DPD screening to prevent toxicity in fluoropyrimidine treated patients

Staveren, M.C. van

Chapter 3

Investigational Medicinal Product Dossier (IMPD) of uracil

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INTRODUCTION

The Investigational Medicinal Product Dossier (IMPD) is the basis for approval of clinical trials by the competent authorities in the EU [1]. The IMPD includes summaries of information related to the quality, manufacturing and control of the Investigational Medicinal Product, data from non-clinical studies and from its clinical use. An overall risk-benefit assessment, critical analyses of the non-clinical and clinical data in relation to the potential risks and benefits of the proposed study are essential parts of the IMPD. In certain situations, e.g. where the Investigational Medicinal Product has already been authorised as a medicinal product in one of the EU Member States or when clinical studies with the IMP have already been approved by a Member State, a simplified IMPD will be sufficient.

The Clinical Trials Directive (2001/20/EC) came into force in April 2001, harmonizing the laws, regulations and administrative provisions of the Member States relating to the implementation of Good Clinical Practice (GCP) in the conduct of clinical trials on medicinal products for human use. Member States were obliged to transform the requirements outlined in the Directive into the respective national laws by May 2004. The Directive introduced a harmonized procedure for the authorization to perform a clinical study in any one of the EU Member States. In addition, it defines the documentation to be submitted to the Ethics Committee as well as the IMPD to be submitted to the competent authority for approval. Thus, an IMPD is requested whenever the performance of a clinical study in any one of the EU Member States is intended [1].

Since uracil has no marketing authorization, an IMPD is required to perform clinical studies with the oral uracil loading dose.

METHODS

Based on the IMPD template [2], all paragraphs were written for uracil. All tests considering impurities, assays and quality were performed by an analytical monograph with the use of liquid chromatography with UV photo diode array detection. Acceptance criteria were derived from the European Pharmacopeia General Monograph 04/2013 Substances for Pharmaceutical Use, H5.10 Control of impurities in substances for Pharmaceutical Use and H2.2.46 Chromatographic Separation Techniques. All tests were performed at the laboratory of the Hospital Pharmacy Meppel-Hoogeveen. The manufacturing of the study drug was

performed by the Hospital Pharmacy Haagse Ziekenhuizen under Good Manufacturing Practice and Good Clinical Practice conditions. Chemical data about uracil and its synthesis route was derived from the supplier and safety data was derived from published animal studies. All studies were performed with an oral powder formulation.

1. Directive, C.T. IMPD. 2006 08.08.2006; Available from: http://www.impd.eu.
2. Directive, E.C.T., IMPD, CCMO, Editor. 2016: Web page. p. 26.

INVESTIGATIONAL MEDICINAL PRODUCT DOSSIER

URACIL

VERSION 4 **MAY 2013**

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1. Introduction

This clinical trial application presents information relating to uracil oral powder in dosages of 700, 800, 900, 1000, 1100 and 1200 mg. Uracil is an endogenous compound, and is used as a substrate for phenotyping of dihydropyrimidine dehydrogenase (DPD). DPD is a key metabolizing enzyme in the metabolic pathway of 5-fluorouracil. Patients with DPD deficiency are at high risk to develop severe toxicity after treatment with 5-fluorouracil or capecitabine. Pre-chemotherapy phenotyping of DPD with uracil may be a feasible method for patient screening. Uracil has been evaluated in several clinical studies [1-6] involving healthy subjects to evaluate the safety and tolerability profile and to assess the pharmacokinetic behaviour of the compound. In one of these studies, uracil was administered orally to 12 healthy individuals in a dose of 500 mg/m^2. No unintended effects or adverse effects were reported during or after the administration. In 4 of these subjects uracil was also administered in a dose of 1000 mg/m^2 [5]. No adverse events were reported. In addition uracil was administered to 8 patients with proven DPD deficiency in a dose of 500 mg/m^2. Physical observation of the patients after administration did not reveal any unexpected side effects or unintended effects [6].

2.1 Chemical pharmaceutical data

2.1.S Drug substance

2.1.S.1 General information

Uracil is a common naturally occurring pyrimidine [7]. Uracil was originally discovered in 1900 and it was isolated by hydrolysis of yeast nuclein that was found in bovine thymus and spleen, herring sperm, and wheat germ [8]. Uracil is a planar, unsaturated compound that has the ability to absorb light [9].

2.1.S.1.1 Nomenclature

Uracil
2,4-dihydroxypyrimidine; 2,4(1H,3H)-pyrimidinedione, 2-oxy-4-oxy pyrimidine, 2,4-pyrimidinediol
CAS number: 66-22-8

2.1.S.1.2 Structure

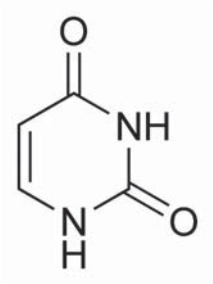


Figure 3.1 Uracil chemical structure.

Chemical formula: $C_4H_4N_2O_2$
Molecular mass: 112.09 g/mol

2.1.S.1.3 General properties

Stereochemistry:	Not applicable
Description:	White to slightly yellowish crystalline powder
Melting range:	335°C
Hygroscopicity:	Uracil is not hydroscopic
pKa (acidic):	9.45
Solubility:	Freely soluble in hot water, sparingly in cold water (100 parts of water at 25°C dissolves 0.358 part of uracil). Almost insoluble in alcohol, ether. Soluble in ammonia water and other alkalies.

2.1.S.2 Manufacture

2.1.S.2.1 Manufacturer

Pharma Waldhof GmbH
Hansaallee 159
D-40549 Düsseldorf
Germany

2.1.S.2.2 Description of manufacturing process and process controls

The uracil used in this study was synthesized by the condensation of maleic acid with urea in fuming sulfuric acid [6].

$$C_4H_4O_4 + CH_4N_2O \rightarrow C_4H_4N_2O_2 + 2\,H_2O + CO$$

Related substances from the synthesis pathway, as supplied by the manufacturer, are listed in Table 3.1.

2.1.S.2.3 Control of materials

Table 3.1 Reagents, solvents and other materials

Material	Grade	Specific test item	Possible impurity
dl-maleic acid	unknown	no	Yes
Urea	unknown	no	Yes
Sulphate	unknown	no	Yes
Methanol-sodium	unknown	no	Yes
Ethyl formate	unknown	no	Yes

2.1.S.2.4 Controls of critical steps and intermediates

Unknown / Not available.

2.1.S.2.5 Process validation and/or evaluation

Unknown / Not available.

2.1.S.2.6 Manufacturing process development

Unknown / Not available.

2.1.S.3 Characterization

2.1.S.3.1 Elucidation of structure and other characteristics

Each batch of uracil is identified by infra red absorption spectrophotometry. The spectrum obtained is compared with a uracil reference standard.

2.1.S.3.2 Impurities

Each batch of uracil is tested on related substances by liquid chromatography with UV photo diode array detection. The sum of areas of any peak corresponding to impurities may not be greater than 1.0%. Furthermore each batch is tested for heavy metals (< 10 ppm) and loss on drying (< 0.5%).

2.1.S.4 Control of drug substance

2.1.S.4.1 Specification

Batches of the active ingredient will comply with the below specification (Table 3.2). Batches will be released only if the impurity profiles can be supported by available non-clinical data.

Table 3.2 Specifications for URACIL drug substance

Attribute	Method	Acceptance criteria
Appearance Dissolution, pH and appearance	Visual observation 0.35 g in 100 ml water	White crystalline powder Conforms colour reference test pH = 5.0–5.5
Identification	IR Absorption	Conforms to the reference spectrum Figure 3.2
Melting point	PhEur	330–340°C
Purity (1) Heavy metals (2) Related substances	 Ph.Eur., Test C HPLC (UV-PDA)	 ≤ 20 ppm Each: ≤ 0.3% Total: ≤ 1.0%
Water	Ph.Eur., loss on drying	≤ 0.5%
Residue on ignition	PhEur	≤ 0.10%
Assay	HPLC	99.0 to 101.0%

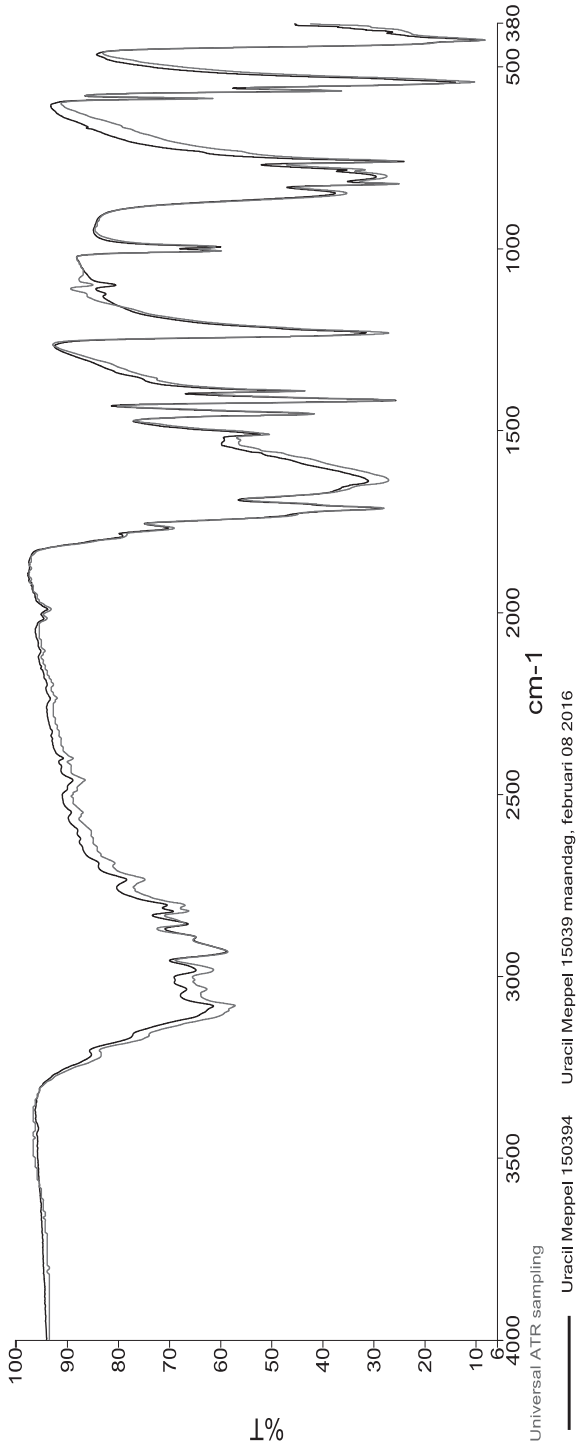
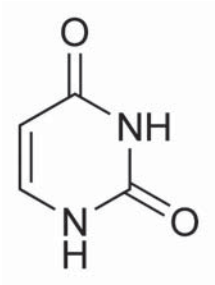


Figure 3.2 IR Absorption spectrum of two batches of uracil.

2.1.S.4.2 Analytical procedures

Monograph Uracil



URACIL

Uracilum

$C_4H_4N_2O_2$ Mr112.09

CAS [66-22-8].

DEFINITION

Uracil contains not less than 98.0 percent and not more than the equivalent of 102.0 percent of 2,4(1H,3H)-pyrimidinone, calculated with reference to the dried basis.

CHARACTERS

A white or almost white, crystalline powder, freely soluble in hot water, sparingly in cold water. Almost insoluble in alcohol.

IDENTIFICATION

Examine by infrared absorption spectrophotometry (Ph.Eur. 2.2.24), comparing with the spectrum obtained with uracil reference standard.

TESTS

Solution S. Dissolve 0.35 g in hot *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY7 or Y7 (*2.2.2, Method II*).

pH (*2.2.3*). The pH of solution S is 5.0 to 5.5.

Related substances. Examine by liquid chromatography (2.2.29).

Test solution. Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 100.0 ml to obtain a solution having a known concentration of about 1 mg per ml.

Reference solution (a). Dilute 1.0 ml of the *test solution* to 100.0 ml with *water R*.

Reference solution (b). Dilute 1.0 ml of *reference solution (a)* to 10.0 ml with *water R*.

Reference solution (c). Dissolve 25 mg of *dihydrouracil reference standard* plus 25 mg of *5-fluorouracil reference standard* and 25 mg of *uracil reference standard* in *water R* and dilute to 100.0 ml.

The chromatographic procedure may be carried out using:

- A stainless steel column 0.250 m long and 4 mm in internal diameter packed with octadecylsilyl silica for chromatography R (5 µm),
- As mobile phase at a flow rate of 0.8 ml per minute a mixture 99 parts 1.5 mM phosphate buffer (pH 5.8) and 1 part methanol R,
- As detector a spectrophotometer set at 205 nm.

Inject 20 µl of *reference solution (c)*. Adjust the sensitivity of the system so that the heights of the three peaks are not less than 20 percent of the full scale of the recorder. The test is not valid unless the resolution between the first and second peak and between the second and third peak is less than 2.5. Inject 20 µl of *test solution*, 20 µl of *reference solution (a)* and 20 µl of *reference solution (b)*. Continue the chromatography for three times the retention time of uracil. In the chromatogram obtained with the test solution: the sum of the areas of any peaks corresponding to impurities is not greater than the area of the principal peak in the chromatogram obtained with *reference solution (a)* (1.0 percent); the area of any peaks corresponding to dihydrouracil and 5-fluorouracil is not greater than the area of the principal peak in the chromatogram obtained with *reference solution (b)* (0.1 percent) and the sum of the areas of such peaks in not greater than three times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 percent). Disregard any peak with an area less than 0.2 times of the principal peak in the chromatogram obtained with *reference solution (b)*.

Heavy metals (*2.4.8*). *Use a platinum crucible.* 1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*). Not more than 0.5 percent, determined on 1.000 g by drying in vacuo at 80°C for 4h.

ASSAY

Test solution. Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 100.0 ml.

Test solution (a). Dilute 1.0 ml of the *test solution* to 100.0 ml with *water R*.

Reference solution. Dissolve 0.10 g *uracil reference standard* in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (a). Dilute 1.0 ml of the *reference solution* to 100.0 ml with *water R*.

The chromatographic procedure may be carried out using:

- A stainless steel column 0.250 m long and 4 mm in internal diameter packed with octadecylsilyl silica for chromatography R (5 µm),
- As mobile phase at a flow rate of 0.8 ml per minute a mixture 99 parts 1.5 mM phosphate buffer (pH 5.8) and 1 part methanol R,
- As detector a spectrophotometer set at 266 nm.

Inject 20 µl of *test solution (a)* and 20 µl of *reference solution (a)*, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity in mg of $C_4H_4N_2O_2$ in the proportion of uracil taken by the formula:

$$10C(r_T/r_{RS}),$$

in which C is the concentration, in µg per ml, of uracil in the *reference solution*, and r_T and r_{RS} are the uracil peak responses obtained from the *test solution* and the *reference solution* respectively.

STORAGE

Store in tight, light-resistant containers.

2.1.S.4.3 Validation of analytical procedures

Standard Operating Procedure: Validation chromatographic analytical methods ZAMH, version 4.

2.1.S.4.4 Batch analyses

One batch is purchased so far for clinical testing. The batch analyses from the producer are shown below:

Table 3.3 Results from two batch analyses uracil

Test	Specifications	Result Batch 1 Lot# 46303900
Assay	Min. 98%	99.75%
Loss on drying	Max. 0.5%	0.15%
Heavy metals	Max. 10 ppm	< 10 ppm
Residue on ignition	Max. 0.2%	0.05%

2.1.S.4.5 Justification of specification

The uracil analytical monograph is partly based on the Ph.Eur. monograph of 5-fluorouracil, and partly on specifications supplied by the manufacturer.

2.1.S.5 Reference standards or materials

The reference standard, Uracil Lot 41K3648 was purchased from Sigma-Aldrich Inc. No additional recrystallization or purification was performed.

2.1.S.6 Container closure system

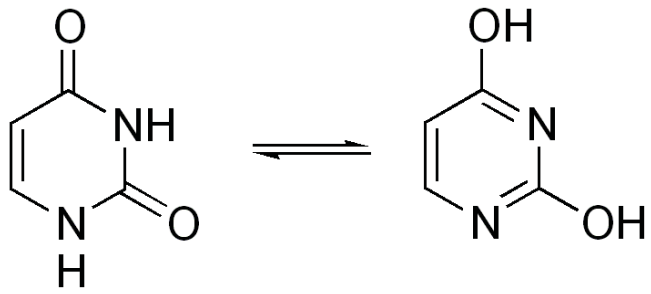
The uracil powder is shipped and stored in bag stored in an airtight container.

2.1.S.7 Stability

Uracil is very stable under normal temperatures and pressures. Uracil is not compatible with strong oxidizing agents. Hazardous polymerization has not been reported.

Uracil undergoes keto-enol tautomeric shifts because of its resonance structures due to the NH2 substituents and OH substituents. Also because any nuclear instability the molecule may have from the lack of formal aromaticity is compensated by the cyclic-amidic stability [8]. The keto tautomer is referred to the lactam structure, while the enol tautomer is referred

to as the lactim structure. These tautomeric forms are predominant at pH = 7. The lactam structure is the most common form of uracil.



Lactam:
Keto Tautomer

Lactim:
Enol Tautomer

Figure 3.3 Tautomeric forms of uracil.

2.1.P Medicinal product

2.1.P.1 Description and composition of URACIL ORAL POWDER

The qualitative compositions of uracil oral powder filled in flasks is listed in Table 3.4.

Table 3.4 Qualitative composition of URACIL oral powder 700, 800, 900, 1000, 1100, and 1200 mg

Component	Reference to standards	Function
URACIL	Uracil Lot 41K3648	Active ingredient
Brown plastic bottle, 300 mL	Ph.Eur.	Container

2.1.P.2 Pharmaceutical development

The formulation used for the clinical trials is uracil powder without additives. In previous clinical studies the same formulation was used.

2.1.P.2.1 Components of the medicinal product

The powder is filled in brown 300 ml plastic bottles without additives.

2.1.P.2.2 Medicinal product

Not applicable.

2.1.P.2.3 Manufacturing process development

No development information needs to be provided.

2.1.P.2.4 Container closure system

No development information needs to be provided.

2.1.P.2.5 Microbiological attributes

Not applicable.

2.1.P.2.6 Compatibility

Not applicable.

2.1.P.3 Manufacture

2.1.P.3.1 Manufacturer

GMP manufacturing:
Apotheek Haagse Ziekenhuizen
Escamplaan 900
2547 EX Den Haag

2.1.P.3.2 Batch formula

This information does not have to be provided.

2.1.P.3.3 Description of manufacturing process and process controls

The production process involves the weighing of uracil powder directly in the container. Only one dosage strength (700, 800, 900, 1000, 1100 or 1200 mg) is produced in each batch. The production takes place under GMP conditions. All containers comply with the specifications (95–105% of declared value).

2.1.P.3.4 Controls of critical steps and intermediates

The weighing of the powder is double checked by two persons. The total batch is released by a qualified person.

2.1.P.3.5 Process validation and/or evaluation

All equipment, documents and procedures used for manufacturing comply with GMP standards.

2.1.P.4 Control of excipients

2.1.P.4.1 Specifications

Not applicable.

2.1.P.4.2 Analytical procedures

Not applicable.

2.1.P.4.3 Validation of analytical procedures

Not applicable.

2.1.P.4.4 Justification of specifications

Not applicable.

2.1.P.4.5 Excipients of human or animal origin

Not applicable.

2.1.P.4.6 Novel excipients

Not applicable.

2.1.P.5 Control of medicinal product

2.1.P.5.1 Specifications (s)

Clinical trial batches of URACIL oral powder 700, 800, 900, 1000, 1100 and 1200 mg will meet the following specifications.

Table 3.5 Release and shelf-life specifications for Uracil oral powder in glass containers

Test item	Method	Acceptance criteria
Description	Visual observation	White powder in brown colloured flask
Identification	IR spectrophotometry	The product IR spectrum is identical to that of the reference spectrum
Content Uniformity	Weighing	Conforms to Ph. Eur.

2.1.P.5.2 Analytical procedures

Document ID: Uracil in plasma with use of HPLC.
Document ID: Standard Operating Procedure uracil with use of (U)HPLC.

2.1.P.5.3 Validation of analytical procedures

Document ID: Validation chromatographic analytic methods ZAMH, version 4.

2.1.P.5.4 Batch analyses

Not applicable.

2.1.P.5.5 Characterization of impurities

Since the active substance is used without additives and no further processing is performed on the uracil powder, no characterization of impurities is performed in the final product.

2.1.P.5.6 Justification of specification(s)

Not applicable.

2.1.P.6 Reference standards

The reference standard, Uracil Lot 41K3648 was purchased from Sigma-Aldrich Inc. No additional re-crystallization or purification was performed.

2.1.P.7 Container closure system

The uracil powder is filled in brown plastic 300 mL bottles.

2.1.P.8 Stability

The stability data for the drug substance show that uracil is intrinsically very stable.

There is no indication that any degradation occurs during storage is closed polypropylene containers.

Stability of uracil solution of 3 mg/ml in water was tested at 30, 50 and 70°C for one week showing stability of concentration (vc 2.3). The shelf life of an uracil solution in water of 3 mg/ml was tested for 26 weeks showing stability of concentration (vc 1.6).

Considering the above, a shelf life of 36 months at room temperature is set for uracil powder in polypropylene bottles. The storage instruction will be to store the bottles below 30°C.

2.2 Non-clinical pharmacology, pharmacokinetics and toxicology

2.2.1 Test materials used in toxicity studies

Uracil powder.

2.2.2 Integrated assessment of the data package

See CAS 66-22-8 [10].

2.2.3 List of studies conducted & references

Table 3.6 LD_{50} values of uracil in different species [9]

Species	Route of administration	LD_{50}
Rat	oral	> 6 g / kg
Mouse	oral	> 8 g / kg
Mouse	parenteral	1513 mg / kg
Dog	oral	> 5 g / kg
Rabbit	oral	> 10 g / kg

2.2.3.1 Safety data regarding long-term (chronic) administration of uracil

Uracil was administered to male and female dogs for 3 months and to male dogs for 12 months at dose levels of 0, 210, 420, 840 and 1680 mg uracil per kg body weight per day by gavage. While there were minor differences seen in food consumption, water consumption and erythroid parameters between the 3-month and the 12-month studies, it was concluded that there were no adverse effects seen neither on these parameters nor on body weight, EKG, clinical laboratory studies and organ weights. Pathological observations did not show treatment-related effects [12].

2.2.4 GLP statement and bioanalytical methods

The non-clinical pharmacology data have been extracted from the CAS database and from peer reviewed publications.

2.3 Clinical data

2.3.1 Clinical pharmacology

Uracil is a non-toxic endogenous pyrimidine and essential part of the structure of RNA.

Uracil and 5-FU are chemically almost alike and both substances are substrates for DPD. Uracil is an endogenous pyrimidine involved in RNA synthesis and, accordingly, an excellent candidate for DPD phenotyping.

2.3.2 Clinical pharmacokinetics

The pharmacokinetics of uracil after oral intake has been established in human volunteers and in patients with DPD deficiency in previous studies [5, 6].

There is no data available of pharmacokinetic parameters in special populations (i.e. age and gender, race, renal insufficiency, hepatic insufficiency).

2.3.3 Human exposure

In a Dutch study in 12 healthy volunteers, no adverse reaction was observed after oral ingestion of 500 mg/m^2 and 1000 mg/m^2 uracil [2, 3]. Oral administration of 6 mg/kg ^{13}C labeled uracil in 255 American volunteers also did not reveal adverse reactions [1, 4].

2.4 Overall risk and benefit assessment

Uracil is an endogenous pyrimidine base and an essential part of the structure of RNA. The LD50 value of uracil is very high and ranges from 6–8 g/kg in rats, mice and rabbits [11]. Chronic oral administration of 1680 mg/kg uracil in dogs during 1 year appeared completely safe [12].

Uracil is commercially marketed in combination with tegafur in the pharmaceutical product UFT® (Merck). This product is registered for the treatment of colorectal cancer. The daily dose of uracil in this commercial formulation is 672 mg/m^2.

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