(54) Title: INTRAVAGINAL USE OF 18-METHYL-15β,16β-METHYLENE-19-NOR-20-SPIROX-4-EN-3-ONES, INTRAVAGINAL RINGS COMPRISING 18-METHYL-15β,16β-METHYLENE-19-NOR-20-SPIROX-4-EN-3-ONES, AND USE THEREOF IN CONTRACEPTION

(57) Abstract: The present invention relates to intravaginal use of 18-methyl-15β,16β-methylene-19-nor-20-spirox-4-en-3-ones of the general formula (I), to an intravaginal ring comprising 18-methyl-15β,16β-methylene-19-nor-20-spirox-4-en-3-ones of the general formula (I) and its use in contraception.
Intravaginal use of 18-methyl-15β,16β-methylene-19-nor-20-spirox-4-en-3-ones, intravaginal rings comprising 18-methyl-15β,16β-methylene-19-nor-20-spirox-4-en-3-ones, and use thereof in contraception

The present invention relates to the subject matter characterized in the patent claims, i.e. the intravaginal use of 18-methyl-15β,16β-methylene-19-nor-20-spirox-4-en-3-ones of the general formula (I), and to an intravaginal ring comprising 18-methyl-15β,16β-methylene-19-nor-20-spirox-4-en-3-ones of the general formula I,

![General formula (I)](image)

wherein R⁶ and R⁷ are each a hydrogen atom or together are an α-methylene group.

The invention therefore relates to the intravaginal use of 18-methyl-15β,16β-methylene-19-nor-20-spirox-4-en-3-one (Compound (A)) and/or 18-methyl-6α,7α,15β,16β-dimethylene-19-nor-20-spirox-4-en-3-one (Compound (B))

![Compound (A)](image)

![Compound (B)](image)

The invention further relates to an intravaginal ring (IVR) comprising the Compound (A) and/or (B) for and its use in contraception.
Introduction

Approaches with hormonal contraceptives are widely accepted by users owing to easy application and high contraceptive reliability. Among them, oral contraception (pill) is the most frequently used contraceptive method in many countries. However, there is a demand for the development of new, long-acting reversible contraceptives (LARC) that require minimal medical guidance and patient compliance compared to oral contraceptives. New LARCs like intrauterine devices (e.g. Mirena®; EP 0652738 B1 and EP 0652737 B1) or subcutaneously placed polymer-based implants (Jadelle®) require less patient compliance compared to oral contraceptives. However they are implanted and removed by an invasive procedure. In contrast intravaginal rings can be inserted into the vagina by the user herself. They provide a continuous release of the active ingredient over a period from several weeks up to one year. Therefore intravaginal rings have been under clinical investigation over the last 45 years. Such intravaginal rings are devices made of flexible and drug-permeable silicone elastomers that release the active agent(s) to the vaginal mucosa for a certain period of time at a defined rate [for details see Brache et al. Contraception 2013; 87: 264-273; Harwood et al. Semin Reprod Med 2001; 19: 381-390]. The first studies on an IVR releasing medroxyprogesterone acetate (MPA) were published by Mishell and Lumkin in 1970 [Mishell and Lumkin. Fertil Steril 1970; 21: 99–103]. Since then, several clinical trials with IVR releasing different progestins such as progesterone, nesterone, levonorgestrel and other progestins were published (see below).

An IVR releasing a combination of ethinylestradiol and etonogestrel (NuvaRing®; EP00876815) was developed by Organon and introduced into the market in the early 1990s. However, estrogens are associated with potential risks such as a slightly increased risk of thrombosis and certain side effects such as loss of libido, nausea, and headache. Therefore, estrogen-free contraceptive methods, like progestin-only Pills (POP’s e.g. Microlut®, Camila®, and others), progestin-only intrauterine devices (e.g. Mirena®), and progestin-only IVRs (e.g. Progering®; Nestorone-ring ; LNG-ring) were developed. Contraceptive efficacy of progestin-only contraceptives like progestin-only pills (POPs) is thought to depend primarily on cervical mucus thickening, making the cervix impermeable for sperm [Roland et al. Fertil Steril 1970; 21:211-16, Katz et al. Adv Contracept 1997; 13: 143-151]. Other discussed mechanisms of action are interference with fallopian tube motility, thus inhibiting egg transport, changes in the uterine endometrium, interfering with blastocyst implantation and ovulation inhibition[Brache et al. Contraception 2013; 87: 264-273; Harwood et al. Semin Reprod Med 2001; 19: 381-390; McCann et al. Contraception 1994; 50 (6 Suppl 1):S1-195]. Nevertheless, contraceptive efficacy is lower than that of combined oral contraceptives. In contrast to estrogen/progestin combinations, contraception with continuously applied progestins are associated with irregular
bleeding and spotting regardless of whether they are applied orally in progestin-only pills or vaginally via an IVR. Irregular bleeding and spotting is the main reason for discontinuation of presently available synthetic progestin-only contraceptives which undermines the use of these valuable contraceptives.

A multicenter clinical trial with a intravaginal ring releasing 20 micrograms per day of levonorgestrel for at least 90 days was performed over 8176.74 woman-months (1005 women) in order to assess contraceptive efficacy and clinical acceptability. The one year pregnancy rate was 4.5 per 100 women (Pearl Index; PI-Index = 4.5). However, the overall discontinuation rate at 1 year was 50.3% and the most common reason for discontinuation was menstrual problems with 17.2%. [Koetsawang et al. Contraception. 1990 Feb; 41(2):105-24.]

The Progereg® (US 5869081) is a doughnut-shaped intravaginal ring with a cross-sectional diameter of 8.4 mm and an overall diameter of 58 mm which releases progesterone at an average rate of 10 mg/day over a 3-month (90-day) period. It consists of a homogeneous mixture of soft, flexible silicone elastomers and micronized progesterone. It has been developed by Population Council as a contraceptive method for lactating woman. The ring is to be used continuously and replaced every 3 months plus two weeks. IVR removal should be rare and include intercourse and ring cleaning, but re-insertion within 2 h is mandatory. The 10 mg/day progesterone release rate results in a progesterone plasma concentration of 10 nmol/L in lactating women which is sufficient to inhibit fertility during breastfeeding (10 – 25 nmol/L plasma concentration of progesterone). The contraceptive efficacy of the 10 mg/day progesterone releasing IVR was studied in several clinical trials. Overall, three of 1466 breastfeeding women became pregnant while using this method during 10,829 women-months of exposure, which is comparable to the efficacy of a copper-T intrauterine device. Although women using the progesterone releasing IVR experienced a lactational amenorrhea of approximately 6 -12 months, vaginal bleeding has been reported to be fairly common in the first 30 days after insertion of progesterone IVR [ Nath et al. Contraception. 2010 Nov;82(5):428-34.]. In a clinical trial with 187 women using the progesterone releasing ring, 68% of the women experienced bleeding and spotting in the first 30 days with a mean number of bleeding and spotting days of 8.1 +/- 4.9. In the following 5 months, more than one bleeding and spotting run per 30-day interval occurred in < 5% and prolonged bleeding lasting more than 10 days occurred in 5.4%. [Diaz et al. Contraception. 1997 Oct;56(4):223-32]. However, there are no data on the contraceptive efficacy and irregular bleeding and spotting in non-lactating woman.
Like progesterone Nestorone (NES) is systemically instable [Noe et al. Contraception. 1993 Dec;48:548-56] and was therefore tested in an IVR releasing 50 µg/day Nestorone in lactating woman. No pregnancies were observed in 555 women-months and postpartum amenorrhea was prolonged while breastfeeding performance and infant growth were not different from those in a previous comparative group of 132 TCu IUD users. The 1-year continuation of use was high (80.4%). In addition the NES-only IVR, releasing 50, 75, or 100 µg/day Nestorone was tested in 178 non-lactating women for 6 month. The 50 µg/day release rate resulted in a 6-month cumulative pregnancy rate of 0.0% and for the higher daily doses the 6-month cumulative pregnancy rate was 1.9% and 2.1%, respectively. However, menstrual disturbances were also associated with this ring, more so in the lower doses, while the higher dose had reduced bleeding.[Brache and Faundes Contraception. 2010 82(4):418-427; US 6,126,958].

In summary, progestin-only IVR are useful contraceptives in lactating women with high contraceptive efficacy and acceptable irregular bleeding pattern. However, in contrast to non-lactating woman lactating woman possess a reduced fertility and a postpartum amenorrhea and hence currently available progestin-only IVR are not suitable contraceptives for non-lactating woman.

Object of invention

It is therefore an object of the present invention to provide a progestin-only contraceptive intravaginal ring which ensures sustained local cervical mucus blockage and contraceptive efficacy in lactating and in non-lactating woman comparable to the contraceptive efficacy of oral contraceptives while systemic effects on the ovarian cycle and the endometrium are minimized, which results in reduced irregular bleeding and spotting.

Description of invention

The object of invention is achieved according to the invention by the intravaginal use of compounds of general formula (I)
wherein R₆ and R₇ are a hydrogen atom or together are an α-methylene group, namely by the intravaginal use of

Compound (A): 18-methyl-15β,16β-methylene-19-nor-20-spiro-4-en-3-one and/or
Compound (B): 18-methyl-6α,7α,15β,16β-dimethylene-19-nor-20-spiro-4-en-3-one.

(A)  
(B)

These progestins employable according to the invention (compounds according to general formula (I), compound (A), compound (B)), and preparation thereof, are described in WO 2008/000521, (EP 2038294, US 7,846,917) with compound (A) being disclosed there only as intermediate.

Furthermore, the use of Compound (A), Compound (B), and further compounds in pharmaceutical preparations for contraception and for therapeutically treatment of premenstrual complaints such as headaches, depressive moods, water retention and mastodynia is described in WO 2008/000521. WO2008/000521 discloses in addition to oral and transdermal dosage forms also parenteral oily injection solutions.

The not yet published patent application PCT/EP2013/058220 discloses the intrauterine use of Compound (A) and Compound (B) for contraception and gynecological treatment with the exception of the treatment of menorrhagia and other uterine hemorrhages, which is described in the non-prepublished patent application PCT/EP2013/058152.
However, WO 2008/000521, PCT/EP2013/058220, and PCT/EP2013/058152 do not describe an intravaginal use, nor the compounds (A) and (B) being employed in an intravaginal ring (IVR).

**Detailed description of the invention**

In a study with intravaginal rings releasing Compound (A), 18-methyl-15β,16β-methylene-19-nor-20-spiro-4-en-3-one, which was placed into the vagina of monkeys, the inventors were able to demonstrate a surprising dissociation of a local action at the cervix mucus and a systemic action as shown by inhibition of ovulation and effects on the cervix and the endometrium. Already at a release rate of 1 μg/day the swim motility of the sperm in the cervix mucus was reduced by approximately 45% and at 3 μg/day by approximately 70% (see Figure 2.2). In addition, the threshold dose for inhibition of mucus ferning was already at 1μg/d (see Figure 2.5). In contrast, no or low systemic effects were observed at 1μg/day or 3 μg/day and only at the higher release rates of 10 and 30 μg/days systemic effects were clearly detectable. The threshold dose for ovulation inhibition was 3 μg/day with an ovulation rate of 60%. Normal menstruation was also observed under IVR treatment at a daily dose of 1μg/d. Higher doses progressively abolished menstruation with a dose dependency identical to that of ovulation inhibition. The number of irregular bleeding and spotting days was unaffected at a release rate of 1 and 3 μg/d of Compound (A). In contrast, doses of 10 and 30μg/d showed a statistically not significant trend for an increased number of irregular bleeding days.

Furthermore, estrogen receptor (ERα) expression, which is a key marker for progestin effects on the endometrium, was unaltered at release rates of 1 and 3μg/d, reduced at 10μg/day, and not detectable at 30 μg/day.

Intravenous administration of 200 μg Compound (A) in postmenopausal women showed that Compound (A) is surprisingly rapidly cleared with a mean clearance of 0.685 L/h/kg, indicating that Compound (A) is systemically instable.

The marked strong dissociation of high local versus low systemic activity and the high gestagenic efficacy of the substances are sufficient for causing a contraceptive action only due to the local effects. Systemically caused side effects, such as those occurring with the use of other gestagens, may thus be prevented or at least greatly reduced. Owing to the high local gestagen concentration, a more rapid onset of contraceptive efficacy and better bleeding control can also be expected.

Owing to the properties of Compound (A) and (B), these are very well suited for intravaginal use in contraception. Preference is given here to intravaginal administration by means of an intravaginal ring.
One embodiment of the invention is intravaginal use of 18-Methyl-15β,16β-methylene-19-nor-20-spirox-4-en-3-ones of the general formula (1),

![Chemical Structure](image)

formula (1)

wherein $R^6$ and $R^7$ are each a hydrogen atom or together are an $α$-methylene group.

18-methyl-15β,16β-methylene-19-nor-20-spirox-4-en-3-one (Compound (A)) and 18-methyl-6α,7α,15β,16β-dimethylene-19-nor-20-spirox-4-en-3-one (Compound (B)) are equally preferred.

![Chemical Structures](image)

(A)  
(B)

A further embodiment of the invention is an intravaginal ring comprising Compound (A) and/or Compound (B).

The terms intravaginal, vaginal ring, and IVR are used synonymously. An intravaginal ring is a substantially ring-shaped polymeric dosage form which provides controlled release of active ingredient(s) to the vagina over extended periods of time in which absorption of the drugs takes place via circumvention of the gastrointestinal tract. The application in an IVR provides a convenient formulation with low variability in drug release rate, avoiding hepatic first-pass metabolism of the drug substance and improving treatment compliance since no daily recall of drug intake is required. In contrast, the contraceptive principle of the progestin-only pill (POP) requires an exact dosing schedule to ensure a reliable contraceptive effect. In that aspect, continuous administration with an IVR is of great advantage.
Intravaginal rings are described in several patents like US 3,545,439, EP 0 050 867, US 3,920,805A, US 4,292,965. IVR’s may comprise a polymer matrix but no membrane or wall encasing said matrix (monolithic dosage form), whereas some other IVR’s comprise a polymer matrix, also named core, encased by a membrane. The core and the membrane can be formed from the same or different biocompatible polymers. Examples of commonly used polymeric materials include, but are not limited to, polysiloxanes, polyurethanes, thermoplastic polyurethanes, ethylene/vinyl acetate copolymers (EVA), and copolymers of dimethylsiloxanes and methylvinylsiloxanes, biodegradable polymers, for example poly(hydroxyalkanoic acids), poly(lactic acids), poly(glycolic acids), poly(glycolides), poly(l-lactides), poly(lactide-co-glycolides), and a mixture of at least two of them.

The core or the membrane or both can comprise the drug substance. A number of different hormone releasing IVR are known from the literature, e.g. Progesterone® (US5,869,081) or the Nuvaring® (EP 0 876 815 B1).

According to the invention, the IVR comprises at least one compartment comprising a core, or a core encased by a membrane, said core and membrane comprising the same or different polymer composition, wherein at least one of said compartments comprises Compound (A) and/or Compound (B).

The IVR can be manufactured in accordance with standard techniques described in the art e.g. US 3,920,805, US 4,888,074, US 4,215,691, WO2010/058070. The design and manufacture of an exemplary IVR is described in example 1. Human size rings can be produced in a similar way as described in example 1. Variations can be made with respect to the surface area of the drug releasing part to adjust it to the desired dose. Adjustment of membrane thickness and material can also be needed.

According to the invention the IVR can be continuously used for at least one month up to one year, preferably for three month to six month. Continuous use includes that the IVR can be taken out intermittently for 1 up to 4 hours for e.g. cleaning or during intercourse. Alternatively according to the invention the IVR can be used in such a way that it is used during the menstruation-free phase of the respective cycle and not used during menstruation; e.g. the IVR is inserted into the vagina after the last day of the menstruation, is left into the vagina for three weeks and taken out for one week before or during menstruation.

The release of a compound from the IVR is described by the release rate. Release rate means the average amount of active drug substance released from the IVR within 24 hours that is available for
absorption by the surrounding tissue. The polymer composition of the core and/or the membrane, membrane thickness, and membrane surface area are the main parameters which influence the release rate. A person skilled in the art will know that the average release rate from IVR can decrease over the period of application. The in vitro release rate is routinely used in the art to characterize hormone-containing IVR. The release rate is determined by analyzing the released amount of a compound in vitro at 37°C in an aqueous solution of 2-HP-β-CD (2-hydroxypropyl-beta-cyclodextrin) in a shaking water bath for the intended time of use of the IVR in vivo. The in vitro release rate at each sampling time point is calculated as released amount (μg) per day (24h) [μg/day or μg/d]. The term dose and release rate are used synonymously in this patent application.

According to the invention, a long-term release IVR is used to ensure constant average release rates over several weeks or months up to one year. Long-term release IVR means any IVR suitable for administration of drugs over a prolonged period of time avoiding fluctuations of drug levels normally induced by short-term release formulations (e.g. tablets, injections, etc.).

A considerably increased potential release of active ingredients shortly after insertion (so called burst effect) of long-term release IVR is known to a person skilled in the art. IVR showing such a burst effect shortly after insertion are also considered to be claimed according to the invention even if during the duration of the burst effect the release rate is increased. Owing to the burst effect, the IVR according to the invention may achieve the desired release rates according to the invention only one, two or three days after insertion of the IVR into the vagina, in exceptional cases only after a week.

To a person skilled in the art, it is known that application of an IVR can lead to a change (decrease) in the daily release rate over the period of administration. IVR which exhibit such a change are considered to be claimed as long as sufficient compound is released.

According to the invention, the release rate of Compound (A) and (B) needs to be high enough to ensure local contraceptive efficacy but low enough to avoid systemic side effects. As shown in the monkey study, this goal is achieved at a release rate of 1 – 3 μg/day of Compound (A) (see Example 2). Taking into account the anatomical differences between monkey and human and the human pharmacokinetics of Compound (A) and (B), a release rate of 1-200 μg/day, preferably of 1-100 μg/day, and more preferably of 2-50 μg/day is suitable for application in human. Such a release rate can be achieved by choosing the appropriate parameters for the design of the membrane and core of the IVR such as polymer composition, membrane thickness, and membrane surface area.
Another embodiment is an intravaginal ring, characterized in that a daily dose of 1-200 μg, preferably of 1-100 μg, and more preferably of 2-50 μg of either Compound (A) or Compound (B) is released from the IVR.

An further embodiment of the invention is a intravaginal ring for use in contraception.

Example 1: Determination of the release rate of an IVR releasing of Compound (A) and production of the intravaginal rings for the in vivo study

For an in vivo study with cynomolgus monkeys, Compound (A)-releasing intravaginal rings adapted to the size of the cynomolgus monkeys were manufactured. The rings had an outer diameter of 14 mm and a cross-section of 2.4 mm.

The rings contained a core of compound (A) and elastomer. Said core was coated by a release-controlling membrane. The intended drug dosages were achieved by appropriate selection of the materials for the core and the membrane and by adjusting the drug concentration and the surface area of the Compound (A)-containing core in combination with the membrane thickness. Suitable selection of these parameters makes it possible to control the release of Compound (A) over periods of more than 3 weeks.

Formulations for four release rates (1μg/d, 3μg/d, 10μg/d, and 30μg/d described in table 1.1) of Compound (A)-releasing rings were produced, with each releasing Compound (A) for at least 3 weeks. Placebo rings were likewise produced. The properties and materials of these IVR are summarized in Table 1.1.
**Table 1.1:** Properties and materials of IVR releasing Compound (A) at 4 different target release rates of 1, 3, 10, 30 μg/day. PTFPMS (poly(3,3,3-trifluoropropylmethyldsiloxane)); PDMS (poly(dimethylsiloxane)); ID (inner diameter of the membrane tube); OD (outer diameter of the membrane tube).

<table>
<thead>
<tr>
<th>Target release rate</th>
<th>Membrane</th>
<th>Membrane tube size</th>
<th>Core</th>
<th>Core tube size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1μg/d</td>
<td>100% Fluorosilicone (PTFPMS)</td>
<td>Length 35mm ID 0.9mm OD 2.4mm</td>
<td>50w-% Compound (A) in PDMS</td>
<td>OD 1.1mm Length 3mm</td>
</tr>
<tr>
<td>3μg/d</td>
<td>100% Fluorosilicone (PTFPMS)</td>
<td>Length 35mm ID 1.0mm OD 2.4mm</td>
<td>50w-% Compound (A) in PDMS</td>
<td>OD 1.1mm Length 11mm</td>
</tr>
<tr>
<td>10μg/d</td>
<td>50% Fluorosilicone (PTFPMS/PDMS 50:50)</td>
<td>Length 35mm ID 1.0mm OD 2.4mm</td>
<td>50w-% Compound (A) in PDMS</td>
<td>OD 1.1mm Length 6mm</td>
</tr>
<tr>
<td>30μg/d</td>
<td>50% Fluorosilicone (PTFPMS/PDMS 50:50)</td>
<td>Length 35mm ID 1.0mm OD 2.4mm</td>
<td>50w-% Compound (A) in PDMS</td>
<td>OD 1.1mm Length 21mm</td>
</tr>
</tbody>
</table>

a) Production of Compound (A)-releasing rings

**Core**

Two core compositions were prepared, one inert core and one Compound (A) comprising core. The matrix of Compound (A) comprising core was made of silicone elastomer (polydimethylsiloxane) and the inert core was made of fluorosilicone elastomer (poly(3,3,3-trifluoropropylmethyldsiloxane)). The Compound (A)-containing core was produced by mixing micronized Compound (A) and the silicone elastomer in a mixer. The Compound (A) content of the mixture was 50% by weight. The mixture was shaped in a mold to give a small elastic rod having a thickness of 1.1 mm and cured (it would also have been possible to achieve this by extrusion through a nozzle). The fluorosilicone elastomer core was extruded to give a small elastic rod having a thickness of 1.1 mm (it would also have been possible to achieve this in a mold).

**Membrane**
Two drug-release-controlling membrane tubes were produced by tube extrusion. One membrane was made of 100% fluorosilicone and the other membrane was made of 50% fluorosilicone containing elastomer (other 50% part being silicone elastomer). The wall thickness of the tube (the membrane thickness) was about 0.7 mm.

5 Assembly of the ring

The Compound (A) core was cut into four lengths: 3 mm (1µg/d), 11 mm (3µg/d) and 6 mm (10µg/d), and 21 mm (30µg/d). In order to fill out the whole ring a 100% fluorosilicone rod as inert segment in the ring core was used. The inert fluorosilicone elastomer core was cut into appropriate lengths so that a total core length of 35 mm was achieved. The membrane tube was cut to a length of 35 mm and swollen in ethyl acetate (100% fluoromembrane) or in ethylacetate/cyclohexane (50% fluoromembrane). The ring was put together by pushing the core segment(s) into the swollen membrane tube. The tube was shaped into a ring by overlapping and gluing. A small PE piece was applied in the ring joint to give better ring shape. For closing the ring joint, a small amount of MED-6655 RTV fluorosilicone was used as glue. After evaporation of the solvents, the tube contracted and compressed the parts tightly.

b) Determination of Compound (A) release rate from the IVR

Method

The release rate of Compound A from the rings was analyzed in vitro at 37°C in a 1% aqueous solution of 2-HP-β-CD (2-hydroxypropyl-beta-cyclodextrin) in a shaking water bath (70 strokes/min). The solutions were changed daily in the first week and twice in the following weeks. The sample solutions were analyzed by UPLC, using a Waters Acquity UPLC BEH C18 1.7µm, 2.1 x 50mm column at 50°C, and acetonitrile/water (52:48) as eluent at a flow rate of 0.6 ml/min. The detection wavelength for Compound A was 243 nm. Three rings were tested in parallel. The in vitro release rate at each sampling time point is calculated as released amount (µg) per day (24h).

20 Results

The rings were tested in vitro for up to 34 days. The starting release rates were about 4 times higher than the final steady release rate which was achieved within one week. The in vitro release rate of Compound (A) is depicted in figure 1.1 and Table 1.2.
Table 1.2: In vitro release rate of Compound (A) from IVR with different target release rates

<table>
<thead>
<tr>
<th>Target release rate</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 13</th>
<th>Day 16</th>
<th>Day 20</th>
<th>Day 27</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 µg/d</td>
<td>5.9</td>
<td>3.4</td>
<td>2.4</td>
<td>1.5</td>
<td>1.4</td>
<td>1.2</td>
<td>1.2</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>3 µg/d</td>
<td>19.8</td>
<td>9.5</td>
<td>6.9</td>
<td>4.3</td>
<td>3.9</td>
<td>3.4</td>
<td>3.5</td>
<td>3.0</td>
<td>2.8</td>
</tr>
<tr>
<td>10 µg/d</td>
<td>37.0</td>
<td>17.6</td>
<td>13.9</td>
<td>11.6</td>
<td>11.4</td>
<td>11.0</td>
<td>11.3</td>
<td>10.6</td>
<td>10.3</td>
</tr>
<tr>
<td>30 µg/d</td>
<td>73.7</td>
<td>43.1</td>
<td>37.8</td>
<td>34.2</td>
<td>34.6</td>
<td>33.6</td>
<td>34.1</td>
<td>32.6</td>
<td>31.9</td>
</tr>
</tbody>
</table>

Example 2: Assessment of Compound (A) administered via an IVR in primates

*Study design*

The aim of the experiment was to characterize the local and systemic effects of Compound (A) administered via an IVR in Cynomolgus monkeys. Cynomolgus monkeys were selected as a non-human primate species because they continuously cycle throughout the year.

As parameters for the **local action** of Compound (A) sperm penetration through cervical mucus and ferning were analyzed.

10 **Sperm penetration through cervical mucus:**

Since cervical parameters (e.g. Insler score as is used in human) have not been validated for this species, the ability of sperm to penetrate samples of diluted cervical mucus, was taken as parameter for mucus thickening.

Sperm-Mucus Penetration tests were performed using the Vanguard distance (the distance from the end of the capillary tube to the furthest traveled sperm, after a set amount of time) as described by Ola Bolarinde [Ola Bolarinde Human Reproduction 2003; 18:1037-1046].

Procedure: Semen was collected from breeding sound males and preserved in Irvine Scientific Refrigeration Medium (catalog number 90129). Prior to sperm-mucus penetration test, refrigerated cervical mucus was allowed to sit at room temperature for 30 minutes. Semen was thawed in a 37°C water bath for 2 minutes and expressed in to a 15 ml polystyrene conical tube and placed in a warm room (30-32°C) where tests were performed. Cervical mucus was drawn up Micro-hematocrit capillary tubes (Fisherbrand, 22-362-574), and mucus was held in capillary tubes with a paraffine/vasoline/wax plug on the top. Capillary tubes were lowered into a conical tube containing
sperm and set for 15 minutes. Each sample was run in duplicate. Capillary tubes were analyzed under a 10x microscope, and a line was drawn where the furthest sperm was seen and the distance measured [mm]. Each test included an internal standard for cervical mucus, which consisted of the TALP (Tyrode’s Albumin-Lactate-Pyruvate )/HEPES (4-(2-hydroxyethyl)-1-piprazineethanesulfonic acid)/ BSA (bovine serum albumin) / Methyl Cellulose media. The TALP/BSA medium, described in Barry Bavister et al. [Barry Bavister, Biology of Reproduction 1983; 28:983-999], was combined with 3mg/ml bovine serum albumen (BSA). Medium control, with four percent Methly Cellulose was added to the medium to thicken the consistency and more closely resemble cervical mucus.

Ferning:

Insler scoring was performed using the guide by Oei et al. [Oei et al. European Journal of Obstetrics & Gynecology and Reproductive Biology 1997; 73:63-66]. A swab was inserted into the vagina and rolled three times across a microscope slide to evaluate ferning. Slides were analyzed the following day under 10x magnification. Ferning was scored 0= no crystallization; 1= atypical fern formation; 2=partial; typical fern formation on parts of slide; and 3= complete; typical fern formation of the whole slide.

Systemic activity was defined as antigonadotropic activity which, at appropriate doses, leads to ovulation inhibition. Parameters analyzed in order to characterize the systemic action of Compound (A) were ovulation inhibition (measured as low serum progesterone levels), effects on the endometrium, and bleeding behavior.

Ovulation inhibition

Successful ovulation was recorded by measuring serum progesterone levels, which rise a few days after ovulation when an active corpus luteum has been formed. A lack of mid-cycle progesterone rise thus indicates ovulation inhibition.

Plasma samples were assayed by imunochemiluminescence assay for estradiol (E2) and progesterone (P). The E2 and P assays were carried out on one of two Roche automatic clinical platforms (Cobas e411 and a E-170). Low limit of detection for the E2 assay was >30 pg/ml. Low limit of detection for the P assay was 0.2 ng/ml. A pool of high and low E2 and P plasma was run in every assay.

Intra-assay coefficient of variation coefficient of variation for E2 was 5.86, Inter-assay CV for E2 was 6.02. Intra-assay CV for P was 6.41, Inter-assay for P was 7.75. Ovarian cyclicity was defined as the increase in E2 in the mid cycle, followed by an increase in P (>1 ng/ml) in the secretory phase.
Ovulation was defined as having a functional luteal phase, with elevated P plus mid cycle levels of E2.

Effects on the endometrium

Needle biopsies of the endometrium were collected. To collect the biopsy, anesthesia was induced in the animals with isoflurane and the monkeys were placed in dorsal recumbency for laparoscopy. During laparoscopy, the uterus was stabilized by the broad ligament alligator forceps. A guide hole into the abdomen was made with a 16 gauge needle and fundus of the uterus was sampled with a 16 ga Tru-Cut® biopsy needle (Baxter Healthcare Corporation, Valencia, CA, USA) as described by Greb et al. [Greb RR et al. Hum Reprod 1997; 12: 1280-1292.].

Immunohistochemical analysis of estrogen receptor alpha expression

7μm cryosections were cut and mounted on Superfrost Plus microscope slides. Immunohistochemistry (IHC) of ERα was done as described by Slayden et al. [Slayden et al. Endocrinology 1995; 136(9):4012-21]. Briefly, after fixation in 2% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA) + 1.22% picric acid (Electron Microscopy Sciences, Hatfield, PA) in 0.1M phosphate buffered saline (PBS), the slides were immersed twice for 2 min each in 85% ethanol + 1.5% polyvinyl pyrrolidone (PVP, 360,000 mol wt, Sigma) at 4°C, rinsed in PBS + 1.5% PVP, and then immersed twice, 7 min each, in 0.37% glycine in PBS + 1.5% PVP. After rinsing again with PBS +1.5% PVP, the slides are immersed in 3% % hydrogen peroxide solution for 45 min, then 0.1% gelatin in PBS + PVP at 4°C. The slides were treated for 20 min with a normal horse serum, incubated with Avidin and Biotin solution (Vector Laboratories, Burlingame, CA) for 15 min respectively and then incubated overnight at 4°C with antibodies against estrogen receptor (ER; clone 1D5; Thermo Scientific; catalog no. MS-354-P0) at a 1:100 dilution, progesterone receptor (PR; Thermo Scientific; catalog no. MS-298-P) at a 1μg/mL dilution and Ki67 (Biogenex, San Ramon, CA; Catalog no. MU370-UC) at a 1:200 dilution. Following this, slides were rinsed several times, 2-3 min each, with 0.1% gelatin containing 0.075% BRIJ L23 solution (Sigma) in PBS. After washing, the sections were reincubated with normal horse serum and then with a biotinylated secondary anti-mouse antibody (Vector Laboratories, Burlingame, CA) for 30 min at 25°C. The slides were rinsed several times with 0.1% gelatin, 0.075% BRIJ L23 solution in PBS and the biotinylated antibody complexes were reacted with an avidin-biotin peroxidase kit (Vector Laboratories, Burlingame, CA).

The avidin-biotin complex was prepared by mixing equal parts of Solution A (0.01%) and Solution B (0.01%) with PBS. Following that, slides were stained with 0.025% 3,3’-diaminobenzidine (Dojindo
DAB, Wako Chemicals, Richmond, VA) in 0.038M Tris hydrochloric acid buffer (Invitrogen) and 0.03% % hydrogen peroxide (Fisher Scientific, Pittsburgh, PA). The slides were rinsed several times with deionized water, and then treated with 0.026% aqueous osmium tetroxide (Electron Microscopy Sciences, Hatfield, PA) for 1 min, rinsed several times with deionized water, postfixed for 3 min in 2% paraformaldehyde + 1.22%picric acid, dehydrated through a series of alcohols and xylenes and coverslipped with Permount (Fisher Scientific, Pittsburgh, PA).

Bleeding behavior:

Each animal was checked by vaginal swab daily for uterine bleeding. Menstruation was defined as uterine bleeding following the decline of progesterone at the end of the cycle. Tampons (2 cm long) were placed in the monkeys’ vagina beginning on the first day of menstruation, and removed and replaced three-times daily for 5 days or until regular menses ended. The tampons were stored at -20°C, then assayed for blood by alkaline haematin spectrophotometry. To do this, the tampons were dried in an 80°C vacuum oven overnight, then combined with 200 mL 5% sodium hydroxide and mixed for 1 hr with a Stomacher 400 Circulator. The resulting extract was filtered and transferred to 96 well Elisa plates. A standard curve (20-600 μl whole blood) plus internal reference standards (100 and 400 μl whole blood) were similarly digested with sodium hydroxide. Estimates of menstrual blood loss were calculated from the standard curve. Inter-assay CV was 5.6, intra assay CV was 1.4 (on whole blood).

Study design

Twenty-five adult cycling cynomolgus monkeys were used for this study. The animals were pair housed assigned to 5 groups (n=5/ group see below). Two menstrual cycles of each animal were controlled for individual cycle length, successful ovulation, sex steroid levels in serum and occurrence of menstruation. Therefore plasma was collected on cycle days 10, 21, 23, 25, 27, and 29 and analyzed for estradiol (E2) and progesterone (P) to monitor ovarian cyclicity. Only animals with normal ovulatory cycles were recruited for the study.

The study consisted of one complete treatment cycle of 28 days and an additional incomplete treatment cycle of 16 days. The aim of the second treatment cycle was to check whether ovulation/progesterone rise in the first treatment cycle was completely blocked or just delayed. That is necessary for the determination of the dose which has local effects on cervical mucus but no systemic effects.
Vaginal rings that release Compound (A) were placed in the vagina of each monkey at day 5 of the first treatment cycle. The ring was replaced by a new ring at day 10 of the second treatment cycle. Doses for the vaginal rings were as follows:

Group 1: 0 µg [placebo control]
Group 2: 1 µg Compound (A)
Group 3: 3 µg Compound (A)
Group 4: 10 µg Compound (A)
Group 5: 30 µg Compound (A)

Animals were tested daily by vaginal swab with a cotton tip to detect frank bleeding and spotting. In the two treatment cycles blood samples (4 ml) were drawn on cycle days 7-9 and continuing every 5 days through the luteal phase for drug level and progesterone/estradiol measurement. Cervix mucus was taken at day 10 of the 2nd treatment cycle, when the IVR was exchanged in order to analyze sperm motility and ferning of the cervix mucus. Endometrial biopsies were collected at the end of the study and analyzed for ERalpha-expression.

Results:

1. Systemic effects

Inhibition of ovulation is a marker for systemic activity of Compound (A). Plasma progesterone levels rises a few days after ovulation when an active corpus luteum has been formed and is therefore an indicator for successful ovulation. A daily dose of 1 µg/day of Compound (A) resulted in normal ovulation in all animals whereas higher doses dose-dependently inhibited ovulation with 30µg/d being fully effective (see figure 2.1).

Disturbance of the menstruation and irregular bleeding and spotting are a marker for systemic activity of Compound (A). All animals menstruated normally in the pretreatment cycles (open bars; see figure 2.2). Normal menstruation was also observed under IVR treatment at a daily dose of 1µg/d. Higher doses progressively abolished menstruation with a dose dependency identical to that of ovulation inhibition (figure 2.2, hatched bars). Compared to the pre-treatment cycles, the number of irregular bleeding and spotting days was unaffected at release rate of 1 and 3 µg/d of Compound (A) (see figure 2.3). In contrast, doses of 10 and 30µg/d showed a statistically not significant trend for an increased number of irregular bleeding days.
Estrogen receptor alpha (ERα) is considered to be a key marker for progestin effects on the endometrium. Immunohistochemical analysis of fine needle biopsies taken from the endometrial of Compound (A) IVR-treated animals for ERα staining is shown in figure 2.4. ERα expression was unaltered at 1 and 3μg/d, indicating no systemic activity of Compound (A). At doses of 10 and 30 μg/day ERα expression was reduced, both in zona functionalis and zona basalis showing that both doses result in systemic effects of Compound (A).

2. Local effect on the cervical mucus

Around ovulation the viscosity of the cervical mucus is reduced to support sperm penetration and motility and hence fertility. Sperm motility in the cervix mucus sampled from the treated animals was reduced by 45% already at the lowest dose of 1μg/d. An sperm motility was completely blocked already at a dose of 10μg/d Compound (A) (see figure 2.5).

Cervical mucus which supports sperm motility shows a characteristic ferning pattern which can be detected with a phase contrast microscope. As shown in figure 2.6, already at a daily release of Compound (A) of 1 μg/day no ferning pattern was detectable. As shown above, the same dose was found to inhibit sperm swim up by almost 50% (figure 2.5)

Example 3: Human pharmacokinetics

A first-in-man clinical study was conducted to investigate the pharmacokinetics of Compound A in 16 healthy postmenopausal women after intravenous administration (over 5 minutes) of 1 single dose of 200 μg Compound (A). Blood samples for pharmacokinetic analysis were collected until up to 96 hours p.a. (pre-dose, 2 min (during infusion), 5 min (end of infusion), 10, 15, 20, 30 and 40 min, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 22, 24, 28, 34, 40, 48, 58, 72, 82 and 96 hours after start of infusion). Pharmacokinetic parameters were calculated with a standard non-compartmental approach using the computer program WinNonlin (Pharsight Corporation).

Geometric mean plasma concentrations of Compound (A) following single intravenous (i.v.) infusion of 200 μg are presented in Figure 3.1. Following intravenous infusion maximum concentrations were reached at the end of the infusion. Maximum concentrations amounted to a mean value of 9.52 μg/L. Following the maximal concentration (C_max), plasma levels showed a triphasic decline with a rapid distribution phase until about 2 hours, a second phase with a slower decline up to about 12 h post dose and the terminal phase with a slow decline to a mean values of 0.0064 μg/L after 72 hours p.a.. Part of the subjects (5 out of 16) showed concentrations values close to the lower limit of quantification
(0.005 µg/L) until 96 hours p.a. The compound was eliminated with a mean terminal half-life of 36.2 hrs. The total systemic exposure amounted to an average of 4.25 µg•h/L. Compound (A) was rapidly cleared with 0.685 L/h/kg.

**Example 4: Mating study in cynomolgus monkeys**

The aim of the study is to identify the minimal release rate of Compound (A) from an IVR that blocks fertility without altering menstrual cycle. Plasma levels of Compound (A) and hormone levels are further outcomes for this experiment. The number of pregnant animals in the verum group are compared to that in the placebo control group. It is anticipated that most of the control animals becomes pregnant within 2-4 menstrual cycles.

Menstrual cycle control will be compared between pre-treatment and treatment cycles (without mating). Moreover, bleeding control will be compared between the individual dose groups and the placebo control group.

The study is performed with cynomolgous monkeys. Females are assigned to 4 groups with 10 animals per group and treated with IVR’s that release Compound (A) with the following release rates:

- **Group 1:** 0 µg (placebo control)
- **Group 2:** 1 µg/day Compound (A)
- **Group 3:** 3 µg/day Compound (A)
- **Group 4:** 30 µg/day Compound (A)

*In a pre-treatment cycle* blood levels of estradiol (E2) and progesterone (P) are measured as described in example 2 in order to determine the baseline levels for normal cycles in each of the animals. These levels are compared to levels for each animal in the treatment cycles in order to identify systemic effects of Compound (A) on ovarian cyclicity. Frequency and intensity of menstrual bleeding can vary greatly between animals. Therefore baseline levels of menstrual spotting and frank menstruation for each animal are determined in the pre-treatment cycle by daily swabbing.

*Treatment cycle without mating:* The animals are treated for 1-2 menstrual cycles without mating. On day 10 of each menstrual cycle the IVR is removed and replaced by a new IVR. Simultaneously to the exchange of the IVR’s, samples of cervical mucus are collected from all IVR treatment cycles including mating cycles and used to measure ferning and sperm motility as described in example 2.

The cervix is visualized with a speculum and the abundance of vaginal spines and gaping of the cervical os is noted. The frequency of menstrual spotting is determined by daily swabbing and compared against the baseline for each animal (determined in the pre-treatment cycle). Blood is
assayed for estradiol (E2) and progesterone (P4) to assess ovarian function (as described in example 2) and compared to baseline levels in the pre-treatment cycle. Based on the study described in example 2, the 30 µg dose is expected to block ovarian cyclicity. If this result can be confirmed in this study group the IVR is removed and the animals are taken out of the study. On day 10, when the IVR is exchanged, the animals are sedated and a sample of cervical mucus is collected in order to determine sperm motility and ferning as described in example 2.

_Treatment cycle with mating:_ Treatment is continued for 2-4 cycles. As before, the IVR is removed and replaced by a new IVR on day 10 of each menstrual cycle. Beginning on day 10 of the cycle, the females are introduced to males for 4 days. After mating, the animals are separated and the females returned to their home cages. Beginning on day 21 of the mated cycle, blood collection resumes and serum is assayed for progesterone as a rapid serological test for pregnancy. Daily swabbing continues through the beginning and then stops briefly at the time of mating to assure maximum chance for embryo implantation to take place. Swabbing for uterine bleeding and spotting resumes in order to detect implantation bleeding. Beginning on day 21, the monkeys undergo Doppler ultrasound (DUS) and contrast enhanced ultrasound (CEU). Doppler ultrasound is carried out on a GE Voluson (General Electric, Milwaukee, WI) and CEU with a Sequoia system (Siemens Medical Systems, Mountain View) every 3 days, until the animals are either: 1) defined as pregnant through the detection of a fetal heartbeat by DUS on two consecutive scans or 2) defined as not pregnant through the detection of two days of vaginal bleeding (i.e. menses) and a serum concentration of P4 <0.5 ng/ml in two consecutive blood samples. CEU also assesses placental blood flow as a sensitive marker of early pregnancy detection. Animals with confirmed pregnancies are removed from the study. Then the frequency of menstrual spotting during the first two IVR cycles is compared against the baseline for each animal.
Description of the figures:

Figure 1.1: Release rate of Compound (A) from different IVR. The release rate of Compound (A) [µg/day] in dependency of the time [days] is shown for four IVR’s with the following target release rates: diamond – 1 µg/day; square – 3 µg/day; triangle - 10 µg/day; cross – 30 µg/day.

Figure 2.1: Ovulation inhibition by Compound (A) IVR in normally cycling cynomolgus monkeys. The ovulation rate in the first treatment cycle [%] is shown in dependency of the Compound (A) release rate (1, 3, 10, 30 µg/day) from the IVR. 100% Ovulation rate means that in all animals of the group a rise in plasma progesterone level was detected.

Figure 2.2 Menstruation under Compound (A) IVR The mean days of menstruation in dependency of applied daily dose is shown. White bars showing the mean days of menstruation of the pre-treatment cycle and the hashed white bars of the first treatment cycle.

Fig. 2.3: Irregular Bleeding under Compound (A) IVR
The mean unscheduled bleeding days in dependency of applied daily dose is shown. Black bars showing the mean days of menstruation of the pre-treatment cycle and grey bars of the first treatment cycle.

Fig. 2.4: Immunohistochemical analysis of Estrogen Receptor Alpha (ERα) expression in endometrium biopsies of animals treated with Compound (A) releasing IVR. Tissue section of immunostained endometrial biopsies from the zona functionalis (upper panel) and zona basalis (lower panel) of animals treated Compound (A) IVR with increasing release rates (0 µg/day (very left row), 1 µg/day, 3 µg/day, 10 µg/day, 30 µg/day (very right row) are shown.

Fig. 2.5: Ex vivo sperm motility in diluted cervical mucus samples. The distance travelled in 10% cervical mucus [mm] in dependence of the applied daily dose of Compound (A) is shown. The shorter the distance the higher the local effect of Compound (A) on the cervical mucus.

Fig. 2.6: Microscopical analysis of cervical mucus from cynomolgus monkeys treated with Compound (A) IVR Microscopic images of cervical mucus sampled from animals which were treated with Compound (A) IVR with increasing release rates (0 µg/day (very left row), 1 µg/day, 3 µg/day, 10 µg/day, 30 µg/day (very right row). The very left image shows a typical ferning pattern of cervical mucus which supports sperm motility.
Fig. 3.1: Geometric mean plasma concentrations of Compound (A) until 72 hours post administration following single i.v. administration of 200 μg. The concentration of Compound (A) [μg/L; logarithmic scale] in dependence of time after administration [h] is shown. LLOQ means lower limit of quantification and is represented by the continuous line.
**Patent claims**

1. Intravaginal use of 18-Methyl-15β,16β-methylene-19-nor-20-spirox-4-en-3-ones of the general formula (1),

   \[
   \text{formula (1)}
   \]

   wherein \( R^6 \) and \( R^7 \) are each a hydrogen atom or together are an \( \alpha \)-methylene group.

   Intravaginal ring comprising 18-methyl-15β,16β-methylene-19-nor-20-spirox-4-en-3-one of the general formula (1)

2. Device for intravaginal release of 18-methyl-15β,16β-methylene-19-nor-20-spirox-4-en-3-one of the general formula (1)

   \[
   \text{formula (1)}
   \]

   wherein \( R^6 \) and \( R^7 \) are each a hydrogen atom or together an \( \alpha \)-methylene group.

3. Intravaginal ring comprising 18-methyl-15β,16β-methylene-19-nor-20-spirox-4-en-3-one of the general formula (1)
wherein $R^8$ and $R^7$ are each a hydrogen atom or together an $\alpha$-methylene group.

4. Intravaginal ring according to Claim 2, comprising 18-methyl-15$\beta$,16$\beta$-methylene-19-nor-20-spiro-4-en-3-one.

5. Intravaginal ring according to Claim 2, comprising 18-methyl-6$\alpha$,7$\alpha$,15$\beta$,16$\beta$-dimethylene-19-nor-20-spiro-4-en-3-one.

6. Intravaginal ring according to Claim 3, characterized in that a daily dose of 1-200 µg of 18-methyl-15$\beta$,16$\beta$-methylene-19-nor-20-spiro-4-en-3-one is released from the ring.

7. Intravaginal ring according to Claim 5, characterized in that a daily dose of 1-100 µg of 18-methyl-15$\beta$,16$\beta$-methylene-19-nor-20-spiro-4-en-3-one is released from the ring.

8. Intravaginal ring according to Claim 6, characterized in that a daily dose of 2-50 µg of 18-methyl-15$\beta$,16$\beta$-methylene-19-nor-20-spiro-4-en-3-one is released from the ring.

9. Intravaginal ring according to Claim 4, characterized in that a daily dose of 2-500 µg of 18-methyl-6$\alpha$,7$\alpha$,15$\beta$,16$\beta$-dimethylene-19-nor-20-spiro-4-en-3-one is released from the ring.
10. Intravaginal ring according to Claim 8, characterized in that a daily dose of 2-200 μg of 18-methyl-6α,7α,15β,16β-dimethylene-19-nor-20-spiroxa-4-en-3-one is released from the ring.

11. Intravaginal ring according to Claim 9, characterized in that a daily dose of 5-100 μg of 18-methyl-6α,7α,15β,16β-dimethylene-19-nor-20-spiroxa-4-en-3-one is released from the ring.

12. Intravaginal ring according to any of Claims 2-10 for use in contraception.
Figures:

Figure 1.1

[Graph showing the concentration of Compound A (µg/d) over time (days).]
Figure 2.1

![Graph showing ovulation rate during first 28 days at different doses.](image-url)
Figure 2.2:
Figure 2.3:
Figure 3.1:

Concentration of Compound (A) [µg/L] vs Time [h]

LLOQ
**INTERNATIONAL SEARCH REPORT**

**International application No**  
PCT/EP2014/072267

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K9/00  A61K31/00  A61P15/18  A61F6/05
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K  A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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**Further documents are listed in the continuation of Box C.**

**See patent family annex.**

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**Date of the actual completion of the international search**  
8 January 2015

**Date of mailing of the international search report**  
15/01/2015

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**Authorized officer**  
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