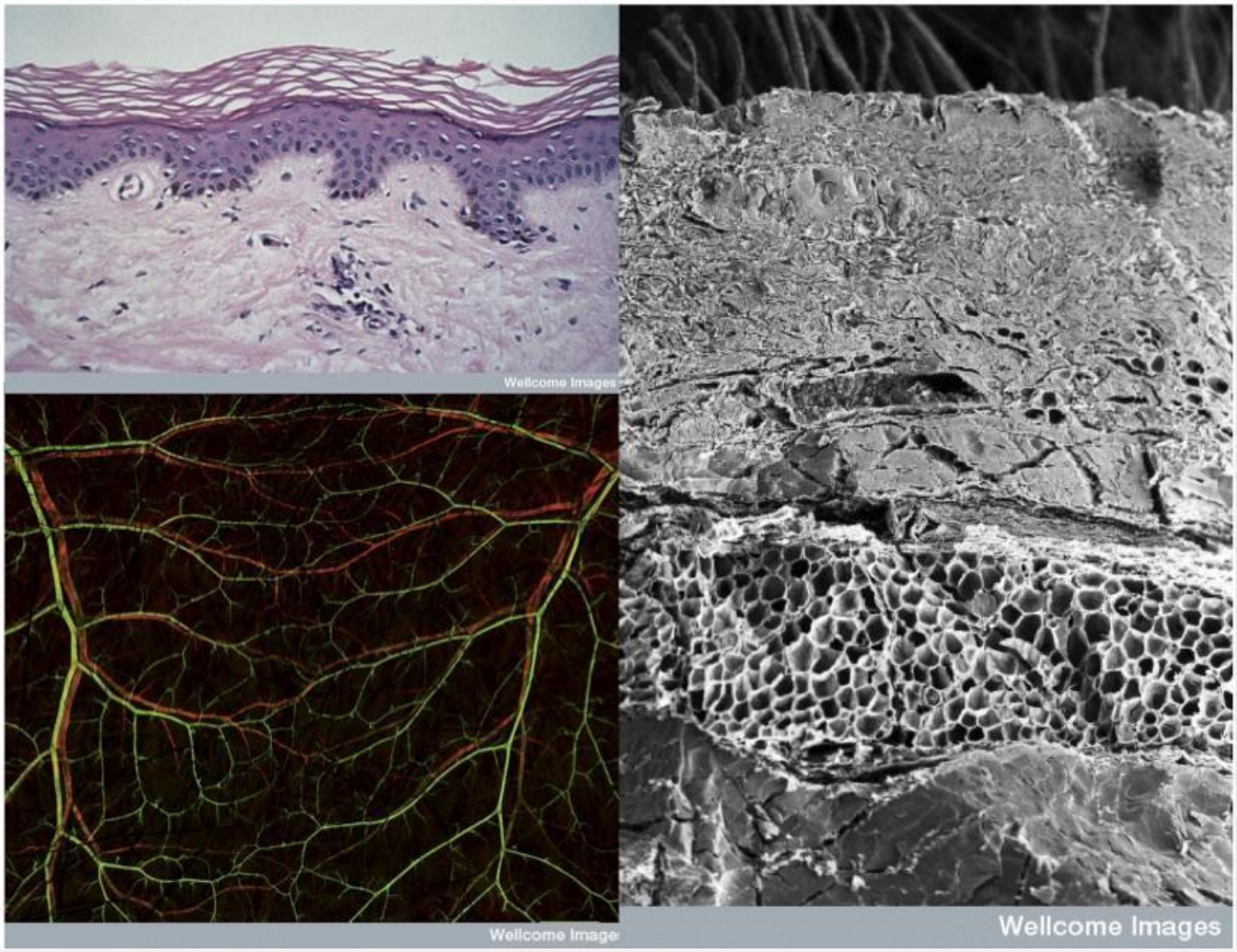
The Scholars Programme



How safe is your shampoo? How the ban on animal testing for cosmetics has changed safety testing.

Dr Stewart

Autumn 2015

Student:

Grade	Marks	What this means
1st	70+	Performing to an excellent standard at first year undergraduate level
2:1	60-69	Performing to a good standard at first year undergraduate level
2:2	50-59	Performing to an excellent standard at GCSE level
3rd	40-49	Performing to a good standard at GCSE level
Workings towards a pass	0-39	Workings towards a pass
Did not submit	DNS	No assignment received by The Brilliant Club

Lateness	
Any lateness	10 marks deducted
Plagiarism	
Some plagiarism	10 marks deducted
Moderate plagiarism	20 marks deducted
Extreme plagiarism	Automatic fail

Name of PhD Tutor	
Title of Assignment	

Name of Pupil	
Name of School	

ORIGINAL MARK / 100		**FINAL MARK / 100**	
DEDUCTED MARKS		**FINAL GRADE**	

Learning Feedback Comment 1 -	
What you did in relation to this Key Learning Priority *Enter feedback here*	**How you could improve in the future** *Enter feedback here*
Learning Feedback Comment 2 –	
What you did in relation to this Key Learning Priority *Enter feedback here*	**How you could improve in the future** *Enter feedback here*
Learning Feedback Comment 3 –	
What you did in relation to this Key Learning Priority **Enter feedback here**	**How you could improve in the future** **Enter feedback here**
Resilience Comment –	
How you showed learning resilience during the course *Enter* *feedback here*	**How you could build learning resilience in the future** *Enter feedback here*

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Course Rationale

In response to growing concerns for consumers about the ethics of animal testing for cosmetics, in the early 1990's the EU set out to stop the use of laboratory animals for the testing of cosmetic products. This was not something that could happen overnight as we need alternative was of making sure that our cosmetics were safe. But how can a few cells in a dish really equate to the effect of our cosmetic production on real people? The course is aimed at students who have a keen interested into how scientist solve complex problems and how we determine if scientific models really predict real world effects.

The initial tutorial will introduce the historical prospective as why animal testing was introduced and what it is used for focusing on the development of topical skin products. Pupils will be challenged to develop their own view on toxicology testing and develop an understanding of the key role skin plays in our body's defences. The tutorials will then go on to explain the development of in vitro/in silico models for toxicity, the limitations and challenges faced in the development of these models. This course looks to develop scientific thinking in the student's and how validated methods are developed and tested. This course looks to develop scientific thinking in the student's and how validated methods are developed and tested. In addition student will be able to develop an appreciation of the ethics concerns faced by scientists and will challenge their personal views of the use of animals in research.

Mark Scheme

Key Skill	1st	2.2
Logical reasoning	☐ There is a clear, logical and structured flow to argument. ☐ Justifications for the study are well reasoned and clearly supported by the literature, but the limitations of the suggested method are also recognised and discussed.	☐ The structure of arguments may be unclear in places and difficult to follow. ☐ Justifications for the study poorly reasoned and weakly or not supported by the literature, but the limitations are not fully identified.
Analysis and evaluation	☐ Critical evaluations of the methods are logical – the limitations and benefits of the methods are identified. ☐ Made logical suggestions for a new study design or was able to successful support the method selected from the literature.	☐ Critical evaluations of the methods are not always logical – the limitations and benefits of the methods are not full identified. ☐ Was able to method selected from the literature, but justification of selection was weak.
Research Skills	☐ All the outside sources are correctly referenced in the agreed format, which is appropriate for the scientific style. ☐ Uses a purposeful but imaginative range of sources; reliable, relevant, and balanced in their choice. ☐ Comprehensive research is evidenced; sources of information go beyond the obvious choices and those suggested throughout the course.	☐ Sources are mostly but not always referenced correctly. ☐ A limited range of sources outside of the course reading has been used. ☐ Sources that have been found outside of the course reading may be unreliable; inappropriate for the subject; or heavily biased; limited attempt made to balance these with other sources.
Language	scientific vocabulary ly. ocabulary shows a g of the concepts xplicitly covered in ider reading without	☐ Uses simple scientific vocabulary effectively but struggles with new vocabulary that is more challenging, in particular new vocabulary from their own wider reading. ☐ Scientific vocabulary may be used incorrectly at times.
Communication	☐ Writing logical and organised such that it does not detract from the content. ☐ Appropriate use of punctuation. ☐ A high level of proficiency in both spelling and grammar. ☐ Writing follows an appropriate scientific style.	☐ Writing is not always logical and organised such that it detracts from the content. ☐ Absence of an appropriate use of punctuation. ☐ Poor use of both spelling and grammar. ☐ Writing follows an appropriate scientific style.

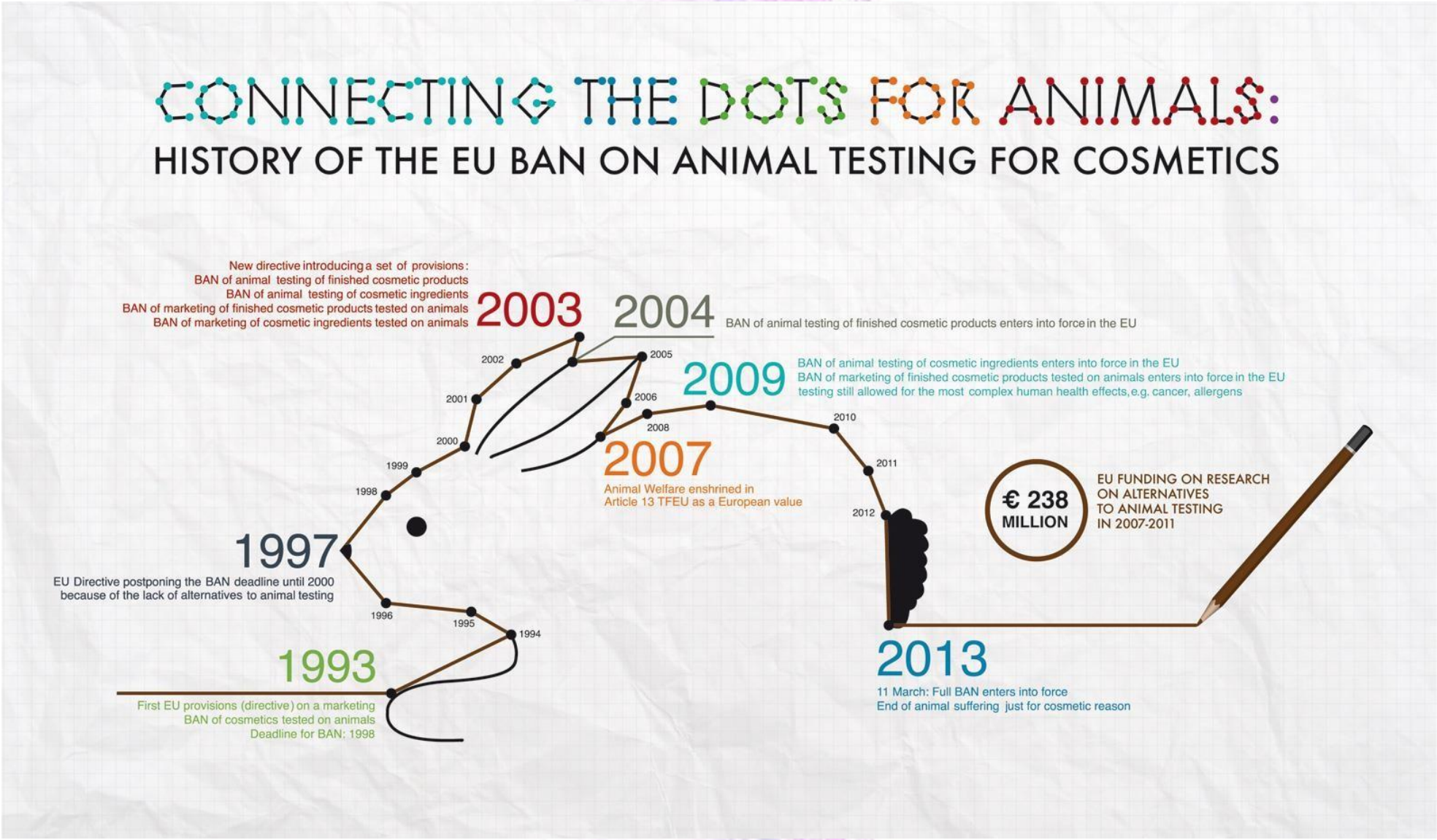
Glossary of Keywords

The table below describes the key word used in the course:

Keywords	Definitions
Ethics	
Allergen	
Corrosion	
Cytokines	
Dermis	
ELISA	
Epidermis	
Franz cells	
In silico	
***In silico* modelling**	
In vitro	
In vivo	
Inflammation	
Interspecies variation	
Intraspecies variation	
Irritation	
Keratinocytes	
Method validation	
Method verification	
MTT assay	
OECD	
Risk assessment	
Skin permeation	

The table below describes the key word used in the course:

Keywords	Definitions
Stratum corneum	
Toxicology	
Viability	


CONNECTING THE DOTS FOR ANIMALS:
HISTORY OF THE EU BAN ON ANIMAL TESTING FOR COSMETICS
New directive introducing a set of provisions:
BAN of animal testing of finished cosmetic products
BAN of animal testing of cosmetic ingredients
BAN of marketing of finished cosmetic products tested on animals
BAN of marketing of cosmetic ingredients tested on animals
2003
2004
BAN of animal testing of finished cosmetic products enters into force in the EU
2009
BAN of animal testing of cosmetic ingredients enters into force in the EU
BAN of marketing of finished cosmetic products tested on animals enters into force in the EU
testing still allowed for the most complex human health effects, e.g. cancer, allergens
2007
Animal Welfare enshrined in
Article 13 TFEU as a European value
1997
EU Directive postponing the BAN deadline until 2000
because of the lack of alternatives to animal testing
1993
First EU provisions (directive) on a marketing
BAN of cosmetics tested on animals
Deadline for BAN: 1998
1994
1995
1996
1998
1999
2000
2001
2002
2005
2006
2008
2010
2011
2012
€ 238
MILLION
EU FUNDING ON RESEARCH
ON ALTERNATIVES
TO ANIMAL TESTING
IN 2007-2011
2013
11 March: Full BAN enters into force
End of animal suffering just for cosmetic reason

Tutorial 1 The purpose and limitation of animal testing

By the end of Tutorial 1 you should be able to answer these questions:

- ☐ Why is animal testing used?
- ☐ What are the scientific limitations of animal testing?
- ☐ What are the ethical issues associated with animal testing?
- ☐ What are the 3Rs?

Activity 1 – The ethical issues associated with animal testing

Considering the class debate on the ethics of animal testing summarise what you feel are the key points of this discussion below:

Activity 2 Defining the 3 Rs

Draw lines to match each of the 3 Rs to the correct definitions then 2 methods of applying each of the principles:

The 3 Rs	Definitions	Methods of applying the principle
Reduce	Is any decrease in the number or severity of 'inhumane' procedures applied to those animals that still have to be used.	Social housing for animals Use of mathematical modeling to demine the number of animals required for an experiment
Refine	The substitution of conscious living higher animals for insentient material.	Toys for animals AMES test uses bacteria to test for chemicals that may cause cancer
Replace	Lower the number of animals used to obtain information.	Using by products of food production (i.e. pig heart) for research Using inbred animals means researchers can get reliable results from fewer of them.

Activity 3 Application of the principles of the 3 Rs

You have just shown you two different movies, they both demonstrate examples of application of the 3 Rs under each of the principles identify were the principles of the 3 Rs where used:

Reduce	Refine	Replace

Tutorial 1 Baseline Test

The homework assignment for the first tutorial is a baseline test to see your initial level of attainment in this subject area. The assignment will test for some or all of the subject specific skills that are required later in the final assignment.
However, it is shorter than the final assignment and is will be an introduction to the subject as well as a challenge!

Do not worry too much about doing 'well' or 'badly' on the baseline test, it takes into account the fact that you may not be familiar with the subject area. It is designed to help you and your PhD tutor identify where you are at the start of the programme and to help you measure your progress along the way.

1.1: Describe the structure and function of key immune cells

The immune response is stimulated in the body by antigens. Antigens are foreign, protein-based substances. They are often found attached to the surface of pathogens such as …… and ……

White Blood Cells

Draw a diagram of phagocyte

Phagocytes destroy pathogens by:

……

……

……

Draw diagram of a lymphocyte:

Lymphocytes destroy pathogens by:

……

……

.....

.....

.....

Compare the functions of B and T lymphocytes:

.....

.....

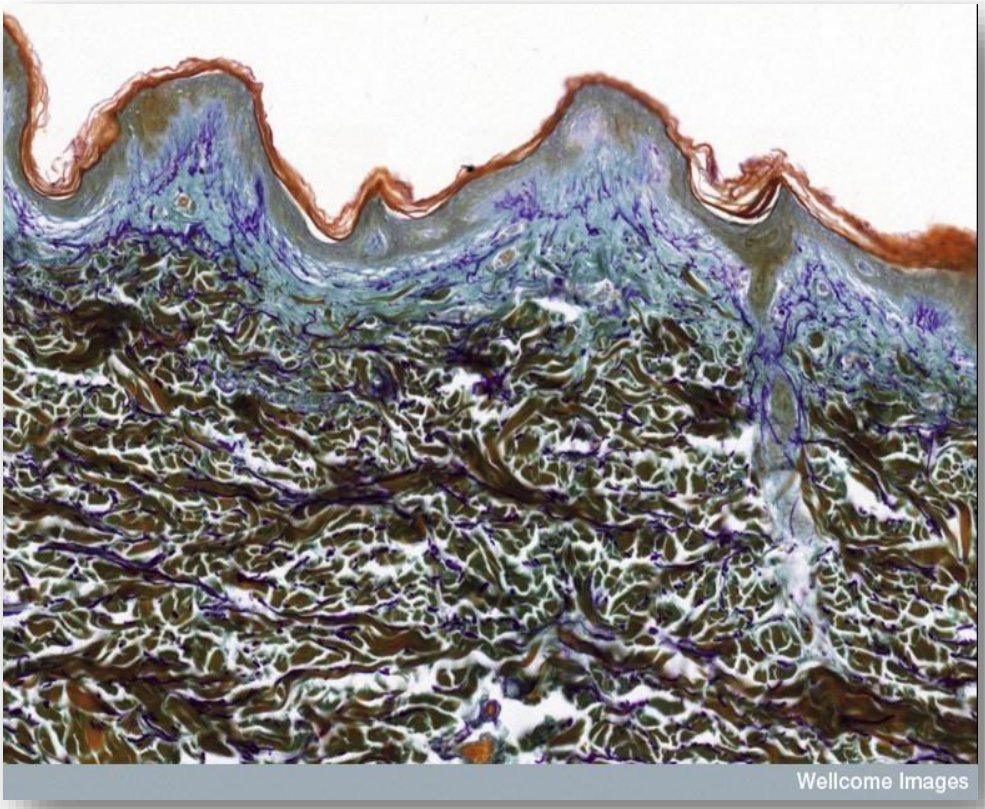
.....

.....

.....

.....

Tutorial 2 Structure and function of human skin



By the end of Tutorial 2 you should be able to answer these questions:

- ☐ Introduce the structure and function of the skin
- ☐ Introduce the types of skins cells
- ☐ Discuss the way application of chemicals to the skin could have a negative outcome.

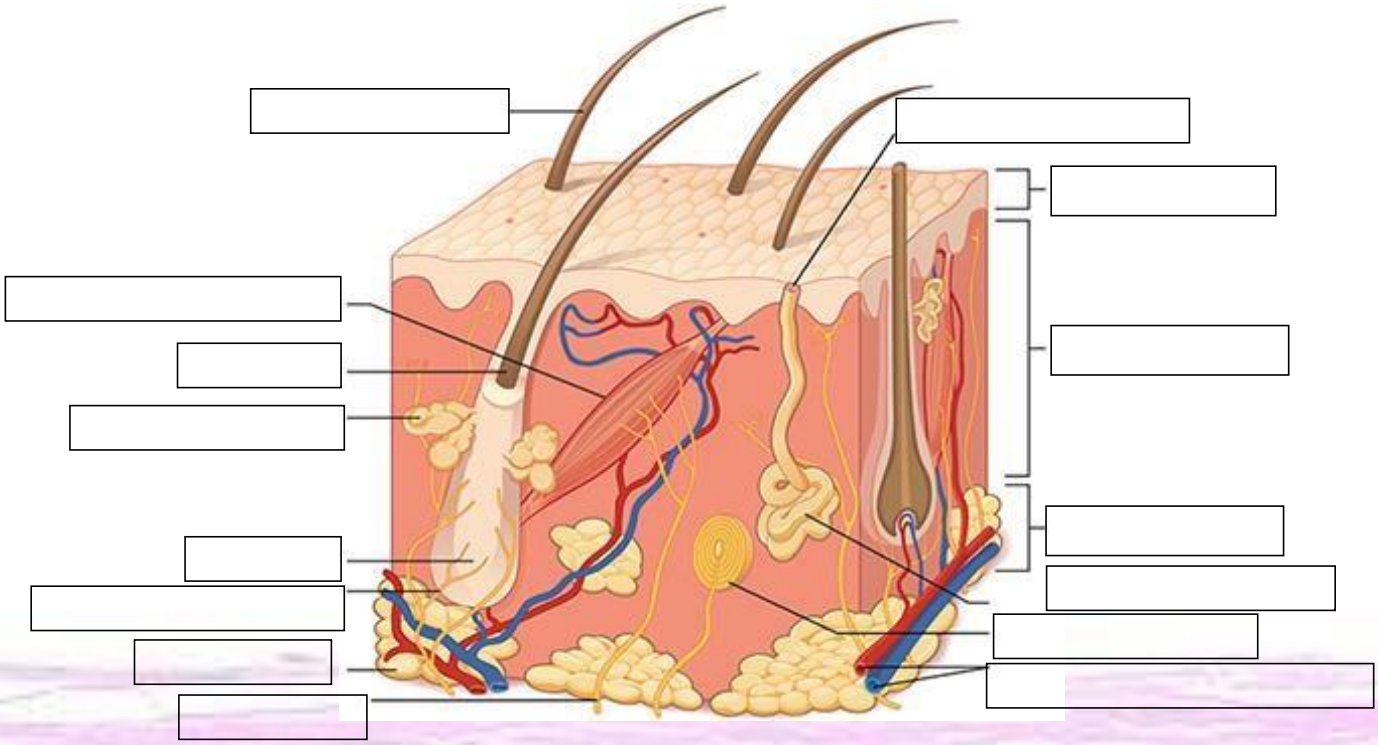
Activity 1 The function of human skin

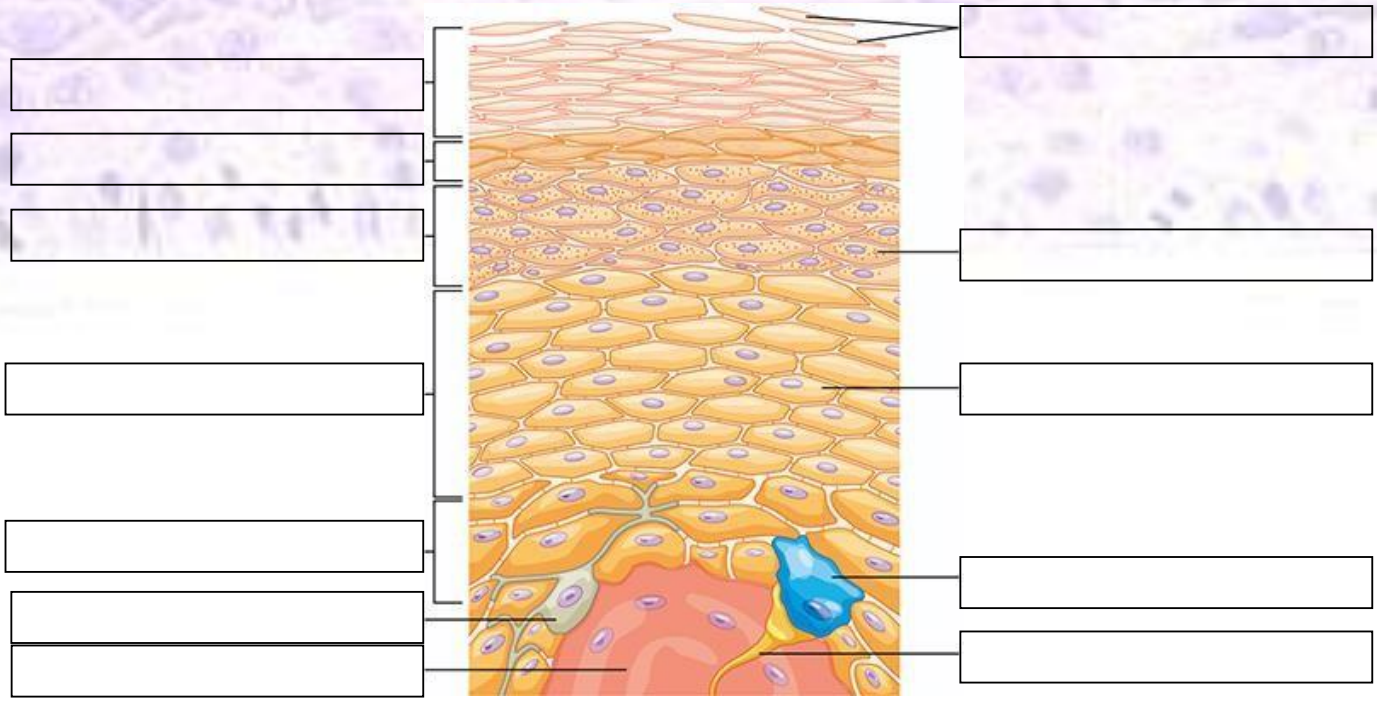
Following the discussion of function of human skin complete the table below to describe the key functions of human skin:

Function	Description

Activity 2 The structure of human skin

Label the diagrams below to describe the structure of skin:





Activity 3 Adverse effects of applications of chemicals to skin

Complete the following descriptions of the skin reactions to chemicals:

Skin Corrosion: The production of irreversible_____to the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to_____.

Skin Irritation: The production of reversible_____changes in the skin resulting in itchiness,_____and_____.

Allergic contact dermatitis: Is caused by contact with a_____that causes a type IV or 'delayed hypersensitivity' reaction. A sensitiser is a substance that can induce an 'over-reaction' of the_____.

Tutorial 2 Homework

The following cell types are components of skin for each cell provide and diagram, the location of this cell in the type in the skin and its function:

Keratinocytes

Location in the skin:_____.

Function.....

.....

.....

.....

.....

.....

.....

Langerhans cell

Location in the skin:_____.

Function.....

.....

.....

.....

.....

Fibroblast

Location in the skin:_____.

Function.....

.....

.....

.....

.....

Melanocytes

Location in the skin:_____.

Function.....

.....

.....

.....

.....

.....

.....

Tutorial 3 – Introduction to the alternatives to animal testing

By the end of Tutorial 3 you should be able to answer these questions:

- ☐ What are types of method used as alternatives to animal testing?
- ☐ What are the benefits of each method?
- ☐ What are the limitations of each method?

Activity 1 – Summary of the method used as alternatives to animal testing

For each of the alternative method determine a positive and negative attributes:

Alternative Method	Benefits of this method	Limitations of this method
Reconstructed human epidermis		
Ex vivo human skin		
Cell based assay		

***In silico* methods**		

Tutorial 2 – Homework

You are to read the following (pages 20 –34) exert for Advances in Dermatological Sciences and answer the following question in no more than 500 words.

Two models are described for the investigation for skin irritation and corrosion *in vitro* you are to determine the method which do you think would be the best option for testing a new moisturiser and why?

1 INTRODUCTION

1.1 Skin irritation and corrosion

Any chemical that is applied to the skin has the potential to induce a skin reaction, these reactions are complicated and are difficult to fully replicate *in vitro* and may exhibit species variations when tested *in vivo*. Skin irritation is the observed response (erythema, edema, pain itching and heat) to chemicals that result in inflammation at the site of application. Skin irritation is defined as reversible damage of the skin following application of a chemical for up to 4 h (1). By contrast, skin corrosion is defined as ''irreversible damage to the skin, namely visible necrosis through the epidermis and into the dermis, following the application of a test substance for a period of 3 min up to 4 h (2).

1.2 Mechanisms of skin irritation

The skin is the largest human organ, with functions that far exceed it's primary role as a protective barrier. The skin is the main target tissue for external toxins, provides protection from environmental hazards, UV-irradiation and water loss. The skin is composed of multiple layers serving multiple essential functions including metabolism of chemicals which come in contact with the skin. Further to this when the skin is exposed to a chemical, specific immunological and histological responses can occur. The skin is composed of three layers all of which are dominantly populated by keratinocytes therefore it is not surprising that keratinocytes are the key player in many skin functions including the immune responses resulting from chemical exposure. In response to physical or chemical stresses, keratinocytes produce and release cytokines including interleukins IL-1α, IL-8, IL-7, IL-15, tumour necrosis factor α (TNF-α), interferon induced protein 10 (IP-10), granulocyte/macrophage colony-stimulating factor (GM-CSF), transforming growth factor (TGF) and other signalling factors which initiate cutaneous inflammation (3).

The mechanism by which chemicals induce skin irritation can vary depending on the innate properties of the chemical. By far the most investigated mechanism by which chemicals initiate irritation is the mechanism that results from exposure to surfactants such as SDS (a

commonly used model irritant (4, 5). Surfactants initiate skin irritation by disruption of cellular membranes resulting in extracellular release of pro-inflammatory cytokines which normally reside within keratinocytes. This in turn results in an inflammatory cascade, from which skin irritation is the result (6). A typical skin inflammatory response is described in Figure 1 and the key cytokines and inflammatory mediators expressed during this reaction.

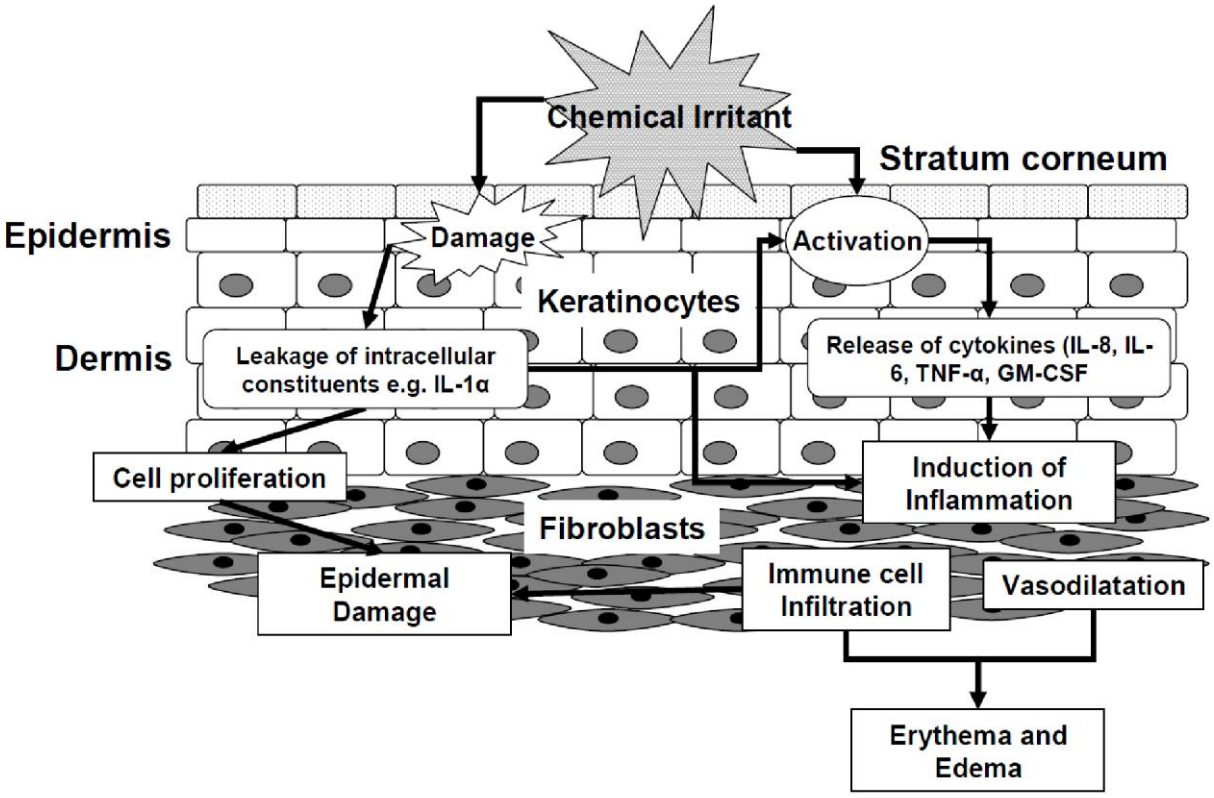


Figure 1. Inflammatory responses in skin

1.3 *In vitro* alternatives to animal testing for skin irritation

The potential for a chemical to cause skin irritation is an important safety consideration and therefore forms part of toxicological routine evaluation. Historically, the evaluation of the potential of a chemical to produce skin irritation has been carried out in rabbits using the Draize skin irritation test according to OECD TG 404 and Method B.4 of Annex V to Directive 67/548/EEC (7). This involves the application of chemicals to the animal's skin and evaluation of visible changes such as erythema and edema. The chemicals are then graded for erythema and edema and a primary irritation index (PII) is calculated as shown in Equation 1.

$$\text{Primary Irritation Index (PII)} = \frac{\sum \text{erythema grades at 24/48/72/ h} + \sum \text{edema grades at 24/48/72/ h}}{3 \times \text{number of animals}}$$

Equation 1. Calculation of Primary Irritation Index

Despite this method being the regulatory accepted method for determining the irritancy of new chemicals it has come under a lot of scrutiny in recent years. From a scientific prospective the relevance of the rabbit test to estimate human skin irritation hazards has been seriously questioned due to species differences in both barrier properties and reactions to certain irritants (8). Additionally over the last few decades there has been increasing pressure from a variety of sources to limit and replace this test particularly with regard to the testing of cosmetic products. This has lead to the development of a number of *in vitro* models to assess skin irritation *in vitro*, the predominate type of model resulting from this are the Reconstructed Human Epidermis (RHE) models. Other possibilities for *in vitro* models which could be further investigated include keratinocyte cultures and *ex vivo* skin models.

1.3.1 Reconstructed Human Epidermis

In an attempt to develop new methods for assessment of the effect of chemicals on the skin, various reconstructed human equivalent models have been developed and used for skin irritancy testing including Skinethic RHE (9), EpiDerm (10), Episkin (11), Prediskin (10), RE-DED (12), Apligraf (13) Skin (14) and LSE / HSE (15). There are two distinct types of models; the first is the epidermal equivalents which are produced by culturing adult human keratinocytes on synthetic matrices. The second are the full skin equivalents which consist of multilayering, differentiating human keratinocyte cultures grown on fibroblasts containing collagen matrices. Both model types use conditions which permit terminal differentiation and the reconstruction of an epidermis with a functional horny layer achieved by growing the tissue at the liquid air interface (6).

Three of these models (EpiDerm™ SIT (EPI-200), SkinEthic™ RHE and EpiSkin™) have validated protocols for the prediction of skin irritation (1) either as full replacements for animal testing or as a partial replacement test, within a tiered testing strategy depending on the specific member countries regulations. The use of RHE models for the prediction of skin irritation testing involves the topical application of test materials to the surface of the epidermis, and the subsequent measurement of its cytotoxic effects, by MTT assay. (18, 19, 20).

Though the general structure, composition and aspects of their biochemistry bare a close resemblance to human skin, RHE models exhibit differences in terms of barrier function which leads to the potential for false positive results. Several studies have investigated the reason for the deficit in barrier function, including the presence of unkeratinized microscopic foci (21) and the presence of desmosomal structures with preserved lamellar appearance demonstrating that the formation of corneosomes is compromised *in vitro* (22) The weakness of the barrier function is improved in the full thickness models were a dermis is also present but despite this deficiencies still persists in barrier function in these models (23). This means there is still both room for improvement in these models but also scope for development of new models.

1.3.2 *Keratinocytes cultures*

Keratinocytes play a pivotal role in inflammatory responses in skin. A number of studies have investigated the possibility of using keratinocytes in culture as a model for the identification of dermal irritants. Eun *et. al.* (1994) compared the effects of irritants on human oral and skin keratinocytes by determining mitochondrial metabolism using the MTT assay and measuring membrane integrity by the LDH assay. They showed that both assays were equally sensitive to the irritants tested. They concluded skin keratinocytes would be a suitable model for evaluating if a chemical would by an irritant on either the skin or oral mucosa (24). Muller-Decker *et. al.* (1994) used pro-inflammatory mediators (IL-1α and proinflamatory eicosanoids) and cell viability in human keratinocytes to develop an assay for skin irritation. They concluded that keratinocytes *in vitro* respond to chemicals of graded irritant potential

with graded release of proinflamatory mediators. They also suggest that for complete assessment of a compound multiple mediator endpoints should be studied (25). Cohen *et. al.* (1991) studied the production and release of IL-1α and prostaglandin E_2 (PGE_2) in response to inflammatory stimuli following UVB irradiation and detergent injury by SDS. They demonstrated both inflammatory stimuli cause an increase in intracellular IL-1α and PGE_2 levels followed by extracellular release. They suggest measurement of both markers both intra and extracellular may provide an *in vitro* test to detect potentially irritant products (26). Gueniche and Ponec (1993) studied the effects of surfactants on keratinocytes and fibroblast cell cultures derived from human skin and SV40 transformed human keratinocytes (SVK14 cells) for *in vitro* screening of skin toxicity. The results of this study showed that changes in the morphology and proliferation of cultured skin cells *in vitro* showed a similar ranking order to *in vivo* skin irritancy data and that nearly all of the surfactants tested increased IL-6 production (27). Together these studies show the enormous potential for using keratinocytes for the evaluation of potential skin irritants.

1.3.3 *Human skin cultures*

In addition to the cells based systems already discussed an alternative model for investigation skin irritation is excised human skin cultures. An excised human skin culture, has the advantages of the presence of differentiating keratinocytes and of the possibility of topical application of both water soluble and insoluble test compounds. The intact stratum corneum provides a physiological barrier between the chemical and living cells. In this organ culture model, *ex vivo* human skin discs are cultured on a micro porous membrane, which allows transport of culture medium through the dermis into the epidermis, whereas the epidermal side remains free of direct contact with culture medium. Therefore test substances can be applied, directly to the skin surface, in a manner similar to those encountered in *in vivo* exposure. Despite the potential of such a model there is little reference to this type of model using human tissue in the literature. There are references in the literature to animal derived skin irritancy cultures including rabbit skin (28) and skin from hairless mice (29). Though these cultures may have use, they have the same issues arising from *in vivo* animal testing, namely potential lack of correlation between animal and human skin and the ethical issue surrounding the use of animals for testing.

Pistoor *et. al.* (1996) investigated the potential use of human skin explant cultures for the identification of contact allergens. In this skin explants were dosed with two dermatological inactive compounds, five irritants and six contact allergens. Post treatment skin explants were immunohistochemically examined, and showed changes in immune cell distribution following exposure to contact allergens that were not seen following exposure to the other chemicals tested (30). Though this study does not deal directly with the potential of *ex vivo* skin for the identification of irritants, it does demonstrate the ability of *ex vivo* cultured skin to display pathophysiological responses to chemicals. Given the species specificity of such a model this would in theory be an ideal *in vitro* method for irritancy testing

2 AIMS

The aim of this study is to evaluate the use of both primary keratinocytes and *ex vivo* human skin to determine the irritant potential of chemicals.

3 METHODS

3.1 Selection of test chemicals

To investigate the relationship between cell viability in Primary Human Epidermal Keratinocytes (neonatal) cells (HEKn cells) and *in vivo* primary irritation scores. HEKn cells were treated with chemicals corresponding to a wide range of primary irritation scores as determined by the Draize test. The chemicals used in this study were primarily selected from the Chemicals Data Bank complied by the European Centre for Ecotoxicology and Toxicology of Chemicals. Additional suitable compounds were selected from published literature (31).

3.2 HEKn toxicity assays

HEKn cells were seeded out into 96 well plates then allowed to adhere for 48h then the medium was replaced with medium containing the required treatments for 24h. The treatment concentrations used varied depending on the chemical be tested and were optimised to produce an IC_{50} value for each chemical. The viable cell number was assessed by MTT

3.3.3 *Determination of viability changes in response chemicals*

Production of IL-8 and IL-1α content was assessed by specific commercially available ELISA kits according to the manufactures instruction.

4 RESULTS AND DISCUSSION

4.1 Development of Keratinocyte model for detection of irritants.

The compounds investigated in this study displayed a wide range of IC_{50} values (0.026 to 67mg/ml) with the most potent irritants displaying the lowest IC_{50} values (Figure 3A). The log IC_{50} values were then plotted against the literature primary irritations score determined by the Draize rabbit skin irritancy test (Figure 3B). The results show a good correlation with an r^2 value of 0.8454 between the primary irritation score and the IC_{50} values determined in this cell based model.

This correlation suggests that this assay makes a good model for skin irritation by using the IC_{50} values in keratinocytes to get an indication of the potential for irritation. The main limitation of this assay is that the test substances must be soluble in the culture media. This is particularly important as often the chemicals used for topical applications are only marginally soluble in aqueous solution. In addition the model doesn't account for the barrier function of human skin therefore the potential for false positives is reasonable high in this model. Therefore an additional model is needed to evaluate chemicals that are not sufficiently soluble in the test media and which a barrier function close to human skin.

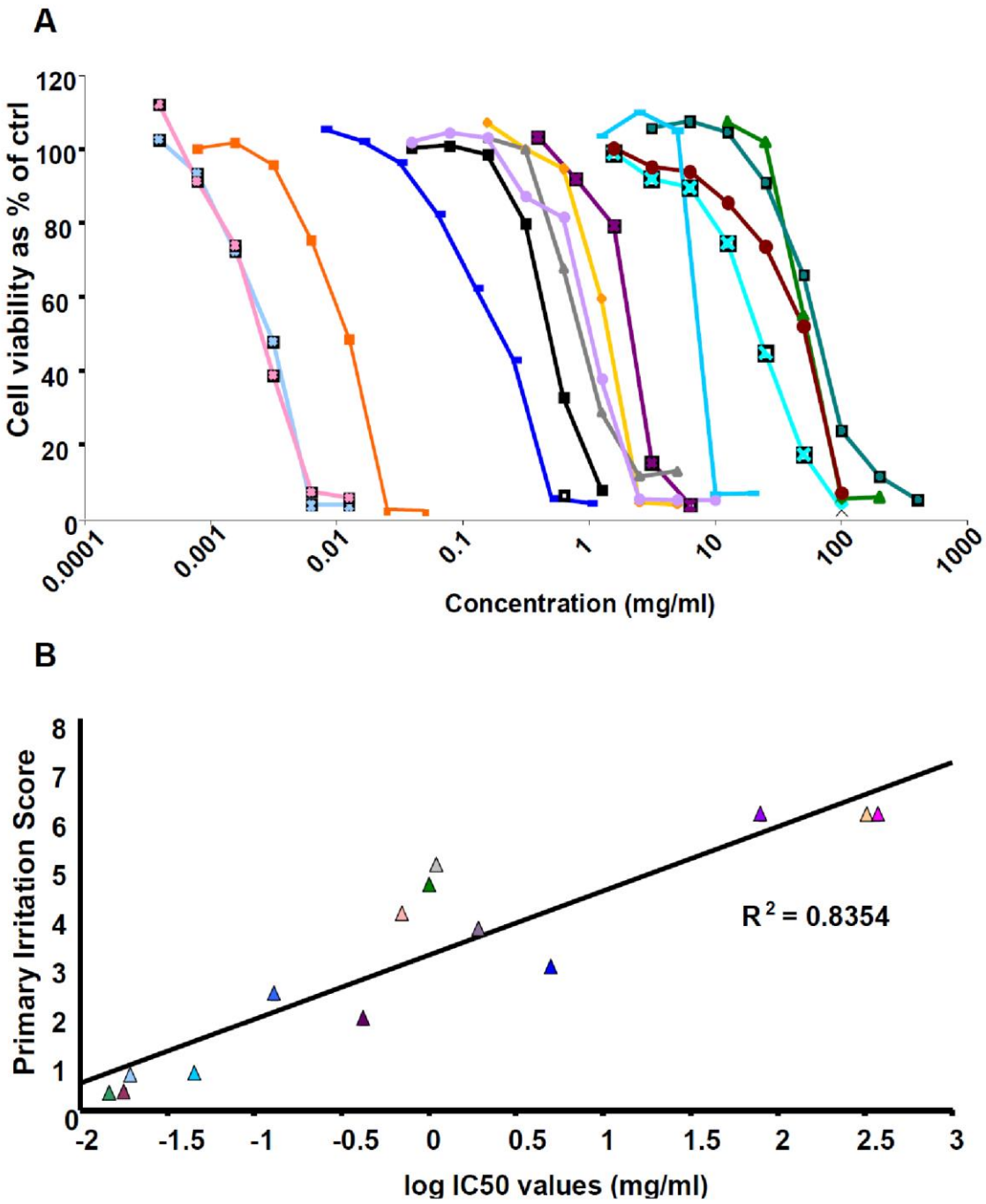


Figure 3. Correlation between HEKn viability and Primary irritation score HEKn cells were treated with the compounds over a range of concentrations to determine the IC_{50} values (n=3). A) Shows the concentration–viability curve for the chemicals tested B) Shows the correlation between the IC_{50} values for each compound and their *in vivo* primary irritation score.

4.2 Development of an *ex vivo* human skin model for determination of dermal irritancy.

The model selected to investigate irritancy of chemicals which have low aqueous solubility in this study was *ex vivo* human skin. This model was selected primarily due it is similarly to the *in vivo* situation particularly in terms of barrier function of the skin. Initially the viability of the skin was assessed to determine the suitability of the method i.e. could the viability of the tissue be maintained in the culture long enough to allow the intracellular increases in cytokines and their extra cellular release to occur. Figure 4 show the results of the viability time course for excised skin, this showed that tissue viability could be maintained in the culture (above 60% of the 24h value at 96 hours) for long enough to allow for the defined dosing protocol which requires the tissue to be incubated for 48 hours post treatment. This time point was selected as starting point for this investigating to mimic the protocols used on RHE cultures which all use an endpoint close to 48 hours. Given the possibility that *ex vivo* skin might be less sensitive than a RHE model, it seemed unlikely that a lower incubation period would yield significant results.

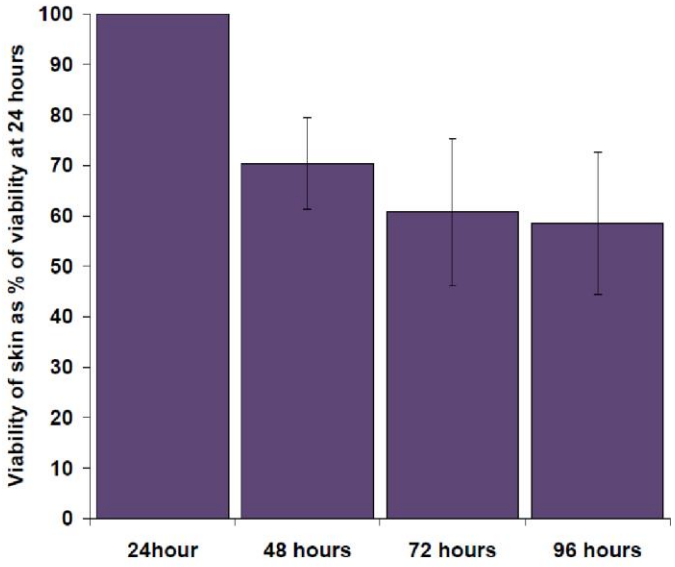


Figure 4. Viability of skin over culture periods Skin was prepared then cultured under standard conditions for 96 hours. For the duration of the culture the skins viability was assessed every 24 hours by MTT assay (n=5 ± SEM).

4.2.1 *Responses of ex vivo skin to application of chemicals*

To determine the ability of *ex vivo* human skin in culture to determine the irritancy potential of chemical substances three endpoints were investigated. The first was the viability of the tissue 48 hours after application of the test substance. The second was to measure the level of the inflammatory cytokine IL-1α in the conditioned media and the third was to measure the levels of IL-8 in the media. IL-1α was investigated as it is one of the most important cytokines in the irritation response. IL-1α is known to be constitutively produced in keratinocytes, but in response to stimulation IL-1α can be released and this is thought to be an essential primary event of the inflammation cascade. This release of IL-1α stimulates further release of secondary mediators, including IL-8. IL-8 is a promoter of dendritic cell migration and also in the recruitment of monocytes and neutrophils all of which are vital components of the inflammation process (32).

The MTT assay results show (Figure 5) that following exposure to most of the irritants tested a significant (greater than 50% compared to negative control) decrease in cell viability was observed. However when chemicals with lower PII's were tested the MTT assay results only displayed slight changes in viability compared to the untreated control. There were two exceptions to this, both of which showed a reduction of only approximately 40% viability despite have PII greater than some of the other chemicals which caused viability reductions considerably higher. This suggests that tissue viability alone is not sufficient to determine if a chemical is an irritant alone in this assay.

In this *ex vivo* cell model, two cytokines (IL-8 and IL-1α) were also measured (Figure 6), and changes in the levels of both were detected following exposure to certain chemicals, but not all chemical which had elevated IL-8 had elevated IL-1α. The differences between the patterns of cytokines which were elevated in response to the different irritants could be suggestive of the mechanism of irritation induced by particular chemicals. As previously mentioned surfactants initiate irritation by release of intracellular IL-1α this is as a result of disruption of cellular membranes. But this is not the case for all irritants, some don't exhibit membrane-damaging characteristics therefore probably do not initiate the inflammatory response solely by the release of IL-1α (6). Other possible mechanisms of initiation of

irritation include the generation of reactive oxygen species (ROS) (33), or effects of compounds on transmembranous receptors resulting in altered signal transduction (34). This suggests that monitoring a range of cytokines could be vital to the identification of all irritants regardless of the mechanism of action.

Additionally the ratio of IL-1α and IL-8 can also be shown to be indicative of differences in terms of the potential of the compound to be a contact sensitizer. Coquette et. al. (2003) used RHE to show that compounds that can act as contact sensitizer show higher levels of IL-8 than IL-1α, with the reverse being true of compounds that act only as irritants (32). The results of this current study show one chemical, a mild skin irritant which is also known to be a mild skin sensitizer (36), shows the highest response in terms of IL-8 release but only a modest increase in IL-1α release. Whereas one of the strong irritants tested showed only an increase in IL-1α release with no effect on IL-8 release. Of course with a low sample set as shown here no full conclusion can be reached at this stage on the full potential for this model for identifying contact allergens.

The addition of the cytokine data can also be used to identify irritants which didn't have significant effects on viability, for example Isostearic acid which only induced a modest decrease in viability but showed elevation in production of both IL-1α and IL-8 reflecting its irritant potential. This would suggest that analysing the cytokine release from this culture can identify irritants that would not be identified by analysing tissue viability alone.

R Phrase	PII	Viability	IL-1α	IL-8
NC	0.33	<50%	-	-
	0.78	<50%	↑	↑
R38	2.92	<50%	↑	↑
R38	3.25	>50%	↓	↓
R38	3.63	>50%	↑	↑
R34	4	>50%	↓	↓
R34	4.11	<50%	↓	↓
	4.33	<50%	↑	↑
R35	5.22	>50%	↓	↑
R38	6.78	>50%	↑	-

Table 1. Summary of test compounds properties. Shows the phases associated with the various compounds (Risk Phrases R34: Causes burns, R35: Causes severe burns, R38: Irritating to skin). The primary irritation indices from Chemicals Data Bank complied by the European Centre for Ecotoxicology and Toxicology of Chemicals. This also table summarises of the data shown in Figures 5 and 6

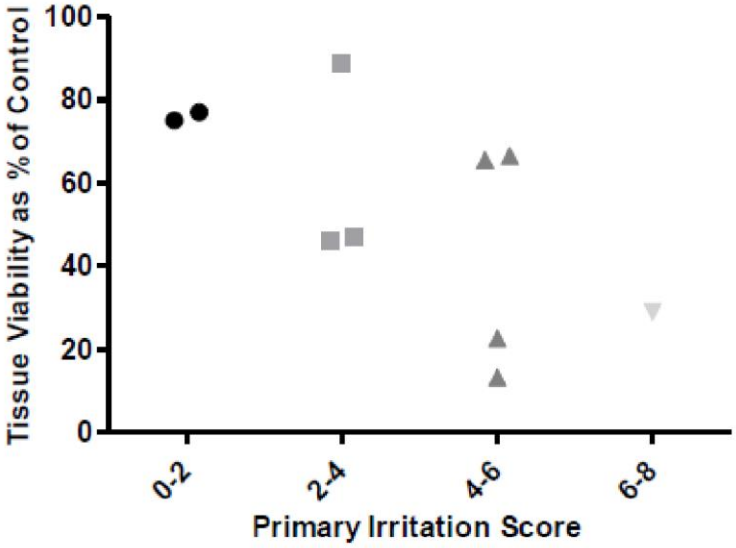


Figure 5. Viability of skin post treatments with chemicals Skin was treated with each chemical for the treatment period, skin was then cultured for an addition 48h before viability was determined by MTT (n=3).

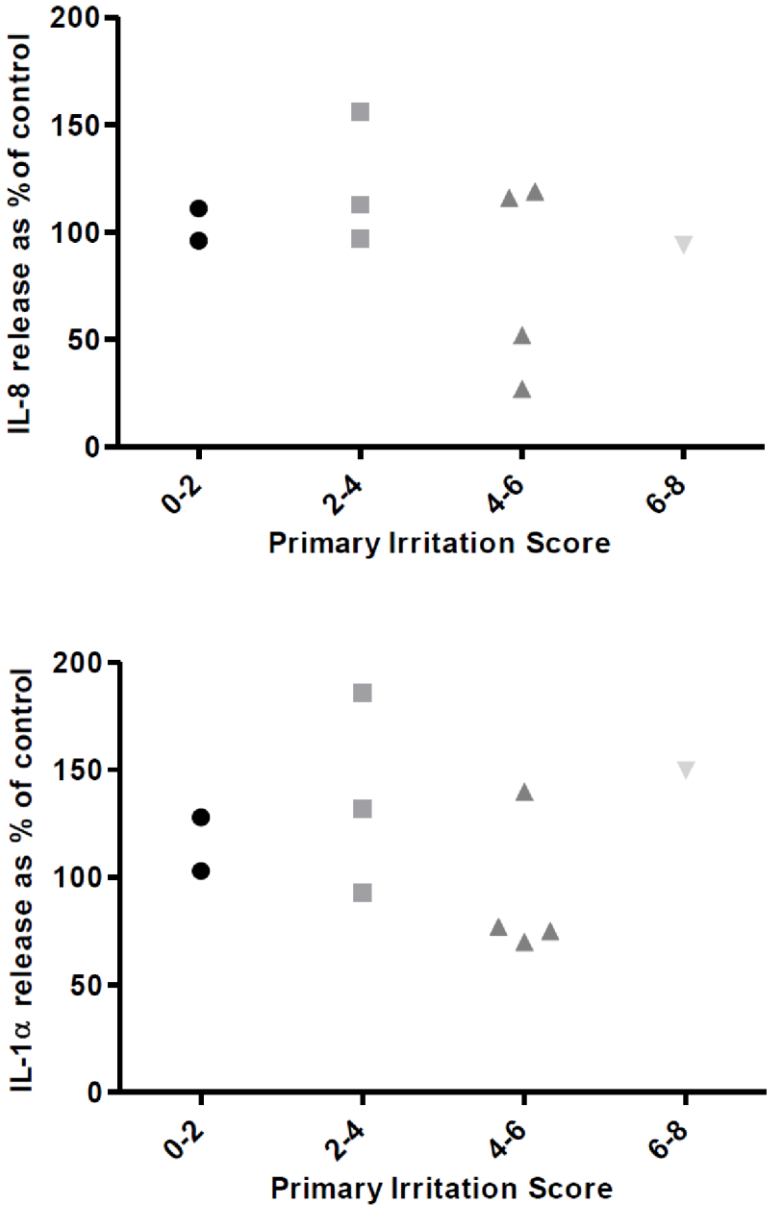
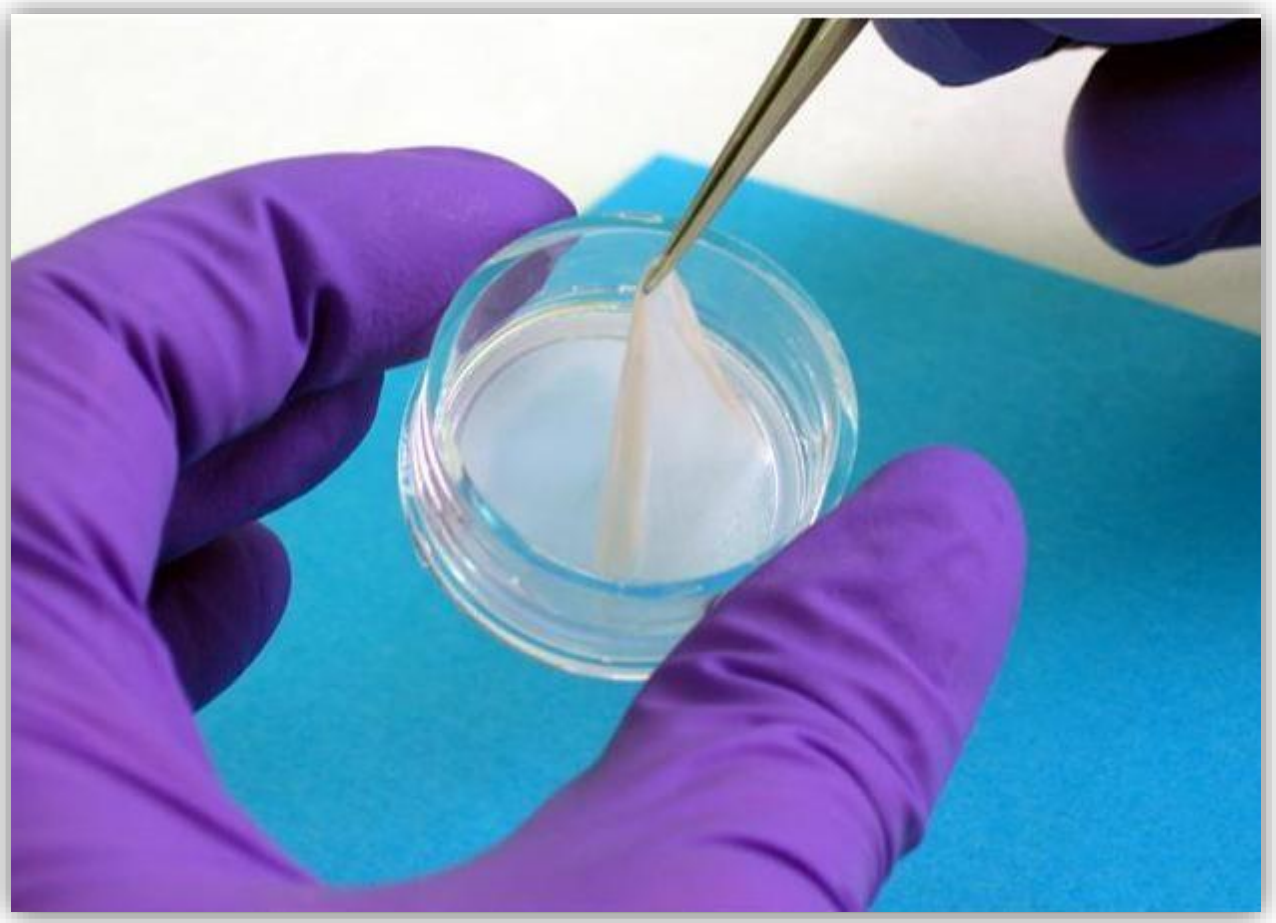


Figure 6. Release of cytokines in response to dermal irritants. Conditioned media from skin cultures 48 hours after chemical expose was assayed for both and IL-1α (A) and IL-8 (B) by ELISA.

Tutorial 4 – The development of in vitro reconstructed skin models

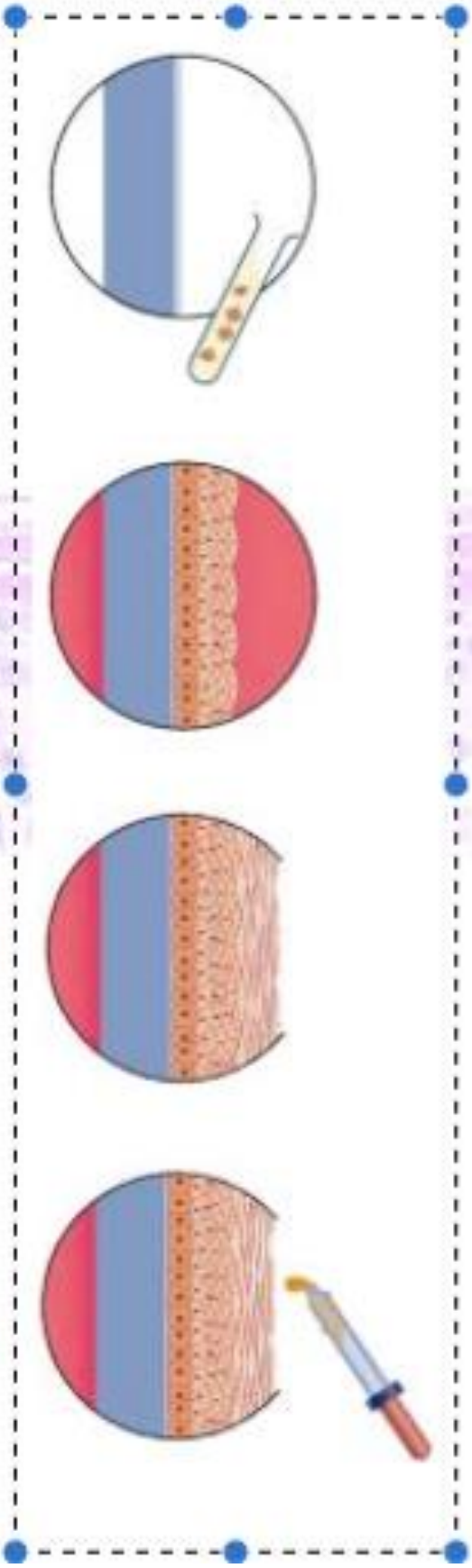


By the end of Tutorial 4 you should be able to answer these questions:

- ☐ What are reconstructed skin models?
- ☐ How reconstructed skin models are used to test for skin irritation?
- ☐ How do scientists evaluate the relevance of *in vitro* methods to the *in vivo* response?

Activity 1: Production of RHE tissues

Annotate the following diagram to describe the production of RHE tissues:



Activity 2: The of RHE tissues

Following during the following video presentation

http://www.jove.com/video/1366/an-vitro-skin-irritation-test-sit-using-epiderm-reconstructed-human

Select one of the days i.e. Tissue conditioning - Day 0, for this day you are to answer the following questions:

What do you think is the key step in the day you have selected?

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Write protocol for the step you have selected

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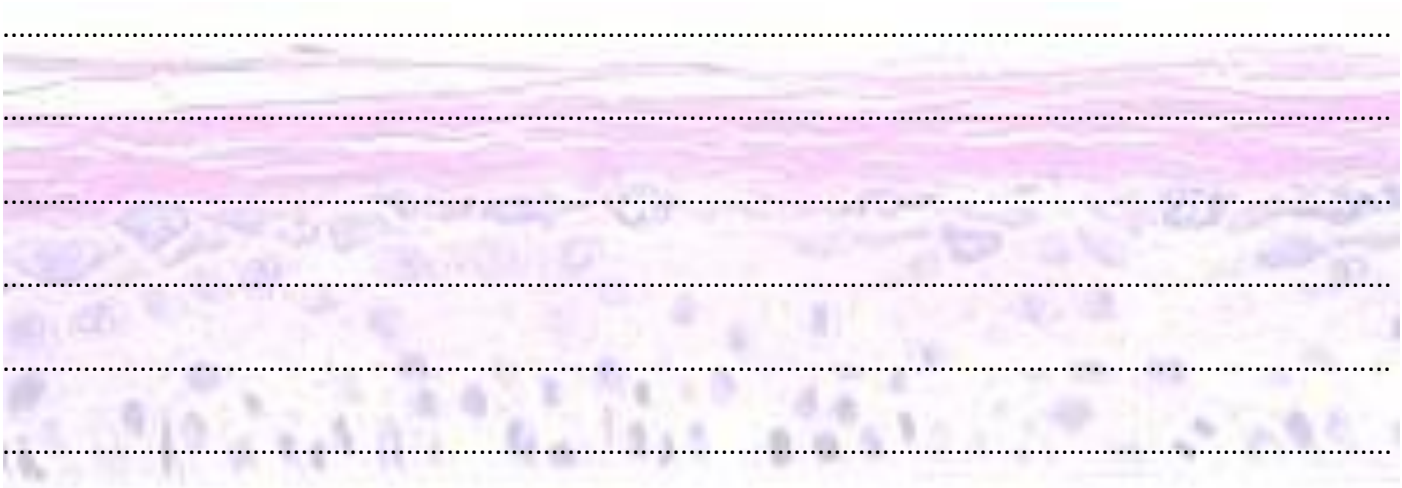

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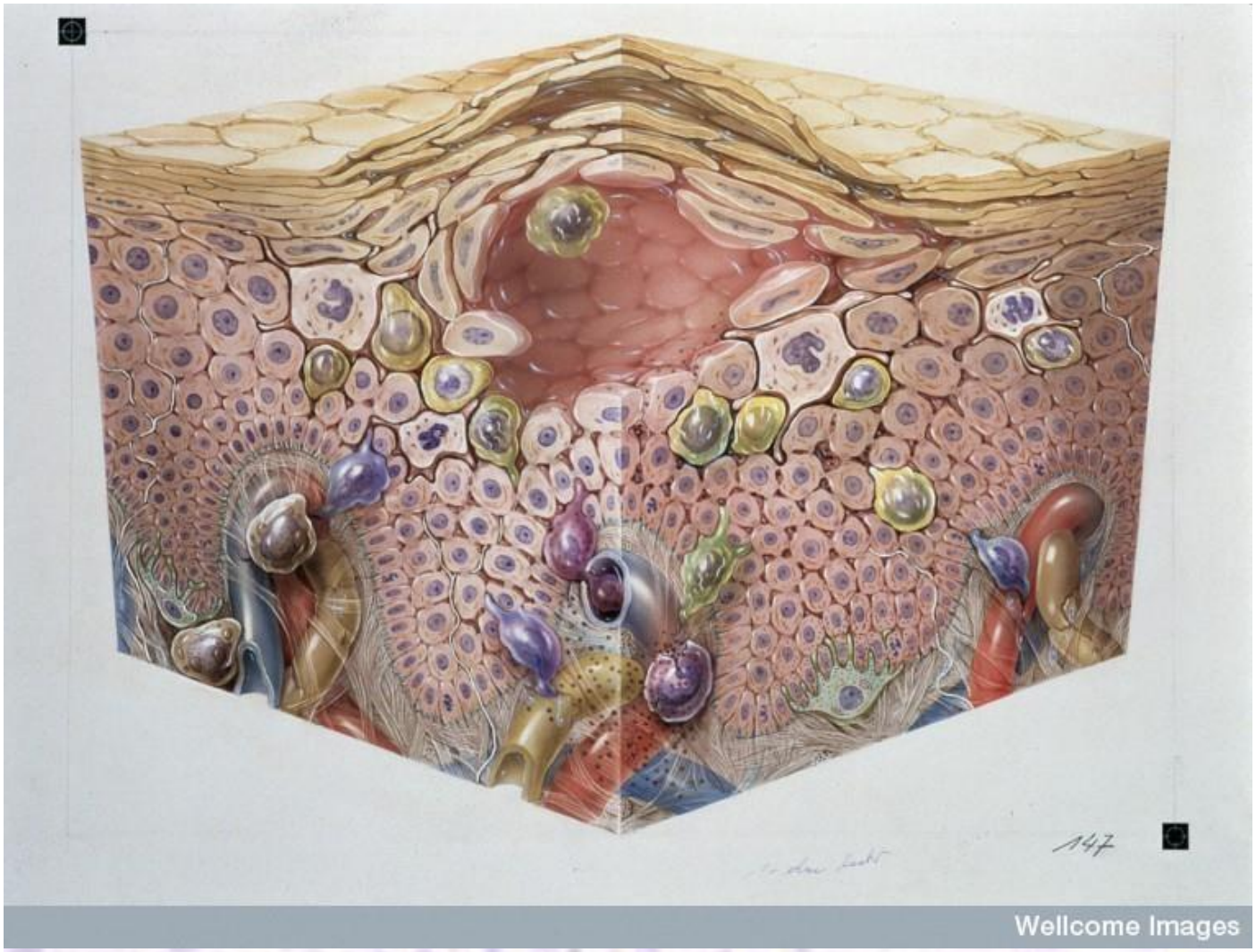
Tutorial 4 – Homework: Draft Assignment

In preparation for you final assignment, you are to evaluate the methods for determining skin irritation. You are to write a short (no more than 200 words) description of each method. In addition you are to complete the table below, giving a minimum of 1 example for each.

Method	Advantage	Disadvantage

Tutorial 5 – Development of your final assignment



What is the Purpose of Tutorial 5?

- Provide feedback on the homework to each other
- To provide guidance for writing the final assignment

Activity 2: Peer assessment of homework 5

You are to read one of the group's homework and complete below:

Name: ……

What did you think went well?

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How do you think this could have been improved?

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Tutorial 5 Homework: Final Assignment

The aim of the final assignment is to prepare a poster summarising your knowledge of in vitro models.

Tutorial 6 Feedback

What is the Purpose of Tutorial 6?

- Reflect on skills learned on the programme with a view to encouraging resilience
- Receive feedback on final assignment

What are the key questions?

- What strengths have I demonstrated in my work and what areas of development are there any left for me to address?
- What steps do I need to take to improve my academic output?

Activity 1– Marking your own

Using the mark scheme, identify three things you did well and three things you could improve.

What went well?

What could be improved?

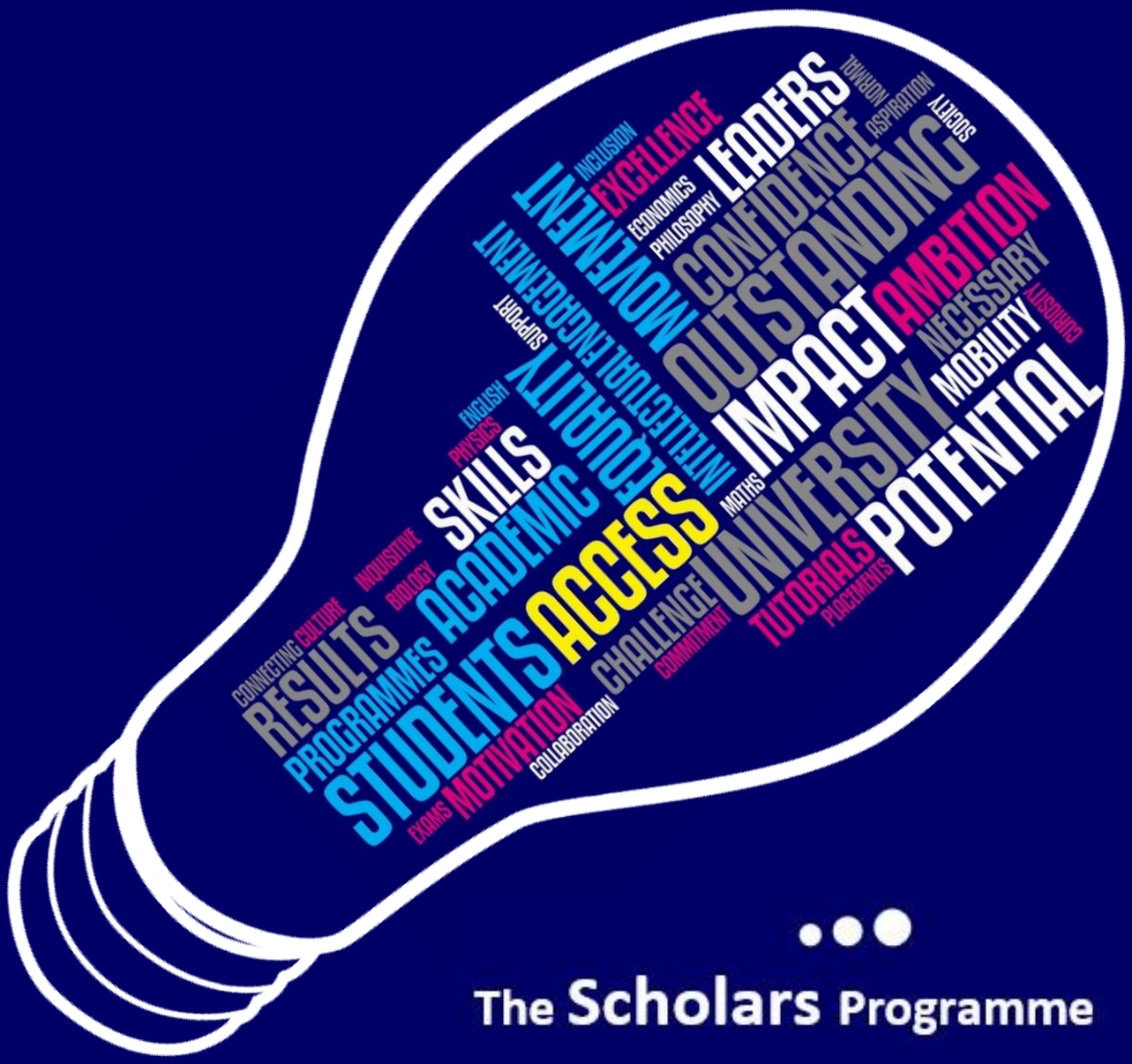
1.

2.

3.

Activity 2 Responding to feedback

Read through your feedback sheet and highlight on your assignment where you received positive feedback (one colour) and areas for improvement (different colour). You might find it helpful to annotate this with a brief comment or two.



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