EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

21 July 2011
EMA/CHMP/668488/2011
Committee for Medicinal Products for Human Use (CHMP)

CHMP assessment report

Mercaptopurine Nova Laboratories

International non-proprietary name: **mercaptopurine**

Procedure No. **EMEA/H/C/002022**

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Product information

Name of the medicinal product:	Mercaptopurine Nova Laboratories
Applicant:	Nova Laboratories Ltd. Martin House Gloucester Crescent Wigston Leicester LE18 4YL United Kingdom
Active substance:	mercaptopurine monohydrate
International Nonproprietary Name/Common Name:	mercaptopurine
Pharmaco-therapeutic group (ATC Code):	Purine analogues (L01BB02)
Therapeutic indication(s):	Mercaptopurine Nova Laboratories is indicated for the treatment of acute lymphoblastic leukaemia (ALL) in adults, adolescents and children
Pharmaceutical form(s):	Oral suspension
Strength(s):	20 mg/ml
Route(s) of administration:	Oral use
Packaging:	bottle (glass)
Package size(s):	1 bottle + 1 bottle adaptor + 2 oral syringes

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List of abbreviations

6-MP	6-Mercaptopurine
ACCIS	Automated Childhood Cancer Information
ALL	Acute Lymphoblastic Leukaemia
ANC	Absolute neutrophil count
ASMF	Active Substance Master File
EFS	Event Free Survival
EP	European Pharmacopoeia
GC	Gas Chromatography
HDPE	High Density Polyethylene
HGPRT	Hypoxanthine-guanine phosphoribosyl transferase
HPLC	High Pressure Liquid Chromatography
IARC	International Agency for Research on Cancer
ICH	International Conference for Harmonisation
IR	Infrared
LOAEL	Lowest Observed Adverse Effect Level
MAA	Marketing Authorisation Application
MS	Mass Spectometry
mTIMP	Methylthioinosine monophosphate
NOAEL	No Observed Adverse Effect Level
NMR	Nuclear Magnetic Resonance
PIP	Paediatric Investigation Plan
PSUR	Periodic Safety Update Report
TGN	Thioguanine nucleotides
TIMP	Thioinosine monophosphate
TPMT	Thiopurine S-methyltransferase
UKALL	United Kingdom Childhood Acute Lymphoblastic Leukaemia Trial
USP	United-States Pharmacopoeia
UV	Ultraviolet
XO	Xanthine oxidase

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Nova Laboratories Ltd. submitted on 2 June 2010 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Mercaptopurine Nova Laboratories, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 22 October 2009.

Mercaptopurine Nova Laboratories was designated as an orphan medicinal product EU/3/09/628 on 30 April 2009. Mercaptopurine Nova Laboratories was designated as an orphan medicinal product in the following indication: Treatment of acute lymphoblastic leukaemia. The calculated prevalence of this condition was 1.2 per 10,000 EU population.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Mercaptopurine Nova Laboratories as an orphan medicinal product in the approved indication. The outcome of the COMP review can be found on the Agency's website: ema.europa.eu/Find Medicine/Human medicines/Rare disease designations.

The legal basis for this application refers to Article 10(3) of Directive 2001/83/EC.

The chosen reference product is:

Reference medicinal product which is or has been authorised in accordance with Community provisions in force for not less than 6/10 years in the EEA:

- Product name, strength, pharmaceutical form: Puri-Nethol 50 mg Tablets
- Marketing authorisation holder: The Wellcome Foundation Limited Trading as GlaxoSmithkline UK
- Date of authorisation: 12 December 1986
- Marketing authorisation granted by: United Kingdom
- Marketing authorisation number: PL 00003/5227R

Reference medicinal product authorised in the Community/Member State where the application is made:

- Product name, strength, pharmaceutical form: Puri-Nethol 50 mg Tablets
- Marketing authorisation holder: The Wellcome Foundation Limited Trading as GlaxoSmithkline UK
- Date of authorisation: 12 December 1986
- Marketing authorisation granted by: United Kingdom
- Marketing authorisation number: PL 00003/5227R

Differences compared to the reference medicinal product: change in pharmaceutical form

The application submitted is composed of administrative information, complete quality data and a bioequivalence study with the reference medicinal product Puri-Nethol instead of non-clinical and clinical data unless justified otherwise.

The applicant applied for the following indication: Mercaptopurine Nova Laboratories is indicated for the treatment of acute lymphoblastic leukaemia (ALL). It may be utilised in induction and consolidation phases of therapy of ALL. It is particularly indicated, however, for the continuous phase of therapy.

Information on Paediatric requirements

Not applicable.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the application contained a critical report addressing the possible similarity with authorised orphan medicinal products.

Market Exclusivity

Not applicable.

Scientific Advice

The applicant received Scientific Advice from the CHMP on 24 July 2008. The Scientific Advice pertained to insert quality, non-clinical and clinical aspects of the dossier.

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Manufacturers

Manufacturer of the active substance

Fermion Oy
Lääketehtaantie 2
Sanginsuu
FIN-90650 Oulu
Finland

Manufacturer of the finished product

Nova Laboratories Ltd.
Martin House
Gloucester Crescent
Wigston
Leicester LE18 4YL
United Kingdom

Manufacturer responsible for batch release

Nova Laboratories Ltd.
Martin House
Gloucester Crescent
Wigston
Leicester LE18 4YL
United Kingdom

1.3. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams were:

Rapporteur: **Tomas Salmonson** Co-Rapporteur: **Pierre Demolis**

The EMA Product Team Leader: Irene Papadouli

- The application was received by the EMA on 2 June 2010.
- The procedure started on 21 July 2010.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 8 October 2010 (Annex 1). The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 8 October 2010 (Annex 2).
- During the meeting on 18 November 2010, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 19 November 2010 (Annex 3).
- The applicant submitted the responses to the CHMP consolidated List of Questions on 11 February2011.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 25 March 2011 (Annex 4).
- During the CHMP meeting on 14 April 2011, the CHMP agreed on a List of Outstanding Issues to be addressed in writing by the applicant (Annex 5).
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 23 May 2011.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of outstanding issues to all CHMP members on 9 June 2011 (Annex 6).
- During the CHMP meeting on 23 June 2011, the CHMP agreed on a 2nd list of outstanding issues to be addressed in writing by the applicant (Annex 7).
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 1 July 2011
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the 2nd List of Outstanding Issues to all CHMP members on 11 July 2011 (Annex 8).
- During the meeting on 21 July 2011, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Mercaptopurine Nova Laboratories on 21 July 2011.
- The CHMP adopted a report on similarity of Atriance, Evoltra, Glivec and Sprycel with Mercaptopurine Nova Laboratories on 17 March 2011 (Appendix 1).

2. Scientific discussion

2.1. Introduction

Problem statement

Acute Lymphoblastic Leukaemia (ALL) is a malignant proliferation of lymphoid cells (blast cells), a biologically heterogeneous disorder among patients. A clinical diagnosis is generally refined using morphologic, immunologic, cytogenetic, biochemical and molecular genetics tests of bone marrow-derived blasts. These tests classify patients into two broad subsets, namely 'T' and 'B' cell, and thence into subtypes of 'T' and 'B' cell leukaemia. The clinical and biological characterisation of a patient's disease are used together to direct treatment and as a guide to prognosis.
ALL is the most common malignancy diagnosed in children, representing nearly one third of childhood cancers. Although a few cases of ALL are associated with inherited genetic syndromes, the cause of ALL remains largely unknown.

The lymph nodes, liver and spleen are the most common sites of extra-medullary involvement in ALL. These organs and tissues are enlarged as a result of infiltration by blasts. Lymph node , hepatic or splenic enlargement may be asymptomatic. However, intra-thoracic lymph node enlargement can present as a mediastinal mass with associated respiratory difficulty. Central nervous system and, in boys, testicular involvement is less common. Signs or symptoms of CNS involvement include headache, nausea and vomiting, lethargy, irritability, neck stiffness and papilloedema. Signs of cranial nerve involvement (the third, fourth, sixth and seventh cranial nerves are most frequently involved) may occur. ALL can also present as an intracranial or spinal mass causing numerous neurological symptoms, most of which are due to nerve compression. Testicular involvement appears as painless testicular enlargement and is most often unilateral.

The achievement of a complete remission is a prerequisite for the long-term survival of patients with acute leukemia. First remissions in ALL using amethopterin, an analogue of methotrexate were demonstrated in 1948 (Sidney Farber). Since then, combinations of various agents have been successful in inducing remissions, such as vincristine and prednisone(or dexamethasone), Daunorubicin and L-asparaginase (or PEG–L-asparaginase). The use of additional drugs, such as the anthracyclines (daunorubicin or doxorubicin) or cyclophosphamide is often reserved for children with high-risk ALL.

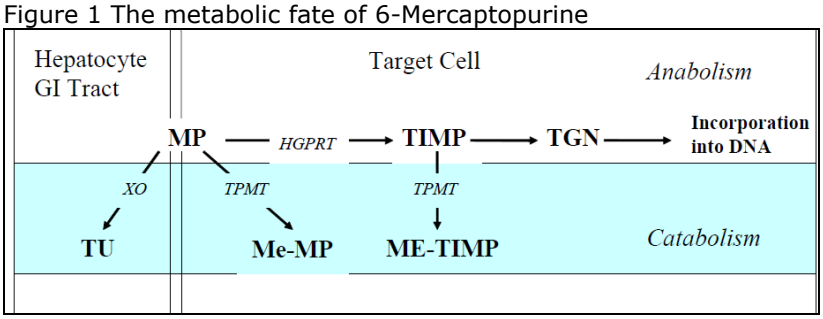
It has been estimated that approximately two to three logs of leukemic blasts are killed during the induction phase of therapy, leaving a residual leukemic burden of approximately 100 million cells. Therefore, additional treatment is necessary to prevent relapse. In the past, children with ALL relapsed within a median of 4 to 6 months when treatment was not continued beyond the remission induction phase. In the late 1960s, investigators at St. Jude Children's Research Hospital developed a "total therapy" approach for the treatment of children with ALL. The model included remission induction, continuation chemotherapy with or without intensification, and preventive CNS therapy. The choice of continuation therapy was empiric. The early St. Jude studies evaluated 6-mercaptopurine, methotrexate, cyclophosphamide, and cytarabine in various doses and combinations. The best outcome was achieved in patients who received 2 to 3 years of daily oral 6-mercaptopurine and weekly methotrexate. Long-term follow-up of patients treated with this latter combination show a 42% disease-free survival rate for children with initial WBC counts of fewer than 25,000 cells per μL, whereas it was only 16% for all other patients. Recent clinical trials have confirmed the importance of intensive re-induction therapy or dose-intensified antimetabolite therapy, even for patients with favorable risk features.

About the product

The development of the purine analogues in cancer chemotherapy (deVita et al 2005) began in the early 1950s with the synthesis of thiopurines. The purine analogues 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG) continue to be used principally in the management of acute leukemia. 6-MP has an important role in maintenance therapy for acute lymphoblastic leukemia (ALL), whereas 6-TG is

active in remission induction and in maintenance therapy for AML. These analogues have a single substitution of a thiol group in place of the 6-hydroxyl group of the purine base. 6-MP is a structural analogue of hypoxanthine, whereas 6-TG is an analogue of guanine. 6-MP is an inactive pro-drug which acts as a purine antagonist but requires cellular uptake and intracellular anabolism to thioguanine nucleotides for cytotoxicity. Finally, azathioprine is a derivative of 6-MP and acts as a prodrug to provide sustained release of 6-MP.

Figure 1 The metabolic fate of 6-Mercaptopurine



6-MP in its monophosphate nucleotide form inhibits *de novo* purine synthesis and purine interconversion reactions, whereas the nucleotide triphosphate metabolites are incorporated directly into nucleic acids. The relative contribution of each of these actions to the mechanism of cytotoxicity is unclear. 6-MP is converted to monophosphate forms by hypoxanthine-guanine phosphoribosyl transferase (HGPRT). The ribonucleotide monophosphate inhibits the first step of *de novo* purine synthesis catalyzed by glutamine phosphoribosylpyrophosphate aminotransferase and block the conversion of inosinic acid to adenylic acid or to guanylic acid. Inhibition of purine nucleotide synthesis leads to the buildup of 5'-phosphoribosyl-1-pyrophosphate, which facilitates the activation of 6-MP and 6-TG to their active nucleotide forms by HGPRT. Both thiopurine ribonucleotide and deoxyribonucleotide metabolites are formed, which can then be incorporated into cellular RNA and DNA, respectively. In some experimental model systems, incorporation of thiopurine nucleotides into DNA correlates with cytotoxicity.

The efficacy of 6-mercaptopurine (6-MP) for the treatment of ALL has been established over many years through a number of national and international trials. Consequently, all treatment protocols used by EU member nations for treating childhood ALL include oral 6-MP. Actual daily doses of 6-MP for the treatment of childhood ALL appear to vary from as little as 7.5 mg to a high of 125 mg. There is no maximum dose of 6-mercaptopurine recommended, as the dosage should be adjusted to suit the individual patient, based on absolute neutrophil count (ANC) and platelet count.

Currently, 6-MP is only available as oral tablet which poses a number of difficulties for the patients and the care giver, especially when used in children. However, for standard maintenance therapy, most children need doses other than that obtained from the 50 mg tablet. In addition, taking whole tablets can be difficult especially in young children and that means that the tablet has to be crushed, which also involves safety risks for the person handling the tablet.

The rationale for the development of Novapurine oral suspension was that a liquid formulation would provide advantages over the single-strength, solid tablet for the treatment of a serious disease in children. The proposed oral suspension offers more flexibility and accuracy in terms of dosing and improved ease of administration (and hence compliance) for children. This is particularly important given that the ideal dosing is to titrate according to haematological values.
Mercaptopurine Nova Laboratories oral suspension (20 mg/ ml) contains 6-MP as monohydrate. The applicant applied for the following indication: *treatment of acute lymphoblastic leukaemia (ALL). It may be utilised in induction and consolidation phases of therapy of ALL. It is particularly indicated, however, for the continuous phase of therapy*. The finally approved indication is: *Mercaptopurine Nova Laboratories is indicated for the treatment of acute lymphoblastic leukaemia (ALL) in adults, adolescents and children*. The dose is governed by cautiously monitored haematotoxicity and the dose should be carefully adjusted to suit the individual patient in accordance with the employed treatment

protocol. Depending on phase of treatment, starting or target doses generally vary between 25-75 mg/m^2 body surface area (BSA) per day.

Type of application and aspects on development

The Applicant submitted on 22nd February 2010 a request for accelerated assessment. The request was rejected since, even though an age appropriate formulation is expected to facilitate the administration of 6-MP to children, the currently available formulation (Puri-Nethol 50 mg tablet) has been used to treat children for many years with good treatment results, and therefore the unmet medical need was questioned.
Mercaptopurine was designated as an Orphan Drug for ALL on 30 April 2009 (EU/3/09/628).

2.2. Quality aspects

2.2.1. Introduction

This Marketing authorization application concerns a centralized procedure for Mercaptopurine Nova Laboratories 20mg/ml oral suspension from Nova Laboratories Ltd. The drug product has been granted orphan medicinal product designation on 30 April 2009.
The application is submitted in accordance with Article 10(3), hybrid application, of Directive 2001/83/EC. The reference medicinal product is Puri-Nethol® 50 mg tablets, from "the Welcome Foundation Limited", authorized in the UK since 12 September 1986.
The finished product is an oral suspension containing Mercaptopurine as active substance.
It is packed in 100 ml glass bottle, with tamper evident, child resistant (CR) closure, supplied with a bottle adaptor (HDPE) and two graduated oral syringes for administration purposes.

The excipients are the following: xanthan gum (viscosity modifier), aspartame (sweetener), concentrated raspberry juice (flavouring agent), methyl parahydroxybenzoate and propyl parahydroxybenzoate (antimicrobial preservative), water (diluent/vehicle).

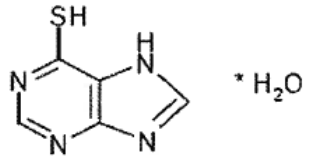
The container closure system consists of a 100 ml glass bottle, with tamper evident, child resistant (CR) closure, supplied with a bottle adaptor (HDPE) and two graduated oral syringes for administration purposes.

2.2.2. Active Substance

The active substance is Mercaptopurine monohydrate. Mercaptopurine is a well-established active substance that has been marketed for several decades as Puri-Nethol® tablets. Mercaptopurine is described in the European Pharmacopoeia (PhEur) and US Pharmacopoeia (USP).

The physico-chemical properties of the active substance (mercaptopurine monohydrate or 6H-Purine-6-thione, 1,7-dihydro-,monohydrate Purine-6-thiol monohydrate) have been adequately detailed including properties such as appearance, melting point, density, solubility, pKa, isomerism (none) and polymorphism (none observed).

The structural formula is shown below:



Manufacture

The description of the manufacturing process includes a micronisation step and the process controls have been provided along with a synthesis chart. Information related to the reagents and raw materials (solvents, catalysts) and conditions of synthesis is considered satisfactory.

Full details of the manufacturing process are presented in an Active Substance Master File (ASMF).

The structure of the active substance has been fully elucidated on one batch of in-house reference material by ^{13}C NMR and ^{1}H NMR (Nuclear magnetic Resonance), MS (Mass Spectrometry), IR (infrared), and UV (ultraviolet) spectroscopy.

A discussion on potential impurities of mercaptopurine including their structure and limits was presented in the application including organic impurities, degradation products and residual solvents.

Specification

Specification of the active substance mercaptopurine includes the following tests: identification (UV Ph.Eur. and IR), sulphated ash (Ph.Eur.), assay (Ph.Eur.), water content (Ph.Eur. Karl-Fischer), organic volatile impurities (GC), residual solvents (GC), related substances (HPLC), particle size distribution (laser diffraction).

Specifications applied by the drug product manufacturer are in accordance with the Ph.Eur. monograph. Additionally, the active substance manufacturer applies internal specifications for related substances, residual solvents, and particle size distribution. Limits for residual solvents are below the ICH Q3C recommended levels.

Regarding the analytical methods, the active substance manufacturer has used the Ph.Eur. methods except for the additional tests, where in-house methods are used and described. The validation of the in-house methods has been presented and followed the ICH guidelines.

Results were presented for 3 commercial scale batches of mercaptopurine from the ASMF holder and from the finished product manufacturer and the results were found in line with the specification.

The results were compliant with the Ph.Eur. Additional specifications for residual solvents are set by the drug substance manufacturer and are in accordance with ICH Q3C. Specifications for particle size were considered acceptable.

The primary packaging is made of transparent polyethylene bags, placed into black bags and then in fibre or plastic drums. The material complies with the Ph.Eur. The in-house specification includes identification by IR and thickness.

The ASMF holder declared that the polyethylene bag is suitable for pharmaceutical materials and provided certificate of analysis from the supplier (stating conformity to the Ph. Eur. monograph 3.1.3) and metal residues analysis results. A certificate of suitability (CEP) has been granted by EDQM for the active substance in the packaging material "transparent polyethylene bag", therefore the information provided was deemed sufficient.

Stability

Stability data have been presented on 10 commercial scale batches kept in the commercial packaging under ICH long term conditions (up to 60 months at 25°C/60%RH) and accelerated conditions (6 months at 40°C/75%RH). In addition, a forced degradation study has been performed and showed that the HPLC method was stability indicating.

Under long-term and accelerated conditions, no significant changes have been noted for assay, water content, and related substances. Results on long term (25°C/60%RH) and accelerated (40°C/75%RH) conditions were conformed to the specification throughout the duration of the study.

A satisfactory re-test period under the recommended storage conditions (room temperature, protected from light) has been justified.

Comparability exercise for Active Substance

Not applicable

2.2.3. Finished Medicinal Product

Mercaptopurine Nova Laboratories 20mg/ml oral suspension is an aqueous suspension of mercaptopurine. It is packed in 100ml amber glass bottles, closed with tamper evident, child resistant cap.

The medicinal product contains the following compendial excipients: xanthan gum (viscosity modifier), aspartame (sweetener), concentrated raspberry juice (flavouring agent), methyl parahydroxybenzoate and propyl parahydroxybenzoate (antimicrobial preservative), water (diluent/vehicle). The composition of the concentrated raspberry juice has been detailed.

The suspension is filled into amber glass bottles at a nominal fill of 100 ml, and closed with a polyethylene screw cap. In use, a polyethylene adaptor is inserted to facilitate the use of a dosing syringe. Graduated 1 ml and 5 ml dosing syringes are provided with each bottle. The bottle and other components are packed in a cardboard carton.

Pharmaceutical Development

Mercaptopurine is a well studied active substance that has been on the market for several decades in the EU in the form of a tablet formulation. It is described as "practically insoluble in water" in the Ph Eur therefore is an ideal candidate for a suspension formulation. Mercaptopurine is labile in both strong acid and base; but in neutral pH it is stable in suspension. Furthermore, the limited solubility reduces the unpleasant taste of the active substance and simplifies the taste masking procedure, and consequently also patient compliance.
The fine particle size distribution of the drug substance, as chosen, helps improving suspension physical stability and the content uniformity of the product, and improved also compliance by eliminating "grittiness" from larger particles.
Since Mercaptopurine is cytotoxic, the formulation design has to take into account minimisation of the potential for unnecessary contact with users and patients during use.

The excipients included in Mercaptopurine 20 mg/ ml suspension are well established compendial excipients (Ph.Eur except the raspberry flavour that complies with the British Pharmacopoeia 1988 monograph). Compatibility of the active substance with the excipients is known due to the applicant's earlier experience of extemporaneous manufacture of mercaptopurine suspensions and has since been supported by stability tests. The strength of the formulation is chosen to give a typical oral drug product dose volume of 1–5 ml.

Excipients used are namely: xanthan gum (viscosity agent), aspartame (sweetener), concentrated raspberry juice (flavouring agent), methyl para-hydroxy benzoate (antimicrobial preservative), propyl para-hydroxy benzoate (antimicrobial preservative),
Concentrated raspberry juice is prepared from the juice of *rubus lutaeus* L. with sucrose added to adjust the weight per ml in the final concentrate. Sulphur dioxide is also added as a preservative and limited without safety concern.
The role of xantham gum and raspberry flavour and their concentration in the finished product are well justified. Xanthan gum modifies the viscosity and thus physically stabilises the suspension to allow accurate dosing.

Aspartame was used to improve the taste. Sucrose as a sweetening agent was not considered appropriate for chronic medication, due to the potential for causing dental caries over the 2 to 3 year treatment period.
The preservatives methyl parahydroxybenzoate and propyl parahydroxybenzoate are included in the formulation, due to the multidose use of the product.
The Applicant intends to introduce a revised preservative system by variation of the Marketing Authorization. Refer to section 2.2.6. Recommendations for future quality development
The analytical procedures used are those described in the Ph.Eur. monograph. Validation was therefore not deemed necessary. No justification of the specification was requested since the excipients are all compendial. Certificates of analysis in line with the specification are included for each excipient.

Although mercaptopurine has been available for several decades in the form of Purinethol tablets, . there was a need for another formulation to address the treatment of paediatric patients. Furthermore, the suspension may also be of help for adult patients with swallowing difficulties.

The proposed formulation is an extension of an already successful "Specials" formulation, which is not authorised but is manufactured under GMP conditions in the UK, developed from it with minimal change from the composition point of view.
The bioavailability of the suspension has been assessed and compared to Purinethol tablets. The suspension was shown to be systemically available faster and more reproducibly.
This suspension should allow more accurate dosing and should be more easily accepted, especially by paediatric patients.

The manufacturing process has been adequately described and can be considered as a standard process. The process is simple and can be divided into three parts: preparation of the base, incorporation of the active substance and filling. The manufacturing process has not changed much since the beginning of the pharmaceutical development. The process allows to maintain the active substance homogeneity until the end of the filling step.

The drug product is filled into 100 ml amber type III glass bottles with child-resistant cap. An adaptor is inserted for in-use dose measurements and graduated 1 ml and 5 ml syringes are provided with each bottle. The container size is chosen to provide sufficient suspension for one month treatment. The description and the choice of the container have been appropriately detailed. A filling overage of 3 ml is added to ensure that a nominal volume of 100 ml can be withdrawn.

The drug product is packaged in a glass bottle with a cap/expanded polyethylene wad. In use the cap is removed, an adaptor for use with HDPE oral dosing syringes is inserted into the neck of the bottle and the original cap is replaced. Graduated 1 and 5 ml dosing syringes are provided with each bottle. No significant interaction between the product and the syringe is anticipated given that the contact period during dosing is brief and that the active substance is highly insoluble in an aqueous vehicle. Stability testing has shown no evidence of interaction between the active substance and the PE compounds of the container closure system.

The bottles are amber soda-lime silica glass and meet the US and PhEur requirements for type III for hydrolytic resistance and for light transmission. Specifications include: appearance, cleanliness, comparison with a reference 100ml bottle, filling volume, hydrolytic resistance test A, and light transmission. Certificate of analysis from supplier includes physical tests and declares compliance with EP requirements as described above and with directive 1935/2004.

The cap is composed of: inner layer of polypropylene (PP), outer layer of high density polyethylene (HDPE), and a liner (wad) of expanded polyethylene. The liner is in direct contact with the medicinal product. The supplier has declared that the outer layer is compliant with the 2002/72/EC directive.

The in-house specifications for the cap include: appearance, cleanliness, comparison with reference cap, cap fit compatibility, identification of polyethylene by IR spectroscopy.
It has been confirmed that the material in the wad, and all other materials in contact with the product, comply with 2002/72/EC.
In addition, the cap is child resistant and complies with the standard EN ISO 8317:2004.

Adventitious agents

Not applicable

Manufacture of the product

The manufacturing process can be summarised as follows: preparation of the parabens solution, then dissolution of aspartame, xanthan gum, raspberry flavour and dispersion of Mercaptopurine, and filling of the bottles. Process parameters have been controlled in a satisfactory manner and in-process controls included tests such as: mixing speed, mixing duration, bottle filling.

Three consecutive batches of mercaptopurine 20 mg/5 ml oral suspension have been manufactured at production scale. Process verification data have been provided including: description of the process parameters, IPC data on the bulk before addition of Mercaptopurine, data recorded on the product after filling (beginning, middle and end of the filling step). The tests included: appearance, pH, density, identification of drug substance, drug substance content, preservatives content, aspartame content, uniformity of mass of delivered dose, dissolution, viscosity and particle size, data on the filling weight. All data complied with the specifications with an adequate intra and inter batch variability. The data presented for the three industrial scale batches can stand for a preliminary process validation study. It can be considered that the manufacturer has good control over the process.

It is declared that Mercaptopurine Nova Laboratories 20mg/5ml does not contain any excipient of human or animal origin. Therefore no TSE risk is anticipated.

Product specification

The release and shelf life specifications of the Mercaptopurine 20 mg/ml suspension are adequate for this pharmaceutical form and the following parameters are tested: appearance (visual), pH (Ph.Eur.), density (Ph.Eur.), uniformity of mass of delivered doses (Ph Eur), viscosity (Ph. Eur.), particle size (Ph. Eur.), dissolution (Ph. Eur.), mercaptopurine content (HPLC), identification (HPLC, BP method), related substances (HPLC), preservative content (HPLC), homogeneity of suspension, aspartame content (HPLC), microbial contamination (Ph.Eur.).

Analytical methods have been described and in-house methods validated in accordance with I*CHQ2* guidelines.

Results for 3 consecutive production scale batches of the medicinal product were presented, representative of the manufacturing process.

Batch analysis results conform to specifications and are consistent, batch-to-batch. Specifications are adequately justified and impurities levels are below the ICH limits and do not present any specific toxicological concern.

Stability of the product

Stability studies have been conducted on 3 production scale batches under ICH long term (5°C) and accelerated conditions (25°C/60%RH , 40°C/75%RH). The batches were studied for 12 months at 5°C and 25°C and 6 months at 40°C.
The following parameters were investigated during those stability studies: appearance, density, uniformity of mass of delivered doses, viscosity, particle size, dissolution, mercaptopurine content, identification, related substances, preservative content, homogeneity of suspension, aspartame content, microbial contamination.
The analytical methods used were the same as those ones used for the control of the finished product.
Post approval, the manufacturer commits to place on stability one batch per year,

The only parameters showing any trend on storage at 25°C/60%RH are aspartame content, viscosity and propyl parahydroxybenzoate content. The reductions were minimal and results remained within the finished product acceptance criteria. Therefore, the product remains in specification for at least a year at 25°C. Preservative efficacy data is included at 12 months for 2 batches performed both by the finished product manufacturer and at an external specialist contract laboratory. The results complied with the requirements of Ph.Eur. In addition preservative efficacy results were also included for one batch after for 18 months at 25°C/60%RH and these results also complied with Ph.Eur.

A change management protocol is in place to re-formulate the preservative system, to eliminate potential toxicity due to propyl hydroxybenzoate, and to improve the robustness of fungal preservation. As an interim measure, agreed at a clarification meeting, a shelf life of 1 year is proposed when the product is stored at a temperature not exceeding 25°C.

In addition, Supportive stability data for the extemporaneous formulation is included in the documentation, covering twelve batches and 3–26 months data under ambient conditions. Assay results were reported as variable, which may be caused by factors outside of the control of the drug product manufacturer. The stability of mercaptopurine in the suspension was unaffected with no evidence of degradation.

The packaging for mercaptopurine oral suspension consists of a 100ml amber glass bottle as the primary container within a cardboard box secondary container. A photo-stability study for the suspension was conducted where samples were exposed to 1.354 million lux hours and 2492 watt hours/m^2. Results indicate that storage in amber glass bottles is sufficient protection for mercaptopurine against photo-degradation. Nevertheless, the data indicate that mercaptopurine is prone to photodegradation. This is in accordance with the storage condition requirement in the Ph. Eur. monograph for mercaptopurine which states that it should be stored protected from light.

In use, the patient dose is titrated so that every patient will be administered a dose tailored to their specific response. The volume of suspension drawn up by each patient therefore varies. An in-use stability study was conducted on two batches of suspension (12 and 46 weeks old) where 0.5ml of Mercaptopurine Nova Laboratories suspension was withdrawn every working day over a time span of 42 days (6 weeks). This would ensure withdrawal of a typical volume on 30 occasions, which mimics one month of openings, from a single bottle of suspension. As the study was conducted over an extended period of time the suspension was subjected to a maximum challenge. There was no detectable bioburden at the end of the study confirming the efficacy of the preservative system. A summary of the data showed there was no loss of potency of mercaptopurine during the study and related substances remained well within specification. All analytical and microbiological parameters remained within specification over the complete study. Microbiological contamination remained at very low levels.

In general the results support the shelf-life and storage conditions as defined in the SPC.

Comparability Exercise for Finished Medicinal Drug Product

Not Applicable

GMO

Not Applicable

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Quality Development

Information on development, manufacture and control of the active substance mercaptopurine and the oral suspension has been presented in a satisfactory manner. The results of test carried out on the active substance and the drug product indicate adequate consistency and uniformity of important quality characteristics and these should lead to a satisfactory and uniform performance in clinical practice.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of the product is considered acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and quality aspects relevant to the uniform performance of the product have been investigated and satisfactorily controlled. There is no concern related to the TSE safety.

2.2.6. Recommendations for future quality development

The applicant should investigate the possibility to eliminate propyl-parahydroxybenzoate from the formulation. The revised formulation will be introduced by a variation as detailed in the Change management protocol presented by the applicant.

2.3. Non-clinical aspects

2.3.1. Introduction

As mercaptopurine is a well-known active substance, non-clinical studies have not been submitted and the application was supported by a literature review. The majority of literature data describing single dose and repeat dose toxicity were reported in a publication (Clarke et al., 1953).

The applicant received Scientific Advice from the CHMP pertaining to the non-clinical aspects of the dossier and more specifically to the need for non-clinical pharmacology or toxicology studies in relation to the excipients intended to be used in the 'to be marketed' formulation.

2.3.2. Pharmacology

Primary pharmacodynamics

In vitro, the cytoxicity of 6-MP was investigated in several human leukaemia cell lines.
Exposure of Molt-4 (human acute lymphoblastic leukemia cell line), CCRF-CEM (T-cell acute lymphocytic leukaemia cell line), HL-60 (acute myeloid leukaemia cell line) and Wilson (Burkitt's lymphoma cell line), to 14C-6-MP (10 µM) over a three hour period resulted in a progressive increase in the intracellular levels of TIMP (predominant metabolite), thioguanosine monophosphate (TGMP) and 6-thioxanthine monophosphate (TXMP). These nucleotide metabolites represented over 80% of the total metabolites derived from 6-MP (Zimm S et al., 1985).

In Molt F4 cells, exposure to mTIMP reduces cell growth and viability, and the depletion of adenine nucleotides is thought to be an important contributor in mTIMP mediated cytotoxicity (Stet EH, 1995).

Results of published studies investigating the relationship between incorporation of TGNs into DNA and cytotoxicity in mouse lymphoma L5178Y cell lines and Molt-4 cells have also been provided. In mouse lymphoma L5178Y cell lines, 0,4% replacement of guanine with TGN was associated with a 99,9% cell kill rate (Tidd DM, 1974). In some studies, 6-MP has been shown to exhibit classical antimetabolite cytotoxicity profiles, with cell kill increasing as a function of both drug concentration and exposure time (Adamson PC et al., 1994). Other studies have found that delayed growth inhibitory effects following a 13 hour exposure time were only found for 6-MP concentrations of 25 -100 µM (Tidd DM, 1974).
Moreover, the Applicant has provided published results of studies investigating the relationship between incorporation of TGNs into DNA and cytotoxicity in mouse lymphoma L5178Y cell lines and Molt-4 cells. In mouse lymphoma L5178Y cell lines, 0,4% replacement of guanine with TGN was associated with a 99,9% cell kill rate (Tidd DM, 1974).
In some studies, 6-MP has been shown to exhibit classical antimetabolite cytotoxicity profiles, with cell kill increasing as a function of both drug concentration and exposure time (Adamson PC et al., 1994).

Other studies have found that delayed growth inhibitory effects following a 13 hour exposure time were only found for 6-MP concentrations of 25 -100 µM (Tidd DM, 1974).
Similarly, for Molt-4 cells, prolonged exposure to 1 µM 6-MP was not associated with cytotoxicity, and exposure times of greater than 8 h with 6-MP concentrations >5 µM were required for significant cell kill (Adamson PC et al.,1994). CCRF-CEM cells too have been shown to require >48 hour exposure to 10 µM 6–MP for any significant cytotoxic effect (da Silva et al., CP 1996).

Results of *in vivo* studies investigating the anti-tumour potency of 6-MP have also been provided. In mice implanted with S-180 tumours, a dose of 50 mg/kg of 6-MP significantly retarded tumour growth and delayed tumour-expansion death from 3 to 6 weeks compared to controls. (Clarke DA et al., 1953). Sugiura (Sugiura K, 1953) showed the activity of MP in altering the growth of a variety of mouse and rat tumours. 6-MP showed activity in a variety of mouse cell leukaemias (Law LW, 1954; Burchenal JH, 1953; Law LW et al., 1954).

Secondary pharmacodynamics

No studies or relevant information on secondary pharmacodynamics were submitted (see discussion on non-clinical aspects).

Safety pharmacology

No safety pharmacology studies and no relevant information from the literature were submitted for mercaptopurine (see discussion on non-clinical aspects).

Pharmacodynamic drug interactions

No studies and no information from the literature were submitted (see discussion on non-clinical aspects).

2.3.3. Pharmacokinetics

No non-clinical pharmacokinetic studies were submitted. Certain aspects of non-clinical pharmacokinetics have been described in the literature as detailed below.

Absorption

No studies and no information from the literature have been submitted (see discussion on non-clinical aspects).

Distribution

An *in vivo* intravenous distribution study in the monkey has been reported (Narang Pk et.al, 1983). Following a single intravenous dose of 6-MP, 6-MP in plasma and CSF had a mean half-life,lambda(z), of 2.9 hours and an apparent volume of distribution (Vd,lambda(z) of 3.00 litres/hr/kg. In the post-distributive phase, the decline of 6-MP concentration from the CSF paralleled that from plasma.

Metabolism

6-MP is a pro-drug and therefore requires metabolism before being able to exert its therapeutic cytotoxic effect. It is activated by hypoxanthine-guanine phosphoribosyl transferase (HGPRT), followed by extensive metabolism to TGNs, before being incorporated into the DNA and RNA. The main enzymes competing for the initial metabolism of 6-MP are HGPRT, thiopurine methyltransferase (TPMT), aldehyde oxidase (AO) and xanthine oxidase (XO). Both XO and AO produce metabolites believed to have little or no cytotoxic action (Coulthard S et al., 2005).

In vivo studies in rat showed 6-MP undergoes a high rate of first-pass metabolism following oral administration (Sasaki H et al., 1987).

In a study performed in rats after repeated administration of 6-MP by ip route for 12 days (Innocenti F et al., 1999), 6-MP was actively metabolised. 6-MP biotransformation can be modulated by agents acting on enzymes of the purine metabolism, resulting in significant changes in erythrocytes and tissue levels of 6-mercaptopurine nucleotides (6-MPN) and the active 6-thioguanine nucleotides (6-TGN).

Excretion

No studies and no information from the published literature have been submitted (see discussion on non-clinical aspects).

Pharmacokinetic drug interactions

In vivo studies in rabbit showed that intravenous co-administration of allopurinol with 6-MP caused a 2-fold increase in the half-life and AUC of 6-MP, a 2-fold decrease in total body clearance and an approximate 3-fold decrease in elimination rate constant. Allopurinol had negligible effects on the pharmacokinetic parameters of the major metabolite, 6-thiouric acid, suggesting that allopurinol serves to increase plasma levels of 6-MP by inhibiting its catabolism and thus contributing to a greater availability of 6-MP to the tissues (Tterlikkis L et Al., 1983).

2.3.4. Toxicology

The pre-clinical toxicology of mercaptopurine was reported in published reports from 1953 and 1954 (Clarke DA et al., 1953; Philips FS et al., 1954; Sugiura K 1953; Burchenal JH 1954; Law LW 1954; Law LW et al., 1954) by the same groups that demonstrated its efficacy in mammalian tumours. All data below come from these reports unless specifically stated otherwise.

Single dose toxicity

Single dose toxicity studies were reported in the literature (Clarke DA et al., 1953). The LD_{50}s of mercaptopurine in mouse, rat and cat by various routes of administration are provided in the following table.

Table 1: Summary of Single Dose Toxicity of mercaptopurine in various animal species

	Route of Administration		
	Estimated LD_{50} in mg/kg		
	Intravenous	Intraperitoneal	Oral
Mouse		227	338
Rat		225	382
Cat	>100		

Regardless of the route of administration, the pattern of mortality following single high doses of mercaptopurine were similar in mice with mortality typically occurring 4 to 7 days after dosing, with animals typically appearing normal for up to 6 hours after dosing. For sub-lethal doses, transient bodyweight losses were noted for up to a week after dosing which were subsequently recovered during the following week. Mortality occurred at an earlier stage in rat, with the majority of mortalities occurring within 24 to 48 hours of dosing. At these high doses, there were severe effects on respiration in rat which was not noted in mouse.

Repeat-dose toxicity

The results of a repeated dose toxicity study performed in dogs and published in the literature have been provided (Clarke DA et al., 1953) as well as data from a small study in monkeys (Product Monograph Purinethol, Canada 2003). Results are summarised in the following table:

Table 2: Summary of repeat-dose toxicity studies provided by the Applicant

Reference	Species/Sex/ Number/Group	Dose (mg/kg) /Route	Duration	Major findings
Clarke et al, 1953	Dog/ 10 mg/kg/day : 4 animals 25 mg/kg/day : 4 animals 50 mg/kg/day : 2 animals	10; 25 or 50 mg/kg/day IV	10 mg/kg/day : 10 days 25 mg/kg/day : 4 days 50 mg/kg/day : 3 days	10 mg/kg/day Weight loss, anorexia and evidence of bone marrow and intestinal injury. *(Recovery was rapid, when treatment was stopped).* 25 mg/kg/day Anorexia, weight loss, leucopenia 1 of the 4 dogs was sacrificed. *The remaining 3 animals showed full recovery within 4 weeks of cessation of the treatment.* 50 mg/kg/day Vomiting within a few hours after the 1st injection and bloody diarrhoea, body weight loss and haemoconcentration. Animals became moribund approximately 4 days after the last injection.and histology showed denudation of the surface epithelium of the intestine with congestion and dilation of capillaries, atypia of glandular nuclei, and leukocytic infiltration of the mucosa. Moderate ↓ in the cellularity of the bone marrow Occasional small areas of focal necrosis in the liver.
Purinethol Product Monograph, Canada 2003	Monkeys/2/ sex/group	20; 40 or 80 mg/kg/day IV	7 or 15 days	**At all doses** Moderate anorexia, slightly decreased activity and slight piloerection. Slight ↑ BUN and serum transaminases ↓ in cell volume , Hb and red cell counts. ↓ WBC count. Pathological changes in lungs and kidneys.

Genotoxicity

As would be expected with a cytotoxic chemotherapeutic, mercaptopurine is clearly genotoxic both *in vitro* and *in vivo.*
A number of genotoxicity studies have been reported in the literature. The Applicant has provided an article of Mosesso P et al, which is a review of data related to the genetic toxicology of 6-MP. Consistent positive results have been observed for point mutation induction (*S. typhimurium*) and DNA damage in bacteria (*Bacillus subtilis, E. coli*). [Mosesso P et al., 1993]. In mammalian cells cultured *in vitro,* including human hepatocytes, positive results were found for the induction of point mutations, chromosomal aberrations and sister chromatid exchange (SCE). [Mosesso P et al., 1993]. In rodents treated *in vivo,* mercaptopurine clearly induced micronuclei, chromosomal aberrations and SCEs in somatic cells and dominant lethals, chromosomal aberrations and SCEs in germinal cells. However, mercaptopurine does not appear to be an inducer of aneuploidy. [Mosesso P et al., 1993].

Carcinogenicity

In the IARC monograph (1987), it is reported that 6-MP was tested by intraperitoneal administration in mice and by intraperitoneal, subcutaneous and intravenous injection in rats. Limitations to the data in all reports precluded evaluation of possible carcinogenicity of this compound.

A 2-year carcinogenicity study published in the literature was also provided to document the carcinogenic potential of 6-MP. In this study (Maekawa A et al., 1990), mercaptopurine was administered to F344 rats via the diet at dose levels of 0, 25 or 50 ppm for 2 years.
In males, there was no significant increase in the incidence of any tumour in the treated groups over that arising spontaneously in the control group.
In females, there were positive trends noted in the occurrence of several tumour types, including C-cell tumours, pheochromotytomas, uterine adenocarcinomas and glioma, with the incidence of C-cell

tumours and pheochromocytomas in the 50 ppm group being significantly higher than in the concurrent control group.
Mercaptopurine is classified by the IARC as 'Group 3', meaning that the evidence of its carcinogenicity in humans is inadequate, even if mercaptopurine is clearly genotoxic in vitro and in vivo.

Reproduction Toxicity

The Applicant has provided an article by Mossesso et al (1993) which is an overview of all available data on 6-mercaptopurine including studies focusing on teratogenic, embryotoxic and reproductive effects of the drug compond.

A study designed to evaluate the effects of chemotherapy on the mouse testis found that mercaptopurine did not affect the viability of differentiated spermatogonia and stem cells as evidenced by testicular sperm head counts on Days 29 and 56 of treatment. However, there were some large round spermatids which were presumed to be diploid and a high frequency of abnormally shaped elongated spermatids (Mosesso P et al., 1993).
Mercaptopurine is embryotoxic in rodents at doses not eliciting maternal toxicity.
In one study mercaptopurine, administered as 2 doses on Days 7 and 8 of gestation, was embryolethal with 50% and 90% of foetuses being resorbed following doses of 5 or 10 mg/kg/dose, respectively (Mosesso P et al., 1993) but no malformations were noted.
Embryolethality and teratogenic effects induced by mercaptopurine in rats dosed on Days 7 and 12, respectively, were also reported (Mosesso P et al., 1993).

Mercaptopurine, when administered to Wistar rats on Days 6-12 of gestation caused 100% death of embryos while lower doses (0.5-0.75 mg/kg) induced anomalies of the nervous system and the eyes. Nervous system anomalies were also induced in Swiss Albino mice dosed on Days 6 to 8 of gestation at doses of 0.5-1 mg/kg.

Limb malformations were seen in both rat (dose of 50 mg/kg on Day 12) and Swiss albino mouse (dose of 60 mg/kg on Day 11), with mandible malformations also observed in mouse on Day 11 of gestation (dose of 60 mg/kg).

In rabbits, and in comparison to mouse and rat, far lower doses of 1 mg/kg were sufficient to induce severe limb malformations.
In addition to a direct lethal effect on foetuses, mercaptopurine reduced the ability of the surviving female offspring (F1 generation) of mice to reproduce upon reaching maturity, and the number of foetuses (live and dead) per pregnant mouse was significantly reduced. Histological examination of ovaries from the F1 offspring revealed reduced numbers of oocytes and ovarian follicles, reflecting the possible reason for small litter sizes in the offspring. Malformations were observed in the second and third generation of offspring (Mosesso P et al., 1993).

Local tolerance

The local skin irritation properties of mercaptopurine were studied as part of a transdermal drug delivery system. No non-clinical studies to assess oropharyngeal toxicity have been submitted (see discussion on non-clinical aspects).

Other toxicity studies

Immunotoxicity

The effects of mercaptopurine on immediate and delayed hypersensitivity were studied in the rabbit (Borel Y, et. al, 1964). Mercaptopurine was show to exert both central and peripheral actions on immune reactions. The central effects were inhibition of humoural antibody formation and suppression of delayed hypersensitivity. Mercaptopurine was also able to block the peripheral manifestations of immunity (skin reactions to antigens) without affecting the immune status of the animal. These various actions of mercaptopurine were brought out under selected conditions of timing, dosage and mode of antigen administration. The processes leading to the development of delayed hypersensitivity were

found to be very sensitive to the effects of mercaptopurine, with 4 days treatment being able to suppress this response without inhibiting humoural antibody production or inflammatory responses. The suppression of skin reactions in hyperimmunized rabbits is presumed by the author of the studies to be due to an anti-inflammatory effect of mercaptopurine and inhibition of the local passive Arthus reaction (local type III hypersensitivity reaction) was a function of the amount of antibody in the test serum.

Studies on impurities / excipients

Hydroxybenzoate Preservatives

The proposed oral mercaptopurine suspension contains methylhydroxybenzoate and propyl-hydroxybenzoate in quantities that are usually recommended for pharmaceutical formulations. There are numerous data addressing the effect of p-hydroxybenzoic acids on spermatogenesis and a LOAEL (lowest observed adverse effect level) value of 12.4 mg/kg has been established for propyl-hydroxybenzoic acid (Johnson and Steer, 2005). The quantity of propylhydroxybenzoate in this formulation is less than 0.25% of LOAEL at doses typically used in the treatment of ALL. In the absence of toxicokinetic data and a sufficient number of animals it is not possible to have a clear idea of the frequency of appearance of observed effects (in particular to specify a threshold of toxicity).

Xanthan gum (Xantual 75)

Xanthan gum (CAS No. 11138-66-2. E415) is a commonly used component of both pharmaceutical products and foodstuffs, having been extensively tested in animals and accepted as a food additive in Europe and USA. The Acceptable Daily Intake (ADI) given by the Food and Agricultural Organization of the United Nations and the World Health Organisation (FAO/WHO) Expert Committee on Food Additives (JECFA) is 'not specified' meaning that, on the basis of available data on safety and tolerance, there is no limit for ADI and the gum may be used at a quantity suitable for its application. The amount of xanthan gum contained in the proposed mercaptopurine oral suspension is normal for pharmaceutical formulations.

Aspartame

Aspartame (CAS No. 22839-47-0, E951) is an artificial non-saccharide sweetener which is a methyl ester of the dipeptide of the amino acids aspartic acid and phenylalanine. It is both widely used as a sugar substitute in food and beverages and used in pharmaceutical formulations as a sweetening agent. It is included in the proposed formulation with raspberry juice concentration to provide a palatable product to be acceptable to children and to help ensure patient compliance. The ADI values specified by the US FDA and the EFSA for aspartame are 50 mg/kg and 40 mg/kg bodyweight, respectively. The amount of aspartame contained in the proposed mercaptopurine suspension is less than 1% of the ADI.

Raspberry juice

The concentrated raspberry juice is a well known and natural (non-synthetic) flavour with a high degree of acceptability.

2.3.5. Ecotoxicity/environmental risk assessment

Table 3: Summary of main study results

Substance (INN/Invented Name): Mercaptopurine			
CAS-number (if available):			
PBT screening		**Result**	**Conclusion**
Bioaccumulation potential- log K_{ow}	Published	= 0,71 [Ni N, Yalkowsky SH. Prediction of Setschenow constants. International Journal of Pharmaceutics (2003) 254: 162-172]. = - 0,17 at pH 5,7 and – 0,37 at pH 7,4 [Hoffman M, Chrzanowska M, Hertman T, Rychklewski J. Modeling of purine derivatives transport across cell membranes based on their partition coefficient determination and quantum chemical calculations. Journal of Medicinal Chemistry (2005) 48 (13) : 4482-4486]. = 0,01 at pH = 7,4 [Hazardous Substances Data Bank of the United States. National Library of Medicine: Hansch C and Leo A. The Log P Database. Claremont, CA: Pomona College 1987].	< LogK_{ow} 4.5 No potential PBT
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$, refined	0,00116	µg/L	< 0.01 threshold

An environmental risk assessment has been performed to evaluate the potential environmental risk in the EU resulting from the use of 6-mercaptopurine monohydrate in Mercaptopurine Nova Laboratories 20 mg/mLOral Suspension. Each 5 mL dose of Mercaptopurine Nova Laboratories 20 mg/mLOral Suspension contains 100 mg 6-mercaptopurine monohydrate.

For the purpose of this environmental risk assessment, the maximum daily dose (DOSEai) of 6-mercaptopurine can be considered to be 150 mg (for a 60 kg adult and a dose of 2.5 mg/kg bodyweight per day). Based on estimations from published epidemiological data (assuming a 100% market share for child and adult ALL) and clinical information, a refined value for market penetration (Fpen) of 0.0000154 for 6-mercaptopurine in Mercaptopurine Nova Laboratories 20 mg/ mL Oral Suspension has been established.
Using the refined Fpen value of 0.0000154, the $PEC_{SURFACEWATER}$ values for 6 mercaptopurine has been calculated to be 0.00116 µg/L.
The log partition coefficient in octanol/water (log Kow) values of 6-mercaptopurine is <4.5, such that this drug substance does not present any hazards with respect to bioaccumulation and persistence. Therefore, 6-mercaptopurine is not classifiable as a Persistent, Bioaccumulative and Toxic (PBT) substance.

2.3.6. Discussion on non-clinical aspects

The pharmacodynamic properties of mercaptopurine are well-known and there is extensive clinical experience with the compound. The Applicant provided a literature review of relevant non-clinical data. This was considered acceptable and the conduct of additional animal studies, including secondary pharmacology, safety pharmacology and pharmacodynamic interaction studies was not considered necessary, as it would not add significantly to the existing knowledge. In terms of safety pharmacology it should be noted that mercaptopurine is not associated with obvious adverse effects on behaviour, respiratory or cardiovascular endpoints in clinical use.

Data on the pharmacokinetics of mercaptopurine are limited but due to the extensive clinical experience with mercaptopurine, the metabolism in humans is well understood.

Overall, the toxicity profile is as expected for this class of compounds. Literature data published mostly in 1953 and 1954 have shown that there are no acute toxicities shown with mercaptopurine and that mortality typically occurs 4-7 days after dosing. In general, disturbances in hematopoietic system, the gastrointestinal tract, and the liver were common findings in animal species tested (rat, mice, dog and monkey). Species specific side effects causing mortalities were also seen such as severe respiratory effects in rats. Additionally myocarditis and pulmonary lesions were reported in rats. Generally, mortalities occurred at earlier stages (24-48 hours of dosing) in rats when compared to mice in which mortalities occurred mostly 4-7 days after the dosing. In both dog and monkey, recovery was rapid upon cessation of dosing with mercaptopurine.

No specific information on non-clinical toxicokinetics from the literature was submitted. The lack of toxicokinetic information from animal studies is considered acceptable given the extensive clinical experience with mercaptopurine.

As it has been established that mercaptopurine is genotoxic, carcinogenic and toxic to reproduction, the safety concerns towards mercaptopurine have already been identified and thus no additional testing in this regard is warranted.

As would be expected with a cytotoxic chemotherapeutic and in common with other antimetabolites, mercaptopurine is mutagenic and causes chromosomal aberrations *in vitro* and *in vivo* in mice and rats. Increases in chromosomal aberrations were observed in the peripheral lymphocytes of leukaemic patients, in a renal cell carcinoma patient who received an unstated dose of 6–mercaptopurine and in patients with chronic renal disease treated at doses of 0.4 – 1.0 mg/kg/day.

A 2 year oral (dietary) carcinogenicity study in rat showed positive trends towards a higher incidence of certain tumours such as C-cell tumors, pheochromocytomas, uterine adenocarcinomas and gliomas, and the incidences of C-cell tumors and pheochromocytomas in the highest dose group were significantly higher than the values in the respective control group. In addition, the total numbers of malignant tumors increased significantly in the female in the high dose group. Most of the tumors showing high incidence in this study are frequently observed as spontaneous lesions in this strain of rats, however the study is of insufficient quality to allow for an adequate assessment. Nevertheless, due to the clear genotoxic potential of mercaptopurine shown both in-vitro and in-vivo, the carcinogenic potential of mercaptopurine cannot be excluded. This is adequately expressed in sections 4.4 and 5.3 of the SPC.

6-MP, in common with other cytotoxics, has shown to be teratogenic in experimental animals at doses similar or greater than those used therapeutically in humans. Increased frequencies of fetal death, central nervous system, facial and limb anomalies were observed among offsprings of mice, rats and rabbits treated at various times during organogenesis. In all species, the degree of embryotoxicity and the type of malformations are dependent on the dose and stage of gestation at the time of administration. In addition to a direct lethal effect on foetuses, mercaptopurine affected the ability of surviving female foetuses to reproduce upon reaching maturity and malformations in the second and third generation of offspring were reported. Mercaptopurine did not affect the viability of differentiated spermatogonia and stem cells in the mouse but did produce some large round spermatids (presumed to be diploid) and a high frequency of abnormally shaped elongated spermatids. The text in section 4.6 and relevant instructions in the package leaflet adequately reflect this information.

No juvenile toxicology studies have been conducted but the clinical experience in this regard is considered sufficient.

The local skin irritation properties of mercaptopurine were studied as part of a transdermal drug delivery system. This study is however not relevant for the current application.
No non-clinical studies have been conducted to assess oropharyngeal toxicity. Given the long history of use of mercaptopurine and the extensive clinical database, the Applicant considers that the non-clinical assessment of local tolerance is unnecessary.

Oropharyngeal mucositis is considered unlikely to be influenced by local exposure to mercaptopurine, but will be influenced by systemic levels of TGN, the active metabolite.

Mercaptopurine has been tested for skin irritation in mouse and human with 23 hour exposure per day for 21 days and shown to be non-irritant.

According to the CHMP Scientific Advice given, data on local tolerance of the new formulation in an adequate animal study would be considered of relevance since the surface of oral, pharyngeal and oesophageal mucosa and peri-oral skin (due to accidental spilling) exposed to dissolved drug is expected to be considerably higher after intake of a liquid formulation than after intake of a tablet. Since these data were insufficient this issue is addressed in the clinical section.

Scientific advice has been sought by the Applicant as to whether there is a need for non-clinical pharmacology or toxicology studies in relation to the excipients intended to be used in the 'to be marketed' formulation. The CHMP advised that the necessity of any excipient used in the formulation has to be demonstrated and the content of all excipients should be restricted to a minimum without affecting the quality, safety and efficacy of the drug product. The discussion can be based on thorough literature research, considerations of the long term administration of the proposed drug product and special emphasis on the intended use in children undergoing chemotherapy. The CHMP also advised the applicant to discuss risk associated with the use of the preservatives propyl parahydroxybenzoate and methyl parahydroxybenzoate at the proposed long term treatment in accordance with the Guideline on "Excipients in the label and package leaflet of medicinal products for human use".

All of the excipients in the liquid formulation of mercaptopurine are considered known and commonly included as food additives and/or as ingredients in pharmaceutical formulations and not associated with toxicity, including any local tolerance effects. However, the choice of preservatives as esters of *p*-hydroxybenzoic acids (parabens), perhaps especially propylhydroxybenzoate (propylparaben) in the paediatric populations is generally questioned.

Parabens are known to have oestrogenic activity (EFSA scientific opinion 2004), and published available toxicological information showed uncertainties regarding parabens as food additives. The conclusion from the expert panel was that dietary administration of propyl paraben (propyl hydrobenzoate) induced adverse effects (sperm cells, impaired spermatogenesis, and reduced testosterone) in male rats at dose levels of 10 mg/kg/day. No ADI could be recommended for propyl paraben because of the lack of a clear NOAEL. The NOAEL for methyl and ethyl paraben was considered to be 1000 mg/kg/day.

Generally, the use of propyl hydrobenzoate in pharmaceuticals for the paediatric population should be avoided because of its endocrine disrupting properties. However, the level of propyl hydrobenzoate exposure for patients treated with Mercaptopurine Nova Laboratories is calculated by the Applicant to be less than 0.25% of the LOAEL which is considered to be low. The applicant has acknowledged the recommendations of the CHMP to minimize or eliminate the content of higher esters of hydroxybenzoate in Mercaptopurine Nova Laboratories. If required, a revised formulation will be introduced by variation of the marketing authorization.

The content of aspartame in the liquid formulation of mercaptopurine is low and at the typical doses prescribed for ALL will be less than 1% of the accepted daily intake (ADI). This is acceptable and in line with the CHMP Scientific advice given.

The estimated UVmax for mercaptopurine seems to be 328nm and biodistribution studies in the pigmented tissues are lacking. Normally, in these situations the lack of phototoxicity studies should be justified but due to the extensive clinical experience with mercaptopurine the lack of photoxicity studies is considered acceptable.

In order to evaluate the potential environmental risk in the EU resulting from the use of 6-mercaptopurine monohydrate in Mercaptopurine Nova Laboratories 20 mg/ mL Oral Suspension, an environmental risk assessment has been performed and submitted.
Based on estimations from published epidemiological data (assuming a 100% market share for child and adult ALL) and clinical information, a refined value for market penetration (Fpen) of 0.0000154 for 6-mercaptopurine in Mercaptopurine Nova Laboratories 20 mg/ mL Oral Suspension has been established. This value is considerably lower than the default value of 0.01 in the CHMP Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use (EMEA/CHMP/SWP/4447/00,

01 June 2006). Using the refined Fpen value of 0.0000154, the $PEC_{SURFACEWATER}$ values for 6-mercaptopurine has been calculated to be 0.00116 µg/L. This value is nearly 10 times lower than the action limit of 0.01µg/L. Therefore, Phase II environmental fate and effects assessments were not considered necessary, which is acceptable and in line with the aforementioned guideline.
In conclusion, mercaptopurine is of negligible risk to the environment from the use of Mercaptopurine Nova Laboratories 20 mg/ mL Oral Suspension for the treatment of ALL, when used in accordance with the Summary of Product Characteristics (SmPC) and the Package Leaflet.

2.3.7. Conclusion on the non-clinical aspects

6-MP has now been used clinically for over 50 years. During this period, many thousands of children with ALL, throughout the world, have been administered 6-MP as part of a chemotherapy regimen. A great deal of understanding of the pharmacology and toxicology of the compound has therefore been acquired. No new preclinical data has been submitted for this new formulation of mercaptopurine and the literature data summarised by the applicant is considered sufficient.

2.3.8. Recommendation for future pre-clinical development

As discussed in section 2.3.6. above, the use of parabens as excipients should be avoided.
The CHMP recommended to the Applicant to investigate the possibility to eliminate propyl-parahydroxybenzoate from the formulation.

2.4. Clinical aspects

2.4.1. Introduction

This is an application for oral suspension containing mercaptopurine. To support the marketing authorisation application the applicant submitted one bioequivalence study with cross-over design under fasting conditions. This study was the pivotal study for the assessment.

The applicant provided a clinical overview outlining the pharmacokinetics and pharmacodynamics as well as efficacy and safety of mercaptopurine based on published literature.

Formal scientific advice was given on clinical aspects by the CHMP for this medicinal product. The submission of only bioequivalence data to support the clinical aspects of this application is in line with this Scientific Advice. For the clinical assessment, the *Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev.1*) in its current version is of particular relevance.

In certain EU countries such as the UK, manufacturers of so called 'specials' have provided unlicensed liquid formulations of 6-MP for some time, including the currently applied formulation of Mercaptopurine Nova Laboratories.

GCP

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

The bioequivalence clinical study was performed at AddClin Research (Pty) Ltd., Pretoria, South Africa. The study was monitored by Shandon Clinical Trials Ltd, Cork Ireland. The applicant has provided a statement to the effect that the study was conducted in accordance with South African law, the European Clinical Trials Directive (2001/20/EC), the ICH Guideline for Good Clinical Practice (CPMP/ICH/135/95, January 17, 1997) and the Declaration of Helsinki Directive 2001/20/EC.

External audits: The clinical site (AddClin in South Africa) was audited by the Medicines Control Council of South Africa (MCC) in November 2005, November 2007 and on 21 - 23 July 2010 (Pending). Shandon Clinical Trials has been audited by the Irish Medicines Board (IMB) most recently in 2004 (GCP), 2007 (GMP) and 2010 (GCP).

Internal audits: The study report informs that on behalf of Nova Laboratories Ltd a GLP compliance audit on 18 Aug, 2009 was performed by Triclinium (a private CRO) at the analytical laboratory (GBN Analytics – division of Parexel (Pty) Ltd., South Africa). Pre-study audits for GCP compliance were performed by Shandon Clinical Trials (accompanied by the Nova Laboratories representative) at AddClin Research (Aug 4-5, 2009) and at Parexel (Pty) Ltd. (Aug 7, 2009). Triclinium performed various monitoring visits at AddClin during the course of the clinical phase of the study. The statistics were quality assured by the Shandon Clinical Trials Ltd. Quality Assurance unit, which also checked the study final report according to ICH GCP.

2.4.2. Pharmacokinetics

The pharmacokinetic properties of 6-mercaptopurine tablet formulation are well known out of the experience with Puri-Nethol 50 mg tablets and are summarised below. A bioequivalence study intended to bridge the existing data with the new liquid formulation was submitted as a basis for this application.

Absorption

About 50% of an oral dose of 6-MP is suggested to be absorbed from the GI tract. However, the absolute oral bioavailability is lower due to high first-pass metabolism, which is also subject to large inter-individual variation. A mean oral bioavailability of 16% has been reported in children, with a range from 5 to 37%. The mean Tmax is 2.2 hours with a range of 0.5 to 4 hours.

6-MP disappears rapidly from plasma, with $t_{1/2}$ values of 20 to 90 minutes reported after intravenous administration. Generally, no parent compound is detected in plasma 8 hr after dose. However, 6-MP is activated intra-cellularly by conversion to cytotoxic ribonucleotide derivates, which have a longer half-life (approximately 5 hours).

In the liver, mercaptopurin is rapidly and extensively metabolised by methylation and oxidation as well as the formation of inorganic sulfates. S-metylated metabolites are formed via tiopurine methyltransferase (TPMT). TPMT activity is highly variable in patients due to genetic polymorphism. In a Caucasian population approximately 89% have normal enzyme activity, 11% intermediate activity and 0.3% low or non-detectable activity. On chronic dosing, low TPMT activity leads to a high accumulation of 6-thioguanine nucleotides in erythrocytes, which has been associated with myelosuppression. The inactive metabolite 6-thio uric acid is formed via xanthine oxidase and is excreted in urine. About 7% of an oral dose has been reported to occur as unchanged parent compound in urine within 12 hours.
The absolute oral bioavailability is low due to high first-pass metabolism, which is also subject to large inter-individual variation. A mean oral bioavailability of 16% has been reported in children, with a range from 5 to 37%. The mean Tmax is 2.2 hours with a range of 0.5 to 4 hours.

The Stockley's drug interaction database reports three food interaction studies in children. In one study, administration of mercaptopurine 15 minutes after a standard breakfast including milk delayed Tmax and reduced the AUC and Cmax by 26% and 36%, respectively. However, in another study a high inter-individual variation was seen, but no clear effect of a breakfast consisting of milk or yoghurt, cereal and sandwiches, on mercaptopurin bioavailability. Yet another study showed a not statistically significant decrease in AUC and/or Cmax by 20-22% after a standardised breakfast, again with a wide inter-individual variation. *In vitro* data indicate that mercaptopurine is catabolised in cow's milk, suggested due to the presence of the mercaptopurine-metabolising enzyme xanthine oxidase which is present in milk.

The Applicant presented a case report (Sofianou-Katsoulis *et al*, 2006) of a patient who during the maintenance phase had elevated peripheral blood counts despite increasing the 6-MP dose to 160% of the calculated dosage for his body surface area (BSA). It was found out that the boy routinely took the

chemotherapy with cow's milk. After changing to water, the 6-MP dose could be reduced to the normal dose (100% of the calculated dose for his BSA). The authors suggest that the reduced bioavailability of 6-MP at administration with cow's milk could be due to the high content of xanthine oxidase in 6-MP. Rivard *et al*, 1989, showed that incubation of 6-MP with pasteurised cow's milk at 37°C for 30 minutes resulted in 30% catabolism of 6-MP. There was no effect if the milk was boiled for 5 minutes. In a study in 17 children with ALL, Riccardi *et al*, 1986, demonstrated that administration of 6-MP with a breakfast, including 250 ml milk, reduced AUC by about 27%. In a study by Lonnerholm *et al*, 1989, on the other hand, there was no difference in the mean bioavailability of 6-MP when taken with breakfast compared with the fasted state (mean ratio 1.03) but the individual fed/fasted ratios varied considerably, between 0.33 and 1.81.

Bioequivalence

Clinical bioequivalence study (SC02808)

In support of the application, one original clinical study report has been submitted. The primary objective of the study was "to evaluate the pharmacokinetic characteristics and compare the bioavailability of a Test formulation (Mercaptopurine Oral Suspension 100 mg/5 ml) and the marketed Reference formulation (Puri-Nethol 50 mg Tablet) using a single-dose, randomised, two-period crossover design".

Methods

Study design

The study was a randomised, two-treatment, two-period, two-sequence single-dose crossover study conducted in 60 male, healthy volunteers under fasting conditions. After an overnight fast, subjects were dosed with either one 50 mg Puri-Nethol tablet (reference) or 2.5 ml of the 6-MP oral suspension 20 mg/mL(test). Subjects were not served any food until 4.5 hours after the dose. Blood-samples were collected pre-dose and at 0.25, 0.5, 0.75, 1.0, 1.33, 1.67, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0 and 12.0 hours after drug administration. The study periods were separated by a wash-out period of at least 72 hours. The clinical part of the study was conducted between Sept 25 and Oct 7, 2009, at AddClin Research (Pty) Ltd., Pretoria, South Africa. The study was monitored by Shandon Clinical Trials Ltd, Cork Ireland. The study was sponsored by Nova laboratories Ltd. The analytical part of the study was conducted between Nov 25 and Dec 7, 2009, at Parexel Bioanalytical Services Division, Mosselbay, South Africa.

Test and reference products

Test product: Mercaptopurine oral suspension, 100 mg/5ml, manufactured by Nova Laboratories Ltd. Leicester, UK, batch No. 0790W001, expiry date: Oct 6, 2009.
Reference product: Puri-Nethol, 50 mg tablet, manufactured by GlaxoSmithKline, UK, batch No. 902797, expiry date: Dec 31, 2013.

Population studied

The study was conducted in healthy, male volunteers. A total of 60 adult healthy volunteers were to be enrolled. Dropouts were to be replaced if the number of subjects completing the study would otherwise be less than 60. However, there were no drop-outs and all 60 subjects completed both study periods and were included in the pharmacokinetic analysis.

Analytical methods

Plasma samples were stored at -70ºC in the clinic until shipped to the analytical laboratory. Plasma concentrations of mercaptopurine were determined with a validated LC/MS/MS method. 6-thioguanine was used as internal standard. Altogether, 1920 plasma samples from 60 volunteers were analysed.

Pre-study validation
The validation range for mercaptopurine was 0.508 - 128 ng/ml for 6-mercaptopurine. The lowest recorded concentration of mercaptopurine was 0.5 ng/mL and the highest was 255 ng/mL. A dilution test showed that concentrations of up to 205 ng/ml of 6-mercaptopurine in plasma could be analysed reliably when diluted into the calibration range. High variability of analyte peak areas was observed in the presence of haemolysed blood (1 %). The internal standard provided sufficient compensation so that the precision of the peak area ratios was well within acceptance criteria.

Specificity was shown employing 6 independent sources of human plasma. Sensitivity at the limit of quantification, 0.508 ng/ml, was shown. Satisfactory between- and within-run accuracy and precision was shown for low, medium and high QC sample concentrations. Dilution integrity was demonstrated. Stability in plasma was demonstrated for 6 h at room temperature, and over 3 freeze-thaw cycles.

Within-study validation
The assay method was subject to partial re-instatement validation in connection with analysis of study samples. The validation range for mercaptopurine was 0.497 ng/ml - 130 ng/ml. Specificity was demonstrated in blank plasma from six sources. Recovery was 87%, 86% and 84% for high, medium and low QC samples, respectively. Satisfactory method performance during study sample analysis was demonstrated. Appropriate batch acceptance criteria were used. Repeated analysis was adequately justified.

Pharmacokinetic Variables

Pharmacokinetic variables were calculated using conventional non-compartmental methods. The primary pharmacokinetic variables were C_{max}, AUC_{0-t} and $AUC_{0-\infty}$.

Statistical methods

The statistical analysis was performed at Shandon Clinical Trials Ltd. To compare the pharmacokinetics of the two products, ANOVA was used on log-transformed AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} and 90% confidence interval for the test/reference ratio were calculated. The protocol stated that the oral suspension and the tablet were to be concluded bioequivalent if the 90% confidence intervals for the test/reference ratio of the population geometric means fell within 80-125% for AUC_{0-t} and C_{max}.

Results

The pharmacokinetic results of the study are presented in Table 4and Figure 1below.

The extent of exposure (AUC) and the peak concentration (Cmax) were approximately 13% and 40% higher, respectively, for the oral suspension than for the tablet. The absorption rate was higher for the suspension, with a point estimate for Tmax ratio (suspension vs. tablet) of 0.48 (90% CI:s 0.28-0.61).

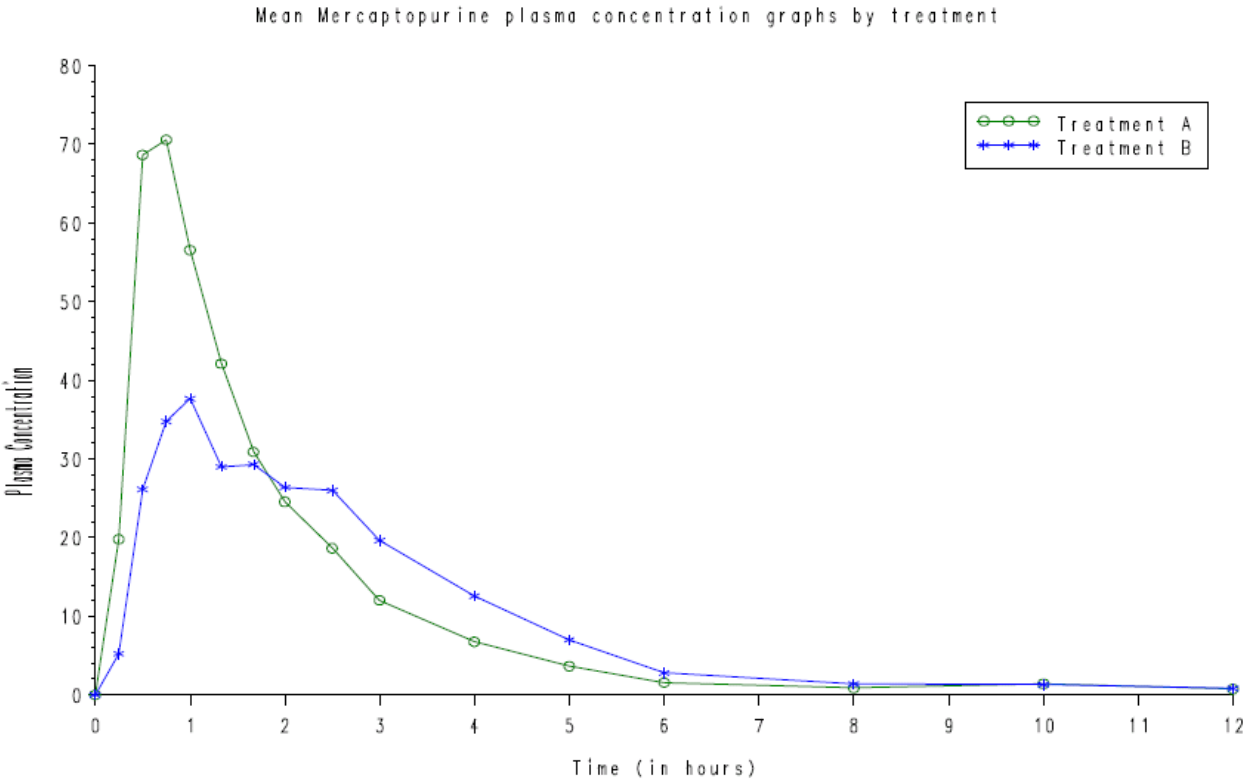
Table 4: Pharmacokinetic parameters, study SC02808

Treatment	AUC_{0-t} ng*h/ml	$AUC_{0-\infty}$ ng*h/ml	C_{max} ng/ml	t_{max} h
Suspension	121.64 ± 36.70	123.31 ± 37.15	86.63 ± 39.65	0.75 (0.25 - 2.50)
Tablet	109.39 ± 43.19	11.92 ± 43.09	68.99 ± 47.49	1.67 (0.50 - 5.00)
***Ratio suspension/tablet (90% CI)**	114.10 107.92 - 120.64	112.65 106.63 - 119.02	139.07 122.39 - 158.03	-

AUC_{0-t} area under the plasma concentration-time curve from time zero to t hours
$AUC_{0-\infty}$ area under the plasma concentration-time curve from time zero to infinity
C_{max} maximum plasma concentration
t_{max} time for maximum plasma concentration

**calculated based on ln-transformed data*

Figure 1: Plot of the mean concentration of mercaptopurine in plasma against time following a single dose of Treatment A (suspension) and Treatment B (tablet)



The inter-subject variability was lower for the suspension (treatment A) than for the tablet (treatment B), as can be seen in Table 5. More specifically, the inter-individual CVs for AUC were 30% and 39% for the suspension and for the tablet, respectively. The inter-subject variation in Cmax was 46% for the suspension and 69% for the tablet. Also the Cmax range was narrower for the suspension, 37.7 - 212 ng/ml vs. 6.72 - 255 ng/ml for the tablet. As a result, the lowest as well as the highest Cmax detected was for the tablet (Figure 2a and b).

Table 5: Inter-individual CVs fro Cmax, AUC0-t, AUC0-∞ and Tmax

	C_{max}		AUC_{0-t}		$AUC_{0-\infty}$		T_{max}	
	A	B	A	B	A	B	A	B
Inter-subject CV %	45.77%	68.83%	30.17%	39.48%	30.13%	38.50%	51.40%	66.19%

treatment A = suspension, test
treatment B = tablet, reference

Figure 2a: Overlaid plots of individual concentrations of mercaptopurine in plasma against time following a single dose of Treatment A (oral suspension)

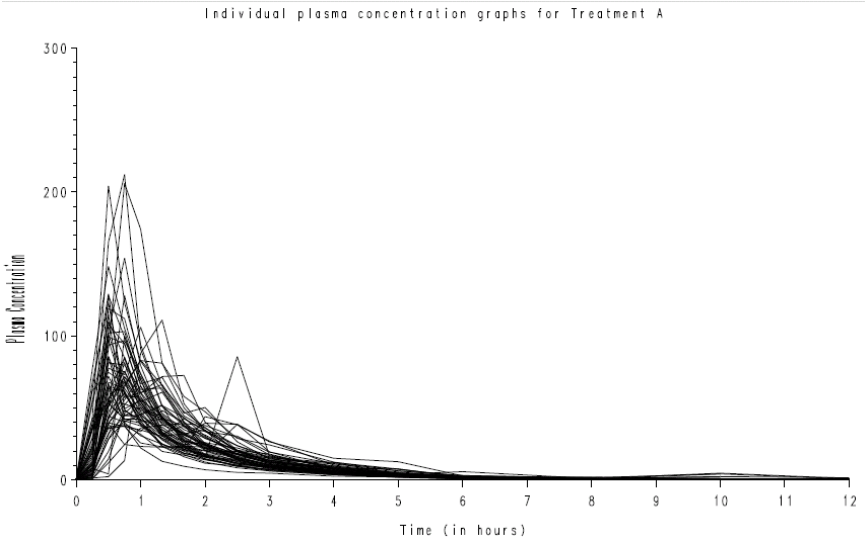
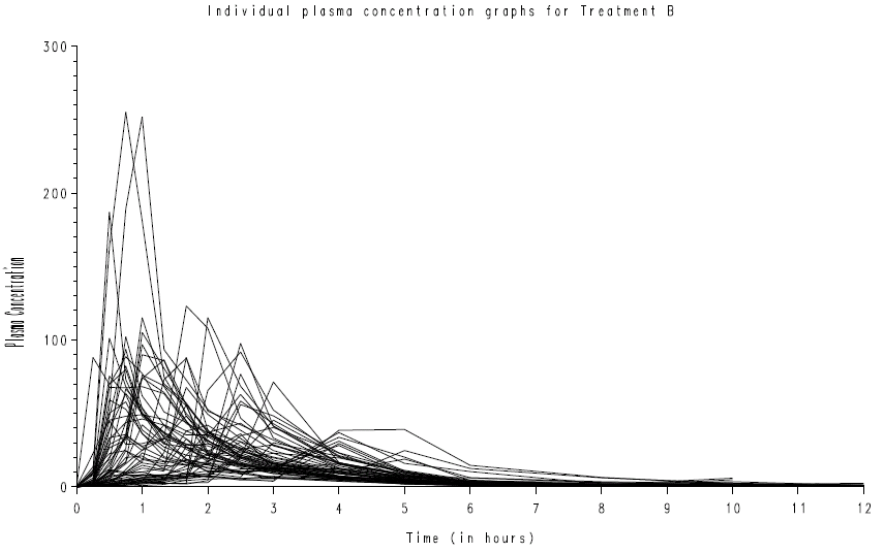


Figure 2b: Overlaid plots of individual concentrations of mercaptopurine in plasma against time following a single dose of Treatment B (tablet)



Elimination

6-MP disappears rapidly from plasma, with $t_{1/2}$ values of 20 to 90 minutes reported after intravenous administration. Generally, no parent compound is detected in plasma 8 hr after dose. However, 6-MP is activated intra-cellularly by conversion to cytotoxic ribonucleotide derivates, which have a longer half-life (approximately 5 hours). The active 6-MP ribonucleotides are formed via hypoxantin guanine phosphoribosyl transferase in a step-wise process. 6-MP ribonucleotide inhibits purine nucleotide synthesis and metabolism which in turn alters the synthesis and function of RNA and DNA.

In the liver, mercaptopurine is rapidly and extensively metabolised by methylation and oxidation as well as the formation of inorganic sulfates. S-metylated metabolites are formed via tiopurine methyltransferase (TPMT). TPMT activity is highly variable in patients due to genetic polymorphism. In a Caucasian population, approximately 89% have normal enzyme activity, 11% intermediate activity and 0.3% low or non-detectable activity. On chronic dosing, low TPMT activity leads to a high

accumulation of 6-thioguanine nucleotides in erythrocytes, which has been associated with myelosuppression. The inactive metabolite 6-thio uric acid is formed via xanthine oxidase and is excreted in urine. About 7% of an oral dose has been reported to occur as unchanged parent compound in urine within 12 hours.

Special populations

There are no specific studies on the pharmacokinetics or safety of 6-MP in patients with renal or hepatic impairment.

2.4.3. Pharmacodynamics

No new pharmacodynamic studies were presented (see discussion on clinical pharmacology).

2.4.4. Discussion on clinical pharmacology

The pharmacological profile of 6-mercaptopurine is well known. The bioavailability of oral 6–mercaptopurine shows considerable inter-individual variability, which probably results from its first-pass metabolism. When administered orally at a dosage of 75 mg/m^2 to 7 paediatric patients, the bioavailability averaged 16% of the administered dose, with a range of 5 to 37%.

Data on the effect of food on mercaptopurine bioavailability appears to be inconsistent and based on the variability observed (in published literature data) it is suggested that the main concern might be milk products, since these contain xanthine oxidase. Although, based on the presented data, it might be difficult to discern the effect of food in general from the effect of milk, recommendations to avoid concomitant intake of milk products were included in the product information. Since drug administration in the fasted state may be difficult, especially in small children, appropriate recommendation that for the individual patient, administration should be standardised with regard to concomitant food, but that concomitant milk products should be avoided is included in the SPC. This is considered adequate, given that the dose will be individually titrated based on haematological response.

Given the divergent results of food interaction studies, and that administration in the fasted state might be difficult, especially in small children, the following recommendations have been introduced in the Summary of product Characteristics and reflected in the Package Leaflet:

Section 4.2:
Mercaptopurine Nova Laboratories may be taken with food or on an empty stomach, but patients should standardise the method of administration. The dose should not be taken with milk or dairy products (see section 4.5). Mercaptopurine Nova Laboratories should be taken at least 1 hour before or 2 hours after milk or dairy products.

Section 4.5 :
The administration of 6–mercaptopurine with food may decrease systemic exposure slightly but this is unlikely to be of clinical significance. Therefore, Mercaptopurine Nova Laboratories may be taken with food or on an empty stomach, but patients should standardise the method of administration. The dose should not be taken with milk or dairy products since they contain xanthine oxidase, an enzyme which metabolises 6–mercaptopurine and might therefore lead to reduced plasma concentrations of mercaptopurine.

The available evidence suggests administration of mercaptopurine in the evening results in greater efficacy and hence reduced risk of relapse, therefore the optimal time of the day for Novapurine intake is in the evening. The following wording is included in section 4.2:

Mercaptopurine displays diurnal variation in pharmacokinetics and efficacy. Administration in the evening compared to morning administration may lower the risk of relapse. Therefore the daily dose of Novapurine should be taken in the evening

As there are no specific studies on the pharmacokinetics or safety of 6-MP in patients with renal or hepatic impairment, the SPC recommends caution in these populations.

The bioequivalence study was conducted in healthy volunteers. Testing of cytotoxic agents in healthy volunteers is considered problematic from the ethical point of view. The Applicant considered the use of healthy volunteers as justified since a single dose was administered on two separate occasions only, while studies in humans and animals have indicated that the effects of 6-MP on the human body are caused by chronic administration. In childhood and adult leukaemia, doses vary from 1.5 - 5 mg/kg/day (continuously). Thus, in an adult male weighing 70 kg the minimum dose will be 105 mg/day. In this study, volunteers were given a single 1 x 50 mg dose of 6-MP (as an oral suspension or tablet) at each of the two dosing visits with a washout period of at least 3 days between visits. The relatively low dose and the dosing schedule was suggested to minimise the risk of any short- or long-term toxicity from two doses of 6-MP. Furthermore, subjects were phenotyped for thiopurine methyltransferase (TPMT) activity. Only subjects with normal or high enzyme activity were recruited into this study.

There have been cases where bioequivalence studies have been conducted in healthy volunteers (e.g. generic versions of 6-MP and azathioprine -a pro-drug of 6-MP tablets) and this approach has been considered acceptable as part of a Marketing Authorisation application. However, since 6-MP is an anti-purine metabolite with a significant toxicity profile, it was appropriate to limit the number of adult healthy volunteers recruited as part of the bioequivalence study.

The Applicant suggested that use of adult instead of paediatric volunteers was justified since 6-mercatopurine as Puri-Nethol (the reference formulation) is used in both adults and children without any difference in dosing, which is given on a mg/m^2 basis.

There are no data to suggest that age is likely to influence the performance in vivo of different 6-MP formulations and hence absorption. A bioequivalence study in children would pose a number of significant challenges:-
- The ALL population is heterogeneous with significant covariates (e.g. co-medications such as methotrexate) which could influence the rate and extent of 6-MP absorption.
- The dosing is individualised; 6-MP is initiated at a dose of 75mg / m^2 during the continuation phase of ALL treatment (i.e. according to the child's body surface area). During the course of therapy, the dose is individualised for each child according to absolute neutrophil and platelet counts. Thus, the possibility of recruiting children that are taking a single (or multiples of) 50mg Puri-Nethol tablet(s) will be extremely limited and present a significant challenge. For the majority of children, the 50mg tablet is split to achieve as near as possible to the desired dose. As already mentioned in the Section 6, this can result in administering a dose which varies significantly from that intended and therefore may bias the trial outcome.
Moreover, it is recognised, that according to "Guideline On The Role Of Pharmacokinetics In The Development Of Medicinal Products In The Paediatric Population" (Doc. Ref. EMEA/CHMP/EWP/ 147013/2004), bioequivalence studies for bridging paediatric clinical documentation between two formulations should preferably be performed in adults, since the applicant can justify that the study results can be extrapolated to the paediatric population. Hence the inclusion of healthy volunteers was considered acceptable provided the risks are acceptable for healthy volunteers.

The results indicated that the extent of exposure (AUC) and the peak concentration (Cmax) were approximately 13% and 40% higher, respectively, for the oral suspension than for the tablet, although the 90% confidence interval for the AUC ratio fell within the normally applied limits for concluding bioequivalence between two products. The absorption rate was clearly higher for the suspension, with a point estimate for Tmax ratio (suspension vs. tablet) of 0.48 (90% CI:s 0.28-0.61). Cmax is approximately 40 % higher with the suspension and therefore switch from tablet to liquid should be done with caution. The following statement is included in section 4.2 of the SmPC:

Switching between tablet and oral suspension and vice versa
A tablet form of 6–mercaptopurine is also available. The 6-mecaptopurine oral suspension and tablet are not bioequivalent with respect to peak plasma concentration, and therefore intensified haematological monitoring of the patient is advised on switching formulations (see section 5.2).

The difference in pharmacokinetic profile between the suspension and the tablet was described in the SmPC, section 5.2, as follows:

*'In a comparative bioavailability study in healthy adult volunteers (n=60), 50mg of Novapurine oral suspension was demonstrated to be bioequivalent to the reference 50mg tablet for AUC, but not Cmax. The mean (90% CI) Cmax with the oral suspension was 39 % (22% - 58%) higher than the tablet although there was less between-subject variability (%C.V) with the oral suspension (46%) than the tablet (69%).'*The elimination half-life of 6–mercaptopurine is 90 ± 30 minutes, but the active metabolites have a longer half-life (approximately 5 hours) than the parent compound. The apparent body clearance is 4832 ± 2562 ml/min/m^2. There is low entry of 6–mercaptopurine into the cerebrospinal fluid.

The main route of elimination for 6–mercaptopurine is by metabolism. The intracellular anabolism of 6-merpactopurine is catalysed by several enzymes to eventually form 6-thioguanine nucleotides (TGNs), but a variety of intermediary TGNs are formed en route to the TGNs. The first step is catalysed by hypoxanthine-guanine phosphoribosyl transferase yielding thioinosine monophosphate (TIMP). 6–mercaptopurine is also subject to S-methylation by the enzyme thiopurine S-methyltransferase (TPMT), yielding methylmercaptopurine, which is inactive. However, TPMT also catalyses the S-methylation of the principle nucleotide metabolite, TIMP, to form methylthioinosine monophosphate (mTIMP). Both TIMP and mTIMP are inhibitors of phosphoribosyl pyrophosphate amidotransferase, an enzyme which is important in de novo purine synthesis. Xanthine oxidase is the main catabolic enzyme and it converts the 6–mercaptopurine into the inactive metabolite, 6-thiouric acid. This is excreted in the urine. Approximately 7% of an oral dose is excreted as unchanged 6–mercaptopurine within 12 hours after administration.

There are individuals with an inherited deficiency of the TPMT enzyme activity who are very sensitive to the myelosuppresive effect of 6–mercaptopurine and prone to developing rapid bone marrow depression following the initiation of treatment with 6–mercaptopurine. This problem could be exacerbated by coadministration with active substances that inhibit TPMT, such as olsalazine, mesalazine or sulfasalazine. Some laboratories offer testing for TPMT deficiency, although these tests have not been shown to identify all patients at risk of severe toxicity. Therefore close monitoring of blood counts is necessary. Substantial dose reductions are generally required for homozygous-TPMT deficiency patients to avoid the development of life threatening bone marrow suppression.

A possible association between decreased TPMT activity and secondary leukaemias and myelodysplasia has been reported in individuals receiving 6–mercaptopurine in combination with other cytotoxics

In terms of other drug interactions, inhibition of the anticoagulant effect of warfarin, when given with 6–mercaptopurine, has been reported. Monitoring of the INR (International Normalised Ratio) value is recommended during concomitant administration with oral anticoagulants.

Cytotoxic agents may decrease the intestinal absorption of phenytoin. Careful monitoring of the phenytoin serum levels is recommended. It is possible that the levels of other anti-epileptic medicinal products may also be altered. Serum antiepileptic levels should be closely monitored during treatment with Mercaptopurine Nova Laboratories, making dose adjustments as necessary.

When allopurinol and Mercaptopurine Nova Laboratories are administered concomitantly it is essential that only a quarter of the usual dose of Mercaptopurine Nova Laboratories is given since allopurinol decreases the rate of metabolism of 6–mercaptopurine via xanthine oxidase. Also other xanthine oxidase inhibitors, such as febuxostat, may decrease the metabolism of mercaptopurine and concomitant administration is not recommended as data are insufficient to determine an adequate dose reduction.

2.4.5. Conclusions on clinical pharmacology

In conclusion, the results of the submitted comparative bioavailability study indicate that the bioavailability from the suspension is in the same range as for the tablet, since bioequivalence was demonstrated for AUC, i.e. extent of absorption. The suspension also appears to perform more

predictable than the tablet. The rate of absorption was higher for the suspension than for the tablet, leading to a higher Cmax.

The pharmacokinetic data are considered sufficient for a new pharmaceutical form.
It is concluded that the pharmacokinetic study shows that the Novapurine oral suspension has reliable pharmacokinetics. The liquid product should also enable more precise dosing. These two factors (reliable pharmacokinetics and precise dosing) should facilitate more predictable dosing on an individual basis.

Pharmacology issues discussed are appropriately reflected in the product information.

2.5. Clinical efficacy

No new clinical efficacy data have been submitted. Reference to literature data and experience with the Puri-Nethol tablet was made. This approach is in compliance with the CHMP Scientific Advice.

2.5.1. Discussion on clinical efficacy

The basic approach to ALL therapy consists of a relatively brief remission-induction phase, followed by intensification (consolidation) treatment and then prolonged maintenance therapy. Although 6-MP may be administered during intensification (consolidation), its main use is during the continuation phase. Daily mercaptopurine and weekly methotrexate constitutes the basis of most continuation regimens. This combination given to the limits of tolerance is associated with improved clinical outcome.

Although all children with ALL require prolonged continuation therapy (2 - 2.5 years), the reasons for this are not well defined. However, decreasing the duration of continuation phase of chemotherapy (to 12 - 18 months) produced worse outcomes (event free survival) overall. While there may be a sub-group of children who do not need prolonged therapy, it is not possible at present to identify them prospectively.

Since thioguanine is more potent than mercaptopurine in model systems and leads to higher concentrations of thioguanine nucleotides in cells and cytotoxic concentrations in cerebrospinal fluid several randomised trials have been done to compare the effectiveness of these two drugs Thioguanine, given at a daily dose of 40 mg/m² or more, produced superior anti-leukaemic responses to mercaptopurine but was associated with profound thrombocytopenia, an increased risk of death in remission, and an unacceptably high rate (10–20%) of hepatic veno-occlusive disease. Although the lower activity of thiopurine methyltransferase is associated with thioguanine-related liver damage, this measure cannot identify reliably patients at risk 6-MP, therefore, remains the drug of choice for acute lymphoblastic leukaemia, although thioguanine could still be given in short-term courses during the intensification phase of treatment.

There is no doubt that 6-MP is considered integral to curing children with ALL. The efficacy of 6-MP for the treatment of ALL is unquestioned and has been established over many years through a number of national and international trials aimed at improving outcomes. Furthermore it would be unethical to conduct any efficacy studies with 6-MP in Europe.

This application is to seek the indication of ALL for an oral 6MP liquid formulation where tablets are the only available formulation so far. This liquid alternative would allow treating patients (including adults) in whom tablets are not appropriate due to difficulties in swallowing. More important, it would solve the problem of dose adaptation in children since the currently available tablets make it necessary to crush or divide tablets with a risk of dosing error related to imprecision.

6-MP is titrated according to haematological response, usually measured by neutrophil and platelet counts. Therefore, the reliability of the formulation from a pharmacokinetic point of view is the key factor in assessing its ability to induce and maintain remission. The use of toxicity as a factor for determining over-dosing or under-dosing is common practice in cancer cytotoxic therapy as some level

of toxicity is required to achieve the desired anti-cancer effect. The toxicity target is therefore more important than the actual dose given. With a liquid formulation an exact measure of fluid corresponding to the dose required is possible.

In this application no further clinical efficacy studies were provided with 6-mercaptopurine because there is enough clinical experience from currently available data. In addition, oral 6-MP in the tablet form (Puri-Nethol) is already licensed in the EU for the treatment of ALL in Children.

The CHMP put special emphasis on the acceptability of the liquid formulation to the paediatric patients. At the request of the CHMP the Applicant collected all data currently available in the EU from use of the proposed oral suspension to provide reassurance that the formulation is accepted by children in terms of palatability which may have implications on compliance (and thereby efficacy).

Data from the availability of the product through continuous supply of the product as a 'Special' to the UK market (unlicensed medicine manufactured for individual patients under the MHRA Specials License) were presented. The Applicant claimed that the palatability and acceptability of Novapurine suspension to children has been demonstrated since there have been no complaints. However, the fact that no complaints have been registered from patients administered with Novapurine as a 'Special' is not sufficient to provide reassurance on Novapurine palatability since this investigation was not planned a priori.

The Applicant has conducted a questionnaire based survey to obtain feedback on the 'collective experience' of the hospital pharmacists, nurses and patients as to the acceptability / palatability of the formulation. The responses were from pharmacists (6), nurses (2) and a combination of pharmacist / nurse (2). The majority (8/10) of respondents stated that Nova's oral mercaptopurine suspension was dispensed for children only in their hospital; in two hospitals it was also dispensed for adults. This survey revealed the acceptability/palatability of the product in the paediatric population.
In addition, palatability of Novapurine was assessed in adults (n=6), on a 7-point scale for bitterness, sweetness, sourness, saltiness. It reveals that the product maintains acceptable palatability throughout its proposed shelf life (0, 6 months, 12 months).
The survey on children was limited to 10 participants (pharmacists, nurses) who relay the children's feeling regarding palatability. This information should have been retrieved from either patients themselves (if age permits) or their carers (parents).

Following CHMP recommendation the applicant proposed to conduct a study to investigate the palatability of the product, in the context of a prospective, open-label, single dose, non-randomised, questionnaire-based survey in children (aged 3-16 years) with acute lymphoblastic leukaemia. The palatability tool for assessing the end points can be chosen following a thorough literature review, but is likely to be based on visual analogue/verbal scales. It is anticipated that young children as well as adolescents will be recruited from leukaemia centres in the UK. A full protocol will be discussed with the CHMP before a study is initiated.

2.5.2. Conclusions on the clinical efficacy

In this application no further clinical efficacy studies were provided with 6-mercaptopurine because there is enough clinical experience from currently available data since oral 6-MP in the tablet form (Puri-Nethol) is already licensed in the EU for the treatment of ALL. This is in line with Scientific Advice given to the Applicant.
At the request of the CHMP the Applicant collected all data currently available in the EU from use of the proposed oral suspension to provide reassurance that the formulation is accepted by children in terms of palatability. This may have implications on compliance (and thereby efficacy) and reassurance is needed that compliance is not lower than it is for the tablet.

The bioequivalence study suggested that the two products have similar bioavailability (AUC) but that absorption was more rapid from the suspension, with a considerably higher Cmax. Given that the 6-MP dose is always individually titrated based on haematological response/toxicity, and since toxicity is coupled to the pharmacological action of 6-MP, it is agreed that additional clinical data to confirm efficacy of the oral suspension are not considered necessary, despite the difference in rate of

absorption compared with the tablet. In this respect, the liquid formulation is suitable even for minimal dose adjustments. However, it should be confirmed that the formulation is acceptable to children.

2.5.3. Recommendations for future clinical efficacy development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

- The Applicant should investigate the palatability of the product, in the context of a prospective, open-label, single dose, non-randomised, questionnaire-based survey in children (aged 3-16 years) with acute lymphoblastic leukaemia. The palatability tool for assessing the end points can be chosen following a thorough literature review, but is likely to be based on visual analogue/verbal scales. It is anticipated that young children as well as adolescents will be recruited from leukaemia centres in the UK. A full protocol will be discussed with the CHMP before a study is initiated.

2.6. Clinical safety

In this application, limited further clinical safety data with 6-mercaptopurine were provided, because there is adequate clinical experience from currently available data. This is in line with the CHMP Scientific advice given on clinical aspects of this application. Limited additional safety data were derived from the previously described bioequivalence study.

60 adult healthy volunteers were included in this study. The Mean age was 22.98 years. Data for all 60 volunteers entered for the study were included in the safety evaluation.

In each of the two study periods, one of the following treatments was administered orally:
Treatment A: 2.5 ml Mercaptopurine 20 mg/mLOral Suspension
Nova Laboratories Ltd., Leicester, UK.
Treatment B: 1 x Puri-Nethol ® 50 mg tablet,
GlaxoSmithKline, UK.
Volunteers were given a single 1 x 50 mg dose of 6-MP (as an oral suspension or tablet) at each of the two dosing visits with a washout period of at least 3 days between visits.
In the bioequivalence study, three adverse events were recorded in three volunteers. One volunteer treated by Mercaptopurine 20 mg/mLOral Suspension had a headache three hours thirty minutes after dosing in Period 2. Two volunteers treated by Puri-Nethol 50 mg tablet had out-of-range blood test results at post study. The case of headache was treated with an icepack. The out-of-range blood tests were repeated, with satisfactory results.
There were no deaths, serious adverse events or significant adverse events.

At pre-study screening 52 volunteers had one or more biochemistry/haematology results slightly outside the laboratory ranges. Ten subjects had a slightly raised total bilirubin pre-study. These total bilirubin increased were not associated with elevated AST or alkaline phosphatase. Post study, 50 volunteers had one or more biochemistry/haematology results slightly outside the laboratory ranges. None of the post study out-of-range results were considered by the Investigator to be clinically significant, with the exception of two possible related events of moderate intensity of reversible elevated AST (>5 x ULN at maximum intensity) in one subject and reversible leucopenia with neutropenia (Grade 1 CTCAE v4). These two volunteers had received the tablet formulation as their second treatment. Repeat tests were carried out for these volunteers, with satisfactory results.

Elevated AST (>5 x ULN at maximum intensity) and reversible leucopenia with neutropenia (Grade 1 CTCAE v4) are two adverse events included in 4.8 section of Puri-Nethol.

Post marketing experience

The issue of local tolerance has been investigated in the pharmacovigilance database of the recent UKALL2003 trial. This is an academic trial comparing the efficacy of different combinatons of chemotherapy for ALL and employs mercaptopurine in the form of both tablet oral suspension. The Applicant was the principle supplier of oral mercaptopurine suspension to the trial until 2008, and afterwards remained one of two suppliers. Data on the incidence of stomatitis were analysed by the trial statistician at the Applicant's request and they are presented in the following table.

Table 6: Incidence of stomatitis in the UKALL 2003

Age Group (years)	Total number of recruited patients	Total Number (%) of Grade 3 / 4 Stomatitis reported[a]	Total number (%) of Grade 3/4 stomatitis during maintenance phase[b]
<5	1270	97 (8.2)	18 (0.3)
5-9	661	48 (7.7)	12 (0.3)
≥10	677	74 (12.2)	5 (0.2)

a. Percentage of all toxicity reports
b. Percentage of toxicity reports during maintenance phase

2.6.1. Discussion on clinical safety

The safety profile is of 6 MP is well known and derives from 50 years-clinical use. The major dose-related toxicities of 6-MP (de Vita et al, 2005) are myelosuppression and gastrointestinal toxicity. Gastrointestinal toxicities include nausea and vomiting, anorexia, diarrhoea, and stomatitis.
Leukopenia and thrombocytopenia are maximal 7 days after treatment. Anaemia is observed less frequently. Full haematologic recovery usually occurs after 14 days. Careful monitoring of haematological parameters should be conducted during therapy. The leucocyte and platelet counts continue to fall after treatment is stopped, so at the first sign of an abnormally large fall in the counts, treatment should be interrupted immediately. Bone marrow suppression is reversible if 6–mercaptopurine is withdrawn early enough. In TPMT-deficient patients, dosage reduction to 5% to 25% of the standard dosage (75 mg/m^2/d) is necessary to prevent excessive toxicity (see also discussion on clinical pharmacology).
Finally, due to myelosuppression, the concomitant use of mercaptopurine with yellow fever vaccine is contraindicated. Moreover, immunisation using a live organism vaccine has the potential to cause infection in immunocompromised hosts. Therefore, immunisations with live organism vaccines are not recommended.
6-MP is hepatotoxic and hepatotoxicity occurs in up to 30% of adult patients and is manifested mainly as cholestatic jaundice, although elevations of hepatic transaminases may also be seen. Hepatotoxicity is usually mild and reversible after discontinuation of 6-MP, but frank hepatic necrosis can occur after high doses of the drug. Combinations of 6-MP with other known hepatotoxic agents should be avoided, and liver function test results should be closely monitored. The mechanism of liver toxicity is not known but may relate to the cytochrome P-450–dependent metabolism of 6-MP to a hepatotoxic metabolite or accumulation of 6-MP metabolites in the liver. Liver function tests should be monitored weekly during treatment. More frequent monitoring may be advisable in those with pre-existing liver disease or receiving other potentially hepatotoxic therapy. The patient should be instructed to discontinue Mercaptopurine Nova Laboratories immediately if jaundice becomes apparent
Similarly, during remission induction when rapid cell lysis is occurring, uric acid levels in blood and urine should be monitored as hyperuricaemia and/or hyperuricosuria may develop, with the risk of uric acid nephropathy. Hydration and urine alkalinisation may minimize potential renal complications.
Pancreatitis has been reported to occur at a frequency of ≥ 1/100 to < 1/10 ("common") in patients treated for inflammatory bowel disease.
One bioequivalence study to bridge the existing knowledge with the new formulation was submitted. Only three adverse events were collected from the bioequivalence study: Headache, elevated AST and leucopenia with neutropenia and these are in line with the known adverse event profile of mercaptopurine.

Since treatment with mercaptopurine per se is associated with toxicity on the oropharyngeal mucosa, potential additional adverse effects caused by topical exposure were discussed as it was questioned whether the pharmaceutical formulation may promote or worsen the potential risk of oral mucositis.

The Applicant was asked to collect all data currently available in the EU from use of the proposed oral suspension to provide reassurance on the local tolerance of mercaptopurine oral suspension with special attention to chemotherapy-induced oral mucositis. The issue of local tolerance has been investigated through the pharmacovigilance database of the recent UKALL2003 trial. The analysis was conducted by the UKALL2003 trial statistician and at the request of Nova Laboratories.
Although a specific evaluation of the effect of oral mercaptopurine formulations (tablet versus suspension) on rates of local toxicity was not an *a priori* objective of UKALL2003, toxicity data collected thus far in excess of 2000 children suggests that potential differences in local exposure to mercaptopurine as a consequence of formulation does not appear to have an effect on the rates of stomatitis, which overall are very low during the maintenance phase. This data was also presented to the PDCO during the PIP application, who consequently waived the need for further evaluation of local tolerance in their final opinion.
The rates of stomatitis are very low (0.2– 0.3%) in all age categories during the maintenance phase of therapy, where mercaptopurine is predominantly administered. The total rates of stomatitis are higher in children over 10 years old, than in children under 5 years old or those aged between 5-10 years. This is not surprising since it is known that incidence of all toxicity is higher in adolescent leukaemic subjects.

The data on local tolerance are also supported by the known pharmacology of mercaptopurine. The anti-proliferative and cytotoxic pharmacology of mercaptopurine requires systemic absorption and activation to thioguanine nucleotides (TGNs) which are principally formed following first pass metabolism in the liver. Oro-pharyngeal toxicity is therefore influenced by systemic exposure and not local exposure and any theoretical local (epithelial) activation of mercaptopurine will be minimal and insignificant relative to the overwhelming levels of circulating TGNs following liver activation (first pass) of an oral dose.

Mercaptopurine Nonva Laboratories contains aspartame (E951), a source of phenylalanine and it may be harmful for people with phenylketonuria.

It also contains methyl parahydroxybenzoate and propyl parahydroxybenzoates which may cause allergic reaction (possibly delayed).

As this medicine contains sucrose, patients with rare hereditary problems of fructose intolerance, glucose-galactose malabsorption or sucrase-isomaltase insufficiency should not take this medicine. Long term use increases the risk of dental caries and it is essential that adequate dental hygiene is maintained.

Gastrointestinal effects, including nausea, vomiting, diarrhoea and anorexia may be early symptoms of overdose having occurred. The principal toxic effect is on the bone marrow, resulting in myelosuppression. Haematological toxicity is likely to be more profound with chronic overdose than with a single ingestion of Mercaptopurine Nova Laboratories. Liver dysfunction and gastroenteritis may also occur. The risk of overdose is also increased when xanthine oxidase inhibitors is being given concomitantly with 6–mercaptopurine.

As there is no known antidote the blood picture should be closely monitored and general supportive measures, together with appropriate blood transfusion, instituted if necessary. Active measures (such as the use of activated charcoal or gastric lavage) may not be effective in the event of 6-mercaptopurine overdose unless the procedure can be undertaken within 60 minutes of ingestion.

Parents and care givers should avoid Mercaptopurine Nova Laboratories contact with skin or mucous membrane. If the suspension comes into contact with skin or mucosa, it should be washed immediately and thoroughly with soap and water.

6MP oral suspension has been developed for children who cannot swallow or for whom precise dosing cannot be achieved by splitting tablets, meaning 3-6 years-old, less than 50 kgs children.
Considering that the weight range for children will be [10-50] kgs, with a 100 mg/5ml concentration and a 2.5 mg/kg posology, the volume administered ranges from 1.25 to 6.5 ml. The applicant has demonstrated precise measurement of the administered volume by the syringes provided. It is agreed

that for doses above 1 ml, 0.2 ml increments will provide sufficient precision in dosage, given the overall pharmacokinetic variability.

As 2 syringes are included in the pack, a risk of dosing error cannot be excluded. Adequate precautions and instructions have been included in the Product Information. So far no such errors have occurred from the availability of the product as 'special' in a number of EU member states. Furthermore, the 1 ml and 5 ml syringes will be provided in different colours. The plunger in the 1 ml syringe will be 'purple' in colour, and in the 5 ml syringe it will be 'white' in colour. This will ensure that the two syringes are clearly distinguishable. The proposed syringes will be available at product launch following MA approval. Surveillance of medication errors as a consequence of syringe confusion are part of the PV strategy.
At the recommendation of the CHMP, the applicant agreed to add overpacks for each syringe on which 'dose less than 1 mL' will be printed on the 1 mL syringe and 'dose more than 1 mL' " will be printed on the 5 mL syringe to clarify to the patient or the caregiver which devices is appropriate for the desired volume to be drawn into the syringe.

2.6.2. Conclusions on the clinical safety

The safety profile of 6 MP derives from 50 years-clinical use. The most frequent adverse reactions include stomatitis, nausea, vomiting, anorexia, gastro-intestinal ulceration and bleeding, hepatotoxicity, bone marrow toxicity and immune suppression.

Based on the pharmacology of 6-mercaptopurine, it is considered unlikely that local exposure would significantly contribute to oro-pharyngeal toxicity. It is suggested that any signals relating to local toxicity issues are handled within the PSUR framework.

The potential risk of dosing is addressed with appropriate instructions and warnings in the product information. Routine Pharmacovigilance strategy will be sensitive to such dosing errors, such as or example, surveillance of medication errors as a consequence of syringe confusion is part of the PV strategy.

2.6.3. Recommendations for future clinical safety development

Furthermore, the CHMP recommends to the Applicant to add overpacks for each syringe on which the text "dose less than 1 ml" would be printed on the 1 ml syringe and "dose more than 1 ml" would be printed on the 5 ml syringe. This will explain the patient or the caregiver which device is adapted to the volume and should be used for withdrawing the liquid.

2.7 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management plan

The CHMP did not require the applicant to submit a risk management plan because it is accepted that risk management plans are generally not required for hybrid products where the active substance has been on the market for a long time, unless a safety concern requiring additional risk minimisation activities has been identified with the reference product. This argument is considered relevant for the substance itself.

As the product is intended for self- (or parent-) administration outside of a hospital setting, risks associated with handling and dosing have been addressed by adequate instructions in the Package Leaflet.

Surveillance of medication errors as a consequence of confusion with the use of syringes is part of the routine Pharmacovigilance strategy.

The CHMP, having considered the data submitted, was of the opinion that routine pharmacovigilance was adequate to monitor the safety of the product.

No additional risk minimisation activities were required beyond those included in the product information.

2.8 User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

3. Benefit-Risk Balance

Benefits

Beneficial effects

6-MP remains an integral part of ALL treatment protocols in children. So far, the only licensed product on the EU market is a 50 mg tablet, which should not be divided, but in practice this has to be done in order to find an appropriate dose for the individual patient.
During therapy of ALL, the dose of 6-MP is individualised, essentially based on haematologic toxicity. Compared with tablets, which is the only currently available licensed formulation, an oral suspension provides better accuracy and ease of administration in small children. From an efficacy as well as safety perspective this is favourable.

Uncertainty in the knowledge about the beneficial effects.

Considering that palatability is sometimes a concern for oral formulations intended for the paediatric population as it may be associated with compliance to the drug, the CHMP recommended to the applicant to perform a survey investigating the palatability of Mercaptopurine Nova Laboratories.

Risks

Unfavourable effects

The dose-limiting toxicity is the haematological effect. As the mercaptopurine dose is individually titrated based on haematological response, no additional *systemic* toxicity is expected from the oral suspension compared with the previously approved tablet. If a switch from tablets to oral suspension, or vice versa, is undertaken, the physician, patients and parents should be made aware that the dose may need to be adjusted.

Uncertainty in the knowledge about the unfavourable effects

From a Quality and Non-clinical perspective, the choice of propylparaben as preservative was questioned, but the exposure of the patient is low, well below the lowest observed adverse event level. Nevertheless, it is recommended to the Applicant to eliminate this preservative in the future.

An increased risk for local toxicity with the Novapurine formulation seems unlikely but has not been prospectively addressed. It is suggested that any signals relating to local toxicity issues are handled within the PSUR framework.

A potential risk of confusion with the inclusion of 2 different syringes in the pack is expected to be well managed by the colour coding of the pack. Signals of such potential errors will be detected through routine pharmacovigilance.

Benefit-risk balance

Importance of favourable and unfavourable effects

The availability of a suspension provides better accuracy and ease of administration especially when used in small children and this is important from an efficacy as well as safety perspective. The haematological toxicity is part of the action of mercaptopurine, well managed by titration of the drug for individual use. The well known toxicity profile of mercaptopurine is expected to be better managed with more accurate dosing achieved with Mercaptopurine Nova Laboratories.

Benefit-risk balance

The benefit-risk balance of Mercaptopurine Nova Laboratories oral suspension 20 mg/ml for the treatment of Acute Lymphoblastic Leukaemia is positive.

Discussion on the benefit-risk balance

Historically, the risk-benefit profile of 6-MP for the treatment of ALL have been well established based on numerous multi-centre, multi-national trials in the EU and other developed countries. Mercaptopurine Nova Laboratories oral suspension ensures better precision and ease of administration than the existing tablet form and importantly, it will provide an alternative formulation for children who are unable to swallow tablets, and for clinicians, an alternative preparation that also allows more flexible dosing.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus decision is of the opinion that Mercaptopurine Nova Laboratories is not similar to Atriance, Evoltra, Glivec or Sprycel within the meaning of Article 3 of Commission Regulation (EC) No. 847/200. See appendix

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Mercaptopurine Nova Laboratories in the treatment of acute lymphoblastic leukaemia (ALL) in adults, adolescents and children is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (See Annex I: Summary of Product Characteristics, section 4.2).

Conditions and requirements of the Marketing Authorisation

Risk Management System and PSUR cycle

The MAH must ensure that the system of pharmacovigilance, presented in Module 1.8.1 of the marketing authorisation, is in place and functioning before and whilst the product is on the market.

The PSUR cycle for the product will follow the standard requirements until otherwise agreed by the CHMP.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Not applicable

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

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