Non-Insecticidal

Insect-Proofing of Wool

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Insect-proofing of wool is usually carried out by application of insecticides during dyeing. Inefficiencies in absorption of insecticide onto wool lead to aqueous effluent containing low levels of insecticide with a significant environmental toxicity to aquatic organisms. Replacing insecticides with non-insecticidal alternatives that target the wool digestion process of insects could greatly reduce the aquatic toxicity of wool processing effluent.

Three classes of non-insecticidal compounds were investigated, including surfactants, naphthalene derivatives, and antimicrobials. Selected compounds were applied to wool fabrics for testing against wool-digesting *Tineola bisselliella* moth and *Anthrenocerus australis* beetle larvae. Anti-feeding effects were measured and used to form hypotheses on molecular shape, size and polarity and their contribution to insect-proofing. The antifungal propiconazole was chosen as the most likely compound to be targeting the wool-digestion process, as seen in *Anthrenocerus australis* larvae.

To elucidate the mode of action of propiconazole on *Anthrenocerus australis*, repellency trials were carried out using control versus treated wool in reversible petri dish, and irreversible olfactometer choice experiments. No repellency effect was detected. Direct contact experiments were carried out by application of propiconazole solutions directly to *Anthrenocerus australis* larvae and by feeding larvae propiconazole-treated wool. No short or long-term toxic effects were detected, and subsequent feeding on untreated wool was not reduced. Gut enzyme activities were measured for *Anthrenocerus australis* fed control wool and compared to larvae fed propiconazole-treated wool. Trypsin, chymotrypsin, and

aminopeptidase activities were significantly (p-value<0.05) reduced 2-2½ fold, although this could not be confidently attributed to enzyme inhibition. Gut morphology was observed in *Anthrenocerus australis* fed control or propiconazole-treated wool using microscopic examination of gut tissue sections prepared in a fixative, and stained to show relevant features of the gut wall and contents. No differences were seen between propiconazole-exposed and control larvae, indicating no cytotoxicity was conferred in the gut region by propiconazole. Lack of observed repellency or toxicity of propiconazole on *Anthrenocerus australis* larvae leads to the hypothesis that the anti-feeding effect may be caused by disruption of gut flora associated with wool digestion.

Dyebath uptake experiments were carried out with propiconazole onto unbacked wool carpet, with susequent bioassays showing low absorption onto wool. Durability testing on the carpet using standard carpet shampoo and light exposure methods, followed by *Anthrenocerus australis* bioassay testing showed an acceptable durability of propiconazole on wool carpet.

Keywords: wool, insect, insecticide, non-insecticidal, mothproof, insectproof, keratin, keratinophagous, propiconazole, gut, enzyme, morphology, histology, repellency, y-tube, Tineola, Anthrenocerus, Daphnia, magna, environment.

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Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction and Literature Review

1.1 Background

The fact that certain insect larvae attack woollen textiles has been long recognised. In the bible, the custom of hoarding costly garments only to have them damaged by moths was cited as a mark of the perishable nature of temporal things:

"*Your riches are corrupted, and your garments are motheaten*"

(James 5:2, 1769 King James Bible)

"*For the moth shall eat them up like a garment, and the worm shall eat them like wool*"

(Isaiah 51: 8, 1769 King James Bible)

However, of the many millions of insect species, only a very small number have the ability to digest and derive nourishment from highly cross-linked protein matter. It is believed that these insects evolved a modified digestive system so that they could exist on a diet of carrion or other dead protein material, such as feathers or insects (Lewis & Shaw, 1987). Although only a few insect species are capable of digesting this material, they are widespread pests of woollen textiles, and cause an estimated one billion dollars worth of damage per annum in the USA alone (Metcalf & Metcalf, 1994). It has been calculated that one moth larva can consume 40 mg of wool in 30 days and the offspring of one adult can consume 42 kg in one year (Townsend, 1983). In fine woollen garments it takes only a small amount of feeding to sever yarn, leaving visible holes which ruin the appearance of the fabric. Carpets will also show visible damage, although they are less sensitive than apparel fabric for a given level of wool consumed.

The current solution to insect attack of woollen textiles involves treatment of wool with insecticides based on chlorinated hydrocarbons, organophosphorus and pyrethroid compounds. Application of insecticides usually takes place during dyeing or scouring, although topical application to finished woollen products is possible. Research into wool

insecticides tends to follow crop protection research, as the volumes required for wool are far smaller, and therefore research funding is comparatively low.

The effects of wool insecticides are not just restricted to wool-consuming insects. Fresh-water crustaceans and fish are also susceptible to these insecticides. Application of insecticide to wool is not usually 100% efficient, and discharge of industrial effluent can cause pollution of waterways and potential toxicity to these aquatic species (Barton, 2000). A non-insecticidal method of protecting wool could greatly reduce the use of these toxic insecticides, resulting in lower environmental toxicity, especially if a mode of action specific to wool digestion is targeted.

1.2 The New Zealand Wool Industry

1.2.1 Value to New Zealand

Wool fibre and woollen manufactured products make up a significant proportion of all New Zealand exports at around 2% (Statistics NZ, Meat and Wool NZ Wool Statistics, 2009). Although the wool industry had been in decline due to low wool prices contributing to decreasing sheep numbers since the 1980s, recent estimates show the average sale prices for New Zealand wool had increased by 32%, and that wool export value had risen by 33% for the year to 30 June 2011 compared to the previous year (Ministry of Agriculture and Forestry, 2011). The reasons for this were mainly falling global production and increased demand, rather than the small increase (<1%) in sheep numbers in 2011. Before this recent upturn in wool prices, the total New Zealand exports of all wool and manufactured wool products for the year to 30 June 2009 were worth $777 million (Meat and Wool NZ Wool Statistics), which represents a 28.5% drop from the $1.088 billion exported five years earlier in 2004 (Meat and Wool NZ Ltd Economic Service, 2008). New Zealand is the third largest producer of wool on a clean basis, which represents 14% of world production (Meat and Wool NZ Ltd Economic Service). Due to the sheep breeds present in New Zealand, most notably Romney, most of this wool is coarse (>31 micron in diameter) and mainly used in carpets (Meat and Wool NZ Ltd Economic Service). Wools of a finer diameter are required for clothing to avoid the characteristic itchy feeling of wool on human skin.

1.2.2 Industry Problems

Presently the wool industry uses synthetic insecticides for insect-proofing (such as a micro emulsion of the pesticide permethrin) applied during the dyeing or yarn scouring processes.

Although most insecticide (98-99%) is absorbed by the wool, some remains in the aqueous effluent and can cause environmental problems when discharged. Insecticides used on wool are very toxic to fish and aquatic invertebrates such as *Daphnia magna* Straus 1820 (water fleas) at the bottom of the food chain in the aquatic ecosystem, which are used as indicators of general toxicity to a wide range of species. In most parts of the world there are strict regulations on insecticide levels in dyehouse effluent. These regulations can cause problems for wool yarn spinners, who are unable to adequately insect-proof yarn without exceeding insecticide effluent limits (Allanach & Shaw, 1989). These problems have contributed to a significant drop in the volume of New Zealand loose wool fibre exports. There was a 42% decline in wool fibre exports to the UK in the four years from 2004-2008 (Meat and Wool NZ Wool Statistics, 2009), partly due to these insecticide problems.

If carpet manufacturers used untreated wool, this would result in extensive damage and consumer dissatisfaction, leading to further recession in the wool industry. A compounding problem with insecticides is that they become less effective over time as the insects become more resistant due to exposure. An example of this is the Australian carpet beetle, *Anthrenocerus australis* Hope 1843, which has become more resistant to permethrin (Barton, 2000), and in 2009 (Woolmark, 2009) required three times more than was needed in 1987 (Woolmark, 1987) for control of wool textile attack.

Controls on effluent toxicity and environmental expectations are growing and the presence of insecticide in wool does not fit well with the clean, green, natural marketing image of New Zealand wool. Many consumers choose wool carpets because of their naturalness and environmental profile, which is adversely affected by the presence of a synthetic insecticide. An eco-friendly replacement for insecticides would help to overcome trade barriers by allowing wool to qualify for eco-labelling and best-practice schemes. New Zealand carpet manufacturers can gain a marketing advantage by becoming licensed by the Environmental Choice New Zealand Trust, as shown on the Environmental Choice New Zealand website http://www.enviro-choice.org.nz/, an environmental labelling programme to help consumers find products that ease the burden on the environment. The Environmental Choice guidelines (Environmental Choice New Zealand Specifications, 2009) include effluent limits on common wool insecticides, which have been difficult for some carpet manufacturers to comply with using current methods.

1.3 The Structure of Wool

1.3.1 Introduction

Wool is a fibrous protein derived from skin cell follicles of domestic sheep *Ovis aries* Linnaeus 1758. The diameter of wool fibre ranges from approximately 15 to 40 μm, depending on the breed of the sheep. Fibre from other species in the subfamily Caprinae is also often referred to as wool. This includes cashmere and mohair from domestic goats *Capra aegagrus hircus* Linnaeus 1758. These fibres, along with human hair, are chemically very similar to wool. Wool, hair, horns, hoofs, feathers, mammalian skin, reptile scales, and tortoise shells are all made of a type of protein called keratin. The group of proteins classified as keratin all contain a high sulphur content, mostly found in the amino acid cystine, although a small amount is also present in methionine (Waterhouse, 1958).

> "*Unstretched wool fibres give a characteristic X-ray diffraction pattern, called an α-pattern, whereas stretched fibres give a different diffraction pattern, the β-pattern. Some keratins, notably feathers, give the β-pattern even when unstretched. Consequently, wool is known as an α-keratin, and feather as a β-keratin. All keratins from mammals are of the α-type, but birds and reptiles can produce both α- and β-types*" (McLaren & Milligan, 1981, p. 1).

Feulghelman (1997) states that there are three cell types produced in the base of the wool follicle in sheep skin. These form the three basic components of wool and hair: the cuticle, the cortex and the medulla.

1.3.2 The Cuticle

The surface of wool fibre is called the cuticle, which accounts for about 10% of the weight of the fibre. The cuticle consists of flattened overlapping scale cells cemented to one another, with the exposed edges of the cells pointing towards the fibre tip (Figure 1.2). In the cuticle, high levels of keratin are present in a physically robust arrangement of polypeptide chains held together by hydrogen bonds, salt linkages, and relatively strong disulphide bonds. Keratin is the insoluble component of wool, 8-16% of which is comprised of the sulphur-containing amino acid cystine (Block & Bolling 1946, as cited in Waterhouse, 1958, p. 208). As cystine is a dicarboxylic diamino acid, it is capable of being incorporated into adjacent polypeptide chains, forming a bridge between those chains with the disulphide bond in the centre (Figure 1.1).

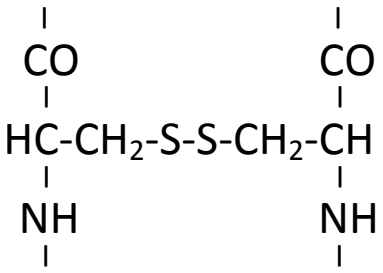


Figure 1.1 Disulphide cross link between polypeptide chains.

Most of the cystine in wool is involved in these bonds, forming a stable three dimensional lattice (Alexander & Hudson 1954, as cited in Waterhouse, 1958, p. 221).

The outer layer of cuticle scale cells is covered by a very thin epicuticle membrane, approximately 3 nm thick. The epicuticle is hydrophobic, and this contributes to the mild water-resistance of clean wool fibre. Despite the hydrophobicity of the wool cuticle, wool fibres are capable of absorbing water vapour at up to almost one third of their own weight without feeling wet, largely due to the hydrophilic properties of the cortex (International Wool Secretariat, 1991). Below the epicuticle is the exocuticle, forming about two-thirds of the scale structure. The exocuticle comprises two layers, known simply as the A and B layers. The A layer contains a higher level of cystine (~35%) than the B layer (~15%). The endocuticle lies below the exocuticle, and has a lower cystine content (~3%), and lower mechanical strength than exocuticle (Feulghelman, 1997). The endocuticle has been found to be more susceptible to enzyme attack than the exocuticle, although performic (peroxy) acid dissolves the exocuticle faster than the endocuticle (Bradbury & Ley, 1972).

1.3.3 The Cortex

The main shaft of the wool fibre, covered by the cuticle, is called the cortex. The cortex consists of spindle-shaped cortical cells around 100 µm long and up to 5 µm across that are tightly packed together in the same orientation as the fibre itself. Cortical cells make up about 90% of the wool fibre and are separated from each other by a cell membrane complex around 25 nm thick. Each cortical cell is made up of rod-shaped microfibrils approximately 10 µm long and 7.3 nm in diameter (Spei & Zahn, 1979). The microfibrils occur in bundles called macrofibrils. In each macrofibril, the microfibrils are separated by a cystine-rich matrix. The quantity and composition of this matrix varies for different keratins, although the microfibrils do not vary (Feughelman, 1997). The cortex can be divided into the

orthocortex and paracortex. These areas can be distinguished under a light microscope due to the different staining characteristics of each region. The macrofibrils are packed more tightly in the orthocortex than the paracortex. In coarse wools, the paracortex forms a tubular structure with the orthocortical cells in the centre, whereas in fine wools the orthocortex is on the outside and the paracortex on the inside of the fibre curvature, or crimp (Maclaren & Milligan, 1981). A diagram showing the general arrangement of the cuticle and cortex, along with magnification of the smaller components is shown in Figure 1.2.

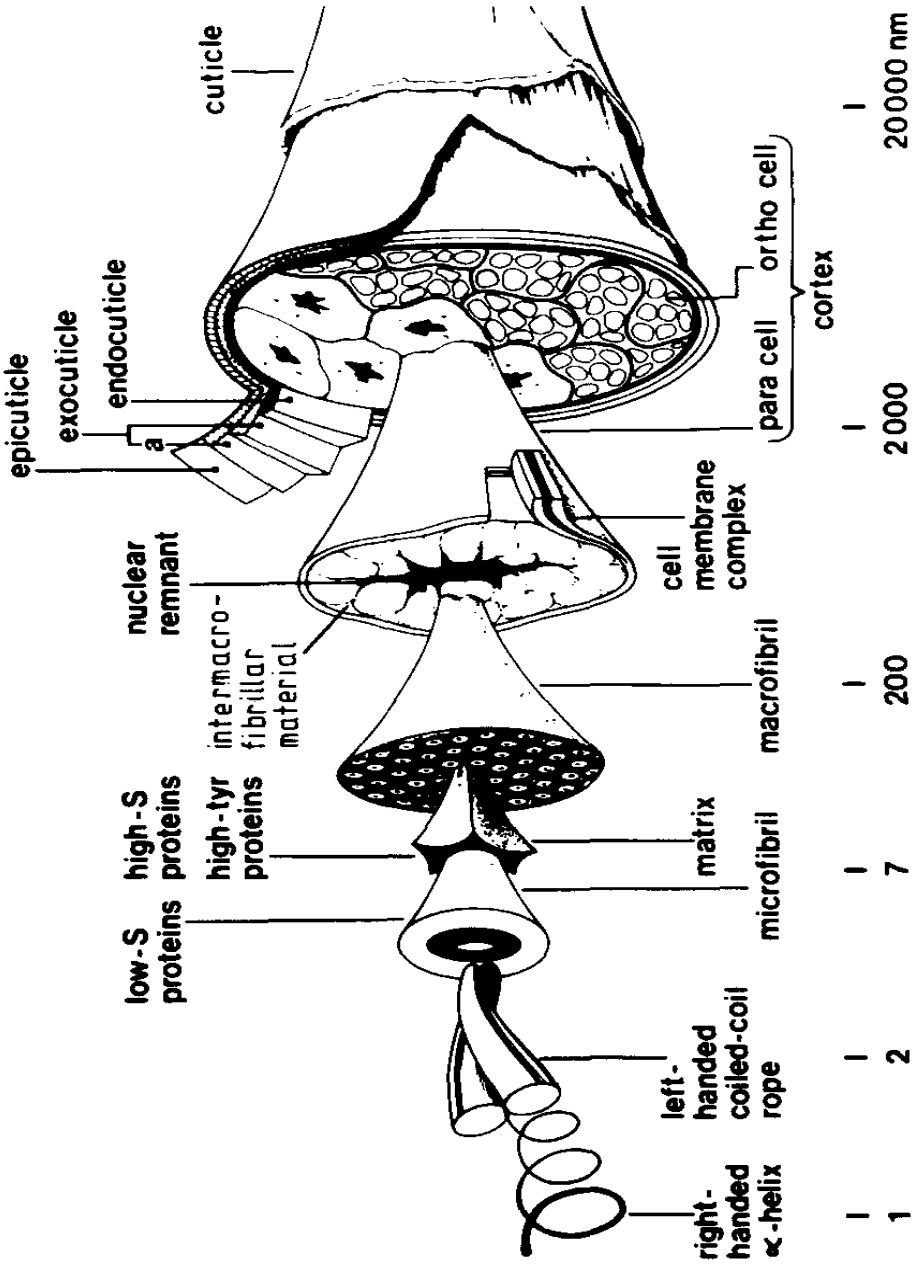


Figure 1.2 The structure of wool (From Spei & Holzem, 1987, p. 965, with kind permission from Springer Science and Business Media).

1.3.4 The Medulla

The medulla is a group of vacuolated cells that are highly resistant to alkali and other keratinolytic agents. They may be present along the axis of coarser α-keratin fibres in a continuous, discontinuous, or fragmented arrangement. The medulla physically represents empty space (air cavities) within the fibre. Medullated wools are often avoided in the textile industry due to coarseness and irregular dye uptake (Feughelman, 1997), but often desired for some rugged carpet styles (Crawshaw, 2002).

1.3.5 Amino Acid Composition of Wool

Wool is comprised of nineteen amino acids, joined in chains 400-500 units long. There are variations in the numbers and arrangement of the amino acids, which give rise to different properties of the proteins. The average amino acid composition of wool is shown in Table 1.1 (Waterhouse, 1958). It can be assumed that there is a degree of experimental error, as the total of all amino acids is almost 110%. Fletcher, Robson and Todd (1963), and Maclaren and Milligan (1981) highlighted the difficulties in measuring some of the amino acids in wool, but the latter concluded there were significant variations between some wool samples, even within the same breed of sheep. It had previously been shown by Ross (1961) that the sulphur content of wool varies according to the season, with lower sulphur content when wool growth was highest and *vice versa*. Changes in the diet of sheep can influence the amino acid composition of their wool, with cystine being paticularly sensitive to dietary variation. When the diet of sheep is supplemented by the addition of sulphur-containing amino acids or casein, the newly synthesised wool shows increases in cystine, proline, and serine, and decreases in aspartic acid and phenylalanine (Gillespie, Broad & Reis, 1969). The only amino acid to contain sulphur, other than cystine/cysteine, is methionine. The sulphur atom in the methionine molecule is positioned between two carbon atoms, and therefore it is not available to form disulphide bonds.

Table 1.1 Average amino acid composition of wool (Waterhouse, 1958).

Amino acid	Composition (%)	Amino acid	Composition (%)
Alanine	4.4	Lysine	3.3
Arginine	10.4	Methionine	0.7
Aspartic acid	7.3	Phenylalanine	3.8
Cystine	12.7	Proline	6.8
Glutamic acid	15.3	Serine	9.4
Glycine	6.5	Threonine	6.8
Histidine	0.7	Tryptophan	0.7
Hydroxylysine	0.1	Tyrosine	4.7
Leucine & Isoleucine	11.3	Valine	4.7

1.3.6 Resistance to Biological Attack

Wool is a fibre grown in outdoor conditions, exposed to water, heat, and bacterial and fungal attack. It is therefore probable that the structure of the wool fibre has evolved to endure these conditions. Many microbes have the ability to produce proteolytic (protein solubilising) enzymes, but few can solubilise keratin. Historically it has been documented that cotton, for example, is more readily attacked by microbes than wool (Hirst, 1923). The intermolecular disulphide bonds of cystine (Figure 1.1, Section 1.3.2) (the dimeric form of cysteine) provide resistance to biological attack, as few organisms have the ability to break this bond. Zaghloul, Embaby and Elmahdy (2011) achieved degradation of wool using a keratinase-producing *Bacillus subtilis* DB 100 (p5.2) recombinant strain bacterium, although this required extensive pre-treatment of the wool by autoclaving, chopping, and treatment with acid and alkali, and three days were required for complete solubilisation. In addition to disulphide bonds, the salt linkages within the wool structure are thought to be important in preventing microbial degradation of keratin (Brown, 1994). These features of the wool structure, along with the hydrophobicity of the wool fibre cuticle, contribute to the resistance of wool to many biological factors.

1.4 Insects Capable of Digesting Wool

1.4.1 Introduction

Insect attack is a major threat to all wool products, particularly as competing synthetic fibres do not suffer this problem. Wool-digesting insects are unusual as they are able to break disulphide bonds in wool during digestion. Of the many millions of insect species, it is generally accepted that there are around 30 moth, 15 beetle, and hundreds of bird-infesting mallophagan lice that have developed the ability to digest and derive nourishment from highly cross-linked keratin (Waterhouse, 1958). Insect pests capable of digesting wool belong to either the Lepidoptera (moth) or Coleoptera (beetle). It should be noted that the adult moths and beetles do not digest wool; it is only the larvae which cause the damage. There are only seven species of each order that are recognised as wool pests of any importance, each marked with an asterisk in Tables 1.2 and 1.3, derived from Waterhouse (1958). Some of the species listed by Waterhouse are junior synonyms or names since replaced by a more senior synonym. Tables 1.2 and 1.3 show these species with correct spelling, using the senior synonym where necessary. Appendix A shows the same species, with any synonyms used by Waterhouse in the right-hand column, along with the original spelling. Species authorities have been given in full in Tables 1.2 and 1.3, and Appendix A, for reasons of clarity as Waterhouse did not include year of publication, and used abbreviations for some authorities.

Table 1.2 Lepidoptera known or suspected to digest keratin (derived from Waterhouse, 1958).

Species	
Tineidae	
Amydria vastella Zeller 1852	*Tenaga inquisitrix* Meyrick 1916
Monopis crocicapitella Clemens 1859	**Tinea columbariella Wocke 1877*
Monopis dicycla Meyrick 1905	*Tinea flavescentella* Haworth 1828
Monopis ethelella Newman 1856	**Tinea pallescentella* Stainton 1851
Monopis ferruginella Hübner 1813	**Tinea pellionella* Linnaeus 1758
Monopis monachella Hübner 1796	*Tinea semifulvella* Haworth 1828
Monopis pseudagyrta Meyrick 1919	*Tinea subalbidella* Stainton 1867
Monopis rusticella Clerck 1796	*Tinea translucens* Meyrick 1917
Monopis trimaculella Snellen 1885	*Tinea trinotella* Thunberg 1794
Monopis weaverella Scott 1858	**Tineola bisselliella* Hummel 1823
Niditinea fuscella Linnaeus 1758	*Trichophaga abruptella* Wollaston 1858
Phereoeca allutella Rebel 1892	**Trichophaga mormopis* Meyrick 1935
Phereoeca uterella Walsingham 1897	**Trichophaga tapetzella* Linnaeus 1758
Praeacedes atomosella Walker 1863	**Oecophoridae**
	**Hofmannophila pseudospretella* Stainton 1849

Table 1.3 Coleoptera known or suspected to digest keratin (derived from Waterhouse, 1958).

Species	
Dermestidae	
**Anthrenocerus australis* Hope 1843	*Attagenus fasciatus* Thunberg 1795
**Anthrenus flavipes* LeConte 1854	*Attagenus lobatus* Rosenhauer 1856
Anthrenus fuscus Olivier 1789	*Attagenus nigripes* Casey 1916
Anthrenus museorum Linnaeus 1761	**Attagenus pellio* Linnaeus 1758
**Anthrenus pimpinellae* Fabricius 1775	**Attagenus piceus* Olivier 1790
**Anthrenus scrophulariae* Linnaeus 1758	*Attagenus schäfferi* Herbst 1792
**Anthrenus verbasci* Linnaeus 1767	**Scarabaeidae**
Attagenus elongatulus Casey 1900	*Deltochilum gibbosum* Fabricius 1775

1.4.2 *Tineola bisselliella:* the Common Clothes Moth

In New Zealand, and globally, the most common pest attacking wool products is *Tineola bisselliella* Hummell 1823, the common (or webbing) clothes moth. This pest is widely used as a bioassay test insect, although it is arguably one of the easiest of the wool-damaging species to control with insect-resist agents. The adults are 6-8 mm long, with a wingspan of 10-15 mm. The upper side of the fore wings is a pale yellow, almost golden colour (see Figure 1.3). The head is covered in long silky hairs which make it appear larger than it really is. When disturbed this moth prefers to run rather than fly and it is said that those seen in flight are males, or females who have laid eggs (Hickin, 1974). The adults are weak fliers and live for two to three weeks, preferring to live and reproduce in darkness.

Tineola lay eggs on woollen cloth and prefer rough surfaces, but the nutrient value of the substrate does not appear to affect ovipositional response (Whitfield, Cole, & Whitney, 1958). Egg laying (oviposition) preference was studied by Kan & Waku (1985) using several different preference tests. Female moths laid their eggs on fleecy substrates irrespective of whether they were wool or cotton. Cotton was preferred, perhaps because of residual grease on the wool. If the wool was solvent extracted in acetone the situation was reversed. *Tineola* were able to sense the gap between fibres by means of mechanoreceptors on the ovipositor. Eggs were preferentially laid in gaps less than 0.3 mm in width and 0.2 mm in depth. Evidence pointed to the importance of tactile, rather than chemical stimuli for determining oviposition preference.

Like that of all other moths, the life cycle comprises of four stages: egg, larva, pupa, and adult. The time taken for the life cycle varies greatly depending on conditions such as temperature, humidity and diet. The life cycle may vary from 48 days to four years (Moncrieff, 1950). A typical life cycle under laboratory conditions may take five to seven weeks. Approximately 50-100 eggs are laid by each female, so *Tineola* can quickly establish themselves if preventative action is not taken. Eggs are typically oval and measure 0.5 mm by 0.3 mm. The adult female will lay her eggs in batches of six to eight over three days. The eggs hatch in 24 days at 15°C, 10 days at 20°C, 7 days at 25°C, and 6 days at 30°C (Hickin, 1974). After this time the embryo larva chews its way out.

Tineola larvae are creamy-white with a golden brown head without eyes (see Figure 1.3). They measure approximately 1 mm by 0.2 mm. They are sensitive to light and have six

thoracic legs, each terminating in a claw. Five abdominal segments each bear a pair of prolegs; those on the last segment being slightly larger are known as “claspers”. *Tineola* pupae are reddish-brown in colour and vary in size depending on the feeding substrate and environmental conditions. They usually range from 3-7 mm in length (Hickin, 1974).

Figure 1.3 *Tineola bisselliella* moth adult (left) and larvae (right). Photos by Ben Smart and Guido Gerding respectively.

1.4.3 Other Significant Moth Species

Many species of *Tinea* (case-bearing clothes moths) exist in sub-tropical and temperate regions of the world; however, *Tinea pellionella*, and *Tinea dubiella* are the species most common in New Zealand. Neither of these species is as important or widespread as *Tineola bisselliella*. *Tinea pellionella* and *Tinea dubiella* adults are similar in appearance, with shiny pale-buff fore wings displaying three faint dark spots. There is a slight dusting of dark scales which gives a darker and duller appearance than *Tineola bisselliella*, and the hind wing is whitish (Ferro, 1978). The eggs of *Tinea pellionella* have longitudinal ridges as opposed to the reticular pattern found with *Tineola bisselliella*, but are otherwise the same (Hickin, 1974). These hatch into larvae closely resembling *Tineola bisselliella* in appearance. *Tinea pellionella* larvae build a silk cocoon at the larval stage, and drag the cocoon with them when travelling using their front legs, which protrude outside the cocoon along with the head, as shown in Figure 1.4. The cocoon acts as a buffer to minimise water loss from larvae during periods of low humidity (Chauvin, Vannier & Guéguen, 1979). Before pupation the cocoon is sealed. Pupae are white, changing later to reddish-brown as observed by Ferro (1978). Hickin noted that when they are ready to emerge from the cocoon, they push their way through the pupal case and silk membrane to emerge as adults.

Figure 1.4 *Tinea pellionella* moth adult (left) and larvae (right). Photos by Machele White and Entomart http://www.entomart.be/INS-0232.html respectively.

Hofmannophila pseudopretella Stainton 1849, the brown house moth, is occasionally a significant pest of wool in moister temperate climates such as New Zealand; however this species is not an obligate keratin feeder, generally preferring a cereal diet (Lewis & Shaw, 1987). Adults have a brown head and thorax. The fore wings can range in colour from dark olive brown to light brown, but are always marked with three black dots in the centre, and fringed with hairs (Ferro, 1978). The larvae are shiny grey-white in colour with dark heads, and are larger than *Tineola bisselliella*, reaching a length of 18-20 mm. A cocoon is spun, although there is less webbing compared to *Tineola* or *Tinea* species.

The tapestry moth, *Trichophaga tapetzella* Linnaeus 1758, is more common in Europe and the United States than in New Zealand. It is easily identified by its white head and distinctive fore wings, the basal area of which are black while the rest is white speckled with black and grey. Larvae usually spin a silken tube in which they live, or alternatively they burrow through the material they live in (Mallis, 1982).

1.4.4 Beetle Species

Carpet beetles are usually surface grazers of textiles, and although they can feed more rapidly and extensively than moth larvae, damage is not usually as obvious during the early stages of attack (Ferro, 1978). The most common beetle pest in Australasia is *Anthrenocerus australis* (Australian carpet beetle). Also common in Australasia are *Anthrenus flavipes* and *Anthrenus verbasci*. The *Anthrenus* genus are are the most common carpet beetles causing damage in Europe and the USA.

The variegated carpet beetle, *Anthrenus verbasci*, is small and black, with adults measuring 2-4 mm in length. The elytra are covered in a variable pattern of white, brown and yellowish scales giving a less regular and paler appearance than that of *Anthrenocerus australis* (see below). In the wild, the adults feed on pollen and nectar, although the larvae often live in nests of birds and bees (Hinton, 1945). The eggs are oval with a mean size of 0.55 by 0.27 mm. They are rough with short spine-like projections at one end (Griswold, 1941). The larvae are 4-5 mm long and brown and hairy (Figure 1.5). The body broadens towards the rear and appears to consist of a series of light and dark brown transverse strips, while long hairs extend from the rear of the larvae with terminal hairs forming white tufts that can be fan-shaped. The life cycle varies between one and three years depending on conditions. Larvae have been recorded surviving up to 10 months without feeding, which is important as the larval stage is generally the overwintering stage for populations in the wild.

Figure 1.5 ***Anthrenus verbasci*** **adult (left) and larva (right). Photos by Pest and Diseases Image Library - http://www.bugwood.org, and Joseph Berger, Bugwood.org respectively.**

Anthrenocerus australis adults are dark in colour, 3 mm long, with a characteristic light coloured hair running in a transverse zig-zag pattern across their back (Figure 1.6). They are strong fliers and feeding mainly on pollen when outside (Ferro, 1978). The larvae are 3-6 mm long, fawn to brown on top with a white underside (Figure 1.6). There is a prominent hairy tuft that protrudes from the last abdominal segment along with three cerci that distinguish *Anthrenocerus australis* from *Anthrenus verbasci*. The eggs are similar in appearance to those of *Anthrenus verbasci*, with around 100 laid over a two week period. In New Zealand only, *Anthrenocerus australis* has evolved significant resistance to permethrin, the most common insecticide applied to wool (Wools of New Zealand, 2009).

Figure 1.6 ***Anthrenocerus australis*** **adult (left) and larva (right). Photos by Frank Köhler (http://www.koleopterologie.de) and Grant Shackell (AgResearch) respectively.**

1.4.5 Mechanism of Wool Digestion

Wool protein digestion involves insect gut enzymes attacking the peptide bonds of the main protein chains. In wool and other animal fibres, the extensive disulphide cross-links between protein chains are thought to prevent enzyme access to the peptide bonds (Lewis and Shaw, 1987). Those few insects that have developed the ability to digest wool have a reducing, and sometimes alkaline, region in the gut capable of breaking the disulphide bonds, allowing normal enzymatic digestion. Insect-proofing strategies that can prevent the disruption of disulphide bonds have the potential to be very specific to wool pests. The main lepidopteran species that attacks wool (*Tineola bisselliella*) has a midgut pH of 9.9, and a reducing potential of -300mV (Linderstrom-Lang, 1936), while the main coleopteran (*Anthrenocerus australis*) has a more neutral pH of 6.8-7.0, and a slightly weaker reducing potential of -190 to -230mV (Waterhouse, 1952a). Wool is fairly resistant to acid, whereas under alkaline conditions of around pH 10 it will partially degrade. Despite the lack of alkalinity and lower reducing potential in the beetle gut, wool is still digested in 8-12 hours at 30°C (Waterhouse, 1952a), compared to 8 hours at 27°C for *Tineola* moth (Day, 1951a). Gut enzymes and a reducing environment appear to be key factors in wool digestion, and are therefore promising targets for insect proofing.

1.5 The Insect Gut of Wool Pests

The fact that so few insects have the ability to digest wool indicates the evolution of a unique digestive system. The enzymatic biochemistry of insect digestive systems is important because it can be exploited to develop novel and specific control agents.

1.5.1 Introduction to Enzymes

Enzymes are proteins that catalyse reactions in which a substrate is converted into another molecule (the end product). Enzymes lower the activation energy of the reaction without undergoing any change themselves, and the rate of reaction is usually directly proportional to the concentration of enzyme (Laidler, 1954). The active site of each enzyme specifically binds to a particular part of the substrate. The active site of the enzyme is usually near the surface of the molecule to allow bonding to occur easily with the substrate. These bonds are reversible allowing continued reactions to occur, without the enzyme being used up. Enzymes are specific to certain reactions and the substrates involved, and within these reactions can show high levels of stereospecificity and regioselectivity (Jaeger & Eggert, 2004). Each different enzyme is active over a specific pH range, with a maximum reaction rate occurring at an optimum pH within this range. An enzyme can be denatured, permanently losing activity, if subjected to extremely high or low pH conditions. Enzyme activity is also dependent on temperature, with most enzyme-catalysed reactions occurring faster at higher temperatures, but with a sudden drop in activity when the temperature is raised high enough to alter the shape of the enzyme (Van den Berg, Vriend, Veltman, Venema & Eijsink, 1998). Other factors that can denature enzymes include freezing, irradiation with ultraviolet light, ultrasonic waves, and chemical agents such as alcohols, urea, guanidine salts and acetamide.

The concentration of the substrate also contributes to the rate of reaction. At low concentrations of substrate, there is a linear correlation with reaction rate, whereas at higher concentrations the rate of reaction gradually becomes independent of substrate concentration due to saturation of the enzyme, leading to a maximum rate of reaction. An enzyme-substrate complex is formed during the reaction, which produces the product of the reaction and regenerated enzyme. This type of behaviour was first interpreted by Michaelis and Menten (1913), as translated by Teich and Needham (1992).

The most relevant group of enzymes involved in digestion of wool are the proteolytic (protease) enzymes. Proteolytic enzymes are catalysts whose biological function is the hydrolytic degradation of proteins, with the basic reaction being:
-CO-NH- + H_2O → -COOH + -NH_2. One group of enzymes, of which pepsin, trypsin, and chymotrypsin are the most important, only act on peptide bonds that are not near to the end of the molecule, and therefore are not close to free amino or carboxyl groups. These

enzymes are known as endopeptidases. Another group of enzymes, of which carboxypeptidase, aminopeptidase, dipeptidase, and leucine aminopeptidase are important. These are only capable of acting on peptide bonds that are at the end of a peptide chain and therefore close to a free amino or carboxyl group. These enzymes are known as exopeptidases (Laidler, 1954).

The Enzyme Commission number (EC number) is a numerical classification for reactions catalysed by enzymes. Table 1.4 summarises the classes of hydrolase reactions acting on peptide bonds (Moss, 2010). Exopeptidases are listed in the range 3.4.11 – 3.4.19, whereas endopeptidases are in the range 3.4.21 – 3.4.25.

Table 1.4 Enzyme Commission (EC) systematic classification of selected hydrolases (Moss, 2010).

EC Number	Systematic name and subclasses
3.4	Peptidases
3.4.11	Aminopeptidases (N-terminal exopeptidases)
3.4.11.3	e.g. cystinyl aminopeptidase
3.4.13	Dipeptidases
3.4.13.18	e.g. cytosol non-specific dipeptidase
3.4.14	Dipeptidyl-peptidases and tripeptidyl-peptidases
3.4.15	Peptidyl dipeptidases
3.4.16	Serine-type carboxypeptidases
3.4.16.6	e.g. carboxypeptidase D
3.4.17	Metallocarboxypeptidases (C-terminal exopeptidases)
3.4.17.1	e.g. carboxypeptidase A [Zn^{2+}]
3.4.18	Cysteine-type carboxypeptidases
3.4.18.1	e.g. cathespin X
3.4.19	Omega peptidases
3.4.21	Serine endopeptidases
3.4.21.1	e.g. Chymotrypsin
3.4.21.4	e.g. Trypsin
3.4.22	Cysteine endopeptidases
3.4.22.2	e.g. Papain
3.4.23	Aspartic endopeptidases
3.4.23.1	e.g. Pepsin A
3.4.23.2	e.g. Pepsin B
3.4.23.3	e.g. Gastricsin (pepsin C)
3.4.24	Metalloendopeptidases
3.4.24.3	e.g. Microbial Collagenase
3.4.25	Threonine endopeptidases
3.4.99	Endopeptidases of unknown catalytic mechanism

1.5.2 Tineidae and Oecophoridae Moth Larvae Gut

The key to the digestion of keratin was discovered by Linderstrom-Lang and Duspiva (1936). They found that wool-consuming insects maintained strongly reducing conditions in the larval midgut, with an oxidation-reduction potential of -300mV for *Tineola bisselliella*. A strongly reducing gut environment allows the reduction of the disulphide bonds in keratin and subsequent enzymatic attack. Wool was found to pass completely through the alimentary tract of *Tineola bisselliella* within 8 hours at 27°C (Day, 1951a), therefore this process of reduction and enzymatic degradation must be fairly rapid. Day also found freshly grown untreated merino wool was much more resistant to *in vitro* digestion by trypsin or proteases extracted from *Tineola bisselliella* than was wool treated with calcium thioglycollate. This chemical reducing agent breaks some of wool's disulphide bonds into sulphydryl groups, which are comparatively easy for enzymes to digest. Wool processing and weathering also reduce some of the disulphide linkages to sulphydryl groups.

Linderstrom-Lang and Duspiva (1936) also found that the foregut and midgut contents of *Tineola* had an alkaline pH of around 9.9. This high pH also has a degradative effect on wool, which is more resistant to acidic conditions due to its iso-electric point of 4.5. Similarly, Waterhouse (1952b) found that *Tineola* had high foregut and midgut pH at 8.0-9.0 and 9.8-10.0 respectively, but also found that the hindgut was acidic at pH 4.6-5.8. In contrast to the reducing mid-gut, the hindgut had an oxidation-reduction potential greater than +250mV. The hindgut acidity was attributed to uric acid which made up 30-40% of the weight of the faeces. Waterhouse (1949) studied the midgut of 40 species of adult Lepidoptera, and two carnivorous lepidopteran larvae. The adult Lepidoptera, if they feed at all, would only consume plant nectar and would therefore presumably derive no benefit from an alkaline gut. Carnivorous insects usually have acidic or neutral midgut contents, yet it was found that the midguts of both larvae and adults in this study were all in the alkaline range. Waterhouse therefore concluded an alkaline gut is a characteristic of the order Lepidoptera, and not dependent on feeding habits. Neither of the two larvae investigated are wool pests, therefore high pH alone does not appear to be sufficent to allow wool digestion.

Powning, Day, and Irzykiewicz (1951) discovered that the activity of a crude proteinase preparation from the gut of *Tineola* was only slightly affected by varying oxidation-reduction potentials from -460mV to +460mV, suggesting that it was not particularly adapted to the oxidation-reduction potential under which it operates. They found a loss of 50% of

enzymatic activity after one minute at 60°C, showing a low heat-stability. Day hypothesised (1951b) that some of the uric acid in the faeces of *Tineola* was produced by the enzyme xanthine oxidase. This was confirmed by qualitative tests that revealed the presence of xanthine oxidase in the gut of *Tineola*. This enzyme therefore helps in the excretion of nitrogen from the *Tineola* gut. Due to the oxidising nature of xanthine oxidase, it is likely this activity is present in the hindgut, which was shown by Waterhouse (1952b) to provide an oxidising environment.

Tineola are able to detoxify a wide range of metals and non-metals, many of which are ordinarily very toxic (Waterhouse, 1952c). Waterhouse fed *Tineola* larvae a diet of yeast/casein or wool with addition of nineteen compounds containing elements known to form insoluble sulphides. They all produced characteristically coloured sulphides in the food undergoing digestion in the midgut. The sulphur from cystine combines with these elements to form sulphides, which are excreted. This was confirmed by measuring cystine levels in the faeces of *Tineola* larvae fed nickel sulphate with wool (Powning, 1953). The control larvae faeces contained 6.7% cystine, whereas those from the larvae fed the diet containing nickel sulphate only contained 1.4% cystine. The following compounds were used in Waterhouse's study: zinc sulphate, ferric chloride, cadmium chloride, thallium acetate, cobalt chloride, nickel sulphide/sulphate, tin (IV) chloride, lead acetate, antimony chloride/sulphide, bismuth nitrate, arsenic oxide, copper sulphate, sodium tellurate/tellurite, osmium oxide, mercuric acetate, silver albuminate, palladium chloride, platinum chloride and gold chloride. Due to their lack of *Tineola* toxicity, these metal compounds are therefore of little use for controlling *Tineola* moth larvae.

Powning (1953) went on to further study *Tineola* excreta, and found that sulphur from wool was excreted mainly as cystine. Of the total water-soluble sulphur excreted, 55% was cystine sulphur, but only 8% was sulphate sulphur. No sulphur dioxide was found to be produced. Most nitrogen excreted was water-soluble as urea made up 3% of the weight of faeces. The amount of urea in the gut was too small (0.14%) to denature keratin. It was concluded neither *Tineola* nor dermestid beetle larvae rely solely on gut alkalinity for the digestion of wool. Crewther and McQuade (1955) showed that very few microbes were present in the gut of *Tineola*, and therefore concluded that bacteria play no part in wool digestion or the maintenance of reducing conditions.

Cysteine is produced in the *Tineola* gut when the disulphide bond of cystine is cleaved via reduction (Figure 1.7). Powning (1954) studied cysteine desulphydrase in different insects, including *Tineola*. The reaction of cysteine losing its sulphydryl group is catalysed by the enzyme cysteine desulphydrase. *In vitro* experiments showed that cysteine desulphydrase liberated hydrogen sulphide from l-cysteine, and that maximum production occured at pH 8.9 for *Tineola* extracts. The enantiomer l-cysteine was used for these experiments. An enantiomer is one of two stereoisomers that are non-superimposable mirror images of each other. Various inhibitors trialled at 0.01 M were shown to reduce cysteine desulphydrase activity including sodium chloride, potassium nitrate, arsenic oxide, sodium hydrogen sulphate, phenylhydrazine and hydroxylamine. Cystine, methionine, homocysteine, glutathionine and thioglycollate did not function as substrates for *Tineola* desulphydrase. Homocysteine is a homologue of cysteine, containing an extra methylene group next to the sulphydryl group.

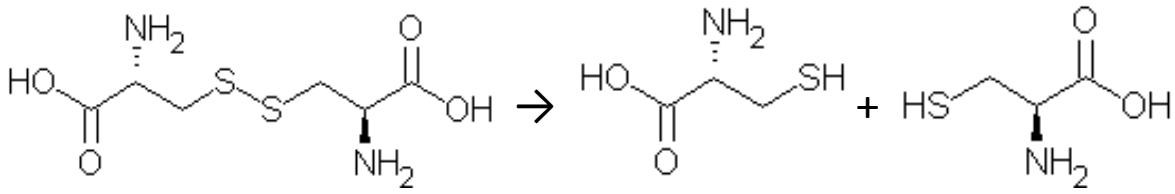


Figure 1.7 Cystine molecule (left) reduced to two molecules of cysteine (right).

Powning and Irzykiewicz (1959) studied cystine reductase in enzyme preparations made from whole *Tineola* larvae. Cystine reductase catalyses the reduction of one cystine molecule to two cysteine molecules (Figure 1.7). Cystine reductase activity was demonstrated using a spectrophotometer to measure the decrease in absorption at 340nm of reduced triphosphopyridine nucleotide (modern name: nicotinamide adenine dinucleotide phosphate, abbreviated to NADPH) in the presence of cystine under anaerobic conditions at the optimum pH 7.3. This decrease in absorption was far lower in the absence of cystine, indicating cystine was reduced by NADPH when both were present. In addition, the production of sulphydryl groups was measured by a colourimetric nitroprusside method, and confirmed by titration with phenyl mercuric nitrate. Powning and Irzykiewicz (1960) extended their study of cystine reductase and also studied glutathione reductases. Whole *Tineola* larva extracts reduced 14 µmoles of cystine per gram of larvae per hour at pH 7.3.

Reduced diphosphopyridine nucleotide (modern name: nicotinamide adenine dinucleotide, abbreviated to NADH)-linked cystine reductase, and NADH and NADPH-linked glutathione reductases of lower activity were also present in *Tineola* extracts.

Powning and Irzykiewicz (1962a) also purified the digestive *Tineola* proteinases for *in vitro* studies more closely resembling the actual gut concentration in the *Tineola* larvae. This was achieved by extraction of insect material at low pH, ammonium sulphate and potassium phosphate fractionations, and elevated temperature. They found freezing/thawing did not deactivate the enzyme. Holding the enzyme at 50°C for one hour at pH 10 (close to the optimum found at pH 9.8) increased the specificity of the enzyme with no destruction of activity. Electrophoresis using paper sprayed with modified azocasein substrate showed that the proteinase moved towards the cathode even at high pH, suggesting a high iso-electric point for the protein. Powning & Irzykiewicz (1962b) later demonstrated that partially purified *Tineola* larval protease anaerobically digested 29.8% of wool at 37°C over 48 hours at pH 10, whereas crystalline trypsin had no effect under the same experimental conditions within the pH range of 6.0 - 9.8. Wool was completely digested in two hours when 0.125 M cysteine was added to the *Tineola* enzyme, showing that reducing conditions are beneficial for wool digestion. The percentage of wool digested was higher at 37°C (29.8%) than at 25°C (8.3%) or 15°C (4.1%). The maximum activity of *Tineola* protease on casein was measured at pH 9.8. Powning (1962) analysed *Tineola* faeces to find they contained 0.28% elemental sulphur, which was consistent with previous work on cysteine desulphydrase (Powning, 1954) and cystine reductase (Powning & Irzykiewicz, 1960).

Ward has completed the most detailed studies of *Tineola bisselliella* midgut enzymes (1972, 1975a-g, 1976). Extracts of whole *Tineola* larvae were fractionated by ammonium sulphate precipitation, ion-exchange diethylaminoethyl cellulose (DEAE) chromatography and acrylamide gel electrophoresis. Ward identified nine serine proteinases (one chymotrypsin-like and eight trypsin-like enzymes) two metalloproteinases, sixteen aminopeptidases and two carboxypeptidases. Of the trypsin-like enzymes, there were four major and three very minor anionic enzymes and one was a cationic enzyme. The trypsin-like enzymes were found to be quite stable at alkaline pH values, but unstable at acid pH, particularly below 4.0. Gel electrophoresis was used to separate these enzymes. The optimum activities of all the gut enzymes investigated were in the alkaline pH range, with very little activity under acidic

conditions. Some of the molecular weights, optimum pH levels and responses to various enzyme inhibitors were recorded, and are summarised in Table 1.5.

Table 1.5 Properties of midgut enzymes from *Tineola bisselliella* moth larvae (selected data from Ward, 1975a-g, 1976).

Enzyme	Molecular weight	pH of optimum activity	Inhibitor/concentration/% inhibition
Major aminopeptidase of low electrophoretic mobility	180,000	8.4	$HgCl_2$/ 2.0mM/ 100% $CuCl_2$/ 2.0mM/ 99% 1:10 phenanthrone/ 2.0mM/ 99% EDTA/ 2.0mM/ 42%
Six aminopeptidases of intermediate electrophoretic mobility	All 240,000	8.2	$HgCl_2$ 2.0mM/ 100% $CuCl_2$ 2.0mM/ 99% $ZnCl_2$ 2.0mM/ 76% 1:10 phenanthrone 2.0mM/ 51% EDTA 2.0mM/ 0%
Three aminopeptidases of high electrophoretic mobility	All 94,000	7.7	$HgCl_2$ 1.0mM/ 99%, 96% $CuCl_2$ 1.0mM/ 95%, 95% $ZnCl_2$ 1.0mM/ 94%, 84% 1:10 phenanthrone 1.0mM/ 74%, 40% EDTA 1.0mM/ 0%, 0%
Major carboxypeptidase	72,000	7.5-7.7	$HgCl_2$ 1.0mM/ 100% $CuCl_2$ 1.0mM/ 94% $ZnCl_2$ 1.0mM/ 56% $CaCl_2$ 1.0mM/ 59% $CoCl_3$ 1.0mM/ 58% 1:10 phenanthrone 1.0mM/ 93% Iodoacetate 1.0mM/ 90% p-Chloromercuribenzoate 0.1mM/ 100% Diisopropylfluorophosphate 1.0mM/ 95% EDTA 1.0mM/ 0%
Major anionic trypsin-like enzyme	Not determined	8.5	$CaCl_2$ 2.0mM/ >95% Urea 4.0M/ 97% Diisopropylfluorophosphate 1.0mM/ 100%
Major metal-chelator sensitive proteinase	24,000	9.4	$HgCl_2$ 1.7mM/ 100% $CoCl_3$ 1.7mM/ 54% $MnSO_4$ 1.7mM/ 62% EDTA 1.7mM/ 100%[1]
Minor metal-chelator sensitive proteinase	Not determined	9.4	$HgCl_2$ 1.7mM/ 100% $CoCl_3$ 1.7mM/ 50% EDTA 1.7mM/ 100%[1]

[1]Can be partially reversed by the addition of calcium, zinc and mercury chlorides.

Hammers, Schmid, Fohles, and Zahn (1985) analysed excreta of *Tineola* and the beetle species *Anthrenus flavipes* (furniture carpet beetle) and *Attagenus piceus* (black carpet beetle) that had been fed untreated wool. The beetle species produced excreta containing more protein (peptide-bound and free amino acids) than the excreta of *Tineola*. This was consistent with the findings of Powning (1953), where *Attagenus piceus* beetle excreta were found to contain approximately twice as much cystine as *Tineola*. Hammers et al. also found that the *Tineola* excreta were high in free cystine, cysteic acid and histidine, whereas the beetle species were high in cystine and arginine. They theorised that it is possible that after reduction of the cystine bonds, cysteine is enzymatically split out and then rapidly oxidised back to cystine, possibly in the acidic hind-gut. *Tineola* larvae fed wool containing 0.1% of the insect resist agent chlorphenylid showed a reduced level of cystine in the excreta, whereas the beetle larvae showed an elevated level of cystine. In the case of *Tineola* larvae, it is possible the reduction in cystine excreted could be analogous to the results of Powning (1953) where nickel sulphate was thought to be detoxified via formation of a sulphide, resulting in less cysteine available to be oxidised back to cystine.

Yoshimura et al. (1988) found two types of L-cysteine lyase (desulphydrase) in *Tineola*, which caused *in vitro* desulphydration of L-cysteine. The first L-cysteine desulphydrase catalysed α,β-elimination of L-cysteine according to the equation L-cysteine + $H_2O \rightarrow H_2S$ + pyruvic acid + NH_3. The second L-cysteine desulphydrase catalysed only β-replacement of L-cysteine, producing H_2S + lanthionine. D-cysteine, L-cystine, L-homocysteine, L-methionine, and L-cystathionine were all inert as substrates. L-cysteine lyase showed optimum activity at pH 8.5-9.0. It was also found that pyridoxal phosphate is the co-enzyme of L-cysteine lyase of *Tineola* larvae. Removing the cysteine lyase activity from *Tineola* larval extracts, while keeping proteolytic activity, resulted in a lower rate of keratin degradation in various concentrations of 2-mercaptoethanol. It was theorised that the likely role of L-cysteine lyase was to maintain reducing conditions in the *Tineola* gut by producing hydrogen sulphide. Christeller (1996) studied the degradation of wool by *Hofmannophila pseudospretella* larvae, a similar moth to *Tineola*. He suspected the chemical species responsible for the low oxidation-reduction potential in the gut of *Hofmannophila* (-200 to -350 mV) was either cysteine or sulphide.

Robinson, Ciccotosto, and Sparrow (1993) found the presence of cysteine lyase/ desulphydrase in *Tineola* larvae, as did Powning (1954). However, contrary to Powning and

Irzykiewicz (1960), no cystine reductase was found. Larvae of another moth, *Tinea pellionella* was found to contain cysteine lyase/desulphydrase, but it was absent in *Anthrenus flavipes* beetle larvae. It was concluded that moths and beetles use different mechanisms to reduce the disulphide bonds of wool and that it was likely that the L-cysteine lyase of Yoshimura et al. (1988) and the l-cysteine desulphydrase of Powning were identical.

Robinson, Ciccotosto, Gaal, and Sparrow (1993) subjected larval extracts to the phase separation technique of Bordier (1981) using the non-ionic surfactant Triton X114 to show that *Tineola* cysteine lyase was partitioned into the detergent-rich phase, away from the smaller protease in the detergent-poor phase. Cysteine lyase/desulphydrase was identified as a potential target enzyme for rational design of new mothproofing agents.

In vitro wool degradation using larval midgut extracts of *Hofmannophila pseudospretella* (Christeller, 1996) was carried out at pH 9.2 and at an oxidation-reduction potential of -200 to -350 mV under anaerobic conditions. Rates of wool degradation were sensitive to oxidation-reduction potential and to the type of reductant. Sulphydryl compounds enabled faster degradation than inorganic sulphur compounds. Only sodium dithionite, used at a lower oxidation-reduction potential than occurs *in vivo*, supported a rate of wool degradation similar to sulphydryl compounds. A two-step pathway was shown to occur for wool digestion, starting with reduction/solubilisation of wool proteins, followed by proteolysis. The reduction/solubilisation step was shown to require the presence of a high molecular weight fraction of the midgut extract and also a reducing agent for maximum rate of wool degradation.

Shannon, Attwood, Hopcroft and Christeller (2001) showed that small numbers of bacteria were present in the midgut of *Hofmannophila pseudospretella*. These bacteria were isolated from the dissected midgut, and three methods were used to identify them. Microscopy, carbohydrate utilisation, and ribosomal sequence data all separated the isolates into the same three classes, identified as the two facultative anaerobes *Lactococcus lactis* and *Carnobacterium piscicola*, and tentatively the aerobe *Bacillus subtilis*. These bacteria were able to grow on selected media, although none utilised wool when added to the media. It was concluded these bacteria were not specifically adapted to the reducing conditions of the *Hofmannophila pseudospretella* midgut.

Hughes and Volger (2006) showed via gene expression from subtracted DNA libraries that serine endopeptidases were by far the dominant group of proteins in the *Tineola* gut. Both major types of serine endopeptidases, trypsin and chymotrypsin, were represented. Carboxypeptidase responsible for hydrolysing the C-terminal end of polypeptide chains was present. All other metabolic enzymes were present in much smaller quantities, including a chitinase and two lipases.

1.5.3 Dermestidae Beetle Larva Gut

Waterhouse (1952a) studied the digestion of wool by three species of dermestid beetle larvae; *Anthrenocerus australis, Anthrenus verbasci* and *Attagenus piceus.* The pH of the midgut was found to be 6.8-7.0 in all three species, with less variation within the midgut than *Tineola.* The oxidation-reduction potential of the midguts were highly reducing at -190 to -230 mV. Most cysteine produced in this reducing environment is not degraded, but is excreted. In *Tineola*, the gut has a cysteine desulphydrase enzyme capable of splitting hydrogen sulphide from cysteine. Beetle larvae do not produce hydrogen sulphide and therefore probably do not have this enzyme, or it is less active at the more neutral gut pH of the dermestid larvae. *Attagenus piceus* beetle larvae faeces contain around 12% cystine, compared to 6-7% for *Tineola* larvae (Powning, 1953). This supports the theory that in dermestid larvae, cysteine is not used to the same extent as in *Tineola* larvae.

Tracheal cells transfer oxygen to tissue of the insect body. Day (1951b) showed that tracheation of the larval midgut of *Anthrenus* and *Attagenus* species is less well developed than that of the *Tineola bisselliella* moth larva. The tracheation of the larval midgut of *Tineola* was shown to be less well developed than most other insects, including lepidopterous species of comparable size. This restricted supply of oxygen in the midgut of wool-digesting species may help to maintain the reducing environment required for wool digestion.

Powning (1953) fed dermestid beetle larvae woollen fabric impregnated with many of the compounds used in the *Tineola* digestion experiments of Waterhouse (1952c). Faeces produced were not any different in colour to those of the control larvae, showing that metal sulphides were not produced. A similar lack of mortality to that shown in *Tineola* suggested that dermestid larvae are capable of detoxifying many elements, including lead and mercury. Instead of insoluble sulphides being produced, the metals form undissociated complexes

with cysteine or cysteine peptides, which are either insoluble or, if soluble, less toxic than the metal in its ionized form.

Other observations made by Waterhouse (1952a) on the dermestid larvae included an acidic hindgut pH of 4.4-4.8 with an oxidation-reduction potential of greater than +260 mV. Food was observed to pass completely through the digestive tract in 8-12 hours at 30°C. Given the work by Powning (1962) showing *Tineola* faeces contain 0.28% elemental sulphur, whereas the faeces of dermestid beetle *Anthrenus flavipes* contain no elemental sulphur, these results support the theory of Waterhouse (1952a) that cysteine desulphydrase is active in *Tineola*, but not in dermestid beetles.

Baker (1986) investigated the difference in enzyme activity between *Tineola* larvae and dermestid beetle larvae *Anthrenus flavipes,* and *Attagenus piceus* at 30°C. Table 1.6 shows specific activities of each type of enzyme. Overall, *Tineola* gut enzymes had greater aminopeptidase activity than the dermestid beetle gut enzymes. General proteinase, trypsin-like and amylase activities were higher in *Attagenus piceus* beetle than in *Tineola* moth, but interestingly *Anthrenus flavipes* beetle showed lower activity than *Tineola* in these assays. When looking at amylase activity, *Attagenus* beetle larvae had almost 17 times the enzyme activity of the *Tineola*, although it should be noted the *Attagenus* larvae were reared on a diet of Purina Lab Chow® with 5% brewer's yeast, whereas the *Tineola* and *Anthrenus* larvae were reared on woollen cloth dusted with brewer's yeast. The Purina Lab Chow® contained approximately 26% protein, 5% fat, and 56% carbohydrate (McManus, 1972), possibly contributing to the higher amylase activity.

In general, *Anthrenus flavipes, Attagenus piceus, and Tineola bisselliella* wool-digesting species showed higher aminopeptidase, trypsin-like and general proteinase activity than four species of granivorous beetles also trialled *Sitophilus oryzae* Linnaeus 1763, *Sitophilus granarius* Hustache A. 1930, *Tenebrio molitor* Linnaeus 1758, and *Tribolium castaneum* Herbst 1797. The granivorous beetles showed much higher amylase activities individually, and as a group, than the three species with high-protein diets. Two granivorous moth species included in the trials (*Plodia interpunctella* Hübner 1813 and *Anagasta kuehniella* Zeller 1879), showed similar amylase and aminopeptidase activities to those of the wool pests, but slightly higher trypsin and lower general proteinase activities. Baker (1986) noted that the role of the hydrolases in contributing to higher trypsin-like activity in these two species was unclear as they did not hydrolyse casein to a similar extent.

Table 1.6 Specific activities (pico-moles/minute/mg protein) of midgut homogenates prepared from three species of wool pests against amylase (starch), proteinase (casein), trypsin (BApNA) and aminopeptidase (LpNA) substrates (selected data from Baker, 1986).

Insect Species	Amylase (starch)	Proteinase (casein)	Trypsin (BApNA[1])	Aminopeptidase (LpNA[2])
Tineola bisselliella	500	138	74	399
Anthrenus flavipes	30	86	62	174
Attagenus unicolor[3]	8,400	172	399	72
Tenebrio molitor	47,000	11	15	4
Sitophilus granarius	104,000	26	19	50
Sitophilus oryzae	137,000	9	3	33
Tribolium castaneum	41,000	25	16	31
Plodia interpunctella	1,900	33	523	165
Anagasta kuehniella	12,000	39	1,534	83

[1]α-N-benzoyl-DL-arginine-p-nitroanilide.
[2]L-leucine-p-nitroanilide.
[3]Synonym of *Attagenus piceus.*

Trivedi, Srivastava, Narain & Chatterjee (1991) observed digestion of wool by *Anthrenus flavipes* larvae using a scanning electron microscope to observe the morphological changes to the structure of wool at various stages through the insect gut. They noticed a large number of bacteria present in the ileum (beginning of hindgut), that were more predominant on the severely degraded wool fragments. They were a rod-shaped type of bacteria called cocobacilli, and not only present on the surface of the wool fragments, but also in the interfibrillar spaces of the cortical cells. Actively feeding protozoa appeared to gradually replace bacteria in the posterior ileum and rectum, which was presumed to be a result of bacterial lysis and protozoan feeding. Starving or feeding *Anthrenus flavipes* non-digestible food materials resulted in fewer bacteria and a greater number of protozoa, which appeared further forward in the ileum. It was postulated that the bacteria may supplement additional nutritional requirements not available in wool, and that proteolytic enzymes involved in wool digestion are likely to originate from the symbiotic intestinal microflora.

Christeller, Markwick, and Burgess (1994) characterized the midgut proteinase activities of the larvae of the moths *Tineola bisselliella* and *Hofmannophila pseudospretella* and the

beetle *Anthrenocerus australis*. The major endopeptidases were serine proteases. Of the serine proteases, all species showed trypsin-like activity whereas only *Anthrenocerus australis* had measureable chymotrypsin-like activity. No significant levels of metalloendopeptidase or cysteine endopeptidase activity were detected. Aspartic acid endopeptidase activity was unlikely to be present in any of these species due to low proteinase activity below pH 5.0. Aminopeptidase activity was present in all larvae. Of the twenty five serine proteinase inhibitors trialled, only a limited number inhibited midgut proteolysis of the lepidopteran larvae, whereas most inhibited midgut proteolysis of the *Anthrenocerus*. Thirteen inhibitors covering the eight most effective for each species are listed in Table 1.7, expressed as a percentage of the control proteolytic activity.

Table 1.7 Effects of serine endopeptidase inhibitors on the proteolytic activity of keratinolytic larval midgut extracts (selected data from Christeller et al., 1994).

	Proteolytic activity as % of control		
Inhibitor	*Tineola bisselliella*	*Hofmannophila pseudospretella*	*Anthrenocerus australis*
Soybean trypsin inhibitor	44 ± 11	42 ± 6	13 ± 2
Taro trypsin inhibitor	44 ± 6	38 ± 4	26 ± 2
Giant taro trypsin inhibitor-1	25 ± 4	31 ± 8	28 ± 2
Giant taro trypsin inhibitor-2	25 ± 5	32 ± 4	21 ± 3
Arrowhead trypsin inhibitor	30 ± 2	37 ± 6	20 ± 8
Erythrena latissima trypsin inhibitor	41 ± 2	51 ± 2	2 ± 1
Human α_2-microglobulin fragment	35 ± 3	43 ± 13	21 ± 5
Lima bean trypsin inhibitor	62 ± 8	64 ± 15	14 ± 2
Wheatgerm trypsin inhibitor 1	36 ± 2	38 ± 3	12 ± 3
Squash phloem trypsin inhibitor	18 ± 1	8 ± 2	14 ± 1
α_1-Antitrypsin (serpin)	13 ± 3	14 ± 3	11 ± 2
Potato proteinase inhibitor-2	60 ± 13	66 ± 8	14 ± 2
α_2-macroglobulin	12 ± 3	18 ± 8	5 ± 2

1.6 Insecticidal Approaches to Insect-proofing

1.6.1 Overview

During the industrial revolution, it was found that wool products could be protected from larval attack by frequent brushing, cleaning or airing, or by storing in cedar wood containers. Cedar wood shavings, camphor and tobacco leaves were all known to repel moths when stored in close proximity to wool (Clarke & Dougall, 1817). It was also known at this time that treatment of wool with turpentine or tobacco smoke had a mothproofing effect, although this was less than ideal due to an unpleasant odour. Unscoured wool was known not to suffer insect attack, and it was claimed "*many people have got rid of the insect by placing unwashed wool in layers among the infected cloth, or rubbing it upon the latter material, so as to communicate its flavour thereto*" (W & R Chambers, 1841, p. 188). The household remedies available at this time were dismissed as ineffective by Waterhouse (1958), although this is likely to refer to the practicality as much as the efficacy.

In the case of wool carpets, insect attack occurs in darker, undisturbed regions underneath furniture, or in low-wear regions near walls. Damp and humid conditions also promote insect attack. Methods for protection of wool carpets are more convenient when applied during the manufacturing process, although spraying insecticide solutions directly or applying during wet cleaning is also possible. Protection applied during manufacture usually occurs in the dyebath, where wool is immersed in water along with dyes and surfactant-based or salt-type auxiliaries before being heated to the boil for 20-30 minutes. Low water solubility of most insecticides promotes absorption into the wool fibre, although some bind to wool in a manner analogous to that of anionic wool dyestuffs, due to a sulphonate residue which imparts water solubility (Lewis & Shaw, 1987).

1.6.2 Insect Resist Agents Used on Wool

One of the first compounds to be used as a mothproofer was the yellow dye, Martius Yellow (2, 4-dinitro-1-naphthol) (Figure 1.8a), which was found to impart protection. Obviously, applications were limited by the yellow colour. Since 1920, many fluoride-based mothproofing agents have been released, but they have low fastness to washing (durability). For wool insecticides, fastness to washing and light is important as it reduces the need for re-application. In 1928, Eulan N (Figure 1.8b) appeared (Hartley, Elsworth & Barritt, 1943), closely followed by other similar triphenyl methane compounds heavily substituted with

chlorine and sulphonated to give water solubility. These were effectively colourless dyes which had moderately good washing fastness.

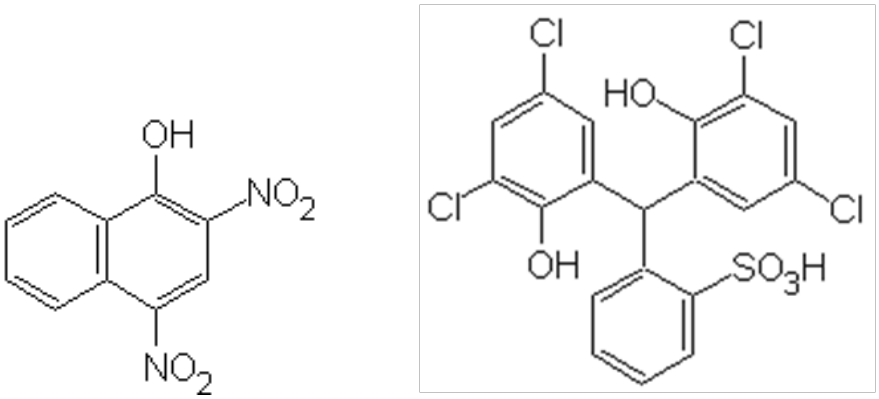


Figure 1.8 (a) Martius Yellow (left), and (b) Eulan N (right).

In 1939, Mitin FF was introduced, containing the substituted urea derivative sulcofenuron (Figure 1.9a). This has good washing fastness, but is relatively expensive (Lewis & Shaw, 1987). Dichloro-diphenyl-trichloroethane (DDT) (Figure 1.9b), was discovered as a potent insecticide in 1939 (Geigy's first 200 years, 1958), and was used in the 1940s through to the 1950s due to mothproofing effectiveness at levels as low as 0.1% on mass of wool (omw), and being relatively inexpensive. The slight volatility of DDT made it impactical for long-term protection of wool (Moncrieff, 1950). Washing fastness was poor, but higher levels were added to wool to compensate for this (The mothproofing of wool, 1949). DDT's environmental impact was studied extensively in the 1960s and it was not used from the 1970s due to high toxicity to aquatic (Cooke, 1972; Harri, Laitinen, & Valkama, 1979; Powell & Fielder, 1982) and bird life (Stokstad, 2007) and some evidence of carcinogenicity to humans (Cohn, Wolff, Cirillo, & Sholtz, 2007).

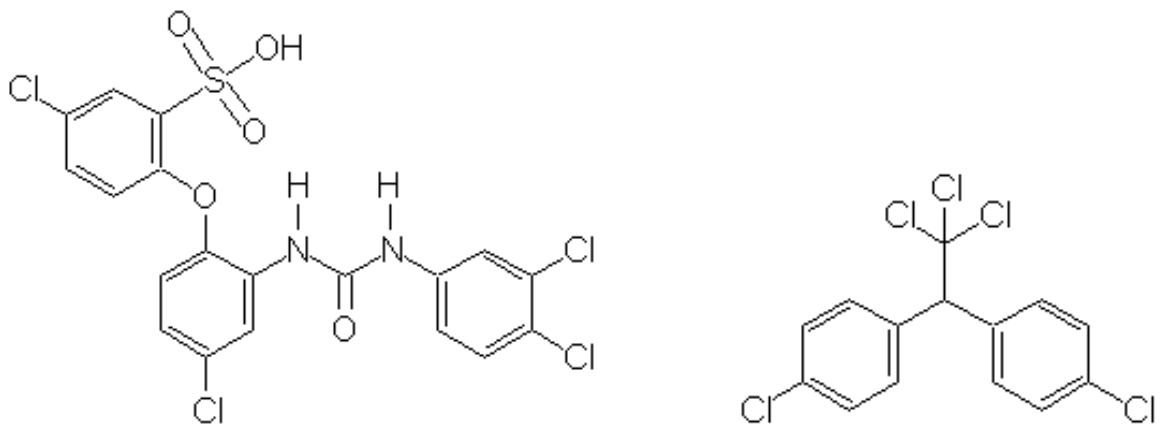


Figure 1.9 (a) Sulcofenuron (Mitin FF) (left), and (b) DDT (right).

Dieldrin (Figure 1.10a) was developed in the 1940s as an alternative to DDT. It was used extensively in the 1960s and 70s (Elliot, Janes, & Potter, 1978), but high mammalian and aquatic toxicity (Cooke, 1972), and an extremely high persistence in the environment (Sharom, Miles, Harris, & McEwen, 1980; Wells & Cowan, 1984) led to a ban. Since 1983, dieldrin has no longer been allowed under the Woolmark® brand for use on wool products (Lewis & Shaw, 1987).

A mothproofer based on chlorphenylid (Figure 1.10b) was introduced in 1958 (Lewis & Shaw, 1987). The chloromethylsulphonamido side chain imparts water solubility under alkaline conditions. When acidified for application onto wool, a milky dispersion of free sulphonamide is formed. The molecule has either five or six chlorine atoms attached to the benzene rings, shown as "x" and "y" in Figure 1.10b. Toxicity to *Rana temporaria* Linnaeus 1758 tadpoles was calculated to be around one half to one fifth that of dieldrin or DDT (Osborn & French, 1981). This compound, the active ingredient of Eulan WA New, was the main product used until 1988, when production was ceased by Bayer because of Federal German regulations forbidding processes that generate dioxins or dibenzylfurans, even though these were removed from the final product (Allanach & Shaw, 1989).

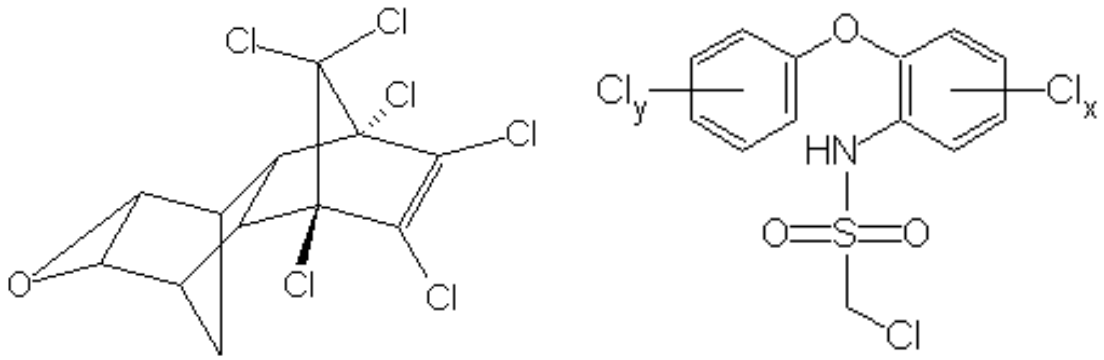


Figure 1.10 (a) Deildrin (left), and (b) chlorphenylid (Eulan WA) (right).

Permethrin (Figure 1.11a), a synthetic pyrethroid first trialled in 1976 (Carter, 1976), was commercially available from 1980 (Lewis & Shaw, 1987), and is still the major wool mothproofer used worldwide today. The molecular structure of pyrethroids is similar to pyrethrins, natural insecticides found in the flower heads of the pyrethrum plant *Chrysanthemum cinerariaefolium*. Permethrin has moderately good wash fastness, is now very cheap, and has low water solubility, allowing good uptake by wool in the dyebath (Carter & Duffield, 1977; Duffield, 1977). Advantages of permethrin over other non-

pyrethroid insect resist agents include effectiveness against the brown house moth *Hofmannophila pseudospretella*, no greater affinity for nylon over wool when dyeing blends, and a high affinity for wool, leading to the ability to be applied in continuous processing such as scouring, where immersion time is relatively short compared to dyebath application. A disadvantage of permethrin is that it has a relatively high toxicity to aquatic invertebrates and this has lead to severe effluent restrictions by UK water authorities (Allanach & Shaw, 1989).

Bifenthrin (Figure 1.11b), also a synthetic pyrethroid, was introduced as a wool insecticide by the Wool Research Organisation of New Zealand in conjunction with a commercial partner in the 1990s to replace permethrin. Advantages include better wash fastness, greater effectiveness at low levels, no resistance problem with beetles and a significantly lower environmental impact than permethrin (Barton, 2000). Disadvantages of bifenthin are that it is more expensive and has higher mammalian toxicity than permethrin.

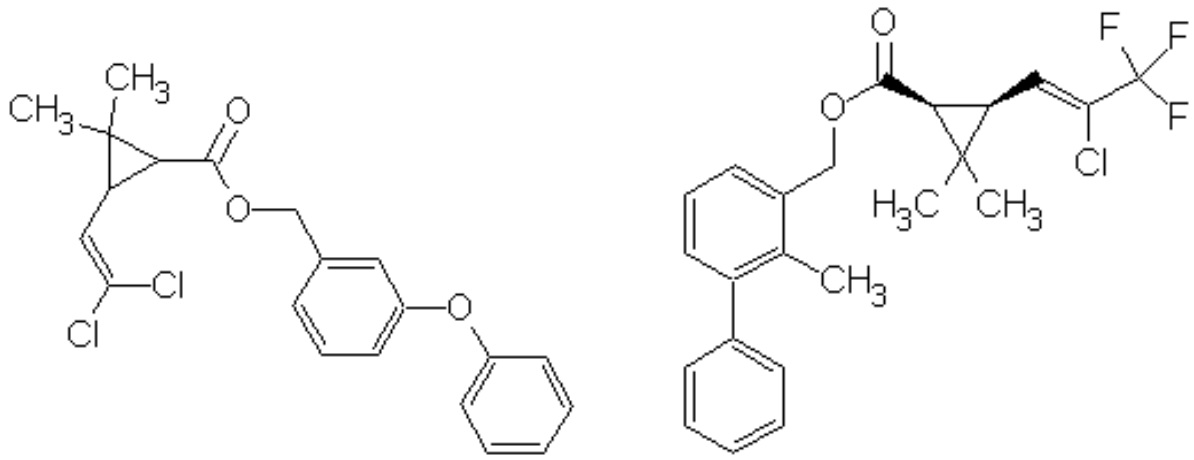


Figure 1.11 (a) Permethrin (left), and (b) bifenthrin (right).

A broad spectrum halogenated pyrrole insecticide, chlorfenapyr (Mystox MP) (Figure 1.12), was introduced by Catomance (UK)/AgResearch in 2007 as an insect resist agent for wool (Mill, 2007). No resistance is expected to develop in wool-damaging insect species in the near future as chlorfenapyr is new to the market. There are very few wool textiles in existence containing this insecticide, and therefore little opportunity exists for insect populations to build up a resistance. The vast majority of protected textiles contain pyrethroid insecticide and at present the only known resistance is by Australian carpet

beetles against permethrin (Barton, 2000). Chlorfenapyr has a similar toxicity to aquatic life to bifenthrin, but better wash and light fastness.

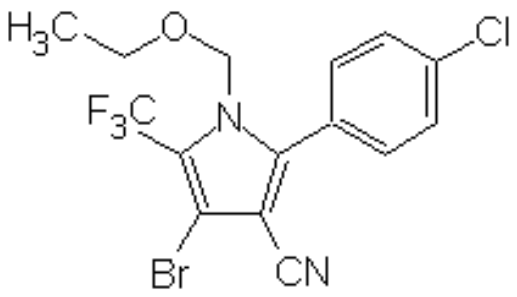


Figure 1.12 Chlorfenapyr (Mystox MP).

1.6.3 Mechansim of Insecticide Action

Permethrin acts as a general contact poison when direct contact with insects occurs, and as a stomach poison when ingested by insects (Tomlin, 1997). When used in an application such as flyspray, the contact effect is the mechanism relied upon to deliver a toxic dose. Wool treated with permethrin poisons wool pests via the gut when the wool is digested and permethrin released. The contact effect is not significant when insecticide is applied in the dyebath, as it is well absorbed into wool fibres, and no toxic effects are noted with insects that do not ingest the fibre (Lewis & Shaw, 1987). Damage to the textile product is not noticeable if the insecticide is present in sufficient concentration to quickly cause mortality or prevent feeding. The mechanism of toxic action of pyrethroids has generally been considered to be interference with the sodium gate in the nerve membrane (Narahashi, 1962). Later studies have shown that permethrin applied to voltage-clamped *Xenopus laevis* (African clawed frog) myelinated nerve fibres results in a delay in the closing of the activation (m) gate, which causes an increased and prolonged sodium tail current (van den Bercken & Vijverberg, 1979; Vijverberg, van der Zalm & van den Bercken, 1982). Vijverberg et al. also showed similarities in the mechanism of DDT and the pyrethroids allethrin and permethrin.

Chlorfenapyr belongs to the pyrrole class of chemistry, derived from dioxapyrrolomycin, and has good efficacy against lepidopteron species (Ahmad, Iqbal & Ahmad, 2003). It is a pro-insecticide, as it is metabolised into an active form after entering the insect. This active form is created in the presence of mixed function oxidases, forming the N-dealkylated analogue of

chlorfenapyr (AC-303,268), which exerts its toxicity through uncoupling of oxidative phosphorylation in rat, fish, and insect mitochondria (Black, Hollingworth, Ahammadsahib, Kukel & Donovan, 1994).

1.7 Non-Insecticidal Approaches to Insect-Proofing

1.7.1 Overview

Avoiding reliance on insecticides would be a significant step forward environmentally for the wool industry. A number of non-insecticidal methods of preventing larval attack on wool have been explored over the years but many of these were less than ideal because either the protection was inadequate or there were deleterious effects on the odour, colour or handle. Many also have poor fastness to washing, shampooing or dry-cleaning.

1.7.2 Surfactants

The first study of the use of surfactants against insects feeding on wool was carried out by Lipson (Lipson, 1955). Certain anionic surfactants applied to wool fabric via dyebath application at 5% omw were found to be effective against *Tineola bisselliella*. The most effective included alkylbenzene sulphonate, secondary alkyl sulphate, and alkyl sulphate. Lipson deduced that the effectiveness of an aliphatic compound is greater when the sulphate group is attached directly to the alkyl chain, and that the chain length is also important. Later work (Freeland & Williams, 1967) showed that a number of cationic and anionic surfactants provided insect-proofing effect against *Tineola bisselliella* and to a lesser degree *Anthrenocerus australis* larvae. Anionic surfactants containing linear alkyl chains were more effective than branched chains, and the length of the chain was most effective at 14-15 carbon atoms. Non-ionic compounds were found to have little insect-proofing effect. Freeland found that linear alkylbenzene sulphonic acid gave the most effective and durable protection when applied via wool dyeing. The mechanism of action of these surfactants has not been investigated.

It has been hypothesised that some herbivorous insects use surfactants present in oral secretions as a repellent to provide protection from predators (Rostas & Blassmann, 2009). It was shown that the oral secretion of beet armyworm larvae (*Spodoptera exigua* Hübner 1808) was highly amphiphilic and capable of wetting the hydrophobic cuticle of predatory ants, causing them to cease attack of the larvae and start extensive self-cleaning. The presence of surfactants was sufficient to explain the defensive character of the oral

secretion, which had previously been attributed to secondary metabolites obtained from the host plant (Sword, 2001). The irritant effect of oral secretions on predatory ants may parallel the toxicity of some anionic surfactants to keratin-digesting insects. Exposure of the ants to oral secretions was not fatal in the experiments by Rostas & Blassman, although they were only run for ten minutes, compared to the two week period for moth and beetle bioassays of Freeland & Williams (1967). The ants may have better sensory mechanisms for avoiding toxic compounds compared to wool-digesting larvae, as it seems unlikely that the ants would ingest toxic doses of oral secretion given their behaviour in the ten minutes they were observed. Alternatively, it may be that surfactants absorbed into wool fibre are more difficult to detect compared to freshly regurgitated oral secretions, and the wetting properties of surfactants are possibly less toxic when released in the insect gut compared to contact with the insect cuticle. It is unlikely that wool or fur would provide insects with secondary metabolites capable of repelling predators, therefore this defence mechanism may not have evolved in keratin-digesting insects. Five lepidopteran and one beetle species were found by Rostas & Blassmann to have amphiphilic properties, but these were all herbivores. Wool-digesting insect larvae may have oral secretions containing surfactants, but these are unlikely to be similar to those found by Freeland & Williams to be toxic.

One negative aspect of the surfactant approach to insect-proofing wool is that the application level required is much higher (2-5% omw) than for regular moth-proofing insecticides, which usually only require around 0.3% omw of a formulation to be applied. These are the levels required to impart an adequate insect-proofing effect to wool. Surfactants do have some toxicity to *Daphnia magna*, although this is usually much lower than for permethrin. The 48 hour LC_{50} for linear dodecyl benzene sulphonate is 5880-6840 µg/l (Maki & Bishop, 1979) compared to 0.2-0.6 µg/l for permethrin (Stratton & Corke, 1981). Allergies or sensitisation are unlikely to develop for surfactants that are well absorbed into wool fibres, and therefore inaccessible to humans.

1.7.3 Mothballs

Although the vapour of camphor had been noted as a moth repellant as early as 1841 (W & R Chambers, 1841), it had later been shown that camphor vapour was only toxic to adult moths, and that larvae remained unaffected (Chauvin & Vannier, 1994). In the early 20th century naphthalene was marketed as mothballs which prevented insect attack whilst imparting a distinctive odour to wool apparel. Naphthalene sublimes to a gas, which repels

clothes moths (Waterhouse, 1958), although it has been shown that both 1,4-dichlorobenzene and the synthetic pyrethroid empenthrin are more effective when used at the same level (Choi, Yu, & Kang, 1996). Mothballs are now made of 1,4-dichlorobenzene due to the flammability of naphthalene (The mothproofing of wool, 1949). The vapours of 1,4-dichlorobenzene are toxic to both adults and larvae of *Tineola bisselliella* (Chauvin & Vannier, 1994).

Non-odorous naphthalene derivatives may have a stronger mothproofing effect that could be worth studying. Mothballs can present a danger to children due to accidental ingestion. Ingested naphthalene, and to a lesser extent 1,4-dichlorobenzene mothballs have been observed to cause hemolytic anemia and methemoglobinemia in humans (Sillery, Lichenstein, Barrueto, & Teshome, 2009). Non-subliming derivatives of naphthalene would be safer and more practical if applied directly to wool during dyeing, possibly acting as gut poisons to insects from within the digested wool fibre.

The mechanism of action of naphthalene on moths does not appear to have been studied, although there has been research into the mechanism of action on mammalian cells, due to widespread human exposure (Wilson et al., 1996). Wilson et al. showed that the cytotoxicity and genotoxicity of naphthalene is associated with the formation of quinones from the metabolite 1-naphthol, rather than the primary metabolite naphthalene-1,2-epoxide. 1,2-naphthoquinone and 1,4-naphthoquinone were shown to be directly toxic to human mononuclear leucocytes and lymphocytes. Although insects were not used in this work, these two naphthoquinones, or closely related compounds, could be tested in initial mothproofing bioassays.

Rivett et al. (1990) showed that two naphthoquinones, juglone and plumbagin (Figure 1.13), with carbonyl groups in the 1,4 positions gave acceptably low mass losses according to Australian Standard 2001.6.1-1980 with *Tineola bisselliella* larvae when applied at 0.4% and 0.6% omw respectively to wool fabrics by padding. Juglone and plumbagin occur as natural products in the roots, leaves, bark, and wood of the English walnut (*Juglans regia*), white walnut (*Juglans cinerea*) and black walnut (*Juglans nigra*) (Inbaraj & Chignell, 2004).

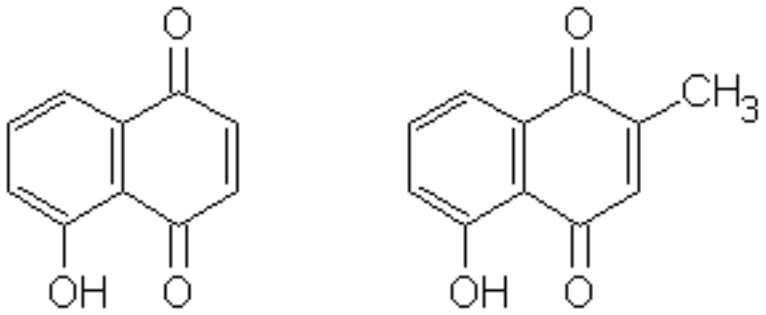


Figure 1.13 Naphthoquinones (a) juglone (left) and (b) plumbagin (right).

1.7.4 Essentials Oils, Acids, and Vitamins

The mothproofing efficacy of some plant-based essential oils has been investigated (Ingham & Sunderland, 2009), showing moderate activity against *Tineola bisselliella* by manuka oil at a level of 6% omw. This provided a borderline mass loss, and 54% mortality against *Tineola bisselliella* according to Wools of New Zealand Test Method 25. Other essential oils trialled with little mothproofing effect included lavender, basil, lemongrass, spearmint, and turmeric. Essential oils are unlikely to have good durability on wool textiles due to their volatility. Despite this, there have been some patented methods for protecting woollen clothing from moth and beetle larvae, including clove essential oil (Riedel, Heller, & Voigt, 1989), and filter paper containing *Juniperus rigida* (Cupressaceae) essential oil (Okano, 1993).

Some essential oils of the aromatic lavender plant (*Lavandula hybrid)*, rosemary herb (*Rosmarinus officinalis)*, and the Tasmanian blue gum (*Eucalyptus globulus)* tree have been shown to be toxic to the bean weevil *Acanthoscelides obtectus* Say 1831 (Papachristos, 2004). A quantitative analysis of each essential oil was carried out using gas chromatography/mass spectrometry (GC-MS) analyses, showing the terpenic constituents, mainly monoterpenoids. The LC_{50} of the sixteen principle monoterpeniods of the essential oils against *Acanthoscelides obtectus* was calculated by exposing insects to varying levels of monoterpenoid vapour for 24 hours. The most effective were terpinen-4-ol, camphor, 1,8-cineole, verbenone, and linalool with LC_{50} values ranging from 0.8 to 7.1 mg/l of air.

Formulations for the eradication of human head lice (*Pediculus humanus capitis* De Geer 1767) are usually based on permethrin or the natural parent compound pyrethrin with the synergist piperonyl butoxide (Burkhart, Burkhart, & Burkhart, 1998). There have been over a

dozen other non-insecticidal formulae available in New Zealand, mostly plant-based essential oils (Hutton, 2005). A recent study of twenty five Argentinean plant-based essential oils found that the vapours of *Cinnamomum porphyrium, Aloysia citriodora,* and *Myrcianthes pseudomato* were all effective in 60 minute knock-down trials of head lice (Toloza, Zygadlo, Biurrun, Rotman, & Picollo, 2010).

Acids most commonly used for pH control during wool dyeing include formic and acetic acid. Although they may be expected to have a neutralising effect on the alkaline mid-gut of *Tineola*, neither of these acids imparts a mothproofing effect on wool. Carboxylic acids with a longer alkyl chain length may impart a mothproofing effect, analogous to the alkyl benzene sulphonates/sulphonic acids investigated by Freeland and Williams (1967), while maintaining the correct acidity level in the dyebath during wool dyeing.

Vitamins are bio-active in mammals and some are known to be toxic in large amounts (Silverio et al., 2003). There is no scientific evidence that ingesting vitamin B1 (thiamine) can be useful in repelling mosquitoes from human skin (Holzer, 2001), although some anecdotal evidence remains on its efficacy (Kodkani, Jenkins, & Hatz, 1999). A range of B vitamins were applied to wool fabric and tested in bioassays with *Tineola bisselliella* (Plant Protection Research Unit, 1994a). The greatest mothproofing effect was found with vitamin B2 (riboflavin), reducing the amount of wool consumed by over half, although not passing the Wools of New Zealand Test Method 25. None of these vitamins caused significant larval mortality.

1.7.5 Antimicrobials

Antimicrobial compounds cover a broad class of chemicals that could interfere with biochemical pathways in the insect digestive system, as they do in bacteria. There could also be an effect on the insect gut flora, as found in the work of Trivedi et al. (1991) with *Anthrenus flavipes*. There appear to be very few studies on the use of antimicrobials to control insects. Careful selection of these compounds would be needed to ensure minimal toxicity to aquatic organisms and mammals. The dry, nutrient-free surface of carpets does not usually harbour mammalian pathogens; therefore cross resistance is not an anticipated problem.

The napthoquinones, plumbagin and juglone (Figure 1.13), are known to have antifungal, antiviral, and antibacterial properties (Inbaraj & Chignell, 2004). They have also been shown to have mothproofing properties (Rivett et al., 1990).

1.7.6 Introducing Non-Reducible Cross-Links into Wool

Altering the chemical structure of wool to confer insect resistance has been attempted by the modification of existing disulphide cross-links, or the introduction of new ones. The resistance of wool to enzymes *in vitro* was studied before and after mechanical damage and reduction of the wool with a thioglycollate solution (Geiger, Patterson, Mizell, & Harris, 1941). It was found that wool was susceptible to pepsin and chymotrypsin after mechanical damage, but only the intercellular substance of the wool fibre was digested, releasing individual cortical and cuticle cells. Breaking of the wool disulphide bonds via the reductive thioglycollate treatment led to almost complete digestion of the wool by pepsin and chymotrypsin, whereas untreated control wool was completely resistant to enzymes. Reduced wool that was re-oxidised showed the same resistance to enzymes as control wool. Reduced wool that had been rebuilt with non-reducible bis-thioether links was even more resistant to chemical attack from alkalis, acids, oxidising/reducing agents, and more stable to biological agents such as enzymes, moths, and carpet beetles (Geiger, Kobayashi, & Harris, 1942). These bis-thioether links are different to the disulphide links usually found in wool, as they contain a linear alkyl moiety between the two sulphur atoms (Figure 1.14). This gave rise to a theory that modification of wool's cystine disulphide cross-links into non-reducible cross-links may impart resistance to enzyme attack *in vivo*, and therefore confer insect resistance on the wool.

$$R\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}R \rightarrow R\text{-}CH_2\text{-}S\text{-}(CH_2)_n\text{-}S\text{-}CH_2\text{-}R$$

Figure 1.14 Cystine residue (left), and bis-thioether cross-link (right).

Geiger and Harris (1942) also investigated the effect of the molecular size of wool proteins on their rate of digestion by pepsin. Wool proteins of varying molecular mass were prepared by reducing wool and making a series of solutions of various concentration before re-oxidising the protein. Protein preparations expected to be of low molecular weight were more rapidly digested than those of high molecular weight. The largest proteins were almost as resistant to digestion as untreated wool. Geiger and Harris concluded that wool was

resistant to digestion by enzymes due to a unique structure consisting of peptide chains joined by disulphide cross-links to form a three-dimensional polymeric network of extremely high molecular weight.

Moncrieff (1950) attempted to alter the structure of the free amino acids present in wool in the hope that it would make the wool less attractive as a food source for insects. He filed a provisional patent (Moncrieff, 1948, as cited in Moncrieff, 1950, p. 125) for a process of treating wool with a dialdehyde solution to react with diamino acid groups (arginine, lysine and histidine) found throughout wool to form modified wool containing a different cross-link involving nitrogen (Figure 1.15). Glyoxal was used as the dialdehyde, and it was found that only around 1% of the level calculated to react with the arginine, lysine, and histidine groups present in wool proteins was required to confer good mothproofing properties to the wool in tests using *Tineola bisselliella* larvae. Moncrieff hypothesised that the mothproofing effect may not have been brought about by the crosslinking of any one amino acid, but rather the increase in the size of the wool molecules due to a higher state of polymerisation.

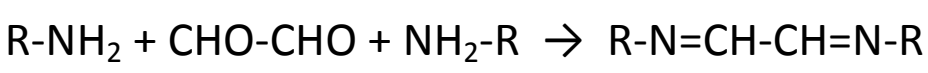


Figure 1.15 Wool polymerisation using glyoxal.

Gibb (1994) later trialled three approaches, the first followed the idea of Moncrieff (1950) using dialdehydes to react with diamino acid residues (lysine, arginine, and histidine) on the wool. A second approach followed the idea of Geiger, Kobayashi, & Harris (1942) involving reduction of cystine residues, followed by a dibromopropane treatment to form bis-thio ether cross-links. The third approach was to treat wool with alkali to form non-reducible lanthionine cross-links (Figure 1.16). Lanthionine is the most common reaction product from the treatment of wool with alkali. It arises from the reaction of alkali with cystine residues. None of these cross-links was known to bring about changes in tactile or olfactory properties of the wool. The presence of new non-reducible cross-links in the treated wool was investigated by measuring their solubility in an alkaline solution containing urea and mercaptoethanol. The mothproofing efficacy conferred by the cross-links was determined by exposing the treated fabrics to *Tineola bisselliella* larvae according to the British Standard test BS 4797:1978, Determination of Resistance to certain Insect Pests. The susceptibility of

the treated wool to proteolytic enzymes in a reducing environment was tested by reducing and treating the wool fabrics in trypsin and chymotrypsin solutions.

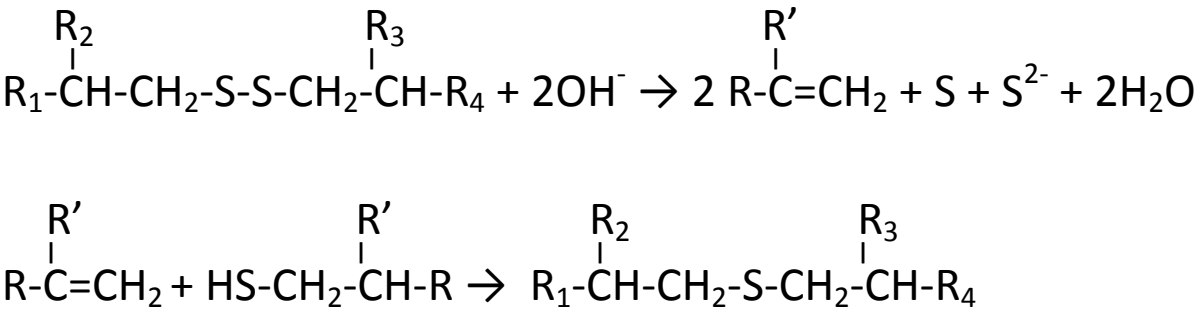


Figure 1.16 Formation of lanthionine cross-link (a) Step 1: cystine residue + alkali → dehydroalanine + elemental sulphur + ionic sulphur + water (top), and (b) Step 2: dehydroalanine + cysteine residue → lanthionine (bottom).

All of the treatments were successful in introducing non-reducible cross-links and in lowering the solubility of the wool, after reduction, in the enzyme solutions. The insect resistance of the wool was generally greater for the treated wool compared to the untreated wool, but was still considered to be unsatisfactory according to the test method used. Combinations of two treatments gave a greater mothproofing effect, therefore Gibb (1994) concluded that the larvae may have more than one pathway for gaining nutrition from wool protein.

1.8 Determination of Insect Resistance

1.8.1 Overview

Ideally, any new insect resist agent should be tested with the major insect pests likely to attack wool. There are various standard methods for determining the insect resistance of woollen textiles. These are very similar in their approach, involving the exposure of the wool product to feeding insect larvae, and an estimation of the damage caused. Wools of New Zealand Test Method 25 is based on the International Organization for Standardization (ISO) Test Method 3998-1977(E). There is also an Australian Standard Test Method AS 2001.6.1-1980 and a British Standard 4797-1978. These methods are generally the same, using eight standardised specimens of textile (fabric, felt, yarn or carpet) for each test treatment, and for the untreated control. The ISO and British methods specify *Attagenus piceus* and *Anthrenus flavipes* beetle larvae, in addition to *Tineola bisselliella* and *Tinea pellionella* moth larvae as the insects that can be used. The Australian Standard uses insect species more

common in Australia – *Anthrenus flavipes* beetle or *Tinea translucens* moth larvae. The Wools of New Zealand method includes all the species above, with the addition of *Anthrenus verbasci, Attagenus pellio,* and *Anthrenocerus australis* beetle larvae*,* and *Tinea dubiella,* and *Hofmannophila pseudospretella* moth larvae. In the Wools of New Zealand method the specimens are conditioned in separate containers at 25 ± 1°C and 65 ± 2% relative humidity (RH) for at least 24 hours (with the exception of bioassays using *Attagenus* species which are held at 27 ± 1°C). There are slight differences in the temperature and humidity used between the test methods. AS2001.6.1-1980 uses 24 ± 2°C and 60 ± 5% RH for both species, whereas the ISO and British standards use 27 ± 1°C for *Attagenus piceus* and *Anthrenus flavipes*, 25 ± 1°C for *Tinea pellionella*, and 24 ± 1°C for *Tineola bisselliella*, and 65 ± 2% RH for all species. After conditioning, the weights of the specimens are then recorded to an accuracy of 0.1 mg. Four of the specimens from each treatment are kept as humidity controls, and the other four are each exposed to 15 insect larvae. After 14 days, the larvae, cast skins, excrement and loose fibres are removed from the specimens before the final weight is recorded. The condition of the larvae is recorded as live, pupating, or dead. The mass loss from each specimen is recorded, and mean values are calculated. Visual assessment of the specimens is carried out (Figure 1.17), and is more important for apparel fabrics than other substrates. For a given mass loss, damage to apparel fabrics is more visible than for carpet, due to the finer fibres and yarns used in fabrics and the pile structure of carpet. The severing of one yarn can ruin the appearance of a wool garment, whereas carpets require several tufts to be severed to cause a noticeable change in appearance. A pass, borderline, or fail result is given for each test treatment based on the mean mass loss and visual assessment. A mean mass loss of under 12 mg on fabrics, or under 15 mg on carpets is required to pass the Wools of New Zealand Test Method 25, although there are also some other requirements for the visual assessments. The other test methods all require mean mass losses of less than 15 mg for a pass. Details of the mass loss and visual assessment calculations, and how they relate to pass, borderline, or fail grades in the Wools of New Zealand Test Method 25 are given in Appendix B.

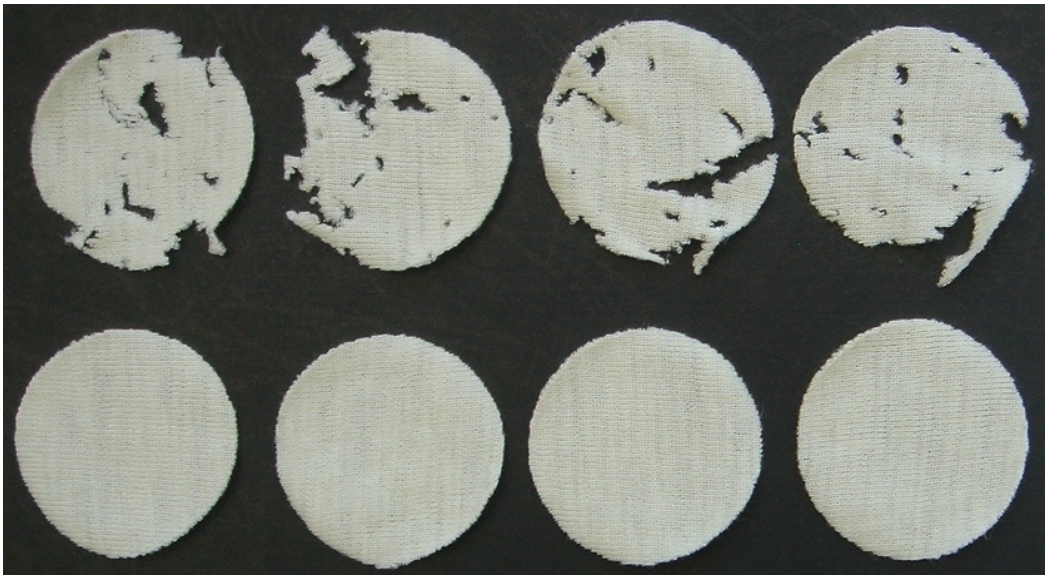


Figure 1.17 Wool fabric discs as used in Wools of New Zealand Test Method 25: four exposed to *Tineola bisselliella* (top) and four humidity controls (bottom).

It should be noted that mass loss was the main criterion used for assessment of treatments in this thesis. Visual assessments are less precise, and can be open to subjective differences in interpretation. The vast majority of wool treated with insect resist agents is used in carpets, where visual assessment is less important than it is for fabrics. However, fabrics were used for this work due to the comparative ease of chemical application and locating insect larvae for assessment. The amount of untreated control wool fabric consumed can vary over time, making comparison of results from different batches of bioassays difficult. This can be largely overcome by comparing the mass loss figures as a percentage of the controls. If larval insect feeding increases over time on the control fabric, it becomes more difficult for a treated fabric sample to pass the test method based on the mass loss data. This is an unavoidable aspect of biological assays, as it is impossible to maintain consistent feeding voracity over time.

Chapter 2

Selection of Compounds and Bioassay Trials

Three classes of compound were selected for investigation – surfactants, naphthalene derivatives, and antimicrobials. Surfactants are suited to insect-proofing due to most having an affinity for wool in aqueous solution, low environmental toxicity compared to insecticides, and being inexpensive (Lipson, 1955; Freeland & Williams, 1967). Many naphthalene derivatives are available, with a variety of functional groups bonded to the naphthalene, allowing elucidation of structure-function relationships based around naphthalene which has a known repellent effect on the common clothes moth. The efficacy of Martius Yellow (Waterhouse, 1958) shows that simple modifications to naphthalene can yield compounds of high efficacy against moth attack of wool. Antimicrobial compounds can be very specific in their mode of action, allowing the targeting of specific biochemical pathways within the insects of interest, possibly directly related to wool digestion. The presence of bacteria in the digestive tract of some wool-digesting insect larvae (Trivedi et al., 1991; Shannon et al., 2001) suggests that antimicrobials may be able to interfere with the gut physiology of these species and possibly also closely related species.

2.1 Application of Compounds to Wool Fabric

To assess the insect protection provided by various non-insecticidal compounds, these compounds were applied to a specific wool fabric that was then exposed to insects to measure the amount of wool eaten and insect mortality. All compounds were sourced from Sigma Aldrich New Zealand, unless otherwise stated. Initial applications were at 3.0% omw onto 100% wool fabric. This was chosen as a concentration high enough to see any anti-feeding effect without being overly impractical due to volumes required in an industrial environment. Fabric construction details were 271 g/m^2, plain weave using single yarns of 125 grams per kilometre, folded together at 100 turns per metre to give a two-fold yarn. The solvents water, ethanol, or acetone were used to dissolve or disperse the compounds before application to the wool fabrics. Visual inspection of the solvent/compound mixtures was made to ensure a clear solution was obtained if possible. A dropper was used to apply solutions of each compound evenly to eight pre-cut wool fabric discs of 38 mm diameter.

After application, the wool fabric discs were left to air-dry at room temperature for at least four hours. After drying, the edge of each wool fabric disc was checked for loose yarn, which was removed before using for insect bioassays. Loose yarns could increase the measured mass loss if shed during the assessment of fabrics, leading to overestimation of the mass loss due to insect larvae feeding.

2.2 Fabric Bioassays using Wools of New Zealand Test Method 25

Insect bioassay trials followed the Wools of New Zealand Test Method 25, based on ISO 3998-1977(E). For each evaluation (including control) there were eight fabric discs of 38 mm diameter. The fabric discs were placed in separate ventilated plastic bottles and conditioned at 25°C and 65% relative humidity (RH) for 24 hours. Insects were reared on a diet slightly different to that specified in the test method, using 16 parts fishmeal to one part brewer's yeast (*Tineola bisselliella*), or 20 parts fishmeal/20 parts oats/20 parts brewer's yeast/one part wool (*Anthrenocerus australis*). Actively feeding larvae were placed on a 1.25 mm mesh sieve, shaken, and exposed to light to encourage larvae to fall through onto a 1.00 mm mesh sieve. Those larvae that did not pass throught the 1.00 mm sieve, described as late instar, were kept and used for experiments. Fifteen insect Iarvae were placed on each of the four fabric discs, while the other four discs were kept as humidity controls. After 14 days the condition of the larvae was assessed, noting mortality and pupation. The discs were brushed clean of larvae, cast skins, excrement and loose fibres. Visual appearance was assessed based on cropping on the edge of the disc, and holes within the fabric. The discs were conditioned for another 24 hours before final weighing. The mean mass loss due to larval feeding was calculated. The variance of mass loss data was analysed using ANOVA to test whether there were any statistically significant differences between the control and treated groups of fabric within a confidence level of 95%. This test method was first used with *Tineola bisselliella* moth larvae, and repeated with *Anthrenocerus australis* beetle larvae using the best performing compounds, as this species is more difficult to control and is a common wool pest in Australasia.

The control fabrics in these bioassays showed low larval mortalities and high mass losses, indicating that the insect colonies were in good condition. Bioassays were run at different times, sometimes overlapping, necessitating a new control for each batch. Results from the same bioassay batch were sometimes split between sections, resulting in the same control data appearing more than once. Pupation occurred in some *Anthrenocerus australis* results,

which may have had the effect of lowering the mass of wool consumed due to pupae not feeding. In these bioassays pupation was fairly consistent, allowing a fair comparison between samples.

2.3 Surfactants

2.3.1 Selection of Surfactants

Previous studies by Lipson (1955), and Freeland and Williams (1967) have identified a number of surfactants effective against wool-digesting insects. Variations on structural features of the surfactants studied by Freeland and Williams were pursued in order to test structure-function relationships. The three general classes of surfactant studied were anionic, zwitterionic, and non-ionic surfactants.

2.3.1.1 Anionic Dodecylbenzene Sulphonic Acid and Structural Analogues

Freeland and Williams (1967) found that anionic linear alkyl benzene sulphonates and sulphonic acids were the most practical group of surfactants for insect-proofing of wool. Using derivatives of these surfactants containing alkyl chains of varying length, they found 14-15 carbon atoms were optimal for mothproofing, closely followed by 12 carbon atoms. As the surfactants containing the 14 and 15 carbon atom chains are now not readily available, the 12 carbon atoms of dodecylbenzene sulphonic acid provided a starting point for the surfactant study. Variations on this molecule included removing the alkyl chain to give benzene sulphonate, removing the sulphonate group to give 1-phenyldodecane, replacing the sulphonic acid with a hydroxyl group to give 4-dodecyl phenol, and testing just the dodecyl alkane group alone. Benzene alone is too volatile for practical mothproofing tests. Obtaining compounds with these structural variations for application to wool and subsequent bioassay should identify which parts of the molecule contribute to the anti-feeding effect.

2.3.1.2 Zwitterionic and Anionic Surfactants

Two linear alkyl sulphate surfactants and a betaine zwitterionic surfactant were obtained for application to wool fabric for bioassays. Sodium lauryl ether sulphate and sodium lauryl sulphate both have a dodecyl alkyl chain. Coco dimethyl betaine has an 11 carbon atom alkyl chain bonded to the positive nitrogen atom, close to a negatively charged carboxylate group. This previously untested zwitterion chemistry, coupled with a similar sized alkyl chain made coco dimethyl betaine a promising lead chemical for bioassays. The zwitterionic N,N-

dimethyldodecyl amine N-oxide was also obtained, due to its simple structure similar to the anionic surfactant sodium lauryl sulphate. N,N-dimethyldodecyl amine, a structual analogue of N,N-dimethyldodecyl amine N-oxide provided a comparison of the same molecular structure without the oxygen atom.

3-(N,N-dimethyl myristylammonio) propane sulphonate is a zwitterionic surfactant containing a 14-carbon alkyl chain, bonded to a quaternary ammonium cation. The sulphonate group is at the opposite end of the molecule to the alkyl chain, as is the case with dodecylbenzene sulphonic acid. The anionic surfactant N-lauroylsarcosine contains an 11-carbon alkyl chain, with a polar carboxylate group at the opposite end of the molecule. The amide group in the middle of this molecule could possibly be cleaved in the protein-digesting insect gut. N-(2-Acetamido)-2-aminoethanesulphonic acid (ACES) is a zwitterionic taurine surfactant. The amide group is at the opposite end of the molecule to the sulphonic acid, and is more polar than the alkyl groups of N-lauroylsarcosine and 3-(N,N-dimethyl myristylammonio) propane sulphonate. N-(2,4-Dinitrophenyl) taurine sodium salt is an anionic surfactant. It is similar to ACES, except that it has a dinitrophenyl group instead of an acetamido group. Nitro groups are present in many neonicotinoid insecticides, including imidacloprid, clothianidin, dinotefuran, thiamethoxam, nithiazine, and nitenpyram.

2-Acrylamido-2-methyl-1-propanesulphonic acid is a hydrophilic, anionic, sulphonic acid acrylic monomer. The acrylamido group may decompose to form ammonia, which in an aqueous environment may disrupt the insect gut enzymes by raising the pH above the optimum.

Amido sulphobetaine-14 (ASB-14) is a zwitterionic surfactant similar to 3-(N,N-dimethyl myristylammonio) propane sulphonate, except with the inclusion of an amido-propane group attached to the alkyl chain. The larger molecular size and reactivity of the amide group were of interest. 3-((3-Cholamidopropyl) dimethylammonio)-1-propanesulphonate (CHAPS) is a zwitterionic surfactant. It has the same molecular structure as ASB-14, with the exception of the 13 carbon alkyl chain of ASB-14 replaced with a large derivative of cholic acid, which is itself a derivative of cholesterol.

2-(N-Morpholino) ethanesulphonic acid sodium salt (MES) is an anionic surfactant with a previously untested morpholine ring. It is commonly used as a biochemical buffer with a pK_a

(logarithmic constant of the acid dissociation constant) of 6.15 at 20°C. This weak acid may be useful in lowering the pH of the alkaline *Tineola bisselliella* midgut.

Methyl 2-sulphooctadecanoate sodium salt is an anionic surfactant with an ester group and a sulphonate group bonded to a secondary carbon atom. Previously selected surfactants only have sulphonate groups bonded to a terminal (primary) carbon atom at the end of an alkyl chain.

Sodium deoxycholate is an anionic surfactant and bile acid with a similar structure to CHAPS. The alkyl chain possesses the sodium salt of a carboxylic acid, without the zwitterionic moiety propyl dimethylammonio-1-propanesulphonate found in CHAPS. This molecule shares a similar structure to the sodium salt of cholic acid, and contains a sterol sub-unit. Sterols are a dietary requirement for all insects (Waterhouse, 1958), as are B-vitamins for *Tineola bisselliella* (Crowell & McCay, 1937) therefore an increased intake of sodium deoxycholate may lead to toxicity in the same manner as vitamin B2 had shown against *Tineola bisselliella* (Plant Protection Research Unit, 1994a). This effect may also be present for wool treated with CHAPS which also contains a cholic acid derivative.

N-(Tris(hydroxymethyl) methyl)-3-aminopropane (TAPS) is an anionic surfactant with a branched alkyl chain, and three primary alcohol groups. This short-chain amino alcohol may affect the solubility of the insect gut contents, or the surface tension of the interface between the gut wall and gut contents. Taurocholic acid is an anionic surfactant with similarities to CHAPS and sodium deoxycholate. The amide and sulphonate groups are separated by an ethyl group, whereas in CHAPS they are separated by a propyl/dimethylammonio/propyl moiety.

L-α-phosphatidylcholine is a phospholipid, but is also classified as a surfactant with lipolytic action (Serra, 2001). It can be described as a phosphoamphoteric molecule due to the phosphate and quaternary ammonium groups acting as basic and acidic groups respectively. This molecule offers a contrast to the zwitterionic ASB-14, CHAPS, and 3-(N,N-dimethyl myristylammonio) propane sulphonate where the anionic groups were positioned at the end of the alkyl chain, and the cationic groups positioned within the alkyl chain, separated from the anionic group by three carbon atoms. These positions were reversed in L-α-phosphatidylcholine, and with only two carbon atoms separating the two groups.

The anionic phenyl-2-amino benzene sulphonate is unique among the sulphonated benzene molecules selected, as the sulphonate group is positioned between two benzene groups via direct bonding to the sulphur and one oxygen atom.

2.3.1.3 Non-Ionic Surfactant

Freeland and Williams (1967) found non-ionic polyoxyethylene nonylphenols and primary alcohol-ethylene oxide biodegradable adducts ineffective at mothproofing. Due to the lack of efficacy against *Tineola bisselliella* compared to anionic and cationic surfactants, no further non-ionic compounds were investigated. A non-ionic surfactant sorbitan monopalmitate, with structural similarities to dodecylbenzene sulphonic acid, was obtained for comparison of bioassay results.

2.3.2 Bioassay Results of Wool Fabric Treated with Surfactants

2.3.2.1 Anionic Dodecylbenzene Sulphonic Acid and Structural Analogues

Removal of either the alkyl chain or the sulphonic acid group from the dodecylbenzene sulphonic acid molecule (benzene sulphonic acid and 1-phenyldodecane respectively) (Figure 2.1b, c) resulted in a loss of mothproofing effectiveness (Table 2.1). The dodecane alkyl chain alone (Figure 2.1e) had no mothproofing effect. Replacing the sulphonic acid group of the dodecylbenzene sulphonic acid molecule with a hydroxyl group (4-dodecyl phenol) (Figure 2.1d) resulted in only a slight loss of activity by way of a slightly higher mass loss and lower mortality. Previous work has also shown that good mothproofing is achieved against *Tineola bisselliella* by replacing the sulphonic acid group with an amino group to give dodecyl aniline (AgriQuality, 2005). It appears that the mothproofing effect of dodecylbenzene sulphonic acid requires the presence of the polar and non-polar entities at each end of the molecule.

Previous bioassays (Plant Protection Research Unit, 1994b) have shown dodecylbenzene sulphonic acid to be less effective against *Anthrenocerus australis* beetle larvae, similar to the results of Freeland and Williams (1967) with the similar *Anthrenus flavipes* beetle larvae.

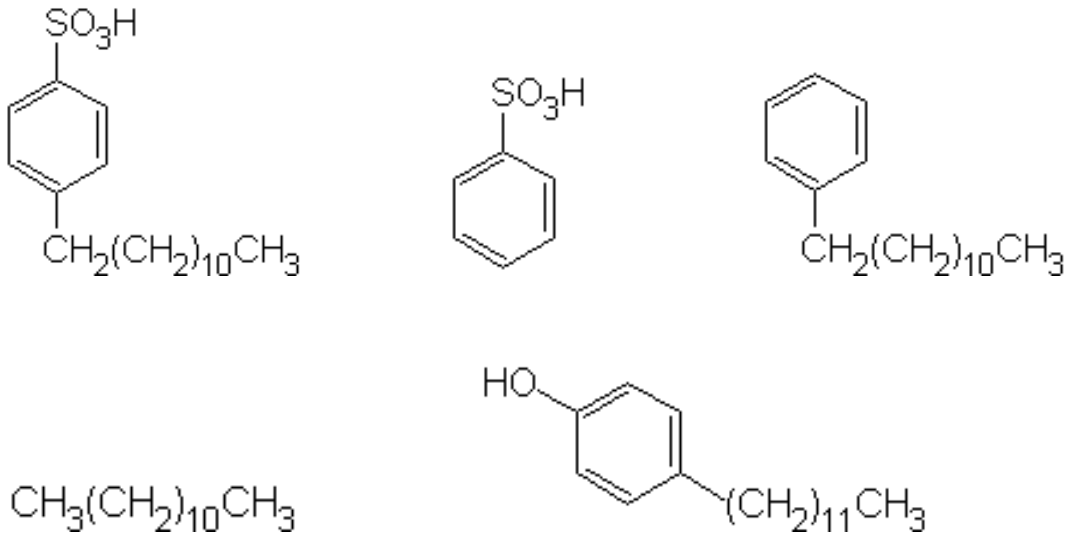


Figure 2.1 Clockwise from top left: (a) dodecylbenzene sulphonic acid, (b) benzene sulphonic acid, (c) 1-phenyldodecane, (d) 4-dodecyl phenol, (e) dodecane.

Table 2.1 Bioassay results of *Tineola bisselliella* on wool fabric treated with dodecylbenzene sulphonic acid and structural analogues.

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	1.7	0.0	76.4 ± 3.3	n.a.	4D	n.a.
Dodecyl benzene sulphonic acid	72.3	1.7	4.0 ± 0.6	5.2	1A	p
Benzene sulphonic acid	0.0	0.0	108.4 ± 9.0	141.9	4D	f
Control	0.0	0.0	116.8 ± 6.6	n.a.	4D	n.a.
1-phenyl dodecane	0.0	0.0	108.0 ± 11.0	92.5	4D	f
Dodecane	3.2	1.7	111.9 ± 4.2	95.8	4A	f
Control	1.9	3.6	51.3 ± 5.7	n.a.	4D	n.a.
4-dodecyl phenol	55.1	1.7	6.1 ± 1.4	11.8	2B	p

[1] As a percentage of the mean voracity control.

2.3.2.2 Zwitterionic and Anionic Surfactants with Tineola bisselliella

Sodium lauryl ether sulphate, sodium lauryl sulphate and coco dimethyl betaine (Figure 2.2a-c) all showed borderline mass loss against *Tineola bisselliella* larvae when applied to wool at 3.0% omw (Table 2.2). They were less effective than dodecylbenzene sulphonic acid applied at 2.0% omw (Freeland & Williams, 1967) due to higher mass losses and lower mortalities against the same species. All three compounds have a non-polar alkyl chain and a polar group at the opposite end of the molecule. The zwitterion chemistry of coco dimethyl betaine did not appear to reduce the anti-feeding effect compared to the two anionic compounds.

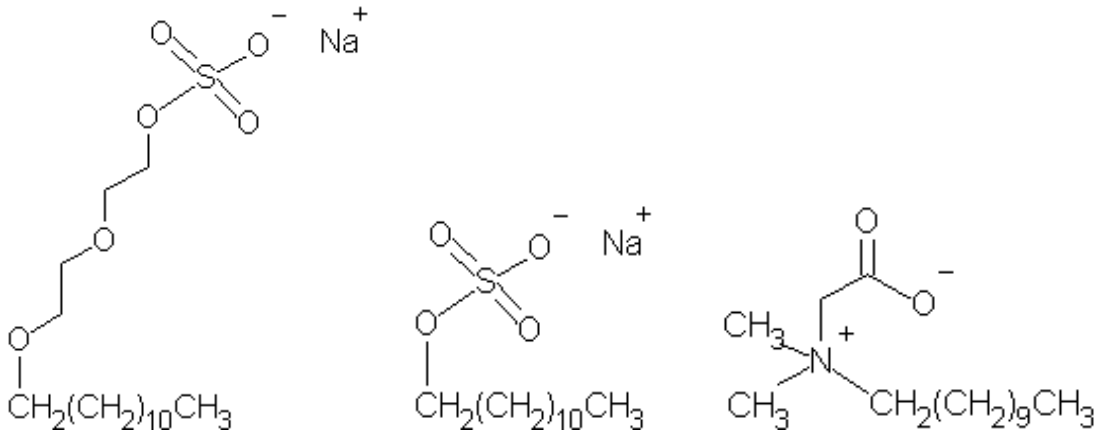


Figure 2.2 (a) Sodium lauryl ether sulphate (left), (b) sodium lauryl sulphate (centre), (c) coco dimethyl betaine (right).

Table 2.2 Bioassay results of *Tineola bisselliella* on wool fabric treated with anionic and zwitterionic surfactants

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	8.3	3.3	85.1 ± 15.1	n.a.	4D	n.a.
Sodium lauryl ether sulphate	12.3	5.0	14.8 ± 1.9	17.4	3C	f
Sodium lauryl sulphate	20.2	0.0	12.6 ± 1.0	14.8	3C	f
Coco dimethyl betaine	10.6	0.0	12.6 ± 1.2	14.8	2B	f

[1] As a percentage of the mean voracity control.

3-(N,N-dimethyl myristylammonio) propane sulphonate (Figure 2.3) treatment at 3.0% omw imparted a high mortality to *Tineola bisselliella* and low mass loss (Table 2.3) to the wool fabric. Freeland and Williams (1967) noted very similar mass losses on fabrics containing 2.0% omw alkylbenzene sulphonates with alkyl chain lengths of 14-15 carbon atoms in length. This showed that the moiety between the alkyl and sulphonate groups can be different to a benzene group, and still retain the mothproofing effect shown by the alkylbenzene sulphonate molecule. As previously shown with coco dimethyl betaine, the zwitterionic structure was also compatible with the mothproofing effect. A bioassay using a lower level of 2.0% omw showed a much higher mass loss and lower mortality (Table 2.3), indicating that the minimum effective rate for 3-(N,N-dimethyl myristylammonio) propane sulphonate against *Tineola bisselliella* was only slightly less than 3.0% omw. The large difference in mass loss results indicated the 3.0% 3-(N,N-dimethyl myristylammonio) propane sulphonate fabric may have been contaminated with a substance toxic to *Tineola bisselliella*, and that this result should be treated with caution.

N-lauroylsarcosine (Figure 2.3) has structural similarities to coco dimethyl betaine (Figure 2.2), although it showed no reduction in mass loss compared to the control fabric (Table 2.3). The presence of an amide group between the head and tail of the molecule, or the weaker polarity of the head of the molecule, may be responsible for the lack of mothproofing effect.

The phospholipid phosphatidylcholine (Figure 2.3), although not a surfactant, was tested alongside the surfactants due to structural features worthy of investigation (Table 2.3). This molecule differs from the zwitterionic surfactants in that a quaternary ammonium cation is present at the head of the molecule rather than nearer the middle, and has fatty acid residue tails. The negatively charged phosphate group in the middle of the molecule gives an opposite charge to the cationic group, and therefore similarity to the zwitterionic surfactants. Freeland and Williams (1967) showed that some quaternary ammonium halides were effective at mothproofing when used at levels as low as 0.2% omw. Phosphatidylcholine had no antifeeding effect, but had the reverse effect of significantly increasing the rate of larval feeding (Table 2.3). It may be that the anionic phosphate group in the centre of the phosphatidylcholine molecule provides an easy point for cleavage, and the separation of the molecule into two parts renders it less effective. The fatty acid residue

tails were unspecified for this molecule and may have been of sub-optimal length for mothproofing.

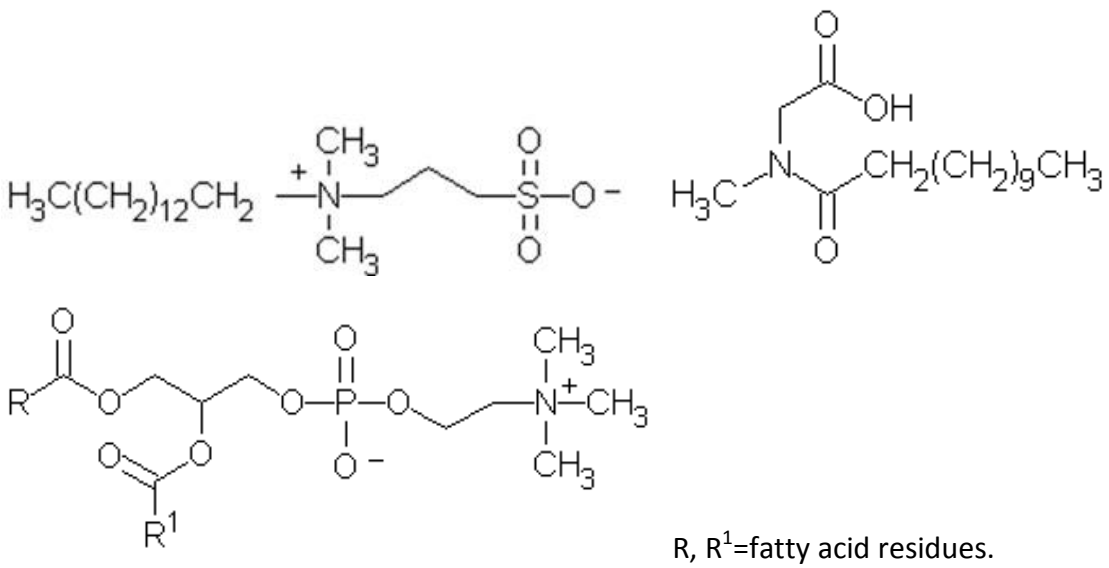


Figure 2.3 (a) 3-(N,N-dimethyl myristylammonio) propane sulphonate (top left), (b) N-lauroylsarcosine (top right), and (c) phosphatidylcholine (bottom left).

Table 2.3 Bioassay results of *Tineola bisselliella* on wool fabric treated with anionic and zwitterionic surfactants and phospholipid.

Treatment	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or border line (b)
Control	1.7	1.7	96.8 ± 4.4	n.a.	4D	n.a.
3-(N,N-dimethyl myristylammonio) propane sulphonate (3.0% omw)	95.0	0.0	4.7 ± 1.1	4.9	1A	p
N-Lauroylsarcosine 3.0% omw	10.0	0.0	84.7 ± 10.7	87.6	4D	f
Phosphatidylcholine (3.0% omw)	1.7	0.0	130.0 ± 7.7	134.3	4D	f
Control	0.0	0.0	51.5 ± 4.2	n.a.	3C	n.a.
3-(N,N-dimethyl myristylammonio) propane sulphonate (2.0% omw)	16.5	0.0	27.4 ± 4.9	53.3	3C	f

[1] As a percentage of the mean voracity control.

Amido sulphobetaine-14 (ASB-14) (Figure 2.4) is a zwitterionic surfactant, which at 3.0% omw gave a marginal pass in terms of a mass loss of 11.4 mg (Table 2.4). This showed lower efficacy compared to the previous result for 3-(N,N-dimethyl myristylammonio) propane sulphonate (Table 2.3) of 4.7 mg mass loss and high mortality. These two compounds differ in that the ASB-14 contains an extra amido-propane moiety at the base of the alkyl chain. This amido-propane moiety could be concluded to lower the efficacy of the ASB-14, perhaps by allowing easier cleavage of the alkyl chain. The similar structural variation of converting an amine to an amide had reduced efficacy of N-lauroylsarcosine compared to coco dimethyl betaine (Tables 2.2 & 2.3).

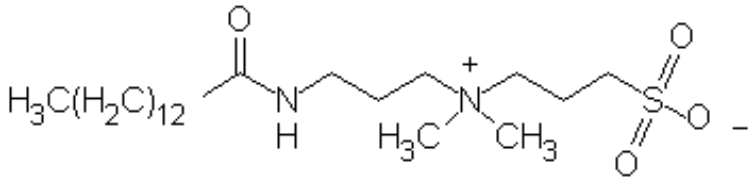


Figure 2.4 Amido sulphobetaine-14 (ASB-14).

A slight anti-feeding effect (Table 2.4) was noted with N-(tris(hydroxymethyl) methyl)-3-aminopropane (TAPS) (Figure 2.5). The polar nature of both ends of the molecule and sub-optimal alkyl chain length appear to prevent TAPS from utilising the same mechanism of action as hypothesised for dodecylbenzene sulphonic acid on *Tineola bisselliella*.

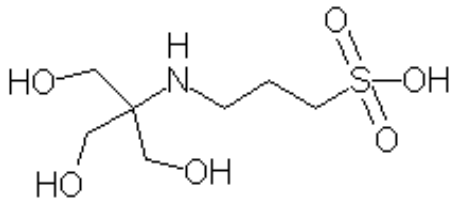


Figure 2.5 N-(Tris(hydroxymethyl) methyl)-3-aminopropane (TAPS).

Methyl 2-sulphooctadecanoate sodium salt (Figure 2.6) showed a slight anti-feeding effect (Table 2.4). The alkyl chain of 16 carbon atoms would be expected to contribute to this effect. Comparison to the structurally similar sodium lauryl sulphate bioassay results show methyl 2-sulphooctadecanoate sodium salt is a less effective anti-feedant. The presence of an ester group near the sulphonate group may contribute to this lower efficacy. The alkyl chain lengths of each molecule are both close to the optimal length discovered by Freeland and Williams (1967) and so would contribute similarly to the anti-feeding effect. This surfactant was the only one in this study to have a sulphonate group attached to a secondary carbon atom that was not part of a benzene group. This reduces the clear distinction between polar and non-polar groups at each end of the molecule, which may have resulted in the reduced efficacy against *Tineola bisselliella*.

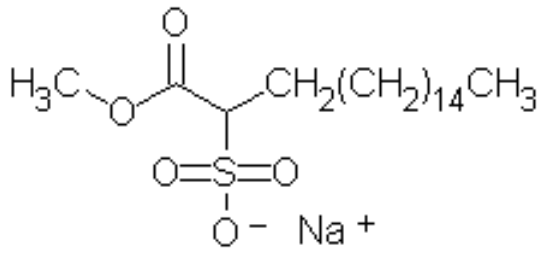


Figure 2.6 Methyl 2-sulphooctadecanoate sodium salt.

The anionic surfactant/bile acid sodium deoxycholate (Figure 2.7) showed a slight anti-feeding effect (Table 2.4). There was no observed increase in mortality of the *Tineola bisselliella* larvae, showing increased sodium deoxycholate intake was not associated with any toxic effects. The hydroxide groups present on the cholic acid derivative at the opposite end of the molecule to the sodium carboxylate group may have disrupted any possible mechanism of action due to neither end of the molecule being non-polar.

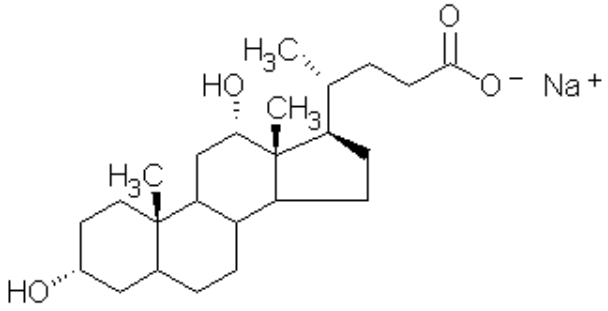


Figure 2.7 Sodium deoxycholate.

The zwitterionic surfactant 3-((3-Cholamidopropyl) dimethylammonio)-1-propanesulphonate (CHAPS) (Figure 2.8) showed a slight anti-feeding effect (Table 2.4). The larger cholic acid derivative moiety of this molecule appears to have been detrimental to the anti-feeding effect compared to the 13 carbon alkyl chain of ASB-14. The zwitterionic head of these two compounds are identical. When comparing the equally poor bioassay result from sodium deoxycholate, it can be seen the different polar heads of these molecules made no difference to the mothproofing efficacy.

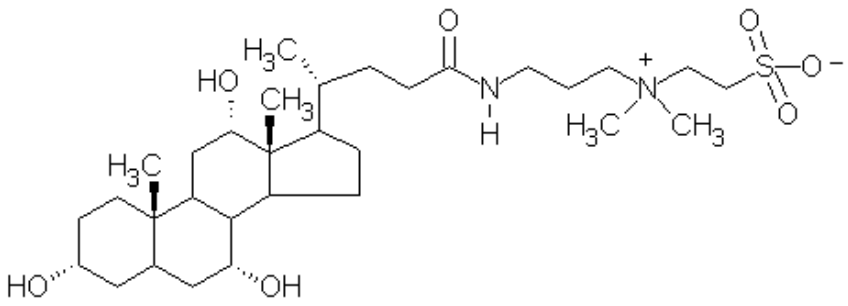


Figure 2.8 3-((3-Cholamidopropyl) dimethylammonio)-1-propanesulphonate (CHAPS).

No anti-feeding effect was noted (Table 2.4) with the anionic surfactant taurocholic acid (Figure 2.9). The only difference between this molecule and CHAPS is the lack of propyl-quaternary ammonium cation moiety. It could therefore be concluded that the quaternary ammonium cation is responsible for the slight anti-feeding effect of CHAPS. If the slightly longer head of the molecule was contributing to this anti-feeding effect, then sodium deoxycholate would most likely not have shown a slight anti-feeding effect.

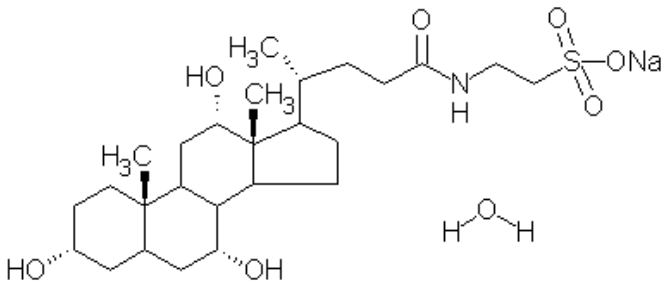


Figure 2.9 Taurocholic acid.

Bioassay results for the anionic surfactant 2-(N-morpholino) ethanesulphonic acid sodium salt (Figure 2.10) showed no mothproofing effect (Table 2.4). The unpaired electrons of the oxygen atom within the morpholine ring would have increased the polarity of the tail of the molecule compared to dodecylbenzene sulphonic acid. A close comparison can be made with the similar molecular structure of benzene sulphonic acid, which also resulted in no antifeeding effect (Table 2.1, Section 2.3.2.1). It appears the similar shape of these molecules does not confer efficacy, despite the variations in polarity. This suggests the efficacy of dodecylbenzene sulphonic acid may not rely solely on variations in polarity from one end of the molecule to the other, but also on the shape of the molecule.

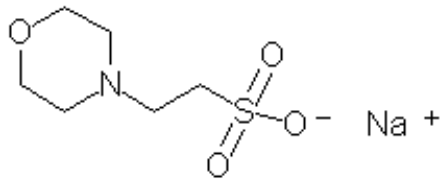


Figure 2.10 2-(N-morpholino) ethanesulphonic acid sodium salt.

As seen in Table 2.4, no antifeeding effect was observed with 2-acrylamido-2-methyl-1-propanesulphonic acid (Figure 2.11). The shape and polarity of this molecule did not contribute to mothproofing. If there was ammonia released from this molecule upon ingestion, it was insufficient to disrupt the digestive process of *Tineola bisselliella*.

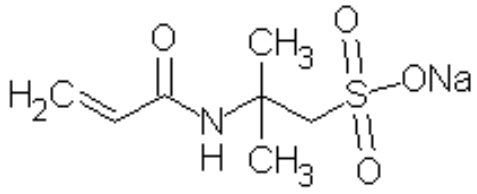


Figure 2.11 2-Acrylamido-2-methyl-1-propanesulphonic acid.

The zwitterionic taurine surfactant N-(2-acetamido)-2-aminoethanesulphonic acid (ACES) (Figure 2.12a) showed no antifeeding effect (Table 2.4). Similarly to 2-acrylamido-2-methyl-1-propanesulphonic acid, the molecular shape and reactivity were not conducive to mothproofing. The anionic surfactant N-(2,4-dinitrophenyl) taurine sodium salt (Figure 2.12b) showed no antifeeding effect (Table 2.4). The nitro groups bonded to the benzene group are also a feature of neonicotinoid insecticides, although this result shows their presence is unlikely to be solely responsible for the efficacy of this class of insecticide.

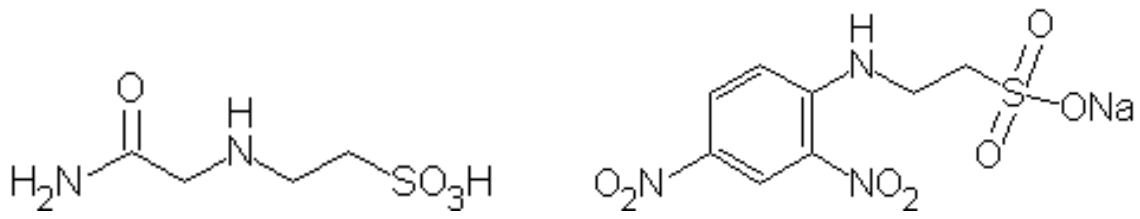


Figure 2.12 (a) N-(2-acetamido)-2-aminoethanesulphonic acid (left), and (b) N-(2,4-dinitrophenyl) taurine sodium salt (right).

Table 2.4 Bioassay results of *Tineola bisselliella* on wool fabric treated with anionic and zwitterionic surfactants and buffers.

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assess-ment	Pass (p), fail (f), or border-line (b)
Control	0.0	1.7	105.9 ± 16.3	n.a.	4D	n.a.
Amido sulphobetaine-14 (ASB-14)	18.3	0.0	11.4 ± 2.2	10.8	1C	f
TAPS[2]	4.9	4.9	63.1 ± 2.5	59.6	4D	f
Methyl 2-sulphoocta-decanoate sodium salt	9.5	0.0	68.1 ± 4.5	64.3	4D	f
Sodium deoxycholate	0.0	0.0	75.3 ± 5.9	71.1	4D	f
CHAPS[3]	5.0	3.3	80.0 ± 4.7	75.6	4D	f
Taurocholic acid	5.0	1.7	90.5 ± 1.5	85.5	4D	f
2-(N-Morpholino) ethanesulphonic acid sodium salt	1.7	6.7	93.4 ± 14.4	88.2	4D	f
2-Acrylamido-2-methyl-1-propanesulphonic acid	5.0	0.0	104.6 ± 3.6	98.8	4D	f
ACES[4]	1.7	1.7	108.3 ± 7.5	102.3	4D	f
N-(2,4-Dinitrophenyl) taurine sodium salt	1.7	0.0	112.9 ± 11.5	106.6	4D	f

[1] As a percentage of the mean voracity control, [2] N-(Tris(hydroxymethyl) methyl)-3-aminopropane, [3] 3-((3-Cholamidopropyl) dimethylammonio)-1-propanesulphonate, [4] N-(2-Acetamido)-2-aminoethanesulphonic acid.

The zwitterionic surfactant N,N-dimethyldodecyl amine N-oxide (Figure 2.13) showed a similar borderline mass loss, but a relatively high mortality (Table 2.5) compared to the two anionic surfactants of a similar size - coco dimethyl betaine and sodium lauryl sulphate (Table 2.2). This showed that the dimethyl amine-N-oxide group had a similar mothproofing efficacy to the sulphate group when bonded to a dodecyl alkyl chain, as in sodium lauryl

sulphate. Another useful comparison can be made between the acetate group of coco dimethyl betaine and the oxide of N,N-dimethyldodecyl amine N-oxide. Aside from these two groups, the only difference between the two molecules is one less carbon atom on the alkyl chain of coco dimethyl betaine. Both compounds imparted similar anti-feeding properties to wool fabrics (Table 2.2 & 2.5), therefore the oxide and carboxyl groups both had a similar effect on *Tineola bisselliella*.

Despite the differences in functional groups of these three molecules, they all have a similar size and shape, which could be optimal for disrupting metabolic processes in the *Tineola bisselliella* larvae. These surfactants may not be involved in any chemical reaction, but may simply hinder the biochemical pathways necessary for normal insect functioning, resulting in a lower rate of feeding.

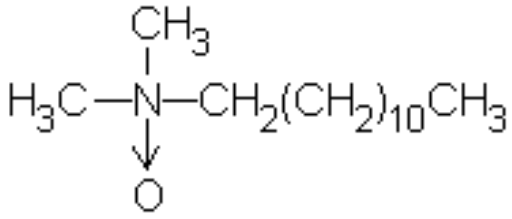


Figure 2.13 N,N-dimethyldodecyl amine N-oxide (DDAO).

Table 2.5 Bioassay results of *Tineola bisselliella* on wool fabric treated with N,N-dimethyldodecyl amine N-oxide (DDAO).

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	8.5	0.0	61.7 ± 14.5	n.a.	4D	n.a.
DDAO	47.6	0.0	14.7 ± 5.5	23.9	1D	f

[1] As a percentage of the mean voracity control.

Phenyl-2-aminobenzenesulphonate (Figure 2.14) contains a sulphonate group between two benzene structures – one bonded to the sulphur and one to an oxygen atom. The synonym 2-aminobenzenesulphonic acid phenyl ester suggests it can also be classified as a sulphonic acid ester, different to carbon-based carboxylic acid esters due to the reactivity of the sulphur atom allowing bonding to an extra oxygen atom. The weak efficacy seen with this

compound against *Tineola bisselliella* (Table 2.6) may be due to the less polar nature of the benzene rings at each end of the molecule and the more reactive ester/sulphonate group in the centre. The amino moiety of 4-dodecylaniline has been shown to be a sufficient substitute for a sulphonic acid group in the case of the efficacious alkylbenzene sulphonic acid compound (AgriQuality, 2005), but bonding to the ortho, or number 2 position of the benzene ring of phenyl-2-amino benzene sulphonate possibly minimises its contribution to mothproofing efficacy. The polar/non-polar moietys of this molecule are not as clearly separated as they are in alkylbenzene sulphonate compounds, possibly contributing to the lower efficacy in this bioassay.

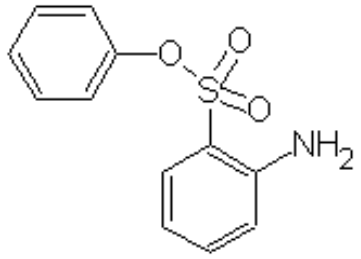


Figure 2.14 Phenyl-2-amino benzene sulphonate.

Table 2.6 Bioassay results of *Tineola bisselliella* on wool fabric treated with phenyl-2-amino benzene sulphonate.

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or border-line (b)
Control	0.0	0.0	85.0 ± 3.7	n.a.	4D	n.a.
Phenyl-2-amino benzene sulphonate	5.0	0.0	65.5 ± 7.8	77.1	4D	f

[1] As a percentage of the mean voracity control.

2.3.2.3 Zwitterionic and Anionic Surfactants with Anthrenocerus australis

The four most effective surfactants from the *Tineola bisselliella* trials (Section 2.3.2.2) were used in bioassays at 3.0% omw against *Anthrenocerus australis* beetle larvae (Table 2.7). N,N-Dimethyldodecyl amine N-oxide was the best performing of these surfactants, passing the test method with negligible mass loss. 3-(N,N-Dimethyl myristylammonio) propane

sulphonate and amido-sulphobetaine-14 both imparted significant reductions in mass loss, although were not close to passing the bioassay.

Coco dimethyl betaine passed the test method, with a low mass loss of 4.7 mg. When expressed as a percentage of the control, this mass loss was slightly lower than that obtained from the same treatment with *Tineola bisselliella* (Section 2.3.2.2, Table 2.2). This is interesting considering that carpet beetles usually require higher levels of insecticide for control compared to moth species. Coco dimethyl betaine was then trialled at a lower level of 2.0% omw, showing a narrow pass (Table 2.7). It can be concluded that the minimum effective rate of coco dimethyl betaine against *Anthrenocerus australis* is slightly below 2.0% omw.

Table 2.7 Bioassay results of *Anthrenocerus australis* on wool fabric treated with zwitterionic surfactants.

Treatment	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or border-line (b)
Control	0.0	7.0	34.9 ± 2.8	n.a.	4B	n.a.
DDAO (3.0% omw)	0.0	3.3	0.7 ± 0.7	1.9	1A	p
Coco dimethyl betaine (3.0% omw)	0.0	1.7	4.7 ± 1.2	13.5	1A	P
3-(N,N-Dimethyl myristylammonio) propane sulphonate (3.0% omw)	0.0	0.0	18.9 ± 2.7	54.1	2D	f
Amido-sulphobetaine-14 (ASB-14) (3.0% omw)	0.0	1.7	23.4 ± 3.1	67.1	1C	f
Control	0.0	0.0	30.9 ± 3.2	n.a.	3B	n.a.
Coco dimethyl betaine (2.0% omw)	0.0	0.0	10.2 ± 1.2	33.2	1A	p

[1] As a percentage of the mean voracity control.

The minimum effective concentration of N,N-dimethyldodecyl amine N-oxide against *Anthrenocerus australis* was investigated by applying this compound to wool fabric at 0.5% and 1.0% omw. Bioassay results showed less than adequate results at these levels (Table 2.8). It was noticed that the cropping around the edge of the wool fabric discs was reduced, but that there were large holes close to the centre. This could have been due to the solvent evaporating faster from the edge of the fabric discs, drawing more N,N-dimethyldodecyl amine N-oxide to the edge, resulting in a lower level in the centre of the disc. To prevent this edge effect in subsequent trials, the method was altered so that the surfactant was applied to a larger piece of fabric, followed by drying and cutting out the discs. Included in this later batch was N,N-dimethyldodecyl amine (DDA) (Figure 2.15), a structual analogue of N,N-

dimethyldodecyl amine N-oxide. The results from this altered application method used to apply DDAO and DDA each at 1.0% omw can also be seen in Table 2.8. The altered application method did not result in a large change in the mass loss, although the visual assessment was more similar to the damage to the control fabric, with most of the feeding restricted to the edge of the wool fabric. The significantly (p-value<0.05) higher mass loss of fabric treated with the same level of N,N-dimethyldodecyl amine showed that the oxygen atom of N,N-dimethyldodecyl amine N-oxide was contributing to the beetle-proofing effect.

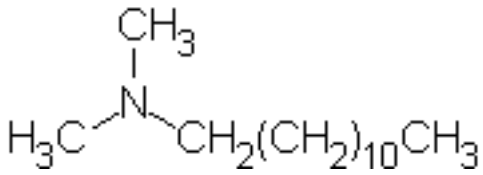


Figure 2.15 N,N-dimethyldodecyl amine (DDA).

Table 2.8 Bioassay results of *Anthrenocerus australis* on wool fabric treated with N,N-dimethyldodecyl amine N-oxide and N,N-dimethyldodecyl amine.

Treatment	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or border-line (b)
Control	0.0	2.3	38.0 ± 0.9	n.a.	3A	n.a.
DDAO (0.5% omw)	0.0	10.4	33.5 ± 2.9	88.0	2D	f
DDAO (1.0% omw)	0.0	8.2	25.5 ± 2.3	67.2	1D	f
(modified application)						
Control	1.8	7.0	37.0 ± 3.6	n.a.	3A	n.a.
DDAO (1.0% omw)	0.0	0.0	27.6 ± 3.6	74.6	4A	f
DDA (1.0% omw)	1.8	0.0	36.3 ± 1.3	98.3	3B	f

[1] As a percentage of the mean voracity control.

2.3.2.4 Non-Ionic Surfactants

Sorbitan monopalmitate (Figure 2.16) showed no mothproofing effect (Table 2.9). This is of interest when compared to the high efficacy of dodecylbenzene sulphonic acid (Section 2.3.2.1, Table 2.1). There are similarities in the size and shape of these two compounds, with each having alkyl chains at one end of their respective molecules and a ring structure at the other end. The ring structure of sorbitan monopalmitate is a five-membered furanose compared to the six-membered benzene in dodecylbenzenesulphonic acid, although it does contain two polar hydroxyl groups. The presence of a carboxylic acid ester group between the alkyl chain and ring structure could allow easier cleavage at this point of the molecule compared to similar compounds without the ester group, such as dodecylbenzene sulphonic acid. Compounds with carboxylic acid ester groups have shown low mothproofing efficacy when looking at previous results with methyl 2-sulphooctadecanoate sodium salt and phosphatidylcholine (Section 2.3.2.2), further strengthening this hypothesis.

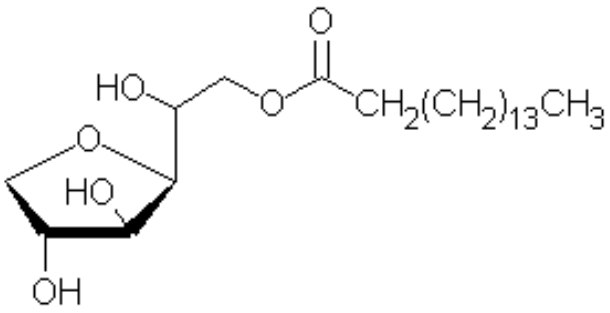


Figure 2.16 Sorbitan monopalmitate.

Table 2.9 Bioassay results of *Tineola bisselliella* on wool fabric treated with sorbitan monopalmitate.

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	1.7	1.7	96.8 ± 4.4	n.a.	4D	n.a.
Sorbitan monopalmitate	0.0	0.0	95.9 ± 3.4	99.1	4D	f

[1] As a percentage of the mean voracity control.

2.4 Naphthalene Derivatives

2.4.1 Selection of Naphthalene Derivatives

The moth repellent naphthalene was considered an obvious choice as the basic entity from which structural variations were explored for mothproofing efficacy. Although naphthalene sublimes from a solid to a gas, there are many stable compounds containing the naphthalene moiety which could be practical in wool dyeing, analogous to Martius Yellow (Figure 1.8a, Section 1.6.2).

The effect of adding sulphonic acid groups to the naphthalene molecule was tested using 1-naphthalene sulphonic acid and 1,3,(6 or 7)-naphthalene trisulphonic acid (trisodium salt hydrate) applied to wool fabrics for bioassay testing. Sulphonic acid groups provide a likely binding site for these molecules with wool, adding to their practicality in wool dyeing. Another variation trialled was the addition of two oxygen atoms, each via double bonding to give a naphthoquinone group, as in 1,4-naphthoquinone-2-sulphonic acid (potassium salt) and 1,2-naphthoquinone-4-sulphonic acid (sodium salt) (Folin's reagent).

The addition of aniline groups to naphthalene compounds was used to determine the effect of an additional non-polar benzene group on mothproofing. 8-Anilino-1-naphthalenesulphonic acid and 4-anilino-1,2-naphthoquinone were obtained for this purpose. α-Naphthyl myristate was used to represent the addition of an ester group between the naphthalene and linear alkyl entities. 2-Naphthyl disulphide was used to test whether the ability of the *Tineola bisselliella* larvae to cleave disulphide bonds would result in a mothproofing effect, presumably by releasing 2-naphthalene thiol into the insect gut.

8-Hydroxy-5,7-dinitro-2-naphthalenesulphonic acid was used to test the effect of nitro and hydroxy groups for comparison when added to the naphthalene sulphonic acid molecule. 1-(3,4-Dichlorophenyl)-3-(1-naphthyl) urea has three groups of interest - naphthalene, urea, and chlorinated benzene.

2.4.2 Bioassay Results of Wool Fabric Treated with Naphthalene Derivatives: *Tineola bisselliella*

Most of the naphthalene derivatives had some antifeeding effect, as shown in Table 2.10. The 1-naphthalene sulphonic acid (Figure 2.17a) showed a moderate mothproofing effect, which was slightly reduced by an increase to three sulphonate groups, as seen in 1,3,(6 or 7)-

naphthalene trisulphonic acid sodium salt (Figure 2.17b). Addition of the quinone structure with carbonyl groups in the 1,4 positions, and using a sulphonate in the 2 position with a potassium counter-ion (1,4-naphthoquinone-2-sulphonic acid) (Figure 2.17c) gave a decrease in efficacy with very little mothproofing effect. It is assumed the sulphonate/potassium salt had little effect on mothproofing as Freeland and Williams (1967) showed that sodium, calcium and lithium sulphonates of dodecylbenzene sulphonic acid were equal to or only slightly less effective than dodecylbenzene sulphonic acid. Changing the carbonyl groups from the 1,4 to the 1,2 positions and moving the sulphonic acid to the 4 position (Folin's reagent) (Figure 2.17d) gave good efficacy as seen by an acceptably low mass loss according to the test method used. Rivett et al. (1990) showed that two naphthoquinones, with carbonyl groups in the 1,4 position, gave acceptably low mass losses on wool; therefore it appears that efficacy depends on aspects other than the position of the carbonyl groups within these naphthoquinone derivatives.

Changing the sulphonic acid group of Folin's reagent to an anilino group (4-anilino-1,2-naphthoquinone) (Figure 2.17e) decreased mothproofing efficacy as seen by a significantly higher mass loss. A similar addition of aniline to the 8 position of 1-naphthalene sulphonic acid (8-anilino-1-naphthalenesulphonic acid) (Figure 2.17f) increased efficacy as seen by a significantly lower mass loss and an increase in mortality. It should be noted that the addition of the aniline group was only beneficial to the mothproofing effect when it did not replace the sulphonic acid group, as was the case for addition to 1-naphthalene sulphonic acid. The coloured nature of 8-anilino-1-naphthalenesulphonic acid and Folin's reagent make them impractical for treating wool commercially.

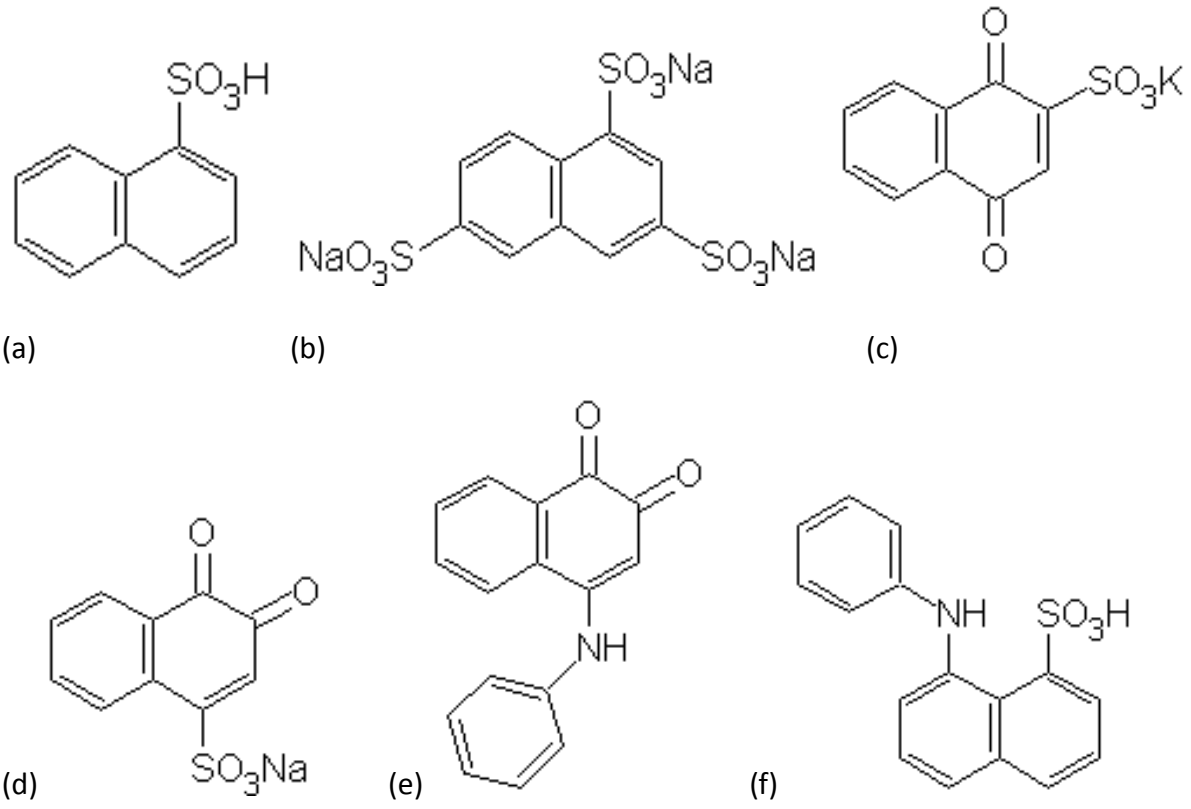


Figure 2.17 (a) 1-naphthalene sulphonic acid, (b) 1,3,(6 or 7)-naphthalene trisulphonic acid (sodium salt), (c) 1,4-naphthoquinone-2-sulphonic acid (potassium salt), (d) 1,2-naphthoquinone-4-sulphonic acid (sodium salt) (Folin's reagent), (e) 4-anilino-1,2-naphthoquinone, (f) 8-anilino-1-naphthalenesulphonic acid.

The lack of efficacy of 8-hydroxy-5,7-dinitro-2-naphthalenesulphonic acid (flavianic acid) (Figure 2.18e) suggests that nitro groups attached to naphthalene were not beneficial for the mothproofing effect (Table 2.10). 2-Naphthyl disulphide (Figure 2.18b) gave a moderate reduction in mass loss, suggesting that either the molecule itself provided a mothproofing effect, or the breaking of the disulphide bond in the moth gut released 2-naphthalenethiol (Figure 2.18c) causing the anti-feeding effect. A later bioassay using 2-naphthalenethiol (Table 2.11) showed higher levels of feeding as a percentage of the control, indicating that 2-naphthalenethiol was less effective than 2-naphthyl disulphide. If the disulphide bond of 2-naphthyl disulphide was broken in the *Tineola* gut, it would be expected that the bioassay results would be similar. The difference in these results suggests that the disulphide bonds were probably unbroken, or only partially broken. As the disulphide bond of 2-naphthyl disulphide was not part of a polypeptide chain as it is in wool, the gut enzymes may have been less effective due to steric or chemical differences between 2-naphthyl disulphide and wool.

The only slight antifeeding effect of α-naphthyl myristate (tetradecanoic acid 1-naphthyl ester), (Table 2.10) is surprising given that it has an alkyl chain of thirteen carbon atoms, which is close to the optimum efficacy of fourteen in the surfactant bioassays of Freeland and Williams (1967). When considering the structure of α-naphthyl myristate it can be seen that the linear alkyl and naphthalene entities are separated by an ester group (Figure 2.18a). This may provide easier separation of the naphthalene from the alkyl group, rendering it less effective than a similar molecule without an ester group. Ester groups were also found to be detrimental to the mothproofing efficacy of surfactants (Sections 2.3.2.2 & 2.3.2.4). Another reason for lack of efficacy could be the lack of polarity at the opposite end of the molecule to the non-polar alkyl chain. The first of these two effects may also explain the total ineffectiveness of sorbitan monopalmitate, which also lacked mothproofing efficacy despite having structural similarities to the effective dodecylbenzene sulphonic acid surfactant (Section 2.3.2.1). Wool fabrics treated with 1-(3,4-dichlorophenyl)-3-(1-naphthyl) urea (Figure 2.18d) showed a moderate mothproofing effect similar to 1-naphthalene sulphonic acid (Table 2.10). This showed that the 3,4-dichlorophenyl urea group is not a worthwhile addition to naphthalene, although the antimicrobial triclocarban, with a very similar structure (Figure 2.19a, Section 2.5.1,), showed very high efficacy (Table 2.16, Section 2.5.2).

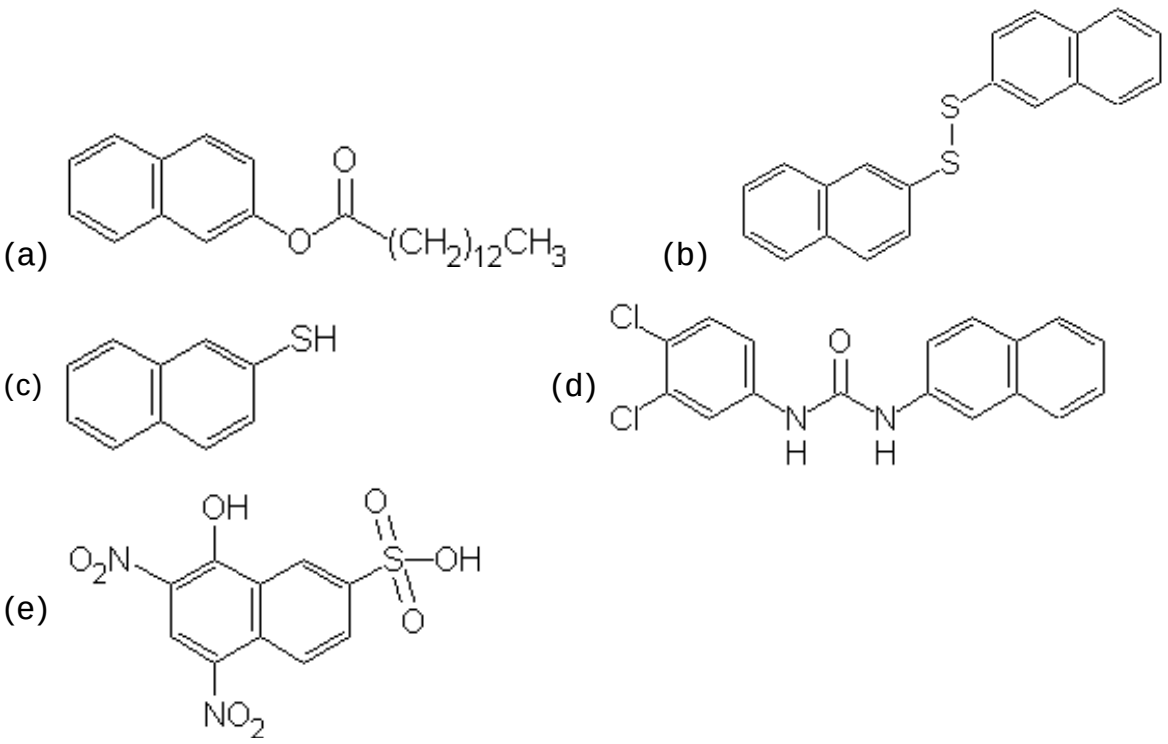


Figure 2.18 (a) α-Naphthyl myristate, (b) 2-naphthyldisulphide, (c) 2-naphthalene thiol, (d) 1-(3,4-dichlorophenyl)-3-(1-naphthyl) urea, (e) 8-hydroxy-5,7-dinitro-2-naphthalenesulphonic acid.

Table 2.10 Bioassay results of *Tineola bisselliella* on wool fabric treated with naphthalene derivatives.

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b).
Control	1.9	3.6	51.3 ± 5.7	n.a.	4D	n.a.
1-naphthalene sulphonic acid	17.5	0.0	18.3 ± 4.6	35.3	4C	f
1,3,(6 or 7)-naphthalene trisulphonic acid	23.9	0.0	26.3 ± 4.5	51.3	4D	f
1,4-naphthoquinone-2-sulphonic acid (potassium salt)	1.9	1.9	45.4 ± 9.5	88.5	4C	f
Control	1.7	1.7	84.9 ± 3.3	n.a.	4D	n.a.
1,2-naphthoquinone-4-sulphonic acid (Folin's reagent)	13.9	0.0	5.8 ± 1.2	6.8	1A	p
Flavianic acid[2]	0.0	0.0	91.5 ± 1.0	107.8	4D	f
Control	1.7	0.0	76.4 ± 3.3	n.a.	4D	n.a.
8-anilino-1-naphthalene sulphonic acid	62.9	0.0	9.2 ± 3.3	12.0	1D	p
Control	0.0	0.0	70.2 ± 3.2	n.a.	3C	n.a.
2-naphthyl disulphide	6.7	0.0	37.4 ± 2.5	53.2	4C	f
α-naphthyl myristate	0.0	0.0	57.4 ± 4.5	81.7	4C	f
4-anilino-1,2-naphthoquinone	1.7	0.0	45.6 ± 3.9	64.9	4D	f
1-(3,4-dichlorophenyl)-3-(1-naphthyl) urea	23.7	0.0	25.8 ± 2.9	36.8	3C	f

[1] As a percentage of the mean voracity control, [2] 8-hydroxy-5,7-dinitro-2-naphthalene sulphonic acid.

Table 2.11 Bioassay results of *Tineola bisselliella* on wool fabric treated with 2-naphthalenethiol.

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b).
Control	1.9	0.0	50.5 ± 2.7	n.a.	3C	n.a.
2-naphthalene-thiol	0.0	0.0	40.5 ± 3.1	80.1	3C	f

[1] As a percentage of the mean voracity control.

2.4.3 Bioassay Results of Wool Fabric Treated with Naphthalene Derivative: *Anthrenocerus australis*

Bioassays using the same test method with *Anthrenocerus australis* larvae were performed using the 8-anilino-1-naphthalenesulphonic acid treated fabric, as this compound provided a good antifeeding effect and high mortality against *Tineola bisselliella* (Section 2.4.2, Table 2.10). At 3.0% omw this compound offered less protection against the beetle larvae with no larval mortality and a higher mass loss, just above the borderline range for the test method (Table 2.12). It may be that naphthalene compounds are less suited to disrupting the neutral beetle midgut than the alkaline moth midgut.

Table 2.12 Bioassay results of *Anthrenocerus australis* on wool fabric treated with 8-anilino-1-naphthalenesulphonic acid.

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b).
Control	0.0	0.0	44.7 ± 5.7	n.a.	4C	n.a.
8-anilino-1-naphthalene-sulphonic acid	0.0	0.0	16.5 ± 2.2	36.9	1B	f

[1] As a percentage of the mean voracity control.

2.5 Antimicrobial Compounds

2.5.1 Selection of Antimicrobial Compounds

A variety of antimicrobial compounds were studied in order to cover a range of different mechanisms of action, to maximise the chances of finding one effective against *Tineola bisselliella* moth larvae. Enzyme inhibition would be an ideal mode of action, as this could inactivate enzymes in the insect digestive tract. Basic similarities in bacterial and eukaryote protein synthesis (Berg, Tymoczko, & Stryer, 2002) would suggest that those antimicrobials with modes of action involving inhibition of protein synthesis could also show efficacy against insects. Berg et al. (2002) noted that eukaryote protein synthesis involved more protein components and was more intricate than prokaryote protein synthesis. These differences would not rule out an effect on insects by antimicrobials designed for use on prokaryotes, as the use of some antibiotics have been shown to have negative side effects on eukaryotes. An example of this includes the use of tetracyclines against bacterial infections in humans, where gastrointestinal and liver disorders, in addition to intracranial hypertension have been documented (Lebrun-Vignes, Kreft-Jais, Castot, & Chosidow, 2012). Antifungal and anti-protozoal compounds show activity against eukaryotic cells, and therefore may be more likely than other antimicrobials to affect insect cells.

Antimicrobial compounds containing multiple benzene groups may be more effective than linear alkylbenzene sulphonate, as insecticides such as bifenthrin, permethrin, sulcofenuron and diflubenzuron contain more than one benzene group. Larger molecules diffuse in and out of wool fibres more slowly than small molecules in aqueous solutions, as is the case for dye molecules. The high temperature and acids used in wool dyeing promote open networks in the wool structure that allow large molecules to slowly enter (Bird, 1947). Slower diffusion of dye and insecticide molecules into wool results in a more even application over the mass of wool in the dyebath. Perhaps more importantly for insect-resist agents, larger molecules have better long-term durability on the wool due to greater entrapment when the dyebath is cooled and neutralised, shrinking the permeable networks within the wool fibre. This was shown to be the case for alkyl sulphate surfactants applied to wool in the dyebath, where longer alkyl chains gave better durability to neutral and alkaline rinses (Holt & Onorato, 1989).

The antimicrobial agent 3,4,4-trichlorocarbanilide, more commonly known as triclocarban, contains two benzene groups (Figure 2.19a), and is identical to part of the sulcofenuron molecular structure (Figure 1.9a, Section 1.6.2). It also contains chlorine groups similar to the insecticide permethrin and an amide group as in diflubenzuron. Triclocarban has been shown to disrupt cell membrane activity via uncoupling of oxidative phosphorylation (Hamilton, 1971). This effect may be useful in disrupting the gut mechanism of keratin-digesting species, as oxidative phosphorylation was first shown in insects by Sacktor (1954). Triclocarban is commonly added to consumer soaps and deodorants, and is predominantly active against gram-positive bacteria (Walsh et al., 2003). The antimicrobial agent triclosan (Figure 2.19b) has a similar molecular structure to that of triclocarban. Triclosan is a broad-spectrum bacteriostatic germicide that has been used in hand soap, toothpaste, fabrics, and plastics for over 30 years.

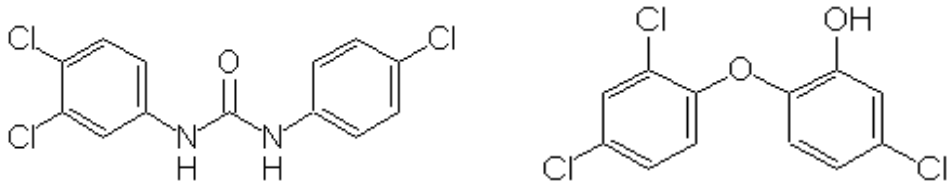


Figure 2.19 (a) Triclocarban (left) and (b) triclosan (right).

The imidazole-based anti-fungal agent miconazole nitrate salt also has a similar molecular structure to triclocarban. It is an enzyme inhibitior and alters cell membrane permeability (Fothergill, 2006), which could be useful for altering the cells of the insect gut wall. Econazole nitrate and sulconazole nitrate (Figures 2.20a and 2.20b respectively), imidazole antifungal compounds similar to miconazole nitrate, were also obtained. The mode of action of azole fungicides is through binding to the heme protein of fungal CYP51 C-14, inhibiting demethylation in ergosterol biosynthesis (Venkatakrishnan, von Moltke, & Greenblatt, 2000). Ergosterol is present in fungal cell membranes, but not in plant or animal cells; therefore it is difficult to hypothesise on the mode of action of azole fungicides in insects.

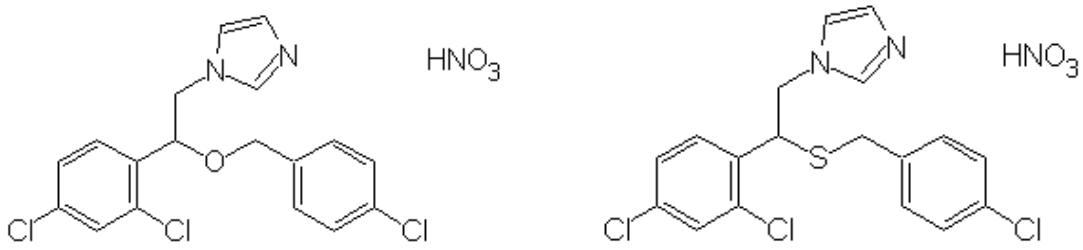


Figure 2.20 (a) Econazole nitrate (left) and (b) sulconazole nitrate (right).

The triazole fungicides tebuconazole, propiconazole and epoxiconazole were obtained from Orion Crop Protection Ltd (New Zealand) as fungicides Compass®, Pro-P™, and Calibre® respectively. These formulations contain quantified levels of fungicide and volatile solvent, but an undefined quantity of surfactant and water. Volatile solvents are removed on drying and are therefore unlikely to contribute any significant insect-proofing effect. Surfactants may have some effect, but this would depend on the quantity of formulation applied to the wool. If low levels of these formulations were found to be effective, it is unlikely that surfactants would contribute significantly to this efficacy, as Freeland and Williams (1967) showed that surfactant levels of 2-5% omw were required to control wool digesting insects. The molecular structures of these three triazole fungicides are shown in Figure 2.21.

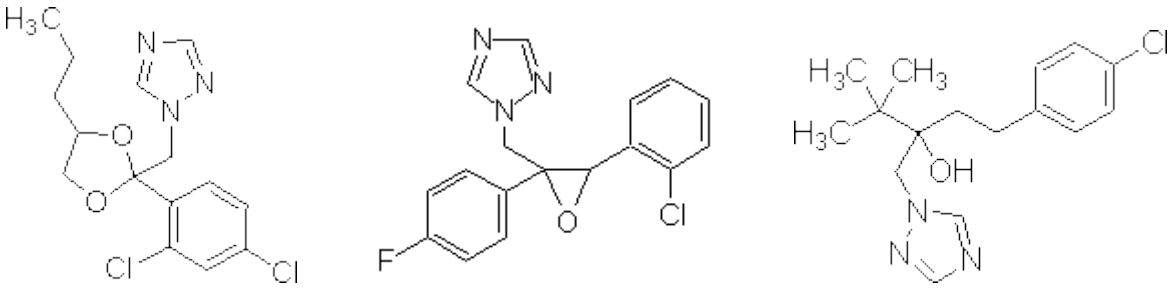


Figure 2.21 (a)Propiconazole (left), (b) epoxiconazole (centre), and (c) tebuconazole (right).

Pentamidine isethionate is a mixture of pentamidine and the anionic isethionic acid (Figure 2.22). It has been widely used as an antimicrobial medicine, for the treatment of several diseases caused by protozoan parasites (Nguewa et al., 2005). Protozoa have been identified in the gut of *Anthrenus flavipes* larvae (Trivedi et al., 1991) and so may also be present in similar wool-digesting beetle species such as *Anthrenocerus australis*.

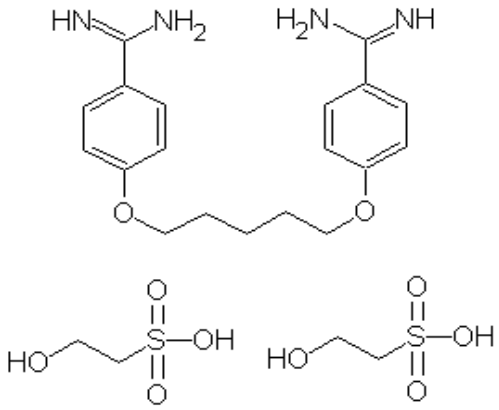


Figure 2.22 Pentamidine isethionate.

Nalidixic acid (Figure 2.23) is a quinolone that inhibits bacterial DNA synthesis (Pedrini, Geroldi, Siccardi, & Falaschi, 1972). There are some similarities between bacterial and insect DNA synthesis, as all cellular life forms synthesize a short RNA primer with a free hydroxyl group which is subsequently elongated by a DNA polymerase (Iyer, Koonin, Leipe, & Aravind, 2005). Although these similarities are on a sub-cellular level, they may be significant enough for nalidixic acid to affect DNA synthesis in insects.

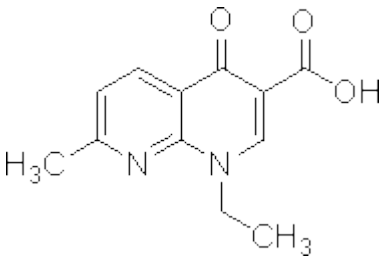


Figure 2.23 Nalidixic acid.

Thiabendazole (Figure 2.24) is an antifungal agent, but is also used medicinally as a chelating agent to bind metals in cases of metal poisoning (Devereux et al., 2004). Metal chelation could be a useful mode of action against insects, as *Tineola bisselliella* gut enzyme inhibition with the metal chelator EDTA has been shown by Ward (1975c, d, g).

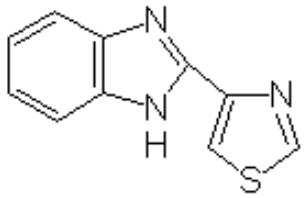


Figure 2.24 Thiabendazole.

Tolnaftate (Figure 2.25) is an antifungal agent that inhibits oxidosqualene cyclase, causing ergosterol depletion and accumulation of squalene oxides in the fungal membrane. (Gupte, Kulkarni & Ganguli, 2002). All insects require a suitable sterol in their diet due to their inability to synthesise sterols (Waterhouse, 1958). Tolnaftate may have a negative effect on the insect species of interest if any symbiotic gut flora were responsible for synthesising sterols.

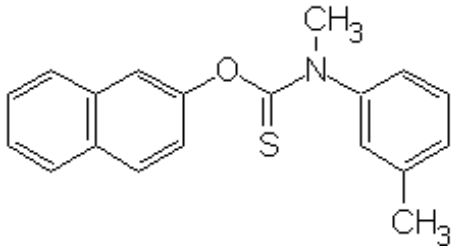


Figure 2.25 Tolnaftate.

The mechanism of action of Clofazimine (Figure 2.26) is not well known (Arbiser & Moschella, 1995). Clofazimine has a broad spectrum activity against Gram-positive but not Gram-negative bacteria, and anaerobic conditions increase the susceptibility of gram-positive bacteria (Van Rensberg, Joone, O'Sullivan & Anderson, 1992). The gut conditions of *Tineola bisselliella* species are generally described as practically anaerobic (Robinson & Nielsen, 1993), therefore this may have an effect on any gut flora of this species. Less well developed tracheation of the the larval midgut of *Anthrenus* and *Attagenus* beetle larvae (Day, 1951b) suggests even more anaerobic conditions in these genera compared to *Tineola bisselliella*.

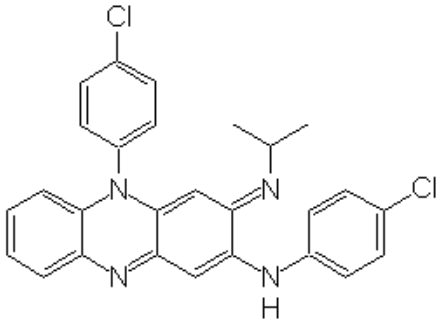


Figure 2.26 Clofazimine.

Sulphacetamide (Figure 2.27) is a sulphonamide antibiotic used for the treatment of acne and dermatitis in humans. It targets folate biosynthesis, although microarray gene expression studies have predicted a mechanism of cell wall synthesis inhibition (Brazas & Hancock, 2005). This broad effect could be useful against insects by affecting any symbiotic organisms present in the gut or externally located, as seen with the ectoparasitic relationship between humans and the organisms causing acne and dermatitis.

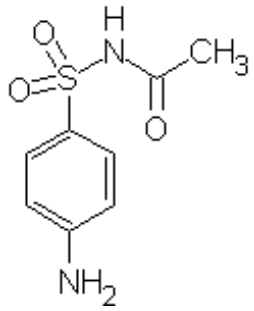


Figure 2.27 Sulphacetamide.

Chloramphenicol (Figure 2.28) is bacteriostatic, specifically inhibiting the synthesis of bacterial protein. This effect has been shown to depend on the steric configuration and conformation of the molecule, and particularly its propanol moiety (Jardetzky, 1963). This stereo-specificity may indicate a “lock and key” type mechanism which relies on the shape of the molecule. The shape of the molecular structure was hypothesised as a possible reason for the efficacy of dodecylbenzene sulphonic acid (Section 2.3.2.2). There is enough similarity in the shape of these two compounds to justify inclusion of chloramphenical in bioassay trials.

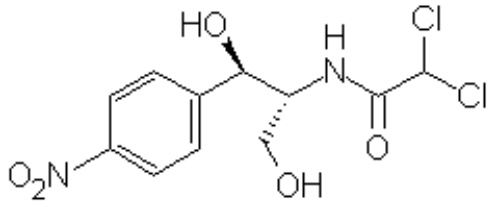


Figure 2.28 Chloramphenicol.

Nicarbazin (Figure 2.29) is an equimolar complex of of N,N′-bis(4-nitrophenyl)urea and 4,6-dimethyl-2-pyrimidinone. Although the precise mode of action is unknown (Davis & Gookin, 2009), there are structural features worthy of investigation. The N,N′-bis(4-nitrophenyl)urea component is similar to triclocarban, except with two nitro groups instead of three chlorine atoms bonded to the phenyl groups. Both nitro groups and chlorine are common features of insecticides, therefore it is possible this combination of structural features may result in efficacy against *Tineola bisselliella* larvae.

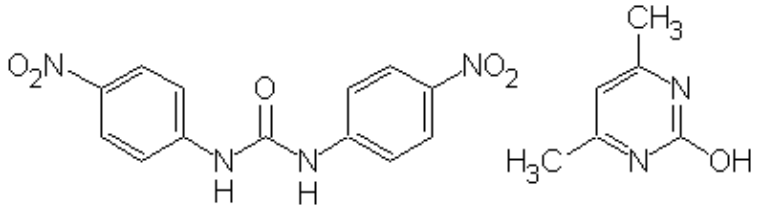


Figure 2.29 Nicarbazin.

Isoniazid (Figure 2.30) is commonly used to treat tuberculosis. The mode of action against *Mycobacterium tuberculosis* involves inhibition of the synthesis of mycolic acid, necessary for the mycobacterial cell wall (Rastogi & David, 1993; Winder & Collins, 1970). This mode of action is probably too specific to be useful against insects, but isoniazid is a general inhibitor of the cytochrome P450 system (Muakkassah, Bidlack, & Yang, 1979; Burke, 1981), a large and diverse group of enzymes, some of which may be present in the target insects.

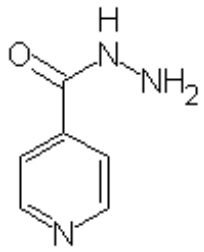
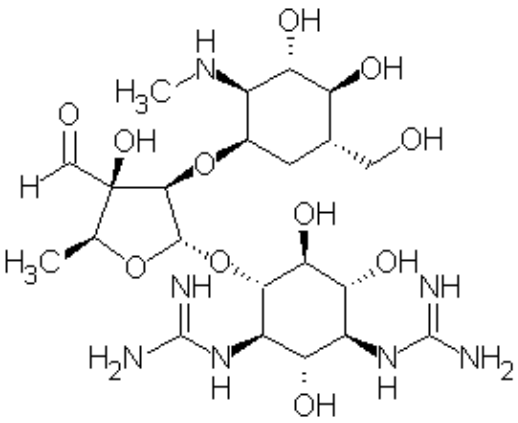


Figure 2.30 Isoniazid.

Streptomycin (Figure 2.31) is a broad-spectrum antibiotic drug that inhibits protein synthesis in bacteria by inhibiting the binding of formyl-methionyl-tRNA to the 30S subunit of the bacterial ribosome, inhibiting both Gram-positive and Gram-negative bacteria (Rastogi & David, 1993), (Sharma, Cukras, Rogers, Southworth, & Green, 2007). Even if this specific mode of action does not give rise to efficacy against insects, the arrangement of most of the nitrogen atoms within the molecular structure is analogous to that of neo-nicotinoid insecticides imidacloprid, clothianidin, dinotefuran, and thiamethoxam. This similarity may result in efficacy against the wool-digesting insect species, as imidacloprid has been shown to have a protective effect on wool fabrics exposed to larvae of *Tineola bisselliella*, *Anthrenus flavipes*, and *Attagenus piceus* (Haas, 1990).



• $1½H_2SO_4$

Figure 2.31 Streptomycin.

Flumequine (Figure 2.32) is a fluoroquinolone antibiotic that inhibits bacterial DNA gyrase (Smith, 1986). The fluorine within this compound could impart a useful mothproofing effect, as some commercial mothproofing agents are based on actives which contain fluorine, such as bifenthrin (Barton, 2000) and chlorfenapyr (Mill, 2007), (Section 1.6.2, Figures 1.11b & 1.12 respectively).

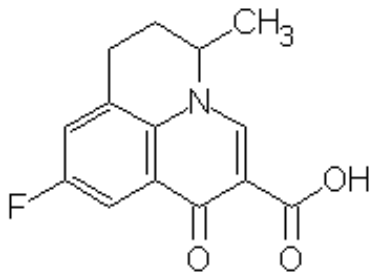


Figure 2.32 Flumequine.

8-Hydroxyquinoline (Figure 2.33) is an antifungal agent with a relatively simple molecular structure, but with many biologically active structural analogues. The quinoline group can be found in a number of synthetic and natural antifungals (Musiol, Serda, Hensel-Bielowka, & Polanski, 2010). 8-Hydroxyquinoline appears to inhibit DNA and RNA synthesis (Mills, 1978). The similarity of this structure to that of the moth repellent naphthalene is of interest and may lead to some direct comparisons to naphthalene derivatives trialled in Section 2.4.2.

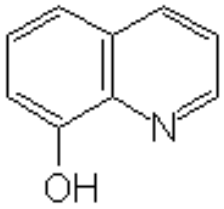


Figure 2.33 8-Hydroxyquinoline.

Theophylline (Figure 2.34) is a phosphodiesterase inhibitor (Essayan, 2001). The molecular structure is the same as that of caffeine, with the removal of one methyl group from the imidazole moiety. Caffeine in plants has been shown to protect against the tobacco hornworm moth species *Manduca sexta* (Linnaeus 1763) by inhibiting phosphodiesterase activity and increasing the intracellular cyclic adenosine monophosphate (Nathanson, 1984). The similar structures and modes of action of these two compounds is an indication theophylline may have an antifeeding effect on *Tineola bisselliella* larvae.

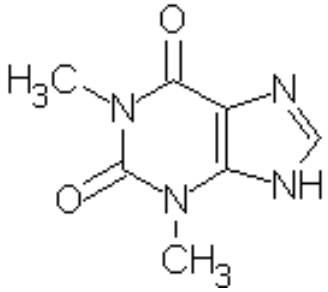


Figure 2.34 Theophylline.

2.5.2 Bioassay Results of Wool Fabrics Treated with Antimicrobial Compounds: *Tineola bisselliella*

Most of the antimicrobials trialled had some mothproofing effect, as shown in Table 2.13. The three imidazole nitrates imparted the greatest mothproofing effect. Of these compounds, the anti-fungal agent econazole nitrate was the most effective, easily passing the bioassay. Sulconazole nitrate showed borderline mass loss, whereas miconazole nitrate fell narrowly over the borderline range. Sulconazole nitrate has the same molecular structure as econazole nitrate, with the exception of a sulphur atom in place of the oxygen (Figure 2.20). Miconazole nitrate differs from econazole nitrate in that it contains an extra chlorine atom, with both benzene rings doubly chlorinated in the ortho, para (2, 4) positions. The similar efficacies of these azole nitrates is not surprising given the similarities in molecular structure.

The efficacy of theophylline against *Tineola bisselliella* was similar to sulconazole nitrate and miconazole nitrate compounds in terms of percentage of control mass loss, although not good enough to pass the bioassay (Table 2.13). The reduction in mass loss and mortality of slightly over 20% suggests that the inhibition of phosphodiesterase mode of action of theophylline may have led to the negative effects on *Tineola bisselliella* larvae.

Azole antifungals are inhibitors of cytochrome P450 mono-oxygenases (McLean et al., 2002). This may be the mechanism of action of the azoles on *Tineola bisselliella*, although this is unlikely to occur in the mid-gut due to the reducing conditions. Of the antimicrobials trialled here, pentamidine isethionate and nalidixic acid were the next best mothproofers behind the imidazoles. The use of pentamidine as an antiprotozoal agent used against human pathogens is common, despite an unknown mode of action (Denise & Barrett, 2001). Nalidixic acid is a topoisomerase inhibitor that inhibits DNA replication in bacteria (Mattern, Paone, & Day, 1982). Microorganisms are not believed to play a part in keratin digestion by *Tineola bisselliella* due to their apparent absence in the mid-gut (Crewther & McQuade, 1955). It seems likely that there are biochemical pathways outside the *Tineola bisselliella* larval mid-gut that are affected by these antimicrobials.

Table 2.13 Bioassay results of *Tineola bisselliella* on wool fabric treated with antimicrobial compounds.

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or border-line (b).
Control	1.7	0.0	58.0 ± 4.1	n.a.	3B	n.a.
Sulconazole nitrate	9.7	0.0	12.2 ± 1.1	21.0	1A	b
Miconazole nitrate	6.8	0.0	17.4 ± 3.3	30.0	2C	f
Nalidixic acid	10.0	0.0	18.9 ± 2.7	32.7	2C	f
Thiabendazole	3.3	0.0	35.4 ± 0.9	61.0	3B	f
Tolnaftate	3.5	0.0	53.8 ± 3.5	92.8	3C	f
Control	0.0	0.0	70.2 ± 3.2	n.a.	3C	n.a.
Clofazimine	23.3	0.0	31.0 ± 4.0	44.2	2C	f
Chloramphenicol	8.3	0.0	32.1 ± 1.3	45.7	2C	f
Sulphacetamide	11.9	0.0	39.8 ± 4.4	56.7	3C	f
Nicarbazin	3.3	0.0	46.1 ± 3.6	65.7	3C	f
Isoniazid	4.2	0.0	48.2 ± 5.5	68.6	4C	f
Streptomycin sulphate	0.0	0.0	60.6 ± 4.6	86.2	4C	f
Control	0.0	1.7	112.5 ± 13.0	n.a.	4D	n.a.
Econazole nitrate	13.3	5.1	7.5 ± 0.4	6.7	2B	p
Pentamidine isethionate	6.7	1.7	20.4 ± 1.4	18.1	1C	f
Flumequine	0.0	1.7	69.4 ± 12.7	61.7	2B	f
8-Hydroxyquinoline	11.7	1.7	102.7 ± 24.1	91.3	4D	f
Control	0.0	0.0	85.0 ± 3.7	n.a.	4D	n.a.
Theophylline	21.7	0.0	20.5 ± 2.0	24.1	3C	f

[1] As a percentage of the mean voracity control.

Following the good results from the imidazole compounds, three triazole compounds were trialled against *Tineola bisselliella* at 3.0% omw. Only expoxiconazole passed the test, whereas propiconazole and tebuconazole showed significant feeding reduction without the required efficacy to pass (Table 2.14).

Table 2.14 Bioassay results of *Tineola bisselliella* on wool fabric treated with triazole compounds.

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	1.7	0.0	106.3 ± 11.5	n.a.	4D	n.a.
Epoxiconazole	18.6	0.0	8.6 ± 2.1	8.1	2A	p
Propiconazole	1.7	0.0	26.0 ± 1.3	24.5	2C	f
Tebuconazole	3.3	0.0	30.5 ± 6.4	28.7	4D	f

[1] As a percentage of the mean voracity control.

Bioassay results for triclosan at 3.0% omw showed no significant efficacy against *Tineola bisselliella* (Table 2.15). Another antimicrobial of similar molecular structure, triclocarban, trialled at 3.0% omw showed very good control of *Tineola bisselliella* due to no significant mass loss and a high mortality (Table 2.15). Lower levels of triclocarban were trialled, yielding a pass at 0.30% omw, and sufficiently low mass loss at 0.05% omw. Both lower levels of triclocarban also displayed high mortalities (Table 2.15). The 48 hour EC_{50} of triclocarban to *Daphnia magna* is 10 μg/L (*Initial Risk-Based Prioritization of High Production Volume (HPV) Chemicals*, 2009), which is considerably more toxic than propiconazole, and closer to that of permethrin.

Table 2.15 Bioassay results of *Tineola bisselliella* on wool fabric treated with triclosan and triclocarban.

Treatment (% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b).
Control	19.0	3.3	58.9 ± 17.1	n.a.	4D	n.a.
Triclosan (3.0%)	12.5	0.0	55.6 ± 15.2	94.3	4D	f
Control	1.7	3.3	66.3 ± 14.6	n.a.	4D	n.a.
Triclocarban (3.0%)	93.0	0.0	0.8 ± 0.4	1.2	1A	p
Control	0.0	0.0	67.4 ± 11.2	n.a.	4D	n.a.
Triclocarban (0.3%)	91.7	1.7	1.8 ± 1.2	2.7	1A	p
Control	0.0	0.0	108.1 ± 9.2	n.a.	4D	n.a.
Triclocarban (0.05%)	51.7	0.0	7.2 ± 0.4	6.7	2C	f

[1] As a percentage of the mean voracity control.

Walsh et al. (2003) found that 0.5 mM of the metal complexing agent ethylenediaminetetraacetic acid (EDTA) can potentiate the activity of triclocarban against *Staphylococcus aureus*, lowering the minimum inhibitory concentration from 0.5 to 0.05 µg/ml. For this reason a combination of these two compounds was investigated with *Tineola bisselliella* to check for a possible synergy. The results in Table 2.16 may show a slight synergy between triclocarban and EDTA. No efficacy for 0.1% omw EDTA alone was noted, but when combined with 0.03% omw triclocarban the mass loss was significantly (p-value<0.05) reduced to 35 mg from 65 mg without EDTA. The mortality also increased from 16.7% to 41.7%, indicating a slight synergistic effect. This possible synergy indicates that the biochemical pathways being disrupted in *Tineola bisselliella* moth larvae may be similar to those disrupted in *Staphylococcus aureus*.

The lack of efficacy of the EDTA-only treatment at 0.1% omw is of interest when considering the work of Ward, as summarised in Table 1.5 (Section 1.5.2). Total inhibition of the major and minor metal-chelator sensitive proteinases, and partial inhibition (42%) of the major

aminopeptidase of low electrophoretic mobility was noted at levels of 1.7 mM and 2.0 mM EDTA respectively. Concentrations of 1.7-2.0 mM EDTA in the *Tineola bisselliella* gut would be likely after feeding on treated wool, as at the level of 0.1% omw EDTA the larval gut contents would need to comprise 50-58% digested wool to achieve this concentration. Hartley, Elsworth & Barritt (1943) showed that under ideal conditions an average *Tineola bisselliella* larva consumes 35 mg of wool in three months (0.39 mg per day). Their test method used half-grown larvae of around 5 mg each, and when considering the digestion time of 8 hours (Day, 1951a), it can be calculated that the average mass of wool in the gut at any point in time would be 0.13 mg. From this it could be tentatively concluded that the metal-chelator sensitive proteinases and major aminopeptidase of low electrophoretic mobility would be inhibited in *Tineola bisselliella* larvae feeding on wool treated with 0.1% omw EDTA. Due to the lack of efficacy seen in Table 2.16, it would seem that *Tineola bisselliella* larvae do not solely rely on these enzymes for digestion of wool.

Econazole nitrate was also tested for a synergy with EDTA, without a significant reduction in mass loss (Table 2.16).

Table 2.16 Bioassay results of *Tineola bisselliella* on wool fabric treated with triclocarban and EDTA.

Treatment	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	0.0	0.0	147.2 ± 3.5	n.a.	4D	n.a.
Triclocarban (0.03% omw)	16.7	5.0	65.2 ± 4.1	44.3	4D	f
EDTA (0.1% omw)	5.0	0.0	167.2 ± 6.9	113.6	4D	f
Triclocarban + EDTA (0.03% + 0.1% omw)	41.7	1.7	35.4 ± 1.3	24.0	3C	f
Econazole (0.5% omw)	0.0	3.3	125.9 ± 13.7	85.6	2D	f
EDTA (0.5% omw)	0.0	5.0	161.0 ± 9.3	109.4	4D	f
Econazole + EDTA (0.5% + 0.5%)	3.3	0.0	109.3 ± 7.3	74.3	4D	f

[1] As a percentage of the mean voracity control.

The mechanism of action of triclocarban with respect to its structural features was investigated. Likely features of the molecule contributing to the efficacy are the chlorine atoms, as many older-generation wool insect-resist agents comprise chlorinated benzene derivatives (Waterhouse, 1958). To test this theory, carbanilide (1,3-diphenylurea) was trialled at 3% omw against *Tineola bisselliella*. Carbanilide (Figure 2.35) has the same molecular structure as triclocarban, but does not contain chlorine. Results for carbanilide show very little anti-feeding effect (Table 2.17). This strongly suggests that the chlorine atoms of triclocarban are involved in the insect-resist effect shown by this compound. Similarly, when looking at bioassay results for the naphthalene compound 1-(3,4-dichlorophenyl)-3-(1-naphthyl) urea (Table 2.10, Section 2.4.2), it appears that replacing one of the chlorinated benzene groups of triclocarban with a naphthalene group results in the loss of most of the mothproofing activity. Given that this molecule represents part of the triclocarban molecule bonded to a naphthalene group, this suggests that the bonding of one

efficacious entity to another does not always result in similar efficacy of the combined molecule.

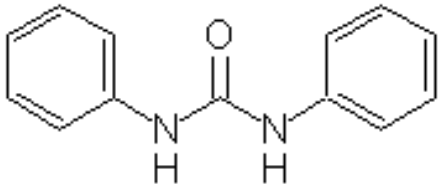


Figure 2.35 Carbanilide.

Table 2.17 Bioassay results of *Tineola bisselliella* on wool fabric treated with carbanilide.

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	0.0	0.0	85.0 ± 3.7	n.a.	4D	n.a.
Carbanilide	10.6	0.0	69.0 ± 12.0	81.2	4D	f

[1] As a percentage of the mean voracity control.

The contrasting ineffectiveness of triclosan suggests that either the positions of the chlorine atoms, or the urea group of triclocarban also contribute to the insect-resist effect. A study of the efficacy of carbanilide derivatives with variations in the position of attached groups was carried out with the bacterium *Micrococcus pyogenes* var. *aureus* (Beaver, Roman, & Stoffel, 1957). The bacteriostatic properties were found to be remarkably specific in that activity was greatly enhanced or completely lost with slight changes in chemical structure. Chlorine atoms in the meta and para (3 & 4) positions increased the bacteriostatic efficacy, whereas chlorine in the ortho (2) position was detrimental to this effect. The 3,4,4'-trichlorocarbanilide and 3,3',4-trichlorocarbanilide molecules had far superior bacterial inhibition than other analogues and homologues. This may also be true of their efficacy against wool-digesting insects. The fact that triclosan has a chlorine atom in the ortho position of one benzene group could contribute to its lack of efficacy against *Tineola bisselliella* (Table 2.15). Contrary to this observation, the most effective of the azole compounds against *Tineola bisselliella* was econazole nitrate, which has chlorine atoms in the ortho and para positions (Figure 2.20). Similarly, the best performing azole against *Anthrenocerus australis* was propiconazole (Section 2.5.3), also with chlorine atoms in these

positions (Figure 2.21). The azole compound tebuconazole has one chlorine atom in the para position only (Figure 2.21), and is less effective against both insect species. It may be that the azole compounds rely on parts of their molecular structure other than the chlorine atoms for efficacy against these insects.

2.5.3 Bioassay Results of Wool Fabrics Treated with Antimicrobial Compounds: *Anthrenocerus australis*

Bioassays using the same Wools of New Zealand test method with *Anthrenocerus australis* beetle larvae were performed on wool fabric treated with econazole nitrate, as this compound had shown high efficacy against *Tineola bisselliella*. At 3.0% omw econazole nitrate offered more protection against beetle larvae compared to *Tineola bisselliella*, with a mass loss of only 1.3 mg despite no larval mortality (Table 2.18). Further bioassays on lower application levels of 1.0% and 0.5% omw econazole nitrate showed a borderline mass loss result at 1.0% omw. The pupation on the control larvae was slightly over 25%, rendering the test technically invalid, although the pupation on the test specimens was low enough to obtain a good idea of the efficacy of this application level (Table 2.18). Sulconazole nitrate was also trialled at 1.0% omw against *Anthrenocerus australis*, showing even greater efficacy than econazole nitrate at 1.0% omw (Table 2.18), and a similar efficacy against the beetle species to that measured against the *Tineola bisselliella* moth larvae at 3.0% omw sulconazole nitrate (Table 2.13, Section 2.5.2).

Table 2.18 Bioassay results of *Anthrenocerus australis* on wool fabric treated with imidazole nitrate compounds.

Treatment	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b).
Control	0.0	0.0	44.7 ± 5.7	n.a.	4C	n.a.
Econazole nitrate (3.0% omw)	0.0	6.7	1.3 ± 0.6	2.8	1A	p
Control	0.0	25.9	42.4 ± 9.5	n.a.	3A	n.a.
Econazole nitrate (1.0% omw)	0.0	17.3	13.8 ± 5.8	32.5	3A	f
Econazole nitrate (0.5% omw)	0.0	17.3	29.2 ± 3.8	68.8	4C	f
Control	0.0	7.0	34.9 ± 2.8	n.a.	4B	n.a.
Sulconazole nitrate (1.0% omw)	0.0	3.5	8.3 ± 1.2	23.8	1A	p

[1] As a percentage of the mean voracity control.

Given the high efficacy of imidazole compounds at 1.0% omw against *Anthrenocerus australis*, three triazole compounds tebuconazole, propiconazole and epoxiconazole were tested in bioassays with this species at 1.0% omw (Table 2.19). Tebuconazole and expiconazole showed showed inadequate protection of wool fabric. Propiconazole treated fabric showed no significant mass loss, and so was tested at the lower level of 0.1% omw (Table 2.20). Although this bioassay failed due to a high mass loss, it was accidentally run for 18 days, and so was a more difficult test to achieve a low mass loss. It was noticed that the wool fabric discs exposed to the *Anthrenocerus australis* larvae suffered more attack in the centre of the discs and less on the outside edges. This could have been a result of the acetone solvent evaporating faster from the edge of the fabric discs, as explained previously with DDAO (Section 2.3.2.3). To prevent this edge effect in subsequent trials, the method was altered so that the solvent/propiconazole was applied to a larger piece of fabric, followed by drying and cutting out the discs. Propiconazole was added to the fabric at

concentrations of 0.2% and 0.3% omw. These fabrics failed the bioassay, although at 0.3% omw propiconazole the fabric was close to a borderline result with a mean mass loss of 15.8 mg (Table 2.20). Visual assessments showed that using the new application method, the feeding damage was restricted to the edge of the wool fabrics, and therefore more in line with the control fabric feeding damage. It could be assumed that the application was more even compared to the previous method of applying to separate individual wool fabric discs. This application method became the standard method used from this point on.

A higher level of 0.4% omw propiconazole was trialled against *Anthrenocerus australis* to ensure that one result gave an acceptably low mass loss. Unexpectedly, the mass loss observed in this bioassay was higher than previously found with 0.3% omw propiconazole (Table 2.20). In order to achieve a smooth dose-response curve, new wool fabric samples were treated with 0.3%, 0.5%, and 0.7% omw propiconazole and subjected to bioassay with *Anthrenocerus australis* larvae. These bioassays showed comfortable passes at all three levels. (Table 2.20). It appeared there were significant variations over time in the repsonse of *Anthrenocerus australis* larvae to propiconazole-treated wool fabric.

Table 2.19 Bioassay results of *Anthrenocerus australis* on wool fabric treated with triazole compounds.

Treatment (1.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or border-line (b)
Control	0.0	17.8	27.6 ± 3.3	n.a.	3A	n.a.
Epoxiconazole	0.0	11.7	18.5 ± 2.8	67.1	3B	f
Propiconazole	0.0	26.7	-1.0 ± 0.7	-3.7	1A	p
Tebuconazole	0.0	20.1	27.3 ± 10.1	98.9	2B	f

[1] As a percentage of the mean voracity control.

Table 2.20 Bioassay results of *Anthrenocerus australis* on wool fabric treated with propiconazole.

Treatment	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	0.0	2.3	38.0 ± 0.9	n.a.	3A	n.a.
Propiconazole (0.1% omw)	0.0	5.5	25.1 ± 1.5	66.0	1D	f
Control	1.8	7.0	37.0 ± 3.6	n.a.	3A	n.a.
Propiconazole (0.2% omw)	0.0	1.7	22.1 ± 2.8	59.7	3A	f
Propiconazole (0.3% omw)	1.6	0.0	15.8 ± 0.7	42.7	2A	f
Control	0.0	0.0	30.9 ± 3.2	n.a.	3B	n.a.
Propiconazole (0.4% omw)	0.0	3.3	21.3 ± 3.4	68.9	3B	f
Control	1.8	0.0	34.9 ± 5.9	n.a.	3A	n.a.
Propiconazole (0.3% omw)	0.0	6.7	4.8 ± 1.7	13.8	1A	p
Propiconazole (0.5% omw)	0.0	1.7	2.5 ± 1.2	7.1	1A	p
Propiconazole (0.7% omw)	0.0	6.7	1.4 ± 0.9	4.0	1A	p

[1] As a percentage of the mean voracity control.

The differences between each set of bioassays made it difficult to determine the minimum effective concentration of propiconazole required to achieve a pass against *Anthrenocerus australis* with the test method, therefore all data were combined to give the best possible estimate. This minimum effective concentration was considered important given the high efficacy of this compound against the *Anthrenocerus australis* larvae, and the lack of efficacy of other non-insecticidal compounds against this species. The negative mass loss shown with propiconazole at 1.0% omw was taken as zero, as an increase in fabric mass is impossible without some error in humidity control or removal of frass. Mass loss was plotted against the concentration of propiconazole added to the wool, and a polynomial trendline was

calculated using Microsoft Excel 2007 (Figure 2.36). An exponential trendline may also have been appropriate, but the data point showing a mass loss of 0 was not compatable with this kind of formula. A linear trendline would also have been less appropriate due to the significantly (p-value<0.05) lower correlation with the data as shown by R^2 values in a sequential F-test. The resulting trendline followed the polynomial formula: mass loss (mg) = $41.256x^2 - 74.65x + 33.446$, where "x" represented the level of propiconazole (% omw). Using this trendline, and the formula to calculate the roots of this quadratic equation $x = (-b \pm \sqrt{(b^2 - 4ac)}) / 2a = (74.65 +/- \sqrt{((74.65)^2 - 4 \times 41.256 \times 21.446)}) / 2 \times 41.256$ as 0.358 and 1.451, it can be estimated that to obtain a pass in Wools of New Zealand Test Method 25 in terms of mass loss (≤ 12 mg) would require no less than 0.36% omw propiconazole. The correlation between the trendline and plotted points of data showed an R^2 value of 0.8631, reflecting the high variability of results between different groups of bioassays, and that the relationship may have been only approximately polynomial. Another method may have been to plot the mass losses as a percentage of the voracity controls. This would raise the complex question of what percentage would be considered a pass under the Wools of New Zealand test method, and so for this reason the absolute mass loss data was used.

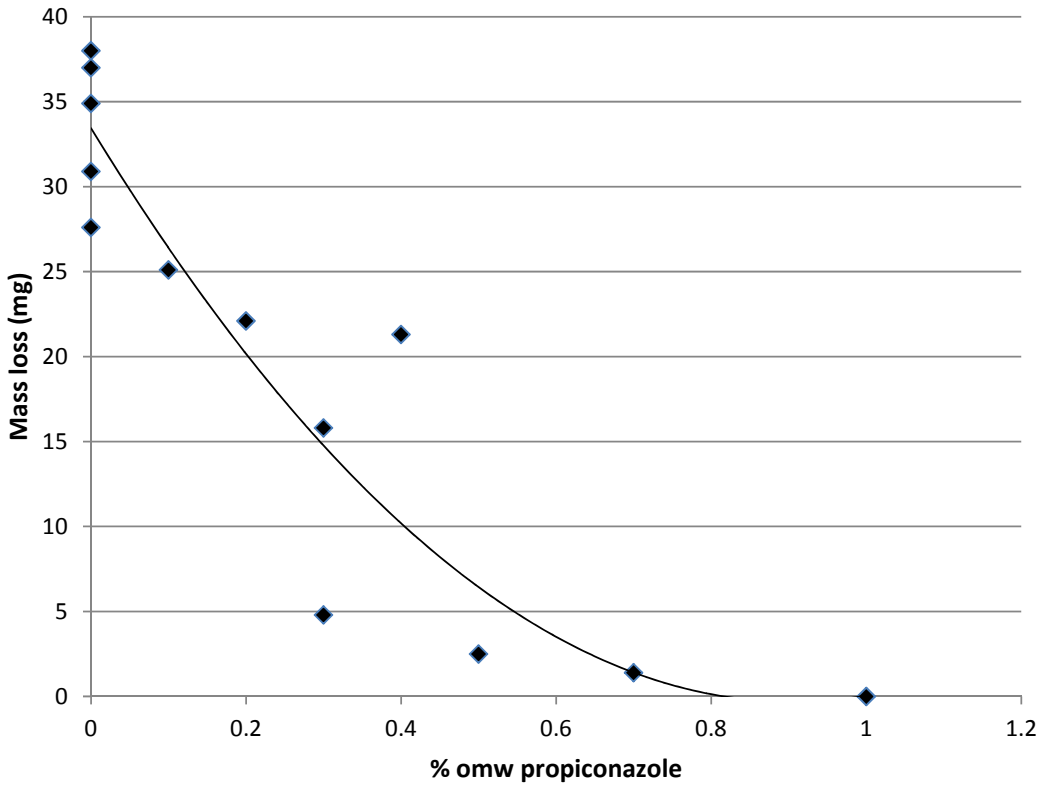


Figure 2.36 Propiconazole level on wool fabric versus mass loss in bioassays with *Anthrenocerus australis* beetle larvae.

Synergies with propiconazole were explored with two additional *Anthrenocerus australis* bioassays. One potential synergy tested was propiconazole with DDAO, one of the more effective surfactants against *Anthrenocerus australis* (Table 2.7, Section 2.3.2.3). When both were added to the same wool fabric at 0.2%, the mass loss was significantly (p-value<0.05) lower than that of the fabric treated with 0.2% propiconazole only (Table 2.21). This was an unexpected result, as DDAO alone at 0.5% omw resulted in only a slight reduction in mass loss (Table 2.8, Section 2.3.2.3). This may have represented a synergy, but was not effective enough to justify further investigation.

The other possible synergy tested was propiconazole with isoniazid, an antitubercular drug. Isoniazid had shown a synergy with the azole fungicides econazole and clotrimazole against *Mycobacterium tuberculosis* (Ahmad, Sharma & Khuller, 2005). Previous bioassay results with isoniazid-only treated wool fabric had shown little effect at 3% omw against *Tineola bisselliella* (Table 2.13, Section 2.5.2), therefore no isoniazid-only fabric was included in this batch. A slightly lower mass loss of the propiconazole-isoniazid treated fabric compared to the mass loss of propiconazole-only treated fabric showed an additive effect was more likely than a synergistic effect (Table 2.21). For ease of comparison, the propiconazole results at 0.2% and 0.3% omw are repeated here as these bioassays were performed at the same time as those of the synergy bioassay testing.

Table 2.21 Bioassay results of *Anthrenocerus australis* on wool fabric treated with propiconazole and possible synergists.

Treatment	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or border-line (b)
Control	1.8	7.0	37.0 ± 3.6	n.a.	3A	n.a.
Propiconazole (0.2% omw)	0.0	1.7	22.1 ± 2.8	59.7	3A	f
Propiconazole (0.3% omw)	1.6	0.0	15.8 ± 0.7	42.7	2A	f
Propiconazole + Isoniazid (both 0.2% omw)	1.7	3.5	16.9 ± 0.2	45.7	1A	f
Propiconazole + DDAO (both 0.2% omw)	1.7	0.0	12.2 ± 1.9	33.0	1A	b

[1] As a percentage of the mean voracity control.

Due to the high efficacy of triclocarban against *Tineola bisselliella*, this antimicrobial was trialled against *Anthrenocerus australis* at 3.0%, 0.5%, and 0.1% omw. The application rate sufficient to impart a borderline mass loss result was 0.5% omw triclocarban (Table 2.22), approximately ten times more than that required for control of *Tineola bisselliella*. These triclocarban treated fabrics resulted in no *Anthrenocerus australis* mortality.

Table 2.22 Bioassay results of *Anthrenocerus australis* on wool fabric treated with triclocarban.

Treatment	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	3.8	9.5	28.7 ± 5.7	n.a.	3C	n.a.
Triclocarban (3.0% omw)	0.0	10.1	7.1 ± 0.6	24.7	1A	p
Control	0.0	25.9	42.4 ± 9.5	n.a	3A	n.a
Triclocarban (0.5% omw)	0.0	10.5	12.2 ± 1.0	28.7	1A	b
Triclocarban (0.1% omw)	0.0	23.9	25.2 ± 4.2	59.5	2A	f

[1] As a percentage of the mean voracity control.

Due to the possible synergy noted between triclocarban and EDTA with *Tineola bisselliella* (Table 2.16, Section 2.5.2), the same combination was tested with *Anthrenocerus australis*. Higher levels of 0.25% omw each were used due to the lower activity of triclocarban against this beetle species compared to *Tineola bisselliella*. No synergy between triclocarban and EDTA was evident from the bioassay results with *Anthrenocerus australis* (Table 2.2.3). High pupation probably reduced the feeding during this bioassay, but the level of pupation was similar for both triclocarban samples, therefore a comparison could still be made.

Table 2.23 Bioassay results of *Anthrenocerus australis* on wool fabric treated with triclocarban and EDTA.

Treatment (0.25% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	0.0	23.0	25.6 ± 2.5	n.a.	3B	n.a.
EDTA	0.0	22.2	34.4 ± 6.7	134.1	3D	f
Triclocarban	0.0	38.8	9.7 ± 1.4	37.9	1A	p
Triclocarban + EDTA (both 0.25%)	0.0	32.1	9.0 ± 1.1	35.0	2B	p

[1] As a percentage of the mean voracity control.

The effect of the anti-protozoal compound pentamidine isethionate was trialled against *Anthrenocerus australis* at 3.0% omw. This compound was of interest against *Anthrenocerus australis* not only due to the good anti-feeding effect on *Tineola biselliella* (Table 2.13, Section 2.5.2), but also due to the documented presence of protozoa in the hind-gut of *Anthrenus flavipes* (Trivedi et al., 1991), a species closely related to *Anthrenocerus australis*. Results showed a low efficacy, suggesting that either protozoa did not exist in the gut of *Anthrenocerus australis* in significant numbers, or that any negative effect on protozoa had no overall effect on the insect host.

Table 2.24 Bioassay results of *Anthrenocerus australis* on wool fabric treated with pentamidine isethionate.

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	0.0	0.0	49.7 ± 2.8	n.a.	3C	n.a.
Pentamidine isethionate	0.0	0.0	47.1 ± 5.5	94.8	4D	f

[1] As a percentage of the mean voracity control.

2.6 Mosquito Repellent and Structural Analogues

2.6.1 Selection of Mosquito Repellent and Structural Analogues

Mosquito repellents may have a mothproofing effect, as the moth repelling compound naphthalene is used in some mosquito-repellent products (Peairs & Cranshaw, 1998), suggesting that there may be similarities between mosquito and moth physiology. The mosquito repellent dimethyl phthalate (Figure 2.37a) has a molecular structure with numerous analogues available. Dimethyl phthalate and structural analogues including phthalhydrazide, phthaldialdehyde, phthalic anhydride, and diisononyl phthalate (Figure 2.37b-e) were applied directly to wool fabrics at 3.0% omw and tested against *Tineola bisselliella* larvae using the Wools of New Zealand Test Method 25.

2.6.2 Bioassay Results of Wool Fabrics Treated with Mosquito Repellent and Structural Analogues

Phthalhydrazide (Figure 2.37b) required dimethyl sulphoxide (DMSO) solvent for fabric application. As DMSO is slow to evaporate, a second control fabric was trialled using only the DMSO solvent, to correct for any effect caused by residual solvent. This DMSO-only control showed some effect on the moth larvae, making assessment of the phthalhydrazide bioassay difficult, although it appeared that phthalhydrazide had no mothproofing effect due to similar results with the DMSO-only control (Table 2.25). Dimethyl phthalate and diisononyl phthalate (Figure 2.37e) showed a similar low efficacy, despite the mosquito-repelling properties of dimethyl phthalate, and the longer alkyl chains of diisononyl phthalate (Table 2.25). Phthaldialdehyde (Figure 2.37c) showed slight activity at 3.0% omw, whereas phthalic anhydride (Figure 2.37d) showed a significant reduction in larval feeding close to that deemed borderline by the Wools of New Zealand test method (Table 2.25).

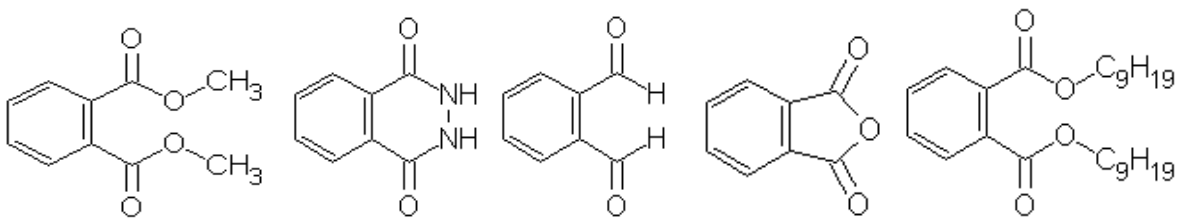


Figure 2.37 From left to right: (a) dimethyl phthalate, (b) phthalhydrazide, (c) phthaldialdehyde, (d) phthalic anhydride, (e) diisononyl phthalate.

Table 2.25 Bioassay results of *Tineola bisselliella* on wool fabric treated with structural analogues of dimethyl phthalate.

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	7.2	6.9	59.9 ± 3.4	n.a.	4A	n.a.
DMSO only	47.1	0.0	14.9 ± 5.9	24.9	3C	f
Phthalhydrazide + DMSO	29.3	1.7	17.3 ± 0.8	28.9	3C	f
Phthaldialdehyde	8.8	0.0	39.0 ± 4.1	65.0	4D	f
Phthalic anhydride	23.3	1.7	16.9 ± 3.8	28.2	4D	f
Control	1.9	0.0	50.5 ± 2.7	n.a.	3C	n.a.
Dimethyl phthalate	3.6	0.0	46.1 ± 1.0	91.3	3C	f
Diisononyl phthalate	0.0	0.0	42.1 ± 1.9	83.3	3B	f

[1] As a percentage of the mean voracity control.

2.7 The Effect of Alkyl Chain Length on Mothproofing

2.7.1 Selection of Compounds Containing Alkyl Chains

The mothproofing results of Freeland and Williams (1967) showed an optimum alkyl chain length for alkyl benzene sulphonates and sulphonic acids of 14-15 carbon atoms. To determine if this is generalisable to other molecular structures, carboxylic acids with alkyl chain lengths ranging from 10 to 18 carbon atoms (decanoic, dodecanoic, tetradecanoic, hexadecanoic, and octadecanoic acids) were obtained. Variations on these molecules also included the one hydroxyl group of dodecanol, the two hydroxyl groups of 1,10-decanediol, and the two carboxylic acid groups of dodecanedioic acid. These compounds were applied to wool fabrics at 3.0% omw and tested according to Wools of New Zealand Test Method 25.

2.7.2 Bioassay Results of Wool Fabrics Treated with Alkyl Compounds

The bioassay results using wool fabric treated with carboxylic acids of varying alkyl chain length showed an optimum mothproofing effect with dodecanoic acid (Table 2.26). Dodecanoic acid, containing a twelve carbon atom alkyl chain, was significantly more effective in reducing larval feeding than decanoic or tetradecanoic acids, which contain ten

and fourteen carbon atoms respectively. Hexadenanoic and octadecanoic acids showed less efficacy, inversely proportional to the longer alkyl chain length. There was no significant mortality with any of the carboxylic acids trialled.

Replacing the carboxylic acid group of slightly efficacious decanoic acid with hydroxyl groups at either end of the alkyl chain removed the anti-feeding effect entirely. Polar entities at both ends of a molecule have been shown to reduce the anti-feeding effect for many compounds. Similarly, the conversion of the primary carbon atom of dodecanoic acid to a carboxylic acid group also reduces the anti-feeding effect to a great extent, most likely due to the carboxylic acid groups at both ends of the molecule (dodecanedioic acid). Replacing the carboxylic acid group of dodecanoic acid with a hydroxyl group reduces most of the anti-feeding effect. This is consistent with the theory that different polarities at either end of a molecule confer a greater mothproofing effect. As the hydroxyl group is less polar than the carboxylic acid, the difference in polarity between the head and tail of the molecule is reduced, resulting in lower efficacy against *Tineola bisselliella*.

Table 2.26 Bioassay results of *Tineola bisselliella* on wool fabric treated with acids and alcohols.

Treatment and (number of carbon atoms) (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	0.0	0.0	140.7 ± 11.0	n.a.	4D	n.a.
Decanoic acid (10)	1.7	1.7	80.2 ± 9.0	57.0	4D	f
Dodecanoic acid (12)	0.0	0.0	53.3 ± 7.0	37.9	4D	f
Tetradecanoic acid (14)	0.0	1.7	76.2 ± 10.8	54.2	4D	f
Hexadecanoic acid (16)	1.7	0.0	78.5 ± 11.0	55.8	4D	f
Octadecanoic acid (18)	0.0	1.7	104.1 ± 10.5	74.0	4D	f
1,10 Decanediol (10)	0.0	1.6	146.3 ± 7.3	104.0	4D	f
Dodecanedioic acid (12)	1.7	1.7	111.7 ± 9.3	79.4	4D	f
Dodecanol (12)	1.7	0.0	97.1 ± 7.9	69.0	4D	f

[1] As a percentage of the mean voracity control.

2.8 Plant-Based Insect Defence Mechanism Involving Jasmonic Acid

Insect attack of plants has been shown to increase the levels of jasmonic acid in plants (Creelman & Mullet, 1995). Jasmonic acid is a plant hormone that regulates signalling networks involved in induced defence responses. These responses include the activation of genes encoding proteinase inhibitor proteins that inhibit digestive enzymes in the herbivorous insect gut. An example of this is caterpillars of the herbivore *Pieris rapae* stimulating production of jasmonic acid in *Arabidopsis thaliana* (De Vos et al., 2006). Other studies have used the application of jasmonic acid to plants to observe reduced feeding effects on insects, such as that of the Pacific spider mite *Tetranychus pacificus* (McGregor 1919) on grapevines (Omer, Thaler, Granett, & Karban, 2000), and the brown planthopper *Nilaparvata lugens* (Stål 1854) on the rice plant *Oryza sativa* (Senthil-Nathan, Kalaivani, Choi,

& Paik, 2009). Application of jasmonic acid to wool was not expected to induce production of protease inhibitors in wool, although a direct effect on wool consuming insects cannot be ruled out due to efficacy seen with other carboxylic acid molecules of a similar size (Table 2.26, Section 2.7.2).

Wool fabrics directly treated with 3.0% omw jasmonic acid showed no mothproofing effect, as shown in Table 2.27. The plant defense mechanism is therefore likely to be entirely due to signalling the production of toxins, without any direct effect of jasmonic acid on insects.

Table 2.27 Bioassay results of *Tineola bisselliella* on wool fabric treated with jasmonic acid.

Treatment (3.0% omw)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	1.7	0.0	58.0 ± 4.1	n.a.	3B	n.a.
Jasmonic acid	0.0	0.0	56.2 ± 3.2	97.0	3C	f

[1] As a percentage of the mean voracity control.

2.9 Conclusions and Comparison of Compounds of Highest Efficacy

2.9.1 Surfactants

Certain structural characteristics of surfactants can be said to contribute to efficacy against insects based on results obtained from bioassays (Section 2.3.2). For anionic surfactants there appears to be an antifeeding efficacy against *Tineola bisselliella* larvae that requires the presence of a polar head and non-polar tail at opposite ends of the molecule. The simple structure of efficacious surfactants such as dodecylbenzene sulphonic acid and structural analogue 4-dodecyl phenol, combined with the evidence of optimum alkyl chain length in Section 2.7.2 and by Freeland and Williams (1967) leads to the conclusion that the mechanism of action on insects may also be a result of the physical shape and size of the molecule rather than a specific chemical reactivity. Variations in shape and size do not exclude a surfactant from imparting an antifeeding effect, but appear to strengthen or weaken this effect. An example of this is sodium lauryl sulphate, which lacks the benzene group of dodecyl benzene sulphonic acid, but is otherwise similar in shape and size. The antifeeding effect on *Tineola bisselliella* of sodium lauryl sulphate was weaker than that of

dodecylbenzene sulphonic acid, but still significant enough for a borderline mass loss at 3.0% omw using Wools of New Zealand Test Method 25.

For surfactants containing an alkyl chain, the strength of the bond between the alkyl chain and the remainder of the surfactant structure appears to be important for insect-proofing. Those alkyl groups bound to a more reactive C=O entity tend to have a lower insect-proofing efficacy, as seen with methyl 2-sulphooctadecanoate sodium salt (Table 2.4, Section 2.3.2.2), N-lauroylsarcosine and phosphatidylcholine (Table 2.3, Section 2.3.2.2), and the non-ionic sorbitan monopalmitate (Table 2.9 Section 2.3.2.4). This suggests that retention of the shape and size of the unreacted molecule in the insect gut is important for insect-proofing efficacy.

Zwitterionic surfactants are unusual in that some, such as N,N-dimethyldodecyl amine N-oxide and coco dimethyl betaine, have a greater anti-feeding effect against *Anthrenocerus australis* beetle larvae than against *Tineola bisselliella* moth larvae. Most compounds, including insecticides, have the opposite order of efficacy against these two species. Hypothesising on reasons for this high beetle efficacy leads to consideration of the effect of gut pH of each species on the surfactants. In the alkaline gut conditions of *Tineola bisselliella*, zwitterionic compounds would predominantly take the anionic form, whereas at the neutral pH of the *Anthrenocerus australis* gut the anionic and cationic would both be present. The presence of cationic groups may give extra functionality to the molecule not present in the anionic form. Antibacterial and antifungal agents are often made from compounds containing cationic groups (Freeland & Williams, 1967), which when present in the beetle gut may play a part in interruption of wool digestion in species that rely on gut microbes for this digestion. *Anthrenocerus australis* may contain the same or similar microbes to those discovered in *Anthrenus flavipes* (Trivedi et al., 1991), leading to susceptibility to some zwitterionic surfactants. The reason cationic surfactants are not used in wool dyeing is due to incompatability with anionic dyeing systems, and as shown by Freeland and Williams, a lower durability to washing in anionic detergents compared to anionic insect-proofers. It may be possible to apply zwitterionic surfactants to wool in a slightly alkaline rinse after dyeing, in an attempt to keep the anionic nature predominant during application. Liberation of the surfactant from wool particles during digestion in the neutral beetle gut could then allow the formation of some cationic groups with the ability to disrupt wool digestion.

2.9.2 Naphthalene Derivatives

Insect-proofing efficacy of naphthalene derivatives appear to follow similar trends to those of surfactants. Compounds with a molecular structure containing a polar head and non-polar tail have a greater anti-feeding effect than those compounds without this combination. A good example of this is the moderate anti-feeding effect against *Tineola bisselliella* of 1-naphthalene sulphonic acid compared to the lack of any anti-feeding effect with 8-hydroxy-5,7-dinitro-2-naphthalene sulphonic acid (Table 2.10, Section 2.4.2). The latter molecule has no non-polar tail with polar groups bonded to both benzene groups within the naphthalene moiety. Another example is the greater antifeeding efficacy of 1,2-naphthoquinone-4-sulphonic acid compared to 4-anilino-1,2-naphthoquinone (Table 2.10, Section 2.4.2). The replacement of a polar sulphonic acid group with a non-polar aniline group leaves the 4-anilino-1,2-naphthoquinone molecule with two non-polar areas and a lesser degree of polarity at the head of the molecule.

Carbonyl groups as seen in naphthoquinone sulphonic acid derivatives appear to confer efficacy depending on their positions on the benzene ring structure. When looking at results of naphthoquinone sulphonic acid derivatives (Table 2.10, Section 2.4.2) it appears that the quinone moeity in the 1,2 spacial arrangement confers a greater efficacy than in the 1,4 arrangement, although specific conclusions are difficult to make due to the work of Rivett et al. (1990), who showed mothproofing efficacy was conferred to wool fabric by naphthoquinones in the 1,4 arrangment.

Theorising on the effect of the reducing conditions in the insect gut on these naphthoquinones, it has been shown 1,2-benzoquinone is produced by oxidation of catechol (Danilewicz, 2007). It is therefore likely that in a reducing environment, such as the *Tineola bisselliella* gut, that 1,2-benzoquinone may revert to catechol. Similarly 1,2-naphthoquinone-4-sulphonic acid may form the naphthalene sulphonate equivalent of catechol (1,2-hydroxynaphthalene-4-sulphonic acid). The hydroxyl groups are likely to be significantly deprotonated, effectively resulting in two negatively charged oxygen atoms in the 1,2 positions and one bonded to the sulphur atom (Figure 2.38). This entity may react with gut enzymes, reducing their ability to digest wool. Alternatively, this reaction may form a temporary bond between the oxygen atoms and amine groups present within the wool particles in the insect gut, analogous to the reaction of vat dyes with wool under reducing conditions (Roessler, Dossenbach, Marte, & Rys, 2002). This could possibly interfere with gut

enzymes by steric hinderance of their reaction with wool, or alternatively may reduce the nutritional value of wool to the insect.

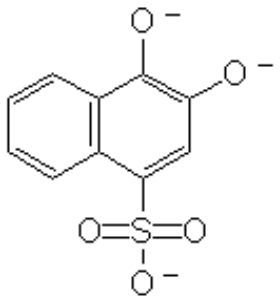


Figure 2.38 Deprotonated 1,2- hydroxynaphthalene-4-sulphonate.

2.9.3 Antimicrobials

Most of the antimicrobials trialled in bioassays were selected on the basis of their mode of action, although some were noted to have structural features similar to other compounds with a known efficacy against insects. The higher efficacy of imidazole compounds against *Anthrenocerus australis* larvae compared to *Tineola bisselliella* leads to the hypothesis that their mode of insect-proofing action is related to antimicrobial action, due to microbes playing a larger role in wool digestion by *Anthrenocerus australis* than in *Tineola bisselleilla*. The high efficacy of the the triazole compound propiconazole against *Anthrenocerus australis* was interesting, considering the other two triazole compounds showed higher efficacy against *Tineola bisselliella* than with *Anthrenocerus australis*. The small number of imidazole and triazole compounds trialled made it difficult to reach firm conclusions, although the mode of action of imidazole compounds may be more broad-spectrum than that of triazoles. The more variable results of the triazole compounds suggest a specific mode of action for each, perhaps affecting a narower range of enzymes or biochemical pathways within either insect species.

The uncoupling of oxidative phosphorylation mechanism of triclocarban is not just restricted to wool-digesting insects, as was deduced from the high toxicity to *Daphnia magna* (*Initial Risk-Based Prioritization of High Production Volume (HPV) Chemicals*, 2009). This non-specific toxicity lowers the environmental suitability of triclocarban as a wool insect-proofing compound. The similarity of the molecular structure of triclocarban to that of the

commercial mothproofing active sulcofenuron, and the lack of efficacy of carbanilide indicates that the mode of action is likely to rely on the chlorine component of triclocarban.

Efficacy of anti-protozoal pentamidine isethionate against *Tineola bisselliella* feeding, coupled with lack of efficacy against *Anthrenocerus australis* suggests a mode of action unrelated to gut microbes due to the apparent lack of flora in the gut of *Tineola bisselliella* (Crewther & McQuade, 1955). The presence of protozoa in the hindgut of *Anthrenus flavipes* (Trivedi et al., 1991), and the similarity to the closely related *Anthrenocerus australis* suggests the possibility that these protozoa could also occur in the latter species, although this cannot be corroborated with the bioassay results from pentamidine isethionate treated wool.

Theophylline showed a moderately strong antifeeding effect against *Tineola bisselliella*, suggesting inhibition of phosphodiesterase enzymes, as was noted by Nathanson (1984) when using caffeine against the tobacco hornworm moth *Manduca sexta* Linnaeus 1763. This effect on *Tineola bisselliella* shows that the wool digestive process is not specifically targeted, as the tobacco hornworm moth feeds on vegetable matter. Similarities in digestive enzymes between the two species may lead to the similar response to phosphodiesterase inhibitors.

2.9.4 Mosquito Repellent Analogues

Although the mosquito repellent dimethyl phthalate had very little mothproofing effect, one of the structural analogues, phthalic anhydride, showed a significant anti-feeding effect close to borderline in terms of mass loss. Phthalic anhydride has two carbonyl groups separated by a single oxygen atom. It is likely that the carbonyl groups would be reduced to singly-bound deprotonated oxygen atoms in the *Tineola bisselliella* mid-gut, as similarly theorised for 1,2-naphthoquinone-4-sulphonic acid (Section 2.9.2). The reactivity of these groups may give rise to specific gut effects, perhaps temporarily binding to the amine groups of wool, as hypothesised for naphthoquinone compounds (Section 2.9.2). The higher mothproofing efficacy of phthalic anhydride compared to the other structural analogues may be due to the oxygen atom between the two carbonyl groups also being deprotonated, giving extra reactivity compared to phthalhydrazide and phthaldialdehyde. The different steric arrangement of the anhydride group may lead to greater mothproofing efficacy compared to the results of analogues containing more oxygen atoms such as di-isononyl phthalate and dimethyl phthalate. It is also possible that the anhydride group is hydrated to two carboxylic

acid groups before entering the alkaline, reducing midgut, which could result in two extra deprotonated oxygen groups. These two extra deprotonated oxygen atoms may not form in di-isononyl phthalate and dimethyl phthalate due to the attached alkyl moietys having less affinity for the basic, reducing species present in the gut lumen compared to the hydrogen atoms possibly liberated from the carboxylic acid groups derived from phthalic anhydride.

2.9.5 Alkyl Chain Moiety

Comparing the mass loss results for decanoic acid and 1,10-decanediol, it can be seen that the single acid group is more effective than two hydroxyl groups at either end of the ten-carbon alkyl chain. When compared to the control mass loss data, 1,10-decanediol showed no antifeeding effect. Dodecanedioic acid has a carboxylic acid at each end of the twelve-carbon alkyl chain, but this proved less effective in bioassays compared to dodecanoic acid, containing a single carboxylic acid group. These three observations all corroborate the hypothesis that having a polar head and non-polar tail is beneficial to the mothproofing effect of a compound. The single hydroxyl group of dodecanol was less effective than the single acid group of dodecanoic acid, most likely due to the greater polarity of the carboxylic acid. Alternatively, the carbonyl group of the acid may add some antifeeding effect due to the extra reactivity associated with the deprotonated oxygen atom as hypothesised for 1,2-naphthoquinone-4-sulphonic acid (Section 2.9.2) and phthalic anhydride (Section 2.9.4). The carbonyl group is the only difference between the dodecanoic acid and dodecanol molecules. The carbonyl group adds acidity to the molecule, although acidity alone is unlikely to be the mechanism of action on *Tineola bisselliella* as treating wool with sulphuric acid alone has no mothproofing effect (Freeland & Williams, 1967).

2.9.6 Selection of Compound for Further Study

When selecting a compound for further study, practicality and environmental toxicity were taken into account by assessing the application level required for protection of wool, and the LC_{50} value of these compounds to *Daphnia magna*. These factors are discussed below for the surfactants, naphthalene derivatives, and antimicrobials of highest efficacy. Mosquito repellent analogues and straight chain acids and alcohols investigated were not effective enough to be considered.

The most effective surfactant for protection of wool fabric against *Tineola bisselliella* moth larvae was dodecylbenzene sulphonic acid. Although this work only evaluated a level of 3.0%

omw (Section 2.3.2.1), levels of less than 2.0% omw were required to achieve mass losses below 12 mg in a 14 day test (Freeland & Williams, 1967). The low toxicity to *Daphnia magna* of dodecylbenzene sulphonic acid, assumed from studies of the sodium salt of this compound showing a 48 hour LC_{50} of 5880-6840 µg/L (Maki & Bishop, 1979), and its high affinity for wool make this a very practical compound for mothproofing. For protection from *Anthrenocerus australis* beetle larvae the most effective surfactant found in this study was the zwitterionic surfactant N,N-dimethyldodecyl amine N-oxide (DDAO). Levels of 2-3% omw were required to achieve a mass loss below 12 mg (Section 2.3.2.3). Toxicity data for DDAO shows a 48-96 hour LC_{50} for *Daphnia magna* of 1000-10800 µg/L (Sanderson et al., 2009). This suggests the overall aquatic toxicity for DDAO is similar to that of dodecylbenzene sulphonate.

The most effective naphthalene derivatives for the protection of wool fabric from *Tineola bisselliella* were 1,2-naphthoquinone-4-sulphonic acid (Folin's reagent), and 8-anilino-1-naphthalene sulphonic acid. Less than 3.0% omw of both of these compounds was required for a mass loss of under 12 mg with *Tineola bisselliella* (Section 2.4.2). The only naphthalene derivative tested against *Anthrenocerus australis* was 8-anilino-1-naphthalene sulphonic acid, of which over 3.0% was required on wool fabric to control this species (Section 2.4.3). The bright colour of these compounds rendered both of them impractical for most scenarios. Aquatic toxicity data is unavailable for these naphthalene derivatives, although published data for 1-naphthol shows that the 48 hour LC_{50} for *Daphnia magna* is 730 µg/L (Leonte, 1973), suggesting that the naphthalene derivatives may be slightly more toxic than the surfactants dodecylbenzene sulphonic acid and DDAO.

The most effective antimicrobial for the protection of wool fabric from *Tineola bisselliella* was triclocarban. Around 0.05% omw triclocarban was required for a mass loss of under 12 mg (Section 2.5.2). The 48 hour LC_{50} of triclocarban against *Daphnia magna* is 10-20 µg/L (*High Production Volume (HPV) Chemical Challenge Program Data Availability and Screening Level Assessment for Triclocarban*, 2002), which is a far higher toxicity compared to the surfactants discussed above, and closer to that of permethrin (Stratton & Corke, 1981). The lack of environmental advantage of triclocarban over insecticides meant that this compound was not worthy of further investigation. Propiconazole was the most effective antimicrobial for protection of wool fabric from *Anthrenocerus australis*. Around 0.36% omw propiconazole was required to reduce feeding of the beetle larvae to 12 mg (Section 2.5.2).

Toxicity data for propiconazole shows a 96 hour LC_{50} to *Daphnia magna* of 4800 µg/L (*Reregistration eligibility decision for propiconazole*, 2006), which is a relatively low toxicity compared to permethrin and naphthalene derivatives.

In further comparison of the different classes of compound investigated, it can be concluded that the weight for weight toxicity of dodecylbenzene sulphonic acid to *Dapnia magna* is probably slightly higher than that of propiconazole when considering the dodecylbenzene sulphonate data is from a 48 hour study (Maki & Bishop, 1979), compared to the 96 hour study for propiconazole (*Reregistration eligibility decision for propiconazole*, 2006). When considering the 0.36% omw application rate of propiconazole required for control of *Anthrenocerus australis*, and also that higher levels of dodecylbenzene sulphonic acid were required for control of the similar *Anthrenus flavipes* beetle larvae (Freeland & Williams, 1967), propiconazole potentially has the lowest environmental impact when used as a beetle-proofing agent. Beetle-proofing agents are possibly in greater demand in parts of the world where keratin-digesting beetle larvae are present, due to their higher tolerance of wool insecticides compared to keratin-digesting moth species.

Comparisons of the environmental impacts of each of the three most effective compounds are illustrated below in Table 2.28. Minimum effective application rates (% omw) were multiplied by a unit proportional to the toxicity to *Daphnia magna* ($1/LC_{50}$), to give a unit proportional to the total toxicity of the level of compound applied (% omw/LC_{50}). As the LC_{50} value for propiconazole was for a 96 hour study (*Reregistration eligibility decision for propiconazole*, 2006), this value of 4800 µg/L was doubled to give an approximation of a figure comparable to the 48 hour studies of triclocarban and dodecylbenzene sulphonate. Calculations were made on the toxicity of propiconazole to *Daphnia magna* when used for protecting wool fabric from *Anthrenocerus australis*, and similarly for dodecylbenzene sulphonate against *Tineola bisselliella*, and triclocarban against both species. As the data in the final row of Table 2.28 show, propiconazole has the lowest impact on *Daphnia magna* when used at the level required to control *Anthrenocerus australis*, whereas dodecylbenzene sulphonate has the lowest impact on *Daphnia magna* when used at the level required to control *Tineola bisselliella*. Triclocarban shows a far greater toxicity to *Daphnia magna* than propiconazole or dodecylbenzene sulphonate when used at levels sufficient to protect wool fabric from either of these insect species.

Table 2.28 Relative impact of insect-proofing compounds on *Daphnia magna* at levels required for protection of wool

	Propiconazole	Triclocarban	Dodecylbenzene sulphonate
48 hour LC_{50} *Daphnia magna* (µg/L)	9600[1]	10	5880
Minimum application level for *Tineola bisselliella* (% omw)	-	0.05	2.0
Minimum application level for *Anthrenocerus australis* (% omw)	0.36	0.50	-
Minimum application level/s (ppm omw)	3600	500 - 5000	20000
Relative impact on *Daphnia magna* (ppm omw/LC_{50})	0.38	50 - 500	3.4

[1]Original LC_{50} data for propiconazole was 4800 µg/L for a 96 hour study (*Reregistration eligibility decision for propiconazole*, 2006).

As a comparison to these non-insecticidal compounds, permethrin has a relative impact on *Daphnia magna* of 88 ppm/LC_{50} at the level required for protection from *Tineola bisselliella*, and 453 ppm/LC_{50} for protection from *Anthrenocerus australis.* These figures were derived from the minimum effective rates of permethrin known to be required to protect wool from these insect species (Wools of New Zealand, 2009). It can be concluded that triclocarban offers no worthwhile environmental advantage over permethrin if used for protection of wool, due to a similar impact on *Daphnia magna*.

Propiconazole was chosen as the best compound for further studies, due to the high efficacy against *Anthrenocerus australis* beetle larvae combined with the low environmental impact evident from the low toxicity to *Daphnia magna* compared to other compounds investigated. *Daphnia magna* water flea is a good indicator of toxicity to a wide range of aquatic species, and so toxicity to other species were not considered. Inhibition of the gut processes involved in wool digestion is a desirable mode of action that is likely to have very little effect on *Daphnia magna* due to this species not digesting wool. The low toxicity of propiconazole therefore made this the most likely of all compounds studied to have a specific effect on wool digestion, and was therefore the best candidate for further research. The anti-feeding effect of propiconazole was most pronounced on *Anthrenocerus australis* larvae, and therefore this species was used for the following studies.

Chapter 3

Behavioural Response of *Anthrenocerus australis* Larvae to Propiconazole

3.1 Introduction

In order to understand the possible reasons for reduced *Anthrenocerus australis* larval feeding on wool fabric treated with propiconazole, several tests were conducted to assess the behavioural reponse of *Anthrenocerus australis* to this compound. Repellency testing was carried out to assess whether the *Anthrenocerus australis* larvae were discouraged from being in close proximity to propiconazole, either with or without the presence of wool. Toxicity testing was carried out to assess whether direct contact with propiconazole resulted in any difference in behaviour or feeding compared to non-exposed *Anthrenocerus australis* larvae.

3.2 Methods

3.2.1 Repellency

Null hypothesis 1: Propiconazole has no repellent effect on *Anthrenocerus australis* larvae.

Chemosensory studies were undertaken to determine whether *Anthrenocerus australis* larvae respond to propiconazole when encountered directly (taste/touch) or indirectly (smell). There were three general methods employed. Two of these involved using wool treated with propiconazole. Wool was of interest, not only because it was the focus of the project, but also because it probably has some characteristic that can be sensed by the larvae to attract them to their food source. The first method, using wool fabrics in a petri dish, involved a reversible choice that could be changed following direct contact with the wool, while the second method used wool fibres in an olfactometer where a non-reversible decision was made before coming into direct contact with the wool. The third method used a petri dish with propiconazole applied to half of the surface area, allowing lavae to make a choice between crawling on the clean or treated glass surface.

Null hypotheses were proposed, and defined as (H1) no repelling effect with direct contact (Sections 3.2.1.1 & 3.2.1.3) and (H2) no repelling effect with olfactory experiments (Section 3.2.1.2). If the null hypotheses were both rejected, then a repelling effect against *Anthrenocerus australis* can be inferred to occur via an olfactory mechanism. If neither of the null hypotheses were rejected, then no repelling effect can be inferred. If the null hypothesis for the direct experiments (H1), but not the olfactory experiment (H2) was rejected, then it can be inferred that propiconazole repels *Anthrenocerus australis* via a contact rather than an olfactory mechanism. It is unlikely that the null hypothesis for the olfactory experiment (H2) would be rejected without this also happening for the contact experiments (H1), as olfactory repellency is likely to be as strong in the contact experiments as it is in the olfactory experiments.

3.2.1.1 Petri Dish: Wool Fabric Experiment

Null hypothesis 1a: Direct contact with propiconazole-treated wool fabric does not repel *Anthrenocerus australis* larvae.

Wool fabric details used in this experiment were as described in Section 2.1. Two wool fabric discs were placed in opposite corners of a square ventilated polycarbonate petri dish (100 × 100 × 17 mm) (Figure 3.1). Initial trials involved two untreated wool fabrics to assess whether there was any bias in the experimental setup. When assessing the repellency of propiconazole, one fabric was an untreated control, and the other was treated directly with 0.3% omw propiconazole by direct application of 1.2% omw Pro-P™ formulation. This experimental method was designed to assess the choice made by the larvae when both fabrics were in the same area, and larvae were free to move between the fabrics. This allowed the larvae to use taste and smell to decide which fabric to consume. Ten *Anthrenocerus australis* larvae were placed on each fabric to assess whether movement between fabrics was likely. Trials then involved placing one larva half way between the two fabrics and observing larval movement. Using more than one larva may have led to some larvae being influenced by others, if for example they had a tendency to follow (or avoid) each other. Placing one larva between the fabrics, where there was no food, forced a decision on the larva, as it was unlikely to remain where it was placed. The petri dish was placed in the dark in an incubator at 25°C and 65% RH. The position of the larva was recorded after 24 hours, allowing enough time for a choice to be made. This method was replicated at least 30 times. After each replicate, petri dishes were wiped with tissue paper

soaked with 100% ethanol, to remove any contaminants from the petri dish, and dried. The position of the petri dish within the incubator and the positions of the control and treated fabrics within the petri dish were alternated to avoid any bias in the experiment. The final positions of the larvae were counted, compared and analysed using analysis of variance (ANOVA) at a 95% confidence level.

Figure 3.1 Wool fabric experiment.

3.2.1.2 Y-tube Olfactometer: Wool Fibre Experiment

Null hypothesis 2a: Propiconazole does not repel *Anthrenocerus australis* larvae by means of olfactory cues.

An experiment was carried out using glass tubing (internal diameter 5 mm) with a Y-intersection leading to two 100 ml plastic bottles, each containing 7.0 grams of either control wool or wool treated directly with 3.0% omw propiconazole, from 12.0% omw Pro-P™ formulation (Figure 3.2). This was the maximum mass of wool that could be pushed into the bottles, and a higher treatment level than used for other experiments, which was expected to maximise the chances of an olfactory response. An *Anthrenocerus australis* larva was placed inside the end of the tube, which was then closed with a breathable gauze cap preventing escape of the larva. The apparatus was then placed in a dark incubator at 25°C and 65% RH for 24 hours before the position of the larva was recorded. This experiment

used one larva for each replicate, following the same reasoning given in the petri dish experiment (3.2.1.1). The experiment was replicated 30 times. After each replicate the interior of the Y-tube was wiped with ethanol-soaked yarn to remove any contaminants, and dried. The position of the apparatus within the incubator and the relative positions of the control and treated wool were changed to avoid any experimental bias. The same statistical analysis used in the petri dish experiment (3.2.1.1) was used on the data collected in this experiment.

With this experiment, each larva had the option of moving towards one of the two plastic bottles, which were either empty, or contained control or treated wool. A decision on where to move was made by each larva at the Y-intersection, presumably using their sense of smell. The lack of food source in the tube where the larvae were introduced was expected to provide motivation for the larvae to move toward the bottles. This apparatus made it difficult for the larvae to move from one bottle to the other, as the openings leading into the bottles were small compared to the bottles themselves. Once the larva entered a bottle, it was considered to have made a non-reversible decision. This was confirmed in preliminary trials showing no larvae moving from one bottle to the other. These preliminary trials also showed that the larvae often took a long time to move from the end of the glass tube, which is why 24 hours was chosen as a suitable length of time to leave the Y-tubes in the incubator before recording the position of each larva.

A possible alternative experimental setup was considered in which air would have been passed slowly through the wools and then through the Y intersection to help the larvae to use their sense of smell to make a decision. This general method of flowing air over an insect to prompt a decision was used by Bunchu, Sukontason, Olson, Kurahashi & Sukontason (2008) on the oriental latrine fly *Chrysomya megacephala* Fabricius 1794. This work involved using a conditioned room, with venting of used air into a fumehood. This methodolgy was rejected for the current project, as the incubator available would not have kept pace with the required velocity and volume of conditioned air being removed for use in the apparatus. This lack of air flow possibly made the test less sensitive, and therefore more difficult to detect an olfactory response. In a real life situation there would be no controlled air flow, so in this way the experiment was a better representation of what would happen to propiconazole treated wool products.

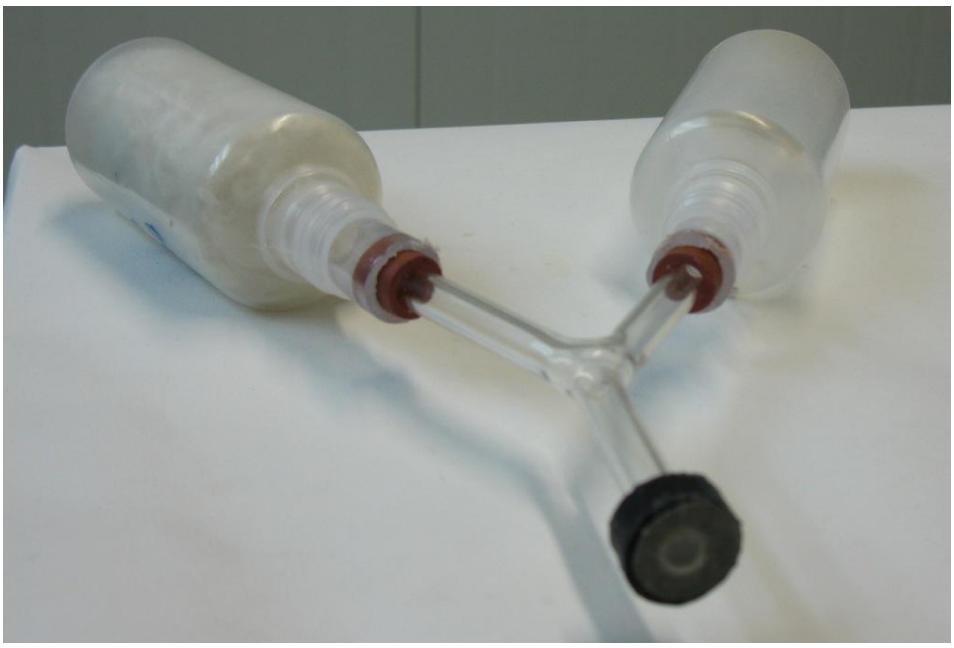


Figure 3.2 Y-tube olfactometer.

3.2.1.3 Treated Surface Application

Null hypothesis 1b: Contact with surfaces treated with propiconazole next to untreated surfaces does not lead to *Anthrenocerus australis* larvae favouring a position on the untreated surface.

This hypothesis was tested in two different ways, using (1) direct application of propiconazole to the surface of a petri dish, and (2) application of propiconazole to filter paper in the base of a petri dish.

Direct application of propiconazole to half of a glass petri dish was used to assess whether the *Anthrenocerus australis* larvae were repelled by the treated glass surface (Figure 3.3). A solution of propiconazole (Pro-P™) was diluted in acetone solvent due to insolubility of propiconazole in water. This formulation was applied to the petri dish using a micropipette. Acetone was not applied to the untreated control half of the dish, as the high volatility of acetone ensured rapid and complete evaporation from the treated side. Intially the concentration of propiconazole was equal to that of standard wool fabric treated at 3.0% omw in terms of mass per unit area. For a fabric of 271 g/m^2 this equated to 8.13 g/m^2 of propiconazole. At this level, and a lower level of 1.36 g/m^2 (equal to 0.50% omw), the larvae had trouble moving over the treated glass surface, becoming stuck in the Pro-P™ residue near the edge of the treated area. When the concentration was reduced to an even lower

level of 0.27 g/m^2 (equal to 0.10% omw), the experiment was successful. One larva was tested at a time in each petri dish by placing each larva on the untreated glass in the middle of the dish near the treated area. After 24 hours in an incubator at 25°C and 65% RH the position of the larva was recorded. No untreated control petri dishes were used in this experiment due to the petri dish and incubator bias already having been well tested in the petri dish wool fabric experiments and Y-tube olfactometer experiments (Sections 3.2.1.1 and 3.2.1.2 respectively). The same replication and statistical analysis of results was used as in the wool fabric experiment in Section 3.2.1.1.

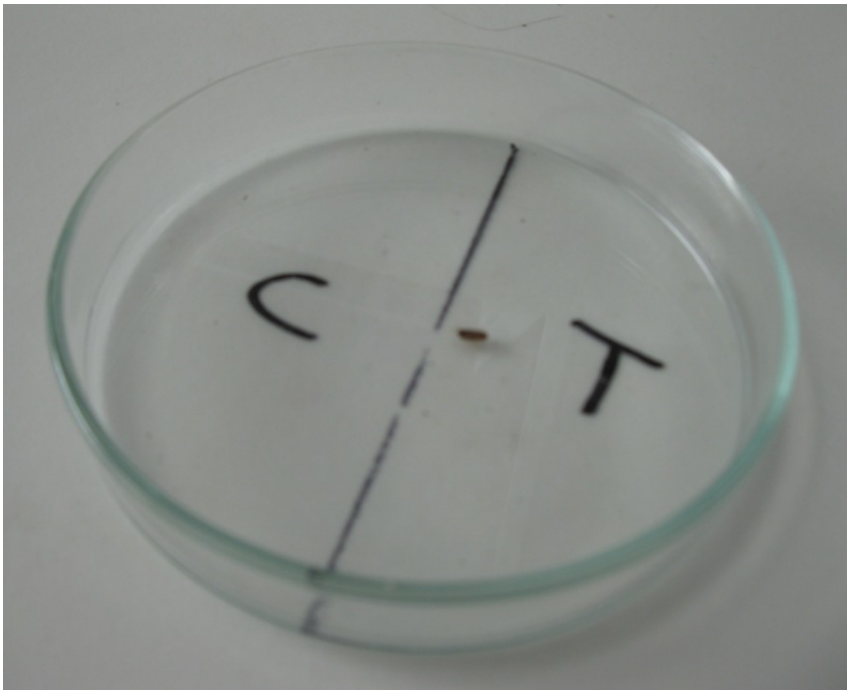


Figure 3.3 Petri dish – half treated with propiconazole.

The surface application experiment was repeated using filter paper (Whatman 452, 90 mm diameter) in the bottom of the petri dish to attain a higher level of propiconazole without the problem of *Anthrenocerus australis* larvae sticking to the glass surface. Initial trials involved untreated filter paper to ensure no directional bias when using the filter paper. Due to the absorbant nature of the filter paper compared to the glass surface previously used, higher levels of 0.60 g/m^2 and 1.36 g/m^2 of propiconazole were applied. In terms of propiconazole per unit area, these concentrations were equivalent to standard wool fabric treated at 0.22% and 0.50% omw, and were used successfully without any restriction of larval movement.

3.2.2 Contact Toxicity

Null hypothesis 3: Propiconazole has no contact toxicity as measured by behaviour, feeding, or mortality after direct application to *Anthrenocerus australis* larvae.

Compounds that specifically target the larval gut are unlikely to have a strong contact effect. This hypothesis was tested using direct application of propiconazole to the *Anthrenocerus australis* larvae. There were two general methods used for the contact toxicity trials. The first involved direct application of propiconazole to *Anthrenocerus australis* larvae, followed by observation of survival and feeding on wool. The second method used wool fabric treated with a moderate level of propiconazole, to encourage enough feeding for any gut effect to take place, followed by feeding on control wool alongside non-exposed larvae to assess recovery and any long-term effects of propiconazole on the larvae.

3.2.2.1 Direct Application of Propiconazole to Anthrenocerus australis *Larvae*

Null hypothesis 3a: Direct application of propiconazole to *Anthrenocerus australis* larvae does not cause symptoms of toxicity.

The toxic effect of direct application of propiconazole to *Anthrenocerus australis* larvae was investigated to determine if propiconazole possesses a general, non-specific toxic mode of action. A series of three concentrations of propiconazole, prepared from Pro-P™ formulation, were each applied directly to the middle of 15 *Anthrenocerus australis* larvae using a micropipette. Propiconazole concentrations were optimised using initial trials to ensure an adequate survival rate for further observation and experimentation. The applications delivered the same dose of propiconazole that each larva would ingest if feeding on wool treated with 0.3% omw propiconazole for 6.7, 13.3 and 20.0 days, based on the amount of feeding observed over these periods of time in the bioassays. This was calculated by using the mean mass loss from a bioassay (Table 2.20, Section 2.5.2), showing that an average *Anthrenocerus australis* larva consumes 75 µg of wool per day if the wool contains 0.3% omw propiconazole. The volume used was 2 µl, as this was the smallest practical volume that could be delivered from a micropipette. Mixtures of water and propan-2-ol (10-50%) were intially used as the solvent for delivery of the propiconazole, as this facilitated easy application due to a lower surface tension compared to water alone. However, initial trials showed that the 50% propan-2-ol increased larval mortality, so the solvent was changed to water alone. Controls included insects to which water, but no propiconazole, was applied (water-only control), and insects to which no water or

propiconazole was applied (no treatment control). It should be noted the no-treatment control larvae underwent slightly less handling with forceps than the treated larvae, although handling with forceps in bioassay testing did not have any noticeable effects on mortality or feeding of *Anthrenocerus australis* larvae as seen in results for untreated contol wool fabrics in Section 2.

Each larva was held with foreceps prior to application of the solution. The 2 µl volume of solution applied was enough to saturate each larva completely. The larvae were left in an open glass petri dish to allow drying of the propiconazole solution. The condition of the larvae, including mortality and response to external stimuli was monitored and recorded in the hour after application, and compared to the controls. The larvae were then placed on untreated wool fabric for 14 days, and a final assessment was made as specified in Wools of New Zealand Test Method 25. The variance of mass loss and mortality was analysed using ANOVA to show whether there were any significant differences between the control and treated groups of larvae within a 95% confidence level.

An additional immersion method was also considered, in which larvae would have been completely immersed in the propiconazole solution. However, after completing the dropper application method it appeared that these two methods were too similar for immersion to be worthwhile, due to complete saturation of the larvae using the dropper method.

3.2.2.2 Recovery of Anthrenocerus australis *Larvae after Ingestion of Propiconazole*

Null hypothesis 3b: Feeding of *Anthrenocerus australis* larvae on propiconazole-treated wool fabric does not cause symptoms of toxicity.

Anthrenocerus australis larvae were selected as previously described (Section 2.2), and 30 were kept in each of four ventilated polycarbonate petri dishes containing wool fabric treated directly with 0.3% omw propiconazole (1.2% omw Pro-P™) in an incubator at 25°C and 65% RH for 14 days. A control group of the same number of larvae were fed untreated wool fabric for the same length of time in the same incubator. The larvae were then used in Wools of New Zealand Test Method 25 on untreated control wool fabrics, using 15 larvae for each of the four replicate fabrics, to ascertain if a full recovery was made. A larger number of larvae were selected for the first stage of the experiment to ensure sufficient numbers of live larvae were available for the Wools of New Zealand test. The same statistical approach

as specified in Section 3.2.2.1 was used to show if any anti-feeding effects were permanent or temporary, in order to draw conclusions on possible modes of action.

3.3 Results of Repellency and Contact Toxicity Experiments

3.3.1 Repellency

3.3.1.1 Petri Dish: Wool Fabric Experiment

This method involved a choice of treated or untreated wool fabric in a petri dish with one larva, repeated multiple times. Results were recorded for beetle larvae, where one of the two fabrics was occupied after 24 hours. In the event of the larvae not occupying either fabric, and remaining on the plastic petri dish, this was recorded as undecided.

The experiment was first carried out with two untreated fabrics in the south-west and north-east corners of the petri dish, and repeated enough times to obtain at least 30 decisions. This was repeated for two untreated wool fabrics in south-east and north-west corners of the petri dish. Results for two untreated wool fabrics are shown below in Table 3.1. There appeared to be no experimental bias due to the incubator, petri dishes or fabrics when observing these results. This was confirmed by Fisher's exact test performed on the proportion of insects that chose the fabric in one area, which showed no significant differences in preference for each area at a confidence level of 95%.

Results using one untreated control fabric and one fabric directly treated with 0.3% propiconazole (1.2% omw Pro-P™) are shown in Table 3.2. Fabrics were alternated between the south-west/north-east and the south-east/north-west positions to obtain a total of at least 30 decisions. There appeared to be no repellency of *Anthrenocerus australis* by wool fabric treated with 0.3% omw propiconazole. Fisher's exact test showed no significant preference for either fabric at a confidence level of 95%.

Table 3.1 ***Anthrenocerus australis*** **beetle larvae positions after 24 hours with two untreated wool fabrics.**

Larva/fabric position	South-east	North-west	Undecided
Number of larvae	16	16	7
Larva/fabric position	South-west	North-east	Undecided
Number of larvae	14	17	5

Table 3.2 *Anthrenocerus australis* beetle larvae positions after 24 hours using untreated wool fabric and 0.3% omw propiconazole treated wool fabric.

Larva choice of fabric	Control	0.3% propiconazole	Undecided
Number of larvae	17	16	7

3.3.1.2 Olfactometer: Wool Fibre Experiment

This method involved single *Anthrenocerus australis* larvae choosing one of two paths that lead to plastic bottles containing either treated, untreated, or no wool. Results were recorded for beetle larvae, where one of the two bottles were inhabited after 24 hours. The beetle larvae sometimes did not move as far as the Y-intersection, therefore they did not make any choice. In this event, the replicate was recorded as undecided and was repeated to gain at least 30 replicates in which a choice was made. Results for two empty bottles are shown below in Table 3.3. Statistical analysis using Fisher's exact test showed that there was no significant preference for either the left or right bottles at a 95% confidence level.

Table 3.3 Y-tube results for *Anthrenocerus australis* larvae with two empty bottles.

Bottle position	Left	Right	Undecided
Number of larvae	19	17	14

Results using one empty bottle and one containing 7.0 g of untreated control wool are shown below in Table 3.4. Fisher's exact test showed no significant preference at a 95% confidence level. It is interesting that there was no preference for the bottle containing wool. This may have been due to the the larvae being unable to sense any difference in smell between the tubes. Alternatively, the larvae may not have been hungry, perhaps due to the change in environment. The significant numbers of undecided larvae not travelling past the Y intersection indicated some reluctance to explore their new environment. This lack of attraction to wool was no hinderance to further studies on the repellency effect of propiconazole, as any effect by wool would only have made interpretation of the propiconazole effect more difficult.

Table 3.4 Y-tube results for *Anthrenocerus australis* larvae with empty bottle and control wool.

Bottle contents	Empty	Control wool	Undecided
Number of larvae	17	14	8

Results using 7.0 g of untreated control wool and 7.0 g of propiconazole-treated wool (3.0% omw) in each bottle are shown below in Table 3.5. Fisher's exact test showed no significant preference for either wool at a confidence level of 95%. Propiconazole appeared to show no repellent effect on the *Anthrenocerus australis* larvae.

Table 3.5 Y-tube results for *Anthrenocerus australis* larvae with control and 3% omw propiconazole-treated wool.

Bottle contents	Control wool	Propiconazole-treated wool	Undecided
Number of larvae	16	16	16

3.3.1.3 Treated Surface Application

Results for the two higher propiconazole levels (8.13 and 1.36 g/m^2) are not shown due to a lack of mobility of the larvae. The propiconazole formulation (Pro-P™) left a sticky residue on the glass surface of the petri dish, resulting in the larvae becoming immobilised. This residue was likely to be a mixture of propiconazole and the proprietary surfactant component. These two components made up 25% and 15-45% of the formulation respectively. The exposed larvae appeared more sluggish than unexposed larvae when nudged with fine tweezers. When removed from the petri dish, most larvae remained sluggish due to the sticky residue on their rear section. It can be said that the Pro-P™ was not repellent enough to stop the larvae from entering the treated area. The results collected using a lower propiconazole level of 0.27 g/m^2 are shown below in Table 3.6. It was clear that the propiconazole formulation had no measureable repellent effect due to the fact that the larvae were exploring the treated area of the petri dish in greater numbers than the untreated area. Fisher's exact test showed significantly (p-value<0.05) higher numbers of larvae on the treated area, which may have been a result of the larvae becoming less mobile as they accumulated more formulation. This was clearly true for the higher levels trialled. If there was any repellent effect, it was small enough to be overcome by the urge of the larvae to explore the petri dish, maybe in search of a food source. The borderline results indicate that the larvae were positioned on the dividing line between treated and control areas.

Table 3.6 Larva position on glass petri dish half treated with 0.27 g/m^2 propiconazole.

Larva position	Control	Treated	Borderline
Number of larvae	9	22	5

Results for this experiment using untreated and half treated filter paper in the bottom of the petri dish are shown below in Tables 3.7 and 3.8 respectively. It was clear from these data that there was no significant (p-value>0.05) bias introduced by the filter paper, and that there was no repellent effect on the *Anthrenocerus australis* larvae from the propiconazole-treated filter paper at either level trialled due to no statistically significant difference in results at a 95% confidence level.

Table 3.7 Larva position on untreated filter paper.

Larva position	North-west	North-east	South-east	South-west	Borderline
Untreated filter paper	7	10	8	7	3

Table 3.8 Larva position on filter paper half treated with 0.60 g/m^2 and 1.36 g/m^2 propiconazole.

Larva position	Control	Propiconazole-treated	Borderline
Number of larvae	16	17 (0.60 g/m^2 propiconazole)	2
Number of larvae	16	15 (1.36 g/m^2 propiconazole)	4

3.3.2 Contact Toxicity

3.3.2.1 Direct Application of Propiconazole to Anthrenocerus australis *Larvae*

Adding 2 µl of distilled water to the *Anthrenocerus australis* larvae resulted in the water beading, with slow wicking into the larvae. Initial experiments showed that 2 µl of 10%, 20%, 30%, and 50% solutions of propan-2-ol were progressively easier to apply, although the solutions did come into contact with the dish, trapping the larvae on the glass at the point of application, until drying occurred. The alcohol content allowed a lower surface tension, reducing beading of the solution above the hydrophobic hairs of *Anthrenocerus australis* larvae. Due to the toxicity of propan-2-ol noted with *Anthrenocerus australis*, distilled water was used as the solvent. After drying, larvae treated with the water-only control appeared to show no mortality and no observable change in mobility or response to external stimulae, such as contact with fine forceps. The same lack of mortality and unchanged mobility/response to external stimulae was also evident in larvae treated with the propiconazole solutions.

Bioassay results for *Anthrenocerus australis* larvae after direct application of propiconazole are shown in Table 3.9. There were no recorded larval mortalities or pupation in this bioassay. There were no statistically significant (p-value>0.05) differences in mass loss between the wool fabrics exposed to treated and untreated larvae, showing that there was a complete recovery made by the larvae after the direct applications of propiconazole and water. This may be an indication that the effect of propiconazole on *Anthrenocerus australis* larvae is not a general one, but rather a gut-specific effect that requires ingestion. An interesting comparison would be to repeat this experiment with permethrin. The low water solubility of permethrin would necessitate using a non-polar solvent, which would be likely to have a toxic effect on the insect larvae, making assessment of the effect of permethrin difficult.

Table 3.9 *Anthrenocerus australis* bioassay results using larvae exposed to direct application of aqueous propiconazole solutions.

Mass of propiconazole (µg)	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control	0.0	0.0	30.9 ± 3.2	n.a.	3B	n.a.
Water only	0.0	0.0	32.5 ± 2.3	105.1	3C	f
1.5 µg + water	0.0	0.0	30.9 ± 2.4	100.1	2C	f
3.0 µg + water	0.0	0.0	31.9 ± 1.3	103.1	2C	f
4.5 µg + water	0.0	0.0	29.1 ± 2.7	94.3	3C	f

[1] As a percentage of the mean voracity control.

The doses of propiconazole applied to *Anthrenocerus australis* larvae have so far been related to the mass of propiconazole consumed due to feeding on wool fabric treated with 0.3% propiconazole. To further quantify the doses of propiconazole applied to *Anthrenocerus australis* larvae, an assumption can be made that the mean mass of larvae used in this experiment was close to 3.6 mg, as was the case for control larvae in Table 4.1 (Section 4.3.1). The three applied amounts of propiconazole shown in Table 3.9 are approximately equivalent to 0.04, 0.08, and 0.13% of the mean mass of each insect larvae. Often lethal dose data (LD_{50}) are given in units of mg/kg body weight. Using these units, the

dermal applications delivered approximately 420, 830, and 1250 mg/kg to the three groups of larvae.

3.3.2.2 Recovery of Anthrenocerus australis *Larvae after Ingestion of Propiconazole*

Results of bioassays using larvae that had previously fed on either untreated wool fabric or wool fabric treated with 0.3% propiconazole showed that the larvae recovered well from exposure to the treated wool (Table 3.10). Not only did these larvae recover, they also consumed significantly (p-value<0.05) more wool than the larvae that had only been exposed to control wool. This effect may have been caused by the lower rate of feeding during the exposure period of the larvae exposed to treated wool, leading to a greater desire for feeding afterwards to compensate for this. It is worth noting the larvae were not slow to feed on the new control wool fabric when it replaced the less edible treated fabric. Despite the ability of these beetle larvae to survive for up to 230 days without feeding (Lamb, 1952) they determined the edibility of the new wool fabric and had consumed around twice the mass of wool than the control group after 14 days.

Table 3.10 Bioassay results of *Anthrenocerus australis* previously fed propiconazole treated wool fabric.

	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or borderline (b)
Control larvae	3.5	8.6	19.0 ± 4.0	n.a.	3B	n.a.
Treated larvae	0.0	3.3	40.6 ± 1.2	213.9	4C	f

[1] As a percentage of the mean voracity control.

3.4 Conclusions from Repellency and Contact Toxicity Experiments

Based on the results from insect repellency and contact toxicity experiments, none of the null hypotheses were rejected. Direct contact with treated wool, glass or filter paper surfaces did not repel the *Anthrenocerus australis* larvae. No repellency was detected using olfactory cues. Direct contact with propiconazole solution or propiconazole-treated wool resulted in no symptoms of toxicity.

The main conclusion inferred from these results is that the effect of propiconazole on *Anthrenocerus australis* larvae appears to be gut-specific, as no toxic effect is evident other than lower feeding rates on propiconazole-treated wool. Even when propiconazole-treated wool is ingested, the larvae are capable of recovering to consume a greater mass of untreated wool to compensate for lower feeding rates while exposed to treated wool. This was not a toxic effect, but rather an increase in feeding voracity due to a previous period with only a contaminated food source available. The gut-specific anti-feeding effect appears to impart no lasting toxic effect on the *Anthrenocerus australis* larvae, allowing the mode of action of propiconazole to be defined as anti-feeding rather than as a general poisoning.

The anti-feeding mode of action may be seen as an advantage in marketing an insect-proofing agent for wool, where the active compound has no contact effect, and is only effective when ingested. This way the product cannot accurately be called insecticidal, as even though the larvae may eventually die from starvation, the mortality is not a direct result of propiconazole poisoning of the insect larvae. A negative aspect of this mode of action could be the wool-digesting insect larvae moving to other wool products in the same area, causing damage to untreated items.

Chapter 4

Effect of Propiconazole on *Anthrenocerus australis* Gut Enzymes

4.1 Introduction

Null hypothesis 3: Ingestion of propiconazole treated wool by *Anthrenocerus australis* does not inhibit gut enzymes associated with wool digestion.

This hypothesis was tested by measuring gut enzyme activities in *Anthrenocerus australis* larvae that had consumed wool treated with propiconazole and comparing these to gut enzyme activities derived from larvae that had consumed untreated control wool for the same length of time. The lack of repellency of propiconazole with *Anthrenocerus australis* larvae as seen in Section 3.3 indicates the anti-feeding effect of propiconazole is most likely to occur as a result of ingestion of the propiconazole treated wool. The ingested propiconazole may be causing a general toxic effect within the insect larvae, or more specifically affecting the enzymes involved in wool digestion. As the enzymes involved in wool digestion by *Anthrenocerus australis* have been previously documented (Christeller et al., 1994), a further study involving propiconazole may lead to greater understanding of the mode of action of this compound in the beetle larva gut.

4.2 Methodology

4.2.1 Insect Collection and Handling

Anthrenocerus australis larvae were selected for this experiment at an age that ensured a sufficient size to minimise the difficulties associated with dissecting small insect larvae and measuring small quantities of gut enzymes. The larvae selection process specified in Section 2.2 was used, although the biggest larvae within this group were not used due to the higher probability of pupation, which would not have allowed the gut to be dissected out. The larvae were fed either untreated control wool, or wool treated with propiconazole 0.3% omw (from direct Pro-P™ application to fabric). Larvae were allowed to feed on these wools for 14 days at 25°C and 65%RH to allow changes in gut enzyme production to occur, but without enough time for this to lead to larval mortality (enzyme activity stops soon after death if the larval gut is not stored in a freezer). This level of propiconazole (0.3% omw) had

been observed in bioassays (Table 2.20, Section 2.5.2) to give a low level of mortality, allowing sufficient numbers of live larvae for the gut enzyme assay. The mass of wool consumed by groups of larvae over 14 days was recorded. Ten groups of 20-22 larvae (five control and five treated groups) were staggered over ten days to allow dissections to take place at the end of each 14 day period. This number of larvae was used in each group to allow for pupation, mortality, and dissection errors while aiming for 10 dissected guts. Initially 20 larvae were used, but this was increased to 22 to give a slightly larger safety margin for accidental gut rupturing during dissection, and greater variety of larvae to choose for dissection.

4.2.2 Enzyme Assays

Gut enzyme assays were first carried out with practice runs looking for trypsin-like activity using N-benzoyl-DL-arginine-*p*-nitroanilide (BApNA) substrate and beetle guts from practice dissections. Trypsin was trialled alongside the beetle gut extracts for comparison. Variation (background noise) in absorbance at 405 nm was a problem that was not solved using a different brand of plate, or by increasing the reaction volume in each well from 105 µl to 210 µl. A general upward trend in absorbance was noticed for the gut extract and trypsin, whereas the blank without enzyme showed very little absorbance as would be expected with slow autohydrolysis. Trypsin present in reaction wells at 0.005 mg resulted in less absorbance than wells containing the gut extract, whereas 0.05 mg trypsin gave greater absorbance. The high variation in absorbances made it impossible to gauge where the enzyme activity was slowing down. This information was necessary, as the initial reaction rate is the best indicator of maximum enzyme activity.

The plate reader was fixed by BMG Laboratories, and the assay method repeated. During this second phase of trials, it was discovered that 105 µl in each reaction well was an insufficient volume to achieve stable absorbance readings. Using 200 µl per reaction well gave stable absorbance readings that could be used to determine maximum reaction rates.

Five untreated control and five propiconazole treated (0.3% omw) wool fabrics were used to support groups of *Anthrenocerus australis* larvae for 14 days. Larvae were weighed before the guts were dissected out with the aid of a light microscope, forceps, and scalpel. Each larva was cooled with ice to reduce movement, then held in a back-down, legs-up position with forceps while the head was removed using the scalpel. The body was carefully opened

by tearing between two pairs of forceps to remove unwanted material. Drying of the gut during dissection (and subsequent enzyme deactivation) was avoided by occasional immersion in 5mM tris(hydroxymethyl)aminomethane (Tris)-HCl buffer at pH 8.0. The midguts were given a brief final rinse in this buffer and stored at -25°C for six months before analysis.

From each group of larvae, ten dissections were carried out, placing five dissected guts in each of two separate eppendorf tubes. Crude extracts were prepared by grinding 5 thawed midguts in 200 µl of extraction buffer (5mM Tris-HCl, pH 8.0, 0.1% Brij-35, 1 mM dithiothreitol) with cooling on ice. Ground samples were centrifuged at 10,000 *g* for 5 minutes, after which the supernatant was transferred to a fresh tube for use in further experiments.

Each sub-group of five guts was sub-sampled by running in duplicate wells on the same serological 96-well flat bottom plate (Jet Biofil® SLP-000-096), giving four reaction wells for each of the ten groups of larvae. Guts from larvae fed control and treated wool were assayed in each of the five rows within the plate, resulting in the use of eight wells per row. Each plate run also included a sixth row of eight blanks containing the same reagents, with the exception of using extraction buffer without gut extract. Three identically prepared plates were run consecutively on the same day for each substrate.

Assay conditions followed those previously published by Christeller et al. (1994). Protease and esterase activities were assessed at 30°C using a FLUOstar Omega (BMG Laboratories) microplate reader, with absorbance filters set at 405 nm with changes in absorbance recorded a total of 20 times over 18 minutes and 22 seconds. Reactions were initiated by the addition of 100 µL of a substrate solution (5 µl substrate stock plus 95 µl 0.1 M Tris-HCl, pH 8.0) to 100 µL of an enzyme solution (5 µl gut extract added to 95 µl 0.1 M Tris-HCl, pH 8.0). Hydrolysis rates were measured for the following enzyme and substrate pairs: trypsin-like activity with 0.50 mM N-benzoyl-DL-arginine-*p*-nitroanilide (BApNA, Sigma), chymotrypsin-like activity with 0.09 mM N-succinyl-alanine-alanine-proline-leucine-*p*-nitroanilide (SAAPLpNA, Sigma), chymotrypsin-like activity with 0.50 mM N-succinyl-alanine-alanine-proline-phenylalanine-*p*-nitroanilide (SAAPPpNA, Sigma), aminopeptidase activity with 0.50 mM L-leucine-*p*-nitroanilide (LpNA, Sigma), and esterase activity with 0.50 mM *p*-nitrophenyl butyrate (pNP butyrate, Sigma). The lower concentration of SAAPLpNA was used due to a limited supply of this relatively expensive substrate. SAAPPpNA was chosen as an alternative

substrate with which to detect chymotrypsin-like enzyme activity. Substrate stock solutions were prepared in N,N-dimethylformamide for *p*-nitroanilide substrates, and methanol for the *p*-nitrophenyl butyrate substrate.

All optical density (OD) data at 405 nm were exported from the FLUOstar Omega to Microsoft Office Excel 2007, and then to a statistical software package SAS version 9.1. All individual rates were first corrected for substrate autohydrolysis by subtracting the rates of the corresponding blank well containing no gut extract. All duplicate reaction rates were checked to ensure they were within 10% of each other. Comparison of mean enzyme activities between larvae fed treated and control wool were then carried out with SAS version 9.1, using a multilevel mixed modelling approach (MMM). Estimated enzyme activity rates were the average of 30 readings taken from two sub-groups from each of five groups repeated on three separate assay plates.

This MMM approach took account of two levels of correlation: (1) correlation between the two sub-samples from the same assay and (2) correlation between the repeated OD readings of each individual well, and modelled these correlations as nested random effects. Because of the requirement to estimate and compare the reaction rates (slopes of the OD reading over time) between the treated and control assay groups, these nested random effects were decomposed into nested random slope effects and nested random intercept effects (i.e. nested random coefficient effects, because both slope and intercept are coefficients in a linear equation). By including these random coefficient effects in analysis to account for the correlations, the MMM approach allowed the estimation of mean reaction rates of the treated and control groups and the most accurate comparison (i.e. with the greatest statistical power). “Correlation between the two subsamples from the same assay” means that the OD readings of two sub-samples from the same assay were expected to be more related (or similar) to each other than the OD readings of sub-samples from another assay. Similarly, “correlation between the repeated OD readings of each individual well” means that the OD reading of one well at a given time is expected to be more related (or similar) to the OD reading of the same well at another time than the OD reading of another well at another time. If these correlations are not negligible, taking account of these correlations leads to smaller error for comparison, and hence, statistically more accurate comparison of the treated and control groups than approaches ignoring the correlations. For all five substrates, the MMM approach found that the above correlations were substantial, as

expected. Therefore, using the MMM approach enabled the most statistically accurate comparison of the treated and control groups.

The conversion of the estimated mean reaction rates of the treated and control groups was calculated using calibration lines obtained from the OD readings of *p*-nitroaniline and *p*-nitrophenol butyrate standards at different concentrations. These standards were run under the same conditions as the test wells. The OD readings of the standards at each concentration were replicated 10 times, including a no concentration level (i.e. concentration = 0). The OD readings of non-zero concentrations were then adjusted by subtracting the OD readings of the zero concentration. The calibration line was then obtained by linear regression:

$$\text{Adjusted OD} = b_1 \times \text{Concentration} + b_0$$

Because the goal was to convert each estimated mean reaction rate (OD/minute) into concentration using the above calibration line, this analysis used an inverse regression method (Lavagnini & Magno, 2007; Massart, Vandeginste, Morgan, Michotte, & Kaufman, 1988), in which the estimated (i.e. converted) concentration *C* is:

$$C = \bar{x} + \frac{1}{b_1}(\text{mean reaction rate} - \bar{y}) = \frac{\text{mean reaction rate}}{b_1} + \bar{x} - \frac{\bar{y}}{b_1}$$

where $\bar{x}$ is the average of all non-zero concentrations of the standards, and $\bar{y}$ is the average of adjusted ODs of all the non-zero concentrations of the standards, with the standard error (SE) of:

$$\text{SE} = \frac{1}{b_1}\sqrt{MSE \times \left(\frac{1}{m} + \frac{1}{n} + \frac{(C-\bar{x})^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}\right)}$$

where *MSE* is the mean square of error of the calibration line (= residual sum of squares / residual degrees of freedom), *m* is the number of replications in the calibration line, *n* is the total number of observations (i.e. adjusted OD readings) in the calibration line, and x_i is the concentration of the *i* th observation.

Conversion of the rate of enzyme reaction from the unit of μM pNA/minute to μM pNA/minute/mg insect, was used to correct for the different mean masses of control and propiconazole-exposed *Anthrenocerus australis* larvae. This was calculated by first dividing the mean enzyme reaction rates and standard errors by the mean mass of larvae used in each reaction well of the gut assays. Although only the insect gut was used in the reaction, the mass of the whole larvae was used in these calculations as it was easier to measure. In this study, the guts of five larvae were ground into 300 μl, and 5 μl of this was used in each well. Therefore the fraction of 5/300 multiplied by five larvae gives $1/12^{th}$ of the mean larval mass used per well. Further calculations were made to convert the concentration "μM" to a number of moles for direct comparison to the work of Christeller et al. (1994). This required the reaction rates and standard errors to be multiplied by 0.0002, as the reaction wells used a volume of 200 μl, or 0.0002 litres. A final step was required to convert micromoles (μm) to nanomoles (nm), achieved by multiplying by 1000.

4.3 Results of *Anthrenocerus australis* Gut Enzyme Assays

4.3.1 Wool Consumption by *Anthrenocerus australis* Used in Enzyme Bioassays

The mass loss of wool fabrics exposed to *Anthrenocerus australis* larvae over 14 days is shown in Table 4.1. The mass of wool consumed per larva was not assumed to be precise, as pupation and death of some larvae occurred at an unknown time during the 14 day feeding period. As shown in Table 4.1 the mean mass of wool consumed per larva in the control samples was approximately seven times as much as for the treated wool fabrics. The mean masses of control *Anthrenocerus australis* larvae of 3.58 mg and propiconazole-exposed larvae of 3.30 mg immediately prior to dissection were similar to the mean mass of 3.57 mg recorded by Christeller et al. (1994).

Table 4.1 Mass of wool consumed by *Anthrenocerus australis* larvae in 14 days before dissection.

Fabric/group	Mass of wool consumed (mg)	Number of larvae	Mean mass of wool consumed per larva (mg/insect)	Mean mass of ten larvae dissected (mg)
Control 1	48.7	20	2.4	3.3
Control 2	43.0	21	2.0	3.9
Control 3	58.7	22	2.7	3.1
Control 4	37.5	22	1.7	3.7
Control 5	49.5	22	2.3	3.9
Treated 1	8.5	21	0.4	3.1
Treated 2	3.9	21	0.2	2.9
Treated 3	4.7	22	0.2	3.4
Treated 4	7.4	22	0.3	3.5
Treated 5	11.1	22	0.5	3.6

Absorbance data from the reactions with five different substrates can be seen in Appendix C. Absorbance data from pNA and pNP standards of different concentrations can be found in Appendix D. Calculation of enzyme reaction rates based on the data for each substrate are presented in the following sections.

4.3.2 *Anthrenocerus australis* Gut Enzyme Activities

The second column of Table 4.2 below shows mean reaction rates (mean slopes of corrected OD over time, OD/minute) of the propiconazole-treated and control groups, estimated from the MMM approach, together with the associated standard error (i.e. standard error (SE) of the mean reaction rate). The MMM approach detected that the difference between the two means for BApNA, SAAPPpNA, SAAPLpNA, and LpNA substrates was statistically significant at the 95% confidence level (p-values < 0.05). Therefore, it can be concluded that with these four substrates the mean reaction rate of the propiconazole-treated group was statistically significantly lower than the mean reaction rate of the control group. The MMM approach detected that the difference between the two means for pNP butyrate was not significantly

different at the 95% confidence level (p-value > 0.05). The p-values for all substrates are shown in the third column of Table 4.2.

The calibration lines for pNA and pNP standards were used to convert OD/minute values to μM pNA/minute using the inverse regression method described in Section 4.2.2. These values, along with the associated standard error, are displayed in the fourth column of Table 4.2. Conversion of these mean reaction rates to nanomoles pNA/minute/mg insect were carried out using the mean larval mass of 3.58 mg for larvae fed control wool and 3.30 mg for those larvae fed propiconazole-treated wool. These values are displayed in the final column of Table 4.2.

Table 4.2. Gut enzyme reaction rates with five substrates, estimated from the multilevel mixed-effect modelling approach.

Fabric/substrate	Mean ± SE (OD/minute)	Significance of OD/minute (p-value)	Mean ± SE (μM pNA /minute)	Mean ± SE (nmoles pNA/minute /mg insect)
Control/BApNA	0.0052 ± 0.0009		0.51 ± 0.45	0.34 ± 0.30
Propiconazole treated/BApNA	0.0022 ± 0.0009	0.022	0.18 ± 0.45	0.13 ± 0.33
Control/SAAPPpNA	0.0903 ± 0.0138		9.87 ± 0.45	6.61 ± 0.30
Propiconazole treated/SAAPPpNA	0.0375 ± 0.0138	0.007	4.07 ± 0.45	2.96 ± 0.33
Control/SAAPLpNA	0.0113 ± 0.0020		1.18 ± 0.45	0.79 ± 0.30
Propiconazole treated/SAAPLpNA	0.0044 ± 0.0020	0.013	0.43 ± 0.45	0.31 ± 0.33
Control/LpNA	0.0053 ± 0.0007		0.52 ± 0.45	0.35 ± 0.30
Propiconazole treated/LpNA	0.0027 ± 0.0007	0.006	0.24 ± 0.45	0.17 ± 0.33
Control/pNP butyrate	0.0227 ± 0.0021		2.95 ± 1.09	1.98 ± 0.73
Propiconazole treated/pNP butyrate	0.0178 ± 0.0021	0.106[1]	2.52 ± 1.09	1.83 ± 0.79

[1] No significant difference at 95% confidence.

The larvae used by Christeller et al. (1994) for their study of *Anthrenocerus australis* were grown on a diet of 1 part fishmeal: 1 part ground oats: 1 part yeast, and were not exposed to wool or any compound intended to alter gut enzyme activity. The *Anthrenocerus australis* larvae in this work were grown on the same diet with the exception of a small addition of untreated wool as specified in Section 2.2, followed by 14 days of exclusive feeding on either control or treated wool. Christeller et al. used BApNA, SAAPLpNA, and LpNA substrates in their study of *Anthrenocerus australis* gut enzymes. They showed a much higher trypsin-like activity of 6.40 $\pm$ 2.30 nmoles pNA/minute/mg insect, a slightly higher SAAPLpNA-hydrolysing activity of 1.26 $\pm$ 0.21 nmoles pNA/minute/mg insect and a slightly higher aminopeptidase-like activity of 0.88 $\pm$ 0.44 nmoles pNA/minute/mg insect in the gut of *Anthrenocerus australis*. Christeller et al. (1994) did not measure SAAPPpNA hydrolysing activity or esterase activity.

4.4 Conclusions from *Anthrenocerus australis* Gut Enzyme Assays

Of the five substrates trialled with *Anthrenocerus australis* gut extracts, four showed a significant reduction in enzyme activity when the larvae were fed on propiconazole-treated wool, compared to untreated control wool. Three of these substrates, BApNA, SAAPLpNA, and LpNA had previously shown corresponding enzyme activities (trypsin, chymotrypsin, and aminopeptidase respectively) important for wool digestion (Christeller et al., 1994). SAAPPpNA substrate also confirmed a strong chymotrypsin-like activity in the extracts. The substrate showing no significant change in enzyme activity, pNP-butyrate, had not previously been noted as important for wool digestion, and so served as a putitative control to ensure the changes in other enzyme activities were not detected in error.

Looking at the magnitude of reduction in enzyme activities corrected for the mean mass of insect larvae as shown in the final column of Table 4.2, it can be seen that excluding pNP-butyrate, enzyme activities in the larvae consuming propiconazole-treated wool were in the approximate range of 39-50% of the activities for larvae consuming untreated control wool. Although the decreases in enzyme activity were statistically significant (p-value < 0.05), this level of decrease cannot confidently be concluded to be a result of enzyme inhibition. A general decline in insect health or gut enzyme activity of the larvae due to decreased feeding could be the cause of lowered enzyme activity. As seen in Table 4.1, when the mass of the larvae was taken into account, the rate of feeding for larvae fed propiconazole-treated wool was around seven times lower than that of control wool. Although these larvae can survive

starvation for up to six weeks with very low mortality (Gerard & Ruf, 1997), it is reasonable to expect a reduction in some gut enzyme activities with a large reduction in feeding.

The lack of significant reduction in esterase activity, as shown with the pNP-butyrate substrate indicates this activity is not affected by the level of wool digestion. To some extent this lack of reduction also shows the general health of the *Anthrenocerus australis* larvae has not declined so far as to affect production of enzymes unrelated to wool digestion. This suggests that the lowered enzyme activity shown with the substrates associated with wool-digesting activity is more likely to be a direct result of the lowered rate of feeding, rather than a general decline in insect health.

The slightly higher chymotrypsin and aminopeptidase enzyme activities found by Christeller et al. (1994) with SAAPLpNA and LpNA substrates are less than two and three times higher respectively than found in this work. The higher rate for SAAPLpNA may be explained by the higher concentration used by Christeller et al. (0.5 mM) compared to this study (0.09 mM), although LpNA concentrations were identical. These higher activities may also be the result of faster dissections or preparation of gut extract for reactions, leading to less deactivation of enzyme. Healthier insect larvae may also lead to higher enzyme activities. Bioassay results show varible feeding rates on control wool (Section 2), which may correspond to variable enzyme activities over time within the same insect colony. The variation between different insect colonies could be even larger. The higher trypsin activity found by Christeller et al. in *Anthrenocerus australis* larvae with BApNA substrate is more difficult to explain due to the approximate 19-fold difference. Christeller et al. used 1.0 mM BApNA compared to 0.5 mM BApNA for this study, which can only partially explain the difference in activity. This does not present a problem for the results given here, as significant differences in trypsin-like activity were found between *Anthrenocerus australis* larvae fed control and treated wool, with a similar magnitude to other enzyme activity differences.

Chapter 5

Gut Morphology of *Anthrenocerus australis*

5.1 Introduction

The anti-feeding effect of propiconazole on *Anthrenocerus australis* has been shown to be related to ingestion of treated wool, without any obvious physical effect on the larvae other than moderately reduced activity of wool-digestion related enzymes (Section 4). This apparent gut-specificity without obvious wool-digesting enzyme inhibition may indicate there is some effect on the cells in the gut region of *Anthrenocerus australis*. A null hypothesis was proposed on this theory.

Null hypothesis 4: Ingestion of propoconazole treated wool by *Anthrenocerus australis* does not visibly alter gut morphology.

The antifeeding effect of propiconazole may be due to a cytotoxic effect on the cells of the digestive tract. This hypothesis was tested by visual comparision of the microscopic structure of *Anthrenocerus australis* midgut sections from larvae that had consumed either propiconazole-treated or untreated wool. Histological techniques involving insect tissue sectioning, staining and transmitted light microscopy were used to highlight features important for comparison.

5.2 Methodology for Assessment of Gut Morphology

Healthy larvae of *Anthrenocerus australis* were fed either untreated control wool fabric, or wool fabric treated with 0.3% omw propiconazole (1.2% omw Pro-P™). The larvae were allowed to feed on the wool for 14 days in a conditioned atmosphere at 25°C and 65%RH. As in previous gut enzyme bioassays, the length of feeding time and concentration of the compound were selected in order to give enough live larvae to study. After the feeding period, six larvae that had been fed control wool and six that had been fed treated wool were prepared for light microscopy by removing their heads with a scalpel, and then using two pairs of forceps to tear small openings along both sides of the thorax, allowing fast penetration of fixative through the hydrophobic cuticle. The larvae were divided into groups of three and immersed in 1 ml of a fixative solution in Eppendorf tubes overnight (5%

formaldehyde, 2.5% acetic acid, 50% ethanol, 2% DMSO). Each group of three larvae was then positioned in one of four cassettes, which were also immersed in the fixative solution. Each cassette was embedded in a paraffin block. Longitudinal tissue sections of 3 μm thickness were cut from the block, along the middle of the thorax, in a horizontal orientation as seen from the usual resting position of the larvae on a flat surface (Figure 5.1).

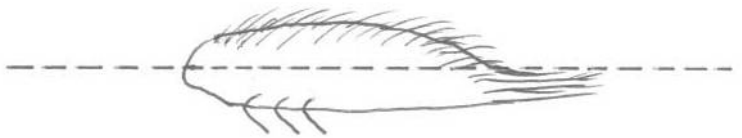


Figure 5.1. Orientation of tissue sectioning on *Anthrenocerus australis* larvae.

The sections were mounted onto glass slides and stained with haematoxylin and eosin. For each of the four blocks, three slides were prepared, each containing three tissue sections of the three insect larvae. The gross anatomy of the digestive tract was examined using a Zeiss (West Germany) microscope with Plan 40 lens, illuminated with bright field, and fitted with an Olympus DP70 digital camera. Images were recorded using the digital camera connected to a computer running Olympus DP controller version 1.2.1.108 and Olympus DP manager version 1.2.1.107 software. Evidence of cytotoxicity on the cells of the digestive tract, or gross morphological changes, were sought after in order to draw conclusions about the mechanism of action of propiconazole. This procedure was repeated three more times, examining a total of 24 larvae fed control wool, and 24 larvae fed treated wool. A total of nine tissue sections were obtained for each larva, with a total of 432 sections viewed over 48 slides.

5.3 Results of Gut Morphology Assessment

Histological photographs of *Anthrenocerus australis* gut sections were variable in quality, with only nine larvae from control wool and twelve larvae from treated wool showing observable features from the gut wall. Of the guts observed, most contained artifacts such as dislocated objects, uneven focus, or cracking of the gut wall, making these sections unsuitable for publication.

Larval gut contents derived from feeding on both control and treated wool (Figures 5.2, 5.3, 5.4, and 5.5) showed a peritrophic membrane around the food bolus, separating the ingested food from the midgut wall. In most sections the peritrophic membrane was ruptured, and gut contents were spread randomly within the gut lumen. The peritrophic membrane is a common feature of insect midguts (Lehane, 1997).

The midgut wall consists of a simple epithelium, composed mostly of simple columnar cells. Columnar cells were typically 25 µm long in the midgut region, although they appeared to be slightly longer in the anterior and posterior sections of the midgut. Columnar cells stained a purple colour. Microvilli approximately 5-12 µm long were clearly visible at the tips of the columnar cells, forming a striated border with the gut lumen. They also appeared longer in the anterior and posterior regions of the midgut and stained a pale shade of red or pink. The base of the midgut wall was composed of a base layer of cells 3-5 µm in length and 1-2 µm wide, aligned in the direction of the gut wall as best seen in Figure 5.4. These cells were evenly spaced, and in most sections stained a red colour. The base of the midgut wall appeared to be attached to the lamina propria, which stained a brighter red than the base of the midgut wall in all sections.

Although columnar cells are the major constituent of the midgut wall, there also appeared to be a small number of shorter, rounder cells present near the base layer most obviously seen in Figures 5.4 and 5.5. These cells stained a darker shade of purple than the surrounding columnar cells. These are most likely to be nidi of regenerative cells, as mentioned in a study of the three dermestid larvae *Anthrenocerus australis*, *Anthrenus verbasci*, and *Attagenus piceus* (Waterhouse, 1952a).

In some images, cytoplasmic spheres were seen between the midgut wall and gut contents of larvae fed both control (Figure 5.6) and treated wool, and although not common there appeared to be slightly more spheres in larvae fed control wool. These spheres were 5-10 µm in diameter, and were more commonly oval in shape when still attached to the columnar cells. As seen to the left in Figure 5.6, a short stem-like structure connected the cytoplasmic spheres to the columnar cells, until release of the spheres into the gut occurred. These cytoplasmic spheres were also observed in the midgut of the wool digesting larvae of *Hofmannophila pseudospretella* (Gerard, 2002), although they were bigger at 60-200 µm in diameter.

No clear differences in the gut wall were evident between larvae fed control or propiconazole-treated wool fabric. Figures 5.2-5.6 below show selected results from the observed tissue sections, and are displayed at 650 × magnification.

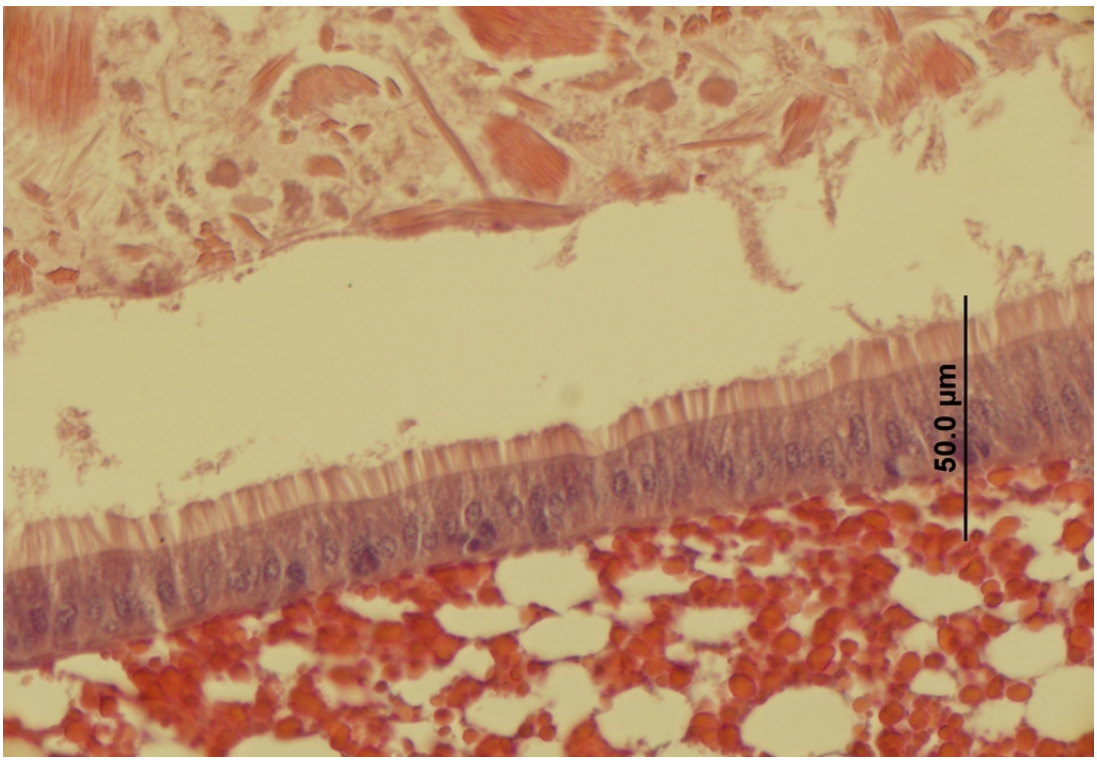


Figure 5.2 Midgut wall section of *Anthrenocerus australis* larva fed untreated control wool.

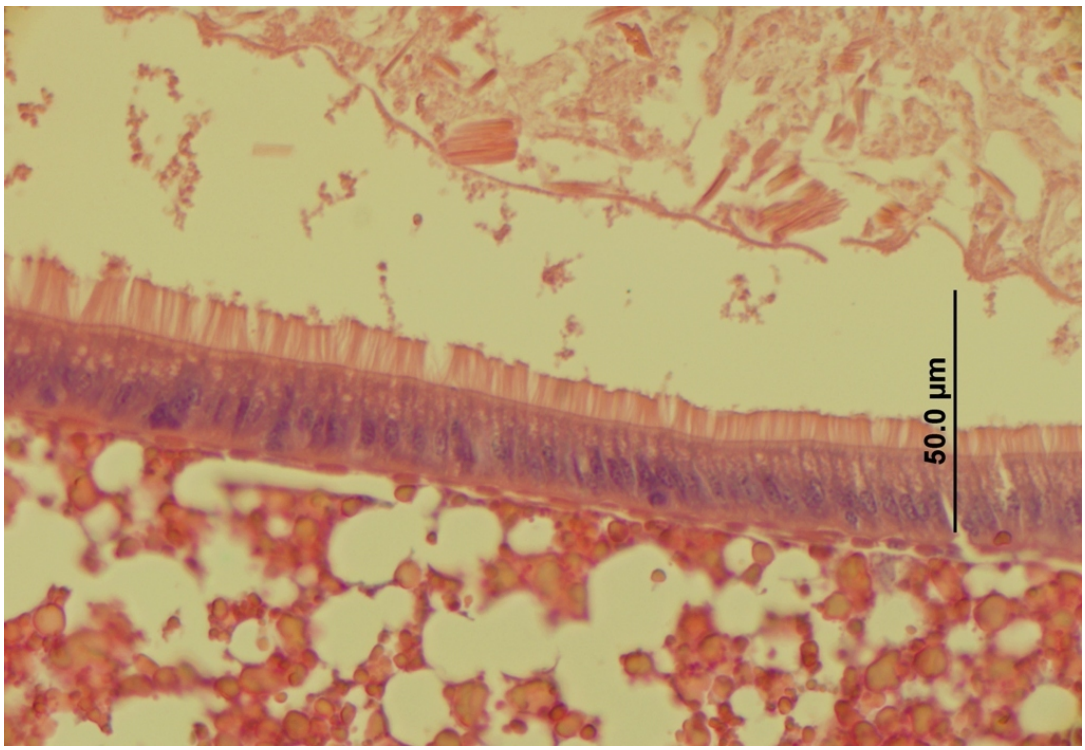


Figure 5.3 **Midgut wall section of *Anthrenocerus australis* larva fed propiconazole-treated wool.**

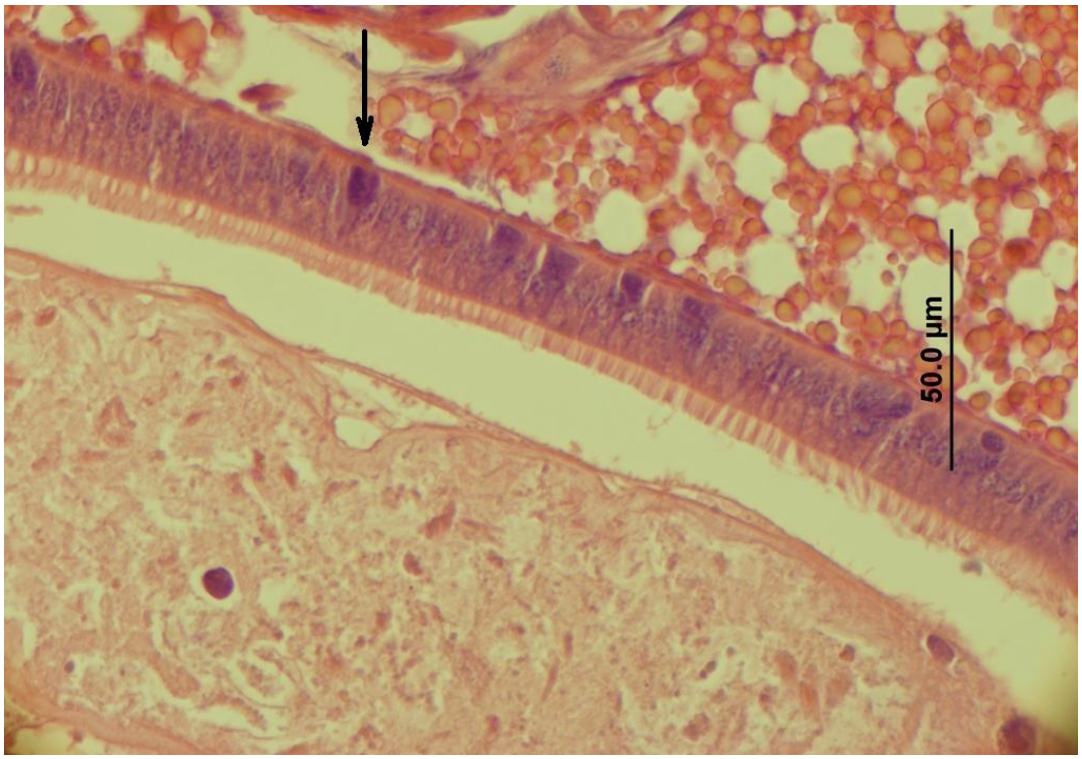


Figure 5.4 **Midgut wall section of *Anthrenocerus australis* larvae fed propiconazole-treated wool, with arrow highlighting one of the regenerative, basally-located cells.**

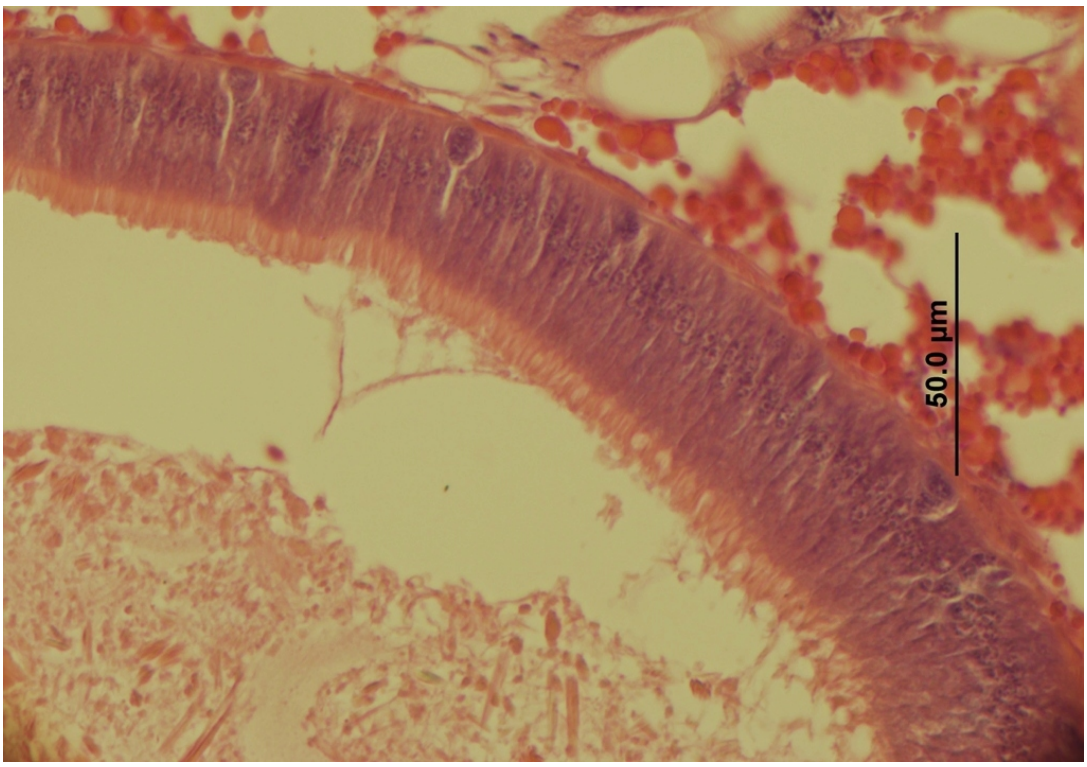


Figure 5.5 **Posterior midgut wall section of *Anthrenocerus australis* larvae fed control wool.**

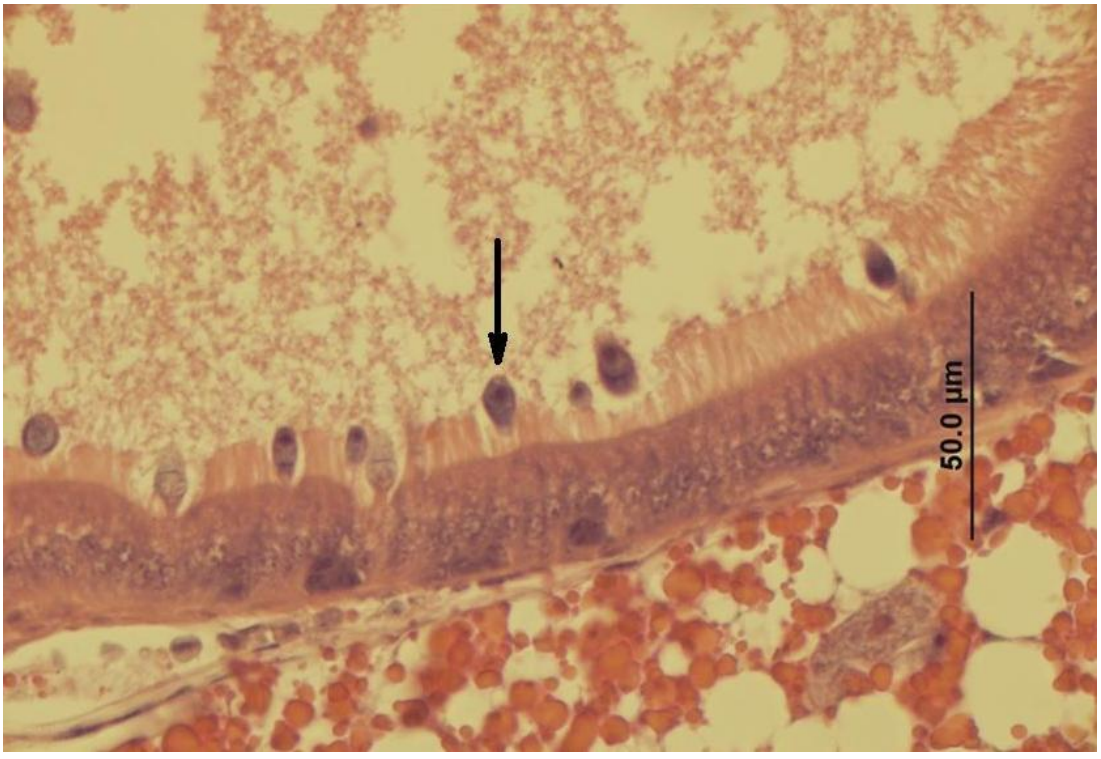


Figure 5.6 **Midgut wall section of *Anthrenocerus australis* larvae fed control wool, with arrow highlightling one of several cytoplasmic spheres amongst the microvilli.**

5.4 Conclusions from Gut Morphology Assessment

The observed features in gut sections from larvae fed either untreated control or propiconazole-treated wool fabric showed no noticeable differences, leading to the conclusion that propiconazole does not alter the gut morphology of *Anthrenocerus australis* larvae. The null hypothesis from Section 5.1 is therefore accepted. The mode of action of propiconazole cannot be attributed to a cytotoxic effect on the cells of the digestive tract of *Anthrenocerus australis*. This suggests that the mode of action of propiconazole may occur at the sub-cellular level involving biochemical reactions, or that if any cytotoxic effect is present, it does not occurr in the gut of the insect. The mode of action of propiconazole on fungi has been described as inhibition of demethylation in ergosterol biosynthesis (Venkatakrishnan, von Moltke, & Greenblatt, 2000). Demethylation may occur in some of the biochemical pathways within *Anthrenocerus australis*, and although this may not necessarily occur in the gut region, it could give rise to the anti-feeding effect observed.

The common clothes moth *Tineola bisselliella* also shares a similar midgut morphology to *Anthrenocerus australis*, as described by Waterhouse (1952a, 1952c, 1952d). Columnar cells with a striated border were common, and were also longer in the anterior and posterior regions of the midgut compared to the middle region. A peritrophic membrane was also present, containing the bolus within the gut lumen. Cigar-shaped black masses were observed between the peritrophic membrane and epithelium of larvae fed a diet rich in nickel (Waterhouse, 1952c), which may be similar to the cytoplasmic spheres seen in *Anthrenocerus australis* (Figure 5.6). The major difference from the *Anthrenocerus australis* gut was the presence of large numbers of goblet cells in the *Tineola bisselliella* gut wall, which appeared to be absent in the beetle species according to Waterhouse (1952a), and confirmed by this work. These goblet cells had no striated border with the lumen. Small numbers of regenerative cells were described as scattered along the entire midgut, positioned along the basement membrane in *Tineola bisselleilla* (Waterhouse, 1952d). No description of the appearance of these cells was given, making comparison to the regenerative cells in *Anthrenocerus australis* difficult due to the low magnification and lack of colour in gut wall photographs.

Many of the features observed in the *Anthrenocerus australis* gut wall sections were similar to those identified in a gut histology study of the wool-digesting moth *Hofmannophila pseudospretella* (Gerard, 2002). As also seen with *Tineola bisselliella*, there were columnar,

goblet, and regenerative cells making up the simple epithelium of the midgut. Cytoplasmic spheres were more common in fed than in starved larvae. The dimensions of the columnar cells of the gut wall in *Hofmannophila pseudospretella* as measured by Gerard were approximately ten times larger than those found here in *Anthrenocerus australis* (Figures 5.2-5.6). Shannon, Attwood, Hopcroft and Christeller (2001) also found a similar gut wall morphology in *Hofmannophila pseudospretella*, and although their work was not focused on cell size, they did show a midgut wall structure approximately five times larger than those observed here in *Anthrenocerus australis*.

The neutral gut conditions in dermestid beetle species may be related to the lack of goblet cells as seen in the gut histology studies. The goblet cells present in the studies of lepidopteran species have been suggested in the case of *Tineola bisselliella* to be involved in regulation of a potassium phosphate buffer system (Kasper, 1978, as cited in Gerard, 2002, p. 21). This may contribute to the high pH of the *Tineola bisselliella* midgut, given the alkalinity of dipotassium and tripotassium phosphate. Kasper also postulated that the columnar cells are involved in elaboration of digestive enzymes in *Tineola bisselliella*, which would suggest that goblet cells do not perform this function. Enzyme activities shown to hydrolyse casein, BApNA, LpNA, and SAAPLpNA were shown in the midguts of both *Tineola bisselliella* and *Anthrenocerus australis* species (Christeller et al., 1994), also suggesting that the goblet cells of *Tineola bisselliella* are not the source of gut enzymes.

Trivedi et al. (1991) showed bacteria in the gut of *Anthrenus flavipes*, and hypothesised that these bacteria may contribute to the wool-digesting enzymes in the lumen. The possibility of gut flora in the similar beetle species *Anthrenocerus australis* was not investigated here, although it is possible that in the absence of highly alkaline midgut conditions, there may be bacteria present in the digestive tract of *Anthrenocerus australis* that could contribute to wool digestion. This extra source of proteolytic enzymes may compensate for the lack of alkalinity, which would be expected to make digestion of wool more difficult for *Anthrenocerus australis* compared to *Tineola bisselliella*. Further work should involve electron microscopy of the gut sections of *Anthrenocerus australis* to check the digestive tract for evidence of bacteria, and metagenomic studies of any gut flora discovered.

Chapter 6

Propiconazole Uptake and Durability on Wool

6.1 Introduction

When evaluating a new insect-resist agent for wool, the most important factors apart from mammalian and environmental toxicity are the application efficiency, and the durability on the wool product. The most convenient method of application for insect resist agents is during wool dyeing. To assess uptake efficiency in the dye bath, the amount of propiconazole remaining on wool after application was measured. Treated wool can be analysed by gas-liquid chromatography (CIPAC, 1995) after an extraction step to remove the propiconazole from the wool. However, there are inefficiencies in solvent extraction of insect-resist agents from wool where residual insect-resist agent remains on the wool after the extraction procedure is carried out. Time-consuming and expensive work is required to quantify this inefficiency. The goal of the analysis of wool would be to determine if sufficient levels of propiconazole are present to protect the associated textile from insect attack. Methods such as high-pressure liquid chromoatography and gas chromatography/mass spectrometry are used for insecticides where routine testing is carried out in order to give timely results for large numbers of samples. However, the use of a bioassay method such as Wools of New Zealand Test Method 25 (or the other similar test methods listed in Section 1.8.1) is the ultimate test of whether a wool textile is protected from insects. The 14 day incubation period in this test makes this a slower method, but for small numbers of samples this is more efficient than setting up an analytical method requiring the purchase of standards, extraction and quantification of propiconazole from wool, and operation of a gas chromatograph.

6.2 Methodology of Propiconazole Application and Fastness Testing

Durability assessment involved comparing the bioassay performance of treated wool carpet with that of wool carpet that had been treated and then exposed to challenges that deplete the level of insect-resist agent on a wool carpet during normal use. Those challenges are simulated by a carpet shampoo treatment followed by light exposure, as specified in Wool of

New Zealand Test Method 28. Any difference in performance of the two carpets can be attributed to propiconazole losses caused by the shampoo and light exposure.

The water solubility of propiconazole is 100 ppm at 20°C (Tomlin, 1997), therefore application levels at higher concentrations than this in the dyebath are expected to show some uptake onto wool. Although water solubility may increase with the elevated temperatures used in wool dyeing, the wool fibre structure becomes more swollen at elevated dyebath temperatures (Bird, 1947) and is more open to large molecules such as dyes and insecticides similar in size to propiconazole.

Propiconazole was applied to a 100% wool tufted carpet with polypropylene primary backing only, in a laboratory-scale piece-dyeing machine. Polypropylene was not expected to react significantly with propiconazole or the dyebath agents due to the relatively inert nature of the hydrocarbon structure compared to wool. Water (500 ml) was heated in a Dyemaster (John Jeffreys Engineering) to 40°C, and the carpet submerged in a metal cage with gentle agitation to remove air bubbles. The mass of wool contained in the carpet was 37.8 g, giving a liquor to wool ratio of 13.2:1. Commonly used dye levelling auxiliaries Lyogen MF (Chemcolour Industries (NZ) Ltd) and technical grade sodium sulphate were added to the liquor at 1.0% and 5.0% omw respectively. The de-aerating agent Chemaf 497 (Chemcolour Industries (NZ) Ltd) was also added to the liquor at 0.2 g/l to reduce bubbles forming on the wool. Sodium acetate was added to the liquor at 1.0 g/l and the pH of the dyebath was set to 4.8 by addition of acetic acid. Pro-P™ was added to the liquor at 2.11% omw, equal to 0.53% omw propiconazole.

The dyebath was heated to 98°C over 45 minutes and held for 30 minutes before cooling to 40°C over 15 minutes, after which the carpet was rinsed in cold water for 5 minutes. After drying, latex backing was applied to the carpet, and the latex cured for 10 minutes at 100°C to solidify the latex, ensuring firm anchoring of each tuft of yarn. Half of this carpet was exposed to shampoo wet extraction cleaning and light exposure as specified in the Wools of New Zealand Test Method 28. This method simulates the factors that may deplete the level of an insect resist agent present on wool during the normal life cycle of a carpet. Exposed and unexposed carpet was tested in bioassays against *Anthrenocerus australis* beetle larvae to assess the fastness of propiconazole to shampoo cleaning and light exposure.

6.3 Results of Propiconazole Application to Wool and Fastness Testing

The bioassay results from the dyebath application of propiconazole to wool carpet showed a significant (p-value<0.05) reduction in wool consumed by the *Anthrenocerus australis* larvae, although not enough to pass the Wools of New Zealand Test Method 25 (Table 6.1). The dyebath application appeared inefficient due to the high mass loss shown with an application level of 0.5% omw propiconazole, compared to direct applications of 0.3-0.4% propiconazole omw passing the test method (Section 2.5.2). It should be noted that the control larvae consumed more wool here (64.1 mg) than in previous propiconazole bioassays (27.8-38.0 mg) shown in Section 2.5.2. for direct application. When comparing the mass losses as a percentage of the control mass losses, the dyebath application still appears less efficient than direct application despite the variable results shown in Section 2.5.2.

The mass loss of control carpet exposed to shampoo and light treatments was significantly higher (p-value<0.05) than the unexposed carpet (Table 6.1). The observation by Day (1951a) that chemically reduced wool is more easily degraded compared to non-reduced wool by *Tineola bisselliella* gut enzyme extract due to a lower occurrence of disulphide bonds may explain this difference, as light exposure of wool is known to reduce the cystine content in wool and therefore the occurrence of disulphide bonds (Weatherall, 1973; Simpson, 2002). Another possibility was that residual carpet shampoo chemical/s on the wool fibres increased their palatability to the beetle larvae. Although this has not been documented in the literature, results from Table 2.1, Section 2.3.2.1 show the presence of the surfactant benzene sulphonic acid increased the palatability of wool to *Tineola bisselliella* and so this possibility should be considered for surfactants present in the carpet shampoo solution. The propiconazole-treated carpets showed no significant difference (p-value>0.05) in mass loss when comparing exposed to unexposed. This suggested the level of propiconazole on the carpet wool, although inadequate for protection, was not noticably reduced by the shampoo and light exposure. The similar mass losses of exposed and unexposed propiconazole carpets suggested that shampoo or light exposure do not make wool more palatable to beetle larvae, therefore weakening the theories on wool weathering and residual carpet shampoo increasing palatability of wool as suggested above.

Table 6.1 Bioassay results of *Anthrenocerus australis* on wool carpets including propiconazole and exposure to shampoo and light.

	Mean % mortality	Mean % pupation	Mean mass loss (mg) (± S.E.M)	Mean % mass loss[1]	Visual assessment	Pass (p), fail (f), or border-line (b)
Control	1.7	0.0	64.1 ± 3.3	n.a.	n.a.	n.a.
Control exposed	1.7	1.8	79.9 ± 7.2	124.6	n.a.	f
Propiconazole	0.0	0.0	46.4 ± 4.9	72.5	n.a.	f
Propiconazole exposed	1.7	0.0	41.8 ± 2.0	65.2	n.a.	f

[1] As a percentage of the mean voracity control.

6.4 Conclusions on Uptake and Durability of Propiconazole on Wool

There appears to be a weak affinity for propiconazole by wool in an acidic dyebath application, perhaps due to lack of suitable reactive groups within the propiconazole molecule. This lack of affinity makes propiconazole unlikely to be a suitable active for an insect-resist dyebath agent for wool. Dyebath-applied propiconazole did not appear to be significantly removed by shampoo and light exposure designed to simulate the factors that usually deplete insect-resist agents in carpets. Some compounds can be broken down by light exposure, or totally removed by shampoo treatment with a wet-vacuum procedure. This suggests that propiconazole, if applied sufficiently to wool, would have acceptable durability as an insect-resist agent. Increasing the concentration of propiconazole in the dyebath to 2-3% omw may have yielded sufficient protection, although this would lead to a higher concentration of propiconazole in the effluent. This may be viable given the low aquatic toxicity of propiconazole, although durability testing would need to be repeated to ensure higher levels were equally resistant to light and shampoo. Alternative application techniques, such as addition of propiconazole to carpet shampoo solution, application to wool during a brief post-dyeing high-pH rinse, or a continuous application to yarn in the final bowl of a chemical yarn twist-setting machine (McKinnon, 1989) may yield higher uptakes that are more efficient and therefore practical.

Chapter 7

Conclusions and Further Work

Many surfactants, naphthalene derivatives, and antimicrobial compounds show insecticidal and anti-feeding effects on the keratin-digesting *Tineola bisselliella* and *Anthrenocerus australis* larvae when applied to wool fabric. Molecular size, shape, and polarity have been linked to anti-feeding effects with these insects when using surfactants, naphthalene derivatives, and linear alkyl carboxylic acids. A non-polar linear alkyl chain of 12 carbon atoms appeared to be optimal for activity against *Tineola bisselliella*, provided it was strongly bound to the rest of the molecule. The head of the molecule required a polar group for efficacy against insects, with higher polarity correlating with higher anti-feeding properties. The difference in polarity from one end of the molecule to the other appeared to be higher in compounds providing a greater anti-feeding effect. The surfactant of highest efficacy against *Tineola bisselliella*, dodecylbenzene sulphonic acid, was anionic in nature, whereas the zwitterionic N,N-dimethyldodecyl amine N-oxide was most effective against *Anthrenocerus australis*, possibly due to the neutral pH of the beetle midgut allowing extra functionality of the zwitterion. The most effective napthalene derivative at confering anti-feeding properties to wool fabric against *Tineola bisselliella*, 1,2-naphthoquinone-4-sulphonic acid (sodium salt), was almost as effective as dodecylbenzene sulphonic acid, but impractical due to colouration of the wool.

Some antimicrobial compounds showed higher insect-proofing efficacy on wool fabrics than surfactants or naphthalene derivatives. Although triclocarban was the most effective moth-proofer for a given level, its high toxicity to *Daphnia magna,* similar to permethrin, indicated it was not likely to be targeting the wool digestion mechanism. The mechanism of action of the anti-fungal econazole nitrate on *Tineola bisselliella* is uncertain, although it was the second most effective mothproofer of wool fabrics. Imidazole nitrate compounds trialled showed higher efficacy towards *Anthrenocerus australis* larvae than *Tineola bisselliella*, leading to the hypothesis that these antifungals were disrupting gut flora of *Anthrenocerus australis* larvae not present in *Tineola bisselliella*. This hypothesis assumes there are gut flora present in *Anthrenocerus australis*, which is possible given the findings of Trivedi et al. (1991) concerning the closely related *Anthrenus flavipes* beetle larvae where cocobacilli

bacteria and protozoa were discovered. The presence of bacteria or protozoa in the *Anthrenocerus australis* gut was not investigated in this work, although using a scanning electron microscope to investigate the *Anthrenocerus australis* gut for bacteria or protozoa would be a logical next step for future work, followed by metagenomic studies of any gut flora discovered.

The most effective antimicrobial against *Anthrenocerus australis* beetle larvae was the triazole antifungal propiconazole. The anti-feeding mode of action of the anti-fungal agent propiconazole on *Anthrenocerus australis* can be described as a reversible, ingestion-related effect. No cytotoxicity was seen in the gut wall of *Anthrenocerus australis* after feeding on propiconazole-treated wool, and no repellency effect was seen in behavioural studies with propiconazole. Four gut enzymes of *Anthrenocerus australis* involved in wool digestion showed trypsin, chymotrypsin, and aminopeptidase activities 2-2½ times lower when larvae were fed on propiconazole-treated wool, although this was likely to have been a result of the lower rate of feeding rather than gut enzyme inhibition. One gut enzyme showed unchanged esterase activity with exposure to propiconazole, indicating not only that this enzyme activity was unrelated to wool digestion, but that the *Anthrenocerus australis* larvae were still reasonably healthy despite the lowered rate of feeding. These observations also contribute to the hypothesis that propiconazole interferes with symbiotic gut organisms in *Anthrenocerus australis* to achieve an anti-feeding effect due to no obvious symptoms of toxicity in the insect itself.

Environmental advantages of some non-insecticidal compounds over insecticides are considerable, and application techniques should be further investigated to provide a viable method for treatment of wool. The greatest environmental advantage discovered with non-insecticidal compounds in this work was achieved when using propiconazole against the *Anthrenocerus australis* beetle larvae, where toxicity to *Daphnia magna* was reduced approximately 1200-fold compared to using permethrin at the required rate. Although propiconazole showed poor affinity for wool in an aqueous dyebath, it could still be a practical beetle-proofing compound if a suitable application method was found. The worldwide trend towards environmentally friendly practices may one day result in commercial applications of non-insecticidal insect-proofers becoming widespread.

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Appendix A

Keratin digesting insects derived from Waterhouse (1958)

Table A.1 Lepidoptera known or suspected to digest keratin

Species	Synonyms
Tineidae	
Amydria vastella Zeller 1852	
Monopis crocicapitella Clemens 1859	
Monopis dicycla Meyrick 1905	
Monopis ethelella Newman 1856	
Monopis ferruginella Hübner 1813	
Monopis monachella Hübner 1796	
Monopis pseudagyrta Meyrick 1919	
Monopis rusticella Clerck 1796	
Monopis trimaculella Snellen 1885	
Monopis weaverella Scott 1858	
Niditinea fuscella Linnaeus 1758	*Tinea/Acedes fuscipunctella* (Haworth 1828)[5]
Phereoeca allutella Rebel 1892	
Phereoeca uterella Walsingham 1897	*Phereoeca walsinghami* (Busch [*sic*] 1933)[3]
Praeacedes atomosella Walker 1863	*Praecedes* [*sic*] *thecophora* (Walsingham 1908)[6]
Tenaga inquisitrix Meyrick 1916	
**Tinea columbariella* Wocke 1877	
Tinea flavescentella Haworth 1828	
**Tinea pallescentella* Stainton 1851	**Acedes pallescentella* (Stainton 1851)[4]
**Tinea pellionella* Linnaeus 1758	
Tinea semifulvella Haworth 1828	*Acedes semifulvella* (Haworth 1828)[4]
Tinea subalbidella Stainton 1867	*Tinea/Scleroplasta liberiella* (Zeller 1879)[7]
Tinea translucens Meyrick 1917	*Tinea metonella* (Pierce & Metcalf 1934)[2]
Tinea trinotella Thunberg 1794	*Tinea/Accedes* [*sic*] *ganomella* (Treitschke 1833)[1]
**Tineola bisselliella* Hummel 1823	**Tineola furciferella* (Zagulyayev [*sic*] 1954)[1]
Trichophaga abruptella Wollaston 1858	
**Trichophaga mormopis* Meyrick 1935	**Trichophaga percna* (Corbet & Tams 1943)[1]
**Trichophaga tapetzella* Linnaeus 1758	
Oecophoridae	
**Hofmannophila pseudospretella* Stainton 1849	

Citations in which synonymy is recognised: [1] Global taxonomic database, 2010, [2] Carter, 1984, [3] Villanueva-Jiménez & Fasulo, 2010, [4] Butterflies and moths of the world, [5] Australian biological resources, 2010, [6] Vieira, Borges, Karsholt, & Wunderlich, 2003, [7] Gaedike, 2007.

Table A.2 Coleoptera known or suspected to digest keratin

Species	Synonyms
Dermestidae	
**Anthrenocerus australis* Hope 1843	
**Anthrenus flavipes* LeConte 1854	**Anthrenus vorax* (Waterhouse 1883)[3]
Anthrenus fuscus Olivier 1789	
Anthrenus museorum Linnaeus 1761	
**Anthrenus pimpinellae* Fabricius 1775	
**Anthrenus scrophulariae* Linnaeus 1758	
**Anthrenus verbasci* Linnaeus 1767	
Attagenus elongatulus Casey 1900	
Attagenus fasciatus Thunberg 1795	*Attagenus gloriosae* (Fabricius 1801)[2]
Attagenus lobatus Rosenhauer 1856	
Attagenus nigripes Casey 1916	
**Attagenus pellio* Linnaeus 1758	
**Attagenus piceus* Olivier 1790	**Attagenus unicolor* (Brahm 1791)[1], **Attagenus megatoma* (Fabricius 1798)[1]
Attagenus schäfferi Herbst 1792	
Scarabaeidae	
Deltochilum gibbosum Fabricius 1775	

Citations in which synonymy is recognised: [1] Bosquet, 1990, [2] Archibald & Chalmers, 1983, [3] Veer, Prasad, & Rao, 1991.

Appendix B

Wools of New Zealand Test Method 25

Mass loss: Dm = (M0 x M3/M2) - M1 where: Dm = mass loss

M0 = initial sample mass (control or test)

M1 = final sample mass (control or test)

M2 = initial sample mass (moisture control)

M3 = final sample mass (moisture control)

Cropping (feeding) on edge of disc:

1 = no detectable damage

2 = very slight visible cropping

3 = moderate cropping

4 = very heavy cropping

Feeding damage on disc:

A = no detectable damage

B = yarn or fibres partially severed

C = a few small holes, yarn or fibres severed

D = several large holes

WNZ TM25 Satisfactory/Unsatisfactory Insect Resistance Criteria for Fabrics (Flat Woven) and Felts:

Definition of borderline resistance:

A tested sample of fabric or felt shall be considered a borderline case if:

a) The mean mass loss of the test specimen is greater than 12 mg but less than 15 mg. Under this condition not more than one specimen may have a loss exceeding 20 mg.

And a visual assessment better than either:

b) Assessed as attack level 2B on two of the test specimens, and the remaining test specimens are undamaged (1A); or

c) Assessed as attack level 3B on any one test specimen, and the remaining test specimens are undamaged (1A), (this indicates uneven insect resist agent application).

The sample shall be considered to have Unsatisfactory Resistance to insect attack when the estimation of holes on any one test specimen is assessed as C or D, or if the attack level is assessed as more severe than that defined as Borderline.

Satisfactory Resistance

A tested sample of fabric or felt shall be considered to have Satisfactory Resistance if the attack level is classified as less than that defined as Borderline.

The sample shall be considered to have Satisfactory Resistance if 90% test insects mortality is achieved irrespective of mass loss or visual assessment.

Unsatisfactory Resistance

A tested sample of fabric or felt shall be considered to have Unsatisfactory Resistance if the attack level is classified as greater than that defined as Borderline. If the estimation of holes is classified as C or D on any one specimen, the sample has an Unsatisfactory Resistance to attack.

Appendix C

Optical Density Data From *Anthrenocerus australis* Gut Enzyme Assays

The second row of all tables represents the time converted to metric minutes. Due to limited column space, blank = blnk.

Table C.1 BApNA Plate 1:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a	0.0985	0.0987	0.1028	0.1079	0.1066	0.1112	0.1154	0.1173	0.1211	0.1231	0.1286	0.1298	0.1318	0.1340	0.1399	0.1406	0.1450	0.1471	0.1504	0.1554
A	2	C1a	0.0865	0.0934	0.0937	0.0970	0.0971	0.1018	0.1069	0.1104	0.1117	0.1142	0.1176	0.1191	0.1227	0.1258	0.1288	0.1327	0.1367	0.1387	0.1434	0.1465
A	3	C1b	0.0984	0.1004	0.1005	0.1077	0.1065	0.1098	0.1129	0.1178	0.1210	0.1208	0.1252	0.1264	0.1278	0.1294	0.1311	0.1326	0.1383	0.1389	0.1397	0.1433
A	4	C1b	0.0915	0.0988	0.0976	0.1030	0.1014	0.1052	0.1087	0.1113	0.1137	0.1137	0.1176	0.1207	0.1221	0.1229	0.1268	0.1294	0.1326	0.1335	0.1356	0.1373
A	5	T1a	0.0766	0.0832	0.0819	0.0828	0.0808	0.0862	0.1085	0.0846	0.0880	0.0865	0.0860	0.0865	0.0878	0.0864	0.0886	0.0872	0.0888	0.0886	0.0885	0.0904
A	6	T1a	0.0823	0.0864	0.0846	0.0869	0.0848	0.0864	0.0869	0.0882	0.0882	0.0865	0.0902	0.0880	0.0873	0.0893	0.0897	0.0895	0.0906	0.0909	0.0906	0.0922
A	7	T1b	0.0750	0.0837	0.0817	0.0830	0.0813	0.0808	0.0815	0.0857	0.0843	0.0823	0.0851	0.0865	0.0862	0.0878	0.0876	0.0879	0.0887	0.0900	0.0907	0.0920
A	8	T1b		0.0834	0.0822	0.0835	0.0816	0.0818	0.0835	0.0860	0.0859	0.0841	0.0869	0.0876	0.0879	0.0889	0.0899	0.0909	0.0930	0.0926	0.0922	0.0941
B	1	C2a	0.1053	0.1095	0.1140	0.1227	0.1236	0.1347	0.1391	0.1474	0.1518	0.1594	0.1641	0.1707	0.1785	0.1857	0.1947	0.2003	0.2092	0.2121	0.2195	0.2270
B	2	C2a	0.0945	0.1028	0.1083	0.1165	0.1207	0.1262	0.1342	0.1420	0.1470	0.1521	0.1614	0.1663	0.1734	0.1806	0.1880	0.1934	0.2030	0.2077	0.2145	0.2230
B	3	C2b	0.0867	0.0918	0.0924	0.0986	0.0955	0.0998	0.1026	0.1042	0.1057	0.1069	0.1100	0.1122	0.1153	0.1160	0.1195	0.1207	0.1240	0.1264	0.1260	0.1314
B	4	C2b	0.0835	0.0872	0.0884	0.0910	0.0912	0.0949	0.0979	0.0999	0.1019	0.1024	0.1058	0.1081	0.1097	0.1120	0.1143	0.1161	0.1197	0.1201	0.1223	0.1256
B	5	T2a					0.0886	0.0887	0.0897	0.0922	0.0905	0.0893	0.0937	0.0903	0.0890	0.0909	0.0914	0.0899	0.0919	0.0917	0.0907	0.0935
B	6	T2a			0.0876	0.0873	0.0866	0.0865	0.0891	0.0885	0.0890	0.0918	0.0912	0.0878	0.0900	0.0917	0.0917	0.0906	0.0913	0.0913	0.0906	0.0927
B	7	T2b	0.0802	0.0860	0.0886	0.0911	0.0921	0.0944	0.0980	0.1021	0.1028	0.1042	0.1098	0.1086	0.1123	0.1152	0.1156	0.1179	0.1214	0.1226	0.1256	0.1301
B	8	T2b	0.0837	0.0969	0.0968	0.0995	0.1007	0.1018	0.1054	0.1083	0.1124	0.1135	0.1191	0.1176	0.1218	0.1241	0.1275	0.1287	0.1303	0.1333	0.1376	0.1402
C	1	C3a	0.1005	0.1097	0.1179	0.1302	0.1354	0.1432	0.1517	0.1633	0.1713	0.1782	0.1865	0.1951	0.2050	0.2132	0.2235	0.2290	0.2421	0.2516	0.2606	0.2690
C	2	C3a	0.1059	0.1167	0.1248	0.1351	0.1406	0.1503	0.1588	0.1684	0.1761	0.1836	0.1940	0.2007	0.2096	0.2191	0.2296	0.2365	0.2486	0.2550	0.2623	0.2733
C	3	C3b	0.0855	0.0907	0.0938	0.0987	0.0963	0.1004	0.1036	0.1084	0.1139	0.1124	0.1156	0.1181	0.1217	0.1241	0.1291	0.1304	0.1339	0.1376	0.1396	0.1448

Table C.1 BApNA Plate 1 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
C	4	C3b	0.0922	0.1012	0.1007	0.1047	0.1052	0.1075	0.1092	0.1147	0.1162	0.1187	0.1237	0.1251	0.1289	0.1302	0.1344	0.1355	0.1420	0.1437	0.1459	0.1484
C	5	T3a	0.0897	0.0940	0.0930	0.0966	0.0932	0.0969	0.0936	0.0984	0.0982	0.0981	0.1018	0.0996	0.1002	0.1002	0.1008	0.1007	0.1011	0.1023	0.1014	0.1038
C	6	T3a	0.0731	0.0761	0.0774	0.0770	0.0769	0.0788	0.0769	0.0811	0.0808	0.0803	0.0822	0.0817	0.0832	0.0857	0.0829	0.0863	0.0885	0.0859	0.0850	0.0890
C	7	T3b	0.0938	0.0970	0.0956	0.0966	0.0944	0.0948	0.0988	0.0984	0.0959	0.0943	0.0956	0.0943	0.0947	0.0930	0.0973	0.0993	0.1008	0.1035	0.1053	0.1056
C	8	T3b	0.0883	0.0954	0.0943	0.0964	0.0951	0.0961	0.0994	0.0972	0.0947	0.0941	0.0971	0.0954	0.0950	0.0953	0.0966	0.0962	0.0970	0.0978	0.0999	0.1010
D	1	C4a	0.0993	0.1060	0.1092	0.1155	0.1196	0.1250	0.1339	0.1403	0.1439	0.1500	0.1571	0.1619	0.1660	0.1722	0.1796	0.1821	0.1903	0.1964	0.2013	0.2065
D	2	C4a	0.1001	0.1088	0.1139	0.1213	0.1230	0.1304	0.1345	0.1428	0.1449	0.1499	0.1583	0.1638	0.1684	0.1728	0.1799	0.1850	0.1907	0.1958	0.2020	0.2078
D	3	C4b	0.0944	0.1027	0.1066	0.1136	0.1162	0.1228	0.1279	0.1344	0.1372	0.1429	0.1507	0.1561	0.1603	0.1658	0.1721	0.1776	0.1837	0.1905	0.1955	0.2004
D	4	C4b	0.0931	0.1024	0.1072	0.1152	0.1156	0.1230	0.1284	0.1336	0.1405	0.1452	0.1534	0.1583	0.1618	0.1681	0.1739	0.1782	0.1864	0.1907	0.1973	0.2023
D	5	T4a	0.1017	0.1140	0.1223	0.1341	0.1405	0.1493	0.1652	0.1723	0.1833	0.1933	0.2040	0.2162	0.2245	0.2357	0.2457	0.2572	0.2700	0.2808	0.2900	0.2995
D	6	T4a	0.1003	0.1121	0.1209	0.1315	0.1394	0.1473	0.1578	0.1685	0.1785	0.1876	0.1977	0.2071	0.2148	0.2262	0.2356	0.2493	0.2566	0.2657	0.2758	0.2857
D	7	T4b	0.0825	0.0893	0.0945	0.0961	0.0992	0.0996	0.1037	0.1057	0.1090	0.1086	0.1143	0.1150	0.1167	0.1186	0.1216	0.1224	0.1266	0.1272	0.1303	0.1340
D	8	T4b	0.0765	0.0825	0.0849	0.0882	0.0882	0.0900	0.0930	0.0977	0.0994	0.0999	0.1058	0.1056	0.1092	0.1094	0.1151	0.1161	0.1176	0.1208	0.1237	0.1250
E	1	C5a	0.1022	0.1122	0.1188	0.1289	0.1345	0.1430	0.1519	0.1606	0.1678	0.1763	0.1850	0.1914	0.1997	0.2110	0.2168	0.2254	0.2348	0.2446	0.2489	0.2627
E	2	C5a	0.0984	0.1088	0.1153	0.1251	0.1285	0.1409	0.1490	0.1590	0.1690	0.1746	0.1840	0.1889	0.1953	0.2061	0.2131	0.2206	0.2315	0.2400	0.2467	0.2560
E	3	C5b	0.0973	0.1042	0.1091	0.1157	0.1190	0.1288	0.1350	0.1417	0.1481	0.1529	0.1604	0.1656	0.1731	0.1788	0.1852	0.1910	0.2003	0.2054	0.2114	0.2199
E	4	C5b	0.0883	0.1148	0.1211	0.1112	0.1172	0.1227	0.1299	0.1387	0.1444	0.1491	0.1581	0.1639	0.1711	0.1762	0.1842	0.1928	0.1988	0.2046	0.2122	0.2190
E	5	T5a	0.1005	0.1087	0.1118	0.1160	0.1189	0.1265	0.1319	0.1406	0.1435	0.1489	0.1546	0.1601	0.1657	0.1692	0.1764	0.1809	0.1894	0.1921	0.1996	0.2049
E	6	T5a	0.0947	0.1030	0.1060	0.1120	0.1161	0.1183	0.1253	0.1331	0.1380	0.1436	0.1505	0.1552	0.1639	0.1674	0.1725	0.1788	0.1863	0.1929	0.1959	0.2032
E	7	T5b	0.0829	0.0839	0.0884	0.0885	0.0872	0.0898	0.0925	0.0931	0.0946	0.0963	0.0991	0.0994	0.1004	0.1028	0.1044	0.1082	0.1090	0.1096	0.1089	0.1127
E	8	T5b	0.0912	0.0914	0.0932	0.0950	0.0954	0.0963	0.0980	0.1014	0.1026	0.1035	0.1061	0.1069	0.1083	0.1122	0.1131	0.1148	0.1183	0.1182	0.1202	0.1234
F	1	blnk 1	0.0849	0.0869	0.0853	0.0861	0.0833	0.0858	0.0870	0.0884	0.0877	0.0869	0.0889	0.0860	0.0875	0.0878	0.0879	0.0859	0.0897	0.0874	0.0887	0.0876
F	2	blnk 2	0.0893	0.0900	0.0897	0.0908	0.0902	0.0906	0.0912	0.0942	0.0914	0.0914	0.0931	0.0914	0.0899	0.0925	0.0926	0.0909	0.0941	0.0898	0.0903	0.0908
F	3	blnk 3	0.0792	0.0834	0.0808	0.0821	0.0823	0.0857	0.0860	0.0870	0.0859	0.0858	0.0870	0.0855	0.0859	0.0872	0.0867	0.0856	0.0871	0.0877	0.0868	0.0866
F	4	blnk 4	0.0843	0.0853	0.0842	0.0847	0.0826	0.0853	0.0868	0.0864	0.0863	0.0861	0.0873	0.0872	0.0862	0.0866	0.0863	0.0873	0.0889	0.0869	0.0854	0.0873
F	5	blnk 5	0.0890	0.0909	0.0893	0.0877	0.0881	0.0878	0.0899	0.0929	0.0923	0.0917	0.0929	0.0917	0.0938	0.0916	0.0925	0.0943	0.0938	0.0913	0.0945	0.0918
F	6	blnk 6	0.0763	0.0796	0.0784	0.0801	0.0771	0.0797	0.0800	0.0835	0.0841	0.0844	0.0861	0.0834	0.0849	0.0848	0.0861	0.0857	0.0867	0.0848	0.0862	0.0865
F	7	blnk 7	0.0846	0.0850	0.0857	0.0844	0.0854	0.0833	0.0861	0.0887	0.0875	0.0879	0.0910	0.0859	0.0877	0.0883	0.0895	0.0918	0.0907	0.0898	0.0926	0.0922
F	8	blnk 8	0.0905	0.0928	0.0884	0.0888	0.0877	0.0857	0.0883	0.0891	0.0896	0.0923	0.0936	0.0901	0.0898	0.0914	0.0912	0.0932	0.0935	0.0916	0.0908	0.0925

Table C.2 BApNA Plate 2:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a	0.0932	0.0973	0.0997	0.1056	0.1059	0.1069	0.1112	0.1132	0.1167	0.1186	0.1222	0.1229	0.1266	0.1299	0.1332	0.1366	0.1406	0.1404	0.1460	0.1486
A	2	C1a	0.0911	0.0934	0.0933	0.0973	0.0997	0.1010	0.1056	0.1088	0.1088	0.1104	0.1162	0.1191	0.1222	0.1232	0.1265	0.1295	0.1318	0.1354	0.1394	0.1414
A	3	C1b	0.0945	0.1006	0.1014	0.1053	0.1078	0.1087	0.1103	0.1098	0.1145	0.1152	0.1189	0.1200	0.1221	0.1247	0.1261	0.1299	0.1320	0.1355	0.1384	0.1396
A	4	C1b	0.0904	0.0961	0.0986	0.1017	0.1041	0.1026	0.1067	0.1096	0.1119	0.1119	0.1164	0.1163	0.1192	0.1212	0.1237	0.1254	0.1275	0.1301	0.1346	0.1380
A	5	T1a	0.0800	0.0808	0.0816	0.0844	0.0823	0.0825	0.0823	0.0823	0.0829	0.0822	0.0855	0.0841	0.0857	0.0835	0.0837	0.0836	0.0836	0.0854	0.0851	0.0855
A	6	T1a	0.0836	0.0830	0.0826	0.0859	0.0834	0.0845	0.0840	0.0874	0.0848	0.0845	0.0865	0.0866	0.0868	0.0877	0.0879	0.0886	0.0871	0.0871	0.0886	0.0897
A	7	T1b	0.0721	0.0759	0.0763	0.0791	0.0770	0.0770	0.0791	0.0798	0.0808	0.0797	0.0808	0.0818	0.0821	0.0816	0.0837	0.0837	0.0824	0.0849	0.0874	0.0853
A	8	T1b	0.0735	0.0792	0.0780	0.0811	0.0813	0.0816	0.0826	0.0833	0.0833	0.0835	0.0831	0.0833	0.0857	0.0863	0.0848	0.0859	0.0858	0.0877	0.0880	0.0871
B	1	C2a	0.0974	0.1052	0.1111	0.1207	0.1258	0.1326	0.1372	0.1439	0.1527	0.1562	0.1650	0.1696	0.1767	0.1813	0.1905	0.1979	0.2025	0.2094	0.2192	0.2222
B	2	C2a	0.0912	0.1014	0.1069	0.1148	0.1200	0.1250	0.1300	0.1370	0.1427	0.1543	0.1567	0.1617	0.1691	0.1755	0.1832	0.1894	0.1967	0.2019	0.2103	0.2154
B	3	C2b	0.0851	0.0891	0.0916	0.0936	0.0940	0.0973	0.0985	0.1011	0.1029	0.1031	0.1044	0.1082	0.1120	0.1122	0.1142	0.1166	0.1183	0.1197	0.1234	0.1237
B	4	C2b	0.0825	0.0874	0.0891	0.0915	0.0941	0.0937	0.0959	0.0982	0.0988	0.1003	0.1050	0.1062	0.1094	0.1093	0.1113	0.1145	0.1146	0.1174	0.1200	0.1216
B	5	T2a	0.0822	0.0840	0.0851	0.0877	0.0874	0.0855	0.0873	0.0860	0.0878	0.0876	0.0874	0.0872	0.0898	0.0879	0.0890	0.0905	0.0912	0.0912	0.0896	0.0889
B	6	T2a	0.0813	0.0853	0.0847	0.0875	0.0862	0.0879	0.0857	0.0867	0.0864	0.0864	0.0852	0.0869	0.0872	0.0881	0.0867	0.0873	0.0869	0.0873	0.0895	0.0871
B	7	T2b	0.0867	0.0894	0.0904	0.0962	0.0950	0.0952	0.0973	0.0979	0.1010	0.1031	0.1088	0.1097	0.1138	0.1141	0.1168	0.1180	0.1207	0.1237	0.1254	0.1275
B	8	T2b	0.0854	0.0922	0.0926	0.0957	0.0965	0.0983	0.1005	0.1042	0.1074	0.1077	0.1112	0.1142	0.1158	0.1177	0.1189	0.1213	0.1246	0.1255	0.1294	0.1297
C	1	C3a	0.0972	0.1074	0.1165	0.1272	0.1339	0.1428	0.1497	0.1593	0.1659	0.1736	0.1877	0.1930	0.2031	0.2109	0.2207	0.2294	0.2384	0.2474	0.2604	0.2680
C	2	C3a	0.1051	0.1141	0.1210	0.1312	0.1388	0.1468	0.1554	0.1631	0.1720	0.1773	0.1892	0.1963	0.2050	0.2131	0.2225	0.2309	0.2393	0.2486	0.2592	0.2665
C	3	C3b	0.0825	0.0883	0.0906	0.0943	0.0956	0.0972	0.1007	0.1041	0.1081	0.1095	0.1134	0.1148	0.1210	0.1242	0.1240	0.1292	0.1311	0.1324	0.1377	0.1391
C	4	C3b	0.0886	0.0944	0.0956	0.1005	0.1015	0.1051	0.1066	0.1109	0.1144	0.1136	0.1209	0.1211	0.1253	0.1276	0.1298	0.1327	0.1341	0.1389	0.1454	0.1444
C	5	T3a	0.0898	0.0925	0.0937	0.0930	0.0925	0.0939	0.0937	0.0951	0.0965	0.0957	0.0968	0.0969	0.0978	0.0956	0.0989	0.0993	0.0973	0.1006	0.1017	0.1000
C	6	T3a	0.0744	0.0770	0.0766	0.0786	0.0773	0.0780	0.0794	0.0781	0.0805	0.0791	0.0838	0.0822	0.0832	0.0815	0.0832	0.0831	0.0843	0.0841	0.0873	0.0855
C	7	T3b	0.0845	0.0856	0.0865	0.0883	0.0893	0.0904	0.0893	0.0885	0.0899	0.0870	0.0904	0.0916	0.0897	0.0912	0.0917	0.0909	0.0898	0.0895	0.0896	0.0895
C	8	T3b	0.0905	0.0935	0.0955	0.0976	0.0955	0.0946	0.0952	0.0950	0.0954	0.0946	0.0981	0.0977	0.0983	0.0959	0.0983	0.0982	0.0967	0.0970	0.1002	0.0979
D	1	C4a	0.0974	0.1029	0.1091	0.1153	0.1208	0.1269	0.1294	0.1352	0.1403	0.1445	0.1548	0.1608	0.1630	0.1663	0.1744	0.1789	0.1863	0.1912	0.1978	0.2013
D	2	C4a	0.1000	0.1062	0.1106	0.1167	0.1232	0.1272	0.1311	0.1402	0.1445	0.1490	0.1553	0.1597	0.1651	0.1701	0.1758	0.1807	0.1857	0.1916	0.1982	0.2023
D	3	C4b	0.0910	0.0990	0.1032	0.1111	0.1147	0.1212	0.1233	0.1300	0.1362	0.1415	0.1498	0.1529	0.1601	0.1653	0.1734	0.1763	0.1819	0.1878	0.1982	0.2000
D	4	C4b	0.0937	0.0998	0.1063	0.1107	0.1159	0.1220	0.1278	0.1333	0.1376	0.1420	0.1498	0.1550	0.1610	0.1664	0.1721	0.1780	0.1813	0.1887	0.1938	0.2004
D	5	T4a	0.0969	0.1088	0.1167	0.1264	0.1346	0.1443	0.1530	0.1633	0.1735	0.1812	0.1946	0.2004	0.2095	0.2187	0.2318	0.2404	0.2474	0.2554	0.2673	0.2789

Table C.2 BApNA Plate 2 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
D	6	T4a	0.0976	0.1084	0.1183	0.1285	0.1384	0.1482	0.1558	0.1683	0.1734	0.1816	0.1965	0.2038	0.2141	0.2227	0.2321	0.2418	0.2524	0.2592	0.2735	0.2821
D	7	T4b	0.0861	0.0897	0.0926	0.0975	0.0960	0.0976	0.1015	0.1021	0.1046	0.1061	0.1096	0.1118	0.1142	0.1153	0.1172	0.1191	0.1215	0.1251	0.1275	0.1280
D	8	T4b	0.0788	0.0831	0.0838	0.0883	0.0883	0.0909	0.0953	0.0959	0.0967	0.0995	0.1011	0.1028	0.1073	0.1070	0.1109	0.1116	0.1130	0.1158	0.1202	0.1196
E	1	C5a	0.1011	0.1106	0.1176	0.1276	0.1310	0.1415	0.1496	0.1573	0.1670	0.1722	0.1833	0.1903	0.2006	0.2083	0.2175	0.2244	0.2332	0.2420	0.2515	0.2605
E	2	C5a	0.0976	0.1067	0.1129	0.1217	0.1297	0.1384	0.1442	0.1526	0.1603	0.1691	0.1754	0.1868	0.1924	0.2018	0.2086	0.2175	0.2278	0.2353	0.2447	0.2522
E	3	C5b	0.0961	0.1027	0.1083	0.1177	0.1201	0.1286	0.1327	0.1392	0.1454	0.1518	0.1597	0.1666	0.1737	0.1784	0.1844	0.1929	0.1987	0.2042	0.2133	0.2171
E	4	C5b	0.0925	0.1008	0.1056	0.1125	0.1186	0.1248	0.1324	0.1387	0.1438	0.1504	0.1576	0.1643	0.1722	0.1776	0.1854	0.1909	0.1969	0.2047	0.2129	0.2189
E	5	T5a	0.1051	0.1136	0.1148	0.1215	0.1256	0.1329	0.1345	0.1437	0.1475	0.1529	0.1605	0.1633	0.1690	0.1761	0.1840	0.1886	0.1944	0.2007	0.2072	0.2134
E	6	T5a	0.0987	0.1051	0.1079	0.1156	0.1181	0.1243	0.1294	0.1342	0.1406	0.1444	0.1516	0.1551	0.1634	0.1689	0.1746	0.1808	0.1827	0.1908	0.1972	0.2043
E	7	T5b	0.0821	0.0848	0.0856	0.0897	0.0904	0.0926	0.0920	0.0935	0.0953	0.0947	0.0990	0.0970	0.1002	0.1008	0.1035	0.1040	0.1040	0.1065	0.1108	0.1115
E	8	T5b	0.0901	0.0900	0.0898	0.0930	0.0923	0.1064	0.0975	0.0991	0.0987	0.0985	0.1106	0.1029	0.1042	0.1041	0.1073	0.1073	0.1149	0.1109	0.1121	0.1134
F	1	blnk 1	0.0847	0.0832	0.0831	0.0850	0.0829	0.0836	0.0829	0.0829	0.0837	0.0826	0.0835	0.0840	0.0841	0.0840	0.0841	0.0838	0.0827	0.0841	0.0869	0.0855
F	2	blnk 2	0.0865	0.0885	0.0876	0.0884	0.0879	0.0880	0.0884	0.0891	0.0891	0.0881	0.0909	0.0902	0.0887	0.0906	0.0895	0.0893	0.0909	0.0898	0.0903	0.0890
F	3	blnk 3	0.0826	0.0829	0.0808	0.0828	0.0830	0.0832	0.0824	0.0837	0.0846	0.0843	0.0852	0.0854	0.0852	0.0846	0.0857	0.0834	0.0853	0.0843	0.0837	0.0843
F	4	blnk 4	0.0847	0.0853	0.0838	0.0864	0.0838	0.0850	0.0846	0.0868	0.0862	0.0846	0.0882	0.0846	0.0868	0.0868	0.0856	0.0851	0.0851	0.0845	0.0878	0.0857
F	5	blnk 5	0.0920	0.0908	0.0884	0.0891	0.0878	0.0908	0.0894	0.0907	0.0902	0.0900	0.0921	0.0908	0.0905	0.0885	0.0896	0.0879	0.0902	0.0913	0.0911	0.0918
F	6	blnk 6	0.0809	0.0814	0.0780	0.0811	0.0807	0.0818	0.0818	0.0819	0.0831	0.0822	0.0834	0.0835	0.0829	0.0825	0.0821	0.0822	0.0814	0.0801	0.0826	0.0816
F	7	blnk 7	0.0848	0.0847	0.0850	0.0863	0.0853	0.0860	0.0856	0.0855	0.0864	0.0854	0.0871	0.0868	0.0872	0.0875	0.0864	0.0859	0.0870	0.0870	0.0889	0.0882
F	8	blnk 8	0.0895	0.0900	0.0894	0.0892	0.0881	0.0881	0.0882	0.0903	0.0909	0.0868	0.0914	0.0894	0.0907	0.0905	0.0882	0.0887	0.0891	0.0905	0.0928	0.0907

Table C.3 BApNA Plate 3:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a	0.0978	0.1003	0.1042	0.1066	0.1100	0.1109	0.1151	0.1180	0.1224	0.1230	0.1274	0.1295	0.1333	0.1375	0.1408	0.1417	0.1453	0.1476	0.1520	0.1544
A	2	C1a	0.0877	0.0937	0.0975	0.1003	0.1036	0.1052	0.1109	0.1126	0.1168	0.1189	0.1212	0.1235	0.1275	0.1314	0.1354	0.1359	0.1395	0.1408	0.1454	0.1495
A	3	C1b	0.1001	0.1039	0.1064	0.1065	0.1106	0.1108	0.1132	0.1165	0.1195	0.1186	0.1218	0.1250	0.1301	0.1313	0.1312	0.1341	0.1353	0.1392	0.1411	0.1427
A	4	C1b	0.0971	0.1011	0.1029	0.1026	0.1063	0.1093	0.1142	0.1164	0.1162	0.1184	0.1230	0.1233	0.1269	0.1281	0.1296	0.1314	0.1353	0.1368	0.1386	0.1431
A	5	T1a	0.0804	0.0862	0.0850	0.0851	0.0849	0.0845	0.0882	0.0887	0.0900	0.0862	0.0890	0.0894	0.0911	0.0908	0.0893	0.0894	0.0896	0.0914	0.0897	0.0897
A	6	T1a	0.0860	0.0874	0.0866	0.0869	0.0860	0.0867	0.0903	0.0908	0.0896	0.0915	0.0913	0.0905	0.0928	0.0929	0.0911	0.0902	0.0918	0.0936	0.0929	0.0923
A	7	T1b	0.0792	0.0848	0.0826	0.0808	0.0840	0.0856	0.0866	0.0880	0.0864	0.0875	0.0885	0.0881	0.0909	0.0900	0.0889	0.0898	0.0912	0.0920	0.0923	0.0929
A	8	T1b	0.0819	0.0886	0.0887	0.0879	0.0907	0.0891	0.0915	0.0919	0.0899	0.0900	0.0935	0.0929	0.0945	0.0939	0.0947	0.0926	0.0952	0.0963	0.0952	0.0969
B	1	C2a	0.1047	0.1134	0.1202	0.1247	0.1321	0.1372	0.1457	0.1516	0.1579	0.1659	0.1710	0.1765	0.1846	0.1905	0.1997	0.2033	0.2116	0.2181	0.2243	0.2333
B	2	C2a	0.0999	0.1093	0.1152	0.1195	0.1273	0.1321	0.1401	0.1466	0.1532	0.1583	0.1669	0.1733	0.1812	0.1866	0.1928	0.1983	0.2087	0.2140	0.2217	0.2266
B	3	C2b	0.0902	0.0973	0.0982	0.0996	0.1016	0.1028	0.1081	0.1080	0.1106	0.1112	0.1146	0.1150	0.1169	0.1194	0.1218	0.1225	0.1255	0.1297	0.1301	0.1319
B	4	C2b	0.0853	0.0914	0.0928	0.0939	0.0972	0.0983	0.1026	0.1056	0.1063	0.1084	0.1098	0.1101	0.1169	0.1163	0.1203	0.1193	0.1230	0.1248	0.1274	0.1304
B	5	T2a	0.0872	0.0907	0.0915	0.0893	0.0886	0.0888	0.0922	0.0922	0.0904	0.0910	0.0925	0.0922	0.0938	0.0928	0.0931	0.0929	0.0919	0.0921	0.0928	0.0923
B	6	T2a	0.0859	0.0910	0.0879	0.0888	0.0887	0.0880	0.0920	0.0901	0.0904	0.0891	0.0938	0.0921	0.0921	0.0914	0.0929	0.0894	0.0927	0.0918	0.0923	0.0910
B	7	T2b	0.0863	0.0940	0.0955	0.0949	0.0949	0.0948	0.1013	0.1039	0.1056	0.1058	0.1131	0.1132	0.1173	0.1173	0.1194	0.1214	0.1252	0.1271	0.1285	0.1310
B	8	T2b	0.0952	0.1016	0.1024	0.1018	0.1057	0.1066	0.1107	0.1118	0.1157	0.1165	0.1223	0.1223	0.1253	0.1266	0.1308	0.1292	0.1320	0.1355	0.1387	0.1398
C	1	C3a	0.1095	0.1199	0.1264	0.1368	0.1452	0.1512	0.1626	0.1702	0.1785	0.1881	0.1975	0.2072	0.2173	0.2248	0.2354	0.2437	0.2531	0.2613	0.2712	0.2785
C	2	C3a	0.1146	0.1239	0.1314	0.1394	0.1474	0.1546	0.1647	0.1733	0.1814	0.1901	0.2001	0.2072	0.2173	0.2247	0.2349	0.2425	0.2521	0.2612	0.2693	0.2786
C	3	C3b	0.0894	0.0940	0.0964	0.0993	0.1020	0.1039	0.1068	0.1089	0.1131	0.1165	0.1178	0.1220	0.1245	0.1276	0.1307	0.1331	0.1373	0.1398	0.1430	0.1457
C	4	C3b	0.0960	0.1002	0.1037	0.1037	0.1077	0.1105	0.1160	0.1178	0.1207	0.1217	0.1258	0.1288	0.1320	0.1346	0.1393	0.1397	0.1437	0.1471	0.1506	0.1521
C	5	T3a	0.0937	0.0969	0.0961	0.0938	0.0975	0.0965	0.0993	0.0988	0.0995	0.0978	0.1011	0.0998	0.1020	0.1009	0.1038	0.1029	0.1030	0.1036	0.1041	0.1030
C	6	T3a		0.0810	0.0815	0.0808	0.0812	0.0805	0.0823	0.0838	0.0848	0.0846	0.0866	0.0843	0.0902	0.0871	0.0874	0.0870	0.0890	0.0907	0.0904	0.0907
C	7	T3b	0.0911	0.0938	0.0931	0.0931	0.0913	0.0920	0.0921	0.0947	0.0956	0.0952	0.0943	0.0935	0.0958	0.0970	0.0966	0.0957	0.0961	0.0964	0.0952	0.0964
C	8	T3b	0.0959	0.1007	0.0979	0.0962	0.0965	0.0955	0.0965	0.1001	0.0994	0.0991	0.0993	0.0971	0.1002	0.1005	0.1013	0.1006	0.1007	0.0992	0.0990	0.0988
D	1	C4a	0.1017	0.1105	0.1142	0.1171	0.1251	0.1290	0.1348	0.1420	0.1478	0.1510	0.1581	0.1636	0.1699	0.1753	0.1815	0.1866	0.1930	0.1988	0.2029	0.2095
D	2	C4a	0.1104	0.1155	0.1195	0.1255	0.1331	0.1368	0.1422	0.1500	0.1553	0.1591	0.1662	0.1701	0.1781	0.1820	0.1895	0.1923	0.1995	0.2034	0.2093	0.2147
D	3	C4b	0.0969	0.1047	0.1088	0.1138	0.1213	0.1242	0.1324	0.1380	0.1404	0.1474	0.1544	0.1591	0.1661	0.1717	0.1764	0.1814	0.1889	0.1920	0.1993	0.2035
D	4	C4b	0.1077	0.1133	0.1160	0.1203	0.1287	0.1335	0.1410	0.1462	0.1505	0.1553	0.1625	0.1673	0.1731	0.1791	0.1842	0.1891	0.1962	0.2019	0.2068	0.2125

Table C.3 BApNA Plate 3 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
D	5	T4a	0.1099	0.1209	0.1301	0.1393	0.1486	0.1559	0.1693	0.1774	0.1872	0.1958	0.2061	0.2174	0.2269	0.2358	0.2470	0.2563	0.2651	0.2738	0.2858	0.2945
D	6	T4a	0.1151	0.1258	0.1348	0.1436	0.1525	0.1590	0.1702	0.1776	0.1887	0.1959	0.2056	0.2139	0.2265	0.2334	0.2447	0.2556	0.2660	0.2740	0.2844	0.2932
D	7	T4b	0.0910	0.0961	0.0984	0.0978	0.1014	0.1028	0.1079	0.1092	0.1110	0.1123	0.1154	0.1188	0.1212	0.1213	0.1257	0.1257	0.1273	0.1299	0.1314	0.1333
D	8	T4b	0.0831	0.0894	0.0922	0.0909	0.0970	0.0955	0.1008	0.1018	0.1041	0.1053	0.1088	0.1103	0.1142	0.1169	0.1170	0.1209	0.1235	0.1266	0.1294	0.1289
E	1	C5a	0.1113	0.1203	0.1276	0.1355	0.1424	0.1492	0.1594	0.1668	0.1774	0.1847	0.1956	0.2021	0.2124	0.2207	0.2300	0.2362	0.2466	0.2559	0.2636	0.2714
E	2	C5a	0.1085	0.1187	0.1261	0.1323	0.1404	0.1477	0.1566	0.1636	0.1744	0.1809	0.1904	0.2007	0.2127	0.2174	0.2267	0.2362	0.2426	0.2559	0.2624	0.2729
E	3	C5b	0.1054	0.1144	0.1174	0.1235	0.1290	0.1354	0.1438	0.1475	0.1554	0.1615	0.1701	0.1747	0.1840	0.1907	0.1955	0.2018	0.2095	0.2142	0.2229	0.2316
E	4	C5b	0.1029	0.1110	0.1170	0.1206	0.1277	0.1327	0.1412	0.1477	0.1540	0.1598	0.1689	0.1729	0.1836	0.1893	0.1964	0.2035	0.2121	0.2159	0.2236	0.2321
E	5	T5a	0.1116	0.1188	0.1214	0.1253	0.1324	0.1375	0.1444	0.1494	0.1545	0.1601	0.1663	0.1727	0.1813	0.1846	0.1944	0.1946	0.2029	0.2103	0.2138	0.2210
E	6	T5a	0.1038	0.1139	0.1170	0.1208	0.1251	0.1338	0.1390	0.1444	0.1489	0.1540	0.1594	0.1648	0.1733	0.1765	0.1844	0.1886	0.1985	0.2010	0.2063	0.2110
E	7	T5b	0.0864	0.0889	0.0912	0.0933	0.0934	0.0924	0.0957	0.0981	0.0990	0.0999	0.1010	0.1026	0.1053	0.1057	0.1094	0.1096	0.1100	0.1114	0.1123	0.1140
E	8	T5b	0.0928	0.0951	0.0972	0.0948	0.0969	0.0986	0.1011	0.1017	0.1051	0.1061	0.1072	0.1090	0.1112	0.1127	0.1129	0.1133	0.1167	0.1171	0.1178	0.1200
F	1	blnk 1	0.0859	0.0857	0.0861	0.0841	0.0867	0.0858	0.0869	0.0880	0.0873	0.0874	0.0875	0.0882	0.0918	0.0867	0.0885	0.0872	0.0892	0.0882	0.0889	0.0874
F	2	blnk 2	0.0886	0.0898	0.0874	0.0878	0.0894	0.0894	0.0906	0.0914	0.0903	0.0915	0.0924	0.0900	0.0930	0.0920	0.0933	0.0907	0.0914	0.0910	0.0904	0.0916
F	3	blnk 3	0.0822	0.0842	0.0849	0.0826	0.0851	0.0844	0.0872	0.0873	0.0878	0.0863	0.0887	0.0871	0.0897	0.0878	0.0890	0.0872	0.0884	0.0876	0.0883	0.0902
F	4	blnk 4	0.0943	0.0978	0.0942	0.0920	0.0958	0.0931	0.0949	0.0964	0.0954	0.0950	0.0957	0.0946	0.0970	0.0954	0.0968	0.0941	0.0963	0.0961	0.0955	0.0942
F	5	blnk 5	0.0982	0.0985	0.0987	0.0951	0.0961	0.0953	0.0979	0.0980	0.0982	0.0990	0.0997	0.0978	0.0995	0.0991	0.0995	0.0974	0.0984	0.0965	0.0971	0.0958
F	6	blnk 6	0.0820	0.0840	0.0833	0.0814	0.0809	0.0814	0.0826	0.0853	0.0862	0.0838	0.0887	0.0853	0.0874	0.0858	0.0881	0.0877	0.0885	0.0862	0.0845	0.0824
F	7	blnk 7	0.0883	0.0903	0.0885	0.0880	0.0887	0.0873	0.0898	0.0921	0.0903	0.0890	0.0916	0.0929	0.0911	0.0905	0.0914	0.0900	0.0918	0.0905	0.0903	0.0905
F	8	blnk 8	0.0936	0.0949	0.0939	0.0915	0.0936	0.0924	0.0957	0.0936	0.0934	0.0949	0.0967	0.0945	0.0968	0.0953	0.0952	0.0941	0.0968	0.0948	0.0928	0.0930

Table C.4 SAAPPpNA Plate 1:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a	0.1807	0.2279	0.2706	0.3210	0.3704	0.4182	0.4680	0.5208	0.5713	0.6263	0.6780	0.7302	0.7858	0.8406	0.8938	0.9475	1.0027	1.0589	1.1121	1.1694
A	2	C1a	0.1696	0.2171	0.2636	0.3107	0.3583	0.4094	0.4593	0.5110	0.5591	0.6148	0.6671	0.7193	0.7728	0.8282	0.8830	0.9350	0.9898	1.0455	1.1003	1.1578
A	3	C1b	0.1713	0.2084	0.2459	0.2874	0.3307	0.3695	0.4130	0.4576	0.4980	0.5435	0.5908	0.6348	0.6813	0.7311	0.7753	0.8218	0.8676	0.9157	0.9643	1.0166
A	4	C1b	0.1667	0.2043	0.2405	0.2798	0.3197	0.3588	0.4028	0.4441	0.4858	0.5296	0.5748	0.6175	0.6622	0.7105	0.7553	0.7996	0.8476	0.8943	0.9386	0.9899
A	5	T1a	0.0830	0.0909	0.0953	0.1028	0.1095	0.1161	0.1253	0.1335	0.1406	0.1519	0.1586	0.1686	0.1768	0.1885	0.1971	0.2066	0.2159	0.2255	0.2347	0.2492
A	6	T1a		0.0988	0.1016	0.1075	0.1153	0.1238	0.1305	0.1394	0.1449	0.1554	0.1638	0.1708	0.1800	0.1913	0.1988	0.2113	0.2190	0.2295	0.2385	0.2514
A	7	T1b	0.0801	0.0880	0.0914	0.1009	0.1062	0.1170	0.1241	0.1333	0.1428	0.1514	0.1636	0.1715	0.1807	0.1916	0.2007	0.2131	0.2221	0.2344	0.2445	0.2584
A	8	T1b	0.0857	0.0941	0.1011	0.1086	0.1170	0.1235	0.1326	0.1402	0.1481	0.1578	0.1700	0.1773	0.1872	0.1975	0.2062	0.2181	0.2386	0.2412	0.2503	0.2626
B	1	C2a	0.2308	0.3016	0.3723	0.4459	0.5207	0.5977	0.6749	0.7532	0.8330	0.9142	0.9935	1.0756	1.1565	1.2435	1.3273	1.4065	1.4899	1.5739	1.6539	1.7372
B	2	C2a	0.2245	0.2981	0.3694	0.4408	0.5137	0.5918	0.6675	0.7456	0.8240	0.9038	0.9869	1.0638	1.1461	1.2314	1.3104	1.3915	1.4740	1.5566	1.6368	1.7224
B	3	C2b	0.1380	0.1681	0.1976	0.2316	0.2633	0.2973	0.3328	0.3674	0.4034	0.4413	0.4772	0.5150	0.5544	0.5946	0.6316	0.6727	0.7125	0.7535	0.7938	0.8352
B	4	C2b	0.1404	0.1734	0.2072	0.2433	0.2788	0.3154	0.3555	0.3914	0.4319	0.4734	0.5127	0.5540	0.5971	0.6412	0.6812	0.7261	0.7692	0.8150	0.8589	0.9049
B	5	T2a	0.0879	0.0848	0.0850	0.0874	0.0868	0.0888	0.0914	0.0930	0.0961	0.0978	0.0979	0.1001	0.1011	0.1058	0.1054	0.1088	0.1099	0.1110	0.1120	0.1179
B	6	T2a	0.0845	0.0836	0.0828	0.0873	0.0871	0.0879	0.0903	0.0923	0.0927	0.0963	0.0968	0.0975	0.0999	0.1047	0.1059	0.1081	0.1098	0.1110	0.1114	0.1156
B	7	T2b	0.1377	0.1689	0.2000	0.2357	0.2687	0.3055	0.3419	0.3805	0.4165	0.4571	0.4946	0.5342	0.5734	0.6181	0.6563	0.6992	0.7415	0.7841	0.8249	0.8718
B	8	T2b	0.1446	0.1803	0.2128	0.2495	0.2836	0.3226	0.3601	0.4001	0.4388	0.4815	0.5214	0.5635	0.6060	0.6504	0.6953	0.7386	0.7823	0.8261	0.8705	0.9199
C	1	C3a	0.3624	0.5005	0.6372	0.7806	0.9230	1.0689	1.2189	1.3682	1.5149	1.6683	1.8158	1.9661	2.1124	2.2614	2.4032	2.5415	2.6795	2.8143	2.9302	3.0725
C	2	C3a	0.3609	0.4929	0.6240	0.7612	0.8940	1.0333	1.1711	1.3126	1.4511	1.5932	1.7311	1.8708	2.0117	2.1513	2.2872	2.4234	2.5567	2.6808	2.7933	2.9362
C	3	C3b	0.1592	0.2001	0.2416	0.2881	0.3327	0.3802	0.4299	0.4798	0.5298	0.5828	0.6354	0.6890	0.7447	0.7993	0.8542	0.9090	0.9675	1.0245	1.0814	1.1411
C	4	C3b	0.1678	0.2137	0.2547	0.3022	0.3475	0.3960	0.4475	0.4979	0.5504	0.6028	0.6564	0.7101	0.7646	0.8232	0.8768	0.9364	0.9941	1.0519	1.1093	1.1712
C	5	T3a	0.1027	0.1086	0.1165	0.1236	0.1315	0.1428	0.1518	0.1607	0.1680	0.1792	0.1897	0.1994	0.2080	0.2209	0.2301	0.2365	0.2631	0.2583	0.2662	0.2810
C	6	T3a	0.0811	0.0921	0.0968	0.1075	0.1159	0.1260	0.1379	0.1450	0.1565	0.1696	0.1765	0.1875	0.1983	0.2114	0.2199	0.2312	0.2426	0.2513	0.2646	0.2793
C	7	T3b	0.0873	0.0897	0.0919	0.0945	0.0965	0.1013	0.1050	0.1068	0.1122	0.1160	0.1185	0.1212	0.1251	0.1314	0.1313	0.1354	0.1412	0.1447	0.1483	0.1517
C	8	T3b	0.0940	0.0959	0.0973	0.1014	0.1037	0.1091	0.1122	0.1163	0.1172	0.1213	0.1240	0.1267	0.1299	0.1360	0.1391	0.1426	0.1459	0.1500	0.1528	0.1572
D	1	C4a	0.2633	0.3520	0.4418	0.5411	0.6359	0.7368	0.8387	0.9428	1.0482	1.1538	1.2601	1.3673	1.4773	1.5910	1.6955	1.8100	1.9158	2.0280	2.1304	2.2422
D	2	C4a	0.2578	0.3405	0.4215	0.5134	0.5995	0.6892	0.7832	0.8779	0.9744	1.0708	1.1665	1.2663	1.3661	1.4696	1.5681	1.6700	1.7684	1.8683	1.9657	2.0734
D	3	C4b	0.2364	0.3149	0.3932	0.4763	0.5601	0.6446	0.7386	0.8223	0.9120	1.0018	1.0931	1.1831	1.2782	1.3754	1.4643	1.5609	1.6559	1.7486	1.8394	1.9430
D	4	C4b	0.2399	0.3157	0.3953	0.4744	0.5590	0.6431	0.7292	0.8180	0.9072	0.9992	1.0861	1.1757	1.2714	1.3661	1.4542	1.5533	1.6457	1.7395	1.8309	1.9299

Table C.4 SAAPPpNA Plate 1 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
D	5	T4a	0.3578	0.4942	0.6304	0.7740	0.9130	1.0612	1.2101	1.3578	1.5074	1.6573	1.8047	1.9511	2.1020	2.2568	2.3931	2.5474	2.6799	2.8156	2.9455	3.0943
D	6	T4a	0.3454	0.4760	0.6085	0.7444	0.8808	1.0190	1.1620	1.3039	1.4461	1.5909	1.7329	1.8762	2.0205	2.1671	2.3072	2.4475	2.5795	2.7153	2.8406	2.9791
D	7	T4b	0.1342	0.1604	0.1853	0.2161	0.2438	0.2750	0.3059	0.3364	0.3676	0.4029	0.4329	0.4641	0.4977	0.5347	0.5656	0.5989	0.6340	0.6683	0.7040	0.7416
D	8	T4b	0.1234	0.1482	0.1722	0.2019	0.2294	0.2591	0.2895	0.3197	0.3526	0.3837	0.4155	0.4493	0.4804	0.5182	0.5494	0.5853	0.6196	0.6576	0.6890	0.7269
E	1	C5a	0.3187	0.4333	0.5521	0.6766	0.8036	0.9299	1.0633	1.1934	1.3296	1.4653	1.6000	1.7372	1.8770	2.0179	2.1510	2.2880	2.4177	2.5515	2.6714	2.8161
E	2	C5a	0.3030	0.4100	0.5196	0.6364	0.7520	0.8720	0.9943	1.1175	1.2410	1.3669	1.4953	1.6223	1.7530	1.8851	2.0111	2.1375	2.2602	2.3909	2.5025	2.6430
E	3	C5b	0.3222	0.4328	0.5501	0.6719	0.7926	0.9167	1.0425	1.1694	1.2987	1.4262	1.5552	1.6836	1.8118	1.9445	2.0736	2.1979	2.3282	2.4518	2.5631	2.7036
E	4	C5b	0.3108	0.4240	0.5426	0.6630	0.7844	0.9082	1.0336	1.1597	1.2878	1.4199	1.5476	1.6770	1.8086	1.9372	2.0673	2.1971	2.3229	2.4456	2.5644	2.7002
E	5	T5a	0.2328	0.3037	0.3768	0.4546	0.5306	0.6103	0.6939	0.7786	0.8637	0.9499	1.0372	1.1282	1.2178	1.3100	1.3994	1.4916	1.5876	1.6782	1.7705	1.8693
E	6	T5a	0.2261	0.2926	0.3625	0.4369	0.5126	0.5897	0.6727	0.7534	0.8375	0.9230	1.0092	1.0940	1.1837	1.2745	1.3650	1.4517	1.5436	1.6351	1.7254	1.8233
E	7	T5b	0.1315	0.1507	0.1733	0.2007	0.2263	0.2528	0.2797	0.3062	0.3332	0.3635	0.3909	0.4190	0.4483	0.4802	0.5100	0.5402	0.5699	0.6013	0.6309	0.6648
E	8	T5b	0.1366	0.1588	0.1831	0.2121	0.2388	0.2693	0.2982	0.3272	0.3569	0.3898	0.4204	0.4506	0.4824	0.5165	0.5494	0.5812	0.6136	0.6476	0.6807	0.7171
F	1	blnk 1	0.0793	0.0811	0.0774	0.0788	0.0820	0.0811	0.0820	0.0816	0.0816	0.0841	0.0810	0.0830	0.0820	0.0850	0.0825	0.0830	0.0835	0.0819	0.0814	0.0848
F	2	blnk 2	0.0848	0.0836	0.0844	0.0839	0.0858	0.0846	0.0862	0.0871	0.0848	0.0864	0.0866	0.0872	0.0872	0.0892	0.0863	0.0868	0.0858	0.0875	0.0864	0.0877
F	3	blnk 3	0.0820	0.0809	0.0789	0.0803	0.0812	0.0801	0.0825	0.0838	0.0814	0.0844	0.0818	0.0818	0.0820	0.0835	0.0827	0.0824	0.0833	0.0828	0.0824	0.0837
F	4	blnk 4	0.0845	0.0829	0.0810	0.0830	0.0809	0.0814	0.0832	0.0822	0.0835	0.0844	0.0835	0.0820	0.0825	0.0862	0.0839	0.0849	0.0842	0.0861	0.0830	0.0861
F	5	blnk 5	0.0926	0.0895	0.0876	0.0902	0.0903	0.0913	0.0893	0.0929	0.0893	0.0913	0.0906	0.0913	0.0901	0.0911	0.0905	0.0906	0.0895	0.0918	0.0884	0.0911
F	6	blnk 6	0.0803	0.0780	0.0772	0.0779	0.0775	0.0774	0.0811	0.0801	0.0798	0.0785	0.0805	0.0812	0.0794	0.0828	0.0787	0.0786	0.0819	0.0831	0.0789	0.0827
F	7	blnk 7	0.0849	0.0833	0.0820	0.0841	0.0816	0.0844	0.0840	0.0843	0.0833	0.0841	0.0853	0.0839	0.0843	0.0875	0.0840	0.0835	0.0844	0.0845	0.0828	0.0868
F	8	blnk 8	0.0963	0.0919	0.0911	0.0908	0.0891	0.0919	0.0914	0.0914	0.0909	0.0916	0.0924	0.0894	0.0910	0.0926	0.0925	0.0907	0.0908	0.0905	0.0909	0.0926

Table C.5 SAAPPpNA Plate 2:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a	0.1868	0.2312	0.2755	0.3216	0.3710	0.4170	0.4684	0.5200	0.5703	0.6219	0.6743	0.7264	0.7785	0.8309	0.8836	0.9381	0.9946	1.0466	1.0994	1.1547
A	2	C1a	0.1800	0.2250	0.2684	0.3193	0.3660	0.4144	0.4657	0.5178	0.5683	0.6204	0.6722	0.7264	0.7808	0.8326	0.8885	0.9421	0.9985	1.0547	1.1052	1.1608
A	3	C1b	0.1783	0.2179	0.2562	0.3007	0.3426	0.3830	0.4220	0.4657	0.5128	0.5558	0.5987	0.6441	0.6912	0.7384	0.7845	0.8305	0.8786	0.9283	0.9744	1.0233
A	4	C1b	0.1743	0.2108	0.2482	0.2922	0.3311	0.3729	0.4163	0.4600	0.5036	0.5466	0.5916	0.6364	0.6842	0.7293	0.7754	0.8232	0.8721	0.9208	0.9666	1.0156
A	5	T1a	0.0871	0.0935	0.0988	0.1050	0.1106	0.1180	0.1246	0.1324	0.1400	0.1512	0.1575	0.1667	0.1755	0.1852	0.1928	0.2035	0.2141	0.2239	0.2334	0.2431
A	6	T1a	0.0905	0.0937	0.0989	0.1076	0.1127	0.1200	0.1275	0.1350	0.1448	0.1547	0.1618	0.1677	0.1772	0.1871	0.1969	0.2087	0.2161	0.2277	0.2355	0.2445
A	7	T1b	0.0803	0.0909	0.0961	0.1067	0.1139	0.1202	0.1314	0.1385	0.1473	0.1601	0.1691	0.1785	0.1875	0.1986	0.2084	0.2189	0.2316	0.2420	0.2536	0.2651
A	8	T1b	0.0866	0.0926	0.0989	0.1069	0.1128	0.1207	0.1290	0.1390	0.1480	0.1587	0.1672	0.1757	0.1887	0.1982	0.2070	0.2195	0.2309	0.2422	0.2527	0.2645
B	1	C2a	0.2773	0.3619	0.4518	0.5450	0.6362	0.7309	0.8251	0.9237	1.0230	1.1234	1.2196	1.3212	1.4206	1.5171	1.6182	1.7171	1.8190	1.9184	2.0148	2.1120
B	2	C2a	0.2880	0.3789	0.4717	0.5658	0.6614	0.7590	0.8557	0.9561	1.0590	1.1596	1.2640	1.3614	1.4660	1.5672	1.6688	1.7717	1.8744	1.9793	2.0734	2.1728
B	3	C2b	0.1450	0.1733	0.2029	0.2304	0.2623	0.2945	0.3276	0.3634	0.3990	0.4349	0.4702	0.5069	0.5455	0.5816	0.6188	0.6594	0.6981	0.7378	0.7749	0.8165
B	4	C2b	0.1373	0.1644	0.1922	0.2199	0.2496	0.2795	0.3108	0.3449	0.3787	0.4117	0.4451	0.4802	0.5167	0.5508	0.5858	0.6244	0.6615	0.6990	0.7349	0.7733
B	5	T2a	0.0823	0.0841	0.0857	0.0876	0.0895	0.0909	0.0909	0.0940	0.0970	0.0996	0.0990	0.1033	0.1044	0.1059	0.1064	0.1096	0.1110	0.1159	0.1144	0.1176
B	6	T2a	0.0821	0.0838	0.0846	0.0855	0.0872	0.0876	0.0892	0.0912	0.0951	0.0971	0.0992	0.1010	0.1023	0.1045	0.1027	0.1079	0.1097	0.1109	0.1110	0.1151
B	7	T2b	0.1411	0.1718	0.2051	0.2402	0.2733	0.3088	0.3456	0.3810	0.4198	0.4593	0.4953	0.5359	0.5794	0.6165	0.6563	0.7012	0.7437	0.7857	0.8247	0.8678
B	8	T2b	0.1439	0.1794	0.2114	0.2454	0.2789	0.3156	0.3527	0.3909	0.4297	0.4694	0.5080	0.5492	0.5901	0.6310	0.6708	0.7163	0.7610	0.8032	0.8443	0.8905
C	1	C3a	0.3852	0.5204	0.6605	0.8047	0.9502	1.0968	1.2466	1.3949	1.5476	1.6989	1.8501	2.0020	2.1493	2.2926	2.4435	2.5868	2.7219	2.8512	2.9832	3.1217
C	2	C3a	0.3971	0.5328	0.6707	0.8124	0.9557	1.1012	1.2444	1.3934	1.5410	1.6888	1.8326	1.9799	2.1237	2.2666	2.4162	2.5575	2.6879	2.8176	2.9474	3.0796
C	3	C3b	0.1663	0.2096	0.2512	0.2989	0.3438	0.3903	0.4419	0.4917	0.5434	0.5981	0.6505	0.7018	0.7584	0.8133	0.8709	0.9263	0.9880	1.0450	1.1018	1.1615
C	4	C3b	0.1736	0.2166	0.2584	0.3045	0.3480	0.3965	0.4463	0.4986	0.5493	0.6014	0.6540	0.7090	0.7652	0.8190	0.8741	0.9337	0.9883	1.0471	1.1046	1.1646
C	5	T3a	0.1041	0.1122	0.1188	0.1266	0.1338	0.1439	0.1488	0.1595	0.1708	0.1786	0.1887	0.1982	0.2079	0.2154	0.2256	0.2364	0.2477	0.2574	0.2633	0.2749
C	6	T3a	0.0862	0.0940	0.1006	0.1107	0.1177	0.1285	0.1352	0.1463	0.1562	0.1662	0.1753	0.1864	0.1963	0.2056	0.2165	0.2260	0.2396	0.2495	0.2602	0.2694
C	7	T3b	0.0906	0.0947	0.0954	0.0976	0.1020	0.1060	0.1068	0.1124	0.1153	0.1193	0.1204	0.1251	0.1291	0.1325	0.1373	0.1410	0.1467	0.1512	0.1497	0.1540
C	8	T3b	0.0900	0.0958	0.0963	0.1014	0.1028	0.1059	0.1105	0.1127	0.1186	0.1212	0.1236	0.1279	0.1315	0.1326	0.1367	0.1417	0.1463	0.1508	0.1533	0.1558
D	1	C4a	0.2665	0.3519	0.4408	0.5352	0.6269	0.7251	0.8229	0.9259	1.0256	1.1293	1.2350	1.3414	1.4481	1.5509	1.6612	1.7674	1.8760	1.9805	2.0857	2.1939
D	2	C4a	0.2761	0.3603	0.4472	0.5391	0.6318	0.7283	0.8228	0.9222	1.0242	1.1232	1.2251	1.3305	1.4354	1.5407	1.6426	1.7513	1.8763	1.9815	2.0714	2.1681
D	3	C4b	0.2450	0.3237	0.4029	0.4864	0.5676	0.6567	0.7416	0.8309	0.9213	1.0128	1.1040	1.1961	1.2897	1.3832	1.4762	1.5731	1.6702	1.7631	1.8572	1.9553
D	4	C4b	0.2528	0.3280	0.4080	0.4923	0.5760	0.6619	0.7512	0.8413	0.9304	1.0232	1.1128	1.2071	1.3011	1.3922	1.4895	1.5869	1.6819	1.7773	1.8737	1.9700
D	5	T4a	0.3610	0.4880	0.6174	0.7535	0.8895	1.0252	1.1700	1.3105	1.4532	1.5983	1.7387	1.8870	2.0269	2.1731	2.3128	2.4552	2.5959	2.7313	2.8620	2.9895

Table C.5 SAAPPpNA Plate 2 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
D	6	T4a	0.3574	0.4890	0.6194	0.7555	0.8901	1.0271	1.1689	1.3111	1.4543	1.5953	1.7386	1.8804	2.0274	2.1704	2.3099	2.4583	2.5886	2.7253	2.8520	2.9774
D	7	T4b	0.1392	0.1666	0.1924	0.2222	0.2530	0.2811	0.3115	0.3429	0.3781	0.4091	0.4407	0.4730	0.5087	0.5413	0.5759	0.6115	0.6469	0.6809	0.7162	0.7504
D	8	T4b	0.1316	0.1567	0.1832	0.2118	0.2404	0.2718	0.2990	0.3322	0.3642	0.3965	0.4312	0.4646	0.4971	0.5318	0.5652	0.6026	0.6374	0.6740	0.7094	0.7430
E	1	C5a	0.3357	0.4516	0.5688	0.6912	0.8173	0.9461	1.0778	1.2115	1.3452	1.4807	1.6179	1.7553	1.8948	2.0306	2.1715	2.3072	2.4415	2.5779	2.7055	2.8247
E	2	C5a	0.3239	0.4390	0.5519	0.6738	0.7928	0.9154	1.0410	1.1695	1.2994	1.4292	1.5614	1.6928	1.8267	1.9563	2.0931	2.2245	2.3508	2.4854	2.6115	2.7255
E	3	C5b	0.3419	0.4522	0.5710	0.6926	0.8153	0.9412	1.0677	1.1959	1.3241	1.4567	1.5866	1.7161	1.8479	1.9788	2.1114	2.2424	2.3734	2.5018	2.6191	2.7332
E	4	C5b	0.3293	0.4474	0.5650	0.6870	0.8107	0.9351	1.0622	1.1908	1.3212	1.4521	1.5831	1.7142	1.8486	1.9766	2.1090	2.2465	2.3706	2.5014	2.6273	2.7374
E	5	T5a	0.2420	0.3137	0.3850	0.4590	0.5344	0.6157	0.6953	0.7791	0.8641	0.9495	1.0356	1.1256	1.2171	1.3040	1.3945	1.4894	1.5806	1.6720	1.7640	1.8545
E	6	T5a	0.2327	0.3041	0.3741	0.4488	0.5261	0.6051	0.6849	0.7666	0.8533	0.9381	1.0249	1.1140	1.2030	1.2918	1.3821	1.4715	1.5648	1.6563	1.7477	1.8380
E	7	T5b	0.1283	0.1492	0.1709	0.1990	0.2202	0.2452	0.2708	0.2983	0.3232	0.3518	0.3773	0.4067	0.4363	0.4606	0.4895	0.5199	0.5488	0.5810	0.6080	0.6387
E	8	T5b	0.1295	0.1543	0.1749	0.1984	0.2223	0.2469	0.2709	0.2975	0.3241	0.3512	0.3765	0.4034	0.4324	0.4593	0.4878	0.5173	0.5461	0.5751	0.6009	0.6312
F	1	blnk 1	0.0794	0.0801	0.0797	0.0808	0.0794	0.0812	0.0802	0.0805	0.0827	0.0845	0.0835	0.0828	0.0830	0.0836	0.0811	0.0829	0.0846	0.0832	0.0821	0.0856
F	2	blnk 2	0.0836	0.0830	0.0827	0.0853	0.0843	0.0850	0.0859	0.0865	0.0882	0.0877	0.0861	0.0868	0.0882	0.0872	0.0858	0.0884	0.0882	0.0893	0.0878	0.0887
F	3	blnk 3	0.0786	0.0784	0.0796	0.0797	0.0796	0.0802	0.0797	0.0807	0.0819	0.0816	0.0804	0.0803	0.0824	0.0797	0.0824	0.0812	0.0836	0.0839	0.0824	0.0830
F	4	blnk 4	0.0830	0.0833	0.0826	0.0824	0.0829	0.0839	0.0824	0.0839	0.0835	0.0843	0.0839	0.0835	0.0853	0.0845	0.0838	0.0841	0.0857	0.0865	0.0844	0.0846
F	5	blnk 5	0.0907	0.0891	0.0896	0.0893	0.0894	0.0884	0.0884	0.0889	0.0906	0.0925	0.0920	0.0888	0.0908	0.0890	0.0902	0.0905	0.0934	0.0929	0.0900	0.0902
F	6	blnk 6	0.0768	0.0781	0.0787	0.0773	0.0785	0.0790	0.0774	0.0801	0.0792	0.0790	0.0798	0.0800	0.0785	0.0800	0.0787	0.0800	0.0821	0.0817	0.0815	0.0808
F	7	blnk 7	0.0842	0.0849	0.0861	0.0838	0.0827	0.0849	0.0862	0.0846	0.0864	0.0841	0.0851	0.0864	0.0855	0.0848	0.0857	0.0854	0.0869	0.0869	0.0858	0.0853
F	8	blnk 8	0.0901	0.0908	0.0902	0.0911	0.0905	0.0899	0.0899	0.0893	0.0899	0.0901	0.0902	0.0911	0.0912	0.0891	0.0891	0.0922	0.0919	0.0927	0.0916	0.0904

Table C.6 SAAPPpNA Plate 3:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a	0.1921	0.2390	0.2845	0.3318	0.3802	0.4283	0.4762	0.5262	0.5766	0.6304	0.6814	0.7357	0.7844	0.8381	0.8929	0.9464	1.0003	1.0509	1.1095	1.1611
A	2	C1a	0.1807	0.2275	0.2747	0.3197	0.3694	0.4167	0.4647	0.5118	0.5630	0.6168	0.6662	0.7187	0.7714	0.8238	0.8765	0.9312	0.9848	1.0374	1.0918	1.1447
A	3	C1b	0.1811	0.2205	0.2562	0.2946	0.3368	0.3758	0.4218	0.4593	0.5026	0.5490	0.5911	0.6397	0.6839	0.7293	0.7747	0.8210	0.8696	0.9156	0.9644	1.0090
A	4	C1b	0.1754	0.2148	0.2523	0.2901	0.3334	0.3736	0.4166	0.4586	0.5029	0.5488	0.5911	0.6383	0.6828	0.7288	0.7759	0.8219	0.8705	0.9168	0.9649	1.0095
A	5	T1a	0.0811	0.0912	0.0977	0.1035	0.1140	0.1190	0.1274	0.1324	0.1408	0.1524	0.1590	0.1683	0.1750	0.1862	0.1956	0.2066	0.2160	0.2246	0.2343	0.2404
A	6	T1a	0.1027	0.1074	0.1122	0.1186	0.1258	0.1326	0.1385	0.1454	0.1536	0.1640	0.1704	0.1804	0.1883	0.1970	0.2050	0.2159	0.2273	0.2351	0.2458	0.2507
A	7	T1b	0.0836	0.0927	0.1001	0.1063	0.1169	0.1244	0.1324	0.1384	0.1507	0.1621	0.1703	0.1802	0.1897	0.1996	0.2105	0.2196	0.2353	0.2431	0.2570	0.2666
A	8	T1b	0.0852	0.0944	0.1019	0.1086	0.1191	0.1297	0.1368	0.1423	0.1523	0.1651	0.1762	0.1855	0.1960	0.2057	0.2141	0.2260	0.2399	0.2500	0.2626	0.2720
B	1	C2a	0.2639	0.3410	0.4193	0.5010	0.5849	0.6669	0.7530	0.8370	0.9249	1.0156	1.1020	1.1908	1.2821	1.3706	1.4620	1.5518	1.6454	1.7304	1.8232	1.9102
B	2	C2a	0.2695	0.3519	0.4330	0.5168	0.6035	0.6910	0.7766	0.8641	0.9530	1.0447	1.1349	1.2268	1.3203	1.4104	1.5059	1.5966	1.6891	1.7819	1.8756	1.9613
B	3	C2b	0.1522	0.1859	0.2194	0.2530	0.2897	0.3275	0.3621	0.3985	0.4369	0.4786	0.5171	0.5577	0.5978	0.6403	0.6807	0.7236	0.7669	0.8077	0.8510	0.8939
B	4	C2b	0.1480	0.1865	0.2211	0.2564	0.2968	0.3356	0.3715	0.4098	0.4504	0.4934	0.5341	0.5776	0.6177	0.6624	0.7070	0.7502	0.7960	0.8396	0.8866	0.9293
B	5	T2a	0.0824	0.0878	0.0869	0.0897	0.0927	0.0957	0.0949	0.0947	0.0977	0.1026	0.1018	0.1041	0.1058	0.1088	0.1092	0.1118	0.1148	0.1158	0.1193	0.1180
B	6	T2a	0.0790	0.0833	0.0853	0.0876	0.0916	0.0935	0.0934	0.0930	0.0965	0.0999	0.0997	0.1033	0.1050	0.1080	0.1089	0.1091	0.1129	0.1127	0.1173	0.1199
B	7	T2b	0.1509	0.1882	0.2253	0.2594	0.3014	0.3398	0.3770	0.4141	0.4572	0.5024	0.5435	0.5874	0.6307	0.6746	0.7190	0.7653	0.8108	0.8555	0.9052	0.9502
B	8	T2b	0.1549	0.1898	0.2263	0.2620	0.3047	0.3430	0.3814	0.4189	0.4607	0.5074	0.5468	0.5933	0.6369	0.6817	0.7256	0.7702	0.8165	0.8621	0.9087	0.9549
C	1	C3a	0.4117	0.5605	0.7059	0.8529	1.0091	1.1611	1.3153	1.4669	1.6243	1.7855	1.9404	2.0973	2.2520	2.4065	2.5594	2.7017	2.8454	2.9900	3.1292	3.2522
C	2	C3a	0.4082	0.5496	0.6909	0.8338	0.9795	1.1276	1.2703	1.4173	1.5642	1.7149	1.8610	2.0083	2.1593	2.3044	2.4509	2.5846	2.7273	2.8565	2.9926	3.1181
C	3	C3b	0.1706	0.2147	0.2605	0.3058	0.3549	0.4042	0.4520	0.5019	0.5554	0.6117	0.6646	0.7190	0.7730	0.8290	0.8867	0.9463	1.0013	1.0599	1.1213	1.1792
C	4	C3b	0.1758	0.2198	0.2622	0.3087	0.3557	0.4066	0.4535	0.5015	0.5547	0.6096	0.6613	0.7162	0.7705	0.8266	0.8835	0.9382	0.9968	1.0522	1.1111	1.1717
C	5	T3a	0.1135	0.1151	0.1209	0.1302	0.1415	0.1485	0.1586	0.1678	0.1770	0.1890	0.1994	0.2092	0.2179	0.2271	0.2360	0.2482	0.2594	0.2698	0.2829	0.2914
C	6	T3a	0.0930	0.0993	0.1056	0.1140	0.1250	0.1323	0.1401	0.1473	0.1561	0.1683	0.1771	0.1857	0.1946	0.2040	0.2118	0.2225	0.2325	0.2424	0.2528	0.2611
C	7	T3b	0.0892	0.0917	0.0945	0.0994	0.1048	0.1076	0.1088	0.1088	0.1139	0.1236	0.1236	0.1292	0.1313	0.1360	0.1402	0.1406	0.1476	0.1490	0.1558	0.1561
C	8	T3b	0.0913	0.0958	0.0986	0.0999	0.1058	0.1107	0.1132	0.1131	0.1171	0.1233	0.1284	0.1318	0.1350	0.1390	0.1408	0.1441	0.1509	0.1509	0.1589	0.1592
D	1	C4a	0.2791	0.3716	0.4618	0.5554	0.6513	0.7517	0.8529	0.9537	1.0578	1.1632	1.2682	1.3784	1.4848	1.5939	1.7025	1.8122	1.9262	2.0281	2.1398	2.2452
D	2	C4a	0.2819	0.3718	0.4616	0.5532	0.6493	0.7473	0.8400	0.9375	1.0392	1.1408	1.2418	1.3485	1.4519	1.5574	1.6621	1.7647	1.8775	1.9755	2.0858	2.1858
D	3	C4b	0.2512	0.3320	0.4099	0.4929	0.5803	0.6653	0.7518	0.8375	0.9270	1.0181	1.1114	1.2025	1.2951	1.3897	1.4823	1.5745	1.6745	1.7664	1.8612	1.9558
D	4	C4b	0.2599	0.3400	0.4211	0.5036	0.5941	0.6790	0.7653	0.8567	0.9469	1.0425	1.1343	1.2304	1.3227	1.4195	1.5133	1.6096	1.7086	1.8046	1.9011	1.9932

Table C.6 SAAPPpNA Plate 3 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
D	5	T4a	0.3776	0.5118	0.6450	0.7823	0.9256	1.0663	1.2087	1.3509	1.4973	1.6480	1.7921	1.9432	2.0893	2.2405	2.3816	2.5227	2.6752	2.8088	2.9433	3.0792
D	6	T4a	0.3632	0.4982	0.6298	0.7635	0.9037	1.0396	1.1784	1.3183	1.4606	1.6069	1.7526	1.8974	2.0412	2.1850	2.3222	2.4603	2.6064	2.7343	2.8691	3.0022
D	7	T4b	0.1387	0.1642	0.1923	0.2224	0.2518	0.2826	0.3116	0.3406	0.3725	0.4061	0.4370	0.4707	0.5055	0.5383	0.5713	0.6049	0.6404	0.6734	0.7087	0.7435
D	8	T4b	0.1319	0.1564	0.1829	0.2120	0.2423	0.2731	0.3011	0.3307	0.3639	0.3991	0.4275	0.4629	0.4968	0.5321	0.5646	0.5995	0.6359	0.6704	0.7053	0.7406
E	1	C5a	0.3533	0.4716	0.5929	0.7192	0.8480	0.9818	1.1149	1.2478	1.3860	1.5286	1.6669	1.8127	1.9489	2.0926	2.2270	2.3702	2.5153	2.6469	2.7869	2.9130
E	2	C5a	0.3334	0.4485	0.5643	0.6845	0.8101	0.9323	1.0571	1.1857	1.3119	1.4458	1.5771	1.7113	1.8437	1.9777	2.1093	2.2387	2.3780	2.5009	2.6321	2.7546
E	3	C5b	0.3416	0.4510	0.5674	0.6846	0.8064	0.9259	1.0480	1.1711	1.2973	1.4282	1.5511	1.6829	1.8111	1.9367	2.0626	2.1896	2.3251	2.4434	2.5664	2.6847
E	4	C5b	0.3423	0.4631	0.5830	0.7045	0.8364	0.9597	1.0861	1.2143	1.3452	1.4816	1.6116	1.7469	1.8803	2.0135	2.1435	2.2740	2.4117	2.5334	2.6618	2.7864
E	5	T5a	0.2562	0.3295	0.4045	0.4849	0.5677	0.6511	0.7377	0.8210	0.9089	1.0002	1.0918	1.1830	1.2783	1.3737	1.4636	1.5590	1.6566	1.7489	1.8474	1.9421
E	6	T5a	0.2422	0.3133	0.3872	0.4645	0.5449	0.6261	0.7070	0.7875	0.8758	0.9653	1.0526	1.1421	1.2324	1.3254	1.4172	1.5093	1.6053	1.6939	1.7884	1.8801
E	7	T5b	0.1332	0.1570	0.1810	0.2079	0.2359	0.2634	0.2891	0.3156	0.3458	0.3766	0.4042	0.4328	0.4657	0.4961	0.5266	0.5564	0.5901	0.6193	0.6530	0.6846
E	8	T5b	0.1347	0.1573	0.1784	0.2033	0.2305	0.2542	0.2806	0.3054	0.3314	0.3617	0.3895	0.4164	0.4463	0.4760	0.5039	0.5322	0.5606	0.5914	0.6245	0.6533
F	1	blnk 1	0.0863	0.0847	0.0853	0.0860	0.0872	0.0876	0.0865	0.0866	0.0873	0.0884	0.0885	0.0882	0.0876	0.0886	0.0885	0.0896	0.0882	0.0876	0.0892	0.0873
F	2	blnk 2	0.0831	0.0849	0.0848	0.0854	0.0880	0.0879	0.0871	0.0863	0.0863	0.0890	0.0893	0.0878	0.0895	0.0891	0.0868	0.0888	0.0902	0.0872	0.0889	0.0863
F	3	blnk 3	0.0787	0.0809	0.0816	0.0819	0.0857	0.0829	0.0835	0.0829	0.0809	0.0841	0.0856	0.0846	0.0842	0.0837	0.0824	0.0834	0.0849	0.0828	0.0852	0.0831
F	4	blnk 4	0.0838	0.0831	0.0838	0.0832	0.0863	0.0856	0.0848	0.0830	0.0828	0.0871	0.0857	0.0869	0.0861	0.0863	0.0852	0.0856	0.0866	0.0853	0.0864	0.0858
F	5	blnk 5	0.0885	0.0896	0.0885	0.0897	0.0917	0.0924	0.0919	0.0907	0.0901	0.0936	0.0918	0.0931	0.0934	0.0930	0.0935	0.0931	0.0946	0.0921	0.0955	0.0915
F	6	blnk 6	0.0792	0.0789	0.0789	0.0783	0.0807	0.0812	0.0811	0.0803	0.0792	0.0825	0.0811	0.0826	0.0819	0.0831	0.0818	0.0817	0.0825	0.0821	0.0835	0.0822
F	7	blnk 7	0.0863	0.0862	0.0862	0.0860	0.0883	0.0871	0.0864	0.0867	0.0854	0.0884	0.0875	0.0872	0.0894	0.0877	0.0873	0.0879	0.0891	0.0867	0.0888	0.0852
F	8	blnk 8	0.0936	0.0963	0.0967	0.0971	0.1014	0.1022	0.1005	0.0989	0.1001	0.1044	0.1022	0.1028	0.1009	0.1030	0.1035	0.1013	0.1042	0.1039	0.1034	0.1028

Table C.7 LpNA Plate 1:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a	0.0871	0.0957	0.0941	0.0961	0.1000	0.1014	0.1053	0.1075	0.1137	0.1150	0.1211	0.1210	0.1261	0.1277	0.1323	0.1356	0.1397	0.1445	0.1441	0.1500
A	2	C1a	0.0790	0.0839	0.0829	0.0875	0.0916	0.0939	0.0970	0.0984	0.1029	0.1059	0.1102	0.1129	0.1164	0.1200	0.1238	0.1267	0.1284	0.1320	0.1340	0.1412
A	3	C1b	0.0935	0.1002	0.1004	0.1041	0.1077	0.1111	0.1147	0.1172	0.1239	0.1297	0.1324	0.1310	0.1374	0.1400	0.1429	0.1486	0.1516	0.1549	0.1570	0.1633
A	4	C1b	0.0905	0.0953	0.0978	0.1012	0.1047	0.1078	0.1122	0.1148	0.1202	0.1212	0.1267	0.1278	0.1326	0.1375	0.1393	0.1433	0.1456	0.1501	0.1517	0.1589
A	5	T1a	0.0744	0.0777	0.0781	0.0795	0.0818	0.0837	0.0851	0.0857	0.0915	0.0906	0.0945	0.0924	0.0961	0.0970	0.0993	0.1010	0.1015	0.1044	0.1043	0.1098
A	6	T1a	0.0767	0.0832	0.0832	0.0823	0.0856	0.0880	0.0901	0.0914	0.0938	0.0938	0.0988	0.0967	0.0992	0.1017	0.1027	0.1058	0.1050	0.1097	0.1088	0.1120
A	7	T1b	0.0700	0.0743	0.0751	0.0761	0.0781	0.0806	0.0832	0.0830	0.0862	0.0886	0.0912	0.0901	0.0936	0.0939	0.0949	0.0968	0.0971	0.1022	0.1006	0.1036
A	8	T1b	0.0720	0.0773	0.0779	0.0800	0.0833	0.0847	0.0859	0.0881	0.0923	0.0910	0.0940	0.0931	0.0973	0.0966	0.1001	0.1008	0.0997	0.1040	0.1034	0.1059
B	1	C2a	0.1012	0.1124	0.1205	0.1298	0.1374	0.1481	0.1571	0.1670	0.1808	0.1875	0.2010	0.2087	0.2202	0.2313	0.2411	0.2537	0.2612	0.2734	0.2837	0.2979
B	2	C2a	0.0983	0.1074	0.1161	0.1228	0.1333	0.1428	0.1511	0.1611	0.1734	0.1807	0.1926	0.2011	0.2107	0.2205	0.2304	0.2417	0.2508	0.2639	0.2723	0.2840
B	3	C2b	0.0790	0.0844	0.0871	0.0890	0.0943	0.0961	0.1010	0.1039	0.1101	0.1103	0.1159	0.1168	0.1227	0.1269	0.1298	0.1333	0.1354	0.1405	0.1414	0.1472
B	4	C2b	0.0782	0.0835	0.0854	0.0875	0.0920	0.0948	0.0969	0.0994	0.1055	0.1066	0.1119	0.1126	0.1206	0.1201	0.1238	0.1278	0.1305	0.1346	0.1350	0.1421
B	5	T2a	0.0903	0.0872	0.0897	0.0990	0.0922	0.0917	0.0925	0.0922	0.0933	0.0946	0.0952	0.0962	0.0951	0.0967	0.0980	0.1008	0.1010	0.0993	0.1013	0.1033
B	6	T2a	0.0990	0.0982	0.1002	0.0999	0.1024	0.1011	0.1038	0.1027	0.1017	0.1049		0.1050	0.1070	0.1071	0.1081	0.1093	0.1095	0.1110	0.1107	0.1132
B	7	T2b	0.0951	0.0965	0.1002	0.1018	0.1022	0.1077	0.1111	0.1126	0.1161	0.1230	0.1259	0.1279	0.1332	0.1347	0.1391	0.1435	0.1468	0.1478	0.1541	0.1582
B	8	T2b	0.0985	0.0993	0.1036	0.1057	0.1079	0.1128	0.1161	0.1194	0.1216	0.1259	0.1322	0.1354	0.1381	0.1425	0.1430	0.1473	0.1503	0.1538	0.1598	0.1618
C	1	C3a	0.0891	0.0953	0.1006	0.1053	0.1120	0.1177	0.1246	0.1298	0.1393	0.1440	0.1532	0.1610	0.1648	0.1721	0.1779	0.1847	0.1897	0.1996	0.2046	0.2128
C	2	C3a	0.0973	0.1037	0.1091	0.1120	0.1273	0.1244	0.1288	0.1350	0.1433	0.1479	0.1562	0.1609	0.1676	0.1729	0.1805	0.1865	0.1906	0.1992	0.2050	0.2129
C	3	C3b	0.0773	0.0830	0.0846	0.0888	0.0919	0.0966	0.0988	0.1018	0.1077	0.1101	0.1146	0.1174	0.1218	0.1269	0.1285	0.1337	0.1384	0.1421	0.1436	0.1490
C	4	C3b	0.0819	0.0856	0.0888	0.0917	0.0960	0.1005	0.1046	0.1069	0.1133	0.1133	0.1197	0.1212	0.1267	0.1307	0.1328	0.1385	0.1396	0.1449	0.1478	0.1556
C	5	T3a	0.0870	0.0909	0.0946	0.0939	0.0971	0.1005	0.1011	0.1016	0.1066	0.1078	0.1107	0.1115	0.1132	0.1148	0.1163	0.1213	0.1224	0.1241	0.1254	0.1293
C	6	T3a	0.0704	0.0744	0.0758	0.0786	0.0826	0.0823	0.0832	0.0860	0.0903	0.0893	0.0949	0.0930	0.0985	0.1003	0.1015	0.1037	0.1053	0.1073	0.1073	0.1139
C	7	T3b	0.0775	0.0797	0.0803	0.0804	0.0850	0.0854	0.0860	0.0866	0.0909	0.0908	0.0944	0.0926	0.0951	0.0951	0.0972	0.0987	0.0995	0.1004	0.1009	0.1042
C	8	T3b	0.0850	0.0882	0.0875	0.0871	0.0901	0.0916	0.0915	0.0929	0.0961	0.0964	0.0988	0.0981	0.1000	0.1006	0.1030	0.1035	0.1038	0.1042	0.1041	0.1097
D	1	C4a	0.0923	0.1011	0.1062	0.1115	0.1180	0.1241	0.1316	0.1369	0.1480	0.1534	0.1643	0.1672	0.1739	0.1834	0.1890	0.1972	0.2031	0.2125	0.2163	0.2261
D	2	C4a	0.0971	0.1044	0.1101	0.1139	0.1193	0.1248	0.1326	0.1388	0.1470	0.1507	0.1607	0.1624	0.1688	0.1788	0.1839	0.1926	0.1963	0.2030	0.2103	0.2190
D	3	C4b	0.0834	0.0896	0.0929	0.0973	0.1018	0.1069	0.1133	0.1155	0.1249	0.1287	0.1371	0.1383	0.1446	0.1511	0.1548	0.1625	0.1661	0.1739	0.1775	0.1847
D	4	C4b	0.0866	0.0943	0.0972	0.1003	0.1046	0.1116	0.1151	0.1214	0.1280	0.1310	0.1388	0.1406	0.1476	0.1530	0.1580	0.1637	0.1679	0.1739	0.1779	0.1853
D	5	T4a	0.0911	0.1018	0.1048	0.1119	0.1187	0.1251	0.1340	0.1402	0.1484	0.1556	0.1648	0.1691	0.1784	0.1853	0.1931	0.2011	0.2144	0.2161	0.2227	0.2328

Table C.7 LpNA Plate 1 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
D	6	T4a	0.0926	0.1002	0.1061	0.1103	0.1173	0.1251	0.1320	0.1375	0.1459	0.1504	0.1597	0.1659	0.1738	0.1820	0.1880	0.1960	0.2000	0.2086	0.2157	0.2280
D	7	T4b	0.0803	0.0834	0.0855	0.0868	0.0905	0.0937	0.0946	0.0959	0.1013	0.1024	0.1067	0.1068	0.1105	0.1121	0.1143	0.1177	0.1193	0.1225	0.1225	0.1304
D	8	T4b	0.0764	0.0796	0.0804	0.0827	0.0855	0.0889	0.0899	0.0908	0.0951	0.0969	0.0998	0.1011	0.1039	0.1064	0.1079	0.1104	0.1128	0.1150	0.1151	0.1209
E	1	C5a	0.0923	0.0965	0.1005	0.1023	0.1084	0.1122	0.1170	0.1228	0.1295	0.1315	0.1405	0.1426	0.1478	0.1538	0.1583	0.1622	0.1681	0.1743	0.1775	0.1846
E	2	C5a	0.0869	0.0916	0.0932	0.0967	0.1014	0.1055	0.1115	0.1129	0.1201	0.1245	0.1299	0.1311	0.1393	0.1434	0.1477	0.1525	0.1544	0.1617	0.1637	0.1722
E	3	C5b	0.1155	0.1178	0.1234	0.1261	0.1290	0.1346	0.1398	0.1417	0.1463	0.1517	0.1582	0.1634	0.1691	0.1725	0.1783	0.1832	0.1880	0.1930	0.2012	0.2063
E	4	C5b	0.0957	0.0988	0.1005	0.1066	0.1092	0.1146	0.1175	0.1236	0.1280	0.1323	0.1381	0.1428	0.1475	0.1539	0.1586	0.1662	0.1696	0.1737	0.1803	0.1866
E	5	T5a	0.0966	0.1026	0.1029	0.1059	0.1113	0.1147	0.1203	0.1238	0.1280	0.1342	0.1398	0.1401	0.1452	0.1510	0.1532	0.1591	0.1622	0.1667	0.1690	0.1759
E	6	T5a	0.0888	0.0948	0.0972	0.0987	0.1048	0.1084	0.1130	0.1172	0.1241	0.1241	0.1314	0.1328	0.1383	0.1435	0.1468	0.1507	0.1546	0.1593	0.1618	0.1708
E	7	T5b	0.0811	0.0863	0.0867	0.0913	0.0956	0.0975	0.1021	0.1061	0.1107	0.1100	0.1175	0.1164	0.1220	0.1238	0.1285	0.1314	0.1324	0.1367	0.1386	0.1457
E	8	T5b	0.0868	0.0914	0.0916	0.0957	0.0998	0.1031	0.1076	0.1092	0.1141	0.1161	0.1213	0.1207	0.1251	0.1286	0.1310	0.1342	0.1382	0.1413	0.1432	0.1490
F	1	blnk 1	0.0763	0.0788	0.0757	0.0769	0.0774	0.0793	0.0777	0.0784	0.0794	0.0785	0.0797	0.0795	0.0804	0.0798	0.0810	0.0810	0.0798	0.0812	0.0788	0.0820
F	2	blnk 2	0.0863	0.0869	0.0869	0.0856	0.0878	0.0867	0.0885	0.0866	0.0906	0.0871	0.0911	0.0888	0.0906	0.0893	0.0891	0.0902	0.0888	0.0910	0.0883	0.0915
F	3	blnk 3	0.0765	0.0764	0.0755	0.0747	0.0754	0.0769	0.0779	0.0766	0.0782	0.0783	0.0800	0.0770	0.0819	0.0787	0.0800	0.0811	0.0790	0.0817	0.0794	0.0819
F	4	blnk 4	0.0841	0.0840	0.0832	0.0834	0.0835	0.0835	0.0844	0.0838	0.0874	0.0848	0.0862	0.0846	0.0861	0.0856	0.0854	0.0878	0.0839	0.0862	0.0847	0.0879
F	5	blnk 5	0.0906	0.0897	0.0910	0.0908	0.0905	0.0897	0.0905	0.0895	0.0935	0.0910	0.0908	0.0902	0.0922	0.0928	0.0932	0.0911	0.0918	0.0926	0.0900	0.0921
F	6	blnk 6	0.0781	0.0776	0.0785	0.0762	0.0790	0.0774	0.0788	0.0783	0.0811	0.0805	0.0802	0.0784	0.0813	0.0821	0.0814	0.0823	0.0796	0.0819	0.0792	0.0844
F	7	blnk 7	0.0868	0.0861	0.0854	0.0857	0.0866	0.0865	0.0870	0.0874	0.0911	0.0862	0.0880	0.0869	0.0896	0.0881	0.0873	0.0886	0.0880	0.0888	0.0876	0.0911
F	8	blnk 8	0.0982	0.1000	0.0998	0.0991	0.0993	0.0999	0.1000	0.1003	0.1025	0.0994	0.1024	0.0994	0.1011	0.1027	0.1008	0.1028	0.1022	0.1017	0.1001	0.1017

Table C.8 LpNA Plate 2:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a		0.0971	0.0999	0.1046	0.1089	0.1109	0.1167	0.1190	0.1263	0.1281	0.1336	0.1364		0.1439	0.1505	0.1522	0.1572	0.1629	0.1670	0.1701
A	2	C1a	0.0915	0.0941	0.0981	0.1026	0.1078	0.1093	0.1165	0.1188	0.1255	0.1300	0.1341	0.1373	0.1446	0.1451	0.1523	0.1556	0.1613	0.1677	0.1711	0.1753
A	3	C1b	0.0984	0.1067	0.1072	0.1087	0.1136	0.1163	0.1196	0.1208	0.1287	0.1291	0.1333	0.1353	0.1396	0.1413	0.1439	0.1491	0.1529	0.1568	0.1601	0.1648
A	4	C1b	0.0935	0.0988	0.1004	0.1052	0.1092	0.1123	0.1159	0.1187	0.1249	0.1234	0.1309	0.1313	0.1369	0.1374	0.1429	0.1461	0.1484	0.1541	0.1559	0.1593
A	5	T1a	0.0831	0.0850	0.0878	0.0897	0.0918	0.0929	0.0949	0.0948	0.0991	0.0983	0.1016	0.1009	0.1037	0.1021	0.1058	0.1073	0.1082	0.1113	0.1120	0.1146
A	6	T1a	0.0880	0.0915	0.0905	0.0959	0.0953	0.0949	0.0988	0.0985	0.1016	0.1003	0.1063	0.1055	0.1095	0.1074	0.1091	0.1105	0.1128	0.1145	0.1182	0.1177
A	7	T1b	0.0780	0.0816	0.0842	0.0865	0.0877	0.0883	0.0903	0.0918	0.0955	0.0925	0.0968	0.0978	0.0999	0.0986	0.1007	0.1013	0.1027	0.1054	0.1067	0.1079
A	8	T1b	0.0807	0.0860	0.0869	0.0909	0.0915	0.0939	0.0943	0.0952	0.0985	0.0996	0.0998	0.1016	0.1060	0.1034	0.1043	0.1061	0.1084	0.1108	0.1126	0.1202
B	1	C2a	0.1057	0.1143	0.1205	0.1318	0.1412	0.1508	0.1600	0.1686	0.1836	0.1884	0.2029	0.2111	0.2228	0.2298	0.2423	0.2529	0.2629	0.2732	0.2848	0.2941
B	2	C2a	0.1023	0.1095	0.1190	0.1280	0.1391	0.1464	0.1554	0.1630	0.1780	0.1836	0.1940	0.2048	0.2157	0.2220	0.2330	0.2439	0.2539	0.2652	0.2743	0.2852
B	3	C2b	0.0897	0.0912	0.0944	0.0974	0.1016	0.1047	0.1080	0.1092	0.1163	0.1171	0.1211	0.1252	0.1271	0.1304	0.1348	0.1370	0.1420	0.1442	0.1485	0.1533
B	4	C2b	0.0859	0.0896	0.0951	0.0980	0.1007	0.1049	0.1080	0.1106	0.1176	0.1173	0.1216	0.1235	0.1268	0.1291	0.1339	0.1374	0.1402	0.1435	0.1470	0.1506
B	5	T2a	0.0846	0.0862	0.0869	0.0897	0.0897	0.0923	0.0940	0.0914	0.0959	0.0924	0.0969	0.0957	0.0997	0.0963	0.0973	0.0995	0.0980	0.1017	0.1024	0.1032
B	6	T2a	0.0847	0.0857	0.0876	0.0886	0.0891	0.0905	0.0929	0.0902	0.0976	0.0932	0.0962	0.0966	0.0987	0.0960	0.0985	0.0991	0.1005	0.0999	0.1016	0.1009
B	7	T2b	0.0836	0.0874	0.0921	0.0962	0.1007	0.1037	0.1078	0.1100	0.1170	0.1157	0.1214	0.1244	0.1294	0.1311	0.1366	0.1390	0.1425	0.1472	0.1522	0.1541
B	8	T2b	0.0896	0.0929	0.0996	0.1023	0.1048	0.1078	0.1134	0.1158	0.1224	0.1233	0.1284	0.1325	0.1368	0.1383	0.1430	0.1449	0.1473	0.1542	0.1585	0.1624
C	1	C3a	0.0965	0.1003	0.1051	0.1124	0.1197	0.1253	0.1313	0.1368	0.1452	0.1493	0.1568	0.1628	0.1702	0.1741	0.1817	0.1885	0.1964	0.2044	0.2102	0.2160
C	2	C3a	0.1022	0.1076	0.1122	0.1204	0.1258	0.1307	0.1385	0.1425	0.1532	0.1548	0.1642	0.1685	0.1748	0.1798	0.1882	0.1936	0.1984	0.2077	0.2153	0.2208
C	3	C3b	0.0852	0.0889	0.0913	0.0958	0.1007	0.1021	0.1062	0.1079	0.1162	0.1165	0.1214	0.1259	0.1276	0.1322	0.1356	0.1389	0.1422	0.1473	0.1516	0.1549
C	4	C3b	0.0897	0.0940	0.0986	0.1019	0.1056	0.1081	0.1113	0.1141	0.1205	0.1218	0.1272	0.1293	0.1343	0.1347	0.1407	0.1436	0.1483	0.1522	0.1580	0.1604
C	5	T3a	0.0939	0.0977	0.0992	0.1039	0.1059	0.1078	0.1113	0.1091	0.1140	0.1136	0.1164	0.1174	0.1205	0.1197	0.1236	0.1264	0.1279	0.1290	0.1304	0.1330
C	6	T3a	0.0822	0.0842	0.0874	0.0886	0.0912	0.0913	0.0942	0.0950	0.1000	0.0998	0.1022	0.1042	0.1069	0.1075	0.1090	0.1115	0.1121	0.1149	0.1174	0.1216
C	7	T3b	0.0881	0.0880	0.0914	0.0941	0.0959	0.0964	0.0996	0.0993	0.1026	0.1026	0.1057	0.1039	0.1067	0.1052	0.1083	0.1093	0.1089	0.1121	0.1154	0.1147
C	8	T3b	0.0913	0.0921	0.0946	0.0988	0.0985	0.0998	0.1021	0.1007	0.1054	0.1048	0.1073	0.1056	0.1079	0.1079	0.1093	0.1094	0.1119	0.1123	0.1141	0.1148
D	1	C4a	0.0997	0.1054	0.1114	0.1160	0.1242	0.1309	0.1379	0.1416	0.1523	0.1567	0.1643	0.1688	0.1799	0.1846	0.1938	0.1993	0.2068	0.2139	0.2229	0.2308
D	2	C4a	0.1031	0.1091	0.1149	0.1203	0.1269	0.1334	0.1405	0.1464	0.1567	0.1586	0.1676	0.1738	0.1811	0.1867	0.1949	0.2007	0.2060	0.2146	0.2227	0.2293
D	3	C4b	0.0910	0.0946	0.0995	0.1045	0.1095	0.1136	0.1222	0.1231	0.1337	0.1344	0.1424	0.1451	0.1535	0.1562	0.1641	0.1679	0.1746	0.1811	0.1865	0.1927
D	4	C4b	0.0932	0.0982	0.1028	0.1074	0.1162	0.1208	0.1246	0.1281	0.1363	0.1393	0.1462	0.1517	0.1576	0.1622	0.1672	0.1736	0.1777	0.1843	0.1894	0.1967
D	5	T4a	0.0994	0.1062	0.1121	0.1205	0.1260	0.1338	0.1417	0.1474	0.1572	0.1612	0.1692	0.1768	0.1867	0.1913	0.2001	0.2067	0.2145	0.2238	0.2314	0.2398

Table C.8 LpNA Plate 2 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
D	6	T4a	0.0959	0.1043	0.1097	0.1193	0.1253	0.1313	0.1394	0.1443	0.1554	0.1616	0.1708	0.1775	0.1853	0.1894	0.1985	0.2055	0.2135	0.2200	0.2286	0.2368
D	7	T4b	0.0899	0.0904	0.0940	0.0970	0.1019	0.1042	0.1064	0.1093	0.1128	0.1127	0.1190	0.1191	0.1229	0.1245	0.1283	0.1304	0.1308	0.1365	0.1368	0.1398
D	8	T4b	0.0882	0.0892	0.0910	0.0948	0.0988	0.1004	0.1026	0.1043	0.1095	0.1086	0.1137	0.1147	0.1178	0.1190	0.1222	0.1239	0.1262	0.1299	0.1320	0.1341
E	1	C5a	0.0968	0.0990	0.1039	0.1098	0.1137	0.1193	0.1241	0.1256	0.1349	0.1366	0.1434	0.1470	0.1523	0.1544	0.1610	0.1658	0.1716	0.1783	0.1829	0.1873
E	2	C5a	0.0940	0.0958	0.1008	0.1057	0.1121	0.1159	0.1203	0.1221	0.1313	0.1315	0.1376	0.1424	0.1471	0.1492	0.1560	0.1611	0.1654	0.1698	0.1763	0.1786
E	3	C5b	0.0990	0.1015	0.1075	0.1138	0.1188	0.1232	0.1302	0.1335	0.1443	0.1438	0.1518	0.1565	0.1631	0.1685	0.1763	0.1794	0.1870	0.1948	0.1988	0.2074
E	4	C5b	0.0967	0.1002	0.1039	0.1122	0.1169	0.1214	0.1266	0.1323	0.1408	0.1443	0.1490	0.1550	0.1629	0.1676	0.1755	0.1794	0.1866	0.1928	0.2004	0.2057
E	5	T5a	0.1007	0.1042	0.1074	0.1127	0.1187	0.1212	0.1251	0.1273	0.1364	0.1360	0.1408	0.1467	0.1509	0.1530	0.1588	0.1608	0.1652	0.1702	0.1752	0.1810
E	6	T5a	0.0955	0.0995	0.1024	0.1065	0.1112	0.1166	0.1206	0.1232	0.1303	0.1325	0.1378	0.1421	0.1468	0.1485	0.1557	0.1570	0.1614	0.1676	0.1710	0.1767
E	7	T5b	0.0930	0.0967	0.0998	0.1035	0.1081	0.1093	0.1122	0.1143	0.1206	0.1210	0.1270	0.1298	0.1317	0.1345	0.1392	0.1419	0.1460	0.1493	0.1534	0.1551
E	8	T5b	0.0961	0.1009	0.1042	0.1073	0.1120	0.1148	0.1180	0.1190	0.1251	0.1266	0.1321	0.1346	0.1383	0.1409	0.1455	0.1493	0.1547	0.1577	0.1595	0.1636
F	1	blnk 1	0.0835	0.0838	0.0823	0.0816	0.0840	0.0837	0.0845	0.0817	0.0848	0.0835	0.0842	0.0852	0.0860	0.0818	0.0839	0.0844	0.0828	0.0832	0.0839	0.0840
F	2	blnk 2	0.0932	0.0909	0.0906	0.0911	0.0918	0.0913	0.0930	0.0908	0.0954	0.0911	0.0934	0.0944	0.0976	0.0914	0.0920	0.0923	0.0909	0.0946	0.0940	0.0935
F	3	blnk 3	0.0840	0.0824	0.0815	0.0850	0.0856	0.0842	0.0834	0.0827	0.0872	0.0855	0.0850	0.0865	0.0861	0.0882	0.0850	0.0842	0.0848	0.0863	0.0871	0.0871
F	4	blnk 4	0.0883	0.0883	0.0873	0.0905	0.0894	0.0899	0.0891	0.0893	0.0932	0.0909	0.0928	0.0917	0.0918	0.0902	0.0913	0.0922	0.0888	0.0911	0.0920	0.0906
F	5	blnk 5	0.0947	0.0944	0.0948	0.0971	0.0968	0.0958	0.0975	0.0957	0.0999	0.1043	0.0976	0.0976	0.0991	0.0965	0.0964	0.0972	0.0966	0.0970	0.0968	0.0976
F	6	blnk 6	0.0857	0.0841	0.0850	0.0852	0.0858	0.0858	0.0870	0.0848	0.0894	0.0950	0.0931	0.0883	0.0884	0.0862	0.0880	0.0896	0.0880	0.0888	0.0887	0.0879
F	7	blnk 7	0.0932	0.0929	0.0917	0.0926	0.0940	0.0936	0.0953	0.0920	0.0967	0.0927	0.0950	0.0934	0.0943	0.0924	0.0939	0.0939	0.0935	0.0963	0.0948	0.0943
F	8	blnk 8	0.0966	0.0959	0.0941	0.0963	0.0971	0.0984	0.0986	0.0959	0.1011	0.0971	0.0992	0.0999	0.0979	0.0982	0.0981	0.0966	0.0970	0.0980	0.0965	0.0979

Table C.9 LpNA Plate 3:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a					0.1126	0.1173	0.1211	0.1230	0.1272	0.1313	0.1351	0.1381	0.1421	0.1453	0.1512	0.1548	0.1571	0.1604	0.1646	0.1693
A	2	C1a					0.1066	0.1098	0.1141	0.1212	0.1208	0.1248	0.1294	0.1328	0.1387	0.1409	0.1477	0.1511	0.1532	0.1584	0.1627	0.1656
A	3	C1b	0.1078	0.1122	0.1110	0.1150	0.1182	0.1213	0.1244	0.1275	0.1310	0.1358	0.1393	0.1414	0.1440	0.1505	0.1526	0.1556	0.1587	0.1638	0.1656	0.1674
A	4	C1b	0.1087	0.1116	0.1138	0.1147	0.1168	0.1216	0.1244	0.1281	0.1327	0.1358	0.1397	0.1422	0.1452	0.1512	0.1552	0.1576	0.1600	0.1649	0.1675	0.1698
A	5	T1a	0.0927	0.0910	0.0920	0.0925	0.0947	0.0978	0.0983	0.1003	0.1003	0.1042	0.1057	0.1042	0.1062	0.1088	0.1104	0.1154	0.1191	0.1182	0.1175	0.1191
A	6	T1a	0.0957	0.0986	0.0965	0.0976	0.0996	0.0996	0.1018	0.1031	0.1079	0.1064	0.1078	0.1088	0.1099	0.1136	0.1160	0.1160	0.1166	0.1188	0.1207	0.1207
A	7	T1b	0.0875	0.0899	0.0906	0.0915	0.0907	0.0949	0.0953	0.0960	0.0979	0.1009	0.1021	0.1025	0.1039	0.1064	0.1071	0.1087	0.1099	0.1116	0.1120	0.1131
A	8	T1b	0.0884	0.0937	0.0919	0.0965	0.0963	0.0993	0.0987	0.0998	0.1004	0.1040	0.1061	0.1060	0.1092	0.1115	0.1135	0.1110	0.1130	0.1150	0.1150	0.1154
B	1	C2a	0.1208	0.1245	0.1319	0.1407	0.1499	0.1625	0.1712	0.1790	0.1893	0.2020	0.2099	0.2189	0.2315	0.2413	0.2497	0.2610	0.2719	0.2822	0.2938	0.3021
B	2	C2a	0.1116	0.1211	0.1285	0.1400	0.1568	0.1632	0.1652	0.1724	0.1845	0.1956	0.2062	0.2144	0.2243	0.2334	0.2460	0.2560	0.2649	0.2801	0.2865	0.2939
B	3	C2b	0.0931	0.0974	0.1000	0.1019	0.1055	0.1102	0.1137	0.1169	0.1195	0.1238	0.1266	0.1283	0.1328	0.1386	0.1411	0.1433	0.1453	0.1501	0.1525	0.1553
B	4	C2b	0.0936	0.0988	0.0974	0.1015	0.1047	0.1081	0.1112	0.1145	0.1178	0.1220	0.1254	0.1272	0.1304	0.1349	0.1401	0.1421	0.1437	0.1473	0.1520	0.1525
B	5	T2a	0.0929	0.0932	0.0932	0.0964	0.0936	0.0958	0.0966	0.0976	0.0979	0.0993	0.1002	0.1004	0.1021	0.1049	0.1053	0.1046	0.1063	0.1067	0.1075	0.1054
B	6	T2a	0.0909	0.0920	0.0911	0.0916	0.0936	0.0944	0.0975	0.0963	0.0974	0.0992	0.0996	0.0981	0.0999	0.1009	0.1051	0.1039	0.1057	0.1060	0.1064	0.1050
B	7	T2b	0.0938	0.0978	0.0981	0.0995	0.1064	0.1087	0.1125	0.1170	0.1182	0.1234	0.1272	0.1298	0.1342	0.1370	0.1442	0.1457	0.1485	0.1536	0.1571	0.1577
B	8	T2b	0.0977	0.1035	0.1022	0.1079	0.1119	0.1155	0.1186	0.1226	0.1321	0.1305	0.1350	0.1375	0.1414	0.1466	0.1503	0.1535	0.1570	0.1615	0.1629	0.1661
C	1	C3a	0.1044	0.1106	0.1248	0.1188	0.1279	0.1322	0.1389	0.1455	0.1514	0.1574	0.1633	0.1712	0.1773	0.1841	0.1899	0.1970	0.2045	0.2115	0.2156	0.2231
C	2	C3a	0.1114	0.1166	0.1190	0.1249	0.1343	0.1375	0.1430	0.1489	0.1526	0.1601	0.1652	0.1713	0.1786	0.1877	0.1920	0.1993	0.2038	0.2126	0.2157	0.2208
C	3	C3b	0.0970	0.0989	0.1001	0.1009	0.1074	0.1093	0.1129	0.1192	0.1199	0.1244	0.1275	0.1326	0.1354	0.1393	0.1425	0.1472	0.1494	0.1531	0.1573	0.1606
C	4	C3b	0.1004	0.1045	0.1059	0.1100	0.1130	0.1170	0.1200	0.1233	0.1267	0.1329	0.1354	0.1383	0.1429	0.1479	0.1493	0.1524	0.1592	0.1635	0.1647	0.1679
C	5	T3a	0.1038	0.1043	0.1076	0.1058	0.1094	0.1116	0.1145	0.1145	0.1172	0.1203	0.1227	0.1246	0.1240	0.1278	0.1302	0.1322	0.1308	0.1367	0.1374	0.1368
C	6	T3a	0.0900	0.0946	0.0921	0.0922	0.0946	0.0981	0.1003	0.1034	0.1024	0.1070	0.1080	0.1072	0.1103	0.1124	0.1143	0.1175	0.1205	0.1215	0.1236	0.1233
C	7	T3b	0.0988	0.0962	0.1008	0.0973	0.0997	0.1013	0.1018	0.1035	0.1042	0.1063	0.1084	0.1058	0.1095	0.1117	0.1112	0.1134	0.1136	0.1173	0.1165	0.1174
C	8	T3b				0.1006	0.1022	0.1031	0.1046	0.1060	0.1066	0.1084	0.1102	0.1079	0.1115	0.1119	0.1111	0.1138	0.1148	0.1171	0.1176	0.1173
D	1	C4a	0.1093	0.1125	0.1192	0.1223	0.1288	0.1392	0.1447	0.1513	0.1550	0.1647	0.1704	0.1771	0.1846	0.1931	0.2007	0.2071	0.2132	0.2212	0.2273	0.2314
D	2	C4a	0.1116	0.1155	0.1208	0.1264	0.1326	0.1395	0.1453	0.1533	0.1573	0.1664	0.1739	0.1778	0.1897	0.1993	0.2002	0.2084	0.2131	0.2231	0.2261	0.2340
D	3	C4b	0.1041	0.1030	0.1073	0.1092	0.1158	0.1220	0.1255	0.1303	0.1357	0.1415	0.1474	0.1517	0.1597	0.1629	0.1678	0.1743	0.1778	0.1862	0.1889	0.1938
D	4	C4b	0.1035	0.1069	0.1094	0.1137	0.1192	0.1254	0.1308	0.1350	0.1393	0.1464	0.1522	0.1546	0.1625	0.1696	0.1728	0.1795	0.1825	0.1905	0.1956	0.1989
D	5	T4a	0.1089	0.1142	0.1205	0.1252	0.1331	0.1413	0.1479	0.1586	0.1609	0.1683	0.1766	0.1827	0.1927	0.1988	0.2064	0.2149	0.2216	0.2305	0.2373	0.2426

Table C.9 LpNA Plate 3 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
D	6	T4a	0.1061	0.1116	0.1177	0.1238	0.1302	0.1384	0.1448	0.1513	0.1584	0.1664	0.1773	0.1803	0.1902	0.1975	0.2032	0.2118	0.2199	0.2281	0.2342	0.2426
D	7	T4b	0.0962	0.0962	0.0991	0.1005	0.1029	0.1079	0.1088	0.1110	0.1143	0.1171	0.1212	0.1226	0.1249	0.1278	0.1291	0.1332	0.1340	0.1396	0.1386	0.1418
D	8	T4b	0.0930	0.0938	0.0962	0.0963	0.0993	0.1047	0.1044	0.1082	0.1086	0.1135	0.1146	0.1177	0.1208	0.1238	0.1269	0.1282	0.1301	0.1365	0.1358	0.1379
E	1	C5a	0.1055	0.1081	0.1107	0.1137	0.1180	0.1237	0.1284	0.1310	0.1354	0.1439	0.1465	0.1524	0.1551	0.1676	0.1671	0.1697	0.1753	0.1810	0.1851	0.1891
E	2	C5a	0.1001	0.1052	0.1085	0.1108	0.1138	0.1189	0.1241	0.1291	0.1326	0.1374	0.1399	0.1446	0.1504	0.1554	0.1628	0.1633	0.1676	0.1746	0.1777	0.1820
E	3	C5b	0.1056	0.1100	0.1152	0.1178	0.1225	0.1283	0.1350	0.1391	0.1488	0.1545	0.1566	0.1624	0.1711	0.1748	0.1808	0.1863	0.1897	0.1988	0.2064	0.2079
E	4	C5b	0.1048	0.1089	0.1135	0.1170	0.1211	0.1272	0.1332	0.1382	0.1429	0.1502	0.1557	0.1608	0.1679	0.1739	0.1793	0.1860	0.1909	0.2001	0.2043	0.2100
E	5	T5a	0.1088	0.1116	0.1146	0.1173	0.1212	0.1271	0.1317	0.1355	0.1411	0.1435	0.1461	0.1506	0.1537	0.1588	0.1648	0.1671	0.1720	0.1789	0.1805	0.1865
E	6	T5a	0.1035	0.1050	0.1117	0.1137	0.1165	0.1212	0.1252	0.1283	0.1318	0.1409	0.1420	0.1442	0.1513	0.1540	0.1580	0.1624	0.1669	0.1717	0.1751	0.1789
E	7	T5b	0.0982	0.1014	0.1019	0.1043	0.1092	0.1098	0.1150	0.1146	0.1203	0.1232	0.1240	0.1303	0.1328	0.1364	0.1392	0.1428	0.1426	0.1485	0.1521	0.1524
E	8	T5b	0.1029	0.1067	0.1046	0.1099	0.1108	0.1160	0.1176	0.1190	0.1222	0.1274	0.1319	0.1314	0.1362	0.1394	0.1424	0.1474	0.1483	0.1515	0.1559	0.1615
F	1	blnk 1	0.0899	0.0903	0.0877	0.0872	0.0878	0.0877	0.0917	0.0879	0.0872	0.0883	0.0881	0.0887	0.0901	0.0906	0.0907	0.0918	0.0879	0.0904	0.0903	0.0880
F	2	blnk 2	0.0964	0.0962	0.0947	0.0944	0.0954	0.0953	0.0963	0.0950	0.0971	0.0977	0.0968	0.0947	0.0968	0.0997	0.1006	0.0989	0.0988	0.0986	0.0981	0.0978
F	3	blnk 3	0.0899	0.0901	0.0891	0.0868	0.0888	0.0910	0.0888	0.0900	0.0883	0.0902	0.0923	0.0904	0.0900	0.0919	0.0928	0.0922	0.0916	0.0911	0.0908	0.0896
F	4	blnk 4	0.0975	0.0948	0.0930	0.0923	0.0930	0.0942	0.0955	0.0953	0.0951	0.0978	0.0958	0.0940	0.0968	0.0976	0.0972	0.0964	0.0962	0.0987	0.0966	0.0952
F	5	blnk 5	0.1009	0.1001	0.0986	0.0972	0.0989	0.1000	0.1006	0.0990	0.1012	0.1016	0.1008	0.1002	0.1004	0.1023	0.1020	0.1024	0.1016	0.1033	0.1035	0.1009
F	6	blnk 6	0.0919	0.0923	0.0883	0.0903	0.0901	0.0910	0.0919	0.0917	0.0900	0.0927	0.0914	0.0923	0.0924	0.0936	0.0933	0.0928	0.0929	0.0945	0.0939	0.0902
F	7	blnk 7	0.0981	0.0979	0.0967	0.0949	0.0959	0.0973	0.0975	0.0962	0.0980	0.0990	0.0991	0.0990	0.0992	0.1006	0.0992	0.1000	0.0989	0.1005	0.0987	0.0973
F	8	blnk 8	0.1058	0.1032	0.1019	0.1020	0.1002	0.1040	0.1029	0.1021	0.1028	0.1032	0.1053	0.1034	0.1035	0.1036	0.1044	0.1049	0.1050	0.1059	0.1044	0.1040

Table C.10 SAAPLpNA Plate 1:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a	0.0664	0.0677	0.0734	0.0756	0.0773	0.0832	0.0886	0.0936	0.0981	0.1004	0.1043	0.1082	0.1138	0.1200	0.1238	0.1278	0.1329	0.1365	0.1407	0.1460
A	2	C1a	0.0521	0.0563	0.0587	0.0611	0.0659	0.0700	0.0769	0.0791	0.0841	0.0880	0.0913	0.0965	0.1013	0.1044	0.1091	0.1118	0.1182	0.1207	0.1251	0.1270
A	3	C1b						0.0769	0.0811	0.0841	0.0890	0.0922	0.0963	0.1006	0.1047	0.1067	0.1133	0.1144	0.1193	0.1214	0.1288	0.1320
A	4	C1b							0.0736	0.0796	0.0823	0.0853	0.0905	0.0930	0.0971	0.0999	0.1044	0.1071	0.1114	0.1129	0.1187	0.1197
A	5	T1a	0.0450	0.0452	0.0460	0.0451	0.0457	0.0462	0.0502	0.0502	0.0507	0.0514	0.0530	0.0525	0.0564	0.0587	0.0576	0.0562	0.0610	0.0612	0.0624	0.0626
A	6	T1a	0.0500	0.0502	0.0508	0.0472	0.0502	0.0503	0.0541	0.0550	0.0557	0.0560	0.0572	0.0575	0.0620	0.0611	0.0638	0.0616	0.0647	0.0666	0.0679	0.0674
A	7	T1b		0.0419	0.0417	0.0435	0.0438	0.0449	0.0474	0.0473	0.0480	0.0492	0.0503	0.0527	0.0550	0.0557	0.0567	0.0573	0.0577	0.0581	0.0598	0.0602
A	8	T1b	0.0460	0.0468	0.0474	0.0470	0.0478	0.0504	0.0508	0.0526	0.0553	0.0539	0.0553	0.0557	0.0581	0.0586	0.0614	0.0602	0.0630	0.0631	0.0650	0.0661
B	1	C2a	0.0678	0.0755	0.0817	0.0883	0.0955	0.1039	0.1163	0.1241	0.1323	0.1384	0.1489	0.1570	0.1657	0.1739	0.1831	0.1914	0.2014	0.2104	0.2181	0.2262
B	2	C2a	0.0660	0.0737	0.0806	0.0887	0.0967	0.1064	0.1149	0.1247	0.1316	0.1396	0.1490	0.1570	0.1683	0.1763	0.1852	0.1934	0.2053	0.2112	0.2212	0.2284
B	3	C2b	0.0515	0.0571	0.0617	0.0648	0.0675	0.0726	0.0791	0.0859	0.0882	0.0905	0.0968	0.1002	0.1067	0.1116	0.1167	0.1199	0.1275	0.1303	0.1369	0.1404
B	4	C2b	0.0554	0.0595	0.0632	0.0641	0.0689	0.0744	0.0843	0.0920												
B	5	T2a	0.0477	0.0488	0.0486	0.0468	0.0478	0.0493	0.0521	0.0507	0.0520	0.0503	0.0528	0.0524	0.0543	0.0513	0.0551	0.0522	0.0533	0.0514	0.0549	0.0527
B	6	T2a	0.0494	0.0503	0.0470	0.0481	0.0486	0.0506	0.0512	0.0526	0.0523	0.0510	0.0512	0.0508	0.0540	0.0527	0.0540	0.0506	0.0525	0.0530	0.0521	0.0507
B	7	T2b	0.0487	0.0514	0.0537	0.0574	0.0592	0.0652	0.0717	0.0765	0.0799	0.0839	0.0897	0.0901	0.0972	0.1010	0.1088	0.1102	0.1148	0.1216	0.1265	0.1295
B	8	T2b	0.0558	0.0594	0.0605	0.0638	0.0657	0.0741	0.0776	0.0819	0.0851	0.0879	0.0967	0.0982	0.1177	0.1234	0.1253	0.1192	0.1215	0.1219	0.1280	0.1296
C	1	C3a	0.0974	0.1176	0.1378	0.1547	0.1771	0.1972	0.2224	0.2446	0.2640	0.2832	0.3063	0.3243	0.3486	0.3679	0.3846	0.4049	0.4238	0.4393	0.4581	0.4773
C	2	C3a	0.0979	0.1159	0.1353	0.1526	0.1728	0.1927	0.2159	0.2350	0.2544	0.2732	0.2944	0.3132	0.3344	0.3517	0.3697	0.3883	0.4067	0.4213	0.4409	0.4578
C	3	C3b	0.0575	0.0621	0.0667	0.0709	0.0768	0.0849	0.0928	0.0991	0.1051	0.1113	0.1180	0.1249	0.1340	0.1403	0.1482	0.1534	0.1632	0.1750	0.1819	0.1896
C	4	C3b	0.0613	0.0674	0.0732	0.0784	0.0848	0.0935	0.1030	0.1090	0.1157	0.1220	0.1299	0.1387	0.1479	0.1550	0.1631	0.1700	0.1812	0.1861	0.1936	0.2039
C	5	T3a	0.0599	0.0614	0.0624	0.0613	0.0618	0.0621	0.0662	0.0661	0.0659	0.0672	0.0662	0.0667	0.0695	0.0701	0.0722	0.0707	0.0725	0.0710	0.0750	0.0721
C	6	T3a	0.0425	0.0428	0.0438	0.0421	0.0466	0.0444	0.0462	0.0479	0.0482	0.0468	0.0500	0.0494	0.0526	0.0523	0.0535	0.0519	0.0543	0.0537	0.0546	0.0547
C	7	T3b	0.0494	0.0518	0.0520	0.0508	0.0505	0.0523	0.0539	0.0551	0.0545	0.0543	0.0561	0.0557	0.0572	0.0583	0.0576	0.0566	0.0579	0.0561	0.0575	0.0577
C	8	T3b	0.0597	0.0600	0.0598	0.0610	0.0631	0.0621	0.0651	0.0651	0.0667	0.0659	0.0647	0.0669	0.0684	0.0673	0.0681	0.0682	0.0705	0.0676	0.0673	0.0673
D	1	C4a	0.0738	0.0798	0.0861	0.0965	0.1044	0.1141	0.1250	0.1357	0.1472	0.1565	0.1665	0.1775	0.1882	0.1995	0.2115	0.2199	0.2329	0.2412	0.2525	0.2624
D	2	C4a	0.0784	0.0876	0.0966	0.1041	0.1156	0.1263	0.1370	0.1471	0.1594	0.1692	0.1810	0.1920	0.2046	0.2132	0.2270	0.2363	0.2482	0.2595	0.2728	0.2820
D	3	C4b	0.0674	0.0759	0.0830	0.0940	0.1023	0.1156	0.1253	0.1361	0.1474	0.1566	0.1684	0.1804	0.1933	0.2046	0.2151	0.2257	0.2420	0.2487	0.2601	0.2709
D	4	C4b	0.0741	0.0848	0.0921	0.1019	0.1097	0.1219	0.1352	0.1459	0.1575	0.1680	0.1800	0.1889	0.2012	0.2114	0.2249	0.2340	0.2474	0.2560	0.2709	0.2797
D	5	T4a	0.0901	0.1071	0.1249	0.1434	0.1621	0.1808	0.2030	0.2212	0.2397	0.2581	0.2770	0.2942	0.3169	0.3337	0.3537	0.3682	0.3890	0.4065	0.4229	0.4403

Table C.10 SAAPLpNA Plate 1 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
D	6	T4a	0.0955	0.1143	0.1326	0.1537	0.1736	0.1934	0.2160	0.2366	0.2557	0.2761	0.2945	0.3156	0.3368	0.3553	0.3753	0.3914	0.4126	0.4306	0.4476	0.4646
D	7	T4b	0.0524	0.0544	0.0533	0.0539	0.0591	0.0615	0.0665	0.0671	0.0706	0.0708	0.0724	0.0734	0.0798	0.0819	0.0821	0.0838	0.0875	0.0902	0.0917	0.0929
D	8	T4b	0.0490	0.0508	0.0507	0.0509	0.0542	0.0555	0.0597	0.0611	0.0661	0.0655	0.0699	0.0705	0.0723	0.0759	0.0796	0.0784	0.0861	0.0840	0.0876	0.0916
E	1	C5a	0.0942	0.1052	0.1200	0.1344	0.1502	0.1679	0.1861	0.2027	0.2208	0.2357	0.2534	0.2723	0.2897	0.3071	0.3227	0.3398	0.3582	0.3712	0.3900	0.4053
E	2	C5a	0.0836	0.0954	0.1074	0.1217	0.1350	0.1493	0.1674	0.1812	0.1947	0.2092	0.2256	0.2440	0.2593	0.2726	0.2928	0.3162	0.3369	0.3414	0.3483	0.3625
E	3	C5b	0.0926	0.1038	0.1174	0.1310	0.1443	0.1591	0.1759	0.1903	0.2074	0.2201	0.2368	0.2544	0.2687	0.2837	0.3018	0.3138	0.3308	0.3437	0.3590	0.3745
E	4	C5b	0.0857	0.1013	0.1154	0.1324	0.1494	0.1623	0.1803	0.1962	0.2137	0.2285	0.2452	0.2634	0.2802	0.2968	0.3137	0.3268	0.3467	0.3622	0.3772	0.3909
E	5	T5a	0.0770	0.0806	0.0858	0.0927	0.0983	0.1067	0.1144	0.1219	0.1294	0.1329	0.1434	0.1500	0.1601	0.1693	0.1768	0.1837	0.1935	0.1996	0.2096	0.2181
E	6	T5a	0.0734	0.0767	0.0797	0.0850	0.0964	0.0981	0.1111	0.1113	0.1203	0.1269	0.1375	0.1459	0.1558	0.1636	0.1734	0.1828	0.1933	0.1986	0.2103	0.2182
E	7	T5b	0.0540	0.0558	0.0563	0.0584	0.0603	0.0618	0.0666	0.0687	0.0692	0.0710	0.0739	0.0749	0.0785	0.0796	0.0847	0.0840	0.0888	0.0916	0.0928	0.0936
E	8	T5b	0.0583	0.0594	0.0601	0.0611	0.0635	0.0651	0.0697	0.0702	0.0715	0.0729	0.0754	0.0778	0.0810	0.0821	0.0870	0.0860	0.0898	0.0911	0.0937	0.0946
F	1	blnk 1	0.0534	0.0497	0.0503	0.0481	0.0484	0.0475	0.0508	0.0536	0.0492	0.0485	0.0504	0.0479	0.0499	0.0493	0.0486	0.0494	0.0496	0.0461	0.0483	0.0481
F	2	blnk 2	0.0579	0.0539	0.0535	0.0526	0.0535	0.0544	0.0563	0.0556	0.0535	0.0533	0.0545	0.0551	0.0557	0.0552	0.0547	0.0539	0.0563	0.0532	0.0542	0.0531
F	3	blnk 3	0.0500	0.0482	0.0474	0.0468	0.0454	0.0467	0.0480	0.0476	0.0479	0.0465	0.0466	0.0464	0.0483	0.0490	0.0480	0.0468	0.0489	0.0469	0.0464	0.0451
F	4	blnk 4	0.0553	0.0559	0.0535	0.0529	0.0534	0.0531	0.0556	0.0541	0.0540	0.0535	0.0545	0.0515	0.0536	0.0553	0.0545	0.0516	0.0542	0.0519	0.0536	0.0555
F	5	blnk 5	0.0640	0.0610	0.0602	0.0596	0.0600	0.0579	0.0611	0.0600	0.0593	0.0605	0.0588	0.0589	0.0605	0.0594	0.0599	0.0577	0.0596	0.0587	0.0584	0.0574
F	6	blnk 6	0.0494	0.0473	0.0464	0.0458	0.0483	0.0454	0.0484	0.0497	0.0484	0.0480	0.0480	0.0461	0.0508	0.0488	0.0490	0.0483	0.0492	0.0469	0.0476	0.0476
F	7	blnk 7	0.0572	0.0547	0.0553	0.0523	0.0519	0.0518	0.0550	0.0543	0.0546	0.0525	0.0545	0.0542	0.0560	0.0540	0.0545	0.0526	0.0560	0.0534	0.0559	0.0526
F	8	blnk 8	0.0710	0.0669	0.0670	0.0660	0.0650	0.0646	0.0680	0.0668	0.0654	0.0647	0.0652	0.0655	0.0686	0.0662	0.0656	0.0645	0.0656	0.0640	0.0635	0.0650

Table C.11 SAAPLpNA Plate 2:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a	0.0636	0.0670	0.0700	0.0718	0.0756	0.0797	0.0839	0.0942	0.0903	0.0958	0.1000	0.1062	0.1085	0.1109	0.1177	0.1233	0.1259	0.1328	0.1352	0.1402
A	2	C1a	0.0502	0.0559	0.0567	0.0582	0.0645	0.0673	0.0718	0.0754	0.0794	0.0837	0.0876	0.0908	0.0938	0.0975	0.1022	0.1067	0.1135	0.1174	0.1198	0.1249
A	3	C1b	0.0589	0.0638	0.0658	0.0679	0.0701	0.0746	0.0782	0.0821	0.0875	0.0885	0.0942	0.0976	0.0997	0.1028	0.1068	0.1105	0.1159	0.1205	0.1227	0.1267
A	4	C1b	0.0504	0.0562	0.0593	0.0585	0.0627	0.0678	0.0682	0.0737	0.0753	0.0807	0.0851	0.0869	0.0898	0.0920	0.0969	0.0990	0.1024	0.1075	0.1092	0.1119
A	5	T1a	0.0425	0.0437	0.0423	0.0436	0.0449	0.0477	0.0461	0.0486	0.0504	0.0505	0.0505	0.0543	0.0539	0.0523	0.0562	0.0566	0.0601	0.0610	0.0619	0.0614
A	6	T1a	0.0446	0.0457	0.0471	0.0470	0.0483	0.0484	0.0500	0.0519	0.0533	0.0538	0.0559	0.0578	0.0589	0.0586	0.0596	0.0611	0.0626	0.0635	0.0653	0.0648
A	7	T1b	0.0423	0.0415	0.0426	0.0425	0.0434	0.0439	0.0444	0.0462	0.0474	0.0479	0.0490	0.0534	0.0534	0.0526	0.0549	0.0583	0.0578	0.0601	0.0635	0.0657
A	8	T1b	0.0450	0.0469	0.0449	0.0454	0.0482	0.0481	0.0477	0.0518	0.0511	0.0517	0.0531	0.0558	0.0569	0.0580	0.0581	0.0602	0.0609	0.0624	0.0641	0.0652
B	1	C2a	0.0673	0.0737	0.0827	0.0901	0.0998	0.1087	0.1153	0.1288	0.1364	0.1459	0.1583	0.1646	0.1744	0.1820	0.1934	0.2045	0.2142	0.2255	0.2325	0.2418
B	2	C2a	0.0634	0.0714	0.0812	0.0874	0.0986	0.1053	0.1160	0.1272	0.1359	0.1432	0.1532	0.1638	0.1736	0.1844	0.1933	0.2049	0.2155	0.2250	0.2342	0.2457
B	3	C2b	0.0557	0.0605	0.0663	0.0710	0.0771	0.0824	0.0901	0.0990	0.1044	0.1090	0.1172	0.1232	0.1290	0.1350	0.1431	0.1503	0.1577	0.1663	0.1713	0.1772
B	4	C2b	0.0542	0.0591	0.0636	0.0682	0.0751	0.0800	0.0867	0.0950	0.1005	0.1054	0.1118	0.1184	0.1244	0.1292	0.1376	0.1447	0.1524	0.1591	0.1648	0.1710
B	5	T2a	0.0480	0.0461	0.0466	0.0454	0.0483	0.0466	0.0471	0.0490	0.0487	0.0484	0.0502	0.0488	0.0505	0.0477	0.0505	0.0505	0.0495	0.0498	0.0497	0.0512
B	6	T2a	0.0458	0.0455	0.0462	0.0460	0.0466	0.0460	0.0476	0.0485	0.0468	0.0463	0.0502	0.0504	0.0490	0.0473	0.0487	0.0498	0.0497	0.0505	0.0494	0.0520
B	7	T2b	0.0527	0.0577	0.0608	0.0654	0.0731	0.0775	0.0833	0.0910	0.0965	0.1018	0.1063	0.1135	0.1198	0.1233	0.1321	0.1391	0.1470	0.1518	0.1582	0.1647
B	8	T2b	0.0574	0.0624	0.0688	0.0721	0.0786	0.0833	0.0866	0.0954	0.1000	0.1072	0.1149	0.1194	0.1258	0.1309	0.1390	0.1452	0.1518	0.1586	0.1645	0.1706
C	1	C3a	0.0871	0.1072	0.1245	0.1432	0.1625	0.1831	0.2023	0.2222	0.2416	0.2632	0.2826	0.3002	0.3201	0.3403	0.3588	0.3787	0.3963	0.4189	0.4395	0.4595
C	2	C3a	0.1073	0.1260	0.1485	0.1685	0.1920	0.2113	0.2340	0.2564	0.2773	0.2994	0.3224	0.3442	0.3619	0.3823	0.4015	0.4215	0.4440	0.4689	0.4909	0.5084
C	3	C3b	0.0590	0.0646	0.0698	0.0768	0.0835	0.0898	0.0975	0.1075	0.1125	0.1198	0.1308	0.1371	0.1447	0.1518	0.1598	0.1703	0.1774	0.1872	0.1946	0.2006
C	4	C3b	0.0623	0.0678	0.0735	0.0812	0.0886	0.0951	0.1037	0.1143	0.1190	0.1279	0.1395	0.1468	0.1544	0.1624	0.1711	0.1826	0.1898	0.2004	0.2081	0.2147
C	5	T3a	0.0593	0.0569	0.0568	0.0570	0.0584	0.0584	0.0600	0.0602	0.0612	0.0640	0.0662	0.0654	0.0622	0.0613	0.0655	0.0660	0.0657	0.0695	0.0678	0.0664
C	6	T3a	0.0431	0.0426	0.0449	0.0456	0.0451	0.0462	0.0479	0.0476	0.0485	0.0481	0.0507	0.0500	0.0512	0.0504	0.0519	0.0531	0.0531	0.0566	0.0544	0.0541
C	7	T3b	0.0507	0.0511	0.0506	0.0498	0.0525	0.0537	0.0522	0.0548	0.0523	0.0544	0.0567	0.0563	0.0556	0.0546	0.0566	0.0546	0.0557	0.0576	0.0580	0.0582
C	8	T3b	0.0574	0.0589	0.0562	0.0563	0.0571	0.0582	0.0595	0.0595	0.0608	0.0583	0.0614	0.0618	0.0603	0.0597	0.0617	0.0625	0.0636	0.0626	0.0645	0.0622
D	1	C4a	0.0786	0.0879	0.0987	0.1106	0.1266	0.1393	0.1523	0.1675	0.1797	0.1935	0.2093	0.2246	0.2385	0.2506	0.2662	0.2813	0.2970	0.3117	0.3258	0.3402
D	2	C4a	0.0801	0.0881	0.0991	0.1105	0.1232	0.1357	0.1485	0.1629	0.1742	0.1870	0.2032	0.2166	0.2292	0.2414	0.2536	0.2680	0.2816	0.2965	0.3094	0.3224
D	3	C4b	0.0603	0.0685	0.0752	0.0825	0.0927	0.1045	0.1105	0.1206	0.1270	0.1391	0.1472	0.1570	0.1682	0.1755	0.1859	0.1969	0.2062	0.2179	0.2275	0.2369
D	4	C4b	0.0662	0.0739	0.0814	0.0904	0.0994	0.1069	0.1170	0.1265	0.1353	0.1464	0.1568	0.1658	0.1764	0.1898	0.2047	0.2150	0.2257	0.2338	0.2388	0.2476

Table C.11 SAAPLpNA Plate 2 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
D	5	T4a	0.0933	0.1108	0.1309	0.1504	0.1716	0.1912	0.2130	0.2343	0.2543	0.2750	0.2976	0.3166	0.3372	0.3552	0.3767	0.3957	0.4166	0.4359	0.4553	0.4726
D	6	T4a	0.0894	0.1072	0.1305	0.1482	0.1665	0.1873	0.2083	0.2297	0.2475	0.2690	0.2925	0.3114	0.3300	0.3474	0.3685	0.3874	0.4068	0.4262	0.4443	0.4609
D	7	T4b	0.0533	0.0519	0.0567	0.0566	0.0582	0.0591	0.0614	0.0671	0.0701	0.0699	0.0752	0.0769	0.0770	0.0811	0.0829	0.0842	0.0889	0.0913	0.0936	0.0962
D	8	T4b	0.0463	0.0479	0.0504	0.0503	0.0534	0.0572	0.0583	0.0620	0.0650	0.0657	0.0696	0.0711	0.0745	0.0758	0.0801	0.0803	0.0834	0.0876	0.0891	0.0958
E	1	C5a	0.1044	0.1193	0.1396	0.1592	0.1824	0.2040	0.2291	0.2506	0.2733	0.2938	0.3175	0.3410	0.3594	0.3801	0.4024	0.4204	0.4451	0.4646	0.4819	0.4992
E	2	C5a	0.0932	0.1087	0.1291	0.1474	0.1694	0.1852	0.2072	0.2291	0.2468	0.2674	0.2902	0.3106	0.3292	0.3487	0.3685	0.3877	0.4075	0.4261	0.4457	0.4609
E	3	C5b	0.0975	0.1137	0.1312	0.1485	0.1688	0.1872	0.2070	0.2290	0.2474	0.2644	0.2844	0.3032	0.3206	0.3396	0.3591	0.3774	0.3987	0.4151	0.4325	0.4501
E	4	C5b	0.0897	0.1075	0.1256	0.1469	0.1664	0.1843	0.2032	0.2251	0.2434	0.2626	0.2838	0.3042	0.3210	0.3405	0.3590	0.3763	0.3966	0.4165	0.4338	0.4505
E	5	T5a	0.0760	0.0852	0.0931	0.1018	0.1137	0.1240	0.1349	0.1487	0.1583	0.1689	0.1823	0.1942	0.2049	0.2169	0.2302	0.2417	0.2537	0.2671	0.2790	0.2910
E	6	T5a	0.0747	0.0862	0.0951	0.1043	0.1182	0.1289	0.1412	0.1570	0.1659	0.1783	0.1918	0.2058	0.2166	0.2304	0.2445	0.2575	0.2703	0.2849	0.2967	0.3102
E	7	T5b	0.0543	0.0557	0.0560	0.0560	0.0630	0.0595	0.0621	0.0672	0.0662	0.0693	0.0723	0.0750	0.0777	0.0790	0.0775	0.0795	0.0818	0.0873	0.0892	0.0885
E	8	T5b	0.0593	0.0594	0.0605	0.0613	0.0637	0.0642	0.0672	0.0692	0.0750	0.0732	0.0763	0.0797	0.0789	0.0820	0.0840	0.0863	0.0897	0.0932	0.0937	0.0946
F	1	blnk 1	0.0477	0.0485	0.0468	0.0461	0.0461	0.0464	0.0456	0.0477	0.0458	0.0467	0.0467	0.0467	0.0466	0.0424	0.0456	0.0458	0.0460	0.0459	0.0453	0.0451
F	2	blnk 2	0.0551	0.0516	0.0526	0.0529	0.0525	0.0512	0.0518	0.0540	0.0520	0.0520	0.0530	0.0519	0.0525	0.0511	0.0526	0.0510	0.0514	0.0539	0.0532	0.0517
F	3	blnk 3	0.0465	0.0464	0.0449	0.0425	0.0462	0.0444	0.0430	0.0464	0.0446	0.0430	0.0450	0.0456	0.0452	0.0435	0.0434	0.0451	0.0445	0.0459	0.0452	0.0441
F	4	blnk 4	0.0544	0.0519	0.0492	0.0507	0.0494	0.0512	0.0509	0.0518	0.0496	0.0505	0.0526	0.0532	0.0491	0.0499	0.0520	0.0513	0.0498	0.0501	0.0518	0.0500
F	5	blnk 5	0.0616	0.0590	0.0584	0.0581	0.0588	0.0581	0.0586	0.0592	0.0567	0.0592	0.0587	0.0572	0.0558	0.0554	0.0592	0.0574	0.0582	0.0588	0.0584	0.0575
F	6	blnk 6	0.0480	0.0463	0.0477	0.0455	0.0453	0.0463	0.0473	0.0488	0.0475	0.0510	0.0557	0.0478	0.0453	0.0452	0.0473	0.0462	0.0475	0.0466	0.0461	0.0455
F	7	blnk 7	0.0531	0.0519	0.0523	0.0516	0.0526	0.0501	0.0512	0.0536	0.0518	0.0523	0.0539	0.0520	0.0500	0.0484	0.0505	0.0513	0.0507	0.0527	0.0531	0.0502
F	8	blnk 8	0.0568	0.0567	0.0548	0.0560	0.0550	0.0563	0.0558	0.0575	0.0543	0.0551	0.0577	0.0567	0.0541	0.0546	0.0549	0.0541	0.0541	0.0548	0.0563	0.0528

Table C.12 SAAPLpNA Plate 3:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a	0.0638	0.0671	0.0703	0.0756	0.0788	0.0831	0.0882	0.0888	0.0961	0.0977	0.1011	0.1067	0.1122	0.1131	0.1184	0.1227	0.1318	0.1344	0.1389	0.1442
A	2	C1a	0.0528	0.0567	0.0625	0.0648	0.0677	0.0736	0.0743	0.0781	0.0827	0.0856	0.0889	0.0929	0.0985	0.1016	0.1054	0.1110	0.1145	0.1178	0.1237	0.1282
A	3	C1b	0.0605	0.0643	0.0694	0.0707	0.0721	0.0759	0.0810	0.0842	0.0872	0.0905	0.0932	0.0969	0.1002	0.1045	0.1092	0.1131	0.1179	0.1199	0.1262	0.1292
A	4	C1b	0.0509	0.0595	0.0612	0.0644	0.0667	0.0706	0.0736	0.0768	0.0792	0.0842	0.0838	0.0884	0.0947	0.0949	0.0988	0.1029	0.1080	0.1080	0.1144	0.1194
A	5	T1a	0.0409	0.0441	0.0425	0.0453	0.0434	0.0459	0.0472	0.0492	0.0486	0.0493	0.0506	0.0522	0.0532	0.0542	0.0549	0.0551	0.0584	0.0567	0.0610	0.0607
A	6	T1a	0.0437	0.0476	0.0472	0.0501	0.0469	0.0497	0.0524	0.0521	0.0544	0.0551	0.0558	0.0584	0.0580	0.0571	0.0572	0.0584	0.0612	0.0604	0.0642	0.0648
A	7	T1b	0.0391	0.0399	0.0421	0.0440	0.0418	0.0450	0.0451	0.0461	0.0491	0.0487	0.0481	0.0487	0.0497	0.0513	0.0513	0.0560	0.0567	0.0551	0.0586	0.0604
A	8	T1b	0.0406	0.0455	0.0466	0.0466	0.0445	0.0484	0.0510	0.0501	0.0524	0.0551	0.0522	0.0539	0.0568	0.0568	0.0569	0.0634	0.0630	0.0614	0.0634	0.0648
B	1	C2a	0.0731	0.0814	0.0887	0.0987	0.1088	0.1174	0.1294	0.1375	0.1464	0.1556	0.1653	0.1753	0.1868	0.1954	0.2070	0.2168	0.2281	0.2363	0.2500	0.2581
B	2	C2a	0.0682	0.0774	0.0850	0.0943	0.1027	0.1116	0.1217	0.1301	0.1372	0.1475	0.1565	0.1670	0.1772	0.1843	0.1952	0.2052	0.2143	0.2253	0.2357	0.2441
B	3	C2b	0.0572	0.0651	0.0691	0.0748	0.0774	0.0844	0.0899	0.0953	0.1014	0.1072	0.1130	0.1184	0.1255	0.1311	0.1368	0.1442	0.1519	0.1564	0.1642	0.1709
B	4	C2b	0.0559	0.0619	0.0667	0.0702	0.0739	0.0791	0.0853	0.0899	0.0963	0.1015	0.1040	0.1108	0.1179	0.1236	0.1282	0.1337	0.1406	0.1465	0.1537	0.1587
B	5	T2a	0.0484	0.0500	0.0501	0.0516	0.0490	0.0513	0.0514	0.0518	0.0531	0.0524	0.0547	0.0529	0.0540	0.0531	0.0534	0.0552	0.0559	0.0535	0.0547	0.0551
B	6	T2a	0.0430	0.0478	0.0469	0.0472	0.0464	0.0468	0.0482	0.0482	0.0494	0.0477	0.0483	0.0475	0.0486	0.0487	0.0487	0.0498	0.0494	0.0499	0.0518	0.0527
B	7	T2b	0.0566	0.0637	0.0673	0.0737	0.0780	0.0836	0.0890	0.0973	0.1006	0.1050	0.1114	0.1169	0.1321	0.1305	0.1349	0.1421	0.1489	0.1542	0.1633	0.1695
B	8	T2b	0.0578	0.0665	0.0721	0.0785	0.0824	0.0895	0.0963	0.1040	0.1108	0.1139	0.1207	0.1265	0.1334	0.1402	0.1455	0.1534	0.1596	0.1654	0.1734	0.1815
C	1	C3a	0.1263	0.1489	0.1735	0.1950	0.2177	0.2431	0.2677	0.2911	0.3138	0.3366	0.3589	0.3795	0.4042	0.4247	0.4458	0.4656	0.4842	0.5014	0.5226	0.5388
C	2	C3a	0.1233	0.1449	0.1671	0.1898	0.2093	0.2309	0.2532	0.2725	0.2957	0.3146	0.3339	0.3545	0.3770	0.3954	0.4144	0.4331	0.4529	0.4694	0.4877	0.5034
C	3	C3b	0.0590	0.0642	0.0717	0.0778	0.0828	0.0880	0.0973	0.1025	0.1095	0.1158	0.1220	0.1284	0.1379	0.1434	0.1508	0.1586	0.1671	0.1734	0.1826	0.1894
C	4	C3b	0.0661	0.0719	0.0814	0.0865	0.0954	0.0995	0.1069	0.1148	0.1227	0.1310	0.1362	0.1439	0.1534	0.1611	0.1697	0.1757	0.1861	0.1927	0.2024	0.2099
C	5	T3a	0.0604	0.0598	0.0593	0.0614	0.0633	0.0639	0.0674	0.0650	0.0657	0.0666	0.0660	0.0666	0.0678	0.0673	0.0680	0.0696	0.0723	0.0689	0.0708	0.0741
C	6	T3a	0.0460	0.0462	0.0484	0.0487	0.0457	0.0506	0.0508	0.0497	0.0503	0.0501	0.0520	0.0521	0.0544	0.0521	0.0531	0.0551	0.0563	0.0547	0.0579	0.0570
C	7	T3b	0.0482	0.0515	0.0498	0.0498	0.0492	0.0499	0.0514	0.0534	0.0513	0.0530	0.0531	0.0539	0.0553	0.0549	0.0563	0.0556	0.0595	0.0553	0.0570	0.0583
C	8	T3b	0.0547	0.0574	0.0573	0.0591	0.0580	0.0586	0.0598	0.0592	0.0618	0.0599	0.0602	0.0604	0.0628	0.0622	0.0612	0.0639	0.0629	0.0619	0.0640	0.0652
D	1	C4a	0.0816	0.0952	0.1061	0.1199	0.1304	0.1429	0.1568	0.1669	0.1808	0.1973	0.2088	0.2206	0.2353	0.2493	0.2627	0.2770	0.2918	0.3037	0.3205	0.3324
D	2	C4a	0.0933	0.1050	0.1181	0.1321	0.1456	0.1595	0.1765	0.1894	0.2065	0.2184	0.2357	0.2489	0.2641	0.2776	0.2905	0.3071	0.3238	0.3344	0.3507	0.3655
D	3	C4b	0.0671	0.0742	0.0815	0.0921	0.0985	0.1080	0.1145	0.1238	0.1325	0.1409	0.1488	0.1590	0.1693	0.1802	0.1871	0.1996	0.2101	0.2181	0.2294	0.2384
D	4	C4b	0.0688	0.0779	0.0869	0.0931	0.1024	0.1072	0.1146	0.1243	0.1327	0.1409	0.1499	0.1590	0.1681	0.1775	0.1855	0.1950	0.2082	0.2149	0.2250	0.2352
D	5	T4a	0.1037	0.1220	0.1421	0.1606	0.1780	0.1972	0.2164	0.2334	0.2543	0.2719	0.2895	0.3099	0.3273	0.3472	0.3644	0.3836	0.4026	0.4188	0.4357	0.4530

Table C.12 SAAPLpNA Plate 3 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
D	6	T4a	0.0970	0.1142	0.1324	0.1506	0.1682	0.1847	0.2039	0.2199	0.2383	0.2571	0.2732	0.2917	0.3102	0.3265	0.3417	0.3610	0.3794	0.3953	0.4146	0.4274
D	7	T4b	0.0509	0.0533	0.0570	0.0584	0.0608	0.0641	0.0664	0.0692	0.0721	0.0773	0.0788	0.0816	0.0859	0.0849	0.0873	0.0913	0.0951	0.0986	0.1012	0.1036
D	8	T4b	0.0490	0.0507	0.0529	0.0549	0.0565	0.0593	0.0616	0.0628	0.0661	0.0691	0.0723	0.0743	0.0803	0.0799	0.0822	0.0863	0.0895	0.0930	0.0938	0.0965
E	1	C5a	0.1098	0.1288	0.1465	0.1679	0.1856	0.2061	0.2277	0.2486	0.2701	0.2896	0.3096	0.3292	0.3510	0.3703	0.3886	0.4083	0.4282	0.4460	0.4633	0.4835
E	2	C5a	0.1000	0.1181	0.1343	0.1527	0.1698	0.1885	0.2072	0.2247	0.2442	0.2624	0.2792	0.2984	0.3174	0.3357	0.3535	0.3717	0.3902	0.4066	0.4247	0.4422
E	3	C5b	0.1005	0.1163	0.1309	0.1480	0.1632	0.1805	0.1938	0.2098	0.2263	0.2422	0.2566	0.2767	0.2927	0.3075	0.3242	0.3419	0.3579	0.3735	0.3908	0.4045
E	4	C5b	0.0923	0.1087	0.1228	0.1390	0.1511	0.1684	0.1864	0.2019	0.2186	0.2337	0.2495	0.2658	0.2820	0.2976	0.3172	0.3332	0.3507	0.3627	0.3788	0.3956
E	5	T5a	0.0749	0.0843	0.0910	0.0978	0.1044	0.1134	0.1217	0.1306	0.1382	0.1473	0.1541	0.1663	0.1763	0.1843	0.1925	0.2063	0.2144	0.2199	0.2284	0.2356
E	6	T5a	0.0701	0.0788	0.0843	0.0906		0.1053	0.1133	0.1205	0.1291	0.1367	0.1431	0.1522	0.1625	0.1686	0.1773	0.1883	0.1971	0.2065	0.2189	0.2257
E	7	T5b	0.0519	0.0543	0.0536	0.0564	0.0571	0.0601	0.0615	0.0654	0.0676	0.0685	0.0696	0.0724	0.0731	0.0762	0.0778	0.0804	0.0825	0.0827	0.0872	0.0903
E	8	T5b									0.0691	0.0704	0.0715	0.0746	0.0772	0.0775	0.0801	0.0828	0.0845	0.0863	0.0887	0.0902
F	1	blnk 1	0.0462	0.0474	0.0478	0.0471	0.0473	0.0460	0.0463	0.0463	0.0464	0.0469	0.0464	0.0454	0.0460	0.0456	0.0439	0.0454	0.0463	0.0462	0.0456	0.0461
F	2	blnk 2	0.0497	0.0514	0.0524	0.0523	0.0529	0.0531	0.0516	0.0515	0.0506	0.0524	0.0512	0.0506	0.0502	0.0519	0.0495	0.0509	0.0510	0.0498	0.0516	0.0516
F	3	blnk 3	0.0444	0.0461	0.0463	0.0468	0.0455	0.0450	0.0462	0.0446	0.0450	0.0446	0.0452	0.0446	0.0458	0.0453	0.0438	0.0451	0.0459	0.0443	0.0451	0.0466
F	4	blnk 4	0.0506	0.0516	0.0533	0.0532	0.0513	0.0511	0.0519	0.0512	0.0531	0.0522	0.0499	0.0503	0.0494	0.0502	0.0505	0.0499	0.0512	0.0507	0.0502	0.0502
F	5	blnk 5	0.0569	0.0576	0.0571	0.0569	0.0567	0.0584	0.0583	0.0563	0.0557	0.0570	0.0561	0.0556	0.0573	0.0558	0.0561	0.0565	0.0566	0.0575	0.0556	0.0568
F	6	blnk 6	0.0446	0.0478	0.0468	0.0481	0.0467	0.0477	0.0478	0.0460	0.0467	0.0471	0.0451	0.0483	0.0455	0.0469	0.0437	0.0462	0.0458	0.0459	0.0449	0.0470
F	7	blnk 7	0.0510	0.0527	0.0523	0.0538	0.0508	0.0516	0.0525	0.0517	0.0514	0.0506	0.0519	0.0509	0.0508	0.0513	0.0502	0.0492	0.0510	0.0587	0.0575	0.0499
F	8	blnk 8	0.0577	0.0586	0.0579	0.0601	0.0590	0.0598	0.0571	0.0583	0.0567	0.0587	0.0562	0.0562	0.0556	0.0549	0.0558	0.0556	0.0558	0.0563	0.0558	0.0571

Table C.13 pNP butyrate Plate 1:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a	0.1198	0.1483	0.1700	0.1980	0.2276	0.2538	0.2854	0.3098	0.3391	0.3685	0.3996	0.4292	0.4568	0.4858	0.5174	0.5448	0.5730	0.6030	0.6325	0.6639
A	2	C1a	0.1038	0.1342	0.1605	0.1856	0.2157	0.2406	0.2707	0.2974	0.3256	0.3543	0.3824	0.4119	0.4410	0.4684	0.5011	0.5286	0.5558	0.5864	0.6135	0.6443
A	3	C1b	0.1152	0.1355	0.1549	0.1788	0.1995	0.2211	0.2438	0.2671	0.2906	0.3134	0.3367	0.3600	0.3855	0.4074	0.4304	0.4540	0.4767	0.5025	0.5227	0.5489
A	4	C1b	0.1100	0.1347	0.1549	0.1796	0.2007	0.2237	0.2454	0.2694	0.2923	0.3188	0.3420	0.3637	0.3865	0.4136	0.4385	0.4605	0.4838	0.5102	0.5348	0.5586
A	5	T1a	0.0896	0.1118	0.1324	0.1508	0.1742	0.1938	0.2141	0.2378	0.2593	0.2793	0.3032	0.3256	0.3466	0.3697	0.3941	0.4145	0.4353	0.4598	0.4808	0.5044
A	6	T1a	0.0967	0.1182	0.1344	0.1532	0.1780	0.1941	0.2155	0.2354	0.2576	0.2789	0.2990	0.3208	0.3392	0.3612	0.3848	0.4052	0.4278	0.4500	0.4727	0.4973
A	7	T1b	0.0893	0.1105	0.1308	0.1513	0.1739	0.1927	0.2140	0.2353	0.2562	0.2762	0.2995	0.3214	0.3401	0.3630	0.3858	0.4058	0.4278	0.4489	0.4717	0.4946
A	8	T1b	0.0948	0.1155	0.1349	0.1578	0.1797	0.2002	0.2242	0.2439	0.2650	0.2898	0.3128	0.3360	0.3568	0.3796	0.4040	0.4266	0.4486	0.4731	0.4933	0.5185
B	1	C2a	0.1189	0.1493	0.1746	0.2038	0.2352	0.2614	0.2933	0.3223	0.3548	0.3818	0.4140	0.4464	0.4745	0.5047	0.5384	0.5683	0.5975	0.6295	0.6599	0.6917
B	2	C2a	0.1127	0.1428	0.1686	0.1960	0.2263	0.2780	0.2919	0.3110	0.3380	0.3672	0.3948	0.4269	0.4537	0.4844	0.5152	0.5443	0.5714	0.6002	0.6294	0.6583
B	3	C2b	0.1004	0.1270	0.1467	0.1705	0.1974	0.2176	0.2423	0.2650	0.2886	0.3126	0.3369	0.3610	0.3849	0.4080	0.4354	0.4570	0.4822	0.5057	0.5309	0.5548
B	4	C2b	0.1017	0.1288	0.1485	0.1724	0.1967	0.2204	0.2446	0.2683	0.2927	0.3161	0.3407	0.3670	0.3902	0.4138	0.4425	0.4643	0.4878	0.5140	0.5382	0.5638
B	5	T2a	0.0962	0.1168	0.1353	0.1515	0.1728	0.1885	0.2084	0.2278	0.2478	0.2684	0.2868	0.3096	0.3265	0.3466	0.3700	0.3882	0.4082	0.4284	0.4486	0.4707
B	6	T2a	0.0997	0.1187	0.1332	0.1528	0.1743	0.1907	0.2094	0.2304	0.2474	0.2681	0.2862	0.3076	0.3242	0.3445	0.3662	0.3844	0.4027	0.4232	0.4432	0.4648
B	7	T2b	0.1100	0.1396	0.1673	0.1984	0.2272	0.2538	0.2854	0.3118	0.3417	0.3724	0.4003	0.4327	0.4607	0.4903	0.5208	0.5536	0.5815	0.6123	0.6394	0.6713
B	8	T2b	0.1250	0.1442	0.1712	0.2016	0.2311	0.2608	0.2899	0.3233	0.3510	0.3817	0.4116	0.4441	0.4744	0.5047	0.5380	0.5674	0.5965	0.6288	0.6675	0.7108
C	1	C3a	0.1340	0.1731	0.2151	0.2569	0.2999	0.3398	0.3831	0.4240	0.4659	0.5087	0.5509	0.5940	0.6368	0.6780	0.7219	0.7629	0.8043	0.8448	0.8889	0.9313
C	2	C3a	0.1327	0.1711	0.2089	0.2475	0.2882	0.3249	0.3642	0.4033	0.4415	0.4824	0.5212	0.5607	0.6001	0.6384	0.6803	0.7178	0.7560	0.7949	0.8353	0.8753
C	3	C3b	0.1150	0.1440	0.1729	0.2018	0.2349	0.2676	0.2963	0.3267	0.3584	0.3887	0.4190	0.4515	0.4827	0.5165	0.5488	0.5771	0.6100	0.6423	0.6742	0.7040
C	4	C3b	0.1156	0.1466	0.1746	0.2037	0.2387	0.2695	0.2983	0.3299	0.3589	0.3918	0.4205	0.4526	0.4840	0.5150	0.5486	0.5788	0.6098	0.6416	0.6733	0.7062
C	5	T3a	0.1277	0.1581	0.1823	0.2080	0.2382	0.2664	0.2953	0.3258	0.3528	0.3832	0.4108	0.4406	0.4721	0.4993	0.5313	0.5571	0.5873	0.6167	0.6447	0.6763
C	6	T3a	0.1101	0.1442	0.1710	0.2031	0.2352	0.2648	0.2944	0.3276	0.3577	0.3899	0.4229	0.4560	0.4887	0.5191	0.5535	0.5829	0.6148	0.6479	0.6813	0.7145
C	7	T3b	0.1047	0.1265	0.1445	0.1656	0.1886	0.2083	0.2302	0.2506	0.2730	0.2953	0.3173	0.3377	0.3603	0.3814	0.4060	0.4254	0.4468	0.4696	0.4917	0.5138
C	8	T3b	0.1123	0.1366	0.1550	0.1773	0.2023	0.2196	0.2430	0.2648	0.2860	0.3059	0.3290	0.3572	0.3776	0.3980	0.4251	0.4438	0.4655	0.4895	0.5121	0.5339
D	1	C4a	0.1437	0.1899	0.2307	0.2772	0.3249	0.3706	0.4147	0.4570	0.5027	0.5517	0.5963	0.6447	0.6898	0.7350	0.7822	0.8260	0.8719	0.9168	0.9618	1.0098
D	2	C4a	0.1409	0.1835	0.2222	0.2647	0.3093	0.3511	0.3924	0.4332	0.4750	0.5206	0.5616	0.6065	0.6489	0.6916	0.7357	0.7761	0.8196	0.8619	0.9041	0.9484
D	3	C4b	0.1193	0.1538	0.1839	0.2170	0.2509	0.2830	0.3166	0.3486	0.3829	0.4171	0.4498	0.4866	0.5179	0.5522	0.5904	0.6212	0.6551	0.6901	0.7239	0.7585
D	4	C4b	0.1283	0.1629	0.1967	0.2313	0.2681	0.3011	0.3375	0.3735	0.4111	0.4463	0.4826	0.5203	0.5548	0.5927	0.6313	0.6652	0.7021	0.7400	0.7760	0.8127
D	5	T4a	0.1346	0.1786	0.2162	0.2605	0.3071	0.3505	0.3920	0.4389	0.4787	0.5214	0.5662	0.6146	0.6566	0.6993	0.7472	0.7897	0.8301	0.8790	0.9210	0.9666

Table C.13 pNP butyrate Plate 1 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
D	6	T4a	0.1440	0.1909	0.2372	0.2840	0.3335	0.3819	0.4264	0.4739	0.5198	0.5678	0.6141	0.6641	0.7095	0.7566	0.8066	0.8538	0.8995	0.9485	0.9937	1.0433
D	7	T4b	0.1206	0.1480	0.1735	0.2035	0.2340	0.2619	0.2907	0.3206	0.3491	0.3788	0.4090	0.4401	0.4692	0.4986	0.5337	0.5610	0.5904	0.6217	0.6514	0.6832
D	8	T4b	0.1177	0.1479	0.1756	0.2071	0.2389	0.2692	0.3020	0.3313	0.3617	0.3933	0.4250	0.4601	0.4904	0.5229	0.5592	0.5908	0.6213	0.6550	0.6865	0.7201
E	1	C5a	0.1429	0.1777	0.2151	0.2520	0.2928	0.3315	0.3707	0.4115	0.4507	0.4903	0.5317	0.5732	0.6114	0.6530	0.6970	0.7328	0.7755	0.8170	0.8558	0.8960
E	2	C5a	0.1341	0.1680	0.2022	0.2377	0.2757	0.3116	0.3478	0.3848	0.4212	0.4596	0.4966	0.5352	0.5723	0.6102	0.6505	0.6850	0.7232	0.7617	0.7997	0.8382
E	3	C5b	0.1408	0.1783	0.2116	0.2497	0.2882	0.3282	0.3674	0.4054	0.4416	0.4821	0.5204	0.5615	0.6018	0.6387	0.6796	0.7172	0.7570	0.7969	0.8351	0.8760
E	4	C5b	0.1329	0.1724	0.2073	0.2463	0.2854	0.3198	0.3580	0.3980	0.4361	0.4737	0.5145	0.5568	0.5939	0.6320	0.6741	0.7114	0.7484	0.7896	0.8294	0.8713
E	5	T5a	0.1314	0.1579	0.1859	0.2136	0.2450	0.2734	0.3037	0.3336	0.3624	0.3928	0.4204	0.4531	0.4824	0.5128	0.5470	0.5734	0.6032	0.6336	0.6659	0.6957
E	6	T5a	0.1293	0.1588	0.1893	0.2204	0.2561	0.2853	0.3176	0.3501	0.3830	0.4122	0.4460	0.4811	0.5125	0.5459	0.5792	0.6093	0.6418	0.6771	0.7090	0.7425
E	7	T5b	0.1145	0.1436	0.1705	0.1990	0.2308	0.2535	0.2832	0.3123	0.3372	0.3672	0.3962	0.4259	0.4547	0.4808	0.5132	0.5390	0.5680	0.5985	0.6245	0.6546
E	8	T5b	0.1273	0.1542	0.1797	0.2066	0.2358	0.2644	0.2885	0.3188	0.3469	0.3766	0.4052	0.4349	0.4593	0.4879	0.5193	0.5451	0.5736	0.6043	0.6326	0.6606
F	1	blnk 1	0.0923	0.1025	0.1103	0.1182	0.1303	0.1386	0.1488	0.1593	0.1707	0.1809	0.1900	0.2050	0.2123	0.2226	0.2363	0.2462	0.2549	0.2670	0.2780	0.2899
F	2	blnk 2	0.1002	0.1114	0.1178	0.1309	0.1411	0.1497	0.1611	0.1700	0.1784	0.1921	0.2016	0.2148	0.2232	0.2337	0.2466	0.2563	0.2671	0.2779	0.2893	0.3017
F	3	blnk 3	0.0903	0.1011	0.1082	0.1174	0.1318	0.1402	0.1520	0.1617	0.1716	0.1825	0.1933	0.2049	0.2135	0.2249	0.2390	0.2469	0.2589	0.2690	0.2814	0.2928
F	4	blnk 4	0.0980	0.1081	0.1152	0.1253	0.1374	0.1471	0.1572	0.1656	0.1757	0.1880	0.1970	0.2097	0.2196	0.2304	0.2433	0.2491	0.2615	0.2734	0.2833	0.2954
F	5	blnk 5	0.1063	0.1162	0.1224	0.1339	0.1452	0.1532	0.1646	0.1745	0.1843	0.1945	0.2048	0.2170	0.2266	0.2368	0.2489	0.2618	0.2694	0.2799	0.2920	0.3021
F	6	blnk 6	0.0899	0.1004	0.1071	0.1194	0.1305	0.1381	0.1483	0.1594	0.1675	0.1806	0.1901	0.2015	0.2118	0.2210	0.2344	0.2430	0.2514	0.2653	0.2757	0.2870
F	7	blnk 7	0.0988	0.1066	0.1163	0.1254	0.1373	0.1469	0.1585	0.1683	0.1765	0.1887	0.1994	0.2111	0.2215	0.2313	0.2447	0.2537	0.2644	0.2765	0.2864	0.2989
F	8	blnk 8	0.1072	0.1168	0.1239	0.1352	0.1484	0.1565	0.1683	0.1785	0.1881	0.2009	0.2102	0.2231	0.2312	0.2431	0.2570	0.2666	0.2755	0.2872	0.2980	0.3102

Table C.14 pNP Butyrate Plate 2:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a	0.1266	0.1490	0.1755	0.2047	0.2318	0.2546	0.2809	0.3092	0.3371	0.3668	0.3955	0.4212	0.4515	0.4789	0.5074	0.5396	0.5653	0.5930	0.6200	0.6466
A	2	C1a	0.1191	0.1424	0.1711	0.1984	0.2273	0.2541	0.2817	0.3068	0.3374	0.3660	0.3956	0.4231	0.4518	0.4854	0.5120	0.5428	0.5704	0.5995	0.6284	0.6565
A	3	C1b	0.1259	0.1460	0.1709	0.1947	0.2200	0.2443	0.2686	0.2941	0.3241	0.3465	0.3731	0.3988	0.4233	0.4512	0.4750	0.5020	0.5260	0.5535	0.5802	0.6055
A	4	C1b	0.1236	0.1463	0.1717	0.1989	0.2248	0.2498	0.2762	0.3030	0.3323	0.3606	0.3842	0.4110	0.4396	0.4669	0.4924	0.5229	0.5485	0.5775	0.6058	0.6311
A	5	T1a	0.1049	0.1219	0.1436	0.1636	0.1862	0.2045	0.2244	0.2459	0.2672	0.2876	0.3103	0.3332	0.3531	0.3773	0.3963	0.4202	0.4393	0.4622	0.4831	0.5054
A	6	T1a	0.1094	0.1283	0.1468	0.1671	0.1862	0.2074	0.2237	0.2455	0.2659	0.2849	0.3089	0.3288	0.3481	0.3699	0.3905	0.4135	0.4329	0.4540	0.4754	0.4948
A	7	T1b	0.1030	0.1230	0.1444	0.1655	0.1889	0.2081	0.2283	0.2492	0.2747	0.2964	0.3187	0.3400	0.3616	0.3843	0.4070	0.4315	0.4514	0.4750	0.4970	0.5166
A	8	T1b	0.1056	0.1263	0.1450	0.1670	0.1885	0.2084	0.2293	0.2500	0.2735	0.2960	0.3164	0.3397	0.3603	0.3846	0.4049	0.4364	0.4493	0.4741	0.4956	0.5155
B	1	C2a	0.1289	0.1546	0.1839	0.2162	0.2443	0.2731	0.3043	0.3308	0.3640	0.3931	0.4246	0.4535	0.4841	0.5177	0.5486	0.5779	0.6086	0.6408	0.6703	0.6994
B	2	C2a	0.1267	0.1524	0.1804	0.2096	0.2395	0.2657	0.2936	0.3215	0.3536	0.3811	0.4133	0.4420	0.4720	0.5012	0.5293	0.5607	0.5907	0.6200	0.6514	0.6773
B	3	C2b	0.1081	0.1269	0.1473	0.1685	0.1875	0.2069	0.2256	0.2453	0.2670	0.2880	0.3103	0.3294	0.3490	0.3717	0.3914	0.4143	0.4349	0.4543	0.4773	0.4976
B	4	C2b	0.1114	0.1284	0.1523	0.1712	0.1937	0.2119	0.2321	0.2534	0.2769	0.2971	0.3189	0.3406	0.3627	0.3858	0.4055	0.4277	0.4484	0.4721	0.4934	0.5138
B	5	T2a	0.1092	0.1253	0.1438	0.1615	0.1812	0.2001	0.2156	0.2366	0.2539	0.2739	0.2937	0.3109	0.3290	0.3512	0.3708	0.3906	0.4084	0.4283	0.4479	0.4663
B	6	T2a	0.1095	0.1275	0.1448	0.1640	0.1832	0.2018	0.2184	0.2386	0.2693				0.3440	0.3583	0.3770	0.3973	0.4159	0.4352	0.4558	0.4759
B	7	T2b	0.1198	0.1479	0.1726	0.2017	0.2286	0.2579	0.2853	0.3125	0.3416	0.3698	0.4011	0.4271	0.4558	0.4901	0.5150	0.5442	0.5715	0.6019	0.6307	0.6594
B	8	T2b	0.1266	0.1552	0.1825	0.2112	0.2425	0.2704	0.2992	0.3288	0.3615	0.3932	0.4231	0.4527	0.4838	0.5141	0.5454	0.5775	0.6054	0.6388	0.6687	0.6989
C	1	C3a	0.1401	0.1759	0.2101	0.2482	0.2878	0.3214	0.3587	0.3966	0.4364	0.4719	0.5121	0.5482	0.5890	0.6228	0.6636	0.7018	0.7376	0.7778	0.8147	0.8513
C	2	C3a	0.1454	0.1790	0.2162	0.2510	0.2889	0.3243	0.3607	0.3960	0.4330	0.4705	0.5079	0.5430	0.5797	0.6180	0.6530	0.6911	0.7268	0.7629	0.8006	0.8331
C	3	C3b	0.1204	0.1480	0.1780	0.2088	0.2390	0.2678	0.2952	0.3269	0.3567	0.3885	0.4197	0.4468	0.4779	0.5121	0.5399	0.5724	0.5997	0.6305	0.6599	0.6897
C	4	C3b	0.1255	0.1543	0.1853	0.2165	0.2464	0.2758	0.3066	0.3359	0.3654	0.3987	0.4307	0.4618	0.4898	0.5232	0.5525	0.5835	0.6140	0.6483	0.6779	0.7060
C	5	T3a	0.1393	0.1669	0.1939	0.2243	0.2535	0.2804	0.3071	0.3369	0.3689	0.4000	0.4281	0.4574	0.4872	0.5193	0.5470	0.5799	0.6075		0.6666	0.6955
C	6	T3a	0.1245	0.1539	0.1822	0.2136	0.2441	0.2756	0.3043	0.3341	0.3664	0.3994	0.4281	0.4570	0.4879	0.5200	0.5525	0.5823	0.6120	0.6425	0.6775	0.7052
C	7	T3b	0.1164	0.1343	0.1530	0.1732	0.1939	0.2146	0.2301	0.2534	0.2726	0.2940	0.3141	0.3354	0.3552	0.3792	0.3970	0.4189	0.4396	0.4616	0.4809	0.5009
C	8	T3b	0.1215	0.1395	0.1570	0.1752	0.1934	0.2133	0.2298	0.2483	0.2708	0.2942	0.3102	0.3292	0.3496	0.3698	0.3907	0.4092	0.4288	0.4518	0.4697	0.4871
D	1	C4a	0.1410	0.1739	0.2085	0.2454	0.2808	0.3164	0.3523	0.3874	0.4264	0.4632	0.4975	0.5352	0.5723	0.6114	0.6462	0.6829	0.7189	0.7571	0.7929	0.8294
D	2	C4a	0.1432	0.1745	0.2079	0.2437	0.2762	0.3088	0.3410	0.3734	0.4103	0.4436	0.4782	0.5127	0.5459	0.5846	0.6156	0.6506	0.6843	0.7198	0.7537	0.7869
D	3	C4b	0.1310	0.1643	0.1970	0.2318	0.2644	0.2978	0.3305	0.3639	0.4002	0.4339	0.4680	0.5034	0.5374	0.5735	0.6057	0.6478	0.6762	0.7110	0.7458	0.7777
D	4	C4b	0.1349	0.1617	0.1909	0.2245	0.2539	0.2854	0.3172	0.3482	0.3800	0.4116	0.4454	0.4791	0.5096	0.5424	0.5745	0.6080	0.6379	0.6719	0.7033	0.7355

Table C.14 pNP Butyrate Plate 2 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
D	5	T4a	0.1404	0.1761	0.2107	0.2500	0.2867	0.3242	0.3593	0.3973	0.4355	0.4726	0.5108	0.5490	0.5884	0.6262	0.6627	0.7036	0.7377	0.7790	0.8145	0.8555
D	6	T4a	0.1434	0.1815	0.2190	0.2607	0.3011	0.3405	0.3780	0.4190	0.4599	0.4989	0.5405	0.5808	0.6234	0.6631	0.7050	0.7512	0.7929	0.8402	0.8828	0.9266
D	7	T4b	0.1315	0.1579	0.1893	0.2175	0.2462	0.2749	0.3054	0.3348	0.3655	0.3965	0.4239	0.4565	0.4868	0.5171	0.5466	0.5797	0.6105	0.6383	0.6713	0.6994
D	8	T4b	0.1263	0.1519	0.1804	0.2103	0.2410	0.2681	0.2954	0.3294	0.3588	0.3892	0.4184	0.4470	0.4799	0.5117	0.5404	0.5752	0.6025	0.6358	0.6638	0.6954
E	1	C5a	0.1512	0.1861	0.2223	0.2622	0.3013	0.3358	0.3733	0.4109	0.4509	0.4901	0.5321	0.5682	0.6083	0.6471	0.6876	0.7275	0.7665	0.8064	0.8443	0.8816
E	2	C5a	0.1400	0.1771	0.2095	0.2464	0.2844	0.3178	0.3540	0.3899	0.4255	0.4659	0.5024	0.5398	0.5771	0.6164	0.6511	0.6909	0.7272	0.7639	0.8003	0.8376
E	3	C5b	0.1503	0.1876	0.2271	0.2593	0.2980	0.3394	0.3692	0.4079	0.4457	0.4847	0.5230	0.5609	0.6116	0.6380	0.6761	0.7138	0.7520	0.7894	0.8287	0.8637
E	4	C5b	0.1455	0.1820	0.2209	0.2610	0.2990	0.3400	0.3806	0.4191		0.4988	0.5388	0.5788	0.6201	0.6614	0.6992	0.7419	0.7815	0.8218	0.8607	0.9004
E	5	T5a	0.1457	0.1747	0.2042	0.2365	0.2694	0.2976	0.3263	0.3584	0.3915	0.4219	0.4554	0.4869	0.5183	0.5523	0.5847	0.6153	0.6447	0.6777	0.7079	0.7388
E	6	T5a	0.1368	0.1663	0.1967	0.2282	0.2627	0.2922	0.3204	0.3528	0.3861	0.4169	0.4485	0.4808	0.5116	0.5443	0.5752	0.6094	0.6385	0.6733	0.7034	0.7348
E	7	T5b	0.1302	0.1542	0.1808	0.2091	0.2398	0.2660	0.2910	0.3213	0.3483	0.3769	0.4070	0.4351	0.4619	0.4927	0.5212	0.5503	0.5778	0.6092	0.6370	0.6620
E	8	T5b	0.1389	0.1635	0.1953	0.2240	0.2549	0.2835	0.3128	0.3449	0.3748	0.4044	0.4353	0.4667	0.4971	0.5273	0.5596	0.5907	0.6213	0.6541	0.6814	0.7112
F	1	blnk 1	0.1000	0.1069	0.1175	0.1275	0.1373	0.1465	0.1543	0.1648	0.1755	0.1856	0.1980	0.2072	0.2168	0.2286	0.2395	0.2488	0.2611	0.2723	0.2810	0.2906
F	2	blnk 2	0.1078	0.1156	0.1250	0.1358	0.1453	0.1550	0.1637	0.1754	0.1863	0.1975	0.2066	0.2155	0.2268	0.2380	0.2492	0.2611	0.2711	0.2824	0.2910	0.2998
F	3	blnk 3	0.0992	0.1064	0.1159	0.1265	0.1359	0.1460	0.1550	0.1660	0.1759	0.1876	0.1968	0.2065	0.2188	0.2270	0.2380	0.2502	0.2582	0.2702	0.2799	0.2879
F	4	blnk 4	0.1053	0.1138	0.1230	0.1327	0.1428	0.1515	0.1610	0.1698	0.1816	0.1925	0.2004	0.2119	0.2216	0.2329	0.2442	0.2550	0.2639	0.2755	0.2866	0.2932
F	5	blnk 5	0.1111	0.1188	0.1281	0.1393	0.1477	0.1574	0.1647	0.1769	0.1862	0.1960	0.2069	0.2165	0.2279	0.2384	0.2485	0.2595	0.2682	0.2805	0.2892	0.2966
F	6	blnk 6	0.1012	0.1077	0.1186	0.1266	0.1394	0.1468	0.1566	0.1650	0.1774	0.1871	0.1961	0.2080	0.2176	0.2285	0.2380	0.2490	0.2590	0.2693	0.2791	0.2875
F	7	blnk 7	0.1070	0.1156	0.1234	0.1343	0.1428	0.1537	0.1597	0.1717	0.1837	0.1921	0.2028	0.2136	0.2233	0.2348	0.2442	0.2546	0.2647	0.2747	0.2857	0.2954
F	8	blnk 8	0.1148	0.1219	0.1312	0.1409	0.1506	0.1603	0.1698	0.1786	0.1906	0.2014	0.2105	0.2204	0.2311	0.2428	0.2538	0.2645	0.2744	0.2860	0.2960	0.3032

Table C.15 pNP Butyrate Plate 3:

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
A	1	C1a	0.1350	0.1595	0.1850	0.2123	0.2373	0.2640	0.2940	0.3226	0.3503	0.3775	0.4085	0.4358	0.4619	0.4907	0.5217	0.5509	0.5788	0.6084	0.6405	0.6678
A	2	C1a	0.1267	0.1550	0.1825	0.2107	0.2378	0.2642	0.2947	0.3220	0.3508	0.3807	0.4094	0.4388	0.4645	0.4947	0.5254	0.5558	0.5852	0.6145	0.6454	0.6744
A	3	C1b	0.1295	0.1536	0.1733	0.1965	0.2191	0.2423	0.2644	0.2901	0.3121	0.3375	0.3621	0.3871	0.4083	0.4315	0.4572	0.4824	0.5073	0.5309	0.5557	0.5803
A	4	C1b	0.1307	0.1540	0.1840	0.2044	0.2270	0.2547	0.2798	0.3098	0.3317	0.3609	0.3884	0.4089	0.4358	0.4622	0.4892	0.5169	0.5429	0.5696	0.5976	0.6205
A	5	T1a	0.1124	0.1327	0.1525	0.1717	0.1892	0.2102	0.2317	0.2508	0.2709	0.2909	0.3138	0.3325	0.3514	0.3722	0.3932	0.4173	0.4395	0.4585	0.4803	0.5008
A	6	T1a	0.1166	0.1347	0.1547	0.1730	0.1905	0.2121	0.2281	0.2500	0.2719	0.2909	0.3108	0.3299	0.3500	0.3706	0.3918	0.4138	0.4372	0.4567	0.4794	0.4978
A	7	T1b	0.1070	0.1277	0.1466	0.1692	0.1872	0.2076	0.2293	0.2513	0.2713	0.2927	0.3143	0.3342	0.3550	0.3774	0.4030	0.4201	0.4420	0.4622	0.4834	0.5053
A	8	T1b	0.1136	0.1352	0.1586	0.1774	0.1977	0.2190	0.2397	0.2635	0.2853	0.3067	0.3313	0.3517	0.3725	0.3956	0.4197	0.4435	0.4676	0.4902	0.5126	0.5369
B	1	C2a	0.1373	0.1650	0.1944	0.2252	0.2476	0.2777	0.3058	0.3376	0.3660	0.3960	0.4262	0.4529	0.4831	0.5114	0.5441	0.5753	0.6050	0.6358	0.6654	0.6976
B	2	C2a	0.1301	0.1558	0.1860	0.2112	0.2380	0.2676	0.2951	0.3242	0.3527	0.3803	0.4112	0.4375	0.4628	0.4945	0.5231	0.5531	0.5830	0.6136	0.6416	0.6713
B	3	C2b	0.1244	0.1486	0.1702	0.1944	0.2193	0.2440	0.2661	0.2898	0.3160	0.3406	0.3675	0.3875	0.4125	0.4371	0.4625	0.4862	0.5138	0.5392	0.5635	0.5867
B	4	C2b	0.1172	0.1403	0.1605	0.1823	0.2038	0.2256	0.2471	0.2703	0.2953	0.3156	0.3406	0.3603	0.3812	0.4042	0.4273	0.4514	0.4753	0.5032	0.5208	0.5431
B	5	T2a	0.1181	0.1388	0.1580	0.1767	0.1954	0.2157	0.2348	0.2537	0.2763	0.2954	0.3166	0.3338	0.3538	0.3751	0.3951	0.4182	0.4381	0.4600	0.4813	0.5013
B	6	T2a	0.1182	0.1369	0.1575	0.1764	0.1934	0.2146	0.2356	0.2546	0.2762	0.2926	0.3156	0.3357	0.3558	0.3738	0.3939	0.4183	0.4381	0.4602	0.4809	0.5001
B	7	T2b	0.1191	0.1409	0.1654	0.1888	0.2108	0.2348	0.2576	0.2845	0.3065	0.3310	0.3532	0.3778	0.4001	0.4238	0.4505	0.4757	0.5007	0.5250	0.5505	0.5739
B	8	T2b	0.1255	0.1502	0.1745	0.1996	0.2241	0.2505	0.2752	0.2991	0.3274	0.3530	0.3785	0.4052	0.4311	0.4557	0.4837	0.5076	0.5385	0.5620	0.5890	0.6148
C	1	C3a	0.1376	0.1672	0.1955	0.2246	0.2518	0.2823	0.3122	0.3414	0.3722	0.4033	0.4329	0.4602	0.4899	0.5186	0.5495	0.5810	0.6116	0.6413	0.6743	0.7014
C	2	C3a	0.1435	0.1763	0.2078	0.2398	0.2689	0.3018	0.3362	0.3664	0.3989	0.4304	0.4651	0.4931	0.5253	0.5569	0.5902	0.6240	0.6572	0.6898	0.7209	0.7516
C	3	C3b	0.1370	0.1711	0.2030	0.2371	0.2680	0.3036	0.3361	0.3706	0.4047	0.4379	0.4721	0.5040	0.5387	0.5699	0.6059	0.6379	0.6744	0.7083	0.7436	0.7756
C	4	C3b	0.1418	0.1787	0.2119	0.2491	0.2820	0.3205	0.3554	0.3924	0.4273	0.4631	0.4995	0.5341	0.5709	0.6037	0.6426	0.6745	0.7151	0.7509	0.7886	0.8225
C	5	T3a	0.1435	0.1692	0.1967	0.2267	0.2531	0.2806	0.3060	0.3365	0.3828	0.3923	0.4223	0.4467	0.4743	0.5052	0.5333	0.5629	0.5927	0.6219	0.6496	0.6766
C	6	T3a	0.1245	0.1535	0.1795	0.2090	0.2360	0.2657	0.2910	0.3200	0.3511	0.3778	0.4090	0.4351	0.4617	0.4937	0.5223	0.5507	0.5813	0.6098	0.6403	0.6677
C	7	T3b	0.1223	0.1423	0.1610	0.1818	0.2011	0.2226	0.2433	0.2641	0.2856	0.3058	0.3282	0.3473	0.3677	0.3874	0.4093	0.4329	0.4529	0.4735	0.4965	0.5154
C	8	T3b	0.1274	0.1475	0.1704	0.1877	0.2050	0.2278	0.2467	0.2679	0.2903	0.3131	0.3299	0.3486	0.3700	0.3912	0.4119	0.4343	0.4579	0.4760	0.4994	0.5206
D	1	C4a	0.1469	0.1846	0.2207	0.2551	0.2918	0.3292	0.3639	0.4033	0.4420	0.4792	0.5175	0.5553	0.5907	0.6290	0.6692	0.7085	0.7477	0.7835	0.8227	0.8606
D	2	C4a	0.1539	0.1882	0.2239	0.2587	0.2926	0.3275	0.3627	0.4025	0.4370	0.4726	0.5083	0.5424	0.5781	0.6149	0.6518	0.6905	0.7257	0.7626	0.7984	0.8332
D	3	C4b	0.1345	0.1663	0.1988	0.2308	0.2614	0.2938	0.3254	0.3597	0.3932	0.4259	0.4585	0.4910	0.5206	0.5566	0.5893	0.6224	0.6567	0.6907	0.7268	0.7602
D	4	C4b	0.1409	0.1728	0.2043	0.2378	0.2702	0.3036	0.3368	0.3703	0.4012	0.4389	0.4704	0.5029	0.5352	0.5702	0.6013	0.6366	0.6719	0.7052	0.7412	0.7730
D	5	T4a	0.1554	0.1999	0.2436	0.2872	0.3322	0.3753	0.4243	0.4665	0.5119	0.5574	0.6013	0.6453	0.6925	0.7342	0.7809	0.8271	0.8710	0.9176	0.9642	1.0082

Table C.15 pNP Butyrate Plate 3 (continued):

Well row	Well column	Con-tent	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8mins 41sec	OD @ 9 mins 39 sec	OD @ 10 mins 37 sec	OD @ 11 mins 35 sec	OD @ 12 mins 33 sec	OD @ 13mins 31 sec	OD @ 14 mins 30 sec	OD @ 15 mins 28 sec	OD @ 16 mins 26 sec	OD @ 17 mins 23 sec	OD @ 18 mins 22 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700	9.667	10.633	11.600	12.567	13.533	14.500	15.467	16.433	17.400	18.367
D	6	T4a	0.1532	0.1956	0.2374	0.2800	0.3221	0.3666	0.4068	0.4506	0.4929	0.5364	0.5786	0.6186	0.6646	0.7042	0.7494	0.7940	0.8399	0.8783	0.9244	0.9646
D	7	T4b	0.1361	0.1645	0.1933	0.2232	0.2510	0.2798	0.3099	0.3387	0.3681	0.4012	0.4305	0.4552	0.4863	0.5145	0.5448	0.5768	0.6048	0.6359	0.6659	0.6945
D	8	T4b	0.1333	0.1612	0.1879	0.2169	0.2468	0.2744	0.3049	0.3369	0.3669	0.3953	0.4281	0.4555	0.4865	0.5174	0.5499	0.5805	0.6128	0.6418	0.6745	0.7047
E	1	C5a	0.1607	0.1969	0.2340	0.2694	0.3084	0.3453	0.3913	0.4256	0.4639	0.5028	0.5429	0.5817	0.6212	0.6580	0.6999	0.7399	0.7795	0.8198	0.8592	0.8983
E	2	C5a	0.1487	0.1827	0.2188	0.2535	0.2875	0.3242	0.3564	0.3938	0.4293	0.4659	0.5030	0.5379	0.5722	0.6089	0.6483	0.6858	0.7218	0.7578	0.7966	0.8323
E	3	C5b	0.1585	0.1923	0.2296	0.2653	0.3015	0.3402	0.3756	0.4130	0.4515	0.4894	0.5294	0.5648	0.5998	0.6370	0.6766	0.7156	0.7531	0.7909	0.8293	0.8676
E	4	C5b	0.1515	0.1901	0.2305	0.2687	0.3072	0.3478	0.3881	0.4305	0.4696	0.5106	0.5511	0.5916	0.6313	0.6723	0.7143	0.7545	0.7942	0.8360	0.8760	0.9179
E	5	T5a	0.1484	0.1803	0.2106	0.2423	0.2749	0.3053	0.3382	0.3723	0.4032	0.4355	0.4697	0.4995	0.5314	0.5645	0.5990	0.6304	0.6649	0.6964	0.7310	0.7613
E	6	T5a	0.1505	0.1841	0.2174	0.2508	0.2863	0.3185	0.3551	0.3918	0.4232	0.4576	0.4964	0.5273	0.5618	0.5960	0.6341	0.6676	0.7058	0.7388	0.7740	0.8079
E	7	T5b	0.1397	0.1687	0.1959	0.2244	0.2518	0.2801	0.3100	0.3396	0.3696	0.3974	0.4270	0.4567	0.4832	0.5149	0.5463	0.5756	0.6047	0.6358	0.6653	0.6954
E	8	T5b	0.1456	0.1768	0.2088	0.2377	0.2661	0.2983	0.3291	0.3592	0.3931	0.4231	0.4545	0.4871	0.5160	0.5499	0.5806	0.6157	0.6462	0.6769	0.7103	0.7409
F	1	blnk 1	0.1139	0.1232	0.1326	0.1442	0.1528	0.1618	0.1735	0.1834	0.1942	0.2044	0.2160	0.2252	0.2348	0.2467	0.2557	0.2694	0.2804	0.2900	0.3011	0.3113
F	2	blnk 2	0.1194	0.1273	0.1352	0.1447	0.1529	0.1632	0.1744	0.1865	0.1955	0.2066	0.2176	0.2263	0.2380	0.2470	0.2588	0.2708	0.2815	0.2914	0.3044	0.3142
F	3	blnk 3	0.1075	0.1162	0.1263	0.1401	0.1469	0.1560	0.1674	0.1777	0.1883	0.1979	0.2115	0.2215	0.2285	0.2386	0.2513	0.2627	0.2760	0.2850	0.2979	0.3066
F	4	blnk 4	0.1128	0.1215	0.1310	0.1418	0.1511	0.1609	0.1717	0.1856	0.1926	0.2033	0.2158	0.2236	0.2337	0.2429	0.2530	0.2674	0.2778	0.2876	0.3012	0.3096
F	5	blnk 5	0.1187	0.1288	0.1384	0.1478	0.1576	0.1674	0.1772	0.1897	0.1973	0.2078	0.2198	0.2284	0.2393	0.2490	0.2624	0.2724	0.2830	0.2932	0.3044	0.3152
F	6	blnk 6	0.1077	0.1190	0.1274	0.1384	0.1484	0.1567	0.1686	0.1799	0.1890	0.1994	0.2110	0.2202	0.2293	0.2396	0.2530	0.2645	0.2745	0.2841	0.2976	0.3062
F	7	blnk 7	0.1143	0.1221	0.1322	0.1419	0.1498	0.1611	0.1710	0.1827	0.1933	0.2029	0.2130	0.2230	0.2338	0.2423	0.2546	0.2658	0.2799	0.2889	0.3007	0.3137
F	8	blnk 8	0.1230	0.1319	0.1401	0.1505	0.1595	0.1697	0.1815	0.1907	0.2021	0.2128	0.2223	0.2329	0.2412	0.2540	0.2634	0.2765	0.2884	0.2994	0.3085	0.3221

Appendix D

Optical Density Data of Standards

The second row of both tables represents the time converted to metric minutes.

Table D.1 Standard Concentrations of p-Nitroanilide

Well row	Well column	pNA conc (µM)	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8 mins 41 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700
C	1	0	0.0531	0.0539	0.054	0.0531	0.0538	0.054	0.0538	0.0551	0.0531	0.0562
C	2	2	0.0742	0.0774	0.0752	0.0762	0.0756	0.0766	0.0747	0.0771	0.0746	0.0776
C	3	4	0.0863	0.0869	0.0883	0.0864	0.0882	0.0858	0.086	0.0887	0.086	0.0853
C	4	6	0.0834	0.084	0.0828	0.0842	0.086	0.0836	0.0852	0.0849	0.084	0.0846
C	5	8	0.1244	0.1258	0.125	0.1258	0.1256	0.1252	0.1253	0.1269	0.123	0.1246
C	6	10	0.1429	0.145	0.1431	0.1451	0.1432	0.1438	0.1432	0.145	0.1431	0.1434
C	7	20	0.2395	0.2389	0.2391	0.239	0.239	0.2394	0.2376	0.2393	0.236	0.2353
C	8	40	0.4187	0.4182	0.4164	0.4172	0.4142	0.4168	0.4141	0.4185	0.4143	0.4147
C	9	60	0.6172	0.6191	0.6154	0.6138	0.6132	0.6141	0.611	0.6118	0.6103	0.6092
C	10	80	0.7966	0.7955	0.7936	0.7917	0.7898	0.789	0.7886	0.7869	0.7848	0.7859
C	11	100	0.9831	0.9803	0.9757	0.9732	0.9739	0.972	0.9712	0.9698	0.967	0.9672
C	12	200	1.8984	1.8894	1.8801	1.8757	1.8744	1.8669	1.87	1.8685	1.8626	1.8638
D	1	400	3.7421	3.7215	3.7007	3.7168	3.6739	3.663	3.6866	3.6745	3.6672	3.7047

Table D.2 Standard Concentrations of p-Nitrophenyl Butyrate

Well row	Well column	pNP butyrate conc (μM)	OD @ 0 mins 0 sec	OD @ 0 mins 57 sec	OD @ 1 min 55 sec	OD @ 2 mins 53 sec	OD @ 3 mins 52 sec	OD @ 4 mins 49 sec	OD @ 5 mins 47 sec	OD @ 6 mins 46 sec	OD @ 7 mins 44 sec	OD @ 8 mins 41 sec
			0.000	0.967	1.933	2.900	3.867	4.833	5.800	6.767	7.733	8.700
C	1	0	0.0597	0.0573	0.0596	0.0602	0.0593	0.0576	0.0574	0.057	0.0596	0.0563
C	2	20	0.2891	0.2885	0.2883	0.2883	0.2847	0.2819	0.2836	0.2813	0.2822	0.2791
C	3	40	0.4708	0.4687	0.4691	0.4657	0.4607	0.4602	0.4605	0.4587	0.4591	0.456
C	4	60	0.7851	0.7784	0.7756	0.7717	0.768	0.7658	0.7635	0.7584	0.7571	0.7539
C	5	80	0.9692	0.9651	0.9624	0.9563	0.9509	0.9463	0.9449	0.9407	0.9405	0.9362
C	6	100	1.2019	1.1942	1.1892	1.1817	1.1756	1.1686	1.1651	1.163	1.1588	1.1545