

SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion and scientific discussion on procedures, which have been finalised before 1 March 2004. For scientific information on procedures after this date please refer to module 8B.

1. Introduction

Helicobacter pylori (*H. pylori*) is associated with gastritis, duodenal and gastric ulcer. In patients with ulcer disease, it is essential to determine the *H. pylori* status. These patients need treatment with effective antimicrobial agents to eradicate the infection and thereby diminish the risk of recurrence of gastric/duodenal ulcer disease. Effective combination therapy with antimicrobial agents and antacids are sufficiently described elsewhere in the literature. Before and after such a therapy, diagnostic methods are used for the determination of the *H. pylori* status to confirm the diagnosis and outcome of therapy respectively.

Standard reliable methods are biopsy-histology, culture methods on endoscopically obtained samples. Radiological methods are used in cases with known or recurrent ulcer disease and serological methods for confirmation of *H. pylori* status. These methods are generally invasive.

In recent years, the non-invasive ¹³C-breath test has gained acceptance as a diagnostic method for *H. pylori* status. This test has been used for this purpose in different modified methods worldwide, as described in the published literature.

The Helicobacter Test INFAI consists of 75 mg ¹³C-labelled urea (99% ¹³C-enrichment) and has been developed “for in vivo diagnosis of gastroduodenal *Helicobacter pylori* infection” in humans. It is intended for oral administration. The diagnostic principle is based upon the urease activity of *H. pylori* in the stomach, whereas other urease-producing bacteria are seldomly found in the gastric flora. The Helicobacter Test INFAI claims to be a standardised alternative of the similar methods used in various studies in the literature.

Two analytical methods for breath analysis have been approved for the 75mg strength, Isotope Ratio Mass Spectrometry (IRMS) and Non Dispersive Infra Red Spectrometry (NDIR), resulting in two separate presentations, differing in the elements of the kit for breath collection.

2. Chemical, pharmaceutical, and biological aspects

The product has been developed as a kit for the in vivo diagnosis of *Helicobacter pylori* infection in the stomach in humans. There are two presentations of the 75mg strength – in each case the precise composition of the kit concerning breath collection elements depends on the subsequent method of breath analysis:

- 1 polystyrol jar (10 ml volume) with polyethylene snap cap containing 75 mg ¹³C-urea powder for producing an oral solution;
- 4-labelled plastic or glass tubes for sampling, storing and transporting the breath samples for IRMS analysis (2 for sampling time: 0-minute value; 2 for sampling time: 30-minute value). Alternatively, breath collection bags for NDIR analysis.
- 1 straw for collection of the breath samples into the corresponding tubes (IRMS);
- patient leaflet;
- data sheet for patient documentation;
- page of labels and sticker.

All components are packed in a hard paper box.

Composition

The composition of Helicobacter Test INFAI consists only of the active substance, 75 mg of ¹³C-labelled urea, a non-radioactive stable isotope in the form of a crystalline powder, with a ¹³C-portion of ≥ 99%. The product is intended for oral administration after reconstitution with tap water. ¹³C-urea is packed in a colourless polystyrene jar closed with a colourless polyethylene snap cap.

The clinical trial formula of the medicinal product is identical to the one intended to be marketed. No development pharmaceuticals have been performed since the finished product contains only the active substance itself. Tap water has been shown to be an appropriate solvent for reconstitution of ¹³C-urea solution.

Method of preparation

The batch size of the finished product consists of about 1000 g ¹³C-urea yielding approximately 13.000 sales units of the finished product.

The only step of the finished product manufacturing is the automatical filling of 75 mg ¹³C-urea into a polystyrene jar with the help of a suitable filling machine or the manual filling on a suitable precision balance, and subsequently sealing of the containers with a snap cap. Individual filling ranged: 71.2-78.8 mg (= 95-105% of the label claim). The manual filling is only practised for small charges (e.g. clinical studies) and the weight uniformity is guaranteed by weighing each vial, which is documented automatically. Finally, the filled jar is labelled and packed into a hard paper box together with other components of the test kit.

Microbial contamination during filling is avoided by laminar airflow conditions. Since using standardised procedure, it is acceptable that no validation data have been submitted.

Control of starting materials

The synthesis of the active substance, ¹³C-urea, is performed following a classical manufacturing route for urea by introducing ammonia, ¹³C-enriched carbon monoxide, hydrogen sulfide, precipitated sulfur and methanol into an one-pot system. The reaction under pressure is followed by some purification steps by means of carbon filtration and passing the product through different types of columns.

Specifications provided by INFAI GmbH. showed that ¹³C-urea complies with the Ph.Eur. monograph for urea. The following tests were performed:

- identity (¹H-NMR, ¹³C-NMR, melting point, Ph.Eur. test C and D),
- purity (clarity and colour of solution, alkalinity, ammonia, heavy metals, biuret, loss on drying, sulphated ash)
- isotope content (¹³C-NMR, ¹⁵N-NMR and MS-),
- microbiology (total bacterial, yeast and moulds, *E. coli* and *Salmonellae*).

The tests for identity, purity and assay were conducted in accordance with Ph.Eur.. Besides, the special NMR and MS tests were adequately validated

The immediate package material is a colourless polystyrene jar with a polyethylene snap cap. The quality of the container and the closure complies with the Ph.Eur. Their dimensions are specified and their harmlessness confirmed. Other parts of the test set are four breath samples containers (10 ml glass Vacutainers® or 10 ml plastic Vacuettes® with a rubber stopper for collecting and storing the breath samples (two 00-minute-values and two 30-minute-values). It was confirmed that the stability of the breath samples is guaranteed over a period of 3 months and the breath sample containers are suitable for air transport conditions. Furthermore, a bendable drinking straw is added to transfer the breath into the sample containers, for which a specification was provided.

Control tests on the finished product

Active substance complied with above-mentioned specifications. Additional tests for the finished product appearance, identity (Ph.Eur. test D and ¹H-NMR), and determination of weight and uniformity of weight, loss on drying, and isotopic content and microbial purity at release were performed. No further tests or additional validation have been performed, since manufacturing only

consists of filling without thermal or mechanical stress to the material, and since either pharmacopoeial methods or control of starting material methods were used.

A standard operating procedure on the determination of weight and uniformity of weight was submitted. To obtain the net value of the content, the weights of the containers before and after filling are compared.

An adequate product specification of the ^{13}C -urea solution with purified water including the following criteria was also provided: colour and clarity, reconstitution time, taste and pH.

Stability

Stability tests on the active substance were performed by CIL (Cambridge Isotope Laboratories) including description, identification, melting point, alkalinity, clarity and colour of solution, ammonium, heavy metals, biuret, loss on drying, sulphated ash and assay following methods from the B.P. 1993. Data analyses of three batches in the intended marketing pack (amber glass, moisture tight closure) at different temperature and relative humidity stored for up to 24 months showed only an increase of moisture especially between the 18th and 24th month. The noticeable increased loss on drying was however within acceptable limits.

Three batches have been stored in the polystyrene jars with polyethylene snap caps at room temperature (15-25°C) over a period of 36 months and tested for characteristics, identity, loss on drying, biuret, ammonia, assay and microbiology. No additional determination of the ^{13}C -isotope content has been performed since ^{13}C -urea is a stable isotope. The results confirm the proposed product shelf life of 3 years when the finished product is stored under “normal conditions” (15-25°C, ambient humidity) in the packaging intended for marketing, as no alteration concerning its quality could be observed.

Furthermore, although the aqueous solution of ^{13}C -urea has to be ingested directly after preparation, a short term stability test on the product solved in 20 ml tap water was performed. No decomposition of ^{13}C -urea up to two hours was observed, which corresponded to the results from published literature.

Other information

The breath sample analysis is an integrated part of the diagnostic kit and therefore also of the dossier. The principles of breath test analysis (description and validation) and test performance were given.

An increase in $^{13}\text{CO}_2$ in breath is detected by isotope-ratio-mass-spectrometry (IRMS) or non-dispersive infra red spectrometry (NDIR) and expressed as absolute difference ($\Delta\delta$ -value in ‰) between the 30-minute- and 00-minute-value (the precise methodology is slightly different in each case). The test indicates the presence of *Helicobacter pylori* if the increase of $^{13}\text{CO}_2$ exceeds the $\Delta\delta$ -value of 4.0‰.

The analysis of $^{13}\text{CO}_2$ in breath sample is an analysis of the isotopometric ratios r_s of $^{13}\text{CO}_2/^{12}\text{CO}_2$ versus that of a reference gas (r_r). The δ -value is expressed as:

$\delta[\text{‰}] = (r_s - r_r / r_r) \times 1000$. The multiplication with 1000 is performed because of the small value. The change in δ -value after exposure to ^{13}C -urease is then $\Delta\delta = \delta_t - \delta_0$.

The methods for determining the $^{13}\text{CO}_2/^{12}\text{CO}_2$ -ratio have been adequately described and validated. They both show high specificity, precision, accuracy, linearity and reproducibility of measurements.

For analysing the collected breath samples, it was agreed that the relevant information concerning the breath analysis should be included in the Summary of Product Characteristics (SPC), and in the Package Leaflet of each presentation as a tear-off part. This will enable qualified laboratories to perform the analysis of the breath samples, provided that a suitably validated method is used.

3. Toxicopharmacological aspects

No pharmacological or toxicological investigations were performed with the particular product, i.e. Helicobacter Test INFAI. However, in support of this application, an extensive literature research about urea was made.

Pharmacodynamics

No suitable animal model for the oral ^{13}C -urea breath test has been described. No preclinical data were submitted. All studies were performed in human and a compilation of the diagnostic potential of urea (in human) is presented together with a description of (human) adverse effects observed.

As described in the published literature, the principle of the ^{13}C -urea oral diagnostic for *Helicobacter pylori* infection of the stomach is based on the high urease activity in these bacteria at low pH. Urease catalyses the formation of carbon dioxide and ammonia from urea. After oral ingestion, ^{13}C -urea reaches the gastric mucosa, then with *Helicobacter pylori* present it will be metabolised in the stomach to ^{13}C - CO_2 and ammonia. The ^{13}C - CO_2 formed is rapidly absorbed locally, distributed within the bloodstream as bicarbonate, and exhaled as ^{13}C - CO_2 via the lungs (see section 4, Overview of Part IV of the dossier). Absorption and distribution of ^{13}C - CO_2 is faster than the urease reaction. Therefore, the rate limiting step is the cleavage of urea by the urease of the *Helicobacter pylori* present. In the exhaled air, the ratio ^{13}C - CO_2 to ^{12}C - CO_2 will increase early after oral administration of ^{13}C -urea.

No preclinical data is submitted to support the statement that ^{13}C -urea breath test will be affected by all compounds known to eradicate *Helicobacter pylori*. This has been addressed in the SPC.

A literature review showed that the stable isotope ^{13}C labelled to urea does not have any pharmacological or toxicological effect.

Pharmacokinetics

Urea is rapidly absorbed after oral dosing. Part of the administered urea will be cleaved into carbon dioxide and ammonia by the *Helicobacter pylori* present in the stomach. Thus only a fraction of the dose reaches the colon unchanged. The carbon dioxide is then rapidly absorbed locally and distributed. The ammonia formed is absorbed but will be completely metabolised by the liver, via aminoacids, to form urea. Due to extensive first pass metabolism of ammonia in the liver, combined with the low urea dose administered with the oral ^{13}C -urea test kit, toxic blood levels of ammonia are not reached.

Urea absorbed enters the physiologic body pool. It is distributed into extracellular and intracellular fluids; concentrations reached are similar in lymph, bile, CSF and blood. Urea crosses the placenta, penetrates the eye, and is expected to be excreted into milk. Highest levels of urea are observed in the kidneys.

Even after parenteral dosing, some urea is apparently hydrolysed within the gastro-intestinal tract to carbon dioxide and ammonia, presumably by bacterial ureases. Urea is predominantly eliminated via renal excretion.

Toxicology

Single dose toxicity - A compilation of published data in rats and mice, showed that adverse effects were only observed after oral administration of doses higher than 5,000-mg/kg body weights.

Repeat dose toxicity - The documentation does not contain repeated dose toxicity studies using the intended route of administration. Two 4 and 35 week studies of the toxicity of urea after repeated dermal application were cited. The data indicated a low potential of subacute and chronic toxicity for urea.

Reproductive toxicity - Animal reproduction studies have not been conducted. However, the toxic potential of 75 mg urea given orally as a single dose during pregnancy may be considered minimal in women.

Genotoxicity - Because of its high-volume industrial production, the genotoxic potential of urea has been investigated extensively in various test systems. In several *in vitro* and *in vivo* studies, it has been shown that urea has a mutagenic potential at concentrations far above the physiological concentrations.

In addition, 3 published studies showed a 7 times increase in the incidence of chromosomal abnormalities in bone marrow cells of Swiss albino mice given oral doses of 500 mg urea per day for 5 days. Evidence of DNA-damaging activity was obtained with urea in concentrations > 0.36 mol/l in the absence of metabolic activation. The mouse lymphoma TK+/- - TK-/- mutation assay test indicated statistically significant increase in the mutation frequency from control values of 80 to 90 up

to 256 in dependence of urea concentration (0.132-0.662 mol/l). These studies further indicate that urea at high concentrations has genotoxic potential.

Carcinogenicity - The carcinogenic potential of urea was investigated as part of a screen of environmental and occupational chemicals. Mice and rats were fed a diet containing 0.45% urea during 12 months. The results indicated that urea was non-carcinogenic. However, the study duration was not sufficiently long to allow a definitive evaluation of its carcinogenic potential. On the other hand, since the *Helicobacter* breath test is only intended for single dose use and the administered dose is considerably lower than physiological concentration of urea in blood, the carcinogenic risk of this medicinal product was regarded as negligible.

Local tolerance - The documentation did not contain data on the effect of urea on the skin of experimental animals.

In summary, the pharmacological and toxic potential of urea (e.g. genotoxicity) is associated with extremely high doses only, in comparison to the dose advised for used in breath testing (75 mg of ¹³C-urea). The preclinical documentation consisted of publications from the scientific literature. The documentation did not contain all formally required studies. The omissions are justified because urea is a well-known compound, which is generated endogenously via the urea cycle by cleavage of arginine to ornithine and urea. The daily endogenous urea production amounts 25-35 g/day. The physiological concentration of urea in blood ranges between 1.7 and 8.3 mmol/l (100-500 mg/l). It is unlikely that oral administration of 75 mg urea, which is the dose to be administered once, will interfere with the endogenous urea concentration or will cause deleterious effects.

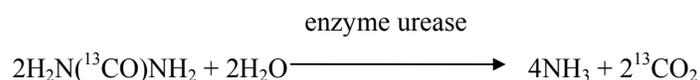
4. Clinical aspects

The clinical pharmacology is mainly based on published literature with some cross-references to clinical trials. The core clinical documentation consists of four “non-controlled” trials, which were performed with the product applied for (#1 to #4). Furthermore, publications were extensively cited to support the clinical experience

Pharmacodynamics

The applied product contains the substrate ¹³C-urea (99% ¹³C and 1% ¹²C) for *Helicobacter pylori*. ¹³C is a natural, stable and non-radioactive isotope, which has no known pharmacodynamic effects. In contrast to the present formulation, the ¹²C isotope is the prevalent one in nature, approximating 99%. The pharmacological properties of ¹³C-urea are identical to those of the natural urea. Due to the single low dose of 75 mg ¹³C-urea used in breath testing, no pharmacodynamic effects are expected.

In case of infection with *Helicobacter pylori* in the stomach, the ¹³C-urea is metabolised by the *Helicobacter* enzyme urease, which liberates ¹³CO₂. Since other urease-producing bacteria are seldomly found in the gastric flora, the presence of urease activity in the stomach is indicative of the presence of *Helicobacter pylori*.



The ¹³CO₂ diffuses into the blood vessels, is transported as a bicarbonate into the lung, and is then liberated as ¹³CO₂ with the exhaled air. The ¹³C/¹²C ratio is measured by IRMS, at 0 and 30 minutes after intake. The change of this ratio is stated as the absolute difference (Δδ-value) between the 00-minute- and 30-minute-value. The cut off point for discriminating *Helicobacter pylori*-negative and positive patients is set to a Δδ-value of 4⁰/₀₀ (see section 2. Overview of Part II of the dossier).

In the case of *Helicobacter pylori*-negative subjects, the ¹³C-urea administered will be absorbed, followed by incorporation into the endogenous urea cycle. Ammonia, which is generated by the bacterial hydrolysis, is included into the metabolism in the form of NH₄⁺. Unabsorbed ¹³C-urea will eventually reach the colon, where it will be cleaved by bacterial urease. It also leads to an increase of the ratio ¹³C-CO₂ to ¹²C-CO₂ in exhaled air, but this will occur later because of the delay caused by gastro-intestinal passage to the colon.

Urea can also be metabolised by gut bacteria. The presence of bacteria other than *Helicobacter pylori* in the upper gastrointestinal tract is very rare. Two main conditions may lead to the presence of bacteria in the stomach: acid antisecretion treatment of gastritis A. However, as the use of urea breath test is contraindicated under the treatment with acid antisecretory agents and the SPC recommends further diagnostic measures for patients with suspected gastritis, the hazard of producing false positive results is avoided.

Pharmacokinetics

The total daily intake of ^{13}C -isotope in food is in average 2.7 g. The addition of 75 mg of ^{13}C -urea, equivalent to 16 mg pure ^{13}C , is not considered to have any biological consequence.

Absorption and distribution of $^{13}\text{CO}_2$ are faster than the urease reaction. Therefore the rate-limiting step in the whole process is the cleavage of urea by *Helicobacter pylori*.

In order to maximise the contrast between *Helicobacter pylori*-positive and negative patients, some test parameters should be adhered to. This can be achieved by choosing the optimal dose of ^{13}C -urea, test meal, threshold level and time schedule for the actions.

^{13}C -urea dose - In the literature, a wide range of ^{13}C -urea doses has been used (75-350 mg) in *Helicobacter pylori*-infected volunteers. A linear increase of $\Delta\delta$ -value with dose was observed. As long as the cut-off range is wide enough to discriminate between *Helicobacter pylori*-positive and negative results, the choice of 75 mg dose is justified. Furthermore, breath tests using 125 and 250 mg doses do not indicate any dose-influence on sensitivity or specificity of the test.

Test meal - Consumption of a test meal before the administration of the ^{13}C -urea will delay gastric emptying, resulting in a prolonged exposure of ^{13}C -urea to the *Helicobacter pylori* urease. The choice of test meal is arbitrary but based on balanced consideration of properties such as acceptability to patients, influence of $^{13}\text{CO}_2$ -exhalation, and effect on the gut motility. With reference to published studies, four different test meals (Meritene, PulmoCare, Citric acid and orange juice) were found to be suitable for ^{13}C -urea breath test. With respect to practicability and acceptability, orange juice was chosen, as the maximum response (highest increase of $^{13}\text{CO}_2/^{12}\text{CO}_2$ -ratio after ^{13}C -urea ingestion in *Helicobacter pylori*-positive patients) was achieved after 30 minutes.

Sampling interval - For the evaluation of ^{13}C -urea breath test, an optimised sampling interval is to be used, to enable a clear discrimination between *Helicobacter pylori* infection and non-infection. Publications showed that two measurements, a 00-minute-value (baseline) and 30-minute-value, were sufficient to provide adequate sensitivity and specificity. As a maximum response was seen 30 minutes after intake; this time point was established as the sampling time for the second test value.

Choice of cut-off value of $4^0/00$ - Although the choice was arbitrary, it has been shown to be a balanced cut-off point with respect to sensitivity and specificity. The validity of this threshold value in comparison to the gold standard (biopsy/culture) was assessed.

Factors influencing the test outcome - As exposure to mouth flora for > 1 minute might influence the increase in $^{13}\text{CO}_2/^{12}\text{CO}_2$ -ratio the first 10 minutes, the recommended ^{13}C -urea dose should be ingested totally and briskly. This effect is however negligible after 15 minutes. Physical stress or exercise is likely to increase $^{13}\text{CO}_2/^{12}\text{CO}_2$ -ratio, as fat and carbohydrates have different ^{13}C -ratio. These differences are negligible compared to the increase of $^{13}\text{CO}_2$ after administration of ^{13}C -urea and lay within the physiological deviation below $2^0/00$. Since the test has to be carried out on empty stomach, a ^{13}C -influence of fat and carbohydrates (with a ^{13}C -content of about 1%) is assumed not to be relevant. The patient must have fasted for 6 hours before the test, preferable overnight.

If these conditions are not fulfilled, the test may lead to wrong results.

Efficacy

In total 561 evaluable patients were involved in four open studies sponsored by INFAI (Table 1). Each patient served as his/her own control. A threshold of $\Delta\delta$ -value of $4^0/00$ was used in these trials, i.e. an increase of $\Delta\delta$ -value by > $4^0/00$ indicated *Helicobacter pylori* infection. Key or quality criteria for efficacy of this diagnostic test, which have to be satisfactorily addressed, are accuracy, linearity, reproducibility, sensitivity and specificity in particular in comparison to the existing gold standards such as bioptic/histologic and culture methods. Patients with unknown *H. pylori* status indicated for

routine endoscopy (for abdominal pain and dyspepsia) were included. Patients who had antibacterial therapy with antiacids (such as bismuth salts, omeprazole, etc.) in the weeks preceding the study were excluded. The breath test was performed after an overnight fast or 2-4 hours after the endoscopic examination or the next morning. The ¹³C-urea dose was ingested 1 minute after the test meal.

Table 1: Quality criteria of breath test versus gold standard*

Clinical Trials (CT)	CT #1	CT #2	CT #3	CT #4 ^b
Number of patients	104	70	194	193
Test meal (200 ml)	PulmoCare	Orange Juice	Orange Juice	Orange Juice
Cut-off point	4‰	4‰	4‰	4‰
Accuracy	94%	99% ^a	98%	97%
Sensitivity	92%	98%	96%	97%
Specificity	97%	100%	100%	97%
Positive Predictive Value	98%	100%	100%	87%
Negative Predictive Value	88%	96%	96%	99%

* Gold standard in CT #1 & CT #2 : histology and/or culture
 CT #3 & CT #4 : histology

a = 97.1% in the statistical report

b = after exclusion of (8) patients with protocol violations.

CT#1 was a single centre intraindividual trial. The aim was to validate the breath test in reference to the combination of histology and culture (gold standards). If one of the reference methods was positive, the patients was defined *H. pylori* positive; if all results were negative, the patient was defined *H. pylori* negative. Of the 110 recruited patients, 104 were evaluable (64 females and 40 males, aged ranging from 17-92 years). Not all formal regulations of GCP have been fulfilled. According to histology and/or culture, 67 patients were *H. pylori* positive and 37 negative. The breath test, in contrast to the gold standard, led to false negative findings in 5 patients, all having $\Delta\delta$ -value clearly below 4‰. The sensitivity for the breath test was 92% and the specificity 97%.

CT#2 was a single centre intraindividual trial. The aim and reference to the gold standards was similar to CT#1. All 70 patients recruited were evaluable, 33 females and 37 males (aged ranging from 18-85 years). The histology and/or culture indicated 47 *H. pylori* positive and 23 *H. pylori* negative patients. The breath test, in contrast to the gold standard, lead to false negative finding in 1 patient having $\Delta\delta$ -value clearly below 4‰. In this single case, the presence of an urease-negative bacterium was suggested, which could be the causative agent of the gastritis. The sensitivity for the breath test was 98% and the specificity 100%. The findings also suggested that neither the breath test nor other methods provide reliable results in case of a low colonisation level.

CT#3 was an epidemiological multi-centre trial. The reference diagnostic methods used were histological colouring of the sample. The primary purpose of this study was to demonstrate a possible connection between dyspepsia and *H. pylori* infection of the stomach in patients aged 18-40 years. The secondary purpose was to validate the breath test in reference to the gold standard (i.e. biopsy followed by histology). Four hundred thirteen patients were evaluable epidemiologically, of which 194 patients for the breath test assessment with unknown *H. pylori* status indicated for endoscopy due to (dyspeptic) complaints in upper abdomen or reflux complaints.

In the epidemiological subgroup, 44-47% of the patients with dyspepsia were reported to have *H. pylori* infection, but due to the non-controlled nature of the study a conclusion could not be drawn.

According to histology, 47% (92/194) of patients were *H. pylori* positive and 53% (102/194) negative. The breath test, in contrast to the gold standard, lead to false negative findings in 4 patients having $\Delta\delta$ -value clearly below 4‰. The sensitivity for the breath test was 96% and the specificity 100%.

CT#4 was a multi-centre trial concerning the diagnostic potential of the ¹³C-urea breath test after eradication therapy. The purpose of this study was to evaluate the quality criteria of the ¹³C-urea breath test in reference to the gold standard (i.e. biopsy followed by histology). Patients indicated for endoscopic control 4-8 weeks after completion of an accepted *H. pylori* eradication treatment were included. Of the 195 enrolled patients (average age 53 ± 15 years), 193 were evaluable for histology and breath test.

According to histology, 81% (156/193) of patients were *H. pylori* negative, whereas the breath test indicated 79% (153/193) of *H. pylori* negative patients. After therapy, the breath test, in contrast to the gold standard, lead to false negative findings in 1 patient having $\Delta\delta$ -value clearly below 4‰, and gave 5 positive findings having $\Delta\delta$ -value clearly above 4‰. The sensitivity for the breath test was 97% and the specificity 97%.

In comparison to the quality criteria of the total population, no significant difference is obvious for *H. pylori* associated disease (gastritis, duodenal and gastric ulcer). In patients with B-gastritis, no negative findings have been reported; in patients with other disease than gastritis or ulcer, no positive findings have been reported. The breath test indiscriminately covers a wide range of *H. pylori* associated gastric and duodenal disease. The ¹³C-urea breath test is a reliable non-invasive method for diagnosis of ongoing *H. pylori* infection in particular in patients with duodenal ulcer. Patients with gastric ulcer, however, should be checked repeatedly, both by means of endoscopy and biopsy, until the ulcer is completely healed in order to exclude the possibility of a carcinoma.

In addition to these above trials, there are supportive studies. Clinical studies cited from the literature show the increasingly wider range of application of the ¹³C-urea breath test. Three eradication studies of other companies, where the ¹³C-urea breath test was used for *H. pylori* detection were also presented. The results indicated that ¹³C-urea breath test is a diagnostic tool useful in eradication therapy. Two scientific studies were reported (#1 and 2) using 4‰ as cut off point. It was shown that in reference to the gold standard, false negative and positive results obtained by using the ¹³C-urea breath test were not in the borderline of the cut off point (4‰).

These scientific and other cited studies can be considered to be supportive of findings in the pivotal clinical trials, although the documentation was not of optimal quality and methods used were not completely standardised according to the applicant criteria in most studies, thus these findings will not be further discussed.

There are insufficient data on the diagnostic liability of the Helicobacter Test INFAI to recommend its use in patients younger than 18 years of age.

In patients with significant gastric resection, false negative results are possible, mainly due to fast gastric emptying. Such patients may require more than one sample being taken and one perhaps before the mentioned 30-minute breath sample. The sensitivity, specificity and usefulness of the ¹³C-urea breath test with the proposed low substrate amount and a single 30 minute post baseline breath sample in this specific population with gastrectomy were not addressed in the present application. There are insufficient data on the diagnostic liability of the Helicobacter Test INFAI to recommend its use in patients with gastrectomy.

It is not expected that the test procedure may be harmful during pregnancy or lactation. However, it is recommended to take notice of the product information of eradication therapy products for their use during pregnancy and lactation.

Since the test is non-invasive and since the substrate including the stable isotope ¹³C does not have any pharmacological or toxicological effect. It may be repeated in the same individual.

Safety

None of the studies performed in more than 9000 patients with the urea breath test, either sponsored by INFAI or cited from literature, reported side effects due to ¹³C-urea.

In summary - ¹³C-urea breath test is a non-invasive method to detect gastroduodenal *H. pylori* infection. The 4 clinical trials show a high efficacy of the breath test with the specified parameters of test meal, dosage and cut off-point, independent of use after or before therapy of *H. pylori* infection. The average sensitivity and specificity for the breath test, compared to bioptic diagnostics of an infection with *H. pylori*, obtained from INFAI sponsored studies was > 95%.

The achieved standardisation of the breath test makes this method suitable for general clinical use especially in patients with known gastritis or gastroduodenal ulcer disease.

The breath test reflects the actual *H. pylori* status and can be used as a control after an eradication therapy. However, for the differential diagnosis before *H. pylori* eradication therapy, invasive endoscopic examination and histologic analysis of biopsy specimens are essential in order to examine the presence of any other complicating conditions (e.g. ulcer, autoimmune gastritis and malignancies).

The suppression/eradication of *H. pylori* may lead to false negative or positive results, therefore the test should be used after at least 4 weeks without systemic antibacterial therapy and 4 weeks after the last dose of acid antisecretory agents.

5. Conclusions

Helicobacter Test INFAI consists of 75 mg ¹³C-labelled urea (99% ¹³C-enrichment).

Satisfactory quality of Helicobacter Test INFAI has been demonstrated, allowing a 3-year shelf life when stored at room temperature, (15 - 25°C).

In view of the fact that urea is physiologically abundantly present and only a small additional amount is to be administered once, it is considered that any additional risk is negligible for this urea breath test. None of the clinical studies performed with the Helicobacter Test INFAI reported side effects due to ¹³C-urea.

Helicobacter Test INFAI is a diagnostic means to detect *Helicobacter pylori* infection in the stomach with a high specificity and sensitivity. However, differential diagnosis with invasive endoscopic methods might be indicated in order to examine the presence of any other complicating conditions, e.g. ulcer, autoimmune gastritis and malignancies.

6. Extension of the indication in adolescents (aged 12-17 years)

Introduction

The prevalence of *H.pylori* infection in young patients is much lower than in adults. In addition, unlike in adults, the infection rarely bears life-threatening risks like gastritis-associated peptic ulcer disease of the stomach and duodenum or gastric carcinoma and gastric lymphoma (MALT). The patients are therefore rarely referred to upper gastrointestinal endoscopy for a confirmation of *H.pylori*, e.g. in search of a possible cause of functional dyspepsia or for diagnosis of chronic active type-B-gastritis. A non-invasive test for *H.pylori* is therefore considered desirable in adolescents. However, neither the ¹³C-urea breath test nor the serologic tests or the antigen stool test have been validated for this so far.

The MAH submitted a Type II variation for an extension of the indication to include adolescents aged 12-17 years. The supporting clinical documentation consisted of one multicentre comparative study in children and adolescents aged 2 -17 years (335 included in the study, 273 included in the analysis), and a clinical expert statement.

Although the clinical study was performed in children and adolescents aged from 2 – 17 years, for children aged 2 - 11 years, the dose of labelled urea was decreased to 45 mg due to lower body mass and to avoid unnecessary cost for labelled urea. The scope of the variation was thus limited to the extension of the therapeutic indication to include adolescents aged 12 – 17 years.

Clinical efficacy

The clinical trial has been performed in accordance with the requirements of Good Clinical Practice. The breath test performance was calculated against the gold standard, which is a combination of histological diagnosis, cultures in different media and a urease test. A *true positive* was defined as a positive culture, or a positive histology and a positive rapid urease test. A *true negative* was defined as a negative culture and negative histology, or a negative culture and negative rapid urease test, or a culture not evaluable and negative histology and negative rapid urease test.

The sensitivity was 96.8 and 97.7 % and specificity was 98.3 and 96.0 % in children and adolescents, respectively. Based on the data there were no major objections preventing an extension of the indication to the age category 12 to 17 years.

The main discussion during the assessment of this application centred on the claimed indication and the posology and method of administration. Regarding the *indication*, it was considered that the new indication should adequately reflect the target population. In view of the fact that gastric colonisation and infection with *Helicobacter pylori* occur both in children and adults, but that children and adolescents are considerably less susceptible to peptic ulcers and other pathological sequelae, the risk to benefit ratio of diagnostic tests and therapeutic regimens for *H. pylori* in children and adolescents are likely to be different from those in adults. Given the relatively low prevalence of *H. pylori* infection in EU countries it is important to recognise that indiscriminate testing in children and adolescents is not recommended, and indeed may threaten the optimal care of these groups of patients. Therefore, any diagnostic test should be employed judiciously and be reserved for children and adolescents who are most likely to derive measurable benefit, such as those likely to have peptic ulcer disease.

Regarding the method of administration, a pilot study showed that the use of citric acid in water as an alternative test meal does not affect the diagnostic value of the test procedure. The lag time for a retest after termination of antisecretory therapy was reduced from 4 weeks to 2 weeks based on data showing that all infected patients had positive results 2 weeks after completion of Lansoprazole therapy, but not at 7 or less days.

There is insufficient data on the diagnostic liability of Helicobacter Test INFAI to recommend its use in patients with gastrectomy and in patients younger than 12 years of age. The relevant warning has been included in section 4.4 of the SPC.

Clinical safety

The database comprises 335 patients, which was considered adequate. No adverse drug reactions were reported and the ¹³C-urea breath test was well tolerated by all patients.

Conclusion

The CPMP considered by consensus that the benefit/ risk profile of Helicobacter Test INFAI for use in patients from 12 to 17 years of age was favourable and issued a positive opinion on the extension of the therapeutic indication to include this population. The European Commission issued a decision to vary the Marketing Authorisation on 23 April 2002.

7. Line Extension for a new 45 mg strength in children aged 3-11 years

Introduction

This application concerns additional 45 mg strength, to be used in children aged 3-11 years. In October 2001 a positive opinion was adopted for a type II variation concerning the use of Helicobacter Test INFAI, powder for oral solution (75 mg) in adolescents aged 12-17 years. The same clinical study, performed in 335 children and adolescents, has been submitted to substantiate the efficacy and safety of Helicobacter Test INFAI for both the type II variation and the present line extension. The

previous assessment of the type II variation has covered the data provided on the use of Helicobacter Test Infai (75 mg), in adolescents aged 12-17 years. The present assessment will focus on the use of Helicobacter Test INFAl (45 mg), in children aged 2-11 years.

The current gold standard for the confirmation of an infection by *H. pylori* consists of the histological diagnosis of biopsies in combination with cultures in different media. A more rapid test is based on the urease activity of *H. pylori* in the biopsy. An important disadvantage of the gold standard method is its invasiveness. Therefore, non-invasive diagnostic modalities, such as serology and urea breath tests, have been developed. A number of serological assays are currently available. However, these have generally been standardised in adults. Studies in children have shown insufficient sensitivity in the diagnosis of *H. pylori* infection in children. The ¹³C-urea breath test is a non-invasive diagnostic test that is based on the detection of the metabolism of urea by the urease of *H. pylori*. An important disadvantage of the non-invasive methods is that it does not allow for the detection of lesions, such as ulcers or neoplasms. Moreover, no antimicrobial susceptibility of *H. pylori* is available.

Chemical, pharmaceutical and biological aspects

Composition

The composition of this line-extension product intended for paediatric use is almost identical to that already authorised for adults. The main difference is in the reduced strength of the product, 45 mg in this case.

As for the adult presentation, it is presented in the form of a multicomponent kit. The active part of the product consists only of the active substance, 45 mg of ¹³C-labelled urea, a non-radioactive stable isotope in the form of a crystalline powder, with an isotopic purity (¹³C) of ≥ 99%. The product is intended for oral administration after reconstitution with tap water. The ¹³C-urea powder is packed in a colourless polystyrene jar closed with a colourless polyethylene snap cap. Other elements in the kit facilitate administration of the dose and collection of breath samples, e.g. bendable straws and glass or plastic containers for breath collection.

Active substance

Information on the manufacture and control of the active substance has been presented in two EDMFs, evaluated to ensure consistency with regard to specifications.

The synthesis of the active substance, ¹³C-urea, is performed following a classical manufacturing route for urea by introducing ammonia, ¹³C-enriched carbon monoxide, hydrogen sulfide, precipitated sulfur and methanol into an one-pot system. Some purification steps by means of carbon filtration and column chromatography follow the reaction under pressure. Proof of structure has been confirmed by a variety of spectroscopic studies.

Active Substance Specification

Specifications show that ¹³C-urea complies with the Ph. Eur. monograph for urea, with the addition of the following relevant tests:

- identity (¹H-NMR, ¹³C-NMR, melting point, Ph. Eur. test C and D),
- purity (clarity and colour of solution, alkalinity, ammonia, heavy metals, biuret, loss on drying, sulphated ash)
- isotope content (¹³C-NMR, ¹⁵N-NMR and MS⁻),
- impurities (sulfur, alcohol insoluble, chloride and methylcarbamide),
- microbiology (total bacterial, yeast and moulds, *E. coli* and *Salmonellae*).

The tests for identity, purity and assay were conducted in accordance with Ph. Eur. and the special NMR and MS tests were adequately validated.

Stability of the active substance

Since in this case the active substance is used as the ‘finished product’ itself, without being compounded into a formulation, investigations into stability have focussed on the stability of the finished product.

Other ingredients

The plastic container for the active substance is composed of materials that comply with the relevant PhEur monographs.

There are no other ingredients *per se* in the finished product, apart from other ‘components’ of the total presentation or kit, i.e. straws and plastic or glass containers for collection of breath samples. These comply with EU food regulations and are CE-certified where relevant.

Product development and finished product

The development and manufacture of the product are very simple. The active substance itself is a crystalline powder, soluble and suitable to be dissolved in domestic tap water for direct oral administration by the patient. Manufacture is basically the automatic filling of 45mg of the active substance into the plastic containers under controlled air-pressure necessary for powdered substances. Microbial contamination during filling is avoided by laminar air flow conditions. Since the process is so simple and standard, minimal validation data have been submitted, and this is regarded as acceptable in this specific case.

The batch size of the finished product consists of about 1000 g ¹³C-urea yielding approximately 22,000 units of the finished product.

The finished product is tested for appearance, identity (Ph.Eur. test for urea and ¹H-NMR), and determination of weight and uniformity of weight, loss on drying, isotopic content and microbial purity at release. The content of active substance at release is controlled to within 95-105% of the nominal amount.

Stability of the Product

Three batches have been stored in the polystyrene jars with polyethylene snap caps at room temperature (15-25°C) over a period of 36 months and tested for characteristics, identity (NMR), loss on drying, biuret, ammonia, assay and microbiology. No additional determination of the ¹³C-isotope content was considered necessary, since ¹³C-urea is a stable isotope and unlikely to change. The results confirm the shelflife and storage conditions as defined in the SPC.

Furthermore, although the aqueous solution of ¹³C-urea has to be ingested directly after preparation, a short-term stability test on ¹³C-urea dissolved in tap water was performed. No decomposition of ¹³C-urea up to two hours was observed, which corresponded to the results from published literature, and is satisfactory to allow oral administration under the conditions of the breath test.

Other information

For information on the principles of breath test analysis see the discussion in the report on the adult presentation, 75mg strength. Note that at the present moment, only IRMS is authorised as an approved method for breath analysis with the paediatric 45mg kit.

An increase in ¹³CO₂ in breath occurs in the presence of gastroduodenal *Helicobacter pylori* and is detected by isotope-ratio-mass-spectrometry (IRMS) and expressed as absolute difference ($\Delta\delta$ -value in ‰) between the 30-minute- and 00-minute-value. The test indicates the presence of *Helicobacter pylori* if the increase of ¹³CO₂ exceeds the $\Delta\delta$ -value of 4.0‰.

The analysis of ¹³CO₂ in breath sample is an analysis of the isotopometric ratios r_s of ¹³CO₂/¹²CO₂ versus that of a reference gas (r_r). The δ -value is expressed as:

$\delta [^0/_{00}] = (r_s - r_r / r_r) \times 1000$. The multiplication with 1000 is performed because of the small value. The change in δ -value after exposure to ^{13}C -urease is then $\Delta\delta = \delta_t - \delta_0$.

The method for determining the $^{13}\text{CO}_2/^{12}\text{CO}_2$ -ratio was adequately described and validated. The standardised mass spectrometry analysis in this case showed good precision, accuracy, linearity and reproducibility of measurements, and these data are included in the SPC.

As for the adult 75mg presentation, it was agreed that the relevant information concerning the analysis of breath samples and testing specifications should be included in the SPC, and also in the package leaflet. The objective is to enable any objectively qualified laboratory to perform an analysis of the breath sample.

Discussion on chemical, pharmaceutical and biological aspects

This is a pharmaceutically simple finished product that requires minimal process validation. Urea is again a simple molecule with no known chemical stability problems. Additionally in this case there is the presence of the isotope ^{13}C but this is well known to be a stable isotope. The pharmaceutical variables were solved for the previously authorised adult version of this product (75mg) and this paediatric form presents no further problems.

The purity of the active substance and the control of the manufacturing processes for the active and the finished product indicate reliable reproducibility, and in turn indicate a reliable product performance in the clinic.

Toxico-pharmacological aspects

Discussion on toxico-pharmacological aspects

No preclinical studies have been performed and the applicant has submitted no preclinical data. In addition, an extensive literature research on this issue has not been carried out and no preclinical expert report was submitted for evaluation. This is nonetheless acceptable for the following reasons:

- The product is a line extension of the existing MA for Helicobacter Test INFAI. The active ingredient is thus well known and already authorised in the EU.
- The line extension under evaluation contains only a very small fraction of the amount of urea physiologically present in the body, and hence no safety risk is expected. When compared with the already marketed 75 mg product, the only change proposed by the applicant is the lower level of ^{13}C -labelled urea, i.e. 45 mg instead of 75 mg. It is well known that only extremely high doses of urea can lead to toxic effects. Regarding the pure, non-radioactive stable isotope ^{13}C , no safety risk is expected, as its levels are also low. Moreover, ^{13}C is a widely used isotope without known toxic effects.

Clinical aspects

The applicant has submitted a single phase III clinical trial (Study UR98/2/001) to evaluate the efficacy and safety of Helicobacter Test INFAI in children and adolescents aged 2-17 years. Given that this application concerns the use of Helicobacter Test INFAI in children aged 3-11 years, the discussion will focus on this subpopulation.

Clinical efficacy

Main study UR98/2/001

Description of the study

This was an open, prospective multicentre study to evaluate the efficacy and safety of Helicobacter Test INFAI. Children aged 2-11 years received a single oral dose of 45 mg ^{13}C -urea per test. An upper gastrointestinal endoscopy including a test for *H. pylori* infection, based on medical need, was required to participate in the trial. No previous *H. pylori* eradication therapy was allowed, nor the

consumption of antibiotics (for other indications), antisecretory drugs, bismuth salts, or sucralfate in the two weeks prior to enrolment.

Primary endpoints/assays

The *primary objective* of the study was to validate the ¹³C-urea breath test in children and adolescents. The ratio ¹³CO₂/¹²CO₂ in exhaled air was measured at baseline and 30 minutes after administration of the appropriate dose of ¹³C-urea. The test was considered positive when the difference between these two ratios exceeded 4‰ (i.e the cut-off value).

The ¹³C-urea breath test was carried out using 100 ml orange juice without sugar as a pre-administered test meal to inhibit gastric emptying.

The diagnostic properties of the ¹³C-urea breath test were compared with a gold standard, namely a combination of diagnostic procedures based on biopsies. A *true positive* was defined when at least one of the following criteria were met: i) positive culture, ii) positive histology and positive rapid urease test. A *true negative* was defined when at least one of the following criteria were met: i) negative culture and negative histology, ii) negative culture and negative rapid urease test, iii) culture not evaluable and negative histology and negative rapid urease test.

A *secondary objective* of the study was the calculation of a receiver-operating-characteristic (ROC) curve of the ¹³C-urea breath test.

Statistical analysis

One-sided 90% confidence intervals for sensitivity and specificity as well as positive and negative predictive values were calculated.

RESULTS *Study populations/accountability of patients*

A total of 335 children and adolescents were enrolled in 12 study centres in Europe (see table below). A total of 204 children in the age category 3-11 years were enrolled. All children with data for the gold standard as well as for the ¹³C-urea breath test were included in the analysis for the primary endpoint (n=180).

	<i>Children (<3 years)</i>	<i>Children (3-11 years)</i>	<i>Adolescents (12-17 years)</i>	<i>Total</i>
Enrolled and analysed for safety	17	204	114	335
Analysed for efficacy (test and reference available)	12	168	93	273

All age groups were adequately represented, with more than 10 patients per year for all ages from 3 to 11 years. The number of evaluable children per centre ranged between n=2 (centre No. 2) and n=54 (centre No. 8) patients. The number of patients from centre no. 8 amounts to 30% of the total evaluable population. In order to assess whether this overrepresentation of study centre no. 8 may have biased the results, one-sided 90 % confidence intervals of sensitivity, specificity, positive predictive value and negative predictive value for centre no. 8 vs. the pooled data of the other centres were calculated. Sensitivity was above 86.6 % in centre no. 8 vs. above 91.8 % in the other centres. Thus, bias is not likely due to the overrepresentation of patients from centre No. 8.

The reasons for endoscopy were available in more than 95% of all children analysed for efficacy. The most frequent reason was abdominal pain (68%). Other frequent reasons were signs of malabsorption, failure to thrive and vomiting.

Efficacy results

Data to compare the results of the breath test with the gold standard were available for 180 children aged 3-11 years. The results in Table 2 show that *H. pylori* infection was found in 63/180 evaluable children (35%) according to the gold standard method. The youngest patients with *H. pylori* infection

were 3 years old. The results of the ^{13}C -urea breath test and the gold standard agreed in 176/180 (97.8%) of the children.

Table 3. Comparison between ^{13}C -urea breath test and gold standard in children (3-11 years)

		<i>Gold standard</i>		
		Positive	Negative	Total
^{13}C -urea breath test	Positive	61	2	63
	Negative	2	115	117
	Total	63	117	180

The results of specificity and sensitivity and the positive and negative predictive values are shown below:

Table 4. Diagnostic properties of the ^{13}C -urea breath test in children (3-11 years)

			<i>90%-CI (one-sided)</i>
Sensitivity	96.8%	(61/63)	$\geq 91.8\%$
Specificity	98.3%	(115/117)	$\geq 95.5\%$
Positive predictive value	96.8%	(61/63)	$\geq 91.8\%$
Negative predictive value	98.3%	(115/117)	$\geq 95.5\%$

Two false-positive test results were observed in children aged 3-11 years (false-positive fraction: $2/117 = 1.7\%$), both concerning 3 year olds. This result appears to confirm recently published results on the diagnostic performance of the ^{13}C -urea breath test in children indicating that the risk of false-positive test results increases with decreasing age.

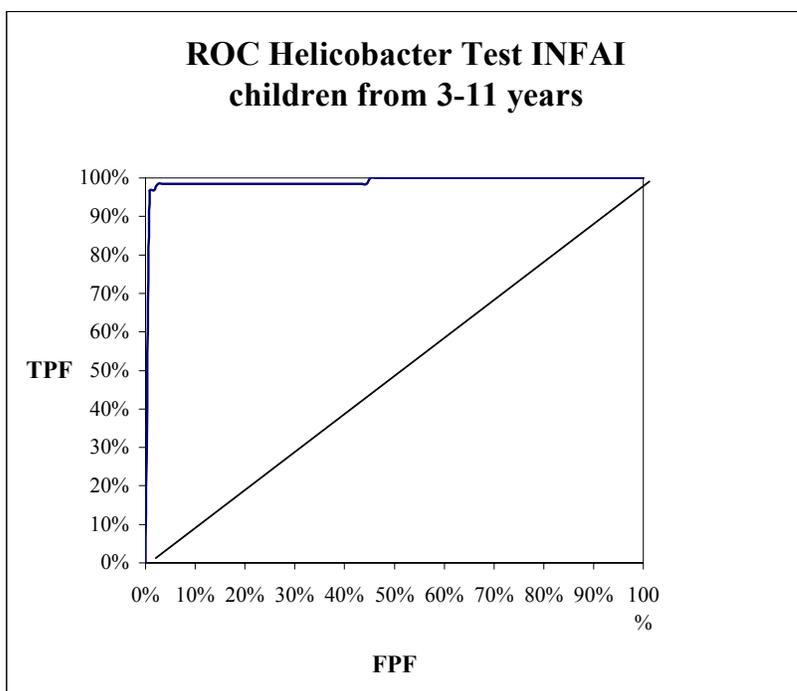
In order to better understand the clinical utility of the ^{13}C -urea breath test in children, the CPMP requested specific ^{13}C -urea breath test results on those children who had peptic ulcers or other pathological conditions at endoscopy. Peptic ulcer disease was diagnosed in 7 children, of whom 5 were *H. pylori* positive, and 2 were *H. pylori* negative. The results are shown in the table below:

***H. pylori* and peptic ulcer disease in children (3-11 years)**

		<i>Peptic ulcer disease</i>		
		Positive	Negative	Total
<i>H. pylori</i> (^{13}C -urea breath test)	Positive	5	58	63
	Negative	2	103	105
	Total	7	161	168

Thus, the prevalence of peptic ulcer disease in the trial population was 4.2% (7/168), and the diagnostic test performance parameters were: Sensitivity 71.4% (5/7), specificity 64.0% (103/161), Positive predictive value 7.9% (5/63) and negative predictive value 98.1% (103/105). The low positive predictive value is due to the very small numbers of children with peptic ulcer disease.

One of the *secondary objectives* of the study was the calculation of a receiver-operating-characteristic (ROC) curve of the ^{13}C -urea breath test. A ROC-curve allows to judge the diagnostic performance of the ^{13}C -urea breath test, including its performance at different cut-off values.



Although the applicant has not provided justification for the 4⁰/₀₀ cut-off point value, the sensitivity/specificity per cut-off point could be calculated from the data provided. Despite the small differences in performance, the table below shows that the > 4.0⁰/₀₀ cut-off point has the best test performance:

Cut-off value	True Positive	True negative
> 3.5 ⁰ / ₀₀	97%	97%
> 4.0 ⁰ / ₀₀	97%	98%
> 4.5 ⁰ / ₀₀	95%	99%
> 5.0 ⁰ / ₀₀	95%	98%

Discussion on clinical efficacy

The results of the study show that, as for adults, the INFAI breath test has a very high sensitivity and specificity with respect to the current diagnostic gold standard. This is reflected in section 5.1 of the SPC.

However, there are no risks known to be associated with the breath test itself. The test should be reserved for children who are likely to derive a benefit, such as those in which endoscopy cannot be performed or does not lead to decisive results or an eradication therapy is to be evaluated.

In order to establish an indication for a diagnostic test it is necessary to assess the diagnostic performance as well as the ability of the agent to provide useful clinical information. The assessment of clinical usefulness includes the evaluation of the impact of the use on diagnostic thinking, therapeutic decisions and clinical outcome, as laid out in the CPMP “Points to consider on the evaluation of diagnostic agents” (November 2001). In the assessment of the clinical usefulness of the urea breath test the epidemiology of *H. pylori* infection in children should be carefully considered. The prevalence of gastric colonisation and infection with *H. pylori* in European children is low, and peptic ulcers and other pathological sequelae associated with *H. pylori* in adults are rare in children. Moreover, the strength of association between *H. pylori* and peptic ulcer disease in children is less clear than in adults and criteria for the use of eradication treatment in *H. pylori* infected children have yet to be clearly established. As a result, the risk to benefit ratio of diagnostic tests and therapeutic regimens for *H. pylori* in children are different from those in adults. Indeed, indiscriminate testing in

children is not recommended, and may threaten the optimal care of this group of patients by exposing them to the risks associated with an unwarranted over prescription of subsequent antibiotic treatment. In the absence of unambiguous criteria for the use of eradication therapy, diagnostic tests should be employed judiciously and be reserved for children who are most likely to derive measurable benefit.

Recent international consensus documents on the approach to *H. pylori* infection in children state that the goal of diagnostic interventions should be to determine the cause of presenting gastrointestinal symptoms, rather than the presence of *H. pylori* infection. Thus, testing for *H. pylori* is recommended only if there is a high probability that the symptoms are due to peptic ulcer disease. If a peptic ulcer is identified during investigation by endoscopy (or upper gastrointestinal barium studies), it is reasonable to test for *H. pylori*. It should be stressed that upper gastrointestinal endoscopy with multiple biopsies remains the optimal approach to the investigation of the pediatric patient with chronic upper abdominal symptoms or suspected peptic ulcer disease. Hence, ¹³C-urea breath tests should not constitute an alternative to upper endoscopy for primary diagnosis of *H. pylori* infection in children.

In the light of the above, the test has been indicated for those children who are likely to derive a benefit, such as those in which invasive tests cannot be performed or when these tests do not lead to decisive results, or to confirm successful eradication of *H. pylori* following therapy, since breath tests are more appropriate than repeat endoscopy in this setting. This is reflected in section 4.1 of the SPC.

Clinical safety

Patient exposure

All children enrolled in the study were eligible for the safety analysis.

Adverse events and serious adverse event/deaths

According to the protocol all unexpected adverse events occurring during the study had to be documented. No adverse events were reported and the ¹³C-urea breath test was well tolerated by all patients.

Discontinuation due to adverse events

None reported

Discussion on clinical safety

No adverse events were reported in the trial and none are expected. The safety profile of this 45 mg line extension is analogous to the excellent safety profile exhibited in the adult and adolescent populations. Hence, there are no safety concerns with regard to this product.

Overall conclusions, benefit/risk assessment and recommendation

Quality

The important quality characteristics of ¹³C-urea are well defined and controlled, and the product is manufactured and controlled in a way that is characteristic of a powder for solution. The specifications and batch analytical results indicate a consistent product, and this in turn is predictive of a uniform clinical performance of the product from batch to batch. There are no unresolved quality issues that have a negative impact on the benefit/risk balance.

Efficacy

The ¹³C-urea breath test is a sensitive and specific diagnostic test to evaluate the presence or absence of *H. pylori* in children aged 3-11 years. Clinical usefulness however is only clear for limited situations.

Safety

No adverse events were reported and the ¹³C-urea breath test was well tolerated by all patients.

Conclusion & Recommendation

The diagnostic performance in children is comparable to that observed in adults and adolescents. There are no safety concerns related to the product. However, differences in epidemiology in children, as well as the strength of association between *H. pylori* infection and peptic ulcer disease in children provide for a different benefit/risk assessment in children compared to adults. This is reflected in the restrictions applied to the indication adopted by CPMP.

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensusby consensus that the benefit/risk profile of Helicobacter Test INFAI for children aged 3-11 in the diagnosis of gastroduodenal *Helicobacter pylori* infection for the evaluation of eradication regimens, or when invasive tests cannot be performed, or when there are discordant results arising from invasive tests was favourable and therefore recommended the granting of the marketing authorisation.