 ± 3.4
30000	41.7 ± 4.6	42.7 ± 5.7

Table 10:- Mean survival data from Figure 6.5 for the HaCaT cells irradiated with UVA in the presence of 10⁻⁶ M of N-acetyl-L-cysteine.

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UVA Dose / (J/m ²)	Cells Plated	Average Colony Count ± S.E	% S.F ± S.E
0	401	102.6 ± 20.1	100
2500	401	45.7 ± 1.6	44.7 ± 1.1
7500	800	56.0 ± 3.2	27.4 ± 1.5
15000	800	72.8 ± 4.3	35.6 ± 1.9
22500	1514	66.6 ± 1.9	17.3
30000	1514	56.0 ± 7.6	14.7 ± 1.9

Table 1:- Survival data for the CHSE cells irradiated with UVA.

UVA Dose / (J/m ²)	Cells Plated	Average Colony Count ± S.E	% S.F ± S.E	% R.S.F ± S.E
0	508	126.4 ± 9.2	100	100
2500	511	95.0 ± 6.3	74.9 ± 4.8	33.5 ± 2.2
7500	503	120.7 ± 7.7	96.7 ± 6.0	26.5 ± 2.1
15000	500	31.5	25.5	9.1
22500	509	55.4 ± 2.1	43.9 ± 1.6	7.6 ± 0.2
30000	500	35.0	28.5	4.2

Table 2:- Residual Survival data for the CHSE cells in Table 1 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Average Colony Count $\pm$ S.E	% S.F $\pm$ S.E
0	408	102.0 $\pm$ 8.6	100
2500	408	47.3 $\pm$ 3.7	46.5 $\pm$ 3.6
7500	816	79.9 $\pm$ 1.9	39.2 $\pm$ 0.8
15000	816	83.2 $\pm$ 4.2	40.8 $\pm$ 2.0
22500	1500	85.5 $\pm$ 9.6	22.9 $\pm$ 2.4
30000	1500	70.5 $\pm$ 10.1	19.1 $\pm$ 2.4

Table 3:- Survival data for the CHSE cells post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Average Colony Count ± S.E	% S.F ± S.E	% R.S.F ± S.E
0	500	139.0 ± 30.2	100	100
2500	500	109.5 ± 11.3	78.9 ± 7.7	36.7 ± 4.5
7500	515	124.1 ± 1.7	86.9 ± 10.5	34.1 ± 4.1
15000	508	41.6 ± 6.2	29.6 ± 4.2	12.1 ± 1.8
22500	503	61.8 ± 2.3	44.5 ± 1.4	10.2 ± 1.1
30000	501	20.0 ± 2.0	14.6 ± 1.0	2.8 ± 0.4

Table 4:- Residual survival data for the CHSE cells in Table 3 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Initial % S.F $\pm$ S.E	Residual % S.F $\pm$ S.E
0	100	100
2500	45.6 $\pm$ 2.3	35.1 $\pm$ 3.3
7500	33.3 $\pm$ 1.1	30.3 $\pm$ 3.2
15000	38.2 $\pm$ 1.9	10.6 $\pm$ 0.9
22500	20.1 $\pm$ 1.2	8.9 $\pm$ 0.6
30000	16.9 $\pm$ 2.1	3.5 $\pm$ 0.2

Table 5:- Mean initial and residual survival data from Tables 1-4 for the CHSE cells irradiated with UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	400	110, 97, 103	100
2500	400	41, 35, 63	44.8 ± 6.7
7500	800	85, 85, 84	40.9 ± 0.1
15000	800	66, 73, 67	33.2 ± 0.8
22500	1600	56, 38, 44	27.0 ± 5.5
30000	1600	60, 48, 71	14.4 ± 1.2

Table 6:- Survival data for the CHSE cells irradiated with varying doses of UVA in the presence of 10^{-6} M of N-acetyl-L-cysteine.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	500	146, 159, 148	100	100
2500	499	150, 150, 140	97.3 ± 1.7	43.5 ± 6.5
7500	498	115, 122, 91	72.6 ± 5.0	29.6 ± 2.0
15000	500	104, 109, 85	65.8 ± 3.8	21.8 ± 5.9
22500	503	164, 161, 168	108.1 ± 1.0	29.1 ± 5.9
30000	501	88, 72, 70	50.6 ± 3.0	7.2 ± 0.7

Table 7:- Residual survival data for the CHSE cells irradiated in table 6 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	403	109, 98, 101	100
2500	403	41, 65, 53	51.5 ± 5.5
7500	806	67, 75, 68	34.0 ± 1.0
15000	806	55, 54, 65	28.1 ± 1.4
22500	1608	71, 79, 72	18.0 ± 0.4
30000	1608	75, 67, 60	16.3 ± 0.8

Table 8:- Survival data for the CHSE cells post exposure to UVA in the presence of 10⁻⁶ M of N-acetyl-L-cysteine.

UVA Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E	% R.S.F $\pm$ S.E
0	501	127, 140, 128	100	100
2500	492	124, 110, 124	92.2 $\pm$ 2.9	47.4 $\pm$ 5.2
7500	502	136, 112	93.9 $\pm$ 6.4	31.9 $\pm$ 2.3
15000	497	146, 130	105.5 $\pm$ 4.3	29.6 $\pm$ 1.9
22500	503	114, 97, 107	80.0 $\pm$ 3.0	14.4 $\pm$ 0.6
30000	498	118, 85, 96	76.0 $\pm$ 6.0	12.3 $\pm$ 1.1

Table 9:- Residual survival data for the CHSE cells irradiated in table 8 after 10 population doublings post exposure to UVA.

UVA Dose / (J/m ²)	Initial % S.F ± S.E	Residual % S.F ± S.E
0	100	100
2500	48.1 ± 4.6	45.4 ± 5.8
7500	37.4 ± 1.5	30.7 ± 2.1
15000	30.6 ± 1.3	25.7 ± 1.6
22500	14.5 ± 1.5	21.7 ± 3.2
30000	15.3 ± 0.8	9.7 ± 0.9

Table 10:- Mean survival data from Figure 6.6 for the CHSE cells irradiated with UVA.

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UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	405	112, 138, 126	100
50	405	107, 113, 95	83.8 ± 3.4
125	810	125, 143, 145	54.9 ± 2.0
250	810	138, 154, 145	58.1 ± 1.5
375	1620	63, 68, 71	13.3 ± 0.3
500	1620	30, 27, 35	6.0 ± 0.3

Table 1:- Survival data for the CHSE cells irradiated with UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	502	126, 137, 140	100	100
50	500	95, 91, 114	74.8 ± 4.3	62.6 ± 4.4
125	510	103, 97, 88	70.4 ± 2.6	38.6 ± 2.0
250	500	78, 90, 87	63.6 ± 2.2	36.9 ± 1.5
375	495	54, 42, 52	37.2 ± 2.3	4.9 ± 0.3
500	503	68, 71, 87	56.0 ± 3.5	3.3 ± 0.2

Table 2:- Residual Survival data for the CHSE cells in Table 1 after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E
0	400	110, 126, 114	100
50	400	96, 92, 100	82.7 $\pm$ 1.6
125	801	51, 46, 50	21.0 $\pm$ 0.5
250	801	42, 43, 46	18.7 $\pm$ 0.4
375	1602	61, 58, 60	12.8 $\pm$ 0.1
500	1602	20, 24, 35	5.6 $\pm$ 0.7

Table 3:- Survival data for the CHSE cells post exposure to UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F $\pm$ S.E	% R.S.F $\pm$ S.E
0	504	135, 132, 129	100	100
50	504	103, 110, 100	79.3 $\pm$ 1.8	65.5 $\pm$ 1.9
125	501	91, 86, 94	69.0 $\pm$ 1.4	14.4 $\pm$ 0.4
250	500	84, 91, 82	65.6 $\pm$ 1.7	12.2 $\pm$ 0.4
375	500	56, 58, 49	41.6 $\pm$ 1.7	5.3 $\pm$ 0.2
500	504	75, 82, 74	58.5 $\pm$ 1.5	7.4 $\pm$ 0.9

Table 4:- Residual survival data for the CHSE cells in Table 3 after 10 population doublings post exposure to UVB.

UVB Dose / (J/m ²)	Initial % S.F ± S.E	Residual % S.F ± S.E
0	100	100
50	83.3 ± 1.9	64.0 ± 3.1
125	38.0 ± 3.3	26.5 ± 1.2
250	38.4 ± 8.2	24.5 ± 0.9
375	13.0 ± 0.2	5.1 ± 0.2
500	5.8 ± 0.4	5.3 ± 0.5

Table 5:- Mean initial and residual survival data from Tables 1-4 for the CHSE cells irradiated with UVB.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	405	104, 103, 100	100
50	405	82, 85, 88	83.2 ± 1.3
125	810	87, 68, 63	37.9 ± 2.6
250	810	56, 50, 57	29.5 ± 1.5
375	1620	90, 97, 100	23.3 ± 0.5
500	1620	65, 61, 68	15.8 ± 0.4

Table 6:- Survival data for the CHSE cells irradiated with UVB and then illuminated with 9,000 (J/m²) UVA to photoreactivate the damage.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	500	98, 87, 116	100	100
50	502	50, 53, 62	54.5 ± 3.0	45.3 ± 2.5
125	500	89, 110, 101	62.3 ± 20.7	22.6 ± 8.0
250	500	85, 76, 79	80.0 ± 2.1	23.6 ± 1.3
375	512	116, 100, 106	104.7 ± 3.7	24.3 ± 1.0
500	504	85, 73, 83	79.6 ± 3.0	12.5 ± 0.5

Table 7:- Residual survival data for the CHSE cells irradiated in table 6 after 10 population doublings post exposure to UVB and then illuminated with 9,000 (J/m²) UVA to photoreactivate the damage.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E
0	400	100,96, 91	100
50	400	82, 80, 87	86.7 ± 0.7
125	801	71, 73, 76	38.2 ± 1.7
250	801	56, 52, 59	29.0 ± 0.8
375	1602	98, 93, 91	23.3 ± 0.5
500	1602	58, 67, 63	15.8 ± 0.4

Table 8:- Survival data for the CHSE cells post exposure to UVB and then illuminated with 9,000 (J/m²) UVA to photoreactivate the damage.

UVB Dose / (J/m ²)	Cells Plated	Colonies Counted	% S.F ± S.E	% R.S.F ± S.E
0	512	93, 100, 103	100	100
50	500	56, 58, 49	56.5 ± 2.3	48.9 ± 2.2
125	505	92, 90, 96	95.5 ± 1.4	36.4 ± 1.0
250	516	78, 84, 86	83.4 ± 1.9	24.1 ± 0.8
375	501	104, 98, 107	104.9 ± 1.8	24.4 ± 0.6
500	503	86, 87, 90	90.7 ± 1.0	14.2 ± 0.3

Table 9:- Residual survival data for the CHSE cells irradiated in Table 8 after 10 population doublings post exposure to UVB and then illuminated with 9,000 (J/m²) UVA to photoreactivate the damage.

UVB Dose / (J/m ²)	Initial % S.F ± S.E	Residual % S.F ± S.E
0	100	100
50	85.0 ± 1.3	47.1 ± 2.3
125	36.9 ± 1.6	30.1 ± 4.5
250	29.2 ± 0.9	23.8 ± 1.0
375	23.9 ± 0.4	24.3 ± 0.8
500	16.0 ± 0.3	13.4 ± 0.4

Table 10:- Mean survival data from Figure 6.7 for the CHSE cells irradiated with UVB and then illuminated with 9,000 (J/m²) UVA to photoreactivate the damage.

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UVA Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	CHSE % positive cells	EPC % positive cells
0	0	0	0	0
2500	64.0 ± 16.3	15.9 ± 2.9	50.2 ± 10.2	100
30000	100	56.3 ± 16.2	100	100
UVA Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	4.6 ± 0.4	0	0
2500	20.6 ± 1.9	38.1 ± 3.1	26.8 ± 12.1	100
30000	100	36.6 ± 22.5	100	100

Table 1:- Showing the % positive *bax* cells post exposure to UVA, initially and after 10 population doublings (passage 1). Errors are expressed as the standard error on the mean for n=3.

UVA Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	CHSE % positive cells	EPC % positive cells
0	0	0	0	0
2500	86.0 ± 24.5	28.7 ± 4.3	35.0 ± 14.0	100
30000	100	33.9 ± 8.4	100	100
UVA Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	7.3 ± 0.8	0	0
2500	38.2 ± 11.7	20.5 ± 1.1	46.8 ± 8.3	100
30000	100	63.2 ± 30.9	100	100

Table 2:- Showing the % positive *bax* cells post exposure to UVA, initially and after 10 population doublings (passage 1). Errors are expressed as the standard error on the mean for n=3.

UVA Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	CHSE % positive cells	EPC % positive cells
0	0	0	0	0
2500	75.0 ± 20.4	22.3 ± 3.6	42.6 ± 12.1	100
30000	100	45.1 ± 12.3	100	100
UVA Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	5.8 ± 0.6	0	0
2500	29.4 ± 6.8	29.3 ± 2.1	36.8 ± 10.2	100
30000	100	49.9 ± 26.7	100	100

Table 3:- Showing the mean % positive *bax* cells from tables 1 and 2 post exposure to UVA, initially and after 10 population doublings (passage 1). This data is shown in Table 5.9 in the results section.

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UVB Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	UVB Dose / (J/m ²)	CHSE % positive cells	EPC % positive cells
0	0	0	0	0	0
100	26.8 ± 6.4	10.6 ± 4.5	50	100	100
700	100	0	500	100	100
UVB Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	UVB Dose / (J/m ²)	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	0	0	0	0
100	100	0	50	100	100
700	100	0	500	100	100

Table 1:- Showing the % positive *bax* cells post exposure to UVB, initially and after 10 population doublings (passage 1). Errors are expressed as the standard error on the mean for n=3.

UVB Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	UVB Dose / (J/m ²)	CHSE % positive cells	EPC % positive cells
0	0	0	0	0	0
100	38.2 ± 3.6	16.6 ± 6.3	50	100	100
700	100	0	500	100	100
UVB Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	UVB Dose / (J/m ²)	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	0	0	0	0
100	100	0	50	100	100
700	100	0	500	100	100

Table 2:- Showing the % positive *bax* cells post exposure to UVB, initially and after 10 population doublings (passage 1). Errors are expressed as the standard error on the mean for n=3.

UVB Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	UVB Dose / (J/m ²)	CHSE % positive cells	EPC % positive cells
0	0	0	0	0	0
100	32.5 ± 5.0	13.6 ± 5.4	50	100	100
700	100	0	500	100	100
UVB Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	UVB Dose / (J/m ²)	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	0	0	0	0
100	100	0	50	100	100
700	100	0	500	100	100

Table 3:- Showing the mean % positive *bax* cells from tables 1 and 2 post exposure to UVB, initially and after 10 population doublings (passage 1). This data is shown in table 5.10 in the results section.

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UVA Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	CHSE % positive cells	EPC % positive cells
0	26.8 ± 4.7	100	10.9 ± 5.3	100
2500	50.6 ± 9.6	0.0	100	100
30000	24.8 ± 9.3	100	100	100
UVA Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	100	12.5 ± 4.7	100
2500	0	100	100	100
30000	0	100	100	100

Table 1:- Showing the % positive *bcl-2* cells post exposure to UVA, initially and after 10 population doublings (passage 1). Errors are expressed as the standard error on the mean for n=3.

UVA Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	CHSE % positive cells	EPC % positive cells
0	39.8 ± 5.7	100	15.3 ± 1.7	100
2500	64.8 ± 8.2	100	100	100
30000	11.6 ± 5.3	100	100	100
UVA Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	100	24.5 ± 6.7	100
2500	0	100	100	100
30000	0	100	100	100

Table 2:- Showing the % positive *bcl-2* cells post exposure to UVA, initially and after 10 population doublings (passage 1). Errors are expressed as the standard error on the mean for n=3.

UVA Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	CHSE % positive cells	EPC % positive cells
0	33.3 ±5.2	100	13.1 ± 3.5	100
2500	57.7 ±8.9	100	100	100
30000	18.2 ± 7.3	100	100	100
UVA Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	100	18.5 ± 5.7	100
2500	0	100	100	100
30000	0	100	100	100

Table 3:- Showing the mean % positive *bcl-2* cells post exposure to UVA, initially and after 10 population doublings (passage 1). This data is shown in Table 5.11 in the results section.

UVB Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	UVB Dose / (J/m ²)	CHSE % positive cells	EPC % positive cells
0	0	100	0	19.8 ± 5.8	100
100	14.9 ± 4.6	100	50	100	100
700	0	100	500	100	100
UVB Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	UVB Dose / (J/m ²)	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	100	0	11.9 ± 5.3	100
100	0	100	50	100	100
700	0	100	500	100	100

Table 1:- Showing the % positive *bcl-2* cells post exposure to UVB, initially and after 10 population doublings (passage 1). Errors are expressed as the standard error on the mean for n=3.

UVB Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	UVB Dose / (J/m ²)	CHSE % positive cells	EPC % positive cells
0	0	100	0	24.2 ± 6.8	100
100	16.1 ± 9.6	100	50	100	100
700	0	100	500	100	100
UVB Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	UVB Dose / (J/m ²)	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	100	0	9.5 ± 3.3	100
100	0	100	50	100	100
700	0	100	500	100	100

Table 2:- Showing the % positive *bcl-2* cells post exposure to UVB, initially and after 10 population doublings (passage 1). Errors are expressed as the standard error on the mean for n=3.

UVB Dose / (J/m ²)	HaCaT % positive cells	HaCaT-ras % positive cells	UVB Dose / (J/m ²)	CHSE % positive cells	EPC % positive cells
0	0	100	0	0	100
100	0	100	50	100	100
700	0	100	500	100	100
UVB Dose / (J/m ²)	HaCaT % positive cells Passage 1	HaCaT-ras % positive cells Passage 1	UVB Dose / (J/m ²)	CHSE % positive cells Passage 1	EPC % positive cells Passage 1
0	0	100	0	0	100
100	0	100	50	100	100
700	0	100	500	100	100

Table 3:- Showing the mean % positive *bcl-2* cells post exposure to UVB, initially and after 10 population doublings (passage 1). This data is presented in Table 5.12 in the results section.

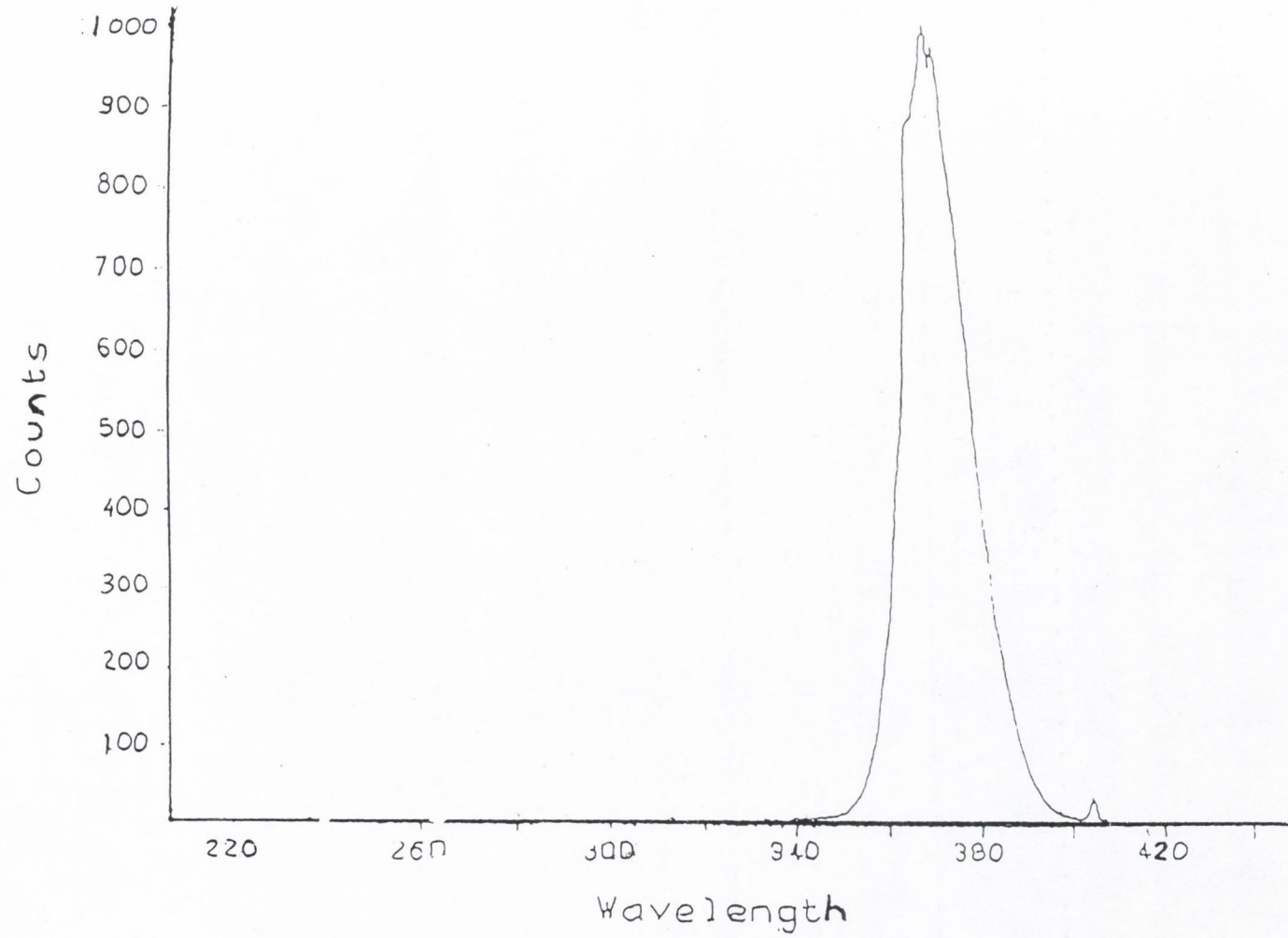


Figure 1:- Spectral output of the UVG-L58 lamp used to irradiate cells with UVA and photoreactivating light.

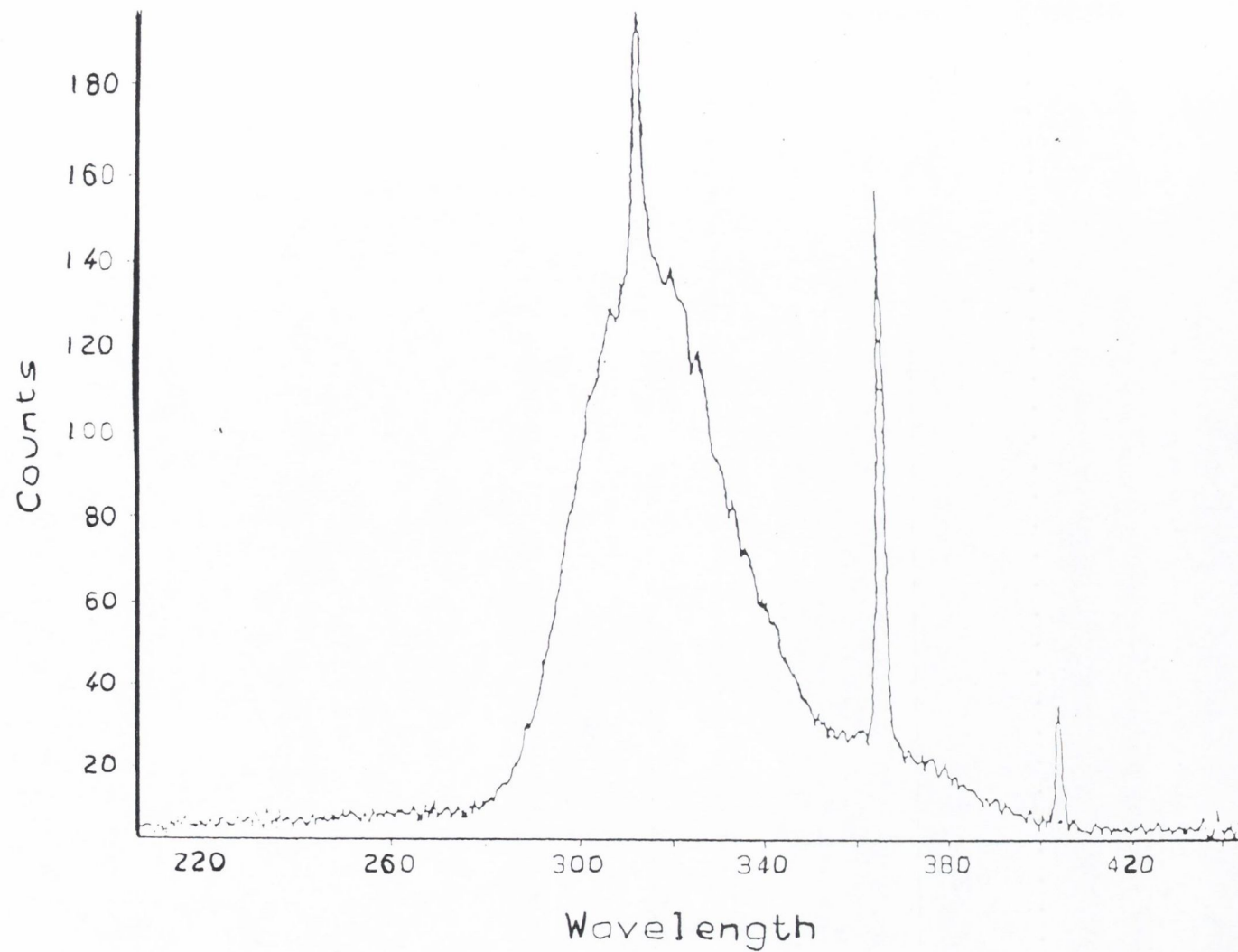


Figure 2:- Spectral output of the UVLM-57 lamp used to irradiate cells with UVB.

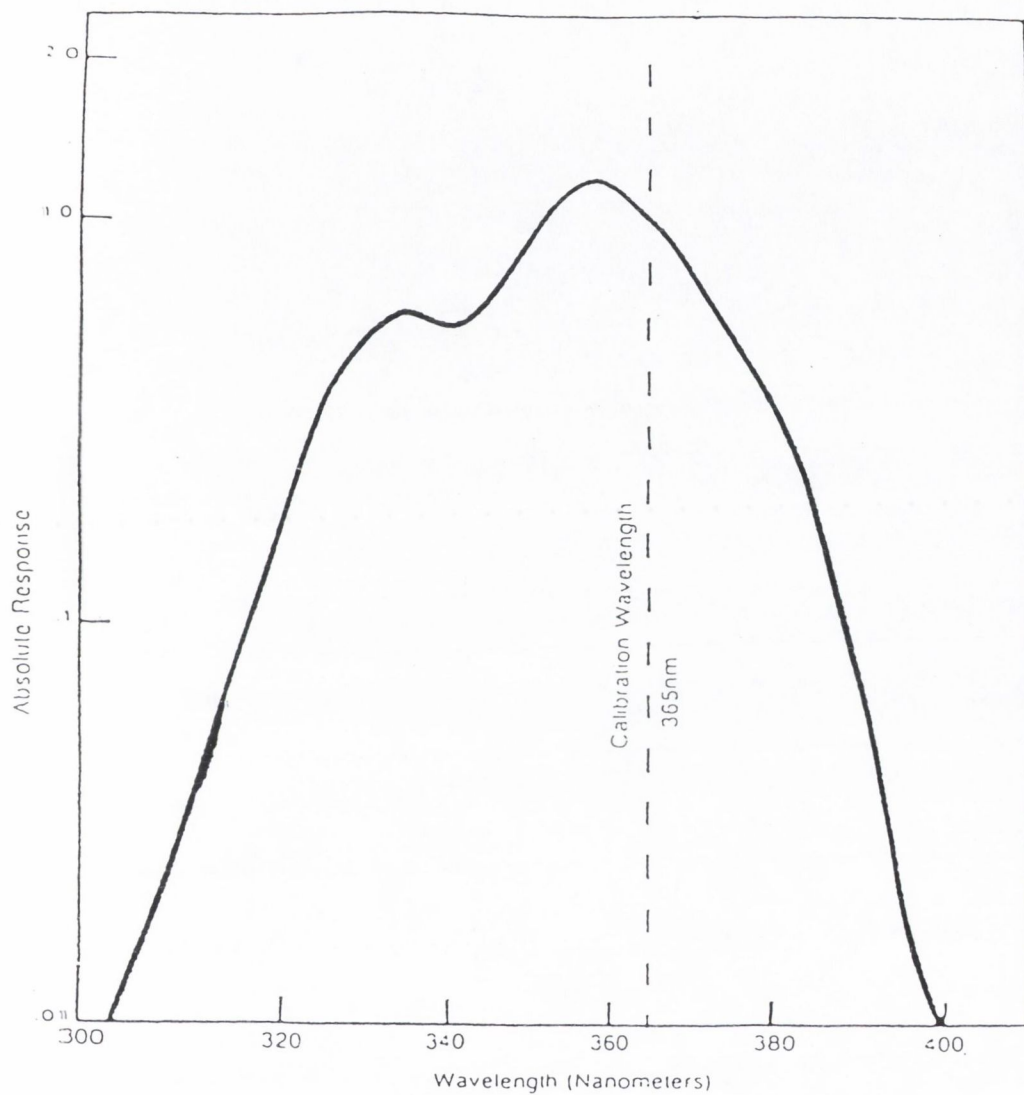


Figure 3:- Spectral response of the UVX-36 long range radiometer used to measure the irradiance of the UVG-L 58 lamp.

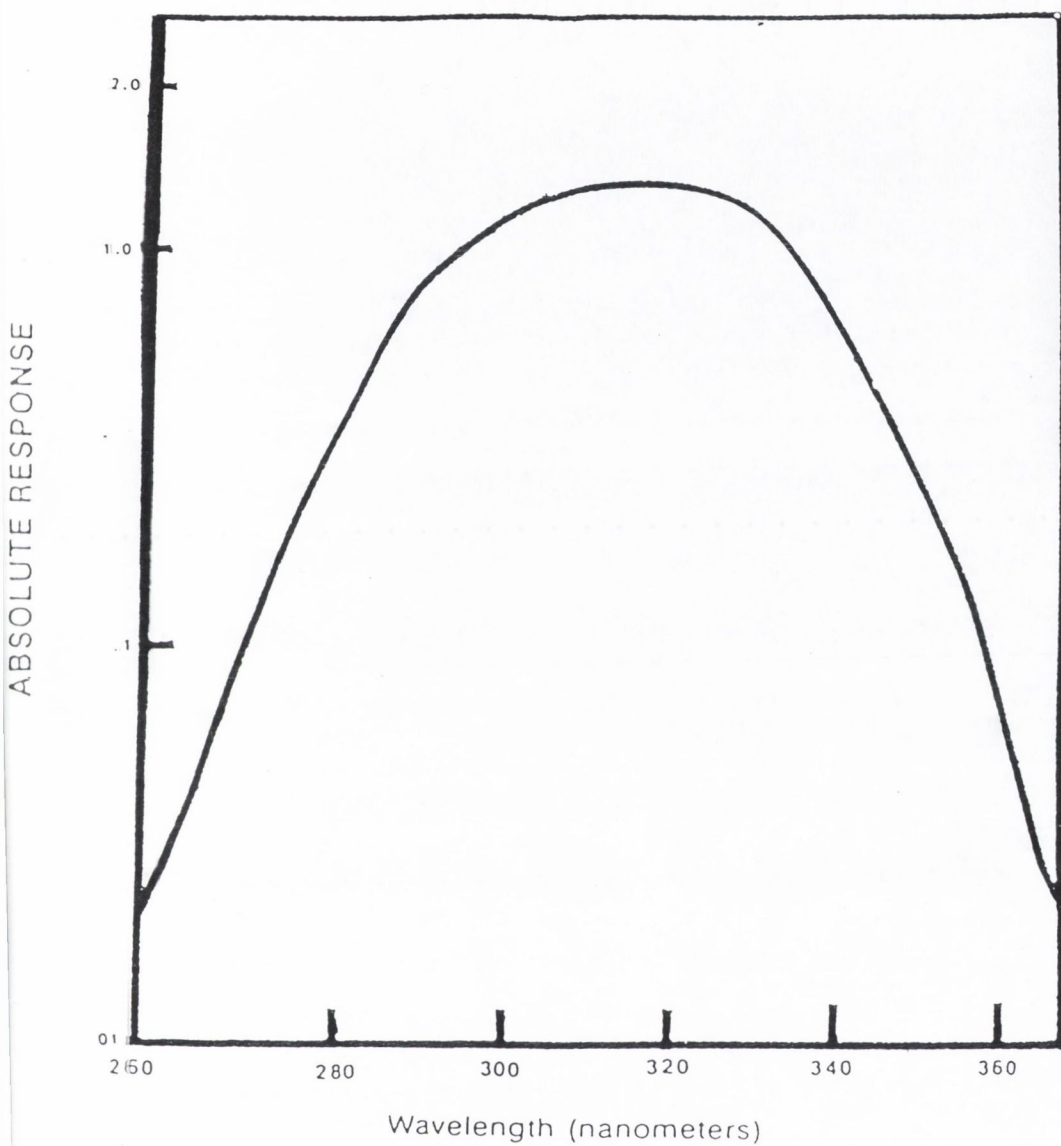


Figure 4:- Spectral response of the UVX-31 mid-range radiometer used to measure the irradiance of the UVLM-57 lamp.