The Effect of Regular Paracetamol
on Bronchial Responsiveness and Asthma Control
in Mild to Moderate Asthma

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Abstract

Over recent decades, the worldwide prevalence of asthma has increased. The reasons for this increase are unknown, which has lead to the search for novel risk factors that may increase susceptibility to atopy and the development of asthma. The growing popularity of paracetamol as a drug to treat pain and fever has occurred contemporaneously with the rising prevalence of asthma, and epidemiological evidence suggests that paracetamol may be a risk factor in the development of asthma and its severity. As a result, there have been numerous calls for randomised controlled trials to be undertaken to investigate the association between paracetamol and asthma, in particular the effect of regular, long-term paracetamol use on asthma severity and control.

This thesis presents the results of a randomised, placebo-controlled clinical trial undertaken to determine the effect of regular paracetamol on bronchial responsiveness and asthma control in adult asthma. In a 12-week, randomised, double-blind, placebo-controlled, parallel-group study, 94 adults with mild to moderate asthma received 12 weeks of either 1 g paracetamol twice daily or a placebo medication twice daily. The primary outcome variable was bronchial hyperresponsiveness, measured as the provocation concentration of methacholine causing a 20% reduction in FEV$_1$ ($PC_{20}$MCh), at baseline and week 12. Secondary outcome variables included FEV$_1$, FeNO, ACQ score, mean morning peak flow, peak flow variability, blood eosinophil, serum IgE and cytokine (IFN-$\gamma$, IL-4, IL-5 and IL-13) levels. 85 participants completed the study. At 12 weeks the mean (SD) logarithm base two $PC_{20}$ was 1.07 (2.36) in the control group (N=54) and 0.62 (2.09) in the paracetamol group (N=31). Although the
mean PC\textsubscript{20} was lower in the treatment group, after controlling for baseline PC\textsubscript{20}, the difference was not statistically significant (paracetamol minus placebo): -0.48 doubling doses (95% CI -1.28 to 0.32), P=0.24. In addition, there were no statistically significant differences in log FeNO (0.09, 95% CI -0.097 to 0.27), FEV\textsubscript{1} (-0.07 L, 95% CI -0.15 to 0.01), ACQ score (-0.04, 95% CI -0.27 to 0.18) or other secondary outcome variables between the paracetamol and placebo groups.

The research described in this thesis demonstrates no statistically significant effect of paracetamol on bronchial responsiveness. There were no significant differences observed in any of the pre-specified secondary outcome variables of asthma control or inflammatory and immunological markers, although undetectable baseline cytokine levels in the majority of trial participants precluded meaningful cytokine analysis. The results of the study require cautious interpretation because the study power to detect the large pre-specified effect of a one doubling-dose reduction in PC\textsubscript{20} was lower than anticipated. However, the results do not rule out a clinically significant effect of paracetamol on bronchial responsiveness, with the 95% confidence interval containing the pre-specified difference of one doubling dose reduction in PC\textsubscript{20}. Furthermore, the point estimate of a reduction in PC\textsubscript{20} of 0.48 of a doubling-dose could potentially be of major public health significance.

As paracetamol is commonly used in all age groups and the global disease burden of asthma is large, the impact of an effect of paracetamol on asthma could be profound. Further adequately powered studies should be performed to determine the effect of paracetamol use on the development of asthma and its severity.
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Oh me! Oh life!... of the questions of these recurring;
Of the endless trains of the faithless, of cities fill’d with the foolish;
Of myself forever reproaching myself, (for who more foolish than I, and who more faithless?)
Of eyes that vainly crave the light, of the objects mean, of the struggle ever renew’d;
Of the poor results of all, of the plodding and sordid crowds I see around me;
Of the empty and useless years of the rest, with the rest me intertwined;
The question, O me! so sad, recurring—What good amid these, O me, O life?

Answer.
That you are here—that life exists and identity;
That the powerful play goes on, and you may contribute a verse.

‘Oh Me! Oh Life!’ by Walt Whitman
(Leaves of Grass, 1892)
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<tr>
<td>ANZCTR</td>
<td>Australian New Zealand Clinical Trials Registry</td>
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<tr>
<td>ACQ</td>
<td>Asthma Control Questionnaire</td>
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<tr>
<td>ALP</td>
<td>Alkaline Phosphatase</td>
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<tr>
<td>ALT</td>
<td>Alanine Transaminase</td>
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<tr>
<td>AMP</td>
<td>Adenosine 5’- Monophosphate</td>
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<tr>
<td>AM404</td>
<td>N-arachidonoyl-phenolamine</td>
</tr>
<tr>
<td>ATS</td>
<td>American Thoracic Society</td>
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<tr>
<td>BHR</td>
<td>Bronchial Hyperresponsiveness</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BP</td>
<td>Blood Pressure</td>
</tr>
<tr>
<td>CCDHB</td>
<td>Capital &amp; Coast District Health Board</td>
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<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CREC</td>
<td>Central Regional Ethics Committee</td>
</tr>
<tr>
<td>FAAH</td>
<td>Fatty Acid Amide Hydrolase</td>
</tr>
<tr>
<td>FBC</td>
<td>Full Blood Count</td>
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<tr>
<td>FeNO</td>
<td>Fractional Exhaled Nitric Oxide</td>
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<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Forced Expiratory Volume in One Second</td>
</tr>
<tr>
<td>GINA</td>
<td>Global Initiative for Asthma</td>
</tr>
<tr>
<td>GGT</td>
<td>Gamma-Glutamyl Transpeptidase</td>
</tr>
<tr>
<td>GP</td>
<td>General Practitioner</td>
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<td>GST</td>
<td>Glutathione S-Transferase</td>
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<tr>
<td>IFN-γ</td>
<td>Interferon-gamma</td>
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<td>IgE</td>
<td>Immunoglobulin E</td>
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<td>Symbol/Abbreviation</td>
<td>Definition</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>ISAAC</td>
<td>The International Study of Asthma and Allergies in Childhood</td>
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<tr>
<td>LABA</td>
<td>Long-Acting Beta Agonist</td>
</tr>
<tr>
<td>LTC4, D4, E4</td>
<td>Leukotriene C4, D4, E4</td>
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<tr>
<td>MCh</td>
<td>Methacholine</td>
</tr>
<tr>
<td>MRINZ</td>
<td>Medical Research Institute of New Zealand</td>
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<tr>
<td>NAC</td>
<td>N-Acetylcysteine</td>
</tr>
<tr>
<td>NAPQI</td>
<td>N-acetyl-p-benzoquinone imine</td>
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<tr>
<td>NO</td>
<td>Nitric Oxide</td>
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<tr>
<td>NSAID</td>
<td>Non-Steroidal Anti-inflammatory Drug</td>
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<tr>
<td>Nrf2</td>
<td>NF-E2-related factor 2</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
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<tr>
<td>PC&lt;sub&gt;20&lt;/sub&gt;</td>
<td>Provocation Concentration (of Methacholine) Causing a 20% fall in FEV&lt;sub&gt;1&lt;/sub&gt;</td>
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<tr>
<td>PEF</td>
<td>Peak Expiratory Flow Rate</td>
</tr>
<tr>
<td>ppb</td>
<td>Parts per billion</td>
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<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
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<tr>
<td>RSV</td>
<td>Respiratory Syncytial Virus</td>
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<tr>
<td>SABA</td>
<td>Short-Acting Beta Agonist</td>
</tr>
<tr>
<td>TRP</td>
<td>Transient Receptor Potential</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine (serotonin)</td>
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Chapter One: Introduction

1.1 Problem Statement

The reasons for the world-wide increase in the prevalence of asthma over recent decades and the international patterns of asthma prevalence are poorly understood and not adequately explained by current knowledge of the causation of asthma (Eder, Ege et al. 2006). This has lead to the investigation of novel risk factors that may increase susceptibility to atopy and the development of asthma. Fifteen years ago, it was first proposed that increasing consumption of paracetamol by children following the discontinuation of the use of aspirin for the treatment of childhood illnesses, because of its association with Reye’s Syndrome, may be responsible for the increasing rates of asthma seen in the United States at that time (Varner, Busse et al. 1998).

Since this hypothesis was proposed, a growing body of evidence suggests that paracetamol may play an important role as a risk factor for the development of asthma, and that increasing world-wide use may have contributed to the increasing global prevalence of asthma seen over the last 40 years (Cohet, Cheng et al. 2004; Beasley, Clayton et al. 2008; Amberbir, Medhin et al. 2010; Beasley RW, Clayton TO et al. 2010). Ecological studies have identified positive associations between per capita consumption of paracetamol and the prevalence of asthma in children and adults (Newson, Shaheen et al. 2000). Childhood asthma risk increases in the offspring of women who consume paracetamol during pregnancy (Eyers, Weatherall et al.
and paracetamol use in the first 12 months of life is associated with an increased risk of wheezing at 3 years (Shaheen, Newson et al. 2002; Amberbir, Medhin et al. 2010), and 6-7 years (Cohet, Cheng et al. 2004; Beasley, Clayton et al. 2008). Cross-sectional surveys in children (Beasley, Clayton et al. 2008), adolescents (Beasley RW, Clayton TO et al. 2010) and adults (Shaheen, Sterne et al. 2000; Davey, Berhane et al. 2005; McKeever, Lewis et al. 2005; Shaheen, Potts et al. 2008) have consistently demonstrated an association between current paracetamol use and asthma in populations with widely differing lifestyles, standards of living, medical practice and availability of paracetamol. However, it has been proposed that these associations may, in part, be due to confounding by indication (Lowe, Carlin et al. 2010; Schnabel and Heinrich 2010; Tapiainen T, Dunder T et al. 2010). Cohort studies in adults have also demonstrated that increasing frequency of paracetamol use is positively associated with newly-diagnosed (adult-onset) asthma (Barr, Wentowski et al. 2004; Thomsen, Kyvik et al. 2008).

In addition to a potential role in the development of asthma, there is also evidence that paracetamol may increase the severity of asthma in those with the disease. The strongest evidence for this comes from the only published randomised controlled trial of the effect of paracetamol use for fever and asthma outcomes, in which asthmatic children experiencing a current febrile illness were randomised to receive either paracetamol or ibuprofen (Lesko, Louik et al. 2002). The children who received paracetamol were more likely to require an outpatient visit for asthma compared to children in the ibuprofen group. The increased risk with paracetamol was dose dependant and related to respiratory febrile illnesses rather than other causes of fever. In further support of an effect of paracetamol on asthma severity, a
United Kingdom-based case-control study, which reported a dose-dependant association between paracetamol use and asthma, and also found a progressively greater risk in those with more severe disease, suggesting an effect on both disease causation and severity (Shaheen, Sterne et al. 2000).

Several mechanisms, in isolation or together, may explain the effects of paracetamol on asthma development and severity. Paracetamol may impair respiratory antioxidant defences by decreasing the amount of reduced glutathione in the lungs (Chen, Richie et al. 1990; Micheli, Cerretani et al. 1994; Rahman and MacNee 2000; Kozer, Evans et al. 2003; Dimova, Hoet et al. 2005; Eneli, Sadri et al. 2005), leading to oxidant-induced inflammation. It is likely that this mechanism is related to gene/environment interactions, as genetic polymorphisms of the glutathione-S-transferase family of genes may influence asthma predisposition (Lenney and Fryer 2007; Imboden, Rochat et al. 2008). Another possible mechanism relates to the effect of paracetamol (Varner, Busse et al. 1998) and depleted glutathione levels (Peterson, Herzenberg et al. 1998; Dimova, Hoet et al. 2005) on Th1-Th2 cytokine response patterns. Further, when paracetamol is administered in therapeutic doses its metabolite N-acetyl-p-benzoquinone imine (NAPQI), may cause neurogenic inflammation in the lungs via the stimulation of transient receptor potential ankyrin-1 (Lenney and Fryer 2007).

The described epidemiological evidence, supported by several biologically plausible mechanisms, has lead to repeated calls for randomised controlled trials to be undertaken to explore the relationship between paracetamol and asthma (Barr 2008; Beasley, Clayton et al. 2008; Farquhar H, Crane J et al. 2009; Amberbir, Medhin et al. 2010; Beasley RW, Clayton
TO et al. 2010; Tapiainen T, Dunder T et al. 2010; Holgate 2011; Johnson and Ownby 2011; McBride 2011). This thesis describes what is believed to be the first randomised, placebo-controlled trial undertaken to investigate the effect of long-term paracetamol on asthma severity and control in patients with asthma.

1.2 Thesis Aim

The aim of this research project was to investigate the hypothesis that regular paracetamol use worsens asthma severity and control in adults with mild to moderate asthma. Specifically, it aimed to address two questions:

1) Does the administration of regular paracetamol in adults with mild to moderate asthma increase bronchial hyperresponsiveness (BHR) and/or worsen asthma control?

2) If an association between paracetamol use and asthma severity and control exists, what are the possible mechanisms through which the association occurs?

A randomised controlled trial (RCT) was undertaken to address these two questions. The results of this research will provide valuable information regarding the relationship between paracetamol and asthma, which will guide future research and may provide evidence on which future guidelines and recommendations are based.
1.3 Thesis Outline

Chapters Two and Three provide a background to the subjects of paracetamol and asthma. Chapter Two describes the history of paracetamol and its mechanisms of actions, therapeutic, pharmacologic and toxicologic properties. Chapter Three defines the disease of asthma and describes its epidemiology and clinical management as relevant to the scope of this research project.

Chapter Four is a review of the literature regarding the paracetamol and asthma hypothesis. It first describes the ecological associations and time trends between the growing international consumption of paracetamol and the increasing prevalence of asthma. It goes on to describe the main mechanisms of action by which an effect of paracetamol on asthma has been proposed. It then discusses the current epidemiological evidence for and against the hypothesis, categorised by life stages into the risk of asthma associated with paracetamol use in pregnancy, infancy, childhood, adolescence and adulthood.

Chapter Five is a continuation of the literature review section of the thesis, in which a more detailed systematic review and meta-analysis is undertaken to further evaluate the evidence regarding intrauterine exposure to paracetamol and the risk of wheezing in offspring. This chapter evaluates the evidence regarding the effect of paracetamol on the causation of asthma, which is likely to be related to its effect on asthma severity and control. Further, the evidence regarding intrauterine exposure to paracetamol is particularly important, as it is without the
issue of confounding by indication, which can make the interpretation of observational data on paracetamol use and asthma difficult to interpret.

Chapter Six describes the design and methodology of the randomised controlled trial investigating the effect of regular paracetamol on airway responsiveness and asthma control in mild to moderate asthma. The results of the study are presented in Chapter Seven, and Chapter Eight comprises a discussion of the study results, methodological issues and recommendations for future research. Chapter Nine summarises and concludes the research project and thesis.
Chapter Two: Background on Paracetamol

2.1 The History of Paracetamol

Paracetamol (acetaminophen) is a member of the aniline family and is the last aniline analgesic still in use today (Bertolini, Ferrari et al. 2006). Paracetamol is a derivative of acetanilide, the first aniline analgesic to be introduced into medical practice during the 1800s (Figure 2.1) (Bertolini, Ferrari et al. 2006). Acetanilide was a popular drug and possessed both analgesic and antipyretic properties; however its unacceptable side effects, most notably cyanosis due to methemoglobinemia, led to a search for safer alternatives (Toussaint K, Yang XC et al. 2010). Some reports suggest that paracetamol was first synthesised in 1852 by Cahn and Hepp (Sneader W 2005), while others claim it was first created at Johns Hopkins University by Morse in 1877, through the inadvertent reaction of p-aminophenol with tin and glacial acetic acid (Ameer and Greenblatt 1977; Prescott 2000; Bertolini, Ferrari et al. 2006). Phenacetin was also discovered at around the same time by Hindsberg and Trupel (Toussaint K, Yang XC et al. 2010). Over the next two decades, both paracetamol and phenacetin were used clinically, however in 1887 von Mering incorrectly declared that phenacetin was less toxic than paracetamol and hence it became more widely used from that point onwards (Bertolini, Ferrari et al. 2006; Toussaint K, Yang XC et al. 2010). In the 1940s, however, two research groups; Brodie and Axelrod from the National Institute of Health (Brodie and Axelrod 1949) and Smith and Williams from St. Mary’s Hospital in London (Smith and Williams 1948), discovered that both acetanilide and phenacetin were broken down in the
body to their active metabolite, paracetamol. It was demonstrated that it was indeed paracetamol that was responsible for the antipyretic and analgesic effects of the analine analgesics, with the toxic side effects mainly being caused by another metabolite of phenacetin, \( p \)-phenitidine (Toussaint K, Yang XC et al. 2010). At this point, the popularity of paracetamol rose. It was first marketed in the United States in 1950 as a combination medicine with aspirin and caffeine, under the trade name TRIOGESIC (Prescott 2000; Toussaint K, Yang XC et al. 2010). However, it was recalled in 1951 after it was inaccurately linked with cases of agranulocytosis. It was marketed again in 1955, under prescription only, as TYLENOL in the United States and PANADOL in the United Kingdom and Australia (Prescott 2000; Toussaint K, Yang XC et al. 2010). It was reintroduced as an over-the-counter medication in the early 1960s and its use became widespread from this point forward (Toussaint K, Yang XC et al. 2010). By 1980 the sales of paracetamol had exceeded those of aspirin in many countries around the world (Bertolini, Ferrari et al. 2006). The rising popularity of paracetamol was in contrast to the demise of phenacetin, which became associated with ‘analgesic nephropathy’ (severe kidney damage from papillary necrosis) (Bertolini, Ferrari et al. 2006). The popularity of paracetamol has continued to increase over recent decades due to the association of aspirin with Reye’s Syndrome in children with viral illness, causing paracetamol to usurp aspirin as the drug of choice for the treatment of pain and fever in children (Prescott 2000).
2.2 Therapeutic Properties and Uses

Paracetamol is an antipyretic and analgesic. It has minimal anti-inflammatory and anti-rheumatic activity (Rang HP, Dale MM et al. 2003). Paracetamol is indicated for mild to moderate pain, for example, headaches, cold, flu, sprains, back pain, dysmenorrhoea, mild arthritic pain, mild surgical procedures, and moderate to severe acute post-operative pain (Barden, Edwards et al. 2004; Bertolini, Ferrari et al. 2006). It is not recommended for treatment of inflammatory conditions due to its poor anti-inflammatory profile. It is used in preference to aspirin in patients receiving anticoagulants or with coagulation disorders (Bertolini, Ferrari et al. 2006) and in any patients with a high risk of gastrointestinal, cardiovascular or renal side effects related to non-steroidal anti-inflammatory drugs (NSAIDs), for example patients with hypertension, renal disease or congestive heart failure.
Ingestion of paracetamol can lead to feelings of relaxation, drowsiness, euphoria or tranquillity (Eade and Lasagna 1967; Abbott and Hellemans 2000).

2.3 Mechanisms of Action

2.3.1 Introduction

Despite the long history and widespread use of paracetamol, its mechanism of action still remains uncertain, although recent emerging evidence suggests that the main mode of analgesic action is in the central nervous system (Bertolini, Ferrari et al. 2006; Smith 2009). Despite this recent progress, however, the exact central pathways responsible for paracetamol’s antipyretic and analgesic effects are still unclear (Smith 2009). This chapter sub-section will provide an overview of current mechanistic theories, including paracetamol’s relationship with the cyclooxygenase pathway, the serotonergic system, the cannabinoid system, and the opioidergic system.

2.3.2 The Cyclooxygenase (COX) Pathway

Sir John Vane first proposed that the mechanism of action of paracetamol was similar to that of aspirin and other NSAIDs (Flower and Vane 1972), the pathway which Vane himself discovered and for which he was awarded a Nobel Price in 1982 (Vane 1971). The proposed
mechanism of action involved blockade of the biosynthesis of prostaglandins through non-selective inhibition of the enzyme cyclooxygenase (COX). COX enzymes catalyse the formation of prostaglandin and thromboxane from arachidonic acid, which in turn act as messenger molecules in the processes of pain and inflammation (Vane 1971; Vane and Botting 1998).

The antipyretic effects of paracetamol were initially thought to be due to the inhibition of COX in the brain (Flower and Vane 1972). More recently, three COX isoenzymes have been discovered; COX-1, COX-2, and COX-3 (Bertolini, Ferrari et al. 2006); COX-1 is constitutive and makes prostaglandins that protect the stomach and kidney from damage. COX-2 is induced by inflammatory stimuli and produces prostaglandins that contribute to the pain and swelling of inflammation, and COX-3 is a splice variant of COX-1 (Vane and Botting 1998). Paracetamol has been shown to have only a weak inhibitory effect on both COX-1 and COX-2 at therapeutic concentrations, and the lack of anti-inflammatory, antiplatelet and gastrotoxic activity of paracetamol can not be explained by its action on these isozymes (Kis, Snipes et al. 2005). A splice-variant of COX-1 (designated COX-3 or COX-1b) was discovered in 2002 and was found to be sensitive to inhibition by paracetamol in canine models, with particular expression in the canine cerebral cortex (Chandrasekharan, Dai et al. 2002; Botting and Ayoub 2005). However, COX-3/1b was later shown to have minimal expression in humans, indicating that it is unlikely to have much clinical relevance (Kis, Snipes et al. 2005) and is therefore unlikely to be a significant mechanism of action of paracetamol.
It has also been proposed that paracetamol may act on the COX enzymes by inhibiting them indirectly (as opposed to directly as is the case with NSAIDs) (Toussaint K, Yang XC et al. 2010). Paracetamol is a phenol and a powerful reducing agent, and may therefore work by reducing COX enzymes to a less active form (Ouellet and Percival 2001). The COX pathway requires a certain concentration of peroxide into order to create COX enzymes, and therefore a co-substrate like paracetamol may also effect the COX cycle by reducing higher enzyme oxidation states via the peroxidase cycle, hence reducing the amount of available peroxide for the cyclooxygenase pathway (Ouellet and Percival 2001). This may explain why paracetamol is not effective at sites of inflammation, where peroxide levels are high or different types of peroxide are produced (Toussaint K, Yang XC et al. 2010).

Overall, however, there is a lack of definitive proof that the analgesic and antipyretic actions of paracetamol work via the COX pathway. This has lead researchers to explore other potential mechanisms of action.
2.3.3 The Serotonergic System

‘5-HT (serotonin) neurons, largely originating in the raphe nucleus in the brain stem, send projections down to the spinal cord that synapse on afferent neurons entering the spinal cord. These descending projections exert an inhibitory (analgesic) effect on the incoming pain signal before it is transmitted to higher CNS (Central Nervous System) centres.’

(Toussaint K, Yang XC et al. 2010)

In the 1990s, it was hypothesised that paracetamol’s mechanism of action may be in the central nervous system (CNS) (Bertolini, Ferrari et al. 2006). Paracetamol was found to reduce nociceptive activity in the rat thalamus in a dose-dependent manner (Carlsson and Jurna 1987) and attenuate human nociception via a central pathway, demonstrated through reduced cerebral potentials and modification of EEG recordings (Bromm, Forth et al. 1992). There has since been considerable research into the effect of paracetamol on serotonin (5-hydroxytryptamine, 5-HT), and evidence exists that at least some part of paracetamol’s analgesic profile is due to its effect on the serotonergic pathway.

It is likely that paracetamol interacts with the serotonin pathway indirectly, as it does not have affinity for 5-HT receptors or neuronal 5-HT reuptake sites (Toussaint K, Yang XC et al. 2010). Research has demonstrated that 5-HT concentrations are raised in the rat brain following paracetamol intake (Pini, Sandrini et al. 1996; Pini, Vitale et al. 1997) and that the analgesic action of paracetamol can be stopped by blockade of the 5-HT receptors in rats (Bonnefont, Chapuy et al. 2005). Further, depletion of cerebral 5-HT levels has been shown to
decrease the efficacy of paracetamol (Pini, Sandrini et al. 1996). Certain serotonin antagonists, such as tropisetron, are able to block paracetamol’s effect (Pelissier, Alloui et al. 1996; Alloui, Chassaing et al. 2002), and the destruction of the serotonin bulbospinal pathway also blocks paracetamol’s analgesic activity (Tjolsen, Lund et al. 1991). In human studies, intravenous application of the 5-HT receptor antagonists tropisetron and granisetron, significantly reduced the analgesic effect of a 1 g dose of paracetamol (as measured by median nerve electrical stimulation or the cold pressor test) (Pickering, Loriot et al. 2006; Pickering, Esteve et al. 2008). While these and other studies have shown a connection between paracetamol and serotonin, the mechanism by which paracetamol acts on the serotonergic descending inhibitory pathway still remains unknown (Smith 2009). There is likely to be cross-over between the serotonergic system and the opioidergic system of analgesic activity (Duman, Kesim et al. 2004).

2.3.4 The Cannabinoid System

The discovery in the 1980s of receptors that mediate the effect of cannabinoids (defined as ‘a group of terpenophenolic compounds present in Cannabis ("Cannabis sativa") and occurring naturally in the nervous and immune systems of animals’ (News Medical 2012)), led to the unveiling of the endocannabinoid system. The endocannabinoid system was thought to be a primitive system, existing in both invertebrate and vertebrate species and involved in a number of physiological processes including pain, motor activity, cognitive function, sleep and appetite (Fride 2002; Toussaint K, Yang XC et al. 2010). The discovery of the first
cannabinoid receptors (CB₁ and CB₂), lead to a search for endogenous ligands and resulted in the detection of the endocannabinoid anandamide, an ethanol amide of arachidonic acid (Fride 2002).

Zygmunt and colleagues demonstrated the link between paracetamol and the cannabinoid system when they proved that paracetamol is metabolised in the brain in a two-step process; firstly by deacetylation to form the primary amine p-aminophenol, and secondly by conjugation with arachidonic acid to form N-arachidonoyl-phenolamine (AM404), the second step being catalysed by fatty acid amide hydrogenase (FAAH) (Zygmunt, Chuang et al. 2000). AM404 is a ligand at CB₁ receptor sites and inhibits the re-uptake of anandamide, leading to an increase in endogenous cannabinoids (Beltramo, Stella et al. 1997; Fegley, Kathuria et al. 2004; Hogestatt, Jonsson et al. 2005). The enzyme FAAH is likely to play an important role as it has been shown to synthesise AM404 from p-aminophenol and arachidonic acid in vitro, and its absence (shown in knockout mice) stops the formation of AM404 in vitro and in vivo (Hogestatt, Jonsson et al. 2005).

Bertolini and colleagues had noted the similarities between paracetamol and the cannabinoids, in particular antinociception caused by activation of spinal serotonin descending projections (due to the exclusive involvement of CB₁ receptors in the case of cannabinoids) (Tjolsen, Lund et al. 1991), and also the reduction of body temperature, and feelings of relaxation, euphoria and tranquillity following consumption of both paracetamol and cannabis (Bertolini, Ferrari et al. 2006). They demonstrated that in rats, the analgesic activity of paracetamol is abated by pre-treatment with selective CB₁ receptor antagonists when given at doses
sufficient to block the analgesic activity of the cannabinoid CB1 agonist, thereby
demonstrating the involvement of the cannabinoid system in the mechanism of analgesic
action of paracetamol (Bertolini, Ferrari et al. 2006; Ottani, Leone et al. 2006).

2.3.5 Other Possible Mechanisms

It is likely that the opioidergic system has some role to play in the analgesic effects of
paracetamol (Smith 2009). Ruggieri and colleagues have demonstrated that the analgesic
efficacy of paracetamol and its metabolite AM404 are both reduced following antagonism of
the μ and κ opioid receptors with naloxone in rats (Ruggieri, Vitale et al. 2008). This was
shown again in a study by Rezende et al, whereby the injection of naltrexone in rats reversed
the hypoalgesia caused by paracetamol (but had no effect on the other drug tested, dypyrone)
(Rezende, Franca et al. 2008).
2.4 Pharmacokinetics

The usual / standard oral dose of paracetamol for both analgesic and antipyretic uses in adults is 650-1000 mg every 4 to 6 hours as needed, up to a maximum recommended dose of 4 g in 24 hours (BNF September 2011). Paracetamol reaches its ceiling effect at 1000 mg in adults (Skoglund, Skjelbred et al. 1991). The recommended oral dose for both analgesic and antipyretic uses in children is 10-15 mg/kg every 4 to 6 hours, up to a maximum of 5 doses per day, (Bertolini, Ferrari et al. 2006).

Absorption

Paracetamol absorption from the stomach is minimal, and most is absorbed through the small intestine (Heading, Nimmo et al. 1973). The onset of analgesic activity is approximately 30 minutes after ingestion, although it depends on the rate of gastric emptying (Prescott 1980). Time to peak concentration after oral administration of regular release tablets is approximately 45-60 minutes, and 30 minutes for liquid paracetamol (Bertolini, Ferrari et al. 2006). The rectal route of administration provides unpredictable levels of absorption, ranging from 24 to 98% (Blume, Ali et al. 1994).
Distribution

The volume of distribution is 0.7 - 1 L/Kg in children and 1 - 2 L/Kg in adults (Bertolini, Ferrari et al. 2006). Paracetamol is distributed evenly throughout most body fluids, with a tissue:plasma concentration of 1:1, except fat and cerebrospinal fluid where it penetrates less effectively (Brodie and Axelrod 1949). It freely crosses the placenta and blood-brain barrier (Bertolini, Ferrari et al. 2006).

Metabolism

Paracetamol metabolism is age- and dose-dependant, however generally it is extensively metabolised, and only 2-5% is excreted unchanged in the urine (Prescott 1980). The plasma half-life is 1.5-2.5 hours, however it can be prolonged in chronic liver disease (Prescott 1980). In the liver, paracetamol is participant to first-pass metabolism and approximately 25% of paracetamol is metabolized during the first passage through the liver (Clements, Heading et al. 1978). In adults, approximately 90% of the paracetamol thus metabolised is through conjugation with glucuronide, sulphate or cysteine into inactive, harmless metabolites. However, approximately 5-15% is metabolised via oxidation through the P450 mixed-function oxidase system, forming the toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQI) (Figure 2.2) (Corcoran, Mitchell et al. 1980; Bertolini, Ferrari et al. 2006). Glutathione, a tripeptide antioxidant, combines with NAPQI to form the non-toxic metabolites cysteine or percaptate, which are then excreted in the urine (Miller, Roberts et al. 1976; Bertolini, Ferrari et al. 2006). Hepatic enzyme induction (for example, in chronic alcoholism) and drugs that induce
cytochrome P450 enzymes (e.g. sulfinpyrazone, isoniazid, anticonvulsants like phenytoin) can increase the rate of paracetamol metabolism and thereby increase the production NAPQI, thus increasing the risk of hepatotoxicity (Prescott, Critchley et al. 1981; Bertolini, Ferrari et al. 2006).

**Excretion**

In the kidneys, paracetamol is excreted via first-order kinetics, so the elimination rate is concentration-dependant (Bertolini, Ferrari et al. 2006). The elimination half-life is 2 to 4 hours in healthy individuals (Albert, Sedman et al. 1974) but it is prolonged in premature infants and newborns (Hansen, O'Brien et al. 1999; Bertolini, Ferrari et al. 2006). Evidence suggesting an increased half-life in the elderly has not been substantiated (Triggs, Nation et al. 1975; Bertolini, Ferrari et al. 2006).
Figure 2.2: Paracetamol metabolism (Bertolini, Ferrari et al. 2006)
(Reproduced with the permission of John Wiley and Sons, Inc.)
2.5 Toxicology

Paracetamol is generally considered a safe drug when used in appropriate doses. The lowest doses reported to have produced toxicity have been 7.5 g in adults and 150 mg/kg in children (Bizovi KE and Smilkstein MJ 2002). As described previously, during paracetamol metabolism glutathione binds with the toxic metabolite NAPQI to produce a non-toxic complex which is then converted to cysteine or mercaptate conjugates and excreted via the kidneys. When an overdose of paracetamol occurs, the supply of glutathione is depleted and when levels of glutathione reach below a critical level (30% of normal stores), free NAPQI rapidly cause damage to hepatic cells leading to cell death (Mitchell, Jollow et al. 1973; Bertolini, Ferrari et al. 2006). Risk of toxicity is increased by chronic alcohol use, increased frequency of paracetamol dosing, prolonged duration of excessive dosing, and use of medications which increase capacity for P450 activation to NAPQI (mentioned above). Serious hepatotoxicity or acute overdose causing death is less common in children, due to the increased supply of glutathione and the regenerative capacity of the liver in children (Lauterburg, Vaishnav et al. 1980). However during excessive repeated dosing in children with acute febrile illnesses, children are at an increased risk of liver damage (Henretig, Selbst et al. 1989; Day and Abbott 1994).

The liver is the organ most affected by paracetamol overdose. Severe cases of overdose lead to fulminant hepatic failure, and can require liver transplantation. Patients present with various combinations of jaundice, encephalopathy, increased intracranial pressure, disseminated
intravascular coagulation and haemorrhage, hyperventilation, acidosis, hypoglycaemia and renal failure (Bertolini, Ferrari et al. 2006). The kidneys are also affected by paracetamol overdose due to the local formation of NAPQI. Severe hepatotoxicity leads to renal dysfunction in approximately 25% of cases and 50% of those with hepatic failure (Wilkinson, Moodie et al. 1977; Makin and Williams 1994; Bertolini, Ferrari et al. 2006). Renal dysfunction may also occur in the absence of hepatic injury (Campbell and Baylis 1992). Injury to other organs is rare. N-acetyl-cysteine (NAC) is a specific antidote for the treatment of paracetamol poisoning. NAC acts as a glutathione precursor, leading to increased glutathione bioavailability (Lauterburg, Corcoran et al. 1983) and can also act as a glutathione substitute and bind with NAPQI to create non-toxic metabolites (Bertolini, Ferrari et al. 2006). However, NAC must be given in a timely fashion, as liver injury may occur from 24 hours after ingestion and fatalities following overdose usually occur within 3 to 5 days of ingestion (Bertolini, Ferrari et al. 2006).
3.1 Definition

One of the difficulties of asthma research is that a clear, universally accepted definition of the disorder is lacking. In 1860 Henry Hyde Salter, a London physician, described asthma as:

“paroxysmal dyspnoea of a peculiar character, generally periodic with intervals of healthy respiration between the attacks”

(Salter 1868)

30 years later Sir William Osler furthered Salter’s description, by connecting the disordered airway function seen in asthma with pathological changes in the lung (Holgate 2004):

“(Asthma is) a neurotic affection characterised by hyperaemia and turgescence of the mucosa of the small bronchial tubes and a peculiar exudate of mucin. The attacks may be due to direct irritation of the bronchial mucosa or may be induced reflexly, by irritation of the nasal mucosa, and indirectly, too, by reflex influences from stomach, intestines or genital organs.”

(Osler W 1892)
Descriptions of asthma continued to be refined over the next century, and the current definition, defined by the Global Initiative for Asthma (GINA), describes it as:

“a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation causes an associated increase in airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment”

(Global Initiative for Asthma (GINA) 2010)

Therefore, asthma is currently defined by its clinical, physiological and pathological characteristics (Global Initiative for Asthma (GINA) 2010). The usual symptoms of asthma include wheeze, shortness of breath, tightness in the chest and cough (Morris 2011). Asthma attacks can be triggered by environmental allergens (such as house dust mite, animal allergens, cockroach allergens and fungi), cold air, exercise, viral upper respiratory infections, gastro-oesophageal reflux disease, chronic sinusitis/rhinitis, aspirin or NSAID hypersensitivity, use of β-adrenergic receptor antagonists, irritants such as household sprays and paint fumes, emotional factors, stress and cigarette smoke (Fireman 2003). Asthma control refers to the extent to which the manifestations of asthma have been removed or reduced by treatment (Taylor, Bateman et al. 2008). It includes the two factors of current clinical control, including symptoms, beta-agonist use and lung function, as well as future risk including exacerbations and decline in lung function (Taylor, Bateman et al. 2008).
3.2 Prevalence

It is estimated that approximately 300 million people are affected by asthma worldwide, with over 250,000 dying each year from the disorder (Global Initiative for Asthma (GINA) 2010). The World Health Organization estimate that 15 million disability-adjusted life years are lost each year due to asthma, contributing 1% of the total global disease burden (Masoli, Fabian et al. 2004). The global prevalence of asthma varies in different populations, but is estimated at between 1 and 18% (Global Initiative for Asthma (GINA) 2010). Asthma rates are higher in developed countries and have been increasing over the last three decades (Eder, Ege et al. 2006). In the United States, asthma prevalence more than doubled between 1980 and 2004 for adults and children alike (Eder, Ege et al. 2006; Centers for Disease Control and Prevention 2007). Similar increases in prevalence have been seen over the last few decades in New Zealand (Holt S and Beasley R December 2001) and Australia (Robertson, Heycock et al. 1991), and over 20 studies from various countries over this time period have consistently shown an approximate 5% increase in asthma prevalence per annum (Burney 2002). Prevalence is also increasing in developing countries as they become more westernised (Masoli, Fabian et al. 2004; Braman 2006). The countries with the highest asthma prevalence are the United Kingdom, New Zealand, Australia, the Republic of Ireland, Canada and the United States (Figure 3.1). Within populations, certain ethnic groups, such as African-Americans and Hispanics, also have a higher prevalence than others (Braman 2006).
Figure 3.1: 12-month prevalence (%) of self-reported asthma symptoms from written questionnaires in 13-14-year-old children in different countries* (ISAAC Steering Committee, 1998)

*Each dot represents the published results of a questionnaire

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These international patterns of asthma prevalence are not adequately explained by our current understanding of the causation of asthma, and the reasons for the increase in asthma prevalence over recent decades is still unknown, despite significant research in this area. Whilst considerable advances have been made in the understanding of the genetics of asthma, it is generally acknowledged that the rise in prevalence has been too rapid to be explained by genetic factors, which would take generations to manifest (Eder, Ege et al. 2006). Changes to diagnostic criteria that occurred from the mid-1980s are likely to have caused some artefactual increase in prevalence (Burney 2002; Braman 2006). However, there has also been an increase in reported symptoms over this time which can not be accounted for by the change in diagnostic practices. Therefore, it is highly unlikely that these diagnostic changes fully account for the increased prevalence of asthma witnessed over the last 3 decades (Burney 2002). Several hypotheses exist to explain the rising prevalence, including environmental factors, obesity and diet, exposure to infections, the hygiene hypothesis and the effect of paracetamol (see section 3.4 below) (Braman 2006).

The prevalence of asthma in New Zealand is amongst the highest in the world (Holt S and Beasley R December 2001). The International Study of Asthma and Allergies in Childhood (ISAAC) (ISAAC Steering Committee 1998; Asher, Barry et al. 2001) measured the prevalence of asthma in New Zealand children, and determined that in the 6-7 year old group, 24.5% reported experiencing wheeze in the previous 12 months and 26.5% reported ever having had asthma. Likewise, in the 13 to 14 year-old age group, 30.2% reported experiencing wheeze in the previous 12 months and 24.4% reported ever having had asthma. The European
Community Respiratory Health Survey (ECRHS) (Burney, Luczynska et al. 1994; Burney, Chinn et al. 1996) collected similar data in New Zealand adults, and determined that the national prevalence of self-reported wheezing in the previous 12 months was 25.7%, and of self-reported asthma was between 14.2 and 17.8% depending on the age group surveyed.

In New Zealand, asthma accounts for 7% of the total number of years lost to disability. It is the highest ranking single disease in terms of years lost to disability in males, and the third highest in women (Ministry of Health 2011). Hospitalisation rates for asthma increased 10-fold in the 1-14 year age group between the late 1960s and late 1980s and, although hospitalisation rates have been steadily decreasing since that time (The Asthma and Respiratory Foundation of NZ 2006), asthma is still the third most common cause of childhood admissions to hospital (Kemp and Pearce 1997; The Children's Social Health Monitor New Zealand 2009). The burden of asthma is disproportionate for Māori and Pacific Islanders, with hospitalisation rates for asthma in Māori twice that of non-Māori and even higher for Pacific Islanders (particularly Pacific Island Children) (Wickens K, Fitzharris P et al. 1998; Tukuitonga, Bell et al. 2000). Impoverishment and socio-economic disadvantage also play a significant role in the severity of asthma symptoms. In one study of adults presenting to an Auckland hospital with severe asthma symptoms, over half of patients surveyed reported experiencing recent financial difficulties and a third reported living in a household reliant on a social security benefit (Kolbe, Vamos et al. 1997).
3.3 Incidence

According to UK data, the incidence of asthma (measured by the number of new presentations of asthma to general practitioners) has increased markedly since 1976 (National Asthma Campaign 2001; Smyth 2002). The incidence of first or new cases of asthma in all age groups peaked in the mid-1990s and, at its peak, the incidence for pre-school children was 11 times higher than the incidence in 1976. Since then the incidence has been steadily decreasing, yet it is still significantly higher now than it was in the 1970’s (Figure 3.2) (Smyth 2002).

![Figure 3.2: Average weekly incidence of first and new episodes of asthma in patients presenting to GPs in England and Wales between 1976 and 2000 (Smyth 2002)](image)
3.4 Provoking Factors

Environmental factors, such as allergens, infections, tobacco smoke, air pollution and diet, as well as host factors such as genetics, obesity, and diet have all been implicated in the development and/or expression of asthma (Global Initiative for Asthma (GINA) 2010). However, none of these theories alone can explain the growing prevalence of asthma around the world or international patterns of asthma prevalence. As a result, novel risk factors, such as the use of paracetamol, are currently being studied. Although a full discussion of the current evidence regarding the influencing factors of asthma is outside the scope of this thesis, below is a summary of current evidence for and against the major host and environmental factors listed above.

Allergens

Exposure to allergens is well known to cause asthma exacerbations, yet their effect on the development of asthma is not yet understood. Whilst it has been hypothesised that increased exposure to indoor allergens such as cat fur and house dust mite may be responsible for the increasing prevalence of childhood asthma (Peat, Tovey et al. 1996), this theory is yet to be proven (Pearce, Douwes et al. 2000). A German cohort study of 939 children found no link between exposure to indoor allergens and the prevalence of asthma, wheeze or BHR in children from birth to age 7 years (Lau, Illi et al. 2000). Another London cohort study of 552 children found no linear relationship between early exposure to indoor allergens and childhood atopy or asthma, but showed instead that the risk of allergy was associated with
birth order and genetics (Cullinan, MacNeill et al. 2004). However, chronic exposure to indoor allergens in sensitised children is associated with an increased severity of asthma, increased BHR and worse lung function (Illi, von Mutius et al. 2006).

**Hygiene Hypothesis**

The hygiene hypothesis is that reduced exposure to microbes in early life, as a result of improved hygiene in modern living, has resulted in increased allergic sensitisation and thus asthma through decreased stimulation and activation of Th1 cells and a reciprocal increased propensity towards Th2 immune responses that involve IgE mediated allergy (Strachan 1989; Strachan 2000; Smyth 2002). The factors implicated in the hypothesis include reduced family size, improved household facilities and personal cleanliness, and subsequent decreased exposure to helminth infection, endotoxins, pets and farm animals (Strachan 2000; Platts-Mills, Erwin et al. 2005).

While immunologically plausible, no inverse relationship between infection and atopy has yet been shown, and the ISAAC study demonstrated that even in countries with very poor hygiene, high rates of asthma are still common (ISAAC Steering Committee 1998; Strachan 2000). However, a higher prevalence of hay fever, eczema and atopy has consistently been demonstrated in children of smaller and more affluent families (Strachan 2000). Further, children with older siblings or who attend day-care in the first 6 months of life are protected against the development of asthma (Ball, Castro-Rodriguez et al. 2000).
The hygiene hypothesis is still contentious and fiercely debated. A definitive relationship between early-life infections, exposure to microbial compounds and the immune system is yet to be developed. Considerable further research is required before definitive implications can be derived (Schaub, Lauener et al. 2006).

**Exposure to Tobacco Smoke**

While there are clear links between exposure to tobacco smoke and worsening of asthma symptoms in people already diagnosed with the condition, the association between tobacco smoke exposure and the development of asthma remains controversial. Exposure to tobacco smoke prenatally and in childhood is related to an increased incidence of non-atopic childhood ‘wheezy bronchitis’ and an increased severity of asthma in children already diagnosed with the disorder (Strachan and Cook 1998). Further analysis suggests that tobacco smoke is likely to be a co-factor in precipitating asthma attacks, as opposed to a cause of asthma itself (Strachan and Cook 1998). Active smoking has been shown to aggravate the decline in lung function in people with asthma, and may increase the severity of the disease and lessen the response to treatment with inhaled corticosteroids (Chalmers, Macleod et al. 2002; Global Initiative for Asthma (GINA) 2010). Active smoking has also been associated with the onset of asthma in adolescence and adulthood, with a dose-response relationship both for duration of smoking and number of cigarettes smoked per day (Strachan, Butland et al. 1996).
Air Pollution

Air pollutants implicated in the relationship with asthma include sulphur dioxide, ozone, nitrogen dioxide and particulate carbons. The link between air pollution and asthma remains controversial and results are difficult to generalise due to geographical variations in air composition (Maynard 2001). Whilst several large studies have failed to demonstrate a higher prevalence of asthma in areas with high levels of air pollution (von Mutius, Martinez et al. 1994; ISAAC Steering Committee 1998), others evidence shows that air pollutants, such as particulate matter and ozone, increase susceptibility to asthma, worsen existing lung inflammation and function and increase health care usage (Tatum and Shapiro 2005). Children living close to major roadways (and thus pollution such as particulate matter from motor vehicles) have been shown to have an increased risk of asthma and are more likely to wheeze and cough, however no increase in bronchial hyperresponsiveness (BHR) or atopic disease has yet been demonstrated (Hirsch, Weiland et al. 1999; Janssen NA, Brunekreef B et al. 2003; Nicolai, Carr et al. 2003).

Obesity

There has been a parallel increase in the prevalence of obesity and asthma in recent decades which indicates a potential link between them (Eder, Ege et al. 2006). Obesity and overweight have been linked to the development of asthma in many studies and weight loss has been shown to decrease asthma symptoms and improve lung function, however the mechanism behind the association remains unclear (Schaub and von Mutius 2005). It is unlikely that reverse causation (asthma symptoms leading to reduced exercise participation and hence
obesity) is the source of the connection, as obesity can often predate the development of asthma (Eder, Ege et al. 2006). It is possible that physical inactivity or other recent changes in our lifestyle and/or diet promote both obesity and asthma (Chinn and Rona 2001).

Diet

Numerous hypotheses have been made between an increasing asthma prevalence and the intake of certain foods and associated vitamins, minerals and fatty acids, however current evidence is inconsistent and inconclusive (McKeever and Britton 2004; Eder, Ege et al. 2006). Breastfeeding as well as avoiding cow’s milk and eggs during pregnancy have not been shown to protect children from asthma (Friedman and Zeiger 2005). An ecological analysis of data from the ISAAC study showed a reduction in symptoms of wheeze, allergic rhinoconjunctivits and atopic eczema with per capita consumption of calories from cereal and nuts, starch, vegetables and vegetable nutrients (Ellwood, Asher et al. 2001). This association may be due to antioxidant effects, or possibly the effect of certain foods on the intestinal microflora (Ellwood, Asher et al. 2001)

Paracetamol

The paracetamol and asthma hypothesis will be discussed in the following chapter.
3.5 Natural History

Due to the heterogeneity of asthma, its progression can be very varied. In over 80% of asthma cases the initial clinical manifestation of the illness occurs during the first 5 years of life (Guerra and Martinez 2002). Despite this, the majority of cases of wheezing that occur before the age of 2 are due to respiratory syncytial virus (RSV) or rhinovirus infection and symptoms do not usually persist after the age of 3 (‘transient wheezing of infancy’) (Guerra and Martinez 2002). However, children who develop wheeze before the age of 3 and who go on to develop persistent asthma symptoms tend to have worse outcomes in lung function than those who develop symptoms later in life (Covar, Spahn et al. 2004). Children under 3 years who are at risk of developing persistent asthma include those who have had four or more episodes of wheezing during the previous year, or either one of the following (National Heart Lung and Blood Institute 2007):

- A parental history of asthma
- A physician diagnosis of atopic dermatitis
- Evidence of sensitivity to aeroallergens

Or two of the following:

- Evidence of sensitisation to foods,
- 4% or more peripheral blood eosinophilia
- Wheezing apart from colds
Over half of the individuals who wheeze frequently in childhood go on to wheeze as adolescents (Guerra, Wright et al. 2004) and the severity of childhood asthma correlates with the likelihood of continuation of the disease into adulthood. Children with mild asthma are likely to have either no asthma or mild asthma as adults (Guerra and Martinez 2002). Chronic asthma, spanning childhood into adulthood, has now been linked with atopy, be that either sensitisation to aeroallergens, high circulating levels of IgE, or both (Guerra and Martinez 2002). There is evidence that adults with asthma have accelerated loss of lung function, although the clinical impact of this decline and whether or not it contributes to fixed airflow obstruction is unknown (National Heart Lung and Blood Institute 2007).

### 3.6 Pathophysiology

Our understanding of asthma has been improving rapidly over recent years, and it is likely that asthma is not a single disease, but ‘a complex of multiple, separate syndromes that overlap’ (Wenzel 2006). The presence of airway inflammation is central to the pathophysiology of asthma. Various inflammatory cells play a role producing mediators which work on target cells of the airways to produce the abnormal pathophysiological features of asthma (Barnes 1996).

Gross features of asthma include overinflation of the lungs, mucous plugs occluding medium and small bronchi and bronchioles, smooth muscle hypertrophy, lamina reticularis thickening, mucosal oedema, epithelial cell sloughing and cilia cell disruption (Fireman 2003; Barrios,
(Kheradmand et al. 2006). Airflow limitation in asthma is caused by a variety of changes in the airway, including bronchoconstriction, airway oedema, BHR and airway remodelling (Fireman 2003). Bronchoconstriction relates to airway narrowing with subsequent limitation of airflow. Allergen-induced bronchoconstriction is caused by IgE-dependant release of mediators such as histamine, tryptase, leukotrienes and prostaglandins from mast cells (Busse and Lemanske 2001). Non-allergen induced bronchoconstriction can also be caused by drugs such as aspirin and other NSAIDs, and other stimuli such as cold air or exercise, which cause bronchoconstriction via a non-IgE mediated pathway (Stevenson and Szczeklik 2006). Airway oedema, mucous hypersecretion, mucous plugs, smooth muscle hypertrophy and hyperplasia all occur when inflammation becomes more persistent (Fireman 2003).

BHR is defined as an exaggerated bronchoconstrictor response to a variety of stimuli and is a major diagnostic feature of asthma (Brannan 2010). The mechanisms of bronchial hyperresponsiveness include inflammation, dysfunctional neuroregulation and structural changes to the airways (Chung and Adcock 2001). Airway remodelling relates to permanent structural changes such as sub-basement membrane thickening, subepithelial fibrosis, and blood vessel proliferation and dilation, which occur in the airway and lead to loss of function which is not fully reversible with treatment (Holgate and Polosa 2006).

Recently, particular emphasis has been placed on the role of the dysfunctional epithelium as a cardinal element to understanding asthma and asthma therapeutics (Holgate 2011). Defects in epithelial barrier function allow the penetration of environmental factors into the airway wall, and impaired repair of the epithelium and repeated distortion of the epithelium through
bronchoconstriction lead to airway remodelling (Holgate 2011). It has been suggested that a greater emphasis on epithelial functioning in the pathogenesis of asthma may lead to more effective novel therapies which increase the airways resistance to the inhaled environment instead of focusing on the suppression of inflammation (Holgate 2011).

3.6.1 Inflammatory Cells in Asthma

The major inflammatory cells involved in airway inflammation in asthma are lymphocytes, mast cells, eosinophils, neutrophils, dendritic cells, macrophages, epithelial cells and airway smooth muscle cells.

Asthmatic airways demonstrate a propensity towards the T helper 2 (Th2) lymphocyte pathway which results in eosinophilic inflammation and secretion of the cytokine cascade characteristic of asthma, including interleukin-4 (IL-4), IL-5, IL-9, and IL-13, which cause overproduction of IgE, increased eosinophils and BHR (Barrios, Kheradmand et al. 2006). Mast cell activation leads to a release of bronchoconstrictor mediators including histamine, cysteiny1-leukotrienes and prostaglandin D2 and can also lead to the release of cytokines which promote inflammation in the airways (Barrios, Kheradmand et al. 2006). Eosinophilic infiltration of the airways of asthma sufferers is a characteristic feature and differentiates asthma from other respiratory conditions; however the role of eosinophils remains uncertain (Barnes 2008). While it was previously thought that the role of eosinophils was associated with airway hyperresponsiveness (Barnes 1996), there role is more likely to be associated with
subepithelial fibrosis, and their presence is a good marker of steroid responsiveness (Barnes 2008). Eosinophil maturation, survival and recruitment in the airways are under the influence of IL-3, IL-5 and GM-CSF (Holgate 2011).

Neutrophil numbers are also increased in the airways of asthmatics, and their presence correlates with disease severity (Fahy, Kim et al. 1995; Barnes 2008). Dendritic cells are macrophage-like cells which act as antigen-presenting cells and bind with allergens on airway surface and then migrate to regional lymph nodes to stimulate the Th2 response (Kuipers and Lambrecht 2004). Macrophages can play both an anti-inflammatory and pro-inflammatory role, however it is likely that in asthma the anti-inflammatory action of macrophages (notably the suppression of lymphocyte function) may be impaired following inhalation of allergens (Spiteri, Knight et al. 1994; Barnes 1996). Macrophages also produce a large number of cytokines which stimulate the inflammatory response. Epithelial cells produce inflammatory mediators such as proinflammatory cytokines, growth factors, endothelins and chemokines which cause the production of more mediators and can directly damage the epithelium itself (Campbell 1997). In asthma, the repair process of damaged epithelial cells may be abnormal (Campbell 1997). The resident cells of the airway, smooth muscle cells, produce their own family of pro-inflammatory mediators which add to the inflammatory pathway of asthma. Smooth muscle cells also undergo proliferation, contraction, activation and hypertrophy which cause airway dysfunction (Borger, Tamm et al. 2006).
3.6.2 Inflammatory Mediators in Asthma

Many mediators are involved in the pathogenesis of asthma, and each mediator has many different effects on the airways, making the identification of the specific role of each individual mediator difficult (Barnes 2008). The major mediators include chemokines, cytokines, cysteinyl-leukotrienes and nitric oxide (Barnes 2008).

Chemokines, in particular eotaxins, play an important role in the onset of asthmatic inflammation by acting as leukocyte chemoattractants, cellular activating factors and histamine-releasing factors (Zimmermann, Hershey et al. 2003). Cytokines are synthesised by several different inflammatory cells (macrophages, mast cells, eosinophils and lymphocytes) and play an important role in chronic inflammation and the determination of severity of disease (Zimmermann, Hershey et al. 2003). The most important cytokines are the lymphokines secreted by T-lymphocytes: IL-3, IL-4, IL-5 and IL-13 (Barnes 1996). Other cytokines (IL-1, IL-6, tumour necrosis factor-α (TNF-α) and GM-CSF are also important in amplifying the inflammatory response (Barnes 1996).

Cysteinyl-leukotrienes LTC₄, LTD₄ and LTE₄ derived from mast cells are potent constrictors of the airway and increase BHR (Barnes 1996). Their role in asthma has recently been confirmed through the development of leukotriene receptor antagonists and the finding that they can be used as supplementary therapy in difficult-to-treat disease (Leff 2001). Nitric oxide (NO) is a potent vasodilator and is produced predominantly by inducible NO synthetase (iNOS) in airway epithelial cells, leading to increased plasma exudation (Barnes and Liew
1995). Measurement of fractional exhaled NO (FeNO) is often used for monitoring disease progress as it is a surrogate marker of airways inflammation (Green, Brightling et al. 2002). This will be discussed further in following chapters.

3.7 Diagnosis, Treatment and Prognosis

3.7.1 Diagnosis

The key diagnostic indicators of asthma are a history of episodic cough, shortness of breath and wheeze associated with airflow obstruction that is reversible either spontaneously over time or in response to treatment (New Zealand Guidelines Group September 2002). Symptoms are often worse in the presence of exercise, viral infection, animals with fur, house-dust mite, mould, smoke, pollen, changes in the weather, strong emotional experiences or menstrual cycles (National Heart Lung and Blood Institute 2007) Acute attacks of wheezing with reduced peak expiratory flow rate (PEF) are highly specific for asthma. Although there is not much diagnostic value in a one-off PEF recording, PEF variability of > 15% is highly specific for asthma (New Zealand Guidelines Group September 2002). The most sensitive test for airway obstruction is spirometry and an FEV₁/FVC ratio of < 70% is diagnostic of obstruction and should be used in the diagnosis of asthma (New Zealand Guidelines Group September 2002). Differential diagnoses include upper respiratory tract infections, post infective BHR, chronic obstructive pulmonary disease (COPD), left ventricular failure, central airways
obstruction or foreign body, vocal cord dysfunction, hyperventilation, bronchiectasis or interstitial lung disease (New Zealand Guidelines Group September 2002).

3.7.2 Treatment

An in-depth discussion of the pharmacotherapeutics and treatment strategies for asthma is outside the scope of this thesis. In summary, however; regular inhaled corticosteroids, such as beclomethasone dipropionate, fluticasone propionate or budesonide, are the mainstay of maintenance treatment in New Zealand and are effective at decreasing symptoms, improving lung function, slowing the rate of airway decline and reducing hospital admissions and risk of mortality (New Zealand Guidelines Group September 2002). Inhaled short-acting β₂ agonists (SABAs), such as salbutamol, are used as required to relieve symptoms, as well as inhaled long-acting β₂ agonists (LABAs), such as salmeterol and formoterol, which improve day and night symptom control, improve lung function, and reduce exacerbation rates (New Zealand Guidelines Group September 2002). The oral methylxanthine, theophylline, is an effective third-line therapy and the inhaled anticholinergic agent, ipratropium, when used in combination with salbutamol, improves clinical outcomes when used in acute exacerbations (New Zealand Guidelines Group September 2002). Leukotriene receptor antagonists show a small additional benefit when used with inhaled corticosteroids, but not to the same extent as the addition of a LABA (New Zealand Guidelines Group September 2002).
3.7.3 Prognosis

A considerable proportion of childhood asthma improves or remits completely by adulthood. It has been postulated that perseverance of symptoms into adulthood is correlated with severity of illness (Guerra and Martinez 2002), however other recent studies have determined that the course of asthma varies according to age and individual variations instead of severity of symptoms (National Heart Lung and Blood Institute 2007). A New Zealand cohort study that assessed lung function in 1,000 children from ages 9 through to 26 years showed that, although poor lung function tracked from childhood through to adulthood, the slope of the curve of FEV₁:FVC in asthmatics and non-asthmatics was parallel and no further deterioration was seen in the asthmatic group after the age of 9 years. This suggests that impairment of lung function occurs in early childhood and is not related to severity of illness after 9 years of age (Sears, Greene et al. 2003).

It is estimated that asthma accounts for 1 in 250 deaths worldwide each year (Masoli, Fabian et al. 2004), however there is considerable regional variation in mortality rates (Braman 2006). Most asthma deaths occur in people aged over 45 years and are due to inadequate medical care or delays in seeking medical help during an attack (Braman 2006). Overall, mortality rates have decreased since the 1980s, which is likely due to improvements in pharmacotherapy. New Zealand experienced two asthma mortality epidemics in the 1960s and 1970s/1980s related to the overuse of two high-dose beta-agonists, isoprenaline and fenoterol respectively (Holt S and Beasley R December 2001), however mortality rates are now similar
to those in other western countries (Asthma and Respiratory Foundation of New Zealand 2002).
Chapter Four: Literature Review – The Association between Paracetamol and Asthma

4.1 Introduction

Our current understanding of the causation of asthma does not adequately explain the global increase in prevalence over recent decades, nor why certain geographical areas contain a higher proportion of asthmatics than others (Beasley, Crane et al. 2000; Eder, Ege et al. 2006). Changes in genetic factors are likely to be too slow in onset to account for the rapid change in prevalence (Eder, Ege et al. 2006), and there remains no robust data linking changes in the environment to increasing asthma incidence and prevalence (Eder, Ege et al. 2006). This has led researchers to hypothesise about possible novel exposures that may increase susceptibility to atopy and the development and severity of asthma. One such risk factor for which there is evidence for a potential role is the increasing use of paracetamol.

Arthur E Varner, an American allergy and immunology specialist, first proposed the paracetamol hypothesis in 1998. Varner noted a previously unappreciated phenomenon that had occurred in the United States (and elsewhere around the world) in the 1980s, whereby paracetamol had replaced aspirin as the drug of choice for febrile children, due to the association of aspirin with Reye’s syndrome (a potentially fatal disorder causing liver and brain damage). Varner proposed that this universal change in fever management in children could have possible immunomodulating effects and lead to an increased predisposition to allergic disease by enhancing the T-helper type 2 (Th2) immunological response (discussed in
more detail in section 4.3.4). Since then, there has been a significant amount of research conducted on the paracetamol hypothesis, and a growing body of evidence exists to suggest that the use of paracetamol may represent an important risk factor for the development of asthma (Newson, Shaheen et al. 2000; Nuttall, Williams et al. 2003; Allmers 2005; Eneli, Sadri et al. 2005; Barr 2008; Beasley, Clayton et al. 2008; Farquhar H, Crane J et al. 2009; Amberbir, Medhin et al. 2010; Beasley RW, Clayton TO et al. 2010; Tapiainen T, Dunder T et al. 2010; Holgate 2011; Johnson and Ownby 2011; McBride 2011).

The importance of this issue is highlighted by the widespread use of paracetamol in both children and adults, and the high and growing prevalence of asthma worldwide. Even a small reduction in the prevalence of asthma would have a large effect on the global burden of disease caused by this common illness, and therefore it is important that any relationship with paracetamol is determined. The mounting body of evidence suggesting an association between the two has lead to debate about the role of paracetamol in the management of childhood illness and created uncertainly in clinical practice (McBride 2011). Some paediatricians are already recommending paracetamol avoidance in children with asthma or at risk for asthma (McBride 2011), and there has been widespread call for further definitive research to be undertaken, in particular randomised controlled trials (RCTs), in order to enable evidence-based guidelines for the recommended use of paracetamol to be made (Barr 2008; Beasley, Clayton et al. 2008; Beasley R, Clayton T et al. 2009; Farquhar H, Crane J et al. 2009; Amberbir, Medhin et al. 2010; Beasley RW, Clayton TO et al. 2010; Farquhar H, Stewart A et al. 2010; Tapiainen T, Dunder T et al. 2010; Holgate 2011; Johnson and Ownby 2011; McBride 2011).
4.2 Ecological Associations and Time Trends

The global rise in asthma prevalence over the last three decades has occurred contemporaneously with growing sales and increasing use of paracetamol worldwide. From the period 1980 to 2003 asthma prevalence in the United States increased from 3.6% to 5.8% (Eder, Ege et al. 2006). Likewise in countries such as Australia, rates of asthma have been steadily increasing over the latter half of the 20\textsuperscript{th} century. In 1964, 19% of Australian children were reported by their parents as having asthma symptoms, yet by 1990 this number had risen to 46%. Studies from New Zealand show a similar phenomenon. Repeat surveys of a rural secondary school in Wairoa, Hawkes Bay, in 1975 and 1989 determined that the prevalence of wheeze had increased from 26% to 34%, and was greater for Maori than non-Maori children (Stanhope, Rees et al. 1979; Shaw, Crane et al. 1990). A similar study of urban New Zealand children living in Upper Hutt, Wellington, likewise determined an increase in asthma prevalence from 7% to 14% from the period 1969 to 1982 (Mitchell 1983). While some of these changes in asthma prevalence may have been attributable to changes in diagnosis and awareness of asthma, it is unlikely to be the only cause of such a widespread change (Eder, Ege et al. 2006; Holt S and Beasley R December 2001).

At the same time, international sales and intake of paracetamol were on the rise. In the United States, sales of paracetamol rose steadily from the 1950’s through to the 1980’s, by which time they matched sales of aspirin (Arrowsmith, Kennedy et al. 1987). By 1990 paracetamol had become the most common medication in the USA, making up approximately 5% of all
medications dispensed (Williams LA, Burke LB et al. 1991). These ecological shifts observed in the USA were mirrored around the world (Beasley, Clayton et al. 2008).

In 1998, Varner and colleagues hypothesised that the decreasing use of paediatric aspirin (and the reciprocal increasing use of paracetamol) had contributed to the increasing prevalence of childhood asthma (Figure 4.1) (Varner, Busse et al. 1998). In 2000, Newson and colleagues used data from the ISAAC study and the ECHRS survey to show a positive ecological association between paracetamol sales and asthma (Newson, Shaheen et al. 2000). Paracetamol sales data were available from 14 countries, and were strongly correlated with gross domestic product (GDP), with paracetamol sales increasing on average across countries by 0.54g/person/year (95% CI 0.15-0.94) for each US$1000 increase in GDP. Paracetamol sales were positively correlated with asthma symptoms, eczema and allergic rhinoconjunctivitis in 13-14 year olds, and with wheeze, diagnosed asthma, rhinitis and bronchial responsiveness in adults (p<0.0005). The prevalence of wheeze increased by 0.52% in 13-14 year olds and by 0.26% in adults for each 10 g per year increase in per capita paracetamol sales. However, ecological analyses must be interpreted with caution, as per the ‘ecological fallacy’, which warns that correlations made at a population level can not necessarily by assumed to apply at an individual level (Piantadosi, Byar et al. 1988).
Figure 4.1: Prevalence of asthma in people under the age of 20 years and total drug store purchases of paediatric aspirin and paracetamol in the USA from 1980 to 1986 (Varner et al, 1998)

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4.3 Paracetamol Use During Pregnancy

A number of studies have been undertaken investigating the effect of intrauterine paracetamol exposure on asthma and wheezing in offspring (Shaheen, Newson et al. 2002; Rebordosa, Kogevinas et al. 2008; Garcia-Marcos, Sanchez-Solis et al. 2009; Kang, Lundsberg et al. 2009; Perzanowski MS, Miller RL et al. 2009; Thengilsdottir H, Goksor E et al. 2010). A
recent systematic review and meta-analysis (Etminan, Sadatsafavi et al. 2009) reported that prenatal paracetamol use by mothers was associated with an increased risk of asthma and of wheezing in children, with odds ratios (OR) of 1.28 (95% CI 1.16 to 1.41) and 1.50 (95% CI 1.10 to 2.05) respectively. However, the study had several weaknesses including that the primary focus of the systematic review was paracetamol use in childhood and adulthood not paracetamol use in pregnancy, the criteria for wheezing and asthma in offspring were not standardised between the studies and detailed study results were not presented.

To extend their report, a systematic review of the association between prenatal exposure to paracetamol and asthma in children was undertaken and the magnitude of the association determined by meta-analysis (Eyers, Weatherall et al. 2011). The details of this systematic review and meta-analysis are discussed in depth in chapter 5.

4.4 Paracetamol Use in Infancy

The Avon Longitudinal Study of Parents and Children (ALSPAC), as well as investigating the role of prenatal paracetamol exposure on wheezing in childhood, also studied paracetamol use in infancy and the association with asthma symptoms. The study found that the use of paracetamol more than once in the first six months of an infants life was also associated with an increased risk of persistent wheezing (wheezing both at 6 months of age and at 3 years) (adjusted OR versus no use 1.64, 95% CI 1.13 to 2.39) (Shaheen, Newson et al. 2002). There was also a weaker association between paracetamol use more than once in the first six months
of an infants life with transient infant wheezing (wheezing at 6 months of age but not at 3 years) (adjusted OR versus no use 1.21, 95% CI 0.99 to 1.48) and late-onset wheezing (wheeze at 3 years but not 6 months of age) (adjusted OR versus no use 1.22, 95% CI 0.97 to 1.54). However, the ALSPAC study design did not allow for further interpretation of these results in terms of possible reverse causation, whereby infants with an atopic tendency may be more likely to display severe eczema symptoms or viral upper respiratory tract infections, leading to higher paracetamol use than non-atopic children.

Similarly, a New Zealand study of approximately 4,000 children reported a link between paracetamol use in infancy and asthma at age 6 to 7 years (Cohet, Cheng et al. 2004). Paracetamol use in the first year of life was associated with current wheezing (OR 1.38, 95% CI 1.04-1.83) and asthma (OR 1.72, 95% CI 1.32 to 2.23) at age 6-7 years. Further, recent use of paracetamol at least once per month was associated with current wheezing (OR 2.10, 95% CI 1.78 to 2.49) and asthma (OR 1.57, 95% CI 1.34 to 1.84). Likewise, an Ethiopian longitudinal birth-cohort study of 780 children found a dose-dependent increased risk of wheeze in children aged 1 to 3 years in those who had consumed paracetamol at the age of 1, with an OR of 1.88 (95% CI 1.03 to 3.44) for those who consumed one to three tablets in the previous month at age 1, and an OR of 7.25 (95% CI 2.02 to 25.95) for those who consumed greater than four tablets in the previous month at age 1 (Amberbir, Medhin et al. 2010).

Phase III of the ISAAC study, which included data from around 200,000 children from 73 centres in 31 countries, provides further evidence of the association between paracetamol use in infancy and asthma in childhood (Beasley, Clayton et al. 2008). In the multivariate
analyses, the use of paracetamol for fever in the first year of life was associated with an increased risk of current asthma symptoms (wheeze in the last 12 months) at age 6 to 7 years (OR 1.46, 95% CI 1.36 to 1.56). The association was present in many countries throughout the world, with varying patterns of childhood febrile illnesses and differences in medical practice and over-the-counter medication use. A similar association was noted with the use of paracetamol for fever in the first year of life and severe asthma symptoms (OR 1.43, 95% CI 1.30 to 1.58), a symptom complex used to identify children with clinically significant asthma. The population attributable risk of severe asthma symptoms due to paracetamol use in infancy was 22% (Beasley, Clayton et al. 2008).

Confounding by indication is defined as being:

‘When a variable is a risk factor for a disease among non-exposed persons and is associated with the exposure of interest in the population from which the cases derive, without being an intermediate step in the causal pathway between the exposure and the disease.’

(Salas, Hofman et al. 1999)

It is important to consider confounding by indication when reviewing the results of the ISAAC study, which may occur in the situation of paracetamol being given for lower respiratory tract infections such as those caused by RSV and rhinovirus, which are associated with an increased risk of asthma in later childhood (Stein, Sherrill et al. 1999; Jackson, Gangnon et al. 2008). In this instance, the use of paracetamol would be positively associated with asthma symptoms in later childhood through the effect of confounding. However, it is
also important to consider that episodes of fever in infancy are associated with a reduced risk of asthma with allergic sensitisation at age 6 to 7 years (Williams, Peterson et al. 2004; Williams, Peterson et al. 2005). In this instance, the use of paracetamol for fever could be associated with a reduced risk of asthma in later childhood, by association. Alternatively, the antipyretic action of paracetamol could potentially contribute to an increased risk of asthma, by reducing the protective effect of fever. Thus the extent to which confounding by indication may have contributed to the association between paracetamol and asthma in the ISAAC study is uncertain (Lowe, Abramson et al. 2009). Furthermore, the presence of confounding does not exclude the possibility of a co-existing causal relationship (Beasley R, Clayton T et al. 2009).

Some studies have failed to show an association between paracetamol use in infancy and asthma. The Melbourne Atopy Cohort Study of 620 infants determined that, although frequent use of paracetamol (on more than 25 days over 2 years) was crudely associated with childhood asthma at age 2 years (OR 1.85, CI 1.18 to 2.89), this association became non-significant when adjustments were made for respiratory tract and gastrointestinal infections and otitis media in the first two years of life (OR 1.30, CI 0.78 to 2.19) (Lowe A, Carlin J et al. 2008). Further, in a United States study investigating the effect of prenatal and infant paracetamol use on asthma symptoms, prenatal use was associated with wheezing in offspring at age 5 (discussed further in chapter 5), however there was no association between childhood use of paracetamol at age 6 months, 1, 2 or 3 years and wheeze at age 5 years (Perzanowski MS, Miller RL et al. 2009).
4.5 Paracetamol Use in Childhood

There has been one randomised controlled trial of paracetamol use in childhood (Lesko, Louik et al. 2002). In the Boston University Fever Study, 1879 asthmatic children, aged between 6 months and 12 years, with a current febrile illness, were randomly assigned to receive either paracetamol or ibuprofen for fever control as required for the following 4 weeks. Children randomised to the ibuprofen group had a significant reduced risk of having an outpatient visit for asthma during the 4 week study period (relative risk 0.56, 95% CI 0.34 to 0.95) compared with children in the paracetamol group. Further, amongst children with a fever related to a respiratory viral illness, the relative risk was even lower in the ibuprofen group (relative risk 0.43, 95% CI 0.24 to 0.79). This was thought to be due to the suppressive effect of ibuprofen on the localised Th2 lymphocyte response that occurs late in viral childhood infections. There was also a dose-response effect noted with a higher cumulative incidence of an outpatient visit for asthma associated with paracetamol doses ≥11 mg/kg than lower doses (6.3 vs. 4.4% respectively). The findings of this study indicate that paracetamol may have a role in increasing the severity of exacerbations in existing disease, in addition to a role in its pathogenesis. Because the study did not include a placebo treatment, it was not possible to determine whether the observed difference in morbidity according to treatment group was attributable to an increased risk with paracetamol or a decreased risk with ibuprofen. This consideration is important as there is evidence that NSAIDs may have some protective effect against the development of asthma (Varner, Busse et al. 1998; Barr, Wentowski et al. 2004;
Barr, Kurth et al. 2007; Barr 2008; Kurth, Barr et al. 2008). It is also important to note that the study was limited to children with an existing

Phase III of the ISAAC study also provides comprehensive evidence of the effect of paracetamol use during childhood on asthma symptoms and severity (Beasley, Clayton et al. 2008). In addition to reporting the effect of paracetamol use in infancy, as discussed earlier, ISAAC phase III also documented current paracetamol use in the 6 to 7 year old age group and the association with current asthma symptoms. The data showed that current use of paracetamol in children aged 6 to 7 years was associated with a dose-dependent increased risk of asthma symptoms, OR 1.61 (95% CI 1.46 to 1.77) and OR 3.23 (95% CI 2.91 to 3.60) for medium and high use versus no use, respectively. Although paracetamol use in infancy was linked with paracetamol use in childhood (i.e. those parents who gave paracetamol to their children as babies were more likely to give it to them as older children), the risk of asthma associated with paracetamol use at age 6 to 7 years was present independently of paracetamol use for fever in the first year of life, and vice versa. Similar dose-dependant associations were observed between current paracetamol use and symptoms of eczema and rhinoconjunctivitis.

Likewise, the ISAAC Phase III study of children aged 13 and 14 years, which analysed data from 322,959 adolescent children from 113 centres in 50 countries (Beasley RW, Clayton TO et al. 2010), determined that recent use of paracetamol was associated with a dose-dependent increased risk of current asthma symptoms, OR 1.43 (95% CI 1.33 to 1.53) and OR 2.51 (95% CI 2.33 to 2.70) for medium and high use versus no use respectively. There was also a dose-dependent association between paracetamol use and risk of current symptoms of
rhinoconjunctivits and eczema. The findings relating to current paracetamol use in childhood are unlikely to be influenced by confounding by indication, however they would be prone to reverse causation if children with asthma were more likely to develop febrile episodes and as a result have greater paracetamol use. However, this would not hold for the observed association between paracetamol use and eczema, independent of asthma, as the symptoms and complications of eczema are not typically associated with the use of paracetamol.

The Melbourne Atopy Cohort Study (Lowe, Carlin et al. 2010) again failed to find an association between paracetamol use in the first two years of life and asthma at age 6-7 years. Increasing use of paracetamol in the first two years of life was shown to be weakly associated with asthma symptoms at age 6-7 years (crude OR 1.18, 95% CI 1.00 to 1.39, per doubling days of use), however this effect was also attenuated following adjustment for frequency of respiratory infections (OR 1.08, 95% CI 0.91 to 1.12) (Lowe, Carlin et al. 2010). Further, paracetamol use for non-respiratory causes was not associated with asthma (crude OR 0.95, 95% CI 0.81 to 1.12) (Lowe, Carlin et al. 2010).

Another study which challenges the paracetamol and asthma hypothesis is by Schnabel and colleagues (Schnabel and Heinrich 2010). Using data from approximately 2296 children, as part of the Influences of Lifestyle-related Factors on the Immune System and the Development of Allergies in Childhood cohort study, the researchers determined that asthmatic children up to the age of 6 years had an average of 1.51 more febrile days (p < 0.01) and 1.27 (p < 0.01) more febrile days due to respiratory tract infection compared to non-asthmatic children. Further, they determined that asthmatic children had, on average, 0.23
more months (p < 0.01) with at least one treatment of paracetamol and 0.16 more months (p < 0.01) with at least one treatment of paracetamol for respiratory tract infections (Schnabel and Heinrich 2010). The authors of this study concluded that it was the increased incidence of respiratory tract infections in children susceptible to asthma which was associated with the development of asthma, as opposed to the use of paracetamol per se, which they thought was likely to have been employed as an antipyretic in the management of the respiratory tract infections.

4.6 Paracetamol Use in Adulthood

The South London Study of dietary antioxidants and asthma from the UK collected information on the use of analgesics in adults aged 16 to 49 years (Shaheen, Sterne et al. 2000). This population-based case-control study showed a dose-dependent association between paracetamol use and asthma, with an OR for monthly users of 1.22 (95% CI 0.87 to 1.72), weekly users of 1.79 (95% CI 1.22 to 4.64) and with a 2.4-fold increased risk in daily users (OR 2.38, 95% CI 1.22 to 4.64). Amongst asthmatics, daily paracetamol use was associated with more severe disease, with ORs of 1.35 (95% CI 0.55 to 3.34), 2.25 (95% CI 0.90 to 5.66) and 8.15 (95% CI 2.84 to 23.40) for mild, moderate and severe asthma respectively. These observations suggest not only that paracetamol use is associated with asthma, but that that it may also play a role in increasing the severity of the disease. The association with paracetamol use was present in both users and non-users of aspirin, and the
frequency of aspirin use was not associated with asthma, or asthma severity, suggesting that the association of paracetamol with asthma was not due to aspirin avoidance by asthmatics.

In a survey of over 7,500 adults and children in Ethiopia (Davey, Berhane et al. 2005), allergic symptoms increased significantly with the frequency of paracetamol use. The ORs for those using more than 3 tablets in the last month compared to no use were 1.89 (95% CI 1.51 to 2.36) for wheeze, 2.52 (95% CI 1.99 to 3.2) for rhinitis and 1.90 (95% CI 1.35 to 2.61) for eczema. There was no observed relationship between paracetamol use and self-reported asthma, but this was likely due to the under-diagnosis of asthma in this study population, particularly in rural areas where healthcare services are lacking. Findings from a nested case-control study of 642 participants suggested that the dose-related associations observed could not be attributed to aspirin avoidance or reverse causation, due to the fact that only 1% of cases reported avoiding aspirin, none took paracetamol for asthma symptoms, and because children under the age of 5 years who commonly experience viral-induced wheeze were excluded. Of interest was the high level of paracetamol use even in this developing country population: 42% of those surveyed reported using paracetamol within the last month, which was similar to the frequency of use observed in the South London Study (Shaheen, Sterne et al. 2000).

The United States Nurses Health Study (Barr, Wentowski et al. 2004) is a prospective cohort study of 121,700 women which has been running since 1976. During 352,719 person-years of follow-up from 1990 to 1996, researchers determined the association of newly diagnosed (adult onset) asthma with frequency of paracetamol use. The multivariate rate ratio for asthma
in participants who used paracetamol for more than 14 days per month was 1.63 (95% CI 1.11 to 2.39) compared with non-users. The positive association of paracetamol use and asthma did not differ significantly between participants who did and did not use aspirin. Paracetamol was commonly used in this cohort with approximately 20% of participants using paracetamol at least 5 days per month and 5% using it more than 22 days per month.

Similarly, in a cohort study of adult twins recruited from the nationwide Danish Twin Registry, there was higher prevalence of new-onset asthma over an 8 year period in participants with a frequent intake of paracetamol (OR 3.03, 95% CI 1.51 to 6.11) (Thomsen, Kyvik et al. 2008). Adjustment for frequent intake of medications other than paracetamol showed a small reduction in effect size (OR 2.16, 95% CI 1.03 to 4.53) consistent with some degree of confounding with other medications and diseases. One drawback of this study, however, was that ‘frequent use’ of paracetamol was self-determined by participants and not quantified, and therefore no dose-response relationship was able to be determined. Restricting the analysis to include only twin pairs discordant for frequent paracetamol intake showed a similar point estimate of risk although not reaching statistical significance (OR 2.00, 95% CI 0.37 to 10.92). There was no association between frequent aspirin use and new-onset asthma in the study population.

In a cross-sectional analysis using the United States Third National Health and Nutrition Examination Survey (NHANES III) (McKeever, Lewis et al. 2005), there was a dose-dependent association between paracetamol use and asthma (Table 3). There was also an association with chronic obstructive pulmonary disease (COPD) (ORs 1.16, 95% CI 1.09 to
1.24) and an inverse association with FEV$_1$, with a reduction of 54.0 ml (95% CI 90.3 to 17.7 ml) when daily users were compared with never users. The specificity of the association with paracetamol (not aspirin or ibuprofen) and the dose response relationship provided support for a causal relationship. The study also observed a strong association between paracetamol use and asthma in the subgroup of participants with non-atopic asthma.

As part of a multicentre case-control study organised by the Global Allergy and Asthma European Network (GA²LEN), the association between frequent paracetamol use and adult asthma was examined in over 500 cases and 500 controls from 12 European centres (Shaheen, Potts et al. 2008). Weekly paracetamol use, when compared with less frequent use, was positively associated with asthma, and the overall effect estimate became stronger after controlling for confounders (adjusted OR 2.87, 95% CI 1.49 to 5.37) (Figure 1). No association was seen between the use of other analgesics and asthma. Information was not collected on indications for use and it is possible that some cases were taking paracetamol for conditions such as migraine, which is more frequently experienced in people with asthma (Strachan, Butland et al. 1996; Davey, Sedgwick et al. 2002), however it is unlikely to confound the association between paracetamol and asthma to a substantive degree, as migraine is uncommon even in asthmatics. In support of this interpretation, the previous UK case-control study reported that frequent paracetamol was taken for migraine in only 0.02% of asthmatics and 0.01% of non-asthmatics (Barr, Wentowski et al. 2004). Once again, it was not possible to determine a dose-response relationship in this study, as the categories for paracetamol use used in the questionnaires were limited.
While the evidence of an effect between paracetamol and asthma is overwhelmingly positive, with only a handful of negative studies published which do not support an association, it is possible that a degree of publication bias does exist. It is, therefore, important for definitive clinical studies to be conducted and published in order to provide conclusive evidence for or against an effect.

### 4.7 Possible Mechanisms

There have been several mechanisms proposed to explain the association between paracetamol and asthma. The most plausible mechanism relates to the effect of paracetamol on circulating levels of glutathione, leading to reduced antioxidant capacity of the body. The effect of paracetamol on neurogenic inflammation, Th1 and Th2 cytokine response patterns, COX-2 activity and the production of prostaglandin E2 have also been implicated. Any changes to respiratory antioxidant defences, inflammatory responses and cytokine levels are likely to affect both the risk of asthma development and the severity or control of current asthma, as discussed in chapter 3. It is therefore likely that any effect of paracetamol on asthma would be expressed through both causation of new onset asthma and worsening of the severity of pre-existing disease.
4.7.1 Glutathione

The glutathione pathway is the main intracellular thiol redox system in erythrocytes and its major function is the detoxification of toxic oxygen metabolites generated during the metabolism of endogenous and exogenous substances, particularly in the lungs (Rahman and MacNee 2000; Kozer, Evans et al. 2003). Asthma sufferers have been shown to have increased concentrations of glutathione in their alveolar fluid and blood which appears to be related to the degree of pulmonary inflammation present and is an adaptive response to associated oxidative stress (Smith, Houston et al. 1993; Reynaert 2011). Airway inflammation in patients with asthma is associated with increased generation of reactive oxygen species (ROS) (Barnes 1990), including superoxide anions, hydrogen peroxide, and hydroxyl radicals by peripheral blood eosinophils and neutrophils (Smith, Houston et al. 1993). ROS produce tissue injury (Freeman and Crapo 1982), smooth muscle contraction (Barnes PJ and Rhoden KJ 1987), BHR (Katsumata, Miura et al. 1990), increased vascular permeability (Del Maestro, Bjork et al. 1981), release of pro-inflammatory mediators (Harlan and Callahan 1984; Sporn, Peters-Golden et al. 1988), and impaired beta receptor function (Nijkamp FP and Hendricks PAJ 1988). Glutathione protects the lungs from the oxidant tissue damage caused by ROS (Kelly 1999).

As described in the previous chapter, paracetamol is metabolised in the liver through the metabolic pathways of glucuronidation and sulphation. The cytochrome P450 system, which metabolises approximately 5% of paracetamol, produces the toxic metabolite NAPQI which is detoxified in the hepatocytes via conjugation with glutathione (Kozer, Evans et al. 2003).
Although erythrocytes synthesise glutathione, there is also an intra-organ glutathione cycle whereby liver cells transport glutathione into the plasma and erythrocytes. Evidence exists that regular paracetamol use decreases reduced glutathione levels in the liver (Trenti, Bertolotti et al. 1992; Nuttall, Khan et al. 2003) and in erythrocytes (Kozer, Evans et al. 2003). It has also been demonstrated that paracetamol use decreases intracellular glutathione levels in pulmonary macrophages in vitro at clinically relevant concentrations (Dimova, Hoet et al. 2005). Further, in rat models, the lungs are the primary organ effected by the glutathione-depleting effects of paracetamol (Micheli, Cerretani et al. 1994). In murine models, paracetamol has been found to deplete both liver and lung reduced glutathione in a dose and time-dependant manner (Chen, Richie et al. 1990). Subsequently, it is possible that paracetamol may impair respiratory antioxidant defences by decreasing the amount of reduced glutathione present in the lungs (Eneli, Sadri et al. 2005). These mechanisms are likely to result from gene/environment interactions, as genetic polymorphisms of the glutathione-S-transferase family of genes may influence asthma predisposition (see Genetics and Glutathione section below) (Lenney and Fryer 2007; Imboden, Rochat et al. 2008).

4.7.2 Genetics and Glutathione

It is possible that the antioxidant genetic status of an individual may modify the effect of paracetamol and determine its effect on asthma status. The glutathione-S-transferase (GST) family of enzymes play a vital role in the antioxidant process, by conjugating glutathione with electrophilic substances that are capable of generating free radicals, thereby detoxifying their
effects (Minelli, Granell et al. 2010). The major gene variants of interest include GSTM1, GSTP1 and GSTT1. 70% of Caucasian individuals display the GSTM1 null mutation, meaning that half the Caucasian population lack a functional copy of the gene (Rogers A and Bunyavanich S 2011). Numerous studies and several systematic reviews and meta-analyses have been undertaken investigating the role of the GSTM1 gene variant in asthma. Although there have been indeterminate results in many populations studied, the GSTM1-null mutation has been associated with increased asthma severity in the setting of increased oxidative stress (for example in the setting of environmental tobacco smoke or ozone) (Rogers A and Bunyavanich S 2011). Likewise, a meta-analysis of the effects of the valine to isoleucine substitution on codon 105 of GSTP1 showed varying results for its significance in the development of asthma (Minelli, Granell et al. 2010), however many studies have demonstrated an effect of the gene variant in a subset of people who were exposed to oxidative stress such as tobacco smoke or air pollution (Rogers A and Bunyavanich S 2011). Although studies have not supported a substantial role of the GST genetic polymorphisms alone in the development of asthma and asthma susceptibility (Minelli, Granell et al. 2010; Rogers A and Bunyavanich S 2011), it is possible that reduced activity of the GST genes, as a result of genetic polymorphisms, combined with reduced levels of glutathione in the body, as a result of paracetamol intake, may lead to an overall decrease in antioxidant capabilities and therefore a higher predisposition towards asthma.

Research has also been undertaken into the role of the transcription factor nuclear erythroid 2 p45-related factor 2 (Nrf2), which is the master regulator of the GST genes. Animal studies have found that Nrf2 knockout mice are particularly susceptible to paracetamol hepatotoxicity
and Nrf2 has been shown to have a central role in preventing oxidative damage in the lungs (Cho, Reddy et al. 2006) and protecting against respiratory disease (Cho and Kleeberger 2010). Disruption of the Nrf2 gene has been shown to cause severe allergen-induced airway inflammation and BHR in mice (Rangasamy, Guo et al. 2005). In humans, polymorphisms in Nrf2 have been linked to acute lung injury (Marzec, Christie et al. 2007) and adult lung function (Siedlinski, Postma et al. 2009).

The Avon Longitudinal Study of Parents and Children (ALSPAC) investigated this hypothesis by stratifying the effect of prenatal and infant paracetamol exposure on asthma phenotypes in 4000 mothers and 5000 children by Nrf2 and GST genotype (Shaheen, Newson et al. 2010). Risk of asthma and wheezing associated with paracetamol use in early pregnancy was increased when maternal copies of the minor T allele of Nrf2 were present; the minor allele being associated with reduced expression of Nrf2. Risk of asthma at 7 years, associated with late gestational exposure to paracetamol, was likewise greater when the maternal GSTT1 genotype was present, and risk of wheezing was increased when the maternal GSTM1 genotype was present. Child antioxidant genotype did not modify the association found between infant paracetamol use and asthma phenotypes, however the relationship between late gestational exposure to paracetamol and infant asthma and wheezing was further enhanced when the GSTM1 genotype was present in the both the mother and the child. No effect modification was seen as a result of maternal or child GSTP1 status.

The proposed explanation behind these findings was that those who were homozygous wild for the GSTM1/T1 genotype were able to conjugate glutathione better than those with the null
gene, and therefore depleted glutathione more extensively when exposed to NAPQI, the toxic metabolite of paracetamol, leading to a reduced antioxidant capacity of glutathione in the lungs (Shaheen, Newson et al. 2010). Further, the relationship between late gestational paracetamol use and asthma and wheezing was further enhanced when the GSTM1 gene was present in both mother and child. Paracetamol crosses the placental barrier and NAPQI is produced by the foetus in late gestation, therefore a similar phenomenon may be occurring in the foetus as in the mother. In another study (Perzanowski MS, Miller RL et al. 2009), the risk of wheeze at age 5 years following prenatal paracetamol exposure was only observed in children with the GSTP1 minor allele. There was also evidence of effect modification by gene variants in GSTT1, but no statistically significant differences with deletions in GSTM1. GSTP is responsible for 90% of GST activity in the lungs (Perzanowski MS, Miller RL et al. 2009), and the authors proposed that the common polymorphism encountered in their study (GSTP1 A105G) could lead to an altered GSTP catalytic capacity both in the lungs and during detoxification of NAPQI (Perzanowski MS, Miller RL et al. 2009).

4.7.3 Neurogenic Inflammation through TRPA1 Stimulation

Another recent mechanistic theory relates to the role paracetamol plays in neurogenic inflammation. Neurogenic inflammation refers to a series of responses mainly at the vascular level throughout different organs and tissues, caused via neuropeptide release from neurons which selectively express the transient receptor potential (TRP) ion channels, in response to various noxious stimuli (Geppetti, Materazzi et al. 2006). Neurogenic inflammation consists
of vasodilation, plasma protein extravasation and leukocyte adhesion to the vascular endothelium of post-capillary venules (Geppetti, Materazzi et al. 2006). In the lungs, neurogenic inflammatory responses include bronchoconstriction, seromucous gland secretion, leukocyte adhesion, plasma extravasation and vasodilation (Geppetti, Tognetto et al. 1999) (Amadesi, Moreau et al. 2001).

Transient receptor potentials (TPR) are a family of ion channels that are currently being widely researched in health due to their interaction with multiple stimuli and their widespread presence in different tissues and organs. Transient receptor potential ankyrin-1 (TRPA1) and transient receptor potential vanilloid-1 (TRPV1) are the two major pro-inflammatory TRP ion channels, and their stimulation in peripheral terminals of primary sensory neurons leads to the release of neuropeptides which mediate neurogenic inflammatory responses (Nassini, Materazzi et al. 2010). TRPA1 and TRPV1 have been implicated in the development of asthma (Caceres, Brackmann et al. 2009) and their agonists, such as capsaicin, acrolein and chlorine, are associated with allergic and occupational asthma (Bautista, Jordt et al. 2006; Caceres, Brackmann et al. 2009). A number of TRPA1 agonists possess an electrophilic carbon atom that undergoes neutrophilic attack by cysteine residues of proteins which is though to be the mechanism by which TRPA1 is stimulated (Hinman, Chuang et al. 2006; Nassini, Materazzi et al. 2010).

NAQPI is highly electrophilic and Italian researchers have proposed that NAPQI may stimulate TRPA1 thereby causing airway neurogenic inflammation (Nassini, Materazzi et al. 2010). They demonstrated that single or repeated therapeutic doses of paracetamol in murine
models produces detectable NAPQI in the lung, and caused increased neutrophil numbers, myeloperoxidase activity, and cytokine and chemokine levels in the airways. They went on to show that NAPQI selectively activates the TRPA1 channel and evokes the release of sensory neuropeptides that mediate neurogenic inflammatory responses in the airways. Further, they demonstrated that antagonism of TRPA1 abated the inflammatory response generated by NAPQI, and that the inflammatory response was absent in TRPA1-deficient mice.

4.7.4 Th1-Th2 Cytokine Response Patterns

Another potential mechanism by which paracetamol may contribute to asthma relates to the effect of paracetamol on the Th1-Th2 cytokine response patterns. The Th1-type lymphocyte response contributes to cell-mediated immunity and delayed hypersensitivity reactions and results from activation by acute bacterial and viral infections. Cytokines involved in the Th1 response include IL-2, IL-12 and interferon-gamma (IFN-\(\alpha\)) (Varner, Busse et al. 1998). The Th2-type lymphocyte response contributes to allergic inflammation involving IgE antibody production and eosinophils, and results from activation by allergens and helminths. Cytokines involved in the Th2 response include IL-4, IL-5, IL-10 and IL-13 (Varner, Busse et al. 1998). An imbalance between the Th1 and Th2 response patterns towards the Th2 response may be critical to the development of allergic disease (Varner, Busse et al. 1998).

This hypothesis suggests that paracetamol fails to block the COX-2 pathway, leading to uninhibited production of prostaglandin E\(_2\) during viral illness. Prostaglandin E\(_2\) promotes the
Th2 pathway and production of IgE and, therefore, a shift in the balance between Th1:Th2 towards the Th2 response occurs (Varner, Busse et al. 1998). The depletion of glutathione in antigen presenting cells in vitro and in vivo in animal models, as occurs during paracetamol metabolism (Dimova, Hoet et al. 2005), has also been shown to cause a shift away from Th1 to Th2 cytokine production which could also pre-dispose to atopic diseases such as asthma (Peterson, Herzenberg et al. 1998; Dimova, Hoet et al. 2005). Paracetamol use may also reduce the cytokine storm that occurs as part of the febrile response. The prevalent cytokines released during a fever include interferon-γ, and IL-2, which have predominant Th1 profiles. However, while paracetamol reduces fever, there is conflicting evidence as to whether it also influences the pattern of cytokine production (Brandts, Ndjave et al. 1997; Pernerstorfer, Schmid et al. 1999) and its effect on cytokines may depend on the cause of the fever.

Paracetamol has been shown to suppress the immune response to, and prolong symptomatic illness from, rhinovirus infections, which are associated with an increased risk of childhood asthma (Lemanske, Jackson et al. 2005). In a randomised placebo-controlled trial of 60 volunteers infected with rhinovirus, the use of paracetamol was associated with suppression of the antibody response and increased nasal signs and symptoms during the infective period. A trend was also seen (although not statistically significant) towards a longer duration of viral shedding in the paracetamol group (Graham, Burrell et al. 1990). Similarly, a recent study of prophylactic paracetamol given at the time of vaccination illustrated that paracetamol given in routine doses is capable of modulating immune responses (Prymula, Siegrist et al. 2009).
The relationship between paracetamol and the Th1-Th2 cytokine pathway is complex and, as described above, there are multiple possible mechanisms by which relationship could exist. Monitoring the cytokine response in asthmatics taking regular paracetamol would lead to a better understanding of this interaction, and has been undertaken in our randomised controlled trial.

4.8 Association with Current Research

The number of epidemiological studies undertaken to investigate the relationship between paracetamol use and asthma is a testament to the importance of the issue. While the majority of studies have demonstrated a positive relationship, some have failed to show a significant association between paracetamol use and asthma. Further, although the many epidemiological studies undertaken are valuable and can demonstrate a relationship between paracetamol use and asthma, they can not prove a causative effect. It is therefore imperative that further research, in the form of randomised controlled trials, is undertaken to investigate the effect of paracetamol use at different stages of life on asthma symptoms and severity, and to determine the magnitude and nature of any risk.

In adults, the opportunity exists to investigate whether the regular use of paracetamol influences asthma severity, as suggested by the UK case-control study which reported a progressively greater risk in those with more severe disease (Shaheen, Sterne et al. 2000), and the Boston University Fever Study which demonstrated that children with asthma who
received acetaminophen were more likely to require an outpatient visit for asthma compared to children in the ibuprofen group (Lesko, Louik et al. 2002).

The randomised controlled trial which is the basis of this PhD thesis is designed to assess if paracetamol increases bronchial hyperresponsiveness and worsens asthma control in adults with mild to moderate asthma.
Chapter Five: A Systematic Review and Meta-Analysis - Paracetamol in Pregnancy and Risk of Wheezing in Offspring

5.1 Introduction

As discussed in chapter 4.3, there is evidence to suggest that the risk of asthma might be increased with exposure to paracetamol in the intrauterine environment. This meta-analysis was undertaken by the investigator to evaluate the complex literature regarding mechanisms for causation, which are likely to also affect asthma severity. It is particularly important to closely review the literature regarding intrauterine exposure to paracetamol and its effect on childhood wheezing, because this pathway of exposure avoids the issue of confounding by indication, which makes the interpretation of infant and childhood paracetamol difficult.

A previous systematic review and meta-analysis (Etminan, Sadatsafavi et al. 2009) reported that the risk of asthma associated with paracetamol use in children and adults was 1.63 (95% CI 1.46 to 1.77). It also reported that prenatal paracetamol use was associated with an increased risk of asthma and of wheezing in offspring, with odds ratios (ORs) of 1.28 (95% CI 1.16 to 1.41) and 1.50 (95% CI 1.10 to 2.05) respectively. However, there were several weakness and flaws in this systematic review and meta-analysis study design. It lacked depth regarding the issue of paracetamol use during pregnancy (it included paracetamol use during childhood and adulthood as well), the detailed findings of the studies were not presented and
the author’s criteria for wheezing and asthma were not standardised between the included studies.

A further systematic review and meta-analysis were undertaken by the investigator (Eyers, Weatherall et al. 2011) in order to comprehensively, and more thoroughly, evaluate the complex literature about the effect of prenatal paracetamol use on wheezing and asthma in offspring.

5.2 Methods

A systematic review and meta-analysis were conducted based on the guidelines outlined in *The PRISMA Statement* (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Moher, Liberati et al. 2009).

5.2.1 Search strategy

The literature search aimed to identify papers written in any language using the health research databases Medline January 1950 to October 2010, EMBASE January 1947 to October 2010, Cochrane Database of Systematic Reviews 2005 to September 2010 and Cochrane Central Register of Controlled Trials 3rd quarter 2010. Studies were searched for using the keywords ‘asthma’ or ‘wheezi’ (and) ‘acetaminophen’ or ‘paracetamol’(and) ‘prenatal’ or ‘pregn$’. There were no limits placed on the search. Search results were reviewed for relevance, and compared with previous meta-analysis for completeness
(Etminan, Sadatsafavi et al. 2009) and the reference lists of the relevant studies were examined.

5.2.2 Study selection

Inclusion Criteria
Studies that met the following criteria were included in the meta-analysis: published randomised controlled trials (RCTs) and non-experimental observational studies that involved the comparison of women using paracetamol during pregnancy with a placebo (RCT) or, in non-experimental observational studies, a control group, and evaluated the effect of paracetamol use during pregnancy on offspring, using wheeze or asthma as a primary outcome. Studies could be presented as full papers or as abstracts. However only studies which presented raw data, or from which raw data was available from the authors on request, were used in the meta-analysis. In the situation where a study was published in different phases, the one with the most complete raw data was used.

Exclusion Criteria
Studies were excluded sequentially based on the following criteria:

- Publications that were reviews, commentaries, editorials or guidelines
- Studies in which asthma or wheeze were not primary or secondary outcomes
- Studies in which paracetamol use in pregnancy was not a primary (or secondary) exposure
- Studies that did not contain raw data for analysis
5.2.3 Data abstraction

The title and abstract of each paper was reviewed and the full paper examined if necessary to determine eligibility for inclusion. The selected publications were examined for study quality and raw data was extracted, including age and number of children with asthma or wheeze, number of women using paracetamol during pregnancy, trimester of pregnancy in which paracetamol use took place (if recorded) and amount of paracetamol used during pregnancy (if recorded). If appropriate data were not included in the studies, the lead author was contacted in an attempt to obtain the raw data. Some studies reported ORs or relative risks for association which were adjusted for multiple different potential confounding variables. The confounding variables differed considerably across the different studies and the tabulated raw data was never presented in relation to the potential confounders in stratified tables. It was, therefore, necessary to calculate unadjusted ORs so that the included studies were comparable. Due to differing categorisation of quantities of paracetamol used and timing of use during pregnancy, all stages of pregnancy were combined and exposure was dichotomised to use/no-use for the purpose of the meta-analysis. If data were presented for two age groups, the older age group was used for the purposes of the meta-analysis. The primary outcome variable was wheeze in the last 12 months prior to interview, defined as ‘current wheeze’.

Additional details relating to a potential dose-response relationship, any effect of family history or genetic status on the association with asthma, the potential effect of paracetamol use on atopic status, bronchial hyperresponsiveness and asthma severity, and the association with other antipyretics/pain relief medications were also recorded if available.
5.2.4 Statistical analysis

Unadjusted ORs were calculated for each included study based on the raw data provided. Odds ratios were pooled by the inverse variance weighted method. Fixed and random effects estimates were produced together with a homogeneity statistic and I-squared estimate. Publication bias was tested by the correlation coefficient between study size and estimate and by examination of a funnel plot. In the Forest plot the size of the boxes is inversely proportional to the variance for each study estimate.

5.3 Results

The outcome of the search strategy is shown in Figure 5.1. There were six studies identified which met the criteria for inclusion in the meta-analysis (Shaheen, Newson et al. 2002; Rebordosa, Kogevinas et al. 2008; Garcia-Marcos, Sanchez-Solis et al. 2009; Kang, Lundsberg et al. 2009; Perzanowski MS, Miller RL et al. 2009; Thengilsdottir H, Goksor E et al. 2010). Three other relevant studies were identified that failed to meet inclusion criteria for the meta-analysis; one publication lacked raw data, which was unable to be obtained following contact with the study’s lead author (Persky, Piorkowski et al. 2008), another publication’s primary outcome variable was bronchial hyperresponsiveness as opposed to wheeze (Bisgaard, Loland et al. 2009) and a third publication was a study with an incomparable design to the other included studies (Koniman, Chan et al. 2007). These three publications will be discussed as part of the systematic review.
The study characteristics are shown in Table 5.1. The age range of offspring was between 30 months and 84 months. For paracetamol use, the stage of pregnancy was combined in five studies, (Rebordosa, Kogevinas et al. 2008; Garcia-Marcos, Sanchez-Solis et al. 2009; Kang, Lundsberg et al. 2009; Perzanowski MS, Miller RL et al. 2009; Thengilsdottir H, Goksor E et al. 2010), however in one study (Shaheen, Newson et al. 2002), paracetamol use in pregnancy was split into early (0-20 weeks) and late (20-32 weeks) stages and the late pregnancy data was used for the analysis. In one study, use of paracetamol during pregnancy was determined during the first and third trimesters and also presented as combined data (Kang, Lundsberg et al. 2009). (All figures and tables reproduced with the permission of Wiley and Sons, Inc).
Figure 5.1: Systematic review search strategy
There was an increased risk of current wheeze in the offspring of women who were exposed to any paracetamol during any stage of pregnancy, with a pooled fixed effects OR of 1.20 (95% CI 1.11 to 1.29) and a pooled random effects OR of 1.21 (95% CI 1.02 to 1.44). Heterogeneity was present (homogeneity test Chi-square 20.9, degrees of freedom = 5, P<0.001, I-squared statistic 76.0 (95% CI 46.2 to 89.3)). Due to the potential difficulty of pooling data from a cross-sectional study (Garcia-Marcos, Sanchez-Solis et al. 2009) with data from cohort studies, a sensitivity analysis was undertaken without the survey data and similar overall results were obtained, with a pooled fixed effects OR of 1.19 (95% CI 1.10 to 1.30) and a pooled random effects OR of 1.22 (95% CI 1.02 to 1.44). Heterogeneity was still present (homogeneity test Chi-square 20.9, df=4, P<0.001, I-squared statistic 80.8 (95% CI 55.2 to 91.8)). There was no evidence of publication bias (test for publication bias P=0.85). The Forest plot is shown in Figure 5.2. The individual findings of the included studies are described below (Table 5.1).
Table 5.1: Characteristics and findings from studies included in the meta-analysis

<table>
<thead>
<tr>
<th>Study [reference]</th>
<th>Design</th>
<th>Child age (months)</th>
<th>Paracetamol in pregnancy</th>
<th>Current Wheeze</th>
<th>Unadjusted OR [95% CI]</th>
</tr>
</thead>
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<tr>
<td>Perzanowski et al.</td>
<td>Prospective cohort</td>
<td>60</td>
<td>Yes</td>
<td>38 (38.4%)</td>
<td>61 99</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>No</td>
<td>41 (20.7%)</td>
<td>157 198</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>297 2.39 (1.40–4.06)</td>
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<tr>
<td>Shaheen et al.</td>
<td>Prospective cohort</td>
<td>30–42</td>
<td>Yes*</td>
<td>587 (15.4%)</td>
<td>3226 3813</td>
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<td></td>
<td></td>
<td>No</td>
<td>608 (11.8%)</td>
<td>4526 5134</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>8947 1.35 (1.20–1.53)</td>
</tr>
<tr>
<td>Thenglisdottir et al.</td>
<td>Prospective cohort</td>
<td>54</td>
<td>Yes</td>
<td>83 (9.3%)</td>
<td>811 894</td>
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<tr>
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<td></td>
<td></td>
<td>No</td>
<td>193 (7.2%)</td>
<td>2485 2678</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>3572 1.32 (1.01–1.72)</td>
</tr>
<tr>
<td>Rebordosa et al.</td>
<td>Prospective cohort</td>
<td>84</td>
<td>Yes</td>
<td>543 (8.0%)</td>
<td>6209 6752</td>
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<td>No</td>
<td>417 (7.9%)</td>
<td>4829 5246</td>
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<td></td>
<td></td>
<td></td>
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<td>11998 1.01 (0.89–1.16)</td>
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<tr>
<td>Kang et al.</td>
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<td>No</td>
<td>922 (69.2%)</td>
<td>411 1333</td>
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<td></td>
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<td>1505 0.85 (0.61–1.19)</td>
</tr>
<tr>
<td>Garcia-Marcos et al.</td>
<td>Cross-sectional survey</td>
<td>36–60</td>
<td>Yes</td>
<td>194 (21.2%)</td>
<td>719 913</td>
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<td>No</td>
<td>147 (18.2%)</td>
<td>659 806</td>
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<td>1719 1.21 (0.95–1.54)</td>
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*Paracetamol use at 20–32 weeks in pregnancy.
Figure 5.2: Forest plot of the association between paracetamol use in pregnancy and current wheeze in childhood *

* For each study the box represents the random effects OR and the line the 95% confidence intervals

Danish National Birth Cohort Study

Rebordosa et al (Rebordosa, Kogevinas et al. 2008) reported data from the Danish National Birth Cohort Study in which information was available on paracetamol use during pregnancy, and asthma symptoms in 66,445 infants at the age of 18 months and 12,733 children aged 7 years. Paracetamol use during pregnancy was associated
with wheezing and asthma, both at 18 months and at 7 years of age. The highest risk was observed for the association between persistent wheeze (children wheezing in the first 18 months of life as well as in the 12 months preceding interview at age 7) and paracetamol use in the first trimester of pregnancy (relative risk 1.35, 95% CI 1.02 to 1.78). At 18 months a significant association with wheeze was observed with paracetamol use during each of the three trimesters, although at age 7 years the association was significant for only first trimester paracetamol use. Use of paracetamol in the first trimester was also associated with severity of asthma attacks at age 7 years, measured as the number of wheezing attacks affecting sleep. There was no significant association between any measure of asthma, at either 18 months or 7 years of age, and prenatal exposure to ibuprofen or aspirin in any trimester. Paracetamol was taken for pain by 91% of women studied, for fever in 16% and for muscle or joint disease in 20%.

**Spanish Infant Survey**

Garcia-Marcos et al (Garcia-Marcos, Sanchez-Solis et al. 2009) reported data from a cross-sectional survey in which parents of 1,741 children aged 3 to 4 years were interviewed. The focus of the study was the effect of asthma status of the mother on the association with wheezing in the preschool child. There was a significant association between the use of paracetamol by the mother at least once per month during pregnancy and risk of wheezing at preschool age (OR 1.71, 95% CI 1.15 to 2.53) but only amongst non-asthmatic mothers (OR 1.74, 95% CI 1.15 to 2.61). There was no association between prenatal paracetamol use and asthma in the
offspring of asthmatic mothers, despite similar use of prenatal paracetamol to non-asthmatic mothers.

**Dominican and African American Birth Cohort Study**

Perzanowski et al (Perzanowski MS, Miller RL et al. 2009) reported a birth cohort study undertaken in an inner city low income Dominican and African American population in the United States. In 301 children aged 5 years, prenatal paracetamol exposure predicted current wheeze (relative risk 1.71, 95% CI 1.20 to 2.42) and the risk increased monotonically with increasing number of days of prenatal paracetamol exposure. Exposure in the second and third trimester increased risk significantly, but the risk associated with use in the first trimester was of lesser magnitude and not statistically significant. An increased risk associated with paracetamol exposure during pregnancy was also identified with persistent wheeze (relative risk 1.65, 95% CI 1.12 to 2.44), and ED visits for wheeze or difficulty breathing (relative risk 2.08, 95% CI 1.35 to 3.21). Prenatal paracetamol exposure predicted serological evidence of atopy (specific IgE antibodies ≥0.35 IU/ml to at least one allergen tested: *D. farinae*, mouse, cockroach, cat and dog) at age 5 years. The risk of wheeze and atopy was modified by the glutathione-S-transferase P1 (GSTP) gene, with a positive association observed only amongst children with the GSTP1 minor allele.
Shahen et al (Shahen, Newson et al. 2002; Shahen, Newson et al. 2005) reported the findings from the Avon Longitudinal Study of Parents and Children (ALSPAC), a prospective cohort study of approximately 14,000 pregnancies and births, with data on the association of prenatal paracetamol exposure with wheezing at two ages in early childhood. Frequent use of paracetamol (most days/daily) in late (20-32 weeks) but not early (0-20 weeks) pregnancy was associated with increased risk of wheeze at age 30-42 months (OR compared with no use 2.10, 95% CI 1.30 to 3.41). Use in both early and late pregnancy was associated with significant increased risk of wheeze at age 81 months (OR compared with no use of 1.81, 95% CI 1.05 to 3.13 and 1.86, 95% CI 0.98 to 3.55 respectively). There was a dose-response relationship demonstrated at both ages, and frequent use in late pregnancy was associated with increased risk of persistent wheezing but not transient infant wheezing. Aspirin use in early and late pregnancy was not associated with wheezing at either age. Use of paracetamol more than once in the first six months of life was associated with a small increase in the risk of wheezing at 30 to 42 months (OR compared with never use 1.29, 95% CI 1.05 to 1.57).

The ALSPAC study also reported the association with atopy assessed by skin prick testing to inhalant allergens and total serum IgE. There was an association between paracetamol use in late pregnancy and total IgE at 7 years, even after controlling for paracetamol and antibiotic use during infancy, although no association with skin prick test positivity.
United States Cohort Study

Kang et al (Kang, Lundsberg et al. 2009) reported data from a prospective study of 2,379 pregnant women, with 1,505 women interviewed when their children were six years old. Paracetamol was more likely to be used in pregnancy by women with diagnosed asthma (OR 1.56, 95% CI 1.24 to 1.96); overall, paracetamol was used by 69% of women during pregnancy. Use of paracetamol did not significantly increase the risk of asthma (OR 0.76, 95% CI 0.53 to 1.10). Paracetamol use during both the first and third trimesters was associated with a significantly reduced risk of asthma (OR 0.59, 95% CI 0.36 to 0.98). There was no evidence of a dose-response relationship.

Western Sweden Cohort Study

Thengilsdottir et al (Thengilsdottir H, Goksor E et al. 2010) reported a prospective, longitudinal cohort study of 8173 children in Sweden of whom 4496 completed follow up at age 4.5 years. In the univariate analysis, they found an increased risk of overall wheezing disorder with both allergy/asthma medication (OR 3.0, 95% CI 2.0 to 4.5) and paracetamol (OR 1.5, 95% CI 1.03 to 2.2). In the multivariate analyses, only the association with maternal allergy/asthma medication remained statistically significant (OR 1.7, 95% CI 1.002 to 2.9). However, the risk of multiple-trigger wheeze was increased by paracetamol, both in the univariate and multivariate analyses (ORs 2.3, 95% CI 1.3 to 4.2 and 2.4, 95%CI 1.2 to 4.9). The risk of episodic viral wheeze was not increased by paracetamol, neither in the univariate nor in the multivariate analysis.
Studies Not Included in Meta-Analysis

Three other relevant studies were identified in the systematic search but did not meet the criteria for inclusion in the meta-analysis.

Persky et al (Persky, Piorkowski et al. 2008) reported data from the Peer Education in Pregnancy Study, a controlled trial of a community-based education intervention, however the raw data necessary for the meta-analysis was unable to be obtained. The study analysed 345 women in the first trimester of pregnancy and followed the offspring during their first year of life. There was a significant increased risk of wheeze in the first year of life in the offspring of mothers who used any paracetamol in mid- to late pregnancy (OR 1.8, 95% CI 1.1 to 3.0), but not early pregnancy (OR 1.0, 95% CI 0.6 to 1.6). Paracetamol use was also associated with a diagnosis of asthma and wheeze that disturbed sleep.

Bisgaard et al (Bisgaard, Loland et al. 2009) reported data from a birth cohort study, the Copenhagen Prospective Study on Asthma in Childhood, which was excluded from meta-analysis because the primary outcome was neonatal bronchial hyperresponsiveness and therefore not comparable to other study outcomes of childhood wheezing and/or asthma. Lung function and bronchial hyperresponsiveness to methacholine were measured in 404 neonates aged six weeks. Maternal intake of paracetamol during the third trimester was associated with a doubling of the bronchial responsiveness in the neonates, however this borderline significance was not maintained in the robust analysis eliminating outliers.
Koniman et al (Koniman, Chan et al. 2007) reported data from a matched patient-sibling case-control study undertaken in Singapore. It was excluded because the variance estimate for the matched case-control study design was deemed to be incomparable to the unmatched data from the cross-sectional and cohort studies included in the meta-analysis. Paracetamol was used in pregnancy in 6 out of 17 children with allergic asthma, but in 0 out of 17 pregnancies of matched siblings without asthma (P=0.03).

5.4 Discussion

5.4.1 Main Findings

The findings from this systematic review and meta-analysis support an association between any use of paracetamol at any stage in pregnancy and the risk of wheezing in offspring aged 2.5 to 7 years of age. The random effects OR for risk of asthma in offspring following paracetamol use in pregnancy, which takes into account the large degree of heterogeneity in the studies analysed, demonstrated a 21% increased risk, with the 95% confidence interval ranging from a 2% to a 44% increased risk. Features of the studies variably included an association with paracetamol use within all trimesters of pregnancy, and associations with persistent asthma, severe asthma and atopy.
The strength of the association was similar to that reported by Etminan and colleagues (Etminan, Sadatsafavi et al. 2009), in which the OR for the risk of asthma and of wheezing with prenatal paracetamol use was 1.28 (95% CI 1.16 to 1.41) and 1.50 (95% CI 1.10 to 2.05). These similar estimates of risk were observed despite the methodological differences between the two meta-analyses. Etminan et al used adjusted ORs or relative risks in preference to our approach of using raw data, and did not standardise the outcome variables. For example, the relative risk for ‘hospitalisation for asthma’ was used in one study (Rebordosa, Kogevinas et al. 2008), the OR for wheezing in the offspring of non-asthmatic mothers (as opposed to all mothers) was used in another (Garcia-Marcos, Sanchez-Solis et al. 2009), and the OR from a third paper is not presented in the original manuscript (Shaheen, Newson et al. 2005). Their measure of paracetamol use in pregnancy was variably frequent or any paracetamol use, whereas we standardised the primary outcome variable to wheeze in the last 12 months and paracetamol use to any use during pregnancy. We used data from the 7 year age group in the Rebordosa et al study (Rebordosa, Kogevinas et al. 2008), whereas Etminan et al used data for the first year of life for which there was considerably greater power. We also included data from the Kang et al (Kang, Lundsberg et al. 2009) and the Thengilsdottir et al (Thengilsdottir H, Goksor E et al. 2010) study which were not published at the time of the Etminan meta-analysis. These differences highlight the major difficulties and limitations in undertaking a systematic review and meta-analysis based on data from studies utilising different methodologies. However, despite the methodological differences, the two meta-analyses have both shown a significant increased risk.
The observation that the association between paracetamol use in pregnancy and childhood asthma was only present in non-asthmatic mothers is important as it indicates that the association is not due to asthmatic mothers (being at greater risk of having a child with asthma) preferentially taking paracetamol due to aspirin avoidance (Garcia-Marcos, Sanchez-Solis et al. 2009). This pattern of behaviour was suggested by the Kang et al study in which it was observed that paracetamol was more likely to be used during pregnancy by mothers who had diagnosed asthma (Kang, Lundsberg et al. 2009).

The association with prenatal paracetamol use may be present as early as 6 weeks of age as suggested by the findings from the Copenhagen prospective study of asthma in childhood (Bisgaard, Loland et al. 2009). In this study, mothers’ use of paracetamol in the third trimester was associated with a doubling of bronchial hyperresponsiveness in the newborn, albeit with wide confidence intervals and failing statistical significance when outliers were removed in a robust test.

In some studies, a progressive increase in risk associated with increasing number of days of prenatal paracetamol exposure (Perzanowski MS, Miller RL et al. 2009), or increased frequency of use (Shaheen, Newson et al. 2002; Shaheen, Newson et al. 2005) was observed. However, a dose-response relationship was not observed by Kang et al (Kang, Lundsberg et al. 2009), or Rebordosa et al (Rebordosa, Kogevinas et al. 2008), with the authors of the latter study proposing that the timing of exposure may be more important than the dose during pregnancy.
5.4.2 Methodological Issues

There are numerous other methodological issues that are also relevant to the interpretation of the findings. The first is that the meta-analysis was conducted using raw, unadjusted data. Data from the individual studies were not presented in cross-tabulated form (stratified by individual potential confounders), which prevented us from adjusting for a group of confounders common to all included studies. It was also not possible to perform the meta-analysis using the reported adjusted ORs, as each study controlled for multiple and varying confounders, for example one study (Shaheen, Newson et al. 2002) adjusted for 22 primary and 7 secondary potential confounders. Therefore, adjusted results were not comparable. As a result, it is possible that the results may be affected by confounding factors such as maternal smoking, respiratory disease in early life, gestational age, breastfeeding status, pet ownership and social class. Despite these possible confounding effects, our findings are similar to those of Etminan and colleagues (Etminan, Sadatsafavi et al. 2009), who used adjusted results in their meta-analysis.

A second methodological issue is that the statistical test for heterogeneity was significant, although all bar one of the studies had a confidence interval consistent with an association. The heterogeneity may be due to the different ages at which wheezing and asthma were measured, and potentially the varying doses of paracetamol used by mothers. We were unable to perform a meta-regression based on study level characteristics to explore this in more detail because of the small number of studies. Because raw data was used in the present analysis, the variability of
confounders for which adjustment was made in the individual studies should not be a cause of the heterogeneity seen in our results.

Assessment of exposure to paracetamol in utero avoided the potential for confounding by indication, which represents the major source of potential bias in studies of paracetamol use in children (Lowe A, Carlin J et al. 2010; Tapiainen T, Dunder T et al. 2010; Strippoli M-PF, Spycher BD et al. 2009). This may occur in the situation of paracetamol being given for lower respiratory tract infections such as RSV or rhinovirus, which are associated with an increased risk of asthma in later childhood (Stein, Sherrill et al. 1999; Lemanske, Jackson et al. 2005; Lowe, Abramson et al. 2009). In addition, paracetamol was primarily used for pain during pregnancy and indications for use did not modify the risk estimates for wheezing in offspring.

Wheeze in the last 12 months was chosen as the primary outcome variable instead of diagnosed asthma because it was recorded in all studies, it is known to carry high prognostic significance for asthma in this age group (Martinez, Wright et al. 1995), and because the diagnosis of asthma is variable in young children. It could be argued that wheezing in the last 12 months may include some children who do not have clinically significant asthma. We addressed this in our review by examining the association with measures of severe asthma such as wheezing attacks affecting sleep, medication use, emergency department visits, and hospital admissions for asthma. Using these criteria, a similar magnitude of risk was observed for both severe asthma and wheeze in the last 12 months (Rebordosa, Kogevinas et al. 2008; Perzanowski MS, Miller RL et al. 2009). In addition, we restricted the analysis to data from the older age group in the Shaheen et al (Shaheen, Newson et al. 2002; Shaheen, Newson...
et al. 2005) and Rebordosa et al (Rebordosa, Kogevinas et al. 2008) studies, in which findings were presented in different age groups in infancy and childhood.

5.4.3 Potential Sources of Error and Bias

It is unlikely that the association reported in the studies could be due to reporting bias, as the hypothesis that paracetamol use at any stage in life, including exposure in utero, may increase the risk of asthma is not widely known by asthma specialists, let alone the lay public. Another potential issue is confounding by paracetamol use in infancy, in that mothers who take paracetamol in pregnancy may be more likely to give paracetamol to their children. If this latter exposure is associated with childhood asthma, as suggested by the worldwide ISAAC study (Beasley RW, Clayton TO et al. 2010), then this could potentially confound the association observed with prenatal paracetamol exposure. This issue was considered in the Danish study, in which the association between paracetamol use during pregnancy and wheezing at 18 months remained significant when the analysis was restricted to children who had only been exposed to the drug during pregnancy (Kang, Lundsberg et al. 2009). Furthermore, the use of paracetamol both pre- and post-natally was associated with a higher risk than only postnatal paracetamol use.

5.4.4 Potential Mechanisms of Action

The effect of the timing of paracetamol use during pregnancy is relevant to the consideration of the potential mechanisms by which paracetamol may have an effect, if the association is causal. Glucuronidation, the main pathway for metabolism of
paracetamol in adults, is markedly reduced in the foetus in the first trimester, whereas GST, which detoxifies the oxidative paracetamol metabolites, is reduced in the third trimester. The balance between these two pathways could determine whether paracetamol exposure in the first or third trimester increased the predisposition to subsequent asthma, through the build-up of toxic oxidative metabolites. This hypothesis would also invoke the concept of programming, in which an insult at a crucial stage of foetal development may predispose to an increased risk of subsequent disease in childhood or adult life (Barker DJP 1994). In the Danish study an association of paracetamol was greater with use in the first trimester, particularly with persistent wheeze in 7 year old children (Rebordosa, Kogevinas et al. 2008). In contrast, Persky et al (Persky, Piorkowski et al. 2008) and Perzanowski et al (Perzanowski MS, Miller RL et al. 2009) reported a greater risk for paracetamol use in the second and third trimesters and Shaheen et al (Shaheen, Newson et al. 2002; Shaheen, Newson et al. 2005) reported an effect with use after but not prior to 20 weeks of pregnancy. In contrast, Kang et al reported no association between asthma and paracetamol use during either the first or third trimester of pregnancy (Kang, Lundsberg et al. 2009).

The potential role of GST in the association between paracetamol exposure in utero and subsequent childhood asthma was suggested by the observation of Perzanowski et al that the risk of wheeze was modified by GSTP1 genetic status (Perzanowski MS, Miller RL et al. 2009; Persky VW 2010). Prenatal paracetamol exposure predicted both current wheeze and seroatopy at 5 years among children with at least one copy of the GSTP1 minor allele (AG, GG) but not among children homozygous for the major allele (AA). GST is involved in the glutathione pathways and the minor allele has
altered conjugation activity, potentially reducing the capacity of the foetus to metabolise paracetamol, leading to a build-up of toxic oxidative metabolites.

5.5 Conclusion

The findings of this study demonstrate a significant increased risk of wheezing in offspring aged 2.5 to 7 years of women who use paracetamol at any stage during pregnancy. Our results, as well as the findings of the previous systematic review and meta-analysis (Etminan, Sadatsafavi et al. 2009), support the need for further definitive research in to the relationship between paracetamol use during pregnancy and asthma in offspring. However, whilst a randomised controlled trial would be the gold standard method to investigate this association, undertaking an RCT in pregnant women would require complex ethical considerations and is unlikely to be feasible. Further research into the effect of paracetamol use in infancy and childhood on the development of asthma, and the effect of paracetamol use in adulthood on asthma severity and control, may provide further vital information regarding the mechanisms by which intrauterine exposure to paracetamol effects the risk of wheezing and asthma in offspring.
Chapter Six: Study Methods

6.1 Outline of Study

As discussed in chapter 3, there have been no randomised, placebo-controlled trials undertaken to assess the effect of long-term paracetamol use on asthma symptoms and severity. One randomised controlled trial, undertaken in children, assessed the effects of paracetamol use on asthma symptoms compared with ibuprofen (no placebo group), and it was, therefore, not possible to determine if the increased risk of asthma exacerbations in the paracetamol group was due to an increased risk with paracetamol or a decreased risk with ibuprofen (Lesko, Louik et al. 2002).

Our study protocol was designed to investigate whether regular paracetamol use in adults increases asthma severity and worsens asthma control, and to investigate any underlying mechanisms of an effect, if demonstrated. It was powered to detect the effect of a one doubling dose reduction in bronchial hyperresponsiveness (BHR), measured via methacholine challenge testing, and was also designed to provide original data to aid in the development of a further definitive study if required.

The study was designed as a double-blind, randomised, placebo-controlled trial, presented below in accordance with the 2010 CONSORT (Consolidated Standards of Reporting Trials) Statement (Schulz, Altman et al.) (Appendix A).
6.2 Study Objectives and Hypothesis

The objective of the study was to determine whether 12 weeks of daily paracetamol use increased BHR in adult patients with mild to moderate asthma. The hypothesis was that long-term daily use of paracetamol would lead to increased BHR and worsening of asthma control, as defined by the results of a methacholine challenge test and secondary outcome variables such as FeNO, the asthma control questionnaire (ACQ), FEV₁, mean morning peak flow and peak flow variability, asthma exacerbations and blood eosinophil, serum IgE and cytokine levels.

6.3 Study Design

The study was designed as a double-blind, randomised (1:1), placebo-controlled, parallel group trial based in Wellington, New Zealand. Participants were randomised to receive one of two treatment regimens for a 12-week period: paracetamol 1 g (2 x 500 mg tablets) taken twice daily, or placebo (2 tablets) taken twice daily.

6.4 Recruitment

Recruitment was planned to take place over a two-year period from June 2009 to June 2011. A variety of recruitment strategies were used to recruit eligible participants. Participant databases from the Medical Research Institute of New Zealand (MRINZ) and the Wellington Asthma Research Group (WARG), University of Otago, were
accessed. Eligible participants (asthmatics between the ages of 18 and 65) were called by telephone and sent study information by mail if interested, then were phoned again for screening approximately one week later and booked in for a screening visit (visit 1) if appropriate.

Patient databases from several Wellington medical centres (including Island Bay Medical Centre, Newlands Medical Centre, Onslow Medical Centre, Port Nicholson Medical Centre, Karori Medical Centre, Ngaio Medical Centre, Brooklyn Medical Centre, Wadestown Medical Centre and Peninsula Medical Centre) were accessed following consent from the general practitioners (GPs) at each clinic. Patients between the ages of 18 and 65 with a diagnosis of asthma, and who were not documented smokers, were sent a letter on behalf of their GP informing them of the study and inviting them to participate. Interested patients were asked to either return a free-post letter expressing interest in the trial, or call a free-phone (0800) number and speak directly with the study principal investigator, and were then sent out study information via mail. They were phoned for screening approximately one week later and booked in for Visit 1 if appropriate.

Advertising was carried out via flyers, newspaper and radio advertisements and Health TV (a lifestyle television channel screened in medical centres and hospital waiting rooms throughout Wellington) advertisements (Appendix B). Interested patients were able to email the principal investigator or call a free-phone (0800) number and speak directly with the study principal investigator, and were then sent study information via mail. They were then phoned for screening approximately one week later and booked in for Visit 1 if appropriate. A press release in March 2010
resulted in a news article in Wellington’s Dominion Post newspaper which also generated recruits for the study (Appendix C).

6.5 Study Participants

The inclusion criteria were as follows:

- Wheeze in the previous 12 months and a doctor’s diagnosis of asthma.
- 18 to 65 years old.
- FEV₁ greater than or equal to 70% predicted at weeks -1 (Visit 1) and 0 (Visit 2). This inclusion criterion was to ensure that all participants had adequate lung function prior to commencement of the trial and methacholine challenge testing, and that volunteers with severe or difficult-to-control asthma were excluded.
- PC_{20} to methacholine (PC_{20} MCh) of 0.125 to 16.0 mg/ml at week 0. This inclusion criterion was to ensure that volunteers with normal bronchial responsiveness (PC_{20} MCh greater than 16 mg/ml (American Thoracic Society 2000) or severe bronchial hyperresponsiveness (PC_{20} MCh less than 0.125 mg/ml (American Thoracic Society 2000)) were not included in the study.
The exclusion criteria were as follows:

- Use of the medications theophylline, ipratropium bromide, tiotropium or leukotriene receptor antagonists more than once in the previous three months. The purpose of this exclusion criterion was to screen out participants with severe or difficult to manage asthma and to minimise the effect of these medications on methacholine challenge testing (American Thoracic Society 2000).

- A screening alanine aminotransferase (ALT) level above the upper limit of the normal reference range or other screening liver function test (LFT) abnormalities considered significant by the investigator. Based on Aotea Pathology standardised reference ranges, the cut-offs for ALT were designated at 30 U/L for women and 40 U/L for men. This exclusion criterion was amended in January 2011 and changed to a screening ALT level above 1.5 times the upper limit of the normal reference range or other screening LFT abnormalities considered significant by the investigator. Based on Aotea Pathology standardised reference ranges, the new cut-offs were designated at 45 U/L for women and 60 U/L for men. This amendment was enacted to reflect changes to standard international criteria for the exclusion of participants screened for enrolment in pharmaceutical studies on the basis of LFTs. The purpose of this exclusion criterion was to screen out any participants with pre-existing liver dysfunction, who would be at a greater risk of developing disturbed liver function if randomised to the paracetamol arm of the study.
• An exacerbation of asthma within the previous two months requiring prednisone, or nebulised bronchodilator. The purpose of this exclusion criterion was to screen out participants with severe or difficult-to-manage asthma.

• Current or past cigarette smoking greater than 10 pack years. Participants who reported “occasional” or “social” smoking behaviour of greater than 10 cigarettes in the previous 3 months were likewise excluded from the study. The purpose of this exclusion criterion was to minimise the likelihood of participants with coexisting COPD taking part in the trial.

• History of allergy or sensitivity to paracetamol or opiates. Paracetamol-naïve participants with a history of allergy or sensitivity to aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) were also excluded. The purpose of this exclusion criterion was to ensure the safety of randomised participants who may be sensitive or allergic to paracetamol or codeine (the analgesic prescribed to participants during the trial if required). Due to the potential for cross-sensitivity between paracetamol and aspirin (Jenkins, Costello et al. 2004), paracetamol-naïve participants with aspirin allergy or sensitivity were also excluded.

• Current or past history of liver disease or volunteers on potentially hepatotoxic drugs, including but not limited to, the following:
  o Amiodarone
  o Chlorpromazine
  o Isoniazid
  o Methyldopa
  o Rifampacin
Valproic Acid

The purpose of this exclusion criterion was to screen out any participants with pre-existing liver dysfunction and/or those at a high risk of developing liver complications if randomised to the paracetamol arm of the study.

- A current history of regular use of paracetamol, in volunteers who were unwilling or unable to discontinue use of this medication during the trial period, where a current history of regular use was defined as use of the applicable medication on more than two occasions per week during the six weeks prior to the first study visit. The purpose of this exclusion criterion was to ensure that any effect measured during the study was due to study treatment and not non-randomised use of paracetamol during the trial period.

- A current history of regular use of aspirin greater than 150 mg/day or regular use of high doses of NSAIDs in volunteers who were unwilling or unable to discontinue use of this medication during the trial period, where a current history of regular use was defined as use of the applicable medication on more than two occasions per week during the six weeks prior to the first study visit. ‘High dose’ NSAID use was defined as use equal to or greater than the recommended daily dose of a standard NSAID (for example: ibuprofen 400 mg taken eight hourly to a maximum of 1200 mg/day). The purpose of this exclusion criterion was to minimise the immunological and inflammatory effects of other analgesics and antipyretics.

- History of alcoholism, or current excessive alcohol intake (defined as greater than 21 standard drinks in a week for men or greater than 14 standard drinks for women). The purpose of this exclusion criterion was to screen out any
participants at a greater risk of developing liver dysfunction if randomised to the paracetamol arm of the study.

- Previous intentional acute overdose of paracetamol, previous suicide attempt or current untreated depression. The purpose of this exclusion criterion was to ensure the safety of all randomised participants, due to the fact that approximately 200 paracetamol tablets were dispensed to each participant randomised to the paracetamol arm of the study at both visit 2 and 3.

- Body Mass Index (BMI) less than 16.0 kg/m². The purpose of this exclusion criterion was to screen out severely underweight participants who are at an increased risk of hepatotoxicity following paracetamol administration (Claridge, Eksteen et al. 2010).

- Pregnant or breast-feeding women and women of child-bearing age not using adequate contraception. The purpose of this exclusion criterion was to prevent any harm to the foetus or child if the participant was randomised to the paracetamol arm of the trial.

- In accordance with ATS guidelines (American Thoracic Society 2000), participants who were unsuitable for BHR challenge testing for reasons including:
  - Moderate to severe airflow limitation
  - Heart attack or stroke within the previous 3 months
  - Uncontrolled hypertension (systolic BP greater than 200 mmHg, diastolic BP greater than 100 mmHg)
  - Recent (within the previous three months) evidence of myocardial infarction or a stroke
  - Inability to perform spirometry or lack of understanding of the procedure
Study participants were seen and data collected at two main centres: Bowen Hospital and Wellington Regional Hospital. At the Wellington Regional Hospital site, the Clinical Measurements Unit was used for the period August 2009 to March 2010, after which point participants were seen at the MRINZ research offices.

6.6 Interventions

Participants were randomised to receive one of two treatment regimens:

1. Paracetamol 1 g dose (2 x 500 mg tablets) taken twice daily, morning and night
2. Placebo (2 tablets) taken twice daily, morning and night

The trial comprised of 4 study visits and an additional 2 to 4 blood tests performed at a community pathology laboratory over a 13 week study period. The details of each visit are outlined below and summarised in Figure 6.1.
Figure 6.1: Study design flow diagram

Abbreviations: SPT = skin prick test, preg test = pregnancy test, FEV₁ = forced expiratory volume in 1 sec, FVC = forced vital capacity, ACQ = asthma control questionnaire, LFTs = liver function tests, FeNO = Fractional exhaled nitric oxide, PEF<sub>var</sub> = peak flow variability, BHR = bronchial hyperresponsiveness, FBC = full blood count
At the outset of the trial the medications used were white Herron Paracetamol Tabsules (500 mg) and matching placebo tablets, supplied by Sigma Pharmaceuticals Australia Pty Ltd. Due to the acquisition by Aspen Asia Pacific Limited of Sigma's Pharmaceutical Division including the Herron products, the original study medication became no longer available for reorder in 2011. Instead, Herron Gold Paracetamol Tabsules 500 mg (and a matched placebo) were substituted. The core tablet formulation ingredients were the same as the original white tablets; however the new stock of tablets was coated with a yellow coating rather than a clear coating. All trial stock was manufactured in accordance with Good Manufacturing Practice (GMP) at GMP licensed facilities, and there was no difference in appearance, taste or odour between the paracetamol and the placebo tablets. The importation and distribution of the medications was approved by the Ministry of Health’s (Medsafe) Standing Committee on Therapeutic Trials (Appendix D).

6.6.1 Visit 1/Screening Visit (Week -1)

A screening visit (visit 1) was held approximately one week prior to the baseline visit (visit 2). Written consent was obtained, participant demographics and a medical history were taken and a brief physical examination (height and weight for BMI, blood pressure, pulse) and spirometry performed.

Bronchodilator reversibility testing was performed by measuring FEV<sub>1</sub> and FVC before and 15 minutes after a 400µg dose of salbutamol, delivered via large volume
spacer (Volumatic GSK, UK), using the method of a vital capacity breath followed
by a breath hold per actuation of 100µg of salbutamol.

The Qoltech asthma control questionnaire (ACQ, Appendix E) (Juniper, O'Byrne et
al. 1999) was self-administered by the participant and their baseline score calculated.
Skin prick testing (SPT) was performed and female participants of child-bearing
potential underwent a urine pregnancy test. A blood sample was taken for
measurement of LFTs (alanine transaminase (ALT), alkaline phosphatase (ALP),
bilirubin, albumin, total protein and gamma-glutamyl transferase (GGT)). A diary was
dispensed to record morning and evening peak expiratory flow rate (PEF) values
(prior to asthma medication use) for one week prior to visit 2 (Appendix F).

Participants who used an ICS remained on a stable dose during the study, in order to
ensure constant anti-inflammatory treatment. If their regular asthma therapy included
the use of a LABA or SABA, they were advised to continue using these as
required/prescribed, except for 48 and 8 hours prior to methacholine challenge testing
respectively, when LABA and SABA use is contraindicated.

**6.6.2 Visit 2/Baseline Visit (Week 0)**

The peak flow diary and meter were collected and checked for completeness and
participants were questioned regarding any asthma exacerbations, changes in health or
regular medications since their first visit (and participant excluded if any exclusion
criteria were met). Baseline assessments of FEV$_1$, methacholine challenge testing (to
determine PC$_{20}$) and FeNO were undertaken and ACQ repeated. Participants who met
all eligibility criteria were dispensed a seven-week supply of study medication (six-weeks of study treatment and an additional week of study treatment in case of a delay in attending the next visit), along with a medication diary for recording doses (Appendix G). A prescription for codeine phosphate (6 x 15 mg tablets) was given to the participant for emergency analgesia during the study period. Participants were advised to contact the study investigator or see their GP if they required further analgesia above the prescribed amount of codeine during the study period. Participants were advised to avoid paracetamol, as well as aspirin and other NSAIDs for the duration of the trial. Randomised participants underwent a repeat blood test for LFTs (ALT, ALP, bilirubin, albumin, total protein and GGT) for the purpose of recording baseline liver function, as ALT may have changed in the week between visit 1 and visit 2. Full blood count (FBC) (eosinophils), total serum IgE level, and serum cytokine measurements (IFN-γ, IL-4, IL-5 and IL-13).

6.6.3 Visit 3 (Week 6)

Participants were questioned regarding any asthma exacerbations, changes in health or regular medications, or any adverse events since their last visit. Compliance with study medication was determined by a review of the medication diary, a count of the units of medication returned at the end of the first 6 week period, and blood paracetamol levels. Reasons for lack of compliance were discussed and participants were re-educated about the importance of compliance with study medication. Measurement of FEV₁, ACQ and FeNO were undertaken and blood tests taken for LFTs (ALT, ALP, bilirubin, albumin, total protein and GGT), paracetamol level, FBC (eosinophils), total serum IgE level and cytokine levels. Participants were given a
further seven-week supply of study medication, a second medication diary to record administered doses, and a diary to record morning and evening PEF values in week 11 of the trial, prior attending visit 4.

6.6.4 Visit 4 (Week 12)

The week 11 peak flow diary was collected and checked for completeness and participants were questioned regarding any asthma exacerbations, changes in health or regular medications, or any adverse events since their last visit. Compliance with study medication was determined by a review of the medication diary, a count of the units of medication returned at the end of the previous 6 week period, and blood paracetamol levels. Methacholine challenge testing was undertaken. Assessments of FEV$_1$, ACQ and FeNO were undertaken and blood tests taken for LFTs (ALT, ALP, bilirubin, albumin, total protein and GGT), paracetamol level, FBC (eosinophils), total serum IgE level and cytokine levels. Female participants of child-bearing potential underwent a final urine pregnancy test to ensure they did not fall pregnant during trial period. A detailed timeline of study procedures is summarised in Table 6.1.

6.6.5 Additional Blood Tests and Liver Function Monitoring

In order to ensure the safety of the treatment regime, blood samples were taken to monitor liver function at all study visits. Additional blood tests to monitor liver function were undertaken at weeks 2 and 4 at community pathology laboratories, and were accompanied by a phone call to the participant to monitor adverse events.
Results of liver function tests were reviewed by data safety reviewers and participants were advised to stop study medication if there was a rise in ALT to greater than or equal to three times the upper limit of normal. Participants who experienced a rise in ALT to between 2 times and 3 times the upper limit of normal remained in the study, however were required to have additional ongoing blood tests to monitor liver function at weeks eight and 10.

A full liver function profile, including ALT, ALP, bilirubin, albumin, total protein and GGT, was performed at each blood test, however ALT was the primary liver function test used to monitor safety and determine ongoing eligibility for the trial. Safety investigators reviewed all ALT results in conjunction with each participant’s full liver function profile, and if any abnormal results were reported, the participant’s health care provider was contacted and informed.

References ranges for ALT were as per the standardised Aotea Pathology reference ranges: for women, normal reference range 0 to 30 U/L and for men; 0 to 40 U/L.
Table 6.1: Schedule of study assessments

<table>
<thead>
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<th>Week</th>
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<th>2</th>
<th>4</th>
<th>6</th>
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<td>4</td>
<td>4</td>
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<td></td>
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<tr>
<td>Skin Prick Tests</td>
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<td>X²</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<td>Urine pregnancy test if applicable</td>
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<td>X</td>
<td>X</td>
<td></td>
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<td>FVC</td>
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<td>Reversibility testing</td>
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<td>Concomitant medications review</td>
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<td>X</td>
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<td>X</td>
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</tr>
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<td>X</td>
<td>X</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Blood (LFTs*)</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Blood (paracetamol level)</td>
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<td>X</td>
<td>X</td>
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<td>X</td>
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</tr>
<tr>
<td>Blood (IgE + FBC + cytokines)</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Trial completion</td>
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<td></td>
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<td></td>
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</tr>
</tbody>
</table>

² Skin prick testing is only to be completed at visit 2 if necessitated by a negative result at visit 1 in participants who have reported use of antihistamine medications; * LFTs to be assayed are as per the Aotea Pathology standard LFT panel and include ALT, alkaline phosphatase, bilirubin and Gamma Glutamyl Transpeptidase (GGTP).
6.7 Outcomes

The primary outcome variable was bronchial hyperresponsiveness, measured as the provocation concentration of methacholine causing a 20% reduction in FEV\(_1\) (PC\(_{20}\) MCh), at week 0 (visit 2/baseline) and week 12 (visit 4) of the trial. Secondary outcome variables included:

- Forced Expiratory Volume in 1 Second (FEV\(_1\)) at 6 and 12 weeks
- Fractional exhaled Nitric Oxide (FeNO) at 6 and 12 weeks
- Asthma Control Questionnaire (ACQ) score at 6 and 12 weeks
- Mean morning peak flow at week 12
- Peak flow variability (PEF\(_{var}\)) at week 12
- Exacerbations of asthma (requiring a doctor visit and need for prednisone or nebulised bronchodilators)
- The effect of paracetamol on liver function, blood eosinophil count, serum IgE, and serum cytokine levels

6.8 Sample Size

A sample size of 60 participants in each group has 80% power at the 5% level of significance to detect one doubling dose difference in PC\(_{20}\) between the groups, with a standard deviation of 1.9 (Pearson, Lewis et al. 2004). To allow for the possibility of up to 10% of study participants withdrawing early from the study, a recruitment target of 66
participants was set for each group. One doubling dose difference in $PC_{20}$ represents a pronounced effect on BHR in an asthmatic population (Lotvall, Inman et al. 1998)

**Interim analyses**

Safety monitoring comprised independent analysis of the rates of severe exacerbations in the placebo and paracetamol groups after approximately 60 participants had completed the study. The pre-specified study termination criterion was to terminate the study had a significant difference in severe asthma exacerbations between the two groups been found.

**6.9 Randomisation**

**Sequence Generation**

A computer generated randomisation schedule was created by a biostatistician (Prof Mark Weatherall).

**Allocation Concealment and Implementation**

The randomisation schedule was administered by a third-party scheme, and was password protected and created without the involvement of the study investigators. Only the study pharmacists (based at Wellington Regional Hospital) had access to the randomisation schedule. In order for the study medication to be dispensed at visit 2, it was necessary for study pharmacists to prepare the study medication prior to final eligibility screening at visit 2. Pharmacists received notification from the study investigators that a participant was due to attend visit 2 (in the form of a pharmacy
medication script which was faxed to pharmacy then sent my mail, Appendix H), and assigned the appropriate treatment group to the participant based on the randomisation schedule. Allocated medication was then delivered to the MRINZ research offices in labelled medication bottles, ready for dispensing to study participants at the end of visit 2 if they were eligible for the study. If a participant failed eligibility criteria at visit 2, the medication was not dispensed, was returned to the pharmacy and the subject was withdrawn from the study. The randomisation code was not re-used.

**Blinding**

The study participants, investigators, participant health care providers and all MRINZ staff involved in the study were blinded as to treatment allocation. Only the Wellington Regional Hospital pharmacists involved in allocation were aware of the treatment allocation. In order to maintain investigator blinding, results of liver function tests were kept from the study investigators and viewed only by the safety data reviewers (Dr Justin Travers and Dr Philippa Shirtcliffe). If further blood tests were needed at weeks eight and 10 due to deranged liver function during the first six weeks of the trial, contact was made with the participant by the safety data reviewer directly so as to maintain investigator blinding. If, during the course of the trial, any participant was found to have deranged LFTs which required their withdrawal from the trial, the study safety investigator contacted Wellington Regional Hospital pharmacy to unblind the participant in order to inform ongoing management of their deranged liver function results. Any unblinding event was notified to the Central Regional Ethics Committee and Medsafe (New Zealand Medicines and Medical Devices Safety Authority).
6.10 Statistical Methods

The primary analysis method was ANCOVA. The logarithm base two PC$_{20}$ for methacholine at 12 weeks was the primary response variable, with the baseline logarithm base two PC$_{20}$ as a co-variate, and a categorical variable for the paracetamol group. The difference in logarithm base two PC$_{20}$ was the doubling dose difference between the two randomised groups. Secondary outcome variables, including FEV$_1$, FEV$_1$ % predicted, ACQ score, FeNO and PEF$_{var}$ were also analysed by ANCOVA. The distribution of FeNO and serum IgE were skewed and normality assumptions for these variables were best met on the natural logarithm scale.

A risk difference and appropriate confidence intervals were calculated for the categorical variable, the number of participants with at least one asthma exacerbation. Simple t-tests were used to compare mean values for ALT, the logarithm transformed eosinophil count and IgE by paracetamol or placebo group, as the latter two had skewed distributions. For those variables with a logarithm transformation, the exponent of the difference in logarithms was interpreted as the ratio of mean values. IFN-\(\gamma\) distributions were compared with a Mann-Whitney U test. For IL-4, IL-5, and IL-13 there were only a few participants with detectable levels and the proportion with detectable levels were analysed with calculation of relative risk or risk difference, if there was a zero cell count, and a Chi-square test.
6.11 Clinical Measurements

6.11.1 Defining the Study Population

6.11.1.1 Spirometry and Bronchodilator Reversibility Testing

Spirometry (lung function testing) is used in the diagnosis of asthma, the assessment of severity and the monitoring of treatment and symptoms (Celli 2000; Global Initiative for Asthma (GINA) 2010). One component of spirometry is bronchodilator reversibility testing, which measures the airways response to an inhaled bronchodilator.

Spirometry was performed to ensure that all participants had mild to moderate airway obstruction, in that any participants with an FEV\textsubscript{1} below 70% predicted were excluded. Predicted values for lung function were taken from tables compiled from the derived equations contained in the report of the working party of the European Community for Coal and Steel (1983). Bronchodilator reversibility testing was performed to further define the study population. During bronchodilator reversibility testing, an improvement in FEV\textsubscript{1} of greater than 12% is generally indicative of asthma, however a proportion of asthmatic patients will not display reversibility at each assessment, particularly those on treatment (Global Initiative for Asthma (GINA) 2010). Spirometry has been shown to be highly reproducible in short (2 week) and long term (8 week) trials of asthma (Faul, Demers et al. 1999).
Pre and post-bronchodilator FEV$_1$ and FVC were recorded at visit one, and pre-bronchodilator FEV$_1$ was recorded at each subsequent visit, using the Micro Medical Microlab spirometer (Micro Medical, Kent, UK) in accordance with ATS standards (Miller, Crapo et al. 2005). Pre-bronchodilator FEV$_1$ was used in all follow-up visits as participants were asked to withhold their bronchodilator prior to methacholine challenge testing. The highest of three reproducible results for FEV$_1$ and FVC were recorded at visit 1 and for pre-bronchodilator FEV$_1$ at subsequent visits. At visit one, after obtaining baseline spirometry, participants were given a dose of 400 µg salbutamol, administered via large volume spacer (Volumatic, GSK, UK), with a vital capacity breath followed by a breath hold per actuation of 100 µg dose of salbutamol. Post-bronchodilator FEV$_1$ and FVC were measured after 15 minutes (McCormack and Enright 2008), and reversibility was calculated as:

\[
\text{Bronchodilator reversibility} = \left( \frac{\text{Pre-bronchodilator FEV}_1}{\text{Post-bronchodilator FEV}_1} \right) \times 100
\]

6.11.1.2 Skin Prick Testing

Skin prick testing (SPT) provides information about the presence of specific IgE to certain allergens, which are introduced into the epidermis of the forearm and lead to a ‘wheal and flare’ reaction (Australasian Society of Clinical Immunology and Allergy 2006 (Revised March 2009)). SPT is a useful test in the investigation of asthma, as a positive test increases the probability of a positive diagnosis in the presence of respiratory symptoms (Australasian Society of Clinical Immunology and Allergy 2006 (Revised March 2009); McCormack and Enright 2008). Epidemiological
evidence suggests that approximately 90% of asthmatics are allergic to common inhaled aeroallergens (Pearce, Pekkanen et al. 1999), and SPT was performed in our study to determine allergic status and to define the study population.

The three allergens most commonly associated with asthma in New Zealand were tested: grass (grass mix #7, Hollister Stier Laboratories, USA), house dust mite (Dermatophagoides pteronyssinus, Hollister Stier Laboratories, USA) and cat pelt (Stallergenes Laboratories, France). Allergens were tested against positive (histamine) and negative controls (Hollister Stier Laboratories, USA). Allergens were kept refrigerated and replaced prior to expiry in accordance with the ASCIA guidelines (Australasian Society of Clinical Immunology and Allergy 2006 (Revised March 2009)).

SPT was undertaken at visit 1 regardless of the use of anti-histamine medication. If a negative result was determined then testing was repeated at visit 2, following three days of withholding anti-histamine medication. Skin was cleaned with water or alcohol and allergens were applied to the volar aspect of the forearm. Position of test sites were marked with pen 2 cm apart and allergens applied using a dropper. Results of wheal size were read at 15 minutes in accordance with ASCIA guidelines (Australasian Society of Clinical Immunology and Allergy 2006 (Revised March 2009)) using a SPT ruler or a regular plastic ruler (measuring length and width and taking the average if irregularly shaped). Results of SPT were interpreted binomially as either positive or negative, based on the formulae (Australasian Society of Clinical Immunology and Allergy 2006 (Revised March 2009)):
Allergen SPT result (mm) – negative control SPT result (mm) ≥ 3mm = positive result
Allergen SPT result (mm) – negative control SPT result (mm) < 3mm = negative result

6.11.2 Clinical and Physiological Measurements

6.11.2.1 Methacholine Challenge Testing

Bronchial hyperresponsiveness (BHR) and airway inflammation are two key pathophysiological features of asthma (Brannan 2010). BHR is defined as an exaggerated response or increased sensitivity to specific bronchoconstrictors (Sterk, Fabbri et al. 1993), which in turn causes airway narrowing and leads to variable airflow limitation and intermittent symptoms such as recurrent episodes of wheezing, breathlessness, chest tightness and coughing (Brannan 2010; Global Initiative for Asthma (GINA) 2010). The mechanisms underlying BHR are not fully understood (Sterk, Fabbri et al. 1993; Global Initiative for Asthma (GINA) 2010), however it is thought that excessive contraction of airway smooth muscle, excessive narrowing of the airways, thickening of the airway wall and sensitisation of sensory nerves by inflammation may all play a role (Global Initiative for Asthma (GINA) 2010).

BHR is assessed by undertaking BHR challenge testing, whereby direct (eg methacholine, histamine) or indirect (e.g. exercise, adenosine 5’-monophosphate) stimuli are applied and the breathing response is recorded under specific measurement conditions and laboratory protocols. BHR testing can be used in the clinical diagnosis of asthma in patients with variable airflow obstruction, and is also a well recognised
test for monitoring asthma severity (Crapo, Casaburi et al. 2000). It is often used in the research setting as an objective outcome measure to determine response to asthma therapies (Kerrebijn, van Essen-Zandvliet et al. 1987; Sterk, Fabbri et al. 1993; Prieto, Berto et al. 1994; Cockcroft, Swystun et al. 1995), during which it has been shown to be highly reproducible in both short and long-term trials of asthma (Faul, Demers et al. 1999; Chinn and Schouten 2005). Serial BHR testing is particularly useful in assessing response to treatment and the effect of exposure to sensitising agents (Woolcock and Jenkins 1990).

Various methods can be used to measure BHR, including direct stimuli from pharmacological agents such as methacholine and histamine, and indirect stimuli such as hypertonic saline aerosols, cold/dry air, exercise and adenosine 5’-monophosphate (Sterk, Fabbri et al. 1993). Direct stimuli act directly on bronchial smooth muscle and cause it to contract, whereas indirect stimuli work through the release of contractile mediators such as histamine, prostaglandin and leukotrienes from inflammatory cells within the airway (Brannan 2010; Sverrild, Porsbjerg et al. 2010). While BHR to direct stimuli is associated with airways inflammation, the mechanism by which the airway narrows does not depend on its presence. Whereas, in the case of indirect stimuli, a positive BHR response requires the presence of inflammatory cells and responsive smooth muscle cells (Brannan 2010). It is for this reason that indirect stimuli have been seen to have a closer relationship to airway inflammation than direct stimuli (Sverrild, Porsbjerg et al. 2010).

Methacholine was chosen as the BHR challenge agent in this study for several reasons. First, methacholine challenge testing has been well standardised and
validated for use in asthmatic patients (Sterk, Fabbri et al. 1993), and has been shown to be highly reproducible in patients with mild, stable asthma (Inman, Hamilton et al. 1998). It is also the most widely used and established technique for BHR challenge testing (American Thoracic Society 2000). Further, most patients with current asthma symptoms should have BHR to methacholine (American Thoracic Society 2000), which was an important consideration in this study due to the criterion which required participants to have BHR to 16 mg/ml methacholine or less for inclusion in the study. Finally, although indirect stimuli have been found to be more closely associated with airways inflammation, due to the fact that FeNO was measured in this study as a marker of airways inflammation, and serum cytokines and blood eosinophils as markers of systemic inflammation, the direct stimulus of methacholine was preferred as it has been better validated for this study population.

Methacholine challenge testing was undertaken using the two-minute tidal breathing dosing protocol (de, Goei et al. 1962) recommended by the American Thoracic Society Guidelines for Methacholine and Exercise Challenge Testing 1999 (American Thoracic Society 2000). The decision to use the two-minute tidal breathing/Wright nebuliser method as opposed to the five-breath dosimeter method was made as it has been shown that the accuracy, safety and precision of the two methods are similar (Wubbel, Asmus et al. 2004; Prieto, Lopez et al. 2008), and that airway responsiveness can be measured as reliably and less expensively using the tidal breathing/Wright nebuliser method (Ryan, Dolovich et al. 1981). Further, due to the bronchoprotective effects of deep inhalations, the 5-breath dosimeter method has been recently found to produce an unacceptable number of false negative challenge results (Cockcroft 2008).
A Micro Medical Microlab spirometer (Micro Medical, Kent, UK) was used to determine FEV1. The spirometer was calibrated and validated using a valid verified 3 L syringe at the start of the study and at 12 monthly intervals and again at the completion of the study as per the manufacturer instructions. An English Wright nebuliser (Roxon Meditec, Montreal, Canada) with a one-way valve was used to deliver the methacholine dose and was powered by dry compressed air from a rotameter. Nebuliser flow rates were calibrated to produce an output of 0.26ml over 2 minutes by interpolation method to ensure outputs remained with +/- 10%, as per ATS guidelines (American Thoracic Society 2000). Flow rates were recalibrated and verified at 6 monthly intervals and again at completion of the study.

Participants were screened for contraindications to methacholine challenge testing prior to commencement (American Thoracic Society 2000), at either study visit 1 or 2 (Table 6.2). Data was collected on factors known to affect bronchial responsiveness, including medication use (short-acting and long-acting beta agonists), caffeine intake and recent viral infection. At visit 1, participants were advised to withhold long-acting beta agonist medication for 48 hours and short-acting beta agonist use for 8 hours prior to challenge testing, and to avoid caffeine intake on the day of the test (American Thoracic Society 2000). If participants had consumed long-acting or short-acting beta-agonists within the specified timeframe prior to methacholine challenge testing, the test was postponed to a later date. Likewise, if the participant was experiencing a current viral infection that had caused (or was considered likely to cause) an FEV1 of less than 70% of predicted on the day of the challenge test, the test
was postponed to a later date. Challenge testing was not postponed if caffeine had been consumed on the day of the test.

Table 6.2: Contraindications for methacholine challenge testing

<table>
<thead>
<tr>
<th>Absolute Contraindications</th>
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<tbody>
<tr>
<td>Severe airflow limitation (ATS specify ( FEV_1 &lt; 50% ) predicted however for the purposes of this study all participants had ( FEV_1 &gt; 70% ) predicted)</td>
</tr>
<tr>
<td>Heart attack or stroke in the last 3 months</td>
</tr>
<tr>
<td>Uncontrolled hypertension (systolic BP &gt;200 mmHg, diastolic BP &gt;100 mmHg)</td>
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</table>

<table>
<thead>
<tr>
<th>Relative Contraindications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate airflow limitation (ATS specify ( FEV_1 &lt;60% ) predicted however for the purposes of this study all participants had ( FEV_1 &gt; 70% ) predicted)</td>
</tr>
<tr>
<td>Inability to perform acceptable-quality spirometry</td>
</tr>
<tr>
<td>Pregnancy or nursing mothers</td>
</tr>
<tr>
<td>Any other safety concern at the investigators discretion</td>
</tr>
</tbody>
</table>

Methacholine (provocholine) was sourced from Methapharm Inc (Ontario, Canada) as 1280 mg vials and was stored at temperatures of between 15°C and 30°C, in accordance with ATS guidelines (American Thoracic Society 2000). Methacholine was diluted with normal saline in a sterile manner, and refrigerated at a concentration of 128 mg/ml for a period of up to three months. Refrigerated methacholine was further diluted for each individual challenge test into doubling concentrations of 0.0125, 0.03, 0.06, 0.125, 0.05, 1, 2, 4, 8, 16, and 32 mg/ml and left to warm to room
temperature for at least 30 minutes prior to each test. Each dilution was recorded using a standardised methacholine preparation schedule (Appendix L).

Basic spirometry was performed prior to challenge testing, and a saline (diluent) dose was given prior to the first methacholine dose. At each inhaled dose, the participant was asked to breath normally though the mouthpiece (with nose clip in place) for two minutes, after which time FEV$_1$ was measured at 30 s and 90 s. The next concentration of methacholine was then administered within 5 minutes of the original dose commencement. If the FEV$_1$ fell by greater than or equal to 20% from baseline (or if participant finished all concentrations without a drop in FEV$_1$), no further medication was given and nebulised salbutamol (5 mg/2.5 ml) was administered immediately, followed by a 10 minute rest period. The participant was monitored and salbutamol nebuliser repeated if necessary until FEV$_1$ was within 10% of post-saline baseline.

Challenge testing was performed within a fumehood to protect the investigator from methacholine inhalation. PC$_{20}$, defined as the provocation concentration of inhaled methacholine required to produce a 20% reduction in FEV$_1$, was calculated using the formula (Cockcroft, Murdock et al. 1983):

$$\text{Logarithmic PC}_{20} = \text{Antilog } [(20-R1)(\log C2-\log C1)/(R2-R1) + \log C1]$$

Where C1 is the methacholine concentration producing less than a 20% reduction in FEV$_1$ and C2 is that producing a greater than 20% reduction in FEV$_1$. R1 and R2 are the percent FEV$_1$ reductions produced by C1 and C2 respectively (Cockcroft,
Murdock et al. 1983). At visit 4, if any participant failed to reach a 20% reduction in FEV₁ at 32mg/ml of methacholine, then their final PC₂₀ was extrapolated using the formula above.

6.11.2.2 Mean Morning Peak Flow and Peak Flow Variability (PEF_{var})

Asthma control relates to the degree to which the symptoms and components of asthma respond to the presence of treatment (Thamrin, Nydegger et al. 2011), and is the primary aim of clinical asthma management (Global Initiative for Asthma (GINA) 2010). The change in the mean value of serial morning peak flow measurements has been shown to be a valuable method of assessing response to treatment and asthma control in clinical trials of asthma (Mitchell, Gildeh et al. 1986). Further, variation in lung function, namely peak flow variability, is an important criterion used to assess asthma control (Global Initiative for Asthma (GINA) 2010; Thamrin, Nydegger et al. 2011). Due to the multidimensional and heterogeneous nature of asthma, there are still no clear guidelines on the assessment of asthma control (Taylor, Bateman et al. 2008; Reddel, Taylor et al. 2009), however PEF_{var} is one way of providing an objective measurement to assess improvement or deterioration in reversible airflow obstruction.

Morning and evening peak flow was recorded for one week preceding the baseline study visit, and in the final week of the study preceding visit 4. Participants were given a peak flow meter (Breath-Alert Peak Flow Meters, Medical Developments International, Australia) at the screening visit and were given both verbal and written instructions on correct technique. They were asked to complete a seven-day diary in which they recorded three consecutive peak flow recordings first thing each morning.
and last thing each night, prior to the use of any asthma medication. The diary was then returned to the study investigator at the subsequent visit.

Mean morning peak flow was calculated as the average of the sum of each best daily morning peak flow recording over the seven-day recording period. $\text{PEF}_{\text{var}}$ was measured as the amplitude as a percentage of the mean over the week, calculated as the difference between the highest and lowest maximum morning and evening PEF results over a greater than 5 days consecutive period, divided by the mean maximum PEF for the week (Higgins, Britton et al. 1993).

6.11.2.3 Asthma Control Questionnaire

As stated above, monitoring asthma control is a fundamental component of asthma management. The Asthma Control Questionnaire (ACQ) (QOL Technologies Ltd) is a well validated and internationally regarded method of monitoring adequacy of asthma control (Appendix C) (Juniper, O'Byrne et al. 1999). The ACQ was designed to measure asthma control in both the clinical research setting and in clinical practice, and measures the full range of control from ‘totally controlled’ to ‘extremely poorly controlled’.

The questionnaire consists of 7 questions measuring five important asthma symptoms (being woken at night by symptoms, waking in the morning with symptoms, limitation of daily activities, shortness of breath and wheeze) as well as bronchodi-lator use. The participant must scale their experience of each symptom and
inhaler use during the previous 7 days using a 7-point scale. The seventh question is completed by the researcher and is a scaled measurement of FEV₁ % predicted at the time of the visit. The questions are equally weighted and the ACQ score is the mean of the 7 questions and ranges from 0 (totally controlled) to 6 (severely uncontrolled). A change in ACQ score of 0.5 is considered the minimal clinically important difference (Juniper, O'Byrne et al. 1999). The ACQ was self-administered by participants prior to any other testing at visit 1, 2, 3 and 4, and was used in the study to monitor any deterioration in asthma control in the paracetamol group compared to the placebo group.

6.11.2.4 Exacerbations

Asthma exacerbations, defined in this study as any deterioration in asthma symptoms requiring the use of prednisone or a nebulised bronchodilator, are an important measurement of asthma control as they constitute the greatest risk to the patient, provoke anxiety in the patient and their family and lead to substantial costs to the health care sector (Reddel, Taylor et al. 2009). Monitoring of asthma exacerbations was used in the study to compare any deterioration in asthma control in the paracetamol group compared to the placebo group.

Participants were questioned at each study visit regarding deterioration in asthma symptoms or any exacerbations requiring a nebulised bronchodilator or oral prednisone at any time since the last study visit. Exacerbations were documented on the study worksheet and were also filed as adverse events (Appendix M). In the case
of an exacerbation, participants were advised to receive normal care from their regular health care provider and to continue taking the study medication.

6.11.3 Inflammatory and Immunological Measurements

6.11.3.1 Fractional Exhaled Nitric Oxide Testing

Fractional exhaled nitric oxide (FeNO) testing is a relatively new, non-invasive clinical test which provides immediate results in the assessment of airways inflammation (Sandrini, Taylor et al. 2009). Nitric oxide (NO) is a widely distributed endogenous regulatory molecule, synthesised by the enzyme nitric oxide synthetase (NOS). The inducible form of NOS (iNOS) is not normally expressed in most tissues, however is stimulated during inflammation by pro-inflammatory endotoxins and cytokines (Shaw, Wilson et al. 2010). NO works in the lungs as a vasodilator, bronchodilator and mediator of the inflammatory response (Lim and Mottram 2008; Sandrini, Taylor et al. 2009; Shaw, Wilson et al. 2010).

NO in exhaled breath comes from several different sources throughout the airways including iNOS (Barnes and Belvisi 1993), cellular sources such as alveolar and epithelial cell surfaces, and S-nitrosoproteins and S-nitrosothiols which may play an important role in NO transportation (Kharitonov and Barnes 2001). FeNO levels are elevated in asthmatic patients (Kharitonov, Yates et al. 1994; Kharitonov and Barnes 2001) and FeNO is a reliable and robust surrogate marker of eosinophilic airway inflammation (Sandrini, Taylor et al. 2009). FeNO, therefore, is an effective measure
of the response to anti-inflammatory asthma therapy, and is a sensitive marker of underlying eosinophilic airways inflammation (Kharitonov and Barnes 2000).

FeNO was measured at visits 2, 3 and 4 in order to assess any difference in airways inflammation between the paracetamol and placebo group. A NiOX Flex chemiluminescence analyser (Aerocrine AB, Stockholm, Sweden) was used for all exhaled NO measurements and was calibrated and maintained as per manufacturer specifications. All exhaled nitric oxide measurements were performed using the online technique with a real-time display of breath profiles. The participant was seated without a nose clip and asked to inhale through the NiOX mouth piece over 2-3 seconds to full lung capacity and then to exhale within the pressure and flow specifications of the NiOX machine (pressure 5-20 cm H20, and flow of approximately 0.05 L/second as per the ATS/ERS guidelines (American Thoracic Society 2005)) for a sufficient amount of time to allow the concentration of NO to be evaluated in a 3 second window of a stable NO plateau. Three viable measurements were taken and the average of three breaths was recorded.

6.11.3.2 Blood Tests

Blood eosinophil count, serum IgE, and serum cytokine (IL-4, IL-5, IL-13, IFN-γ) levels were monitored throughout the study at visits 2, 3 and 4, to determine whether a difference in systemic immunological responses existed between the paracetamol group and the placebo group. Markers of systemic immunological responses were important to determine any effect on Th1/Th2 balance. IL-4, IL-5 and IL-13 are Th2 dependent cytokines, whereas IFN-γ is Th1 dependent. Raised blood eosinophil and serum IgE levels, regulated
by IL-5 and the IL-4/IL-13 cytokine cascade respectively, are closely correlated with asthma severity and control (Bousquet, Chanez et al. 1990; Kabesch, Schedel et al. 2006; Global Initiative for Asthma (GINA) 2010; Holgate 2011). Levels of IFN-γ are decreased in asthmatic airways, and reduced levels of IFN-γ are correlated with disease severity (Chung and Barnes 1999)

All blood samples were sent to Aotea Pathology for processing or storage (Aotea Pathology Certificate of Accreditation, Appendix I). Liver function tests, serum IgE and eosinophils levels were processed by Aotea Pathology. The blood samples for measurement of cytokines were frozen and stored at Aotea Pathology, and subsequently sent to the Wellington Asthma and Respiratory Group laboratory (University of Otago) for processing. Concentrations of IFN-γ, IL-4, IL-5 and IL-13 were measured by ELISA (Quantikine, R&D Systems, Minneapolis, MN), according to the manufacturer’s protocols. The ELISA minimum detectable level for IFN-γ was 12.5 pg/ml, for IL-4 was 27.7 pg/ml, for IL-5 was 3.5 pg/ml and for IL-13 was 55.3 pg/ml. Cytokine levels below the detection limit were assigned values of two thirds of the lowest detectable levels (IFN-γ: 9.4 pg/ml; IL-4: 20.8 pg/ml; IL-5: 2.6 pg/ml; IL-13: 41.6 pg/ml).

Blood samples collected during office hours were delivered immediately to Aotea Pathology. Any blood tests taken after 4pm were stored in the MRINZ laboratory over night before being sent to Aotea Pathology the next morning. LFTs, serum IgE, paracetamol level and cytokine samples were centrifuged at 3000 rpm for 10 minutes using a Labofuge 400R Thermoscientific centrifuge (Thermo Electron LED GmbH, Langenselbold, Germany). LFT, serum IgE and paracetamol level samples were then
refrigerated, and cytokine samples were separated, and serum retained and stored in a
-20°C freezer.

6.12 Ethical Approval and Trial Registration

The study was approved by the Central Regional Ethics Committee (CREC), Wellington, New Zealand on 19th June 2009 (ID number: CEN 08/12/070, Appendix J) and prospectively registered with the Australian New Zealand Clinical Trials Registry on 7th July 2009 (Clinical Trial Number: ACTRN12609000551291, Appendix K). The study was conducted under the strict ethical and quality standards of Good Clinical Practice (GCP) (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use 1996; New Zealand Medicines and Medical Devices Safety Authority October 2010). All participants read a detailed participant information sheet (Appendix L) and had the study explained verbally by a study investigator before consenting to participate in the study and signing the study informed consent form (Appendix M).

6.13 Data Management

All data collected from each participant was recorded on standardised study worksheets (Appendix N) and stored in a secure, locked facility within the MRINZ. Collected data was entered into the study database (Microsoft Access) by study investigators immediately after each study visit and stored on a secure server.
6.14 Monitoring of Compliance

Compliance with study medication was determined by a review of the medication diary, a count of the units of medication returned at the end of the first and second 6-week study period, and blood paracetamol levels taken at weeks 2, 6 and 12.

At visit 3 and 4, participants returned their medication diary (Appendix G) and any remaining study medication from the first or second 6-week period of the study. All participants were given 188 tablets of study medication at visit 2 and visit 3, which equated to 6 weeks of fully compliant medication dosing and one extra week in case of a delay in follow-up. The number of days that the participant had been randomised, and therefore the number of doses of study medication expected to have been consumed over that time period were calculated based on the entries in the medication diary. The remaining tablets of study medication were then counted and were compared with the number of tablets expected to be returned.

For example, when calculating compliance at visit 3; if a participant had been randomised for 43 days, and their first dose was in the evening of visit 2 and their last dose was in the morning of visit 3, then they would have been expected to have taken 84 doses of study medication (168 tablets). This means that there should have been 20 tablets (188-168 tablets) returned at visit 3. If, when the remaining tablets were counted at visit 3, there were 28 tablets returned, this means that 8 tablets (therefore 4
doses) were missed during the 6 week study period. The participant took 80 out of 84 doses and was, therefore, 94% compliant.

Blood paracetamol levels were also monitored, both to ensure compliance in the paracetamol group, and to ensure compliance with not taking any non-randomised paracetamol in the placebo group. The blood samples for the measurement of paracetamol levels were forwarded on to Capital & Coast District Health Board (CCDHB) laboratory by Aotea Pathology for processing. The CCDHB laboratory minimum detectable level for paracetamol was 30 μmol/L.

6.15 Monitoring of Adverse Events

All participants were screened for adverse events, including asthma exacerbations, visits to GP or after-hours clinic and yellowing of the skin or abdominal pain, and serious adverse events, including hospital admissions or life-threatening events, at each study visit. Participants were not asked to report the use of cough and cold preparations as these do not influence the primary outcome variable (American Thoracic Society 2000). Participants were also phoned during week 2 and week 4 of the study to monitor adverse events and ensure that safety blood tests were undertaken. All adverse events were recorded on MRINZ Adverse Event forms (Appendix O) and stored in the study file. All serious adverse events were recorded on MRINZ Serious Adverse Event forms (Appendix P) and notified to the Central Regional Ethics Committee via the CREC serious adverse event notification form (Appendix Q).
6.16 Funding

The principal investigator of the study (S. Eyers) was funded through a Health Research Council of New Zealand Clinical Research Training Fellowship 2009-2012. Further funding for the study was provided through a Health Research Council of New Zealand Project Grant 2010, a Wellington Medical Research Foundation Grant in Aid of Research 2009, and a University of Otago Research Grant 2009.
Chapter Seven: Study Results

7.1 Participants

A CONSORT flow diagram of study participation is shown in Figure 7.1. Recruitment commenced in June 2009 and ended in September 2011. The planned recruitment period of two years was extended by three months due to difficulties in recruitment. Between 6,000 and 8,000 recruitment letters were sent out to non-smoking, adult asthmatics in the Wellington region via the GP network as discussed previously. In addition, over 500 adult asthmatics were contacted through the MRINZ and WARG asthma recruitment databases.

From this initial recruitment drive, there were 724 patients assessed for eligibility at phone screening. Of these 724, 486 did not progress past phone screening. 200/486 declined to participate, and 286 were excluded due to failure to meet inclusion criteria or fulfilment of exclusion criteria. Of these, 128 did not have mild to moderate asthma or had a history of smoking. Seventy-four had contraindications to taking paracetamol or were already using the medication regularly. Thirty-six were excluded due to safety reasons such as a history of a suicide attempt, history of liver disease or being pregnant or breastfeeding. A full list of participants excluded at phone screening is shown in Table 7.1.
Table 7.1: Participants excluded at phone screening

<table>
<thead>
<tr>
<th>N</th>
<th>Reason for Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>Declined to participate</td>
</tr>
<tr>
<td>56</td>
<td>Smoker/ex-smoker</td>
</tr>
<tr>
<td>39</td>
<td>Contraindication to trial/paracetamol (incl: planned surgery, use of contraindicated meds, chronic pain conditions, allergy to NSAIDS/paracetamol)</td>
</tr>
<tr>
<td>37</td>
<td>Asthma too severe</td>
</tr>
<tr>
<td>35</td>
<td>Currently using regular paracetamol</td>
</tr>
<tr>
<td>35</td>
<td>Asthma too mild/no asthma</td>
</tr>
<tr>
<td>34</td>
<td>Unable to travel to study site</td>
</tr>
<tr>
<td>18</td>
<td>History suicide attempt/current depression</td>
</tr>
<tr>
<td>10</td>
<td>Breastfeeding, pregnant, no contraception</td>
</tr>
<tr>
<td>8</td>
<td>Trial period ended</td>
</tr>
<tr>
<td>7</td>
<td>History of liver disease</td>
</tr>
<tr>
<td>6</td>
<td>Age outside range</td>
</tr>
<tr>
<td>1</td>
<td>High weekly alcohol intake</td>
</tr>
</tbody>
</table>
There were 238 participants who progressed through to the screening visit (visit 1/week -1). Of these, 25 were excluded due to safety reasons and 24 did not have mild to moderate asthma or had a history of smoking. Five participants were lost to follow-up following visit 1. A full list of participants excluded at visit 1 is shown in Table 7.2.

Table 7.2: Participants excluded at visit 1

<table>
<thead>
<tr>
<th>N</th>
<th>Reason for Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>FEV$_1$&lt;70% predicted</td>
</tr>
<tr>
<td>11</td>
<td>Raised ALT</td>
</tr>
<tr>
<td>10</td>
<td>History suicide attempt/current depression</td>
</tr>
<tr>
<td>5</td>
<td>Lost to follow up/withdrew consent</td>
</tr>
<tr>
<td>4</td>
<td>Smoker/ex-smoker&gt;10py</td>
</tr>
<tr>
<td>3</td>
<td>Contraindication to trial/paracetamol (incl: planned surgery, use of contraindicated meds, chronic pain conditions, allergy to NSAIDS/paracetamol)</td>
</tr>
<tr>
<td>2</td>
<td>High weekly alcohol intake</td>
</tr>
<tr>
<td>1</td>
<td>No contraception</td>
</tr>
<tr>
<td>1</td>
<td>High blood pressure</td>
</tr>
<tr>
<td>1</td>
<td>Age outside range</td>
</tr>
</tbody>
</table>
There were 181 participants randomised prior to visit 2 based on initial eligibility at visit 1; 91 to the paracetamol group and 92 to the placebo group. 53 out of 91 participants allocated to the paracetamol group and 34 out of 92 allocated to the placebo group were withdrawn at the second visit as they did not meet the eligibility criteria at visit 2. 68 participants had a PC$_{20}$ to methacholine greater than 16 mg/ml and three participants had a PC$_{20}$ to methacholine below 0.125 mg/ml. Nine participants were lost to follow-up or withdrew consent and seven had an FEV$_1$ less than 70% predicted. No study medication was dispensed to the participants who were withdrawn at the second visit. A full list of participants excluded at visit 2 is shown in Table 7.3.

**Table 7.3: Participants excluded at visit 2**

<table>
<thead>
<tr>
<th>N = 87</th>
<th>Reason for Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td>PC$_{20}$ &gt; 16 mg/ml</td>
</tr>
<tr>
<td>9</td>
<td>Lost to follow up/withdrew consent</td>
</tr>
<tr>
<td>7</td>
<td>FEV$_1$ &lt; 70% predicted</td>
</tr>
<tr>
<td>3</td>
<td>PC$_{20}$ &lt; 0.0125 mg/ml</td>
</tr>
</tbody>
</table>
Figure 7.1: Participant flow diagram
As a result, medication was dispensed to 36 participants in the paracetamol group and 58 participants in the placebo group who commenced the intervention phase following visit 2. The demographic characteristics of the participants in whom study medication was dispensed is shown in Table 7.4. The mean age of participants was 40 years and there were 35 male participants. Mean BMI was 26.7 kg/m$^2$. Approximately 30% of study participants were prescribed inhaled corticosteroids, 94% were prescribed SABAs and 18% were prescribed LABAs. Approximately 90% of participants were atopic to either cat fur, grass or house dust mite on skin prick testing.

Table 7.4: Baseline demographic characteristics of study participants
Data are presented as mean ± SD, unless otherwise indicated.

<table>
<thead>
<tr>
<th></th>
<th>Paracetamol Group</th>
<th>Placebo Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>36</td>
<td>58</td>
</tr>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age, y ± SD</td>
<td>41.5 ±13.9</td>
<td>38.3 ± 12.5</td>
</tr>
<tr>
<td>Male sex, No. (%)</td>
<td>15 (41.7)</td>
<td>20 (34.5)</td>
</tr>
<tr>
<td>Weight, Kg ± SD</td>
<td>75.1 ± 16.7</td>
<td>77.4 ± 17.8</td>
</tr>
<tr>
<td>Height, m ± SD</td>
<td>1.7 ± 0.1</td>
<td>1.69 ± 0.11</td>
</tr>
<tr>
<td>BMI, Kg/m$^2$ ± SD</td>
<td>25.9 ± 4.2</td>
<td>27.1 ± 5.8</td>
</tr>
<tr>
<td><strong>Medication Use</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICS, No. (%)</td>
<td>9 (25%)</td>
<td>20 (34%)</td>
</tr>
<tr>
<td>SABA, No. (%)</td>
<td>33 (92%)</td>
<td>55 (95%)</td>
</tr>
<tr>
<td>LABA, No. (%)</td>
<td>9 (25%)</td>
<td>8 (14%)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI = Body Mass Index, ICS = Inhaled Corticosteroid, SABA = Short-Acting Beta Agonist, LABA = Long-Acting Beta Agonist.
The baseline characteristics of the participants in whom study medication was dispensed is shown in Table 7.5. Participants had a baseline mean ACQ score of 0.86 (SD 0.59). Baseline mean FEV$_1$ was 3.11 L (SD 0.83) overall, representing 94% of predicted FEV$_1$ values (SD 12.0). The baseline mean FeNO was 48.9 ppb (SD 41.3). The baseline mean Log FeNO was 3.61 ppb (SD 0.75). The raw baseline mean PC$_{20}$ was 4.29 mg/ml (SD 4.54), representing a baseline mean logarithm base 2 PC$_{20}$ of 1.17 mg/ml (SD 1.80). Whilst IL-4, IL-5 and IL-13 were measured at baseline, only a small proportion of participants had detectable levels (Table 7.8) and hence these values were not reported as they were not representative of the overall group mean.
Table 7.5: Baseline characteristics of study participants

<table>
<thead>
<tr>
<th></th>
<th>Paracetamol Group#</th>
<th>Placebo Group#</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>36</td>
<td>58</td>
</tr>
<tr>
<td><strong>Clinical and Physiological Measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV$_1$, L ± SD</td>
<td>3.09 ± 0.78</td>
<td>3.12 ± 0.87</td>
</tr>
<tr>
<td>FEV$_1$ % Predicted ± SD</td>
<td>94.1 ± 11.3</td>
<td>94.0 ± 12.4</td>
</tr>
<tr>
<td>Bronchodilator Reversibility (%)</td>
<td>9.1 ± 6.0</td>
<td>7.8 ± 5.5</td>
</tr>
<tr>
<td>PC$_{20}$ MCh, mg/ml ± SD</td>
<td>4.14 ± 4.42</td>
<td>4.39 ± 4.66</td>
</tr>
<tr>
<td>Log 2 PC$_{20}$ MCh, mg/ml ± SD</td>
<td>1.30 ± 1.50</td>
<td>1.09 ± 1.96</td>
</tr>
<tr>
<td>Mean Morning Peak Flow</td>
<td>424.0 ± 83.8</td>
<td>419.5 ± 92.3</td>
</tr>
<tr>
<td>PEF$_{var}$, %, ± SD</td>
<td>19.0 ± 9.3</td>
<td>22.2 ± 10.5</td>
</tr>
<tr>
<td>ACQ Score ± SD</td>
<td>0.93 ± 0.63</td>
<td>0.82 ± 0.56</td>
</tr>
<tr>
<td><strong>Inflammatory and Immunological Measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPT Cat, No. (% +ve)</td>
<td>20 (55.6)</td>
<td>33 (57.9)</td>
</tr>
<tr>
<td>SPT Dust, No. (% +ve)</td>
<td>30 (83.3)</td>
<td>52 (91.2)</td>
</tr>
<tr>
<td>SPT Grass, No. (% +ve)</td>
<td>25 (69.4)</td>
<td>38 (66.7)</td>
</tr>
<tr>
<td>SPT Positive, No. (% +ve)</td>
<td>33 (91.7)</td>
<td>55 (96.5)</td>
</tr>
<tr>
<td>SPT Negative, No. (% +ve)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>FeNO, ppb ± SD</td>
<td>44.9 ± 39.2</td>
<td>51.3 ± 42.6</td>
</tr>
<tr>
<td>Eosinophils, x 10$^9$/L, ± SD</td>
<td>0.26 ± 0.12</td>
<td>0.32 ± 0.17</td>
</tr>
<tr>
<td>IgE, kU/L, ± SD</td>
<td>518.4 ± 705.7</td>
<td>480.4 ± 914.0</td>
</tr>
</tbody>
</table>

Abbreviations: FEV$_1$ = Forced Expiratory Volume in 1 sec, SPT = Skin Prick Test, PC$_{20}$ MCh= Provocation Concentration of Methacholine Causing a 20% fall in FEV$_1$, PEF$_{var}$ = Peak Flow Variability, ACQ = Asthma Control Questionnaire, FeNO = Fractional Exhaled Nitric Oxide, IFN-γ = Interferon-gamma

#Mean unless otherwise stated
Following commencement of the intervention phase, five participants were withdrawn from the paracetamol group. Two withdrew at the participant’s own discretion, one was excluded due to a raised ALT greater than three times the upper limit of normal (119 IU/L), one was lost to follow-up and one was excluded due to intercurrent illness. Four participants were withdrawn from the placebo group; two were excluded due to a raised ALT greater than three times the upper limit of normal (207 and 227 IU/L respectively), one withdrew at the participant’s own discretion and one was lost to follow-up. In all, 85 participants completed the study and were included in the analyses.

7.2 Primary Outcome Variable

The main results for primary and secondary outcome variables are shown in Table 7.6 and 7.7. The baseline mean PC\textsubscript{20} in the paracetamol group was 4.14 mg/ml (SD 4.42) and at week 12 was 5.89 mg/ml (SD 16.6). The baseline mean PC\textsubscript{20} in the placebo group was 4.39 mg/ml (SD 4.66) and at week 12 was 6.62 mg/ml (SD 12.4). Following transformation on the logarithmic scale, the baseline mean logarithm base 2 PC\textsubscript{20} in the paracetamol group was 1.30 (SD 1.50) at baseline and at week 12 was 0.62 (SD 2.09). The baseline mean logarithm base PC\textsubscript{20} in the placebo group was 1.09 (SD 1.96) at baseline and at week 12 was 1.07 (SD 2.36). After controlling for the baseline PC\textsubscript{20}, the difference (expressed as a doubling dose reduction, paracetamol minus placebo) was not statistically significant: -0.48 (95% CI -1.28 to 0.32), P=0.24 (Figure 7.2).
Figure 7.2: Log base 2 PC20 versus time
Mean ± one standard deviation
Solid line = control, dashed line = paracetamol
7.3 Secondary Outcome Variables

The baseline mean FEV$_1$ in the paracetamol group was 3.06 L (SD 0.73) and at week 12 was 3.01 L (SD 0.74). The baseline mean FEV$_1$ in the placebo group was 3.05 L (SD 0.83) and at week 12 was 3.07 L (SD 0.86). After controlling for the baseline FEV$_1$, the difference was not statistically significant (paracetamol minus placebo): -0.07 L (95% CI -0.15 to 0.01), P=0.08 (Figure 7.3).

Figure 7.3: Pre-bronchodilator FEV1 versus time

Mean ± one standard deviation
Solid line = control, dashed line = paracetamol
The baseline mean FeNO in the paracetamol group was 44.9 ppb (SD 41.3) and at week 12 was 49.9 ppb (SD 33.4). The baseline mean FeNO in the placebo group was 51.3 ppb (SD 39.2) and at week 12 was 49.9 ppb (SD 36.1). The baseline mean logarithm FeNO in the paracetamol group was 3.53 ppb (SD 0.71) at baseline and at week 12 was 3.69 (SD 0.70). The baseline mean logarithm FeNO in the placebo group was 3.66 ppb (SD 0.78) at baseline and at week 12 was 3.65 ppb (SD 0.76). After controlling for the baseline log FeNO, the difference was not statistically significant (paracetamol minus placebo): 0.09 (95% CI -0.097 to 0.27), P=0.36 (Figure 7.4).

Figure 7.4: Log FeNO versus time

Mean ± one standard deviation
Solid line = control, dashed line = paracetamol
The baseline mean ACQ score in the paracetamol group was 0.81 (SD 0.47) and at week 12 was 0.88 (SD 0.56). The baseline mean ACQ score in the placebo group was 0.93 (SD 0.59) and at week 12 was 1.03 (SD 0.71). After controlling for the baseline ACQ, the difference was not statistically significant (paracetamol minus placebo): -0.04 (95% CI -0.27 to 0.18), P=0.71 (Figure 7.5).

Figure 7.5: ACQ Score versus time

Mean ± one standard deviation
Solid line = control, dashed line = paracetamol
The baseline mean morning peak flow in the paracetamol group was 424.0 (SD 83.8) and at week 12 was 417.1 (SD 82.3). The baseline mean morning peak flow in the placebo group was 419.5 (SD 92.3) and at week 12 was 417.5 (SD 85.9). After controlling for the baseline mean morning peak flow, the difference was not statistically significant (paracetamol minus placebo): -9.6 (95% CI -28.2 to 9.1), P=0.31 (Figure 7.6).

Figure 7.6: Mean morning peak flow versus time

Mean ± one standard deviation
Solid line = control, dashed line = paracetamol
The baseline mean $\text{PEF}_{\text{var}}$ in the paracetamol group was 19.0% (SD 9.30) and at week 12 was 20.4% (SD 10.3). The baseline mean $\text{PEF}_{\text{var}}$ in the placebo group was 22.2% (SD 10.50) and at week 12 was 21.7% (SD 11.7). After controlling for the baseline $\text{PEF}_{\text{var}}$, the difference was not statistically significant (paracetamol minus placebo): 0.21% (95% CI -4.30 to 4.80), $P=0.93$ (Figure 7.7).

**Figure 7.7: PEF\text{var} versus time**

Mean ± one standard deviation
Solid line = control, dashed line = paracetamol
There were three asthma exacerbations in the placebo group and none in the paracetamol group, an absolute difference of 5.6% (95% CI -0.5 to 11.7%). There was no statistically significant difference from baseline to week 12 between the paracetamol and placebo group in logarithm eosinophil count or logarithm IgE levels. Log eosinophil (paracetamol minus placebo): -0.056 (95% CI -0.25 to 0.14), P = 0.57; log IgE (paracetamol minus placebo): 0.098 (95% CI 0.009 to 0.21), P = 0.073.

IFN-γ, IL-4, IL-5 and IL-13 levels were measured throughout the trial, however the results were unreliable due to the small number of participants who had detectable levels at any point in during the study, and were hence not reported (number of participants with detectable levels of cytokines, Table 7.8).

Measured via pill count and medication diary review, medication compliance was 93.2% in the control group and 90.8% compliance in the paracetamol group, difference 2.4% (95% CI -1.0 to 5.8), P = 0.17. Ten out of 32 participants in the paracetamol group had detectable levels (greater than 30 μmol/L) of paracetamol in blood tests at week 2, 11 out of 31 at visit 3 and 12 out of 31 at visit 4. No participants in the control group had detectable levels of paracetamol (greater than 30 μmol/L) in blood tests at week 2, visit 3 or visit 4.
### Table 7.6: Results (1)

<table>
<thead>
<tr>
<th>Variable (Mean (SD) unless otherwise stated)</th>
<th>Baseline</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo= 58</td>
<td>Placebo= 54</td>
</tr>
<tr>
<td></td>
<td>Paracetamol = 36</td>
<td>Paracetamol = 31</td>
</tr>
</tbody>
</table>

Comparison of Variables Visit 2 to Visit 4
Paracetamol minus placebo (95% CI)
(Adjusted for Visit 2)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Comparison</th>
<th>Placebo</th>
<th>Paracetamol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC&lt;sub&gt;20&lt;/sub&gt;, mg/ml</td>
<td>4.39 (4.66)</td>
<td>4.14 (4.42)</td>
</tr>
<tr>
<td></td>
<td>Log 2 PC&lt;sub&gt;20&lt;/sub&gt;, mg/ml</td>
<td>1.09 (1.96)</td>
<td>1.30 (1.50)</td>
</tr>
<tr>
<td></td>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;, L</td>
<td>3.05 (0.83)</td>
<td>3.06 (0.73)</td>
</tr>
<tr>
<td></td>
<td>FeNO, ppb</td>
<td>51.3 (42.6)</td>
<td>44.9 (39.2)</td>
</tr>
<tr>
<td></td>
<td>Log FeNO, ppb</td>
<td>3.66 (0.78)</td>
<td>3.53 (0.71)</td>
</tr>
<tr>
<td></td>
<td>ACQ score</td>
<td>0.93 (0.59)</td>
<td>0.81 (0.47)</td>
</tr>
<tr>
<td></td>
<td>Mean morning peak flow, L/min</td>
<td>419.5 (92.3)</td>
<td>424.0 (83.8)</td>
</tr>
<tr>
<td></td>
<td>PEF&lt;sub&gt;vars&lt;/sub&gt;, %</td>
<td>22.2 (10.50)</td>
<td>19.0 (9.30)</td>
</tr>
<tr>
<td></td>
<td>Exacerbations</td>
<td>N/A</td>
<td>3 (5.6)</td>
</tr>
</tbody>
</table>

Abbreviations: over page
Table 7.7: Results (2)

<table>
<thead>
<tr>
<th>Variable (Mean (SD))</th>
<th>Baseline</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo = 58</td>
<td>Placebo = 54</td>
</tr>
<tr>
<td></td>
<td>Paracetamol = 36</td>
<td>Paracetamol = 31</td>
</tr>
</tbody>
</table>

Comparison of Variables Visit 2 to Visit 4
Paracetamol minus Placebo (95% CI, p-value)
(Adjusted for Visit 2)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Paracetamol</th>
<th>Paracetamol minus Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophil x 10^9 ppb</td>
<td>0.32 (0.17)</td>
<td>0.26 (0.12)</td>
<td>-0.06 (0.15)</td>
</tr>
<tr>
<td>Log Eosinophil x 10^9 ppb</td>
<td>-1.27 (0.53)</td>
<td>-1.41 (0.47)</td>
<td>-0.14 (0.06)</td>
</tr>
<tr>
<td>IgE, kU/L</td>
<td>480.4 (914.0)</td>
<td>518.4 (705.7)</td>
<td>0.098 (0.21)</td>
</tr>
<tr>
<td>Log IgE, kU/L</td>
<td>5.29 (1.30)</td>
<td>5.28 (1.52)</td>
<td>0.098 (0.21)</td>
</tr>
</tbody>
</table>

Abbreviations (Table 7.6 and 7.7):

PC_{20} MCh = Provocation Concentration of Methacholine Causing a 20% fall in FEV\textsubscript{1}, FEV\textsubscript{1} = Forced Expiratory Volume in 1 sec, FeNO = Fractional Exhaled Nitric Oxide, ACQ = Asthma Control Questionnaire, PEF\textsubscript{var} = Peak Flow Variability, Exacerbations = Asthma Exacerbations, IgE = Immunoglobulin G.
### Table 7.8: Number of participants with detectable levels of cytokines

<table>
<thead>
<tr>
<th>Variable (detectable level)</th>
<th>N/N (%)</th>
<th>Paracetamol</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ (12.5 pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 2</td>
<td>21/36 (58.3)</td>
<td>33/58 (56.9)</td>
<td></td>
</tr>
<tr>
<td>Visit 3</td>
<td>25/29 (86.2)</td>
<td>40/55 (72.7)</td>
<td></td>
</tr>
<tr>
<td>Visit 4</td>
<td>26/31 (83.9)</td>
<td>44/53 (83.0)</td>
<td></td>
</tr>
<tr>
<td>IL-4 (27.7 pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 2</td>
<td>1/36 (2.8)</td>
<td>3/58 (5.2)</td>
<td></td>
</tr>
<tr>
<td>Visit 3</td>
<td>4/29 (13.8)</td>
<td>1/55 (1.8)</td>
<td></td>
</tr>
<tr>
<td>Visit 4</td>
<td>2/31 (6.5)</td>
<td>2/53 (3.8)</td>
<td></td>
</tr>
<tr>
<td>IL-5 (3.5 pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 2</td>
<td>1/36 (2.8)</td>
<td>2/58 (3.5)</td>
<td></td>
</tr>
<tr>
<td>Visit 3</td>
<td>3/29 (10.3)</td>
<td>4/55 (7.8)</td>
<td></td>
</tr>
<tr>
<td>Visit 4</td>
<td>3/31 (9.7)</td>
<td>0/53 (0)</td>
<td></td>
</tr>
<tr>
<td>IL-13 (55.3 pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 2</td>
<td>0/36 (0)</td>
<td>4/58 (6.9)</td>
<td></td>
</tr>
<tr>
<td>Visit 3</td>
<td>1/29 (3.5)</td>
<td>3/55 (5.5)</td>
<td></td>
</tr>
<tr>
<td>Visit 4</td>
<td>1/31 (3.2)</td>
<td>7/53 (13.2)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: IFN-γ = Interferon gamma, IL-4/5/13 = Interleukin 4/5/13
7.4 Liver Function Monitoring

The baseline mean ALT in the paracetamol group was 21.4 IU/L (SD 7.9) and at week 12 was 25.4 IU/L (SD 9.7). The baseline mean ALT in the placebo group was 19.4 IU/L (SD 7.5) and at week 12 was 19.0 IU/L (SD 6.0). After controlling for the baseline ALT, there was a statistically significant difference between the two groups (paracetamol minus placebo): 6.3 IU/L (95% CI 2.9 to 9.7), P < 0.001.

Figure 7.7: ALT versus time
Mean ± one standard deviation
Solid line = control, dashed line = paracetamol
(Note: scale of ALT is because of outlying value of 227)
7.5 Calibration and Validation and Storage

Manufacturer used-by dates were adhered to for methacholine, saline, SPT allergens and pregnancy test kits. Opened vials of methacholine and SPT allergens were kept refrigerated as per manufacturer specifications, with a temperature range over the testing period of 3.7 to 7.2 ºC. There were four spikes in refrigerator temperatures outside of the range during the testing period; two spikes to 8.2 ºC, one to 9 ºC and one to 12 ºC. These four incidences occurred during monitoring or restocking of refrigerator and all lasted for under 20 minutes.

Dry methacholine, salbutamol, saline and pregnancy tests were all kept in storage at temperatures in keeping with manufacturer specifications, with a temperature range over the testing period of 20.1 to 25 ºC. There were four spikes in dry store temperatures outside of the range during the testing period; two spikes to 26.2 ºC which lasted for 12 hours, one to 26.7 ºC which lasted 36 hours and one to 27.2 ºC which lasted 36 hours, all of which occurred as a result in air-conditioning malfunction in the dry store room.

Wright nebulizer flow rates were altered at two points throughout the trial to maintain accurate output. Otherwise, flow rates and outputs remained stable for the duration of the trial. There was less than +/- 3% difference to airflow at all calibrations of the spirometer.
8.1 Main Findings

This double-blind, randomised, placebo-controlled, parallel group pilot study found no statistically significant reduction in PC$_{20}$ with 12-weeks of paracetamol treatment. However, the results did not rule out a possible effect, with the 95% confidence interval containing the pre-specified difference of one doubling dose reduction in PC$_{20}$. There were no significant differences observed in any of the pre-specified secondary outcome variables of asthma control or inflammatory and immunological markers.

Although the pre-specified one doubling dose worsening in PC$_{20}$ was not demonstrated, the reduction in PC$_{20}$ of 0.48 of a doubling dose could nevertheless be clinically significant. Previous research has highlighted the importance of interpreting negative studies of BHR in terms of the change in PC$_{20}$ the study was powered to achieve (in our case, a large change), versus what is considered to be a clinically significant change in PC$_{20}$ (Inman, Hamilton et al. 1998). As shown by Mitchell (Mitchell 1989) (Figure 8.1), a small reduction in PC$_{20}$ can lead to an increase in the frequency of severe asthma in the population, which is potentially of major public health significance. Represented on the log scale for BHR to histamine, a small reduction in the dose of histamine causing a 20% reduction in FEV$_{1}$ (represented on the x-axis), causes a much larger effect on the frequency distribution of severe asthma
in the population, compared with mild to moderate asthma (represented on the y-axis) (Figure 8.1).

Figure 8.1: Frequency distribution of asthma severity as measured by the cumulative dose of histamine which causes a 20% fall in FEV₁ (Mitchell 1989). (Reproduced with the permission of BMJ Publishing Group Ltd.)
Likewise, although the FeNO results were not statistically significant, a non-statistically significant increase of 0.09 (9%) in FeNO in the paracetamol group compared to the placebo group from baseline to week 12 was demonstrated. Given that the mean FeNO value of 48.9 ppb was greater than the 35 ppb limit that is indicative of asthma in a steroid naïve patient with respiratory symptoms (Lim and Mottram 2008), and that a change in FeNO of 4 ppb is considered clinically significant (Keen, Olin et al. 2007), the difference of 9% observed between the two study groups would have been clinically relevant if statistically significant. Given the confidence interval which shows a possible effect size from between a 27% worsening in FeNO in the paracetamol group to a 10% improvement in FeNO in the paracetamol group, a true difference between the two groups can not be ruled out. No significant effect was seen in the systemic inflammatory and immunological markers, including IgE and eosinophils, however cytokine results were unable to be analysed because of the low number of participants with detectable levels.

Of interest, was the fact that there was three times the number of asthma exacerbations in the placebo group compared to the paracetamol group, however this difference was not statistically significant. The results of the liver function monitoring showed a statistically significant increase in mean ALT levels of 6.3 IU/L from baseline to week 12 in the paracetamol group compared with the placebo group. This demonstrates that paracetamol, taken at half the recommended daily dose over a 12 week period, has an effect on liver transaminases. This has important implications for future study designs in which this dose of paracetamol or greater may be used over a similar time period. However, mean ALT levels remained within the normal limits in both study groups, and only elevations in ALT of greater than three times the upper
limit of normal are indicative of hepatotoxicity (Watkins, Lkaplowitz et al. 2006). Therefore, while a statistically significant difference in mean ALT between the two groups was observed, the difference is not clinically significant. One participant in the paracetamol group and two participants in the placebo group had increases in ALT to greater than three times the upper limit of normal, and these three participants were excluded from the study and referred for consultation and follow up with their general practitioner. While it is interesting that the placebo group had a higher number of participants with an ALT greater than three times the upper limit of normal, the study was not sufficiently powered to analyse this further.

Important baseline participant characteristics were incorporated as co-variates in the multivariate analysis. Differences in baseline characteristics were not analysed statistically because the study was designed to detect a nominated difference in primary and secondary outcome variables and hence the comparison of baseline variables would be likely to introduce a large number of unquanifiable type II errors. Further, testing all possible baseline variables grossly inflates the type I error rate for the study as a whole.

8.2 Comparison with Previous Studies

There have been no randomised placebo-controlled trials published which have investigated the effect of long term paracetamol treatment on adult asthma severity and/or control, and as such, the results of this study are the first of their kind. An abstract, presented to the European Respiratory Society in 2010 (Kodgule, Kapoor et
al. 2010), reported the interim findings of 14 of a planned 30-participant randomised
placebo-controlled pilot study of adult asthmatics receiving 2 g per day of
paracetamol for 15 days. A significant increase in total airways resistance (R5Hz) and
proximal airways resistance (R20Hz,) measured by impedance oscillometry, was
demonstrated in the paracetamol group (0.29 Hz, 95% CI 0.04 to 0.54; p=0.02 and
0.17 Hz, 95% CI 0.06 to 0.30, p=0.008 respectively). There was a small decline in
FEV₁ in the paracetamol group (114 ml) compared with the placebo group (-30 ml)
but this difference was not statistically significant. No significant changes were found
in PC₂₀ MCh. Notwithstanding the limitations of such a low-powered study, and
pending publication of the full study findings, these interim results suggest that
paracetamol may have adverse effects on airway function.

The only other published randomised controlled trial of paracetamol and asthma was
the Boston University Fever Study (Lesko, Louik et al. 2002), which was undertaken
to investigate the effect of short-term paracetamol use on childhood asthma. In this
study, 1879 asthmatic children, aged between six months and 12 years, were
randomly assigned to receive either paracetamol or ibuprofen for fever control when
required over a 4 week period. Children randomised to the ibuprofen group had a
reduced risk of having an outpatient visit for asthma during the 4 week study period
(OR 0.56, 95% CI 0.34 to 0.95) compared with children in the paracetamol group.
There was also a dose-response effect noted, with a higher cumulative incidence of an
outpatient visit for asthma associated with paracetamol doses of 11 mg/kg or more.
Because the study did not include a placebo treatment, it was not possible to
determine whether the observed difference in morbidity according to treatment group
was attributable to a positive effect of paracetamol or a negative effect of ibuprofen.
This consideration is important as there is evidence that non-steroidal anti-inflammatory drugs may have some protective effect against the development of asthma (Varner, Busse et al. 1998; Barr, Wentowski et al. 2004; Barr, Kurth et al. 2007; Barr 2008; Kurth, Barr et al. 2008).

8.3 Methodological issues

8.3.1 Study Power

Several methodological issues are relevant to the interpretation of the study findings. Most important, this trial was powered only to determine whether there was a pronounced effect on BHR of at least one doubling dose reduction in PC$_{20}$ Methacholine. There were several main factors that affected the study’s ability to achieve the designated power. First, it was not possible to achieve the planned sample size of 132 participants, despite a rigorous recruitment campaign during which approximately 6,000 to 8,000 adult asthmatics in the Wellington region were contacted, and over 700 volunteers were screened. Of those screened, approximately one-third declined to participate. This was likely due to the heavy time commitment of the study and the high number of blood tests required, as well as a reticence to take medication if not required for health reasons. Approximately another third failed eligibility because their symptoms were either too mild or too severe, or they had a history of smoking which predisposed them to concomitant COPD. These subjects were excluded in order to attain an appropriate study population of mild to moderate asthmatics. The majority of other volunteers who were not eligible for the study failed
to meet important safety eligibility criteria, due to factors such as contraindications to paracetamol use, risk to foetus, or risk of hepatotoxicity. It was necessary to exclude these subjects to ensure that the study was safe and ethically sound.

The second factor affecting the study power was that the variability in PC$_{20}$ from baseline to week 12 was larger than anticipated. We experienced a pooled SD of 2.27 doubling doses compared to the SD of 1.9 used in the sample size calculation, which was derived from previous studies (Chinn and Schouten 2005). The larger variability was likely due to the length of the study period, as it has been suggested that there is an increase in within-person variation in PC$_{20}$ with increasing study length, although this has not been formally correlated (Chinn and Schouten 2005). This issue may be dealt with by designing a study of shorter duration, as short-term studies have been found to have excellent repeatability, with within person standard deviations of approximately 0.5 in studies of up to two weeks duration (Chinn and Schouten 2005).

The third factor affecting the study power was that the randomisation process resulted in an unbalanced distribution between the two treatment groups, with 36 participants recruited to the paracetamol group and 58 to the placebo group. It was necessary to notify the hospital pharmacy several days prior to a participant’s second visit, in order to allow the pharmacy adequate time to prepare the study medication so that it was available immediately after visit 2, once participants were shown to be eligible for the study. This required randomising participants prior to their final screening at visit 2. It was anticipated that most participants who passed the eligibility criteria at visit 1 would also pass the eligibility criteria at visit 2; however, the number of participants who had a PC$_{20}$ greater than 16 mg/ml and failed screening as a result was
underestimated. This led to an unequal number of participants in the two study groups, and although the assignment of treatment groups remained a random process, it is likely that this quirk of randomisation caused some impact on the study power. This difficulty with randomisation may have been avoided by undertaking a cross-over study design, however this was not considered because of concerns over the safety of repeat BHR testing in our study population, as well as the potential for a cross-over effect of paracetamol on primary and secondary outcomes.

**8.3.2 Study Participants**

The first issue to consider is external validity. Our age criteria were wide (18 to 65 years), and participants were recruited through a broad range of recruitment strategies, including from 9 medical centre databases from different geographical areas throughout Wellington, newspaper and radio advertising, Health TV and research databases. Participants also had a range of asthma severity from mild through to moderate: although no severe asthmatics were eligible for the trial (based on exclusion criteria of a history of an asthma exacerbation in the previous two months requiring prednisone or a nebulised bronchodilator, an FEV₁ less than 70% predicted or a PC₂₀ less than 0.125 mg/ml at visit 2). Over 80% of asthmatics are classified as having mild to moderate asthma, and thus the severity of asthma in our study population was representative of the majority of asthma sufferers (Dusser, Montani et al. 2007). Baseline characteristics of the study participants were clearly described, allowing clinicians to determine the generalisability of the study findings to their patients (Rothwell 2005). Further, the primary outcome variable was a well
recognised diagnostic and research tool for asthma (Sterk, Fabbri et al. 1993), and therefore is an outcome that is relevant and understandable for physicians.

Although there were a large number of exclusion criteria for the study, some of the exclusion factors affected small numbers only, such as high alcohol intake (number excluded = 3), low BMI (number excluded = 0) and elevated blood pressure (number excluded = 1). As described in section 8.3.1, the most common reasons for ineligibility were failure to meet asthma criteria or safety issues such as risk of hepatotoxicity, risk to fetus or offspring in pregnant or breastfeeding women, or contraindications to paracetamol. It was important to exclude participants with these attributes due to safety reasons and for the internal validity of the trial. However, because the four study visits were performed in Wellington, it was difficult for volunteers living outside of Wellington to take part in the study. The single-site study design and the urban asthmatic population studied may limit the generalisability of the findings of the study to international and/or rural populations. Also, despite a large recruitment campaign of 27 months duration, only 94 participants were randomised. This was despite approximately 20 to 30% of the adult asthmatics in the Wellington population being contacted to take part in the trial, based on an asthma prevalence of 20% (Holt S and Beasley R December 2001), and a population of approximately 140,000 adults between the ages 18 and 65 years. This low recruitment rate has the potential to affect the generalisability of the study, as it is possible that the participants who volunteered to take part were in some way different from the rest of the adult asthmatic population. Further, the results of the study will not be generalisable to patterns of paracetamol intake other than regular, long-term use, such as the pattern of intermittent use of high-dose paracetamol during periods of self-limiting illness.
A further issue to consider is the comparability of the treatment and control groups. As described above, there was an uneven distribution of study participants between the paracetamol and placebo groups. However, participants in each group had comparable baseline FEV$_1$, PC$_{20}$, ACQ and bronchodilator reversibility results. After the second visit, exclusion & withdrawal rates were equivalent between the two groups, with five in the paracetamol group and four in the placebo group, and medication compliance (measured via pill count and diary check) was equivalent in the two groups.

**8.3.3 Study Design**

Our 12-week dosing period was chosen based on evidence that regular, long-term use of paracetamol is associated with an increased risk of asthma in adults (Shaheen, Sterne et al. 2000; Barr, Wentowski et al. 2004; McKeever, Lewis et al. 2005; Shaheen, Potts et al. 2008; Thomsen, Kyvik et al. 2008) and that chronic ingestion of therapeutic doses of paracetamol can reduce serum antioxidant capacity in as little as two weeks (Nuttall, Khan et al. 2003). It also reflected the common pattern of paracetamol use for long-term relief of chronic pain such as that caused by arthritis. We had originally considered using the maximum recommended dose of paracetamol (4 g/day), however we chose to administer half this dose due to concerns of liver toxicity. These concerns were based on a previous clinical trial of paracetamol in healthy participants taking 4 g/day for 14 days, in which the incidence of ALT elevations to more than three times the upper limit of normal was 31 to 44% (Watkins, Lkaplowitz et al. 2006). The decision to administer only half the
recommended maximum daily dose was supported by our findings, which showed a statistically significant elevation in ALT (of 6.2 IU/L) in participants who took 2 g paracetamol per day for 12 weeks.

Methacholine challenge testing was chosen as the primary outcome variable as it represents the most widely used, reproducible and validated direct measure of BHR (Juniper, Frith et al. 1978; Sterk, Fabbri et al. 1993; Chinn and Schouten 2005). However, there is evidence to suggest that indirect stimuli (such as adenosine monophosphate (AMP), hypertonic saline or exercise challenge), may better reflect airways inflammation and are more responsive to changes in asthma severity that may occur as a result of pharmacological therapy (Joos, O'Connor et al. 2003). However, as we also measured FeNO as a marker of airways inflammation, and serum cytokines and blood eosinophils as markers of systemic inflammation, we preferred to use the direct measure of methacholine challenge.

One issue which arose during the study regarding methacholine challenge testing was the large number of participants who failed to reach \( \text{PC}_{20} \) by the 16 mg/ml concentration (thereby displaying normal bronchial responsiveness (McCormack and Enright 2008)). Considering that all participants were required to have a doctor’s diagnosis of asthma and asthma symptoms (wheeze) within the previous 12 months in order to be enrolled in the study, the pre-test probability of asthma in the study population (the likelihood that the participant had asthma before the methacholine challenge test results were considered) was relatively high compared with the general population (American Thoracic Society 2000). However, following methacholine
challenge testing during which a PC$_{20}$ higher than 16 mg/ml is obtained, post-test probability of asthma is low (Figure 8.2).

Figure 8.2: Curve illustrating pre-test and post-test probability of asthma after a methacholine challenge test with four PC$_{20}$ values (American Thoracic Society 2000)

It is well recognised that asthma is over-diagnosed (Burney 2002) and, because we sought to recruit mild asthmatics, it is likely that a proportion of our volunteers with ‘mild asthma’ did not in fact have the disease. However, several other factors may explain the high rate of negative BHR results seen. First, it is possible that bronchial responsiveness was suppressed in the study population, in whom a proportion was using regular ICS therapy. The ATS recommend against withholding anti-inflammatory medication prior to BHR testing and it was important that the participants remained on stable ICS doses throughout the study. However, anti-inflammatory medication has been shown to decrease bronchial responsiveness (Juniper, Kline et al. 1990). In participants without current symptoms, it is also possible that testing occurred during a period of low aeroallergen exposure when symptoms and hyperresponsiveness were low (American Thoracic Society 2000). Finally, a small proportion of asthmatics (usually those with occupational asthma) may only react to a specific sensitiser, and therefore have a negative response to methacholine challenge testing.

Another issue relevant to the interpretation of the study results is the low number of participants who had detectable serum interleukin levels, both at baseline and at the times measured throughout the study. Our decision to monitor cytokines was based on previous asthma studies, which have shown significant changes in cytokine levels due to various interventions (Vliagoftis, Kouranos et al. 2008; Li and Brown 2009). However, most of our study population had undetectable levels of cytokines at baseline and therefore it was not possible to undertake any meaningful analysis of the IFN-γ, IL-4, IL-5 and IL-13 measurements. The low levels of interleukins may
possibly have been due to the stability of asthma symptoms in our study population, as the inclusion criteria of the study required participants to have stable, non-severe asthma. It is also possible that blood levels of cytokines did not adequately reflect cytokine levels in the respiratory system, as experimental studies have determined that cytokine responses are often compartmentalised to the lung (Nelson, Wald et al. 1999). However, other research has demonstrated significantly increased blood levels of IL-4 and IL-5 at various time points during allergic eosinophilic inflammation (Ohkawara, Lei et al. 1997).

8.3.4 Medication Compliance

One source of particular interest in the study was the results relating to medication compliance. When compliance was measured via pill count and diary check, the mean compliance in the two groups over the 12 week study period were similar; 92.5% in the paracetamol group and 93.6% in the placebo group. However, when paracetamol blood levels were checked at week 2, visit 3 and visit 4, only approximately one third of the paracetamol group had detectable levels of paracetamol in the blood at any time.

The reasons for this discrepancy may be multiple. The CCDHB laboratory cut-off for a detectable paracetamol level is set at 30 μmol/L, due to the nature of clinical paracetamol level testing which assesses toxicity (where the range of blood paracetamol levels indicating treatment is in excess of 1000 μmol/L four hours following ingestion (Daly, Fountain et al. 2008)). The CCDHB laboratory also sets this cut-off limit for clinical trials, as the reliability of results below this level have not
been proven following functional sensitivity studies for this paracetamol assay (Filipo Faiga, Section Head Biochemistry, Laboratory Services, CCDHB, personal communication, 25/3/12).

A peak concentration of 80 μmol/L can be expected one hour after ingestion of 1 g of paracetamol (Gibb and Anderson 2008). Given that the half-life of paracetamol is between 1.5 to 2.5 hours and the fact that participants were dosed approximately 12 hourly, it is possible to estimate the blood paracetamol level in our participants on the day of a blood test (Figure 8.3). Figure 3 shows that at 3 hours after ingestion of 1 g of paracetamol, the blood paracetamol level can be below 30 μmol/L. Considering that participants were instructed to take their study medication morning and night, we can assume that many participants had blood tests taken outside of this three hour window following paracetamol ingestion. Further, because blood tests were not taken at a specified time (for example: time of peak concentration), it is possible that many of the paracetamol levels were troughs. This may be an adequate explanation for the low number of participants in the paracetamol group with recordable levels of paracetamol in their blood.
Figure 8.3: Estimated blood paracetamol level versus time following ingestion of 1 g paracetamol given a half-life of 2hr

It is also possible that the high number of non-detectable paracetamol level readings was due to non-compliance in the paracetamol group. However, our liver function monitoring data showed a statistically significant increase in ALT in the paracetamol group compared to the placebo group at week 12 of the study compared with baseline, which would have been unlikely if only one-third of the paracetamol group were compliant with their study mediation throughout the study period. Notwithstanding the limitations of the paracetamol level measurements, the fact that no participants in the placebo group had detectable levels of paracetamol at any time is supportive evidence of their compliance with not taking any non-randomised paracetamol during the study period.
8.3.5 Clinical Measurements

There was one significant change to the protocol during the testing period, which involved the amendment of the exclusion criterion regarding liver function abnormalities. In January 2011, changes to standard international criteria for the exclusion of participants screened for enrolment in pharmaceutical studies on the basis of LFTs were noted. The original protocol specified the exclusion of any participants with an ALT above the upper limit of the normal reference range (30 U/L for women and 40 U/L for men). However, new criteria specified that participants with an ALT within 1.5 times the upper limit of normal are able to participate in pharmaceutical research. Consequently the protocol and the relevant exclusion criteria were modified. Participants who had been excluded from the study at visit 1 due to an ALT result between 1 to 1.5 times the upper limit of normal were re-contacted for rescreening if interested.

8.3.6 Potential Sources of Error and Bias

This issue of recruitment bias is discussed above in section 8.3.2. Participant and investigator bias were effectively minimised as much as possible through the study’s double-blind design. Allocation concealment ensured that study investigators were blinded at the point of randomisation, and this was continued throughout the trial, through the use of safety investigators who checked blood results and communicated directly with participants if there were any safety concerns. This ensured that the study investigators were unable to see the results of blood tests, in particular liver function tests, which may have revealed which treatment the participant was
receiving. Study investigators remained blinded until after the last participant had finished the trial, at which time the randomisation schedule was delivered to MRINZ from the study pharmacy, and each participant was matched to their corresponding treatment group in the study database. Participant bias was further minimised through the use of custom-manufactured paracetamol and placebo tablets that were identical in appearance, taste and odour.

All clinical measurements undertaken in the trial have been previously validated and are known for their reliability and repeatability in clinical research (see Chapter 6). Further, validity was maximized by ensuring all equipment used for measuring primary and secondary outcome variables was calibrated and serviced as per manufacturer specifications, and by ensuring all materials were stored appropriately.

### 8.4 Possible Mechanisms

The main finding of this study was that there was no significant effect of paracetamol on BHR to methacholine. Consistent with this were the findings showing no significant differences in any of the measurable secondary clinical, inflammatory and immunological outcome variables.

One mechanism which has been postulated in the paracetamol and asthma hypothesis is that paracetamol may impair respiratory antioxidant defences by decreasing the amount of reduced glutathione present in the lungs. Previous research has shown that regular therapeutic doses of paracetamol can reduce systemic antioxidant capacity (in which glutathione plays a significant role) in as little as two weeks (Nuttall, Khan et
al. 2003), potentially leading to reduced antioxidant capacity in the lungs and subsequent oxidant-induced inflammation. This is supported by research which has shown that therapeutic levels of paracetamol deplete intracellular glutathione in pulmonary macrophages and type II pneumocytes in vitro. However, depletion of glutathione in antigen presenting cells leads to inhibition of Th1 cytokines in favour of Th2 cytokines (Peterson, Herzenberg et al. 1998), and theoretically this should be reflected in the levels of IL-4, IL-5 and IL-13 in the blood, as well as serum IgE and eosinophil levels resulting from changes in cytokine levels. While the study results do not indicate such a mechanism, this could be due to lack of power, and it would be important for immunological and inflammatory markers to be monitored in any future studies in order to gain further understanding of this complex interaction.

There was no statistically significant difference in FeNO from baseline to week 12, and the interpretation of the cytokine results is limited by the low number of participants with detectable levels throughout the study. It is, therefore, not possible to determine if paracetamol influenced the Th1/Th2 balance. It was also not possible to determine whether paracetamol use may have lead to neurogenic inflammation of the airways through the stimulation of the neurogenic inflammatory pathway, through transient receptor potential ankyrin-1 stimulation by NAPQI, the paracetamol metabolite. This is a specific molecular pathway, which is activated following therapeutic doses of paracetamol, and is distinct from the known toxic effects of NAPQI, which occur following paracetamol overdose. TRPA-1 stimulation mediates a non-eosinophilic inflammatory response, encompassing protein extravasation, neutrophil infiltration and increased myeloperoxidase activity. Other substances which are TRPA-1 stimulated and have been implicated in the pathogenesis or
provocation of asthma include isocyanates, aldehydes, cigarette smoke and chlorine (Bautista, Jordt et al. 2006; Caceres, Brackmann et al. 2009). A primary role of TRPA-1 in asthma is also suggested by the observation in animal models that pharmacological blockade or genetic depletion of the TRPA-1 channel diminishes allergen-induced airways inflammation and BHR (Caceres, Brackmann et al. 2009; Nassini, Materazzi et al. 2010).

8.5 Recommendations for Future Research

The findings of this study provide valuable information on which the design of a further definitive study should be based. A trial of similar design, utilising the same duration and dose of paracetamol and with BHR testing to methacholine as the primary outcome variable, would require a sample size of approximately 650 to attain adequate power to detect a difference in BHR to MCh of 0.5 doubling doses. Likewise, a study using FeNO as the primary outcome variable would require a sample size of between 1612 and 2120 to detect a 10% difference in FeNO between the two groups, based on the standard deviation range of 0.68 to 0.78. Alternatively, a study of short-term use of paracetamol at higher doses could be undertaken, to more closely replicate the common use of paracetamol for relief of fever or pain in self-limited illnesses. A cross-over study of shorter duration, with a sample size of 140 participants, would be adequately powered to detect a 0.5 doubling dose reduction in $\text{PC}_{20} \text{MCh}$ and a 10% change in FeNO, based on a standard deviation of 1.98 and 0.40 respectively.
As discussed above, consideration would also need to be given to the choice of primary outcome variable, and in the case of BHR, whether methacholine or an indirect measure such as AMP or hypertonic saline would be a more clinically relevant responsive measure of asthma severity. Further monitoring of inflammatory and immunological markers would be important in order to determine the mechanism of effect. Total antioxidant capacity, perhaps measured by enhanced chemiluminescence, may be a useful test to more directly measure the effect of paracetamol on antioxidant status and glutathione.

An important issue to note is that our study investigated the effect of paracetamol on asthma severity and not the effect of paracetamol on asthma pathogenesis. As discussed in Chapter 5, the effect of paracetamol use on the development of asthma is of paramount importance, and this could be assessed through a study of infants. Such a study could be designed as a randomised, open-label, parallel group study of paracetamol as required for fever and pain in infants following admission to hospital with a common childhood illness such as bronchiolitis. Children could be randomised to either liberal or restricted use of paracetamol in the three months following hospitalisation, and followed-up monthly via phone calls and house visits to assess usage of the study medication. The primary outcome variable could be symptoms of wheeze in the last 12 months at age 3 years, and secondary outcome measures could include atopy and eczema prevalence at 3 years of age. This study design would draw on the results of the Boston University Fever Study (Lesko, Louik et al. 2002), which demonstrated a larger effect of antipyretic medication on asthma symptoms in children with a current respiratory febrile illness, as opposed to fever from other
causes. It would also be more representative of the typical intermittent exposure of children to paracetamol during brief periods of unwellness, as opposed to regular, long-term paracetamol use that may be seen in adults.

Whilst undertaking such a study would pose certain difficulties, particularly ethical considerations regarding the use of placebo or restricted medication in unwell children and the effect this may have on consent rates, these issues could be assessed in a feasibility study. An appropriate feasibility study could include a questionnaire of parents of children admitted to hospital with bronchiolitis, regarding their willingness to participate in the study were it to take place. It could also gather data on the number of admissions of bronchiolitis into hospital during a specified time period, which, in conjunction with the expected consent rate, could assist in developing a properly powered study.

Whilst this study does not provide definitive evidence on which to base changes to clinical guidelines for the use of paracetamol in asthmatics, neither do the results adequately demonstrate the safety of paracetamol in the asthmatic population. Until definitive evidence has been attained, it has been proposed that health care professionals recommend asthmatic patients to use paracetamol sparingly and only as required for high fever or substantial pain (McBride 2011).
In conclusion, this thesis has analysed current evidence regarding the paracetamol and asthma hypothesis, and has described the first randomised, placebo-controlled trial of its kind undertaken to explore the effect of regular paracetamol on bronchial responsiveness and asthma control. The study showed no significant effect on BHR and asthma control following 12 weeks of treatment with paracetamol at half the maximum recommended therapeutic daily dose. However, this interpretation is limited by low power and the upper confidence interval limits did not rule out a clinically relevant effect. Furthermore, the point estimate of a reduction in PC$_{20}$ of 0.48 and an increase in FeNO of 9% could potentially be of major public health significance. Given the common usage of paracetamol in all age groups including pregnancy and the global burden of asthma, the impact of such an effect could be profound. It is recommended that further adequately powered studies are undertaken to determine the effect of paracetamol use on the development of asthma and its severity.
References


Australasian Society of Clinical Immunology and Allergy (2006 (Revised March 2009)). "Skin Prick Testing for the Diagnosis of Allergic Disease, A Manual for Practitioners."


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Tapiainen T, Dunder T, et al. (2010). "Adolescents with asthma or atopic eczema have more febrile days in early childhood: a possible explanation for the connection between paracetamol and asthma?" J Allergy Clin Immunol 125(3): 751-2.


# Appendices

## Appendix A: CONSORT Checklist

<table>
<thead>
<tr>
<th>Section/Topic</th>
<th>Item No</th>
<th>Checklist item</th>
<th>Reported on page No</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Title and abstract</strong></td>
<td>1a</td>
<td>Identification as a randomised trial in the title</td>
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<td></td>
<td>1b</td>
<td>Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)</td>
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<tr>
<td><strong>Introduction</strong></td>
<td>2a</td>
<td>Scientific background and explanation of rationale</td>
<td>92</td>
</tr>
<tr>
<td>Background and</td>
<td>2b</td>
<td>Specific objectives or hypotheses</td>
<td>93</td>
</tr>
<tr>
<td>objectives</td>
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<td></td>
<td></td>
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<tr>
<td><strong>Methods</strong></td>
<td>3a</td>
<td>Description of trial design (such as parallel, factorial) including allocation ratio</td>
<td>93</td>
</tr>
<tr>
<td>Trial design</td>
<td>3b</td>
<td>Important changes to methods after trial commencement (such as eligibility criteria), with reasons</td>
<td>96</td>
</tr>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Participants</td>
<td>4a</td>
<td>Eligibility criteria for participants</td>
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<tr>
<td></td>
<td>4b</td>
<td>Settings and locations where the data were collected</td>
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<tr>
<td><strong>Interventions</strong></td>
<td>5</td>
<td>The interventions for each group with sufficient details to allow replication, including how and when they were actually administered</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outcomes</td>
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<td>Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed</td>
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<td>6b</td>
<td>Any changes to trial outcomes after the trial commenced, with reasons</td>
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</tr>
<tr>
<td><strong>Sample size</strong></td>
<td>7a</td>
<td>How sample size was determined</td>
<td>108-109</td>
</tr>
<tr>
<td></td>
<td>7b</td>
<td>When applicable, explanation of any interim analyses and stopping guidelines</td>
<td>109</td>
</tr>
<tr>
<td><strong>Randomisation:</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sequence generation</td>
<td>8a</td>
<td>Method used to generate the random allocation sequence</td>
<td>109-110</td>
</tr>
<tr>
<td></td>
<td>8b</td>
<td>Type of randomisation; details of any restriction (such as blocking and block size)</td>
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<tr>
<td>Allocation concealment</td>
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<td>Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned</td>
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<tr>
<td>mechanism</td>
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<td></td>
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<tr>
<td>Implementation</td>
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<td>Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions</td>
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<tr>
<td>Blinding</td>
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<td>If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how</td>
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<td></td>
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<td>If relevant, description of the similarity of interventions</td>
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<table>
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<th>Section</th>
<th>Subsection</th>
<th>Description</th>
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<td>Statistical methods used to compare groups for primary and secondary outcomes</td>
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<td>12b</td>
<td>Methods for additional analyses, such as subgroup analyses and adjusted analyses</td>
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<tr>
<td>Results</td>
<td>13a</td>
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<td></td>
<td>13b</td>
<td>For each group, losses and exclusions after randomisation, together with reasons</td>
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<td>14a</td>
<td>Dates defining the periods of recruitment and follow-up</td>
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<td></td>
<td>14b</td>
<td>Why the trial ended or was stopped</td>
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<td>A table showing baseline demographic and clinical characteristics for each group</td>
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<td>16</td>
<td>For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups</td>
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<tr>
<td></td>
<td>17a</td>
<td>For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)</td>
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<td></td>
<td>17b</td>
<td>For binary outcomes, presentation of both absolute and relative effect sizes is recommended</td>
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<td>Ancillary analyses</td>
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<td>Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory</td>
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<tr>
<td>Harms</td>
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<td>All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)</td>
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<tr>
<td>Discussion</td>
<td>20</td>
<td>Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses</td>
<td>155-166</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>Generalisability (external validity, applicability) of the trial findings</td>
<td>157-159</td>
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<tr>
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<td>22</td>
<td>Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence</td>
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<tr>
<td>Other information</td>
<td>23</td>
<td>Registration number and name of trial registry</td>
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<td></td>
<td>24</td>
<td>Where the full trial protocol can be accessed, if available</td>
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</tr>
<tr>
<td></td>
<td>25</td>
<td>Sources of funding and other support (such as supply of drugs), role of funders</td>
<td>128</td>
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</tbody>
</table>
Appendix B: Recruitment Advertisement

It’s not easy being Green

LUCINDA STANILAND

While small actions on their own are not the solution, they are the foundation on which the bigger changes, the societal and governmental ones, must be based. So, in the face of the climate crisis and the ‘why bother? attitude’, what should we do? Salient writer Lucinda Staniland thinks that you should do something, because, well, it does matter.

Change how you shop and become a conscious consumer
Buy less and buy better quality. Do you really need this? Be a conscious consumer, one who chooses their purchases wisely and goes for locally sourced and sustainably produced products. Vote with your dollars and you let businesses know that producing more sustainable and eco-friendly products is the only way to move forward.

Change the way you eat
We all have to eat and the majority of us are lucky enough to have a large degree of choice in regard to what we put in our mouths. One way to green your diet is to eat less meat and animal products. Most production accounts for 80% of global greenhouse gas emissions and uses a lot of resources, making meat a very carbon-intensive product. To reduce your impact on carbon emissions and the planet’s resources you could consider going meat-free for one meal a week, or becoming a full or part-time vegetarian. Eat local, eat seasonal, and eat organic. It makes a lot of sense to eat this way because it’s cheaper, healthier and tastier, as well as being better for the environment. Growing your own food is awesome too.

Get curious, get informed, and get inspired
Now is the time to learn. The more you know the more power you have to change things. And you needn’t be too depressed, because the people that are involved in creating change are incredibly inspiring. Yes, things are bad, but the work that is already being done to change the direction we are headed is amazing. Check it out.

Get involved and become part of the solution
Link up with environmental and community groups. By getting involved with a group you can meet like-minded people, build community, and work together to make bigger and more impactful changes than you could ever could on your own. The University Club, Genex, is a great place to start. Other groups that are active in Wellington are Generation Zero, 350 and Climate Justice, as well as community gardens like Kel’te Aro and Invermay gardens. Look up their websites to find out more.

Vote
The fact that we, as citizens of a democratic country, have a right to vote is not something we should ever take for granted. Sure, we are all very busy and important people, who don’t have a lot of time to sit around reading politicians’ bullshit promises, but we owe it to ourselves to make the effort to find out what party policies mean for our future. Make climate change a priority in your voting decisions, as all parties, green or not, should have effective strategies in place to get New Zealand down to zero carbon emissions.
Appendix C: Dominion Post Newspaper Article

Study aims to let asthmatics breathe easy over painkillers

Kate Newton

A WORLD’S FIRST study to find out if a common painkiller makes asthma worse is under way in Wellington.

The study, led by scientists at the Medical Research Institute, will seek to find out if paracetamol — long considered the “safe” painkiller for asthmatics — makes the condition harder to control if taken too often.

Sixty people with asthma will be given either paracetamol or a placebo twice a day for 12 weeks and monitored to see if their airways get more “nasty”, causing them to narrow and provoke asthma attacks.

Institute director Richard Beasley said medical researchers around the world were waiting to see what the study found.

“The international community is really interested in this. “Asthma has become more common over the past 50 years, including in New Zealand, but we don’t know why that is.”

Studies had already shown that asthma rates went up when paracetamol was used more often but, until now, no-one had studied whether the two were linked or if it was just a coincidence.

“We know [paracetamol] is safe given as a one-off use. [But] no-one has actually done clinical trials to see what happens if you take it regularly.”

MEDICAL RESEARCH INSTITUTE DIRECTOR RICHARD BEASLEY

We know [paracetamol] is safe given as a one-off use. [But] no-one has actually done clinical trials to see what happens if you take it regularly.’

If the study showed that long-term use of paracetamol did make asthma worse, then further studies were likely to look at whether paracetamol could actually cause people – especially children – to develop asthma.

Until the research was complete, Dr Beasley encouraged asthmatics to continue using paracetamol rather than aspirin, ibuprofen or other painkillers.

“It remains the safe medication to use. We’re not discounting the possibility that paracetamol may even be protective.”

The study, which will be carried out at Wellington Hospital during the next 18 months, has received $400,000 in funding from the Health Research Council, Otago University, the Asthma and Respiratory Foundation and Wellington Medical Research Foundation.

New Zealand has the second-highest asthma rate in the world, with one in six children estimated to suffer from the disease.

Ten volunteers have been recruited for the study but researchers are looking for 50 more participants.

Volunteers can contact researchers on 0800 700 771.
Appendix D: Medsafe Approval for Use of Study Medication

2 June, 2011

Ms Tanya Baker
Medical Research Institute of New Zealand
99 The Terrace
Wellington

Dear Ms Baker

Clinical Trial on Herron Paracetamol (Tabsules)
Protocol Number: PA01

I am pleased to advise you that this clinical trial has been approved by the Director-General of Health.

You are therefore authorised to distribute Herron Paracetamol (Tabsules) for the purposes of this clinical trial to the following approved investigator(s):

<table>
<thead>
<tr>
<th>Approved Investigators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr Kyle Perrin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wellington Hospital</td>
<td></td>
</tr>
<tr>
<td>Riddiford Street</td>
<td></td>
</tr>
<tr>
<td>Newtown</td>
<td></td>
</tr>
<tr>
<td>WELLINGTON</td>
<td></td>
</tr>
</tbody>
</table>

Please note that it is your responsibility to obtain ethics approval before your trial can commence in New Zealand.

Legal reporting and record keeping requirement

It is a requirement of the Medicines Act 1981 that you

1. report the progress of the trial to the Director-General of Health at six monthly intervals;

2. report the results of the trial to the Director-General of Health on completion of the trial;
3. Report serious adverse reactions which occur during the trial to the Director-General in accordance with the requirements of the Guideline on the Regulation of Therapeutic Products in New Zealand, Part 11: Clinical trials – regulatory approval and good clinical practice requirements;

4. Keep complete and accurate records of all quantities of the trial medicine supplied during the trial;

5. Ensure that every label on every package or container of the trial medicine bears the words "To be used by qualified investigators only" or words of similar meaning.

Additional reporting requirements
If a patient of a medical practitioner who is not an investigator is a trial subject, that medical practitioner should be kept informed of the progress of the trial.

Importation of the trial medicine
If requested, you should present this letter to New Zealand Customs as evidence that the Ministry of Health has no objection to the importation of this clinical trial medicine.

In all further correspondence concerning this medicine, please quote the file reference TT50-5849/3 (1202).

Yours sincerely

[Signature]

Dr Alexander Bolotovski
for Director-General of Health
Appendix E: Asthma Control Questionnaire

ASTHMA CONTROL QUESTIONNAIRE (ACQ)

ENGLISH VERSION FOR NEW ZEALAND

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QOL TECHNOLOGIES Ltd.

For further information:
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Fax: +44 1243 573680
E-mail: juniper@qoltech.co.uk
Web: http://www.qoltech.co.uk

This translation has been made possible through a grant from GLAXOSMITHKLINE
Translated by MAPI RESEARCH INSTITUTE
Senior Translator: Ray Kirk

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NOVEMBER 2001
### Asthma Control Questionnaire

**Patient ID:** ______________________

(English Version for New Zealand) **Date:** ______________________

---

**Instructions**

Please answer questions 1 - 6.

Please circle the number of the response that best describes how you have been during the past week.

1. **In general, during the past week, how often were you woken by your asthma during the night?**
   - 0 Never
   - 1 Hardly ever
   - 2 A few times
   - 3 Several times
   - 4 Many times
   - 5 A great many times
   - 6 Unable to sleep because of asthma

2. **In general, during the past week, how uncomfortable were your asthma symptoms when you woke up in the morning?**
   - 0 No symptoms
   - 1 Very mild symptoms
   - 2 Mild symptoms
   - 3 Moderate symptoms
   - 4 Quite severe symptoms
   - 5 Severe symptoms
   - 6 Very severe symptoms

3. **In general, during the past week, how limited were you in your activities because of your asthma?**
   - 0 Not limited at all
   - 1 Very slightly limited
   - 2 Slightly limited
   - 3 Moderately limited
   - 4 Very limited
   - 5 Extremely limited
   - 6 Totally limited

4. **In general, during the past week, how much shortness of breath did you experience because of your asthma?**
   - 0 None
   - 1 A very little
   - 2 A little
   - 3 A moderate amount
   - 4 Quite a lot
   - 5 A great deal
   - 6 A very great deal

---

Page 1 of 2
5. In general, during the past week, how much time did you wheeze?
   0  Never
   1  Hardly any of the time
   2  A little of the time
   3  A moderate amount of the time
   4  A lot of the time
   5  Most of the time
   6  All the time

6. On average, during the past week, how many puffs/inhalations of your reliever (eg. Ventolin/Bricanyl) have you used each day?
   (If you are not sure how to answer this question, please ask for help)
   0  None
   1  1 - 2 puffs/inhalations most days
   2  3 - 4 puffs/inhalations most days
   3  5 - 6 puffs/inhalations most days
   4  9 - 12 puffs/inhalations most days
   5  13 - 18 puffs/inhalations most days
   6  More than 18 puffs/inhalations most days

To be completed by a member of the clinic staff

7. FEV\textsubscript{1} pre-bronchodilator: .........................
   0  > 95% predicted
   1  95 - 90%
   2  89 - 80%
   3  79 - 70%
   4  69 - 60%
   5  59 - 50%
   6  < 50% predicted

FEV\textsubscript{1} predicted:..........................
\( FEV\textsubscript{1}\% \text{ predicted} : \)
(Record actual values on the dotted lines and score the FEV\textsubscript{1} % predicted in the next column)
Appendix F: 

**Peak Flow Diary**

Please keep this booklet in a safe place and bring it with you to your next appointment with your study investigator.

**Instructions:**
- Please record your peak flow rates twice daily, morning and evening.
- The peak flow meter will be provided.

<table>
<thead>
<tr>
<th>Date</th>
<th>Morning</th>
<th>Evening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>7am</td>
<td>7pm</td>
</tr>
<tr>
<td>Day 2</td>
<td>8am</td>
<td>8pm</td>
</tr>
<tr>
<td>Day 3</td>
<td>9am</td>
<td>9pm</td>
</tr>
</tbody>
</table>

Please record your peak expiratory flow rates (PEFR) each morning and evening. You should record your PEFR after inhaling your usual morning inhaler medication, and this is your "FIRST THING in the morning" and "LAST THING at night" before going to bed.

Please keep this questionnaire for record purposes.
### Appendix G: Medication Diary

<table>
<thead>
<tr>
<th>Week</th>
<th>Day of Week</th>
<th>Time</th>
<th>Action</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>Mon</td>
<td>9:00 AM</td>
<td>Take Pill X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wed</td>
<td>1:00 PM</td>
<td>Take Pill Y</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fri</td>
<td>5:00 PM</td>
<td>Take Pill Z</td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>Sun</td>
<td>7:00 AM</td>
<td>Take Pill A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tue</td>
<td>12:00 PM</td>
<td>Take Pill B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thu</td>
<td>6:00 PM</td>
<td>Take Pill C</td>
<td></td>
</tr>
<tr>
<td>Week 3</td>
<td>Sat</td>
<td>8:00 AM</td>
<td>Take Pill D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fri</td>
<td>10:00 PM</td>
<td>Take Pill E</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sun</td>
<td>2:00 PM</td>
<td>Take Pill F</td>
<td></td>
</tr>
</tbody>
</table>
**Appendix H: Pharmacy Clinical Trial Prescription Form**

<table>
<thead>
<tr>
<th>DATE</th>
<th>MEDICATION</th>
<th>DOSE</th>
<th>ROUTE</th>
<th>INSTRUCTIONS/NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Effect of Regular Paracetamol on Airway Responsiveness and Asthma Control in Mild to Moderate Asthma – MHRNZ Protocol No. P10**

**CAPITAL & COAST (WELLINGTON HOSPITAL)**

**PHARMACY CLINICAL CHECK**

**DELIVER TO** [Pharmacy Name]

**DOCTOR'S SIGNATURE & PAGER #**

**PHARMACY CHECK**

---

*Patient should be instructed to reduce all remaining tablets at the next scheduled visit. Return these tablets to the Pharmacy for accountability.*
Appendix I: Aotea Pathology Certificate of Accreditation

<table>
<thead>
<tr>
<th>Schedule to</th>
<th>CERTIFICATE OF ACCREDITATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Client</td>
<td>Aotea Pathology Limited</td>
</tr>
<tr>
<td>Client Number</td>
<td>7202</td>
</tr>
<tr>
<td>Address</td>
<td>Level 6, CMC Building, 89 Courtenay Place, Te Aro, Wellington, 6011</td>
</tr>
<tr>
<td>Telephone</td>
<td>04 381-5900</td>
</tr>
<tr>
<td>Fax</td>
<td>04 381-5048</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://www.apath.co.nz">www.apath.co.nz</a></td>
</tr>
<tr>
<td>Authorised Representative</td>
<td>Ms Vicki McKnight</td>
</tr>
<tr>
<td></td>
<td>Quality and H &amp; S Manager</td>
</tr>
<tr>
<td>Programme</td>
<td>Medical Testing Laboratory</td>
</tr>
<tr>
<td>Accreditation Number</td>
<td>396</td>
</tr>
<tr>
<td>Date of Accreditation</td>
<td>19 December 1989</td>
</tr>
<tr>
<td>Conformance Standard</td>
<td>NZS/ASO 15189:2007</td>
</tr>
<tr>
<td></td>
<td>Medical Laboratories - Particular requirements for quality and competence</td>
</tr>
<tr>
<td>Services Summary</td>
<td>Aotea Pathology</td>
</tr>
<tr>
<td></td>
<td>Biochemistry</td>
</tr>
<tr>
<td></td>
<td>Cytology</td>
</tr>
<tr>
<td></td>
<td>Haematology</td>
</tr>
<tr>
<td></td>
<td>Histology</td>
</tr>
<tr>
<td></td>
<td>Immunology/Seroology</td>
</tr>
<tr>
<td></td>
<td>Microbiology</td>
</tr>
<tr>
<td></td>
<td>Molecular Pathology</td>
</tr>
<tr>
<td></td>
<td>Patient Services</td>
</tr>
<tr>
<td></td>
<td>Specimen Services</td>
</tr>
</tbody>
</table>

Authorized: General Manager

Issue 22 Date: 09/06/09 Page 1 of 2
Appendix J: CREC Ethical Approval

Dr Kyle Perrin
Medical Research Institute of New Zealand
PO Box 10056
The Terrace
Wellington

Dear Dr Kyle Perrin,

CEN/08/12/070 - The effect of regular paracetamol on airway responsiveness and asthma control in mild to moderate asthma

The above study has been given ethical approval by the Central Regional Ethics Committee.

Approved Documents:
- Participant Information Sheet, version 4, dated 10 June 2009
- Participant Informed Consent Form, version 4, 10 June 2009

Accreditation
The Committee involved in the approval of this study is accredited by the Health Research Council and is constituted and operates in accordance with the Operational Standard for Ethics Committees, April 2006.

Final Report
The study is approved until 15 September 2010. A final report is required at the end of the study. The report form is available on http://www.ethicscommittees.health.govt.nz and should be forwarded along with a summary of the results. If the study will not be completed as advised, please forward a progress report and an application for extension of ethical approval one month before the above date.

Amendments
It is also a condition of approval that the Committee is advised if the study does not commence, or is altered in anyway, including documentation eg advertisements, letters to prospective participants.

Please quote the above ethics committee reference number in all correspondence.

The Principal Investigator is responsible for advising any other study sites of approvals and all other correspondence with the Ethics Committee.

It should be noted that Ethics Committee approval does not imply any resource commitment or administrative facilitation by any healthcare provider within whose facility the research is to be carried out. Where applicable, authority for this must be obtained separately from the appropriate manager within the organisation.

Yours sincerely,

Sonia Scott
Central Regional Ethics Committee Administrator
Email: sonia_scott@moh.govt.nz
Appendix K: ANZCTR Trial Registration

ANZCTR
Australian New Zealand Clinical Trials Registry

Request Number: 083544
ACTRN Number: ACTRN1260900051291
Trial Status: Registered
Date Submitted: 6/07/2009
Date Registered: 7/07/2009
Date Last Updated: 20/10/2011
Registration Type: Prospective registered

Page 1

Public title: The effect of regular paracetamol on asthma symptoms in mild to moderate asthma
ANZCTR registration title: A randomised, double-blind, placebo-controlled study to investigate the effect of regular paracetamol on airway responsiveness and asthma control in mild to moderate asthma
Secondary ID: PA01
UTN:
Trial acronym:

Page 2

Health condition(s) or problem(s) studied:
Asthma
Condition category: Respiratory
Condition code: Asthma

Page 3

Description of intervention(s) / exposure: Subjects randomised to the intervention group will receive paracetamol tablets (1g dose) twice daily for 12 weeks.
Intervention code: Treatment: drugs
Comparator / control treatment: Subjects randomised to the control group will receive placebo tablets (calcium hydrogen phosphate-cellulose dummy pills made to match a 500mg paracetamol tablet) twice daily for 12 weeks.
Control group: Placebo
Page 4

Primary outcome:
Bronchial hyper-responsiveness assessed using a methacholine challenge test.

Timpoint:
12 weeks after commencement of treatment.

Secondary outcome 1:
Forced expiratory volume in 1 second (FEV1) as assessed by spirometry.

Timpoint:
6 and 12 weeks after commencement of treatment.

Secondary outcome 2:
Asthma symptom control as assessed using the Asthma Control Questionnaire score.

Timpoint:
6 and 12 weeks after commencement of treatment.

Secondary outcome 3:
Fraction of exhaled nitric oxide (FeNO) measured using a chemiluminescence analyser.

Timpoint:
6 and 12 weeks after commencement of treatment.

Secondary outcome 4:
Exacerbations of asthma requiring a visit to a doctor and the need for prednisone or nebulised bronchodilators (this data will be collected during study investigator interviews of the subjects, and from a review of subject completed questionnaires and General Practitioner reports).

Timpoint:
Monitored throughout the 12 weeks of the study.

Secondary outcome 5:
Mean morning and evening peak flow as measured by the subject using a peak flow meter and calculated from 3 morning and 3 evening readings.

Timpoint:
Peak flows will be measured for the first 7 days of the study compared with the measures taken during the last 7 days of the study (week 11-12).

Page 5

Key inclusion criteria:
1. Wheeze in the past 12 months and a doctor's diagnosis of asthma 2. Baseline FEV1 greater than or equal to 70% predicted 3. Provocative concentration of methacholine required to achieve a 20% fall in FEV1 (PC20 methacholine) of between 0.125-16.0 mg/ml

Minimum Age:
18 Years

Maximum Age:
65 Years

Genders:
Both males and females

Healthy volunteers?
No

Key exclusion criteria:
1. Patients taking theophylline, ipratropium bromide, tiotropium or leukotriene receptor antagonists regularly in the previous 3 months.
2. An exacerbation of asthma within the previous two months requiring prednisone or nebulised bronchodilator. 3. Current or past cigarette smoking greater than 10 pack years (pack years are calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked).
4. History of allergy or sensitivity to paracetamol or opiates or a history of allergy or sensitivity to aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) in subjects who have never taken paracetamol. 5. Current or past history of liver
disease or on potentially hepatotoxic drugs. 6. Current use of regular paracetamol, or aspirin greater than 150 mg/day, or high doses of NSAIDs in patients who are unable to discontinue this use during the trial. 7. History of alcoholism, or current excessive alcohol intake. 8. Previous intentional acute overdose of paracetamol, previous suicide attempt or current depression. 9. Evidence of malnutrition or Body Mass Index (BMI) < 16 kg/m2. 10. Pregnant or breastfeeding women or women of child-bearing age not using adequate contraception. 11. Subjects with a screening alanine aminotransferase level above the normal reference range or other screening liver function test abnormalities considered significant by the investigator. 12. Subjects unsuitable for bronchial hyperresponsiveness challenge testing.

Page 7

Study type: Interventional
Purpose of the study: Treatment
Allocation to intervention:

Describe the procedure for selecting a subject and allocating the treatment (allocation concealment procedures):

Blinded (masking used)

Who will be masked/blinded:
The people receiving the treatment/s
The people administering the treatment/s
The people assessing the outcomes

Assignment:
Parallel

Other design features (specify):

Type of endpoint(s):
Safety

Page 8

Phase: Phase 4
Anticipated or actual date of first participant enrolment: 13/08/2009
Target sample size: 132
Recruitment status: Closed: follow-up continuing

Page 9

Funding source 1:
Charities/Societies/Foundations
Name:
Medical Research Institute of New Zealand
Address: Level 7, CSB Building, Wellington Hospital, Riddiford Street, Newtown, Wellington 6021
Country: New Zealand

Funding source 2: Charities/Societies/Foundations
Name: Wellington Medical Research Foundation Incorporated
Address: PO Box 51 211 Wellington 5249
Country: New Zealand

Funding source 3: Government funding body e.g. Australian Research Council
Name: Health Research Council of New Zealand
Address: Level 3, 110 Stanley Street Auckland 1010
Country: New Zealand

Primary sponsor: Charities/Societies/Foundations
Name: Medical Research Institute of New Zealand
Address: Level 7, CSB Building, Wellington Hospital, Riddiford Street, Newtown, Wellington 6021
Country: New Zealand

Secondary sponsor: None
Name:
Address:
Country:

Other collaborator: University
Name: Wellington Asthma Research Group
Address: University of Otago Wellington School of Medicine and Health Sciences 23 Main Street Newtown Wellington 6021
Country: New Zealand

Page 9

Has the study received approval from at least one ethics committee? Yes

Ethics Committee name: Central Regional Ethics Committee
Address: PO Box 5013 Wellington 6145
Country: New Zealand
Date of approval: 19/06/2009
HREC Number: CEN/08/12/070
Countries of recruitment: Outside Australia New Zealand

Brief summary: The number of people with asthma has been steadily increasing for many years in most countries of the world including New Zealand,
but researchers are not sure why. Some studies have suggested that one reason could be the increasing use of paracetamol. We are aiming to find out if giving paracetamol to people with mild asthma has any effect on their asthma.

Trial website:
Presentations / publication list:

Page 10

Contact person for public queries
Name: Sally Eyers
Address: Medical Research Institute of New Zealand, Level 7, CSB Building, Wellington Hospital, Riddiford Street, Newtown, Wellington 6021
Country: New Zealand
Tel: +64 4 805 0239
Fax: +64 4 389 5707
Email: sally.eyers@ccdhb.org.nz

Contact person for scientific queries
Name: Sally Eyers
Address: Medical Research Institute of New Zealand, Level 7, CSB Building, Wellington Hospital, Riddiford Street, Newtown, Wellington 6021
Country: New Zealand
Tel: +64 4 805 0239
Fax: +64 4 389 5707
Email: sally.eyers@ccdhb.org.nz

Contact person responsible for updating information
Name: Tanya Baker
Address: Medical Research Institute of New Zealand, Level 7, CSB Building, Wellington Hospital, Riddiford Street, Newtown, Wellington 6021
Country: New Zealand
Tel: +64 4 805 0246
Fax: +64 4 389 5707
Email: tanya.baker@mfinz.ac.nz
Appendix L: Participant Information Sheet

Participant Information Sheet

Study title: The effect of paracetamol on asthma symptoms

Introduction
You are invited to take part in a clinical research study. Please take as much time as you need to read this information sheet carefully to determine if the study is of interest to you. You may wish to discuss the information in this sheet with your family or whanau. Please ask us if you have any questions about the study. Your involvement in this study is voluntary and you have the right not to take part and to withdraw at any time.

What is the aim of the study and what is being tested?
The number of people with asthma has been steadily increasing for many years in most countries including New Zealand, but doctors are not sure why. Research has suggested that one reason could be the increasing use of paracetamol tablets.

We are aiming to find out if giving paracetamol tablets to people with mild asthma has any effect on their asthma symptoms.

Where will the study be conducted?
132 people will take part in this study. It will be conducted at Wellington Hospital, Riddiford Street, Newtown, Wellington and at the Medical Research Institute of New Zealand rooms located within the Wellington P3 Research Unit at Bowen Hospital, Churchill Drive, Crofton Downs, Wellington. You can choose which of these clinics you would prefer to attend.

What will the study involve?
One of the study doctors will talk to you about the study and you will be given a copy of this information sheet to read and discuss with your family or whanau. If you are willing to participate in the study you will be asked to sign an Informed Consent form. This form shows that you have been given all the information about the study and that you understand what is involved. You will be asked to sign this form before any of the study tests take place. If you have any questions at any stage please ask the study doctor.

This study requires 4 visits to the research clinic over a period of approximately 13 weeks. The first, second and fourth visits will take about 1 hour and a half and third visit will take about 45 minutes.

If you choose to participate, at the first visit you will be asked some questions about your health. You will have your weight and height measured, as well as your blood pressure and pulse. Tests of your breathing will be done to see how your lungs are working and a blood sample will be taken to check your liver function. You will complete a questionnaire about your asthma and you will have a skin prick test to determine if you have certain allergies to common things like house dust mites, cats and grass. If you are a woman of child-bearing age you will have a urine pregnancy test. You will be given a peak expiratory flow meter and
over the next week you will be asked to measure your peak flow in the morning and evening and record it in a diary provided. You will need to bring this diary back to the next visit.

If the initial blood test is normal you will come for a second visit. You will have breathing tests, another blood test and you will be asked to fill out a questionnaire about your asthma symptoms. You will then be randomly assigned to receive either:

- Paracetamol tablets twice a day for 12 weeks
  
  Or

- Placebo tablets twice a day for 12 weeks

A placebo is a “dummy drug”, in this case it will look and taste exactly the same as the paracetamol tablets. Using a placebo means that neither you nor the study doctors will know if you are receiving paracetamol so that your response can be monitored without any bias. “Randomly” means it is a matter of chance (like flipping a coin), so you have a 1 in 2 chance of receiving the placebo. You will be given a 6 week supply of tablets and a medication diary which you will use during the study to record the time you take the study medication each day, and also any asthma symptoms you have during the study period.

You will have a third visit with the study doctor after 6 weeks for breathing tests, a blood test, another asthma questionnaire and to receive more tablets. Your medication diary and any remaining medication from the previous 6 weeks will be reviewed and any problems or new symptoms will be identified and discussed.

In the week before your last visit (11 weeks into the study) you will be asked to record your morning and evening peak flow readings again in a peak flow diary. You will be telephoned by the study investigator to be reminded you of this. You will need to bring the diary with you to your last visit.

The last visit will be after you have been taking the study medication for 12 weeks and the breathing tests, an asthma questionnaire and a blood test will be repeated. Your medication diary and any remaining study medication will be reviewed again. Women of child-bearing age will have another pregnancy test to ensure that they have not fallen pregnant during the study period.

You will also need to have blood tests in between your visits to the clinic at 2 and 4 weeks (and depending on your results possibly at 8 and 10 weeks). These can be done at an Aotearoa Pathology blood testing centre in the area most convenient to you. During the study, one of the study investigators will phone you at regular intervals (every 2 weeks or thereabouts) to check in how you are doing and to remind you to go for your blood tests.

During this study you will be asked not to take paracetamol if you have pain or a fever. We will give you a prescription for codeine, another pain medicine, to use instead. You will be issued with a card noting all your current medications. If any other doctors prescribe you new medicines during the study you will need to note them on the card. You need to advise the study doctor during your next visit or phone call of any new medicines.

**What tests and procedures will be carried out?**

**Spirometry**

We will measure how your lungs are working by asking you to blow into a machine called a spirometer as hard as you can. This is similar to a “peak flow” and will be done at all four visits. At the first visit spirometry will also be performed before and after a dose of bronchodilator medication.
**Nitric oxide measurement**

By breathing into this machine we can measure the amount of a gas called nitric oxide in your lungs. Nitric oxide tells us how much inflammation is present in the air passages. This will be done at visits 2, 3 and 4.

**Bronchial hyper-responsiveness**

The study investigator will ask you to breathe in something called methacholine which is a mild irritant to the air passages. The amount you inhale will start very small but each new dose will contain more methacholine. A spirometry test will be done after you inhale each dose of methacholine. The investigator will stop the test when your spirometry result drops by 20%. This test is commonly performed in the respiratory clinic on asthmatics, and tells us how sensitive your air passages are. It may make you feel slightly wheezy, but you will be closely monitored by the study doctor and at the end of the test you will be given a nebuliser if necessary. This test will only be done at the visits 2 and 4.

If you take asthma medication such as salbutamol (Ventolin) or terbutaline (Bricanyl) you will need to stop this medication 8 hours before attending visit 2 and visit 4. If you take asthma medication such as salmeterol (Seretide) or formoterol (Foradil) you will need to stop this medication 48 hours prior to attending visit 2 and 4. You will also need to avoid any food or drink containing caffeine on the day of the test.

These issues will be discussed with you during the first visit and the study investigator will remind you of what you need to withhold.

**Blood tests**

During the study you will have at least 6 blood tests. These are taken from a vein in the arm by a qualified person. The blood will be tested for:

1. The amount of paracetamol in the body
2. To make sure your liver is not affected by the tablets
3. To show the ability of your liver to process medicines (a genetic test)
4. To measure markers of inflammation in the body

If your liver is affected by the tablets then you will need at least 2 extra blood tests. Your blood samples will be disposed of at the end of the study.

**Skin Prick Testing**

A small drop of test solution containing a potential allergen is placed on your forearm and a small scratch is made on the skin. A reaction (usually less than 2cm in size) may occur on the skin like a rash and the size of this reaction can help us identify what you are allergic to. This test will only be performed at your first visit. If you take anti-histamine medication usually, it is important that you stop the medication 5 days before the first visit as it may affect the results of the skin prick test. If you are unable to stop your anti-histamine medication for any reason, please discuss this with the study investigator. You will be reminded of this by a study investigator prior to commencing the study.

**What are the possible risks and discomforts?**

The spirometry and nitric oxide breathing tests do not cause any discomfort. The methacholine inhalation test may cause coughing or may make you feel wheezy. These symptoms are short lived and resolve with nebuliser treatment. With blood tests, there is
always the risk of momentary discomfort, and sometimes there is bleeding, swelling or a bruise where the needle goes in.

Although paracetamol is a safe and commonly used medicine, in a small number of cases it can affect the liver. To minimise this risk we are using a dose of 2 grams a day, which is half the recommended maximum dose of 4 grams a day. We are excluding people at risk of liver problems and will do tests of your liver and will be able to stop the tablets if your liver is affected. Women who are currently pregnant or breast feeding will be excluded from this study. Other women of child bearing age can take part if they are using adequate contraception.

There is also a chance that you might have a worsening of your asthma during the study. If this occurs you can still continue with the study if you choose to and have the remaining tests performed. Throughout your time in the study you will receive normal care from your usual doctor, and if you agree we will notify your GP that you are participating.

**What are the possible benefits?**

Although you will not personally receive any direct benefit by being in the study, you will be contributing valuable research information that may help us to better understand the causes of asthma.

**Will taking part cost anything?**

There will be no costs to you as a result of being involved in the study. You will be given compensation for your travel costs and inconvenience in attending the research clinic.

**Participant rights and study withdrawal**

Participation in this study is entirely voluntary and you do not have to take part. Your decision whether or not to participate will not affect your health care in any way or your future relations with the hospital. During the study you will be kept informed of anything that may influence your decision to continue to participate in the research.

If you agree to participate, you may withdraw from the study at any time. If you refuse to participate or if you choose to withdraw (at any time) this will not affect your health care or any benefits to which you are otherwise entitled.

Your participation in the study may be stopped for the following reasons:

- If you don’t follow the investigator’s instructions.
- The investigator decides it is in the best interest of your health and welfare to discontinue.
Compensation for injury

In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation and Compensation Act. ACC cover is not automatic and your case will need to be assessed by ACC according to the provisions of the 2002 Injury Prevention Rehabilitation and Compensation Act. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators.

If you have any questions about ACC, contact your nearest ACC office or the investigator.

Confidentiality and data privacy

If you decide to participate in the study, the study doctor and MRINZ staff will collect medical and personal information about you as part of doing the study.

By agreeing to take part in this research, you will allow your medical information and results to be seen by people who check that the research was done properly.

No material which could personally identify you will be used in any reports on this study. Your personal information (for example your gender, age and medical conditions) and other information will be identified by a number (i.e. coded). The study records will be stored securely in locked offices during the course of the study and archived in a locked cabinet for a minimum of 10 years after the study finishes. The records will then be confidentially destroyed.

Will I be able to find out the results of the study?

Yes, you will be able to find out the results of the study when it is completed.

Where can I get more information about the study?

You can call the researcher whose details are at the bottom of this information sheet. An interpreter can be provided.

Ethical Guidelines

This study has been reviewed and approved by the Central Regional Ethics Committee.

Patient’s Rights

If you have any queries or concerns regarding your rights as a participant in this study, you may wish to contact a Health and Disability Services Consumer Advocate at telephone number: 0800 423 638.
Contact

If you have any questions about the study you can contact one of the study doctors:

**Primary Contacts:**
Dr Sally Eyers  
Telephone: 0800 700 771

Mathew Williams  
Telephone: 04 920 8864

**Principal Investigator:**
Dr Kyle Perzin  
Telephone: 04 805 0147

Fax: 04 389 5707
Appendix M: Participant Consent Form

Participant Informed Consent Form

Study title: The effect of paracetamol on asthma symptoms

Participant Number: 

REQUEST FOR AN INTERPRETER

<table>
<thead>
<tr>
<th>Language</th>
<th>Translation</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>English</td>
<td>I wish to have an interpreter.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maori</td>
<td>E hiahia ana ahau ki tetahi kaiwiakamaori/kaiwhaka pakeha korero.</td>
<td>Ace</td>
<td>Kao</td>
</tr>
<tr>
<td>Samoan</td>
<td>Oute mana'o ia iai se fa'amatala upu.</td>
<td>Joe</td>
<td>Leai</td>
</tr>
<tr>
<td>Tongan</td>
<td>Oku ou fiema'u ha fakatorulea.</td>
<td>Jo</td>
<td>Ikai</td>
</tr>
<tr>
<td>Cook Island</td>
<td>Ka inangaro au i tetai tangata uri reo.</td>
<td>Ae</td>
<td>Kare</td>
</tr>
<tr>
<td>Niuean</td>
<td>Fia manako au ke faka'aoega e taha tagata fakahokohoko kupu.</td>
<td>E</td>
<td>Nakai</td>
</tr>
</tbody>
</table>

Participant Initials: 

Please tick to indicate consent to the following:

- [ ] I agree to take part in the research study titled above and have had time to consider participation.
- [ ] I have read and understand the Participant Information Sheet version 8 dated 21 April 2010. I have had the opportunity to discuss this study with the study investigator and have had time to consider whether or not to participate.
- [ ] I have had the opportunity to use whanau support or a friend to help me ask questions and understand the study.
- [ ] I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and the information sheet.
- [ ] I consent to MRINZ staff collecting and processing my information, including information about my health.
If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be used. □

I agree to an approved auditor appointed by MRINZ, Central Regional Ethics Committee, or a regulatory authority or their approved representative reviewing my relevant medical records for the sole purpose of checking the accuracy of the information recorded for the study. □

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without giving a reason. This will not affect my continuing health care. □

I understand that my participation in this study is confidential, and that no material which could identify me personally will be used in any reports on this study. □

I understand that my involvement in this study will be stopped if it should appear to be harmful. □

I understand that I will receive compensation for travel costs upon completion of the trial and that this amount may be reduced if I do not complete the trial. □

I know who to contact if I have any side effects or if anything occurs which would be a reason to withdraw from the study. □

I agree to my GP being notified of my participation in this study. □

I would like to be advised of the study results YES/NO

*This project has been approved by the Central Regional Ethics Committee. This means that the Committee may check that this study is running smoothly and that the study has followed appropriate ethical procedures. If you have any concerns about the study, you may contact the Central Regional Ethics Committee on (04) 496 2403.*

**Statement by Participant:**  *I hereby consent to take part in this study.*

Name of Participant: ___________________________ Date of Birth: __________

Signature of Participant: ___________________________ Date: __________

**Statement by Investigator:**  *I have fully explained and discussed with the participant the nature, purpose, demands (and possible effects) of the study*

Name of Investigator/Co-investigator: ___________________________

Signature of Investigator/Co-Investigator: ___________________________

Date: ___________________________

Paracetamol/Asthma study  Informed Consent Form version 8 21 April 2010  Page 2 of 2
# Appendix N: Study Worksheets

## Paracetamol and Asthma Study Worksheet

<table>
<thead>
<tr>
<th>Patient ID Number:</th>
<th>Investigator Initials:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Date:</td>
<td></td>
</tr>
</tbody>
</table>

## Subject Information Sheet

<table>
<thead>
<tr>
<th>Patient Name:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex:</td>
<td></td>
</tr>
<tr>
<td>Date of Birth:</td>
<td></td>
</tr>
<tr>
<td>Age at First Visit:</td>
<td></td>
</tr>
<tr>
<td>Address:</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phone Number/s:</th>
<th>Hm:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Work:</td>
</tr>
<tr>
<td></td>
<td>Mobile:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GP Name:</th>
<th></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>GP Contact Details:</th>
<th></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Emergency Contact Person:</th>
<th>Hm:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Work:</td>
</tr>
<tr>
<td></td>
<td>Mobile:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Emergency Contact Numbers:</th>
<th>Hm:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Work:</td>
</tr>
<tr>
<td></td>
<td>Mobile:</td>
</tr>
</tbody>
</table>
Paracetamol and Asthma Study Worksheet

<table>
<thead>
<tr>
<th>Patient ID Number:</th>
<th>Investigator Initials:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Date:</td>
<td></td>
</tr>
</tbody>
</table>

Visit One (Week -1)

I, ........................................, confirm that this clinical trial was fully discussed with the subject. The subject has given fully informed consent for participation in study. The consent form was signed prior to any study-specific procedures commencing.

Signature:........................................ Date:........................................

Past Medical History:

Asthma History:
Age of asthma diagnosis: ............ YRS

Experienced wheeze in the last 12 months: YES / NO

Any exacerbations in prev two months (req prednisone or nebulised bronchodilator): YES / NO

History of allergic rhinitis or hay fever: YES / NO

Cardiovascular History:
History of hypertension: YES / NO

History of MI or stroke in past 3 months: YES / NO

Alcohol/Liver History:
Average weekly alcohol intake:

History of alcoholism: YES / NO

History of liver disease: YES / NO

Smoking History:
Current Smoker (>10 in last 3 months) or Non-smoker: SMOKER / NON-SMOKER

Ex-Smoker: YES / NO

If Yes, >10 pack years: YES / NO

Current Depression, Previous Suicide Attempts or History of Paracetamol Overdose? YES / NO

Current Medications:

*** Time since last dose of short-acting bronchodilator:
Paracetamol and Asthma Study Worksheet

Patient ID Number: 
Investigator Initials: 
Date: 

History of sensitivity to paracetamol, aspirin or NSAIDs: YES / NO

Currently using paracetamol, aspirin or NSAIDs on > 2 occasions/week and unable/unwilling to stop: YES / NO

Female Subjects: 
Currently breastfeeding or pregnant: YES / NO

Current form of contraception: 

Urine Pregnancy Test: POSITIVE / NEGATIVE

Peak Flow Meter and Diary Issued and Explained: YES / NO

Examination Findings:

<table>
<thead>
<tr>
<th>Height:</th>
<th>BMI:</th>
<th>BP:</th>
<th>ACQ Score:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weight:</th>
<th>Pulse:</th>
<th>Predicted FEV1:</th>
<th>FEV1 % Predicted:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bronchodilator Reversibility:

<table>
<thead>
<tr>
<th>Attempt</th>
<th>PRE</th>
<th>POST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEV1</td>
<td>FVC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FEV1</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Best result</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Blood Tests Taken: LFTs

Skin Prick Testing:

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Size of Wheal (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass Mix #7</td>
<td></td>
</tr>
<tr>
<td>Dermatophagoides pteronyssinus</td>
<td></td>
</tr>
<tr>
<td>Cat Pelt</td>
<td></td>
</tr>
<tr>
<td>Glycerine Negative Control</td>
<td></td>
</tr>
<tr>
<td>Histamine Positive Control</td>
<td></td>
</tr>
</tbody>
</table>

Eligible: YES / NO
Reason: 
Visit completed as per protocol: YES / NO

Investigator Signature: Date: 

Paracetamol and Asthma Study Worksheet 27-8-10 Page 3 of 10
Paracetamol and Asthma Study Worksheet

Inclusion/Exclusion Criteria Checklist
(All boxes should be ticked for inclusion in study)

☐ Wheeze in previous 12 months and a doctor diagnosis of asthma
☐ No exacerbation of asthma requiring prednisone or nebulised bronchodilator within the previous two months
☐ 18-65 years old
☐ BMI > 16.0 kg/m²
☐ Baseline FEV₁ ≥ 70% predicted
☐ Not regularly using paracetamol, NSAIDs or aspirin (>2 occasions per week for 6 weeks leading up to study), or willing to stop regularly using these drugs for duration of trial
☐ Not currently taking following medications:

<table>
<thead>
<tr>
<th>Amiodarone</th>
<th>Metyldopa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpromazine</td>
<td>Isoniazid</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>Rifampacin</td>
</tr>
<tr>
<td>Theophylline</td>
<td>Cholinesterase inhibitor medication</td>
</tr>
<tr>
<td>Ipratropium Bromide</td>
<td>Tiotropium</td>
</tr>
<tr>
<td>Leukotriene receptor antagonists</td>
<td></td>
</tr>
</tbody>
</table>

☐ Not a current (>10 in last 3 months) or past cigarette smoker > 10 pack years
☐ No history of allergy or sensitivity to paracetamol, aspirin, NSAIDs or opiates
☐ No past history of liver disease, alcoholism or excessive alcohol intake
☐ No previous intentional overdose of paracetamol, previous suicide attempt or current depression.
☐ Using adequate contraception and not currently pregnant or breast feeding or post-menopausal
☐ BHR Requirements: No uncontrolled hypertension or recent MI or stroke within the last 3 months
☐ Screening ALT within normal reference range

Investigator Signature:................................. Date: .......................
### Paracetamol and Asthma Study Worksheet

**Patient ID Number:**

**Investigator Initials:**

**Date:**

---

**Week 0 Telephone Reminder for BHR**

(Investigator to call subject if there is a long gap between V1 and V2, or if investigator feels reminder phone call is warranted/needed, otherwise write 'not attempted' and sign and date page.)

#### ATTEMPT 1: Date of Attempt: ……………………..

<table>
<thead>
<tr>
<th>Spoke to Subject</th>
<th>Message Left</th>
<th>Unable to Make Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Subject reminded to stop SABA 8 hours prior to BHR: **YES / NO**
Subject reminded to stop LABA 48 hours prior to BHR: **YES / NO**
Subject reminded to avoid caffeine on day of test: **YES / NO**

**Investigator Signature: ……………………..**

**Date: ……………………..**

---

#### ATTEMPT 2: Date of Attempt: ……………………..

<table>
<thead>
<tr>
<th>Spoke to Subject</th>
<th>Message Left</th>
<th>Unable to Make Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Subject reminded to stop SABA 8 hours prior to BHR: **YES / NO**
Subject reminded to stop LABA 48 hours prior to BHR: **YES / NO**
Subject reminded to avoid caffeine on day of test: **YES / NO**

**Investigator Signature: ……………………..**

**Date: ……………………..**

---

#### ATTEMPT 3: Date of Attempt: ……………………..

<table>
<thead>
<tr>
<th>Spoke to Subject</th>
<th>Message Left</th>
<th>Unable to Make Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Subject reminded to stop SABA 8 hours prior to BHR: **YES / NO**
Subject reminded to stop LABA 48 hours prior to BHR: **YES / NO**
Subject reminded to avoid caffeine on day of test: **YES / NO**

**Investigator Signature: ……………………..**

**Date: ……………………..**
Paracetamol and Asthma Study Worksheet

<table>
<thead>
<tr>
<th>Patient ID Number:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Investigator Initials:</td>
<td></td>
</tr>
<tr>
<td>Date:</td>
<td></td>
</tr>
</tbody>
</table>

**Visit Two (Week 0)**

Time of Visit 2: ..........AM/PM

Peak flow weekly diary Week -1/ Week 0 completed and collected: YES / NO

Reason if not completed or collected:

Any serious adverse events (including hospital admission or life-threatening event) or adverse events (including asthma exacerbations, visits to GP or after-hours clinic, yellowing of the skin or abdominal pain) since Visit 1: YES / NO

If so, has the appropriate form been completed: YES/ NO

Any changes to current medications since Visit 1: YES/ NO

If so, what were the changes:........................................

If randomised:

Study medication and diary issued and explained: YES/ NO

Prescription given for codeine: YES/ NO

Forms given for week 2 and week 4 blood tests: YES/ NO

**Blood Tests Taken:**

<table>
<thead>
<tr>
<th>LFTs</th>
<th>Genetic Analysis</th>
<th>Full Blood Count, IgE, Cytokines</th>
</tr>
</thead>
</table>

**Results from Visit Two:**

<table>
<thead>
<tr>
<th>FeNO 1</th>
<th>FeNO 2</th>
<th>FeNO 3</th>
<th>FeNO (average)</th>
<th>ACQ Score</th>
<th>FEVI (best)</th>
<th>FEVI%Predicted</th>
<th>BHR (PC20)</th>
<th>Amp%Mean*</th>
</tr>
</thead>
</table>

*Amp%Mean* = (max best PEFR of week - min best PEFR of week) / mean of all best morning and evening recordings of week) x 100

**Eligible:**

YES / NO

**Reason:** ........................................................................................................

**Randomised:**

YES / NO

**Visit completed as per protocol:**

YES / NO

**Investigator Signature:** .................. Date: ..................
Week 2 Telephone Reminder for Community Blood Test
(Speak to subject or leave a message to remind about blood test and to call MRINZ if there any problems. If no
message service/unable to make contact, continue to recall up to three times during the week.)

**ATTEMPT 1:** Date of Attempt: ..................

<table>
<thead>
<tr>
<th>Spoke to Subject</th>
<th>Message Left</th>
<th>Unable to Make Contact</th>
</tr>
</thead>
</table>

Any issues or problems: YES/NO

...................................................................................................................................................
...................................................................................................................................................
...................................................................................................................................................

Investigator Signature: .................. Date: ..................

**ATTEMPT 2:** Date of Attempt: ..................

<table>
<thead>
<tr>
<th>Spoke to Subject</th>
<th>Message Left</th>
<th>Unable to Make Contact</th>
</tr>
</thead>
</table>

Any issues or problems: YES/NO

...................................................................................................................................................
...................................................................................................................................................
...................................................................................................................................................

Investigator Signature: .................. Date: ..................

**ATTEMPT 3:** Date of Attempt: ..................

<table>
<thead>
<tr>
<th>Spoke to Subject</th>
<th>Message Left</th>
<th>Unable to Make Contact</th>
</tr>
</thead>
</table>

Any issues or problems: YES/NO

...................................................................................................................................................
...................................................................................................................................................
...................................................................................................................................................

Investigator Signature: .................. Date: ..................
Week 4 Telephone Reminder for Community Blood Test
(Speak to subject or leave a message to remind about blood test and to call MRC/NZ if there are any problems. If no message service/unable to make contact, continue to recall up to three times during the week.)

ATTEMPT 1: Date of Attempt:......................

Spoke to Subject ☐  Message Left ☐  Unable to Make Contact ☐

Any issues or problems: YES/NO
........................................................................................................
........................................................................................................

Investigator Signature:.........................  Date: ......................

ATTEMPT 2: Date of Attempt:......................

Spoke to Subject ☐  Message Left ☐  Unable to Make Contact ☐

Any issues or problems: YES/NO
........................................................................................................
........................................................................................................

Investigator Signature:.........................  Date: ......................

ATTEMPT 3: Date of Attempt:......................

Spoke to Subject ☐  Message Left ☐  Unable to Make Contact ☐

Any issues or problems: YES/NO
........................................................................................................
........................................................................................................

Investigator Signature:.........................  Date: ......................
Paracetamol and Asthma Study Worksheet

Visit 3 (Week 6)

Any exacerbation of asthma in previous 6 weeks requiring prednisone or nebulised bronchodilator: YES / NO

Any serious adverse events (incorporating hospital admission or life-threatening event) or adverse events (including asthma exacerbations, visits to GP or after-hours clinic, yellowing of the skin or abdominal pain) in the last 6 weeks: YES / NO

If so, has the appropriate form been completed: YES / NO

Any changes to current medications in last 6 weeks: YES / NO

If so, what are the changes:

Date and Time of last medication dose: DATE............. TIME .............AM/PM

Medication diary completed and collected: YES / NO

Study medication and diary issued for following 6 weeks: YES / NO

Week 8 and 10 blood forms given (to be used only if needed): YES / NO

PEFR meter and diary given and reminder to record PEFR at Week 11 – Week 12: YES / NO

Blood Tests Taken:

<table>
<thead>
<tr>
<th>Paracetamol Level</th>
<th>LFTs</th>
<th>Full Blood Count, IgE and Cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEV1 1</td>
<td>FEV1 2</td>
</tr>
<tr>
<td></td>
<td>FEV1 3</td>
<td>FEV1 (best result)</td>
</tr>
<tr>
<td></td>
<td>FEV1 % Predicted</td>
<td></td>
</tr>
</tbody>
</table>

Results from Visit Three:

FeNO 1
FeNO 2
FeNO 3
FeNO Average
ACQ Score

Compliance Check:

<table>
<thead>
<tr>
<th>No of Days in Study (W 1-6)</th>
<th>No of Doses Missed (sheet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of Doses Expected</td>
<td>No. of missed doses (bottle)</td>
</tr>
<tr>
<td>No. of tablets left in bottle</td>
<td>% Compliance (from bottle)</td>
</tr>
</tbody>
</table>

Visit completed as per protocol: YES / NO

Investigator Signature:.............................. Date: ......................
**Visit 4 (Week 12)**

Time of Visit 4: ..............AM/PM

Any exacerbation of asthma in previous 6 weeks requiring prednisone or nebulised bronchodilator? YES / NO

Any serious adverse events (including hospital admission or life-threatening event) or adverse events (including asthma exacerbations, visits to GP or after-hours clinic, yellowing of the skin or abdominal pain) in the previous 6 weeks: YES / NO

If so, has the appropriate form been completed: YES/ NO

Any changes to current medications in last 6 weeks? YES / NO

If so, what are the changes: .................................................................

Medication diary completed and collected: YES / NO

Peak flow weekly diary Week 11/ Week 12 completed and collected: YES / NO

Date and Time of last medication dose: DATE............. TIME .............. AM/PM

**Female Subjects:**

Urine pregnancy test: POSITIVE / NEGATIVE

**Blood Tests Taken:**

- LFTs
- Full Blood Count, IgE and Cytokines
- Paracetamol

<table>
<thead>
<tr>
<th>Results from Visit Four:</th>
<th>ACQ Score</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>FeNO 1</td>
<td>FEV1 (best result)</td>
<td></td>
</tr>
<tr>
<td>FeNO 2</td>
<td>FEV1% Predicted</td>
<td></td>
</tr>
<tr>
<td>FeNO 3</td>
<td>BHR (PC20)</td>
<td></td>
</tr>
<tr>
<td>FeNO Average</td>
<td>Amp % Mean</td>
<td></td>
</tr>
</tbody>
</table>

**Compliance Check:**

<table>
<thead>
<tr>
<th>No of Days in Study (W 7-12)</th>
<th>No. of tablets left in bottle</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No of Doses Expected</td>
<td>No. of missed doses (bottle)</td>
<td></td>
</tr>
<tr>
<td>No of Doses Missed (sheet)</td>
<td>% Compliance (from bottle)</td>
<td></td>
</tr>
</tbody>
</table>

Visit completed as per protocol: YES / NO

Investigator Signature: .................. Date: ..................

Paracetamol and Asthma Study Worksheet 27-8-10
<table>
<thead>
<tr>
<th>Vial Label</th>
<th>Confirm Dilution</th>
<th>Mix Well (tech.)</th>
<th>Solution</th>
<th>Dilution to Vial</th>
<th>Add ml</th>
<th>Vial and Add ml</th>
<th>Remove 3 ml</th>
<th>Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 16 ml/μl (μl)</td>
<td>E 8 ml/μl (μl)</td>
<td>C 4 μl/μl (μl)</td>
<td>D 2 ml/μl (μl)</td>
<td>F (0.5 ml) (μl)</td>
<td>G (0.25 ml) (μl)</td>
<td>H (0.125 ml) (μl)</td>
<td>I (0.0625 ml) (μl)</td>
<td>J (0.03125 ml) (μl)</td>
</tr>
</tbody>
</table>

**Microhemolysis Preparation Schedule (Procoholemic 1280mg) Vial 2**

- **Sample Expwy:**
  - Sample Expwy 1
  - Sample Expwy 2
- **Batch of Vial Expwy:**
- **Vial Number:**
- **Lot Number:**
- **Prepared By:**
- **Subject Number:**
| B          | 10 mL/mL (gm) | 2 mL/mL (gm) | AV Take 1st from Pool, Mix Dilution (mix well) Add Dilution to Vial  | Take 1st from Pool, Mix Dilution (mix well) Add Dilution to Vial |
|------------|----------------|--------------|-----------------------------------------------------------------|-----------------------------------------------------------------
| V          | 12 mL/mL (10cm) in 2 mL/mL (g) Pool | 10 mL/mL (10cm) in 2 mL/mL (g) Pool | | |

**Melatonin Preparation Schedule (Procolone I280mg) Vial 4**

- **Prepared By:** [Name]
- **Date of Vial Expiry:** 31/12/2023
- **Vial Number:** [Number]
# Methacholine Challenge & FeNO Worksheet

**Week 2 / Week 12 (circle one)**

<table>
<thead>
<tr>
<th>Date of Visit</th>
<th>Technician</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject ID</td>
<td>Doctor</td>
</tr>
<tr>
<td>Date of Birth</td>
<td>Time/date last SABA</td>
</tr>
<tr>
<td>Age at V2</td>
<td>Date/time last LABA</td>
</tr>
<tr>
<td>FEV1 Predict</td>
<td>Date/time last caffeine (tea, coffee, cola, choc)</td>
</tr>
<tr>
<td>Nebuliser</td>
<td>Recent viral infection YES/NO</td>
</tr>
<tr>
<td>Flow Rate (L/min)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spirometry (FEV1)</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>Best</th>
<th>% Predicted¹</th>
<th>20% Fall FEV¹²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline³</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Consult MRINZ SOP for methacholine challenge V4 for details.
³ Must have three readings (two at minimum) within 5% to calculate PC20 value

Time of First Measurement: _____ AM/PM

## Mch Conc (mg/ml)

<table>
<thead>
<tr>
<th>Mch Conc (mg/ml)</th>
<th>30sec FEV1</th>
<th>90sec FEV1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

² Only required if poor quality 1st or 2nd blow
³ %R = % reduction in FEV1 based on best post-saline best FEV1

Concentration of Mch at which PC20 reached: __________

PC20: __________

Salbutamol (5mg/2.5ml at 6L/min) neb given following last dose: YES / NO
Time salbutamol given: _____ AM/PM
Time of post-salbutamol spirometry: _____ AM/PM

<table>
<thead>
<tr>
<th>Spirometry</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>Best</th>
<th>% Predicted³</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

³ Must be greater than 90% of best post-saline FEV1 at end of study

Comments:

________________________________________  Investigator Signature: __________

---

Paracetamol and Asthma Study  Methacholine Worksheet  Version 3  2/6/10

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### Appendix O: MRINZ Adverse Event Form

<table>
<thead>
<tr>
<th>Event</th>
<th>Outcome</th>
<th>Severity</th>
<th>Relationship</th>
<th>Date</th>
<th>Product</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Event:** Moderate asthama (Protocol No. P401)

**Study Title:** The effect of regular participation on anxiety, responsiveness, and asthma control in mid to

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**Adverse Event Form**

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**Institute of Medical Research**

---
### Appendix P: MRINZ Serious Adverse Event Form

<table>
<thead>
<tr>
<th>Event</th>
<th>Date of Event</th>
<th>Date of Report</th>
<th>Participant initials</th>
</tr>
</thead>
</table>

#### Outcome
- o Death
- o Life-threatening
- o Serious
- o Moderate
- o Mild
- o Other: 

#### Study Product
- o Study (protocol No. P001)

#### Relationship to Study Product
- o Patient-specific/Study-specific
- o Study-specific
- o Other:

#### Summary
- o Yes
- o No

Please indicate SAE category from the following choices:

- Death
- Discontinuation of the Study Product
- Hospitalization
- In-patient: in-patient stays of 4 or more days
- Emergency Department: visits to ED
- Other: 

Date of Event: 

Date of Report: 

Participant initials: 

Study: Protocol No. P001

Serious Adverse Event Form
## Appendix Q: CREC Serious Adverse Event Notification Form

### NOTIFICATION/RECEIPT OF SERIOUS ADVERSE EVENTS

<table>
<thead>
<tr>
<th>Dr Sally Eyers</th>
<th>Name of Principal Investigator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical Research Institute of New</td>
<td>Address</td>
</tr>
<tr>
<td>Zealand</td>
<td>(for ease of return in a window</td>
</tr>
<tr>
<td>Private Bag 7902</td>
<td>envelope, please complete in the</td>
</tr>
<tr>
<td>Wellington 6242</td>
<td>left hand box)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study Title</th>
<th>Site:</th>
</tr>
</thead>
<tbody>
<tr>
<td>The effect of regular paracetamol</td>
<td>Wellington Hospital</td>
</tr>
<tr>
<td>on airway responsiveness and asthma</td>
<td></td>
</tr>
<tr>
<td>control in mild/moderate asthma</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ethics Ref No:</th>
<th>Investigator Brochure:</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEN/08/12/070</td>
<td>N/a.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protocol No:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PA01 version 12</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reference/Addendum/</th>
<th>Date Reported</th>
<th>Event</th>
<th>Study related?</th>
<th>Report attached?</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFR #/Safety report no</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Information sheet changes required? No (If yes, attach new version)

Investigator’s Signature: __________________________ Date: ______________

Name: __________________________________________

Investigator Comments: **(must be completed)**

---

**NOTED by ………………… …. ETHICS COMMITTEE:**

Chairperson/Deputy Chairperson: __________________________ Date: ______________

Name: __________________________________________

Ethics Committee Comments: ____________________________

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