

Memorandum

Date: November 16, 2011 2012 FEB 28 P 2: 06
From: Fred Hines, Consumer Safety Officer, New Dietary Ingredient Review Team,
Division of Dietary Supplement Programs, Office of Nutrition, Labeling and
Dietary Supplements, HFS-810
Subject: 75-Day Premarket Notification of New Dietary Ingredients
To: Dockets Management Branch, HFA-305


Subject of the Notification: *E. coli* strain Nissle 1917

Firm: Medical Futures Inc.

Date Received by FDA: August 15, 2011

90-Day Date: November 13, 2011

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and
Cosmetic Act, the attached 75-day premarket notification and related correspondence for the
aforementioned substance should be placed on public display in docket number
FDA-1995-S-0039 as soon possible since the 90-day date is November 13, 2011. Thank
you for your assistance.


Fred A. Hines, DVM, CSO

FDA-1995-S-0039

RPT733



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
5100 Paint Branch Parkway
College Park, Maryland 20740

OCT 28 2011

Sujitha Muthuswamy
Regulatory Affairs/Quality Assurance Associate
Medical Futures Inc.
16, Sims Cres, Unit 29
Richmond Hill, Ontario
Canada - L4B2P1

Dear Sujitha Muthuswamy:

This is to inform you that the notification, dated August 8, 2011 pursuant to 21 United States Code (U.S.C.) § 350b(a)(2)(section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act)) was received and filed by the Food and Drug Administration (FDA) on August 15, 2011. Your notification concerned “*Escherichia coli* strain Nissle 1917” (*E. coli* strain Nissle 1917) which you identify as a new dietary ingredient that you intend to market in a dietary supplement product called “Mutaflor®.”

According to your notification, “Mutaflor®” is in capsule form. The recommended level of consumption will be “[a]dolescents (12-17 years old) and adults and adults (*sic*) (18 years old and over): One capsule per day during the first 4 days. Two capsules daily started on day 5.”

Under 21 U.S.C. 350b(a), the manufacturer or distributor of a dietary supplement containing a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under 21 U.S.C. 350b(a)(2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is considered to be adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

Your notification concerning “*Escherichia coli* strain Nissle 1917” does not comply with the requirements of Title 21 of the Code of Federal Regulations (CFR) 190.6 and is incomplete. Under 21 CFR 190.6(b)(3) an NDI notification must include the level of the new dietary ingredient in the dietary supplement. Your notification does not provide the level of the *E. coli* Nissle 1917 that will be present in the dietary supplement in milligrams or other appropriate units of mass. Under 21 CFR 190.6(b)(4) any reference to published information offered in support of the notification shall be accompanied by reprints or photostatic copies of such references. If any part of the material submitted in a foreign language, it shall be accompanied by an accurate and complete English translation. Your notification contained an abstract authored by Trager *et al.* that was not in English and was not accompanied by either an English translation or a reprint or photostatic copy of the publication.

In addition, FDA has examined the information in your notification and determined that your ingredient, *E. coli* Nissle 1917 is not a dietary ingredient within the meaning of 21 U.S.C 321(ff)(1) that may be lawfully used in dietary supplements. The term “dietary supplement” is defined in 21 U.S.C. 321(ff). A dietary supplement means, among other things, a “product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients: (A) a vitamin; (B) a mineral; (C) an herb or other botanical; (D) an amino acid; (E) a dietary substance for use by man to supplement the diet by increasing the total dietary intake; or (F) a concentrate, metabolite, constituent, extract, or combination of any ingredient described in clause (A), (B), (C), (D), or (E).”

While your notification contains information about the adverse event profile of Mutaflor® during clinical trials and post market surveillance, it is unclear whether the flatulence, diarrhea, and other symptoms described as intolerance reactions, while perhaps acceptable for a prescription drug being used to treat severe gastrointestinal disease, can be distinguished from the gastrointestinal symptoms associated with non-commensal *E. coli* strains. Many but not all of the genetic and phenotypic characteristics of pathogenic *E. coli* have been identified¹. Therefore, it is unclear that the information presented about *E. coli* Nissle 1917 is adequate to demonstrate that your isolate is a non-pathogenic *E. coli* that can be a dietary ingredient as opposed to a pathogenic *E. coli* that would be considered a poisonous or deleterious substance under the conditions of use recommended or suggested in the labeling of your dietary supplement. Given that *E. coli* Nissle 1917 is not a “dietary ingredient,” a dietary supplement that contains it is adulterated under the Act (sections 402(a)(1), 402(f)(1)(A), and 402(a)(3) of the Act). The introduction or delivery for introduction into interstate commerce of any food that is adulterated is prohibited (section 402(f) of the Act).

Because the information in your submission indicates that *E. coli* Nissle 1917 is not a dietary ingredient, we are providing no response with respect to whether there is an adequate basis of safety for your product of commerce under 21 U.S.C. 350b(a)(2) (section 413(a)(2) of the Act). However, please note that, under 21 C.F.R. 190.6(f), failure by FDA to respond to a notification under section 350b(a)(2) does not constitute a finding by the agency that a new dietary ingredient or the dietary supplement is safe or is not adulterated under 21 U.S.C. 342 (section 402 of the Act). Therefore, insofar as it might be argued that your product is a dietary

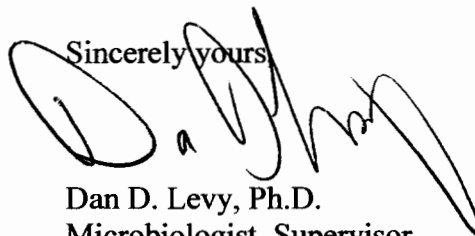
¹ J.B Kaper, J.P. Nataro and H.L.T. Mobley. Pathogenic *Escherichia coli*. Nature Reviews Microbiology 2: 123-40, 2004.

ingredient, it could be deemed to be adulterated under 21 U.S.C. 342(f)(1)(B) (section 402(f)(1)(B) of the Act). In any event, you are not prohibited from submitting a new pre-market notification for *E. coli* Nissle 1917 under 21 U.S.C. 350b(a)(2).

It is possible that a recently enacted law may affect the legal status of dietary supplements containing *E. coli* Nissle, *E. coli* Nissle 1917 or Mutaflor®. Section 301(ll) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 331(ll)) prohibits the introduction or delivery for introduction into interstate commerce of any food (including a dietary supplement) that contains a drug approved under 21 U.S.C. 355, a biological product licensed under 42 U.S.C. 262, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our review of your notification, FDA did not consider whether section 301(ll) or any of its exemptions apply to dietary supplements containing *E. coli* Nissle, *E. coli* Nissle 1917 or Mutaflor®. Accordingly, this response should not be construed to be a statement that a dietary supplement containing *E. coli* Nissle, *E. coli* Nissle 1917 or Mutaflor®, if introduced or delivered for introduction into interstate commerce, would not also violate section 301(ll).

Your notification will be kept confidential for 90 days after the filing date of August 15, 2011. After the 90-day date, the notification will be placed on public display at FDA's Docket Management Branch in docket number FDA-1995S-0039 (formerly docket number 95S-036) as new dietary ingredient notification report number 733. Prior to that date, you may wish to identify in writing specifically what information you believe is trade secret or confidential commercial information and an explanation of the basis for this belief.

If you have any questions concerning this matter please contact Dr. Fred Hines, Consumer Safety Officer, New Dietary Ingredients Review Team, at (240) 402-1756.

Sincerely yours


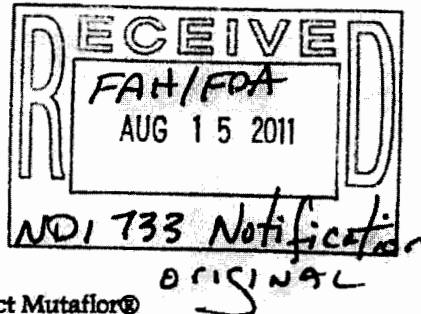
Dan D. Levy, Ph.D.
Microbiologist, Supervisor
New Dietary Ingredient Review Team
Division of Dietary Supplement Programs
Office of Nutrition, Labeling and Dietary Supplements
Center for Food Safety and Applied Nutrition



Medical Futures Inc

16 Sims Cres Unit 29
Richmond Hill ON
L4B 2P1 Canada

To: Division of standards and Labelling Regulations
Office of Nutritional Products, Labelling and dietary Supplements (HFS-820)
Centre for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD, 20740-3835



Aug 08, 2011

Sub. Pre-Market Notification for the Product Mutaflor®

Dear Sir/Madam,

Please find enclosed two copies of our Pre-Market Notification and attached studies for a New Dietary Ingredient in accordance with the code of Federal Regulations, 21 CFR 190.6

Should you have any questions or concerns, I can be reached at (905) 731-0294 x112 or via facsimile at (905) 731-2873

Sincerely,

Sujitha Muthuswamy
Regulatory Affairs/Quality Assurance Associate
Medical Futures Inc



Medical Futures Inc

16 Sims Cres Unit 29
Richmond Hill ON
L4B 2P1 Canada

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Sincerely,

Sujitha Muthuswamy
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Medical Futures Inc



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16 Sims Cres Unit 29
Richmond Hill ON
L4B 2P1 Canada

Pre-Market Notification for a New Dietary Ingredient

Date: Aug 08, 2011

To: Division of standards and Labelling Regulations
Office of Nutritional Products Labelling and dietary Supplements (HFS-820)
Centre for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park MD 20740-3835

To Whom It May Concern,

Pursuant to the Code of Federal Regulations 21CFR190.6. Medical Futures Inc. (currently operating at the address above) is issuing this premarket notification of its intent to manufacture and market a product, Mutaflor with the active ingredient, *Escherichia coli* strain Nissle 1917 This notification is to consider *Escherichia coli* strain Nissle 1917 as a new dietary ingredient We take this action with the understanding that Medical Futures Inc will not market this product in U S for a period of at least 75 days succeeding this notification and acceptance by the agency

Distributing Company Name and Address.

Medical Futures Inc
16, Sims Cres, Unit 29,
Richmond Hill, ON,
Canada - L4B2P1

Manufacturing company and address.

Ardeypharm GmbH,
Loerfeldstr. 20,
58313 Herdecke, Germany



Medical Futures Inc

16 Sims Cres Unit 29
Richmond Hill ON
L4B 2P1 Canada

Product details.

1) Active Ingredient.

Escherichia coli strain Nissle 1917

2) Non-Medicinal Ingredients.

Maltodextrin, Talc, methacrylic acid-methylmethacrylate copolymer (1:1), macrogol (4000), triethyl citrate, glycerol 85%, titanium dioxide, iron(III)-oxide, gelatine, white beeswax, carnauba wax, shellac, purified water

3) Taxonomy.

Probiotic, strain Nissle 1917 of the species *Escherichia coli* (*E.Coli*) belongs to the family of Enterobacteriaceae.

4) Quantity of Product per Unit.

1 gastro-resistant hard capsule contains *E.Coli* strain 1917 corresponding to 2.5×10^9 viable cells (CFU)

5) Recommended use.

- Helps maintain a healthy balance of "friendly" bacteria in the digestive tract or maintains the balance of the intestinal flora.
- Crowd out harmful microbes
- Protects against occasional digestive upsets
- Promotes digestive functioning and gastrointestinal health
- Keeps intestines functioning well
- Reduction of abdominal pain/cramps.

6) Recommended doses.

Adolescents (12-17 years old) and adults and adults (18 years old and over), One capsule per day during the first 4 days. Two capsules daily started on day 5.

7) Product Safety and Scientific studies.

Please see the attachment # 1

Sincerely,

Colin Campbell

CEO

Medical Futures Inc.

Product Safety and Scientific studies

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A. History.

This particular *E. coli* strain was isolated by Professor Alfred Nissle in 1917 in the First World War from the feces of a soldier who, in contrast to all of his comrades, did not develop enterocolitis in the region of Dobrudsha on the Balkan Peninsula, highly contaminated by enteropathogens at that time. From 1917, the research continued based on its potential to protect from presumably infectious gastroenteritis. Initial therapeutic success was noted in the management of gastrointestinal infectious disorders and infections affecting the urinary tract; the focus shifted later to chronic inflammatory conditions.

Mutaflor is a well tolerated product for years and the safety profile is well described in the clinical review by Schultz 2008.

B. **Safety overview including Clinical studies.**

The subject of this application, Mutaflor® capsules, is a probiotic containing *Escherichia coli* strain Nissle 1917 as the active substance which is licensed and distributed in Germany and some other European countries (see the previous marketing experience)

Besides the safety summaries presented below, this application for marketing authorization additionally integrates bibliographical data from recent experimental pre-clinical studies on *Escherichia coli* strain Nissle 1917.

The following expert report consists of a review of standard reference works and of results of pharmaco-toxicological, microbiological, and other investigations on *Escherichia coli* strain Nissle 1917, most of them published in peer-reviewed journals, which confirm that the information on the product given conforms to scientific state of the art and currently accepted practice.

The assessment of safety of *Escherichia coli* strain Nissle 1917 (EcN) is based on:

- 1) the **extensive nonclinical (toxico-pharmacological) data base**, which comprises microbiological serological, biochemical and molecular-biological studies which prove, that EcN does not bear any toxicologically relevant properties. A short summary is given below:
 - Preclinical tests for pharmacodynamic, pharmacokinetic and toxicological classification have shown that the *E. coli* strain Nissle 1917 is not absorbed, does not possess any invasive properties (TRÖGER ET AL., 2003) and is only to be encountered in the habitat provided (intestinal tract)
 - EcN possesses two small cryptic plasmids, the detection of which was accomplished with the use of standard molecular genetics methods. These plasmids are genetically stable and are not transferable to other *E. coli* strains. The DNA sequences of both plasmids are elucidated (BLUM-OEHLER ET AL., 2003)
 - EcN does not bear any transferable plasmids for antibiotic resistance or virulence factors. It is a bad recipient for transferable plasmids and for Shiga-toxin-carrying bacteriophages and is thus, according to state-of-the-art, protected against assimilating toxin-specific genes. The strain can thus be classified as genetically stable. It does not carry genes for hemolysin nor for pathogenic types of fimbriae and is sensitive to serum (GROZDANOV ET AL., 2002)
 - Strain-specific DNA sequences from both plasmids, which have not been found so far in other *E. coli* strains, can be used in a multiplex PCR method to specifically identify *E. coli* Nissle 1917 (EcN). It can be detected in culture as well as in feces samples (BLUM-OEHLER ET AL., 2003)
 - In terms of its molecular genetics, EcN is characterized by a strain-specific DNA banding pattern in pulsed-field gel electrophoresis (analysis according to OTT ET AL., 1991; ZINGLER ET AL., 1992, 1993).
 - *E. coli* strain Nissle 1917 is sensitive to sulfonamides and antibiotics, which are directed against gram-negative bacteria.
 - *E. coli* strain Nissle 1917 reacts sensitive to serum
 - *E. coli* strain Nissle 1917 shows no immunotoxic properties
 - EcN can be generally recognized as safe, because the tests described in the Expert Report on Toxicology and Pharmacology do not provide grounds for any other assessment, i.e. EcN does not produce any toxins associated with pathogenic *E. coli* strains, hemolysins, is not uropathogenic, does not possess any pathogenic adhesion factors.

- According to article 5, para. 2, with regard to the appendix I, Part B, of the German "Gentechnik-Sicherheitsverordnung (GenTSV)" [Genetic Technology Safety Act] the *E. coli* strain Nissle 1917 was allocated to the lowest risk group 1, comprising bacteria serving as acceptor organisms (cf. app. 1). According to article 7, para. 1, of the German "Gentechnikgesetz (GenTG)" [Genetic Technology Law] risk group 1 is the safest. Additionally, the German "Zentrale Kommission für Biologische Sicherheit (ZKBS)" [Central Commission for Biological Safety] considered the *E. coli* strain Nissle 1917 with respect to its nonpathogenicity and its extensive and highly sophisticated characterization options to belong to the safest risk group 1, concerning both the genetic and the functional level.

- 2) the clinical safety study "Tolerance of Mutaflor® in Healthy Volunteers (MU 9810)" which is summarized below.

Common characteristics of the study

Study design and study centers

This study was conducted as a monocentric, placebo-controlled randomized, double blind and parallel-group-study. In this study healthy volunteers were allocated to either the Mutaflor® - or the placebo-group.

Aim of the study

In preparations containing physiological intestinal bacteria, such as *E. coli* strain Nissle 1917, good compatibility is to be expected. To substantiate this, the *E. coli* strain Nissle 1917 was administered over a broad dosage spectrum in the investigation below.

Target criteria

The number of adverse events should be recorded. Furthermore, defecation frequency and stool consistency were documented. Clinically relevant alterations in laboratory values (blood count) or in various clinical parameters, such as pulse and blood pressure were recorded.

Conduct of the study

20 healthy subjects were recruited for this study, whereby 12 had been allocated randomly to the Mutaflor® group and 8 to the placebo group. Daily administration of Mutaflor® 100 mg capsules (=Mutaflor®) was carried out in increasing amounts from 1 to 9 capsules a day, whereby each dose was taken over a period of 4 days. The duration of the study was 20 days.

Results safety

Apart from one individual, non-causal rhinopharyngitis, adverse events occurred as gastrointestinal disorders. They were recorded as nausea, flatulence, diarrhea, convulsive pain in the lower abdomen as well as stomach cramps. They were all of a minor nature, with the exception of one severe case of diarrhea. All volunteers recovered from their complaints without any special measures.

A "probable" causal connection was surmised for 3 volunteers in the Mutaflor® group; a "possible" connection for one other volunteer. Here, it was a case of slight to medium-severe flatulence.

Biocompatibility in the Mutaflor® group was judged by the investigator to be *very good or good* in 83.3 % of the cases. The same judgment was given by 91.6 % of the subjects.

The groups under treatment were compared with regard to the frequency of adverse events using "Fischer's exact test". The differences between the Mutaflor® group and the placebo group were statistically not significant. Defecation frequency and stool composition likewise did not show any statistically significant differences between the groups. Relevant changes of laboratory values (blood count) or of various clinical parameters such as pulse and blood pressure, were likewise not recorded.

This clinical study did not reveal any hitherto unknown risks connected to the oral intake of Mutaflor®. No risks are entailed in administering Mutaflor® with a daily dose of up to 9 capsules Mutaflor®, which surpasses the maximum daily dose of 4 capsules, as recommended in the treatment of chronic constipation, by the factor of 2.25. The observed mild flatulence is not a clinically relevant side effect, especially since it usually disappears after an adaptation period or by reducing the daily dose.

- 3) the documentation of **adverse events (AEs), which occurred within the clinical pharmacology and efficacy studies.**

Undesirable effects attributed to the use Mutaflor® are rarely observed. Most of the adverse events attributed to Mutaflor® belong to the MedDRA system organ class (SOC)

“Gastrointestinal Disorders” The most frequently observed undesirable effect is flatulence. Its frequency can be reduced by spreading the daily dosage over the day. Alternatively, it is recommended to start with Mutaflor® mite capsules in steps with increasing doses.

Further undesirable effects belonging to the SOC “Gastrointestinal Disorders” were changes in stool consistency and/or frequency, abdominal pain, borborygm, nausea and meteorism. It should be remarked, that in patients with ulcerative colitis it is difficult to assess whether adverse events such as changes in stool consistency or frequency or abdominal pain are related to the intake of Mutaflor® or whether they are symptoms of the disease. Anyway, their frequency is classified as very rare (< 1/10 000) in the current SPCs and Package Information Leaflets (PILs) in Germany, the reference member state.

Observed undesirable effects of the SOC “Skin and Subcutaneous Tissue Disorders” were urticaria, desquamation of the skin and erythema. An AE assessed as causally related to the intake of EcN was headache, belonging to the SOC “Nervous System Disorders”. The frequency of all these undesirable effects was also classified as very rare (< 1/10 000) in the current SPCs and PILs in Germany, the reference member state.

It should be noted that the intensity of the AEs mentioned above was almost mild to moderate, thus being of minor clinical relevance.

Since particular AEs did not occur, special approaches to monitor them were not necessary.

2 deaths occurred in the study MU 9400 “Adhesive *Escherichia coli* in Inflammatory Bowel Disease – The Effect of Replacement with Non-Adhesive Organisms”. 1 patient of each treatment group (Mutaflor® vs mesalazine) died after withdrawal from the study. Both deaths were not related to the study medications. No deaths occurred in the other studies.

Other serious AEs were recorded in the study MU 9400. In both treatment groups (Mutaflor® vs mesalazine) 1 serious AE was recorded which was not related to study medications.

Serious adverse events were also recorded in study MU 9507 (Mutaflor®-group, 7 sAEs; mesalazine-group, 6 sAEs). None of these was attributable to study medications.

Concerning other significant adverse events, e.g. discontinuation of a study due to AEs or additional concomitant medication, was of no clinical relevance with regard to Mutaflor®.

In clinical studies dosage modifications were not done. Obvious clinically relevant changes or pathologic trends for the comparison between the Mutaflor® and the control groups (placebo, mesalazine, lactulose) as well as between admission and last examination could not be observed in any of the investigated laboratory parameters

Post-Marketing-Experience

The clinical safety aspects of Mutaflor® have become very well known since Mutaflor® has been permanently used over several decades since 1917 in Germany. PMS-data therefore provide important additional data for the assessment of safety, since the extent of exposure of subjects to Mutaflor® is much larger than that of clinical trials. These data are summarized below.

In summary, no regulatory actions relating to safety were taken during the period from 01/1995 to 07/2006 (data lock point)

In the time from 01/1995 up to 07/2006 about 162 220 820 capsules Mutaflor® were distributed. During this period 140 adverse events which occurred in 75 patients were reported to Ardeypharm GmbH. Depending on the prescription behaviour of the physician the daily dose of Mutaflor® can differ between 1 – 4 capsules. Therefore, the frequency of adverse events – predominately gastrointestinal disorders and skin and subcutaneous tissue disorders –, which have been reported to Ardeypharm GmbH since 1995, was set into relation to capsules sold in Austria, Czech Republic, Germany, Slovak Republic and Switzerland. The corresponding quotient (adverse events/sold capsules Mutaflor®) amounts to nearly 8.63×10^{-7} , which proves the excellent safety profile.

The results of the post marketing surveillance study "A retrospective, multicentric survey on the treatment of intestinal diseases with Mutaflor®" further support the good safety profile of Mutaflor®. Here, apart from efficacy, the compatibility of a Mutaflor®-therapy in functional as well as in inflammatory bowel disease was assessed by 167 doctors on 1.074 patients. Therapeutic indications treated were acute or chronically recurring diarrhea, chronic constipation, irritable bowel disease, ulcerative colitis and Crohn's disease. In more than 90 % of the cases, compatibility was assessed as "very good" or "good". Adverse effects, which required treatment or led to therapy being discontinued, were reported in 1.5 %. Interaction with additional or accompanying medicine did not occur. The data have been published (SCHUETZ, 1989).

In the PMS-study MU 0323, a total of 3807 adults and children with various indications were analyzed. In the subpopulations described here, 98 patients (2 adolescents, age: ≥ 12 years and < 18 years; 96 adults, ≥ 18 years) with ulcerative colitis in remission and 306 patients (10 neonates/infants/small children (age: ≤ 23 months), 75 children (age > 23 months and < 12 years), 8 adolescents (age: ≥ 12 years and < 18 years) and 213 adults) suffering from chronic constipation were analyzed. Besides the already mentioned side effects of the SOC "Gastrointestinal Disorders" no other AE were recorded.

There were no differences in the patterns of AE documented in the clinical studies. The prospective studies MU 9400 and MU 9507 are appropriately designed to be acceptable as a proof for the safety in long-term use according to the ICH-Guideline E1 ("The extent of population exposure to assess clinical safety for drugs intended for long-term treatment of non-life-threatening conditions"). When no serious AE is observed in a one-year exposure period this number of patients can provide reasonable assurance that the true cumulative one year incidence is no greater than 3%. There are no indications of intrinsic factors such as age, gender, height, weight, other illnesses and body dysfunctions influencing the safety of Mutaflor[®].

E. coli strain Nissle 1917 does not produce toxins, is not serumsensitive and not invasive. Since EcN is not systemically available embryotoxic effects can be excluded. A transfer of EcN from the intestine to body fluids such as breast milk is not to be expected. Furthermore, in the clinical studies conducted with preterm and full-term infants no indications for toxic effects were observed (CUKROWSKA ET AL., 2002; LODINOVA-ZADNIKOVA ET AL., 1992; LODINOVA-ZADNIKOVA & SONNENBORN, 1997; SCHROEDER, 1992). For the treatment of pregnant or lactating women suffering from ulcerative colitis or chronic constipation, Mutaflor[®] fills a therapeutic gap.

C. Risk Information

Safety pharmacology

E. coli strain Nissle 1917 (EcN) does not belong to any of the pathogenic types of *E. coli* bacteria. Since EcN is a normal intestinal bacterium which after oral administration is restricted to the area of the intestinal mucus layer and the gut lumen, a systemic exposure or distribution to other organs or tissues does not occur. Absorption from the gut (in the pharmacological sense) of orally administered EcN bacteria does not take place.

Hemolysin formation

Bacterial toxicity can also be detected *in vitro* studying the ability of certain *E. coli* pathotypes, e.g. UPEC strains to damage or destroy erythrocytes (hemolysis). Such kind of bacteria produces the protein toxin α -hemolysin. Therefore, strain Nissle 1917 was tested for hemolytic activity using established *in vitro* microbiological toxicity tests on special blood agar plates (Columbia blood agar, enterohemolysin agar). No hemolytic activity of EcN on blood agar plates could be detected. Thus, the *E. coli* cytotoxin α -hemolysin, characteristic of UPEC strains, is not produced by EcN.

Invasiveness

Certain *Escherichia coli* bacteria are capable of penetrating into eukaryotic cells. This feature is called invasiveness. A special colicin J5-test was used to demonstrate that the *Escherichia coli* strain Nissle 1917 is not invasive. In genuine invasion tests using HeLa cell cultures, no invasiveness of EcN into the eukaryotic cells was detected, so that *E. coli* strain Nissle 1917 can be assumed to be non-invasive. This was later verified in cell cultures with intestinal epithelial cells of the INT407 cell line (ALIENHOEFER ET AL., 2004; BOUDEAU ET AL., 2003; OELSCHLAEGER ET AL., 2001). Mainly invasion factors and other effector molecules are injected by pathogenic bacteria into eukaryotic cells with the help of type-III-secretion systems. The fact that in *E. coli* strain Nissle 1917 no type-III-specific genes were detected (GROZDANOV ET AL., 2004) corresponds to the fact that the strain is incapable of invading eukaryotic cells (ALIENHOEFER ET AL., 2004; BOUDEAU ET AL., 2003; OELSCHLAEGER ET AL., 2001).

Genetic stability, plasmid content and genome structure

A probiotic bacterium serving as the active substance of a pharmaceutical, such as *E. coli* Nissle 1917, should be genetically stable, should not function as an element of horizontal gene transfer nor contain any plasmids carrying genes for antibiotic resistances.

The methods of molecular genetics can be employed to determine whether certain bacteria represent a key position in horizontal gene transfer by readily incorporating extraneous DNA and passing it on. Experiments show that a low-level recipient behaviour of *E. coli* strain Nissle 1917 confirms its genetic stability. On the other hand, the EcN strain also does not act as a donor strain for DNA transfer to other bacteria.

E. coli Nissle 1917 contains no plasmids that can either be mobilized or transferred to other bacteria by conjugation processes. In addition, *E. coli* Nissle 1917 does not contain any bacteriophages which could be capable of transferring genes of the EcN strain to other bacteria (GROZDANOV ET AL.,

2004). In summary, it can be said that *Escherichia coli* strain Nissle 1917 shows no donor activity and only very limited recipient activity for foreign DNA. These biosafety properties predestine this *E. coli* strain for use in viable form as a microbial agent with probiotic qualities. This is especially confirmed by the designation of EcN as a strain belonging to biohazard group 1, i.e. as a strain of *Escherichia coli*, like for example the *E. coli* K-12 strain, with no features of pathogenicity (Judgement of the German ZKBS [Zentrale Kommission für Biologische Sicherheit], the German Central Commission for Biological Safety)

When the genome pattern of strain Nissle 1917 is compared with that of other pathogenic and non-pathogenic *E. coli* strains of the same serotype, no similarity to extraintestinal pathogenic O6.K5 strains can be found, which also correlates with the *in vitro* and *in vivo* findings on the non-pathogenicity of the EcN strain.

Interactions.

E. coli strain Nissle 1917 is a physiological, non-invasive bacterium which is not absorbed and the distribution of which is restricted to the gastrointestinal tract. A systemic exposure or distribution to other organs or tissues does not occur. Therefore, this section is not applicable.

Previous marketing experience

Mutaflor® is marketed as a probiotic in several European countries as shown below. Further, its under evaluation with Health Canada as a Natural Health Product.

A marketing authorisation was granted in the following countries.

Product	Country	Date of Registration	Registration Number
Mutaflor mite, Capsules (formerly Mutaflor 20 mg)	Austria	21 01 2002	1-24346
Mutaflor, Capsules (formerly Mutaflor 100 mg)	Austria	21 01 2002	1-24345
Mutaflor mite, Capsules	Czech Republic	31.07 2000	49/441/00-C
Mutaflor, Capsules	Czech Republic	31 07 2000	49/442/00-C
Mutaflor, Capsules (formerly Mutaflor 100 mg)	Germany	02.06 1978 08 06 2004	0091994/018139 6091994 00 00
Mutaflor mite, Capsules (formerly Mutaflor 20 mg)	Germany	02 06 1978 08 06 2004	0092002/018138 6091994 01 00
Mutaflor, Suspension	Germany	02 06 1978	0092019/018137
Mutaflor, Suspension	Hungary	16.04 2004	OGYI-T-9379/01-02; OGYI-T-9380/01-02
Mutaflor mite, Capsules	Hungary	16.04.2004	OGYI-T-9381/01

Mutaflor, Capsules	Hungary	16.04.2004	OGYI-T-9382/01-02
Mutaflor, Capsules	Slovakia	28.04.2000	44/0144/0-S
Mutaflor mite, Capsules	Switzerland	17.02.2005	00684
Mutaflor, Capsules	Switzerland	17.02.2005	00684

D. Conclusions

In summary, the documentation at hand shows that from immunological and microbiological as well as from pharmacological and toxicological points of view *E. coli* Nissle 1917 (Mutaflor®) is safe for humans. The pharmacokinetic properties of EcN and the documented non-pathogenicity and non-toxicity, coupled with immunomodulatory, anti-inflammatory effects, its ability to stabilize the intestinal barrier function and the capacity to antagonistically replace pathogenic bacteria in the gut provides the rational base for the good biocompatibility and efficacy of this probiotic *E. coli* strain in humans. This even allows the application of Mutaflor® during pregnancy and lactation or in patients with impaired liver or kidney function.

It can be stated that the *E. coli* strain Nissle 1917 is a naturally occurring *Escherichia coli* variant that expresses a number of vitality factors and therefore is able to efficiently colonize the intestinal ecosystem. The strain is, however, not capable of acting as a pathogen.

Since the now 90 years of clinical experience with Mutaflor® in human medicine, and because various gnotobiotic and conventional animal models had revealed no evidence whatsoever of any toxic effects of the EcN strain, it is very safe for the human gut.

E. References

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