NON- CLINICAL OVERVIEW (CTD MODULE 2.4)

Paracetamol Tablets 500 mg

Table of Contents

4.1 Overview of Non-clinical Testing Strategy

4.1.1 Overview

This application is being made in accordance with article 10.1of Directive 2001/83/EEC. The current application does not include a comparative bioavailability or bioequivalence study, but reference is made to fulfilling all requirements for a biowaiver. This Marketing Authorisation only includes a comparative dissolution profile study. It was not considered necessary that a bio-equivalence study be carried out; as this preparation contains Paracetamol as an active ingredient which is a well established molecule. Therefore a biowaiver study has been performed. The data in support of biowaiver is presented in this application. It is based on a claim of essential similarity with Panodil Tablets 500 mg which is marketed in Denmark. The Marketing Authorisations for Panodil Tablets were first granted in 1974 and are held by GlaxoSmithKline Consumer Healthcare A/S.

Paracetamol has antipyretic and mild analgesic actions together with some anti-inflammatory activity. These effects are thought to be related to inhibition of prostaglandin synthesis. Paracetamol is indicated for the treatment of mild to moderate pain including headache, migraine, neuralgia, toothache, sore throat, period pains, aches and pains, symptomatic relief of rheumatic aches and pains, and of influenza, feverishness and feverish colds.

In accordance with the requirements for such an application, this Marketing Authorisation Application does not include any nonclinical studies that have been conducted by the applicant. The following nonclinical overview is compiled from a review of the literature and existing toxicological data, considered within the context of the Quality (Module 3) and Clinical (Module 5 and Module 2.5) information available for these products.

In this application, both the test and reference products contain 500 mg of the drug substance, paracetamol. The excipients used in paracetamol tablets are maize starch, potassium sorbate, purified talc, stearic acid, polyvidone, soluble starch, hydroxypropylmethylcellulose, triacetin. All of the excipients are well known and well established, and controlled by their respective Pharmacopoeial monographs and therefore do not present any safety concerns.

The related substances, monitored and controlled as part of the drug substance and drug product specifications, are part of an in-house monograph for paracetamol. Analysis of Panodil Tablets using the analytical methods used for quality control of related substances in the drug product, identified a similar level of impurities to that found for paracetamol tablets, and hence the impurities in the drug substance and product can be considered controlled to acceptable levels.

(Current ATC code: NO2BE01: Other analgesics and antipyretics: Anilides)

4.1.2. Literature Search Strategy

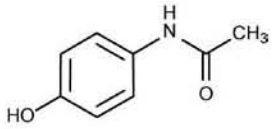
In the present nonclinical overview of paracetamol, reference will be made to relevant published scientific literature, with special emphasis to detailed pharmacological monographs, to provide an integrated and critical assessment of the nonclinical evaluation of the medicinal product.

Toxicological and pharmacological aspects of this application are limited to those known for the drug substance. They are discussed in more detail below.

4.2. Pharmacology

4.2.1 Chemistry of Paracetamol

Figure 1



Chemically, paracetamol is N-(4-hydroxyphenyl)acetamide; CAS Registry no 103-90-2. Its empirical formula is $C_8H_9NO_2$ =151.2. The structure of paracetamol is presented in Figure 1 [redacted]

4.2.2 Mechanism of Paracetamol

4.2.2.1 Overview

The mechanism of action of paracetamol is still not completely understood. Paracetamol has antipyretic and mild analgesic actions together with some anti-inflammatory activity. These effects are thought to be related inhibition of prostaglandin synthesis. In this respect paracetamol has greater tissue selectivity than aspirin and the non-steroidal anti-inflammatory drugs. The reason for this difference is unknown [redacted]

4.2.2.2 Antipyretic and Analgesic activity: in-vitro studies

This hypothermic effect of paracetamol was reduced in COX-1 but not in COX-2 gene-deleted mice. These results support the view that analgesia and hypothermia due to paracetamol are mediated by inhibition of a third COX isoenzyme (designated COX-3). In cultured mouse macrophages, COX-2 is induced by treatment with LPS or with high concentrations of diclofenac. Diclofenac-induced COX-2 is inhibited with low concentrations of paracetamol, whereas LPS-induced COX-2 is insensitive to paracetamol inhibition. The mechanisms of induction and possibly the functions of these two COX-2 enzymes are also different [redacted]

4.2.2.3 Antipyretic and Analgesic activity: in-vivo studies

Evidence shows in COX·2 knockout mice strongly suggests that prostaglandin synthesized by COX·2 mediate the febrile response. [redacted] have postulated that fever results from induction of COX·2 by endotoxin in endothelial cells of hypothalamic blood vessels and prostaglandins formed by this enzyme penetrate into the organum vasculosum laminae terminalis to produce fever. Thus, paracetamol may inhibit the enzyme in endothelial cells or exert its

antipyretic action centrally. However, a peripheral site for the antipyretic action has also been proposed. This postulates that endotoxin increases formation of prostaglandins in the central nervous system by stimulating receptors on sensory fibers of the vagus nerve. Sectioning of the vagus below the diaphragm in rats or guinea pigs prevents endotoxin-induced fever, induction of IL-lβ and increase in hypothalamic PGE2 levels. Perhaps this vagal sensory mechanism contributes to the genesis of fever but does not account entirely for the febrile response [redacted]

The early experiments of on the cross-perfused dog spleen indicated a peripheral rather than a central site for the analgesic action of paracetamol. It was subsequently generally accepted that the analgesic action of all NSAIDs was due to inhibition of prostaglandin formation at peripheral sites and that the only animal models which demonstrated their effect were the mouse and rat abdominal constriction tests. More animal tests have provided evidence for a central analgesic action of paracetamol. Perhaps low doses of paracetamol have a peripheral analgesic action by inhibiting COX-I and high doses act centrally by suppressing a paracetamol-sensitive COX-2 [redacted]

Paracetamol is generally considered to be a weak inhibitor of the synthesis of prostaglandins (PGs). However, the *in vivo* effects of paracetamol are similar to those of the selective cyclooxygenase-2 (COX-2) inhibitors. Paracetamol also decreases PG concentrations *in vivo,* but, unlike the selective COX-2 inhibitors, paracetamol does not suppress the inflammation of rheumatoid arthritis. It does, however, decrease swelling after oral surgery in humans and suppresses inflammation in rats and mice. Paracetamol is a weak inhibitor of PG synthesis of COX-I and COX-2 in broken cell systems, but, by contrast, therapeutic concentrations of paracetamol inhibit PG synthesis in intact cells *in vitro* when levels of substrate arachidonic acid are low (less than about 5 μmol/l). When the levels of arachidonic acid are low, PGs are synthesised largely by COX-2 in cells that contain both COX-l and COX-2. Thus, the apparent selectivity of paracetamol may be due to inhibition of COX-2-dependent pathways that are proceeding at low rates. This hypothesis is consistent with the similar pharmacological effects of paracetamol and the selective COX-2 inhibitors. COX- 3, a splice variant of COX-I, has been suggested to be the site of action of paracetamol, but genomic and kinetic analysis indicates that this selective interaction is unlikely to be clinically relevant. There is considerable evidence that the analgesic effect of paracetamol is central and is due to activation of descending serotonergic pathways, but its primary site of action may still be inhibition of PG synthesis. The action of paracetamol at a molecular level is unclear but could be related to the production of reactive metabolites by the peroxidase function of COX-2, which could deplete glutathione, a cofactor of enzymes such as POE synthase [redacted]

4.2.2.2 Antinociceptive action of paracetamol

Acetaminophen (paracetamol) is a widely used analgesic, but its sites and mechanisms of action remain incompletely understood. Recent studies have been separately implicated spinal adenosine A(1) receptors (A(1)Rs) and serotonin 5-HT(7) receptors (5-HT(7)Rs) in the antinociceptive effects of systemically administered acetaminophen. Study was done to determine whether these two actions are linked by delivering a selective 5-HT(7) R antagonist to spinal cord of mice and examining nociception using formalin 2% model. In normal and A(1)R wild type mice, antinociception by systemic (i.p.) acetaminophen 300mg/kg was reduced by intrathecal (i.t.) delivery of the selective 5-HT(7)R antagonist SB269970 3μg. In mice lacking

A(1)Rs, i.t. SB269970 did not reverse antinociception by systemic acetaminophen, indicating a link between spinal 5-HT(7)R and A(1)R mechanisms. We also explored potential roles of peripheral A(1)Rs in antinociception by acetaminophen administered both locally and systemically. In normal mice, intraplantar (i.pl.) acetaminophen 200µg produced antinociception in the formalin test, and this was blocked by co-administration of the selective A(1)R antagonist DPCPX 4.5µg. Acetaminophen administered into the contralateral hindpaw had no effect, indicating a local peripheral action. When acetaminophen was administered systemically, its antinociceptive effect was reversed by i.pl. DPCPX in normal mice; this was also observed in A(1)R wild type mice, but not in those lacking A(1)Rs. In summary, we demonstrate a link between spinal 5-HT(7)Rs and A(1)Rs in the spinal cord relevant to antinociception by systemic acetaminophen. Furthermore, we implicate peripheral A(1)Rs in the antinociceptive effects of locally- and systemically-administered acetaminophen [redacted]

4.2.3 Other Pharmacological Actions

Paracetamol antinociception is through interference with serotonergic descending pain pathways. This mechanism does not refute arguments that its primary site of action may still be inhibition of PG synthesis. An elegant model where paracetamol acts as a reducing co-substrate on the POX site of the PGHS enzyme when combined with the 'peroxide tone' of different cells, explains paracetamol's lack of platelet and anti-inflammatory effects. An active metabolite has been identified in mice. This metabolite (p-aminophenol) is then conjugated with arachidonic acid by fatty acid amide hydrolase to form AM404. AM404 exerts effect through CB receptors. It may also work through PGHS, particularly in areas of the brain with high concentrations of fatty acid amide hydrolase. Currently, the role and activity of this metabolic product have only been identified in mice and its role in human's unquantified [redacted]

Apart from its antipyretic and mild analgesic actions, paracetamol has no other important pharmacological effects and does not affect platelet function and haemostasis [redacted]

A study was done to investigate whether acetaminophen has an impact on the progression of renal failure. Acetaminophen (150mg/kg/day or 750mg/kg/day) or indomethacin (5mg/kg/day) was orally administered to adenine-induced chronic renal failure model rats for 4weeks. The plasma concentrations of acetaminophen and its metabolites were measured during the treatment period; renal function and oxidative stress in the rats were also monitored. Indomethacin significantly decreased the survival rate of renal failure model rats. In contrast, both low (150mg/kg) and high (750mg/kg) doses of acetaminophen improved the survival rate. The progression of renal failure was attenuated by acetaminophen (750mg/kg) after administration for 2weeks. The metabolites of acetaminophen were found to accumulate in plasma. Plasma glutathione concentration had significantly recovered after acetaminophen administration. Acetaminophen has no effect on the progression of renal damage in adenine-induced renal failure model rats. This result is in part due to acetaminophen's antioxidant activity. These results suggest that acetaminophen is a suitable analgesic agent for treating CKD patients [redacted]

4.2.4 Safety pharmacology of Paracetamol

Immunization with complete Freund adjuvant (CFA) or incomplete Freund adjuvant (IFA) is commonly viewed as painful, yet rodents may not receive analgesics due to concerns that these

drugs affect the desired immune responses. Here we tested the hypothesis that pain associated with immunization with CFA or IFA in mice can be relieved without compromising the effectiveness of the immune response. After subcutaneous immunization in the leg with antigen in CFA or IFA, mice were assessed for signs of pain by using behavioral tests, including unrestricted locomotion in an open field, forced running on an automated treadmill, and voluntary wheel running. Effects of the analgesics acetaminophen, meloxicam, and buprenorphine on behavioral and antibody responses were assessed after primary and secondary immunization with the model antigen ovalbumin and after repeated immunization with a limiting dose of recombinant protective antigen from Bacillus anthracis. Open field activity and the distance traveled during forced gait analysis and voluntary wheel running both decreased after immunization. Treatment with each of the analgesics normalized some but not all of these behaviors but did not decrease the mean or maximal antibody titer after primary or repeated immunization with a moderate dose of ovalbumin or after repeated immunization with a limiting dose of protective antigen. In summary, after immunization with CFA or IFA, mice showed behavioral responses suggestive of pain. Acetaminophen, meloxicam, and buprenorphine attenuated these effects without decreasing antibody responses. Therefore, the use of these analgesics for managing rodent pain associated with CFA- or IFA-containing vaccines can be encouraged [redacted]

In animals, chronic administration of ethanol causes microsomal enzyme induction with increased toxic metabolic activation of paracetamol and enhanced hepatotoxicity. Conversely, the acute administration of ethanol inhibits the potentially toxic oxidative metabolism of paracetamol and protects against liver damage. This protective effect disappears when the ethanol is eliminated and the time interval between the intake of ethanol and paracetamol is critical. The interactions between paracetamol and ethanol do not seem to be specific for any one isoform of cytochrome P450 [redacted]

4.2.5. Toxicity pharmacology of Paracetamol

Paracetamol is rapidly absorbed from the gastrointestinal (GI) tract with peak concentrations achieved within 90 minutes of a therapeutic dose. The presence of food in the stomach may delay the peak but not the extent of absorption. Distribution is rapid with a volume of distribution (Vd) of about 0.9 L/kg and minimal protein binding at therapeutic concentrations. The half-life of paracetamol is 2.0 to 2.5 hours. With hepatic injury, the half-life is prolonged to more than 4 hours. Paracetamol undergoes extensive hepatic metabolism. Approximately 85% of a therapeutic dose undergoes phase II conjugation to sulfated and glucuronidated metabolites that are renally eliminated. Of these two pathways, glucuronidation is predominant in adults, whereas sulfation predominates in children up to about 12 years of age. Up to 10% of paracetamol undergoes phase I oxidation to a reactive intermediate, N-acetyl-para-benzoquinone imine (NAPQI), which is normally conjugated with glutathione to nontoxic cysteine and mercapturate metabolites. Cytochrome 2E1 is the primary cytochrome p450 (CYP) enzyme responsible for this oxidation. At supra therapeutic doses of paracetamol (> 4 g), sulfation becomes saturated with proportional increases in both glucuronidation and, more significantly, oxidation to NAPQI. Smaller proportions of paracetamol are eliminated unchanged in the urine and by ring oxidation to a catechol derivative. At toxic doses of paracetamol, the continued production of NAPQI

eventually results in the depletion of glutathione. Once glutathione stores have been depleted by about 70%, NAPQI binds to cellular proteins and leads to cell injury. Glutathione depletion is only one of a cascade of intracellular events that includes mitochondrial oxidative stress, generation of reactive oxygen and nitrogen species, activation of stress proteins and gene transcription mediators, and mobilization of the liver's innate immune system. The balance between these numerous pathways ultimately determines whether there is recovery or cell death. Mitochondrial failure seems to be the terminal event heralding cell death. Although apoptotic pathways are activated, cell death is typically necrotic because mitochondrial failure precludes ordered cell death. The role of these various pathways in hepato-cellular injury remains an area of active research. Zone hepatocytes, rich in CYP2E1, are most susceptible to injury and this leads to the characteristic centrilobular pattern of hepatic necrosis seen with paracetamol. Patients on CYP2E1-inducing agents, such as ethanol, isoniazid, or St. John's wort, may be at an increased risk of toxicity because of increased NAPQI production, although there is no compelling data that this occurs at therapeutic dosages of paracetamol. Recommended maximum therapeutic dosages of paracetamol are 4 g daily in an adult and 50 to 75 mg/kg/d in children. A single acute ingestion of greater than 7.5 g in an adult or 150 mg/kg in children has been considered potentially toxic, although these thresholds are probably conservative. Single acute ingestions of less than 200 mg/kg in young children (age < 6 years) are unlikely to result in toxicity. Asymptomatic elevations of aminotransferases are sometimes seen with chronic use at the maximum recommended daily dose of 4 g. These elevations are typically less than 3 times the upper limit of normal, although occasionally greater. The clinical importance of these elevations during therapeutic use is uncertain. A prospective study in healthy adults consuming up to 8 g/d for 3 days did not find any toxicity. A recent expert panel's guideline to assist poison information specialists in the management of paracetamol exposures also provides some guidance on doses that should be of concern. Adults and children older than 6 years with an accidental acute ingestion of at least 10 g or 200 mg/kg, whichever is less, within an 8-hour period warrants further evaluation at a health care facility. For children younger than 6 years, the criterion was 200 mg/kg or more within an 8-hour period. The referral recommendations after RSTI in adults and children older than 6 years were at least10 g or 200 mg/kg, whichever is less, in a single 24-hour period or 6 g or 150 mg/kg, whichever is less, per 24-hour period for 48hours or longer. For children younger than 6 years, the criteria following RSTI were (1) 200 mg/kg or more over a single 24-hour period, (2)150 mg/kg or more per 24 hours for the past 48 hours, or (3) 100 mg/kg or more per 24 hours for 72 hours or more. For populations that may be at greater risk of toxicity, a lower threshold for evaluation was recommended. These at-risk groups include pregnancy, prolonged fasting, chronic alcoholism, and chronic use of isoniazid. For these populations, the threshold for referral is ingestion of more than 4 g in 24 hours or greater than 100 mg/kg in 24 hours, whichever is less. Immediate evaluation is required for any patient with an intentional overdose, when child abuse or neglect is a concern, and for any patient with symptoms suggesting hepatic injury [redacted]

4.3 Pharmacokinetics

The pharmacokinetics of paracetamol has been comprehensively studied in humans. Paracetamol is rapidly and comprehensively absorbed after oral administration, with peak plasma concentrations occurring between 15 minutes and 2 hours after ingestion. Dissolution and gastric emptying are rate limiting steps: the mean half time of absorption from the upper small intestine is only 7 minutes. The absolute oral bioavailability is about 80% and is independent of dose in the range 5-20 mg/kg. Paracetamol is not bound to plasma proteins to any extent and the volume of distribution is about 0.9l/kg. The concentrations of paracetamol in saliva are similar to those in plasma. Concentrations in whole blood are up to 20% higher and in breast milk about 20% lower. Paracetamol crosses the placenta [redacted]

The mean plasma paracetamol half-life after a therapeutic dose is 2.3 hours in healthy adults with a range of 1.5-3.0 hours. It varies relatively little between individuals, and is not prolonged to a clinically significant extent at the extremes of age. Paracetamol is extensively metabolised in the liver and the total body clearance is about 5 ml.min-1.kg-1. The clearance of paracetamol is reduced and the half-life increased following a hepatoxic overdose. Prolongation beyond 4 hours usually indicates impending liver damage [redacted]

Some 2-5% of a therapeutic dose of paracetamol is excreted unchanged in the urine. Its renal clearance is about 10 ml/min and is weakly dependent on urine flow but not on pH. The kinetics of paracetamol elimination has been investigated in patients with renal, hepatic, thyroid and gastrointestinal disease.

No clinically significant changes were observed except in patients with severe acute and decompensated chronic liver disease in whom the half-life was considerably prolonged. [redacted] .The pharmacokinetic parameters of paracetamol (acetaminophen) are altered in patients with severe liver disease, but the short-term use of this drug at reduced doses (2 grams daily) appears to be safe in patients with non-alcoholic liver disease.([redacted]

In patients with chronic renal failure there was a marked accumulation of paracetamol conjugates. In epileptic patients receiving anticonvulsants which cause microsomal enzyme induction, the paracetamol half-life is reduced by about 20% ([redacted]

Paracetamol undergoes extensive biotransformation in the liver and the major metabolites are inactive phenolic sulphate and glucuronide conjugates. A minor degree of saturation of sulphate conjugation can be demonstrated within the therapeutic dose range and this pathway (but not glucuronide conjugation) is completely saturated following overdosage. A small fraction of the dose is converted by cytochrome P450-dependent mixed function oxidase to *N-acetyl-P*-benzoquinoneimine (NAPQI), a reactive potentially cytotoxic arylating intermediate which is normally conjugated with glutathione (GSH) and excreted in the urine as mercapturic acid and cysteine conjugates of paracetamol.

Glutathione is depleted following overdosage and the reactive metabolite binds covalently to hepatic macromolecules causing irreversible damage and necrosis [redacted]

The major metabolites of paracetamol following a therapeutic dose are as follows: glucuronide conjugate (55%), sulphate conjugate (30%), mercapturic acid conjugate (4%) and cysteine conjugate (4%). Other minor metabolites have been identified [redacted]

The glutathione conjugate may be excreted in bile but is then largely degraded by intestinal peptidases and the products reabsorbed [redacted]

Although the pathways involved in the metabolism of paracetamol appear to besimilar in humans and experimental animals, the overall pattern of metabolite excretion shows species differences. Humans predominantly excrete the glucuronide conjugate which usually accounts for 50-60% of total urinary metabolites; approximately 30% of the drug is excreted as the sulphate conjugate, 20% as the cysteine and mercapturic acid conjugates combined and only small amounts are excreted as parent compound. Mice excrete glucuronide and sulphate conjugates in a similar pattern to that of humans. In contrast, rats excrete significantly more of the sulphate conjugate than the glucuronide conjugate in urine: the amount of parent compound excreted by rats is comparable to that of mice. The metabolite excretion pattern in hamsters appears to be more similar to that of mice and humans than to that of rats.

Where adequate data are available for various species or strains, the pattern of paracetamol metabolite excretion has been observed to exhibit dose-dependent changes. At non-toxic doses approximating therapeutic levels, paracetamol sulphate and glucuronide are the predominant metabolites in all experimental species. At higher doses, the percentage of administered doses converting to the sulphate conjugate decreases, signalling saturation of the sulphation pathway, and the excretion of mercapturic acid and other sulphur-containing conjugates from the 3-glutathionyl and/or cysteinyl conjugates increases, indicating an increase in the amount of parent compound converted to NAPQI [redacted]

The study was done to evaluate the bioavailability of a drug from rapidly disintegrating tablets prepared using fine spherical crystalline cellulose (PH-M-06®) and spherical sugar granules (Nonpareil®, NP). Rapidly disintegrating tablets containing acetaminophen as the model drug in combination with a mixture of NP-108 (purified D-mannitol) and PH-M-06 were prepared. Plasma concentration profiles and pharmacokinetic parameters of acetaminophen in rabbits were investigated after oral administration of the prepared tablets. No significant difference in C_{max} and $AUC_{0\text{-}inf}$ of acetaminophen between rapidly disintegrating tablets and conventional tablets was observed after direct administration of these tablets into the stomach of rabbits [redacted]

4.4 Toxicology

The toxicity of paracetamol has been extensively studied in laboratory animals and has provided a sound basis for the prediction of toxicity in man. The GLP status of the studies is not reported.

4.4.1 Acute Toxicity

The oral LD_{50} values in the rat are reported to be 1944 mg/kg (MSDS, 2012). Oral LD_{50} values are around 400 to 900mg /kg bw (body weight) in the mouse and more than 2000mg/kg bw in the rat, rabbit and guinea pig. Lethal oral doses in the dog and cat are around 500mg/kg bw and more than 50mg/kg bw respectively. [redacted]

The rat LD_{50} was found by [redacted] to be 3.71 + 0.83 g/kg body weight. At doses less than the LD_{50}, half the deaths occurred after 24 hours, and at doses greater than the LD_{50} three-quarters of the deaths occurred within the first 12 hours. Within 5 hours the animals became listless, hypokinetic and responded less to stimuli. The tail tended to be extended in a Straub-like reaction, some tremor appeared and there was a mild degree of pallor of the lips, eyes and paws. At 24 hours clinical signs included loss of body weight, decrease in food and water intake, hypothermia and aciduria, listlessness, decreased response to stimuli, tremor, tail extension and pallor. Gross findings at autopsy included inflammation of the cardiac and pyloric stomach, small bowel, thymus and brain.

4.4.2 Repeat Dose Toxicity

Fourteen day and 13 weeks repeat dose studies were conducted by the National Toxicology Department for the US Department of Health and Human Services [redacted] Paracetamol was administered in the diet to groups of *F344/N* rats and B6C3F1 mice of each sex. In the 14-day studies rats were fed diets containing 0,800, 1,600,3,100,6,200 or 12,500 ppm paracetamol, and mice were fed diets containing 0, 250,500, 1,000,2,000 or 4,000 ppm paracetamol. There were no deaths among any groups during the study; the final mean body weight *of* male rats that received 12,500 ppm was significantly lower than that *of* the controls. Final mean body weights *of* male and female mice and female rats that received paracetamol were similar to those *of* the controls. Food consumption by male and female rats that received 12,500 ppm paracetamol was lower than that *of* the controls; food consumption by all other exposed groups was higher than that *of* the controls ([redacted]

In the 13-week studies rats and mice were fed diets containing 0, 800, 1,600, 3,200, 6,200, 12,500 or 25,000 ppm paracetamol. Two male and two female rats, and one male and one female mouse that received 25,000 ppm, and two male mice that received 12,500 ppm died from paracetamol-related toxicity before the end *of* the studies. Final mean body weights of male and female rats and mice that received 12,500 or 25,000 ppm were lower than those of the controls. The patterns of food consumption and reduced body weights that occurred among rats and mice that received diets containing 12,500 *or* 25,000 ppm were indicative *of* poor food palatability. Paracetamol-related lesions were observed in the liver (necrosis, chronic active inflammation, hepatocytomegaly), kidney (tubule cast, tubule necrosis, tubule regeneration), reproductive organs (atrophy *of* testis, ovary and uterus), thymus and lymph nodes (lymphoid depletion) *of* rats that received 25,000 ppm, and *of* the liver (chronic active inflammation, hepatocytomegaly) and testis (atrophy) *of* male rats receiving 12,500 ppm. Compound-related lesions in mice were found in the liver (hepatocytomegaly, focal calcification, pigmentation, necrosis) *of* males that received 6,200, 12,500 or 25,000 ppm and females that received 12,000 or 25,000 ppm. This study was used to determine the dose for 2- year carcinogenicity studies. Dose selection for the 2-year studies was based on reduced body weights and the liver lesions observed in rats and mice at 12,500 and 25,000 ppm [redacted]

In large toxic doses paracetamol causes acute centrilobular hepatic necrosis in animals [redacted]. There are considerable species differences in susceptibility and acute hepatotoxic doses in hamsters, humans, mice and rats are about 150, 250, 300 and 3000 mg/kg [redacted]

Paracetamol hepatotoxicity is mainly mediated by N-acetyl-p-benzoquinone imine (NAPQI), which is generated by oxidation of paracetamol by P450 enzymes such as Cyp1a2, Cyp2e1, and Cyp3a4 [redacted]

Hepatotoxicity results from production *of* the intermediate alkylating metabolite N-acetyl-Pbenzoquinoneimine (NAPQI). NAPQI then binds to cell constituents to cause cell damage. Species susceptibility to paracetamol depends on the capacity *of* each species for NAPQI formation [redacted]

Paracetamol causes methemoglobinaemia and oxidative haemolysis in dogs and cats but not normally in humans, even after overdosage [redacted]

Stmin-dependent cataract formation and other ocular abnormalities have been described in induced mice [redacted]

Evidence suggests that coexistent hepatitis C virus (HCV) infection increased the risk of acetaminophen-induced acute liver injury, and was associated with an increased risk of progression to acute liver failure. Little is known about possible mechanisms of enhanced acetaminophen hepatotoxicity in HCV-infected subjects. In this study, we tested a hypothesis that HCV-Tg mice may be more susceptible to acetaminophen hepatotoxicity, and also evaluated the mechanisms of acetaminophen-induced liver damage in wild type and HCV-Tg mice expressing core, E1 and E2 proteins. Male mice were treated with a single dose of acetaminophen (300 or 500 mg/kg in fed animals; or 200 mg/kg in fasted animals; i.g.) and liver and serum endpoints were evaluated at 4 and 24h after dosing. Our results suggest that in fed mice, liver toxicity in HCV-Tg mice is not markedly exaggerated as compared to the wild-type mice. In fasted mice, greater liver injury was observed in HCV-Tg mice. In fed mice dosed with 300 mg/kg acetaminophen, we observed that liver mitochondria in HCV-Tg mice exhibited signs of dysfunction showing the potential mechanism for increased susceptibility [redacted]

4.4.3 Reproductive Toxicity

Paracetamol was tested for its effects on reproduction and fertility in CD-1 mice, following the RACB (Reproductive Assessment by Continuous Breeding) protocol [redacted]). Data on body weights, clinical signs, and food and water consumption from a 2 week dose-range-finding study (Task 1) were used to set exposure levels for the Task 2 continuous cohabitation phase at 0.25%, 0.5%, and 1.0% in the diet. Food consumption was reduced only in females at the top dose level, by 10-20%. Measured body weight and food consumption allowed exposure to be estimated as nearly equal to 370, 770, and 1400 mg/kg/day. During Task 2,4animals died: 2, 1 and 1 each in the low, middle, and high dose groups, respectively. During Task 2, the number of litters/pair decreased by 3% for the high dose group. No changes were noted in the number of pups/litter, viability, or in adjusted pup weight. The slight reduction in number of litters/pair was judged to be too small to yield a detectable change during the statistically-less-powerful Task 3 crossover mating, so no crossover test was conducted.

For the FI evaluation, the last litter in Task 2 from all dose groups was nursed to weaning, and reared on the diet consumed by their parents. FI pup body weights were reduced at all doses for both sexes by nearly equal to 6-18%. Pup body weight gain to weaning was also reduced for the

medium and high dose males (17% and 34%), and for females at all doses (10-28%). All dose groups were reared consuming the same diet provided to their parents. The body weight differences that were seen during nursing were reduced, but still present, at the time of mating. At the FI mating, the F2 pup weight adjusted for litter size was decreased by 11 % at the high dose level. No other reproductive endpoints were affected. After the F2 pups were delivered and evaluated, the FI adults from only the control and high dose groups were killed and necropsied. Compared to controls, the high dose males weighed 10% less, while organ weights were not affected. Sperm abnormalities increased from 7% (controls) to 16% at the high dose. High dose females weighed 8% less, while adjusted liver weight was increased by 10%. In summary, the greatest toxicity produced by paracetamol in the diet of Swiss mice was on the growing neonate (reduced weight gain during nursing). Fertility endpoints (ability to bear normal numbers or normal-weight young) were generally not affected.

In a similar study, paracetamol was evaluated for reproductive toxicity in Swiss CD-1 mice using a continuous breeding protocol [redacted]. Paracetamol was administered in the diet at 0, 0.25, 0.5, and 1.0% (w/w), which represented average daily intakes of 0, 357, 715, and 1430 mg/kg/day, respectively. Exposure of parental (Po) breeding pairs to 1% paracetamol in the diet for 14 weeks during cohabitation significantly decreased the number of litters per pair, and reduced, although not significantly, the number of live pups per litter. Importantly, 6 of 19 high-dose Po pairs failed to produce a fifth litter, and this fully accounted for the diminished number of litters in this group. In addition, the fifth litter that was produced by the 13 high-dose Po pairs averaged only about 9 live pups per litter, which correspondingly reduced the overall group average for this parameter. In comparison, the control and two lower-dose Po pairs produced 11 or 12 live pups per litter on average. Although the birth weights for F1 pups in the final litter were unaffected by prenatal paracetamol exposure, postnatal growth was adversely affected as evidenced by retarded weight gain as measured at 28 and 74 + 10 days of age for all three dietary levels. At 1% paracetamol this weight gain effect was more pronounced at Day 28 than at Day 74 + 10, suggesting that nursing pups may have been exposed to higher concentrations or may be more sensitive to paracetamol and/or an active metabolite than were the young adults. A mating trial of F1 pairs at 74 + 10 days of age indicated that mating, fertility, and reproductive performance were normal, except that the birth weight of F2 pups was significantly depressed at 1 % paracetamol. This latter effect may have been attributable to maternal toxicity in that body weight and relative pituitary weight were significantly decreased, and relative brain and liver weight significantly increased, in high-dose F1 females. In addition, body weight was significantly reduced in the high-dose F1 males at necropsy. No treatment-related effects on reproductive organ weights and no gross or histological changes in the reproductive tissues of the high-dose F1 male and female mice were observed.

Continuous exposure of FI males at 1 % paracetamol, however, significantly increased the percentage of abnormal epididymal sperm, while sperm density and percentage of motile sperm were unaffected. Collectively, these data indicated that continuous exposure to 1 % paracetamol via the diet (1.43 g/kg/day) led to cumulative effects on reproduction in the Po pairs, to retarded growth and abnormal sperm in the FI mice, and to reduced birth weight of F2 pups.

A study was conducted to evaluate the anti-reproductive effect of paracetamol in male rats [redacted]. Male rats were orally administered daily with 500 mg/kg or

1000 mg/kg of paracetamol for 30 consecutive days. Their sexual behaviour and fertility were evaluated using receptive females. At 2 hours after treatment, sexual behaviour was not inhibited but on Day 30 both doses of paracetamol caused marked impairment of libido (assessed by % mounting, % intromission and % ejaculation), sexual vigour (number of mounts and intromissions and copulatory efficiency) or sexual performance (intercopulatory interval). In mating experiments, the fertility (in terms of quantal pregnancy, fertility index, implantation index and number of implants) was significantly reduced. All these effects were reversible. The anti reproductive effect was not due to a general toxicity but due to an increase in pre-implantation losses resulting from oligozoospermia, impairments of normal and hyper-activated sperm motility, and reduction in the fertilising potential of spermatozoa. The authors concluded that long-term use of high doses of paracetamol may be detrimental to male reproductive competence.

Acetaminophen, suspended in Tween 80 solution, was administered once a day, orally by a stomach tube to pregnant Wistar rats from day 8th to 14th of pregnancy at the dose: 3.5 (P1), 35.0 (P2), 350.0 mg/kg (P3). The pregnant females were terminated on day 21st of pregnancy and the number of corpora lutea, implants, resorptions, and fetuses was counted. The fetuses and the placentas were weighed and the length of the fetuses and their tails were checked. The slides of the fetal liver were examined via light microscopy in four stains: hematoxylin and eosin, silver Gomorii, van Giesson, and periodic acid-Schiff. There was a statistical ($p < 0.05$) difference in fetal body length in group P3 without any macroscopic malformation, except for the non-statistical number of subcutaneous ecchymoses. Histological adaptive changes of the fetal liver were observed occasionally in all the studied groups. In conclusion, the oral administration of acetaminophen caused an embryotoxic effect in the highest doses without any macroscopic malformation, and only slightly impaired morphology of the rat fetal liver [redacted].

These pharmaceutical compounds have been associated with congenital cryptorchidism in humans, the best-known risk factor for low semen quality and testicular germ cell cancer. Furthermore, some of these mild analgesics exert potent anti-androgenic effects in the male rat and several endocrine-disrupting compounds, known to alter masculinization, have also been shown to be potent inhibitors of prostaglandin (PG) synthesis similar to mild analgesics. Using a 3-day ex vivo organotypic model system based on gestational day 14.5 rat testes, we herein show that testosterone production was inhibited by paracetamol, at doses of 0.1 μm to 100 μm. Similar results were obtained for aspirin (1-100 μm) and indomethacin (10 μm). The production of the other Leydig cell hormone, Insl3, was not disrupted by exposure to paracetamol. Investigations of the gross anatomy of the testis as well as Leydig cells number and rate of gonocyte apoptosis after the 3 days of ex vivo differentiation showed no significant effect of the analgesics tested compared with controls. These data indicate therefore that mild analgesics specifically inhibit testosterone production in rat foetal testes in vitro and that these compounds had no effect on gonocyte survival. Parallel determinations of prostaglandin D2 (PGD2) production indicated that the effects of paracetamol and aspirin on PGD2 and testosterone were not connected, whereas the effects of indomethacin were correlated. We conclude that mild analgesics exert direct and specific anti-androgenic effects in rat foetal testis in our experimental setup and that the mechanism of action is probably uncoupled from the inhibition of PG synthesis [redacted]

Paracetamol (acetaminophen) is a widely used over – the - counter analgesic and antipyretic drugs. Several studies have reported the toxic, gastrointestinal and musculoskeletal effect of this drug, but there is scanty information on its effect on blood chemistry and reproduction in albino rats. This study was designed to investigate the effect of this drug on haematological and reproductive parameters in male albino rats. Paracetamol (7.5 mg/kg BW) was administered to the rats for 42 days (six weeks) for haematological and andrological study. Distilled water (0.5 ml) served as control. Red Blood Cell (RBC) and Total White Blood Cell (TWBC) counts were determined using haemocytometer. PCV was determined by micro-haematocrit method. Semen analyses were done microscopically. Data were analysed using student's t -test at $p<0.05$. Treatment of rats with paracetamol caused decrease in PCV and RBC counts relative to the controls. Treatment of rats with paracetamol also caused significant decrease in sperm motility and sperm count, but did not produce any pathological lesions on the testes. These findings indicate that paracetamol caused deleterious effect on the blood chemistry and reproductive parameters in male albino rats [redacted]

The mechanism by which acetaminophen (APAP) causes liver damage evokes many aspects such as, drug metabolism, oxidative chemistry and genetic-predisposition. In this study, we leverage the relative resistance of female C57BL/6 mice to APAP-induced liver damage (AILD) compared to male C57BL/6 mice in order to identify the cause(s) of sensitivity. Furthermore, we use mice that are either heterozygous (HZ) or null (KO) for glutamate cysteine ligase modifier subunit (Gclm), in order to titrate the toxicity relative to wild-type (WT) mice. Gclm is important for efficient de novo synthesis of glutathione (GSH). APAP (300 mg/kg, ip) or saline was administered and mice were collected at 0, 0.5, 1, 2, 6, 12, and 24 h. Male mice showed marked elevation in serum alanine aminotransferase by 6 h. In contrast, female WT and HZ mice showed minimal toxicity at all time points. Female KO mice, however, showed AILD comparable to male mice. Genotype-matched male and female mice showed comparable APAP – protein adducts, with Gclm KO mice sustaining significantly greater adducts. ATP was depleted in mice showing toxicity, suggesting impaired mitochondria function. Indeed, peroxiredoxin-6, a GSH-dependent perox-iredoxin, was preferentially adducted by APAP in mitochondria of male mice but rarely adducted in female mice. These results support parallel mechanisms of toxicity where APAP adduction of peroxiredoxin-6 and sustained GSH depletion results in the collapse of mitochondria function and hepatocyte death. We conclude that adduction of peroxiredoxin-6 sensitizes male C57BL/6 mice to toxicity by acetaminophen [redacted]

4.4.4 Mutagenicity

Paracetamol was tested for mutagenic activity in the Ames *Salmonella* plate incorporation assay using strains TA98, TAI00, TA1535, TA1537 and TA1538 in four separate tests: without metabolic activation, and in the presence of a rat, hamster or mouse liver post-mitochondrial supernatant (8-9, Aroclor 1254- induced) [redacted]. Treatment of all five strains of *salmonella* under all four metabolic conditions did not induce any appreciable increase in revertant colony counts, as compared to the negative controls. Therefore, paracetamol did not show any mutagenic potential under these conditions of testing. In a similar study, paracetamol was examined for mutagenicity in the mammalian *Salmonella/microsome* screening test [redacted]

Paracetamol was tested in 6 strains of *Salmonella typhimurium* (TAI535, TA1537,TA1538, TA100, TA97 and TA98) in the presence and absence of a rat-liver microsome activation

system. Paracetamol did not show any evidence of mutagenic activity at concentrations ranging from 0.1 to 50 mg per plate.

Studies with paracetamol indicate some evidence for clastogenicity, particularly in *in vitro* mammalian test systems. Induction of chromosomal aberrations [redacted] and sister chromatid exchanges [redacted] have been reported in cultured mammalian cells in the absence of exogenous metabolic activation. A small, dose-related increase in frequency of micronuclei was observed in cultured rat kidney cells in the absence of exogenous metabolic activation [redacted]

In studies conducted for the National Toxicology Program, USA, paracetamol was not mutagenic in *Salmonella typhimurium* strains TA100 TA1535, TA1537, or TA98 with or without S9. In cytogenic tests with Chinese hamster ovary cells, paracetamol induced sister chromatid exchanges and chromosomal aberrations in both the presence and absence of S9 ([redacted]

In a further study, paracetamol and its major ultimate reactive metabolite, Nacetyl-Pbenzoquinone imine (NAPQI) were studied for their genotoxic potential [redacted] Neither paracetamol nor NAPQI were found to cause mutations in *Salmonella typhimurium,* whereas NAPQI was severely cytotoxic to the bacteria. Radiolabelled paracetamol was found to bind covalently to DNA added to mouse-liver microsomal incubations at a rate of 2.6 pmoles/mg DNA/min. Paracetamol also bound covalently to hepatic DNA at a level of 15 pmoles/mg DNA after a hepatotoxic dose of paracetamol to mice. NAPQI caused extensive DNA single-strand breaks as evidenced by alkaline elution of DNA from treated Reuber hepatoma cells. This effect occurred at concentrations which later resulted in cytotoxicity. Paracetamol was shown to induce increased DNA-repair synthesis in isolated mouse-liver cells in monolayer culture, at concentrations where cytotoxicity was also evident. Increased DNA-repair synthesis occurred at lower paracetamol concentrations in cells isolated from mice pre-treated with phenobarbital. The authors concluded that taken together, these data show that paracetamol can cause DNA interaction leading to damage at levels which are cytotoxic.

4.4.5 Carcinogenicity

In the first published report involving long-term exposure to paracetamol [redacted] groups of 30 male Sprague-Dawley rats received diets containing 5,350 ppm phenacetin, 5,350 ppm phenazone, 1,020 ppm caffeine, a mixture of a pair of these drugs, or a mixture of all three drugs. An additional 30 male rats received 5,350 ppm paracetamol in the diet. An animals were maintained on these diets over their lifespans, which ranged from group means of 92 to 104 weeks (94 weeks for the controls). The group exposed to paracetamol had a mean lifespan of 93 weeks. Group mean body weights at week 74 showed that the mean body weight of the exposed group was similar to that of the control group, but the mean body weights of all other treated groups were significantly less than those of the control group. Animals from all groups had some degree of renal papillary hyperplasia and bladder hyperplasia; however the incidence and severity were increased in animals that received phenacetin either alone or in combination with the other agents. Neoplasms of the renal pelvis, bladder and liver occurred in groups that received phenacetin, phenazone, and combinations of phenacetin, phenazone and caffeine. The overall incidence of papillary hyperplasia was slightly increased among exposed rats (57%) compared to controls (43%); the severity of this lesion in exposed rats was also slightly greater

than in the controls. Four animals from the group that received paracetamol had bladder papillomas, as did two of the control animals. The authors concluded that paracetamol demonstrated no toxic or carcinogenic effect.

A study of paracetamol for possible carcinogenicity was conducted by administering paracetamol in pelleted diets to male and female F344/DuCrj rats [redacted] Groups of 50 rats were administered paracetamol at one of two doses, either 4,500 or 9,000 ppm for males and 6,500 or 13,000 ppm for females. The rats were treated for 104 weeks, and then observed for 26 weeks.

Control groups of 50 rats were fed basal diet throughout the study. Mean intakes of paracetamol by low- and high-dose rats were 195.4 and 402.1 mg/kg/day in the males, and 335.7 and 688.0 mg/kg/day in the females, respectively. The survival rates at week 104 of each group were 86 to 90% in the males and 80 to 82% in the females. No pathologic or statistical evidence of induction of tumours by paracetamol was found. The authors concluded that under the conditions of this study, paracetamol is not carcinogenic to F344/DuCrj rats of either sex.

In a further dietary study, groups of male and female Leeds strain rats were fed diets containing either 0,5,000 or 10,000 ppm paracetamol for up to 18 months [redacted]). At the 10,000 ppm dosage level, 20% of rats of both sexes developed neoplastic nodules of the liver, a statistically significant incidence. These rats also showed gross enlargement of their livers and an increase in foci of cellular alteration, the latter also being observed in the low dosage male rats. Papillomas of the transitional epithelium of the bladder developed in all paracetamol treated groups, and three rats bore bladder carcinomas. However, significant yields of bladder tumours were only obtained from low dosage females and high dosage males. Additionally, 20 to 25% of paracetamol-treated rats developed hyperplasia of the bladder epithelium, which was not coincident with the presence of bladder calculi. A low yield of tumours at various other sites also arose following paracetamol feeding. An electron microscope study of the livers of paracetamol-treated rats revealed ultrastructural changes in the hepatocytes that resemble those that result from exposure to a variety of known hepatocarcinogens.

In a study in mice groups of male and female IF strain mice were fed a diet containing either 0.5% or 1.0% paracetamol for up to 18 months [redacted] Among male mice fed the higher dose, the total liver cell tumour incidence was 87%, 21.7% developing hepatocellular carcinomas: both yields were statistically significant. The corresponding incidence in high dose females was 19.2% and 4.3%, respectively, only the former being significant. Foci of cellular alteration were also present in the livers of high dose mice of both sexes and also in those of the low dose males.

In another study with mice, groups of 105 male and 105 female B6C3FI mice. received 3,000 or 6,000 ppm paracetamol in feed for 133 weeks, and groups of 50 male and 50 female mice received control diets [redacted]. The survival rates and mean body weights of exposed and control animals were similar throughout the study. No neoplasms were associated with exposure to paracetamol.

In a further study with mice, paracetamol was fed to groups of 60-120 male B6C3FI mice at dietary concentrations of 5000 or 10,000 ppm from 6 weeks of age for periods of up to. 70 weeks to study the hepatotoxic effects of paracetamol [redacted] To test for potential liver tumour-promoting effects of paracetamol, *N*-nitrosodiethylamine (DEN) was injected intraperitoneally at 40 mg/kg into additional groups of 30-60 male B6C3FI mice at 4 weeks of age. Two weeks later some mice received paracetamol at dietary concentrations of 5000 or 10,000 ppm. Mice were sacrificed either at 24 weeks after DEN injection or after 22 or 70 weeks of paracetamol exposure. The livers were weighed and prepared for qualitative and quantitative histological evaluation of focal hepatocellular proliferative lesions (FHPL) including microscopic hyperplastic foci and neoplasms by automated image analysis. At 24 weeks the incidence and number of FHPL per square centimeter were significantly increased only in DEN-treated mice receiving 10,000 ppm paracetamol. Chronic hepatotoxicIty was mild at this time. At 72 weeks paracetamol alone had no effect on the incidence or number of naturally occurring liver tumours despite severe chronic hepatotoxicity and suppression of body weight gain in mice receiving 10,000 ppm and only mild toxicity at 5000 ppm. There were histological findings suggesting that the chronic hepatotoxicity had, in part, a vascular pathogenesis. The authors concluded that this study provided evidence against the hypothesis that chronic hepatotoxicity, in and of itself, results in an increased incidence of naturally occurring liver tumours in mice.

In a two-year carcinogenicity study conducted by the US National Toxicology Program [redacted]), diets containing 0, 600, 3,000 or 6,000 ppm paracetanl01 were given continuously to groups of 60 rats and mice of each sex .for up to 104 weeks. After 65 weeks of exposure, 10 animals from each group were evaluated for histopathology and for haematology, urinalysis, and clinical chemistry parameters. Survival and mean body weights of rats that received paracetamol were similar to those of the controls throughout the study. The average severity of nephropathy was increased in exposed male and female rats. In males this was associated with an increased incidence of parathyroid hyperplasia (renal hyperparathyroidism). The incidence of focal renal tubule hyperplasia was also increased in exposed male rats. The incidence of mononuclear cell leukaemia was increased in exposed female rats and was significantly increased in the 6,000 ppm group *(9/50; 17/50; 15/50; 24/50)*. Survival of exposed and control mice was similar throughout the study. Mean body weights of mice that received paracetamol were generally lower than those of the controls throughout the study. Although the incidence of thyroid follicular cell hyperplasia increased with dose among groups of exposed male and female mice, there was no increase in the incidence of follicular cell neoplasms. Renal tubule hyperplasia occurred in one low-dose and two high dose males and a renal tubule adenoma was present in one low-dose and one high-dose male. It was concluded that under the conditions of these 2-year feed studies, there was no evidence of carcinogenic activity of paracetamol in male F344/N rats that received 600, 3,000 or 6,000 ppm. There was equivocal evidence of carcinogenic activity of paracetamol in female F344/N rats based on increased incidences of mononuclear cell leukaemia. There was no evidence of carcinogenic activity of paracetamol in male and female B6C3F I mice that received 600, 3,000 or 6,000 ppm.

The single cell gel electrophoresis (comet) assay is a simple and effective method for detecting DNA damage in cells with or without the capability of cell division. Methyl methanesulfonate (MMS), as a genotoxic compound that reacts with DNA directly, was confirmed for its DNA damage potential by in vivo comet assay in multiple organs such as liver, kidneys and bone

marrow in mice and paracetamol, a widely used analgesic drug, was evaluated for whether it possesses DNA damage potential or not. These results suggest that the in vivo comet assay might be used to detect the DNA damage induced by MMS and the subsequent DNA repair in mouse liver, kidneys and bone marrow. APAP at the highest dose induces DNA damage in liver. Blood chemical results may indicate that the DNA damage by paracetamol treatment was attributable to hepato-cytotoxicity, because DNA damage and hepato-cytotoxicity were detected at the same doses[redacted]

It has been suggested that paracetamol reduces the risk for ovarian cancer. We examined the association between prescription use of paracetamol and ovarian cancer risk in a nationwide case–control study nested within the Danish female population. Case patients (n = 3471) were all women with a first diagnosis of epithelial ovarian cancer during the period from 2000 to 2009. Population control subjects (n = 50576) were selected by risk set sampling. Data were derived from prescription and other nationwide registries. Conditional logistic regression was used to estimate odds ratios (ORs) and two-sided 95% confidence intervals (CIs) for ovarian cancer associated with use of paracetamol or nonaspirin nonsteroidal anti-inflammatory drugs (NSAIDs). All statistical tests were two-sided. Use of paracetamol was associated with a reduced odds ratio for ovarian cancer (OR = 0.82; 95% CI = 0.74 to 0.92; P < .001) compared with nonuse, and the odds ratio decreased further with long-term (≥10 years), high-intensity paracetamol use (OR = 0.45; 95% CI = 0.24 to 0.86; P = .02). Use of nonaspirin NSAIDs was not associated with ovarian cancer risk[redacted]

4.4.6 Summary of Toxicology

Paracetamol has been extensively studied in animal toxicology studies. Hepatotoxicity occurs following administration of large doses. At non-hepatoxic doses paracetamol has very little toxicity. The mechanism of hepatoxicity has been investigated and is known to result from the production of the intermediate N-acetylP-benzoquinoneimine (NAPQI). Genotoxic effects of paracetamol have been reported in the literature, but only at hepatotoxic doses, and some old longterm toxicity animal studies have shown an increased incidence of liver and bladder tumours at hepatotoxic doses.

4.5 Integrated Overview and Conclusions

This formulation of paracetamol tablets is essentially similar to that of the reference product, Panodil Tablets, and there is no reason to suppose that this paracetamol tablet formulation will affect the drug substance or excipients in any way. All excipients in the formulation proposed for marketing are well established and do not give rise to safety concerns. The *in vitro* dissolution profiles and impurity profiles show that Paracetamol Tablets are essentially similar to Panodil Tablets. Toxicological effects of paracetamol are minimal except in large doses where hepatoxicity is the main effect. Paracetamol has been used for the proposed indications for many years, and therefore its therapeutic use and c1inical safety profile are both well established at therapeutic doses. incidence of thyroid follicular cell hyperplasia increased with dose among groups of exposed male and female mice, there was no increase in the incidence of follicular cell neoplasms. Renal tubule hyperplasia occurred in one low-dose and two high dose males and a renal tubule adenoma was present in one low-dose and one high-dose male. It was concluded that under the conditions of these 2-year feed studies, there was no evidence of carcinogenic activity of paracetamol in male F344/N rats that received 600, 3,000 or 6,000 ppm. There was equivocal

evidence of carcinogenic activity of paracetamol in female F344/N rats based on increased incidences of mononuclear cell leukaemia. There was no evidence of carcinogenic activity of paracetamol in male and female B6C3F I mice that received 600, 3,000 or 6,000 ppm.

4.6 List of Literature Citations