

## SCIENTIFIC DISCUSSION

### PRODUCT PROFILE

<b>Product name:</b>	INCURIN
<b>Procedure No.:</b>	EMA/V/C/047/01/0/0
<b>Applicant company :</b>	INTERVET INTERNATIONAL B.V. Wim De Körverstraat 35 5831 AN Boxmeer The Netherlands
<b>Active substances</b>	Estriol
<b>Proposed International Non-proprietary Name:</b>	Estriol
<b>Pharmaceutical form:</b>	Tablets
<b>Strength</b>	1% w/w
<b>Presentation:</b>	PVC/aluminium blisters
<b>Package size:</b>	30 tablets per blister
<b>Target species:</b>	Dogs (bitches)
<b>Withdrawal period:</b>	N/a
<b>Route of administration:</b>	Oral administration
<b>Product type:</b>	Pharmaceutical
<b>Therapeutic indication:</b>	The treatment of hormone-dependent urinary incontinence due to sphincter mechanism incompetence in ovariectomised bitches.

## **BACKGROUND INFORMATION ON THE PROCEDURE**

### **1. Submission of the dossier**

The company Intervet International submitted an application to the EMEA on 2 July 1998 for the granting of a Community marketing authorisation for Incurin in accordance with Council Regulation (EEC) No 2309/93. The application was validated on 14 July 1998.

During its meeting of 5-7 May 1998, the Committee for Veterinary Medicinal Products appointed Dr. G. Conti as Rapporteur and Dr. J. Dichtl as Co-rapporteur for the assessment of the application.

## SCIENTIFIC DISCUSSION

### 1. INTRODUCTION

Incurin contains the natural hormone estriol as active principle. It is identical to Ovestin tablets, a product for human use, which is marketed by NV Organon, a sister company of Intervet within AKZO NOBEL. Ovestin tablets 1 and 2 mg, have been marketed since 1958 and the product is registered in about 55 countries, including all EU countries, for indications related to the peri- and post-menopausal period in women. Urinary incontinence is one of these indications. Intervet has developed Incurin as a treatment for urinary incontinence resulting from sphincter mechanism incompetence (SMI) in bitches.

Estriol is an oestrogenic compound and binds to the oestrogen receptor. However, unlike other oestrogens (for instance oestradiol and ethinylestradiol), the nuclear occupancy time of estriol is short. Estriol is, therefore, a short acting oestrogen. As a consequence estriol does not show the toxic effects common to oestrogens in the dog: the development of pyometra and bone marrow suppression. This was confirmed in a 90-day safety study in dogs, with doses up to 5 times the intended maximal clinical dose of estriol. In this study no indication for the development of pyometra and bone marrow suppression was observed.

The term urinary incontinence is used to describe the condition of involuntary leakage of urine. Urinary incontinence is known to occur in women as well as in bitches. In women the incidence is highest after the menopause and in the bitch after ovariectomy. Both conditions are accompanied with a decrease of the endogenous oestrogen production. The urethral sphincter contains oestrogen receptors and oestrogens appear to be effective in the treatment of urinary incontinence. These findings strongly suggest a relationship between the incidence of urinary incontinence and oestrogen depletion. The action of oestrogens via the oestrogen receptors in the urethral sphincter is most probably the pharmacological basis for the effectiveness of oestrogens in the therapy of urinary incontinence due to sphincter mechanism incompetence (SMI).

Clinical studies showed the sensitivity of incontinent dogs to estriol to be variable and therefore a dose has to be assessed on an individual basis and dosing should not be per kg body weight but per animal. It is advised to start dosing with 1 mg/dog/day (1 tablet). In case of no or insufficient response the dose can be increased to 2 mg. In case of a good response the dose can be decreased to 0.5 mg. If one of the dosages is effective it can be attempted to give that dose every other day.

## 2. OVERVIEW OF PART II OF THE DOSSIER: ANALYTICAL ASPECTS

### II.A QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

#### 1. Composition of the veterinary medicinal product

Active Substance(s)	Grade	% w/w
Estriol	Ph.Eur.	1
<b>Other Substances</b>		
Excipients		To 100.0

#### 2. Container

The product is presented as 100 mg tablets packed in blister package (PVC/Aluminium foil) containing 30 tablets per strip. The strips are packed in cardboard boxes, which are wrapped in polypropylene film..

#### 3 Development Pharmaceutics

Formulation development has not been described in detail and the inclusion of each of the constituents has not been discussed. The formulation used is identical to the formulation of Ovestin, a product for human use on the market for over 40 years. The development of Ovestin/Incurin therefore dates to the late sixties, when it was not customary to perform an extensive development pharmaceutics program.

### II.B METHOD OF PREPARATION

The method of manufacture consists of the mixing of estriol with a previously prepared mixture (granulate) of excipients. The obtained granulate is compressed to tablets using a tableting machine, and subsequently packed mechanically into the push-through strips and placed inside the cardboard boxes. A flow-sheet diagram detailing the whole process is presented.

Validation of the production process was not performed at the time of registration of Ovestin (Incurin). Retrospective validation on 7 recent batches (1997) has been performed on the following parameters: (a)content uniformity ; (b)dissolution rates in water; (c) tablet mass; (d) disintegration time

The data presented show that the specifications set for the dissolution testing are complied with. Nevertheless, according to the available data, a lower dissolution time could be foreseen. In order to be able to evaluate correctly the data, intermediate results were presented (45 min, 60 min). The analytical method used for such determination has now been validated according to ICH guidelines and is considered acceptable.

## II.C. CONTROL OF STARTING MATERIALS

### 1. Active Ingredient

INN:	Estriol
Chemical name:	Estra-1,3,5(10)-triene-3,16,17-triol,(16 $\alpha$ ,17 $\beta$ )-
CAS number:	50-27-1
Classification:	Oestrogenic hormone
Physical form:	White to almost white crystals or powder
Structural formula:	see below
Molecular formula:	C <sub>18</sub> H <sub>24</sub> O <sub>3</sub>
Molecular weight:	288.39
Degree of impurity:	Typical lots of estriol contain >97 % estriol. The remaining materials consist principally of processing solvents, ash and synthesis-related impurities.
Impurities:	Related substances (total) 2 % maximum Solvents 0.1 % maximum Ash 0.1 % maximum
Loss on drying	0.5% maximum
Physical properties:	
Melting point:	Approximately 280°C
Boiling point:	N/A
Vapour pressure:	N/A
Solubility in water:	practically insoluble
Solubility in organic solvents:	ethanol (100%): 0.2% v/v acetone:soluble chloroform:soluble dioxane:soluble diethyl ether:soluble fixed oils:soluble
Density:	N/A
Refractive index:	N/A
Rotation:	+54 to +62 ° calculated on dry substance
Pharmacological group:	Estriol is a hormone belonging to the oestrogen group

An Applicant's part of the Drug Master File was included in the dossier and the full Drug Master file was made available to the Rapporteur, Co-Rapporteur and the EMEA Secretariat.

Estriol is produced from estrone in a multi step synthesis. Only the last step is described, consisting of a reduction with sodium borohydride in methanol. Estriol is purified by treatment with charcoal in methanol and crystallisation from methanol. The structure is proved by IR, proton and carbon NMR, UV, and MS. All spectra are presented both pictorially and in tabular form and the interpretations presented are consistent with the proposed structure.

The reference standard used for the TLC and HPLC analysis is a working standard (batch 003224) assayed in comparison to the USP reference standard; for all other analyses the EP reference standard is used. The method of characterisation of the reference standard batch is described.

The specifications of estriol comply with the specifications as described in the EP for estriol. They include as additional requirements the methanol content (estriol) and the particle size (estriol micronised).

Analytical methods comply with the analytical methods as described in EP, except for the HPLC method for the assay of estriol, the TLC method for identity, purity and related substances, the GLC method for methanol content and the microscopic particle size determination. These four methods are described and the first three are validated.

Data on impurities are derived from the analysis of 4 batches.

Batch analysis data were presented, showing the conformity of 5 batches to the EP. Data also include as additional requirements the results on the methanol content (estriol) and on the particle size (estriol micronised).

## **2. Other Ingredients**

The other ingredients are adequately controlled either by the application of pharmacopoeial monograph requirements or suitable manufacturer's specifications for which full details are provided of the analytical procedures and relevant validation data. Certificates of analysis are also included.

## **I 3. Packaging**

The specifications and routine tests for the packaging materials have been provided. The tablets are packed in push-through strips (PVC/Aluminium foil) containing 30 tablets per strip. The strips are packed in cardboard boxes. The boxes are wrapped in polypropylene film

## **II.D. CONTROL TESTS AT INTERMEDIATE STAGES OF THE MANUFACTURING PROCESS**

Not applicable.

## **II.E. CONTROL TEST ON THE FINISHED PRODUCT**

### **1. Specifications and routine tests**

The finished product specifications include tests for average tablet mass, uniformity of mass, uniformity of content, disintegration time, microbiological contamination. The release limits for the estriol content are set at  $\pm 5\%$  nominal, whereas limits of  $-8$  to  $+5\%$  nominal are applied throughout the shelf-life.

An isocratic, reversed phase HPLC procedure with UV detection is used to identify and assay estriol. Validation data have been provided and the identity of estriol is also checked by means of a satisfactorily validated TLC method.

All other specifications cited above are checked according to methods described in Ph. Eur. Batch analysis data are provided for 3 production batches and the test for uniformity of content was performed on two additional batches.

The friability was tested for 3 recent batches according to Ph.Eur. 1997:2.9.7. 20 tablets per batch were used and the results provided. The range of 25 – 70 N was considered too wide and therefore the Applicant has provided a commitment that the specifications for hardness will be reconsidered based on the outcome of the study into the improvement of the breakability of the tablets, which will be performed.

An HPLC method for the determination of the active compound and degradation products was developed and validated. The method and validation were presented. Degradation products are determined.

## **II.F. STABILITY**

### **1. Stability Tests on the Active Ingredient**

Data on the stability of commercial scale batches were presented. Three batches have been stored at temperature conditions between 15 and 25°C. The Committee considered that the Applicant had provided sufficient data to justify a retest period of 3 years for the active ingredient, especially given the long experience of production.

### **2. Stability Tests on the Finished Product**

The following parameters are checked throughout shelf-life in order to meet the specifications: characters, disintegration time, dissolution rate, hardness, estriol assay, content of related substances. Analytical methods either conform to Ph. Eur. or are the same described earlier in the dossier. The containers used are the same which will be marketed. Stability tests have been performed in the dark and under the following conditions:

- (1) 4°C ambient relative humidity (RH)
- (2) room temperature (15-25°C)
- (3) 25°C 55% RH
- (4) 30°C 45% RH
- (5) 30°C 75% RH

The assay results of the batches used for long term studies, having different storage times, have been analysed statistically by linear regression analyses.

## **3. OVERVIEW OF PART III OF THE DOSSIER: TOXICOLOGICAL AND PHARMACOLOGICAL ASPECTS**

### **III.A SAFETY**

#### **3.1 Pharmacodynamics**

Urinary incontinence is known to occur in bitches and urethral sphincter mechanism incompetence is commonly and usually associated with ovariectomy. Several studies have demonstrated the relationship between the incidence of urinary incontinence and oestrogen depletion. Repeated administration (three months) of 1 mg estriol per dog produced beneficial effects in bitches showing involuntary leakage of urine.

Oestrogens exert their pharmacological effect via the interaction with specific oestrogen receptors in target tissues. In spite of similar cellular mechanisms and receptor affinity, estriol binds the nuclear-bound receptors for a shorter period of time than oestradiol. The possible reasons are the relatively rapid dissociation rate of estriol from the receptor, the rapid dissociation of the estriol receptor complexes from their nuclear binding sites and the rapid elimination of oestradiol from the body. Consequently, estriol may be considered a short-acting oestrogen rather than a weak oestrogen and this may explain the lack of a full uterotrophic effect. However, estriol is able to elicit a full vaginotrophic response after short-term nuclear occupation.

Oestrogen receptors are also present in the female lower urinary tract and this is most probably the pharmacological basis for the effectiveness of oestrogens in the therapy of urinary incontinence. The efficacy of estriol in the therapy of urinary incontinence, observed in woman, probably means that short term nuclear occupation apparently induces, as in the vagina, a full response on the urethral sphincter. A study performed on 9 ovariectomised bitches suggests, on the basis of the urodynamic parameters, that Incurin may improve urethral closure and bladder storage dysfunction in dogs with

urinary incontinence by increasing the functional urethral length and the bladder threshold pressure and decreasing bladder compliance.

### **3.2 Pharmacokinetics**

A single dose study using a high oral administration of 4 mg estriol (one case) or 6 mg estriol (11 cases) was performed.

A multiple dose study using the oral administration of the maximal intended clinical dose of 2 mg for 7 days was conducted in 6 bitches.

A multiple dose study using the oral administration of 2 mg for 7 days was also performed in 9 ovariectomised bitches.

No data regarding distribution and metabolism in the dog are available.

#### *(a) Absorption*

Single and multiple dose pharmacokinetic studies demonstrate that, after oral administration, estriol is rapidly absorbed in the bitch. The maximum plasma concentration ( $C_{max}$ ) are reached within 1 hour. The multiple dose study performed at the highest dose proposed (2 mg/dog/7 days), shows that steady state is reached rapidly and no accumulation occurs. Over the range of 2 to 6 mg/dog kinetics are linear.

#### *(b) Distribution*

The whole-body autoradiograph study (performed in mice) did not show any radioactivity accumulation in organs or tissues. Most radioactivity was found in the gall-bladder and gastrointestinal tract, thus demonstrating the extensive entero-hepatic circulation and urinary elimination.

#### *(c) Metabolism and excretion*

Biotransformation data in the dog are not available. However estriol metabolism has been studied in humans, where oxidation of estriol to 16-oxo-estriol takes place. The metabolite is then converted to 16 $\beta$ -hydroxy-estriol (16-epi-estriol), which can further conjugate with glucuronate or sulphate.

The autoradiographic study performed in mice indicated a rapid entero-hepatic recirculation and urinary elimination (high intestinal and bladder level of radioactivity 30 minutes after subcutaneous administration). Entero-hepatic recirculation has been demonstrated in the dog by multiple dose kinetic study.

### **3.3 Toxicology**

Oestrogens are quite well tolerated drugs, however some general effects related to their endocrine activity and some other effects in the dog may be observed.

Among general effects observable in all mammalian species, the induction of vulva and mammae swelling and vaginal discharge may be considered. These effects may be normally present during the proestrus in the bitch. Other side effects, specifically observed in the dog, may be the induction of pyometra and bone marrow suppression. Pyometra may be caused by long-term hormonal imbalance in dogs and the exposition to exogenous oestrogens may induce such endocrine alteration. Incurin is indicated for use in ovariectomised bitches only.



The dog is very sensitive to bone marrow suppression produced by oestrogen, showing a transient early leucocytosis followed by leucopenia and associated with severe thrombocytopenia and decrease in erythrocyte number. Bone marrow suppression has been observed as a long-term effect produced by high dosages of oestrogens in the dog. Because of the short-acting properties of estriol, a single therapeutic daily dose of estriol does not cause severe side effects such as bone marrow suppression. In rodents and rabbits high doses of oestrogens may induce embryonic death or rib abnormalities.

### **3.3.1 Single dose toxicity**

Two acute toxicity trials have been performed in rats and LD<sub>50</sub> after oral administration was >2 g/Kg, thus suggesting that the acute toxicity of estriol is low.

### **3.3.2 Repeat Dose Toxicity**

A 13-week target animal safety study has been performed using different dosages of estriol (2, 6 and 10 mg/dog/day, respectively). Dose dependent clinical signs (swelling of the vulva and vaginal discharge) were observed and dose dependent post-mortem changes were histologically found in the uterus, ovaries and mammary glands. No signs of pyometra was observed in treated animals. Regarding haematology, a mild and transient increase in mean leucocyte number was observed in the dogs treated with the higher dosage. Histological examination, performed after 13 weeks of treatment, did not reveal any pathological pattern.

A further study, in which ovariectomised bitches were treated with 1 mg of Incurin/dog/day, has been performed. No haematological parameter alterations and clinical signs (with the exclusion of swelling of the vulva and mammae) have been observed.

### **3.3.3 Tolerance in the target species**

see Part IV.

### **3.3.4 Reproductive toxicity (inc. teratogenicity)**

#### *(d) Reproduction studies*

Specific studies on the possible effects of estriol on fertility have not been carried out as Incurin is an estriol formulation intended for the treatment of urinary incontinence, a condition only occurring in ovariectomised bitches.

#### *(e) Embryotoxicity, foetotoxicity and teratogenicity*

For the above mentioned reasons, specific studies on embryotoxicity, foetotoxicity and teratogenicity have not been performed in the target species. However, data generated in laboratory animals were available in the literature.

The oral administration of doses up to 1 mg of estriol/Kg/day from day 6 to day 15 of pregnancy did not induce embryonic mortality and teratogenic effects in rats. In this study, rib abnormalities have been observed at 2 mg of estriol/Kg/day (2/144 fetuses) and at higher dosages.

In a second study the EDL<sub>50</sub> (50% embryoletality dose) for several oestrogens was measured in rats. For oestradiol, ethynylestradiol, oestrone and estriol the EDL<sub>50</sub> was found to be 0.5, 0.8, 1.0 and 4.5 mg/Kg/day, respectively.

Embryoletality has been also determined in the hamster and the EDL<sub>50</sub> for oestradiol, ethynylestradiol, oestrone and estriol resulted to be 1.0, 2.5, 4.0, and 30 mg/Kg/day, respectively.

### 3.3.5 Mutagenicity

Estriol has been assayed in the micronucleus test in rats and in the Salmonella/mammalian-microsome mutagenicity test (Ames test). In these tests estriol did not show mutagenic activity.

### 3.3.6 Carcinogenicity

Estriol is an oestrogen with certain properties which are different from those of other oestrogens, in particular oestradiol-17 $\beta$ . In his review Bergink (1980) links these differences with the pharmacodynamics (i.e. receptor interactions) and pharmacokinetics (protein binding and metabolism) of these oestrogens.

In rats, a single daily dose of estriol has been found to be fully oestrogenic on the short-term oestrogenic responses, e.g. on uterine water uptake, and on early protein synthesis, but only weakly oestrogenic on the long-term responses, such as uterine growth. At the same time, estriol is an antagonist of the long-term effects of other oestrogens; when administered in conjunction with oestradiol-17 $\beta$ , it has been found to partially inhibit the oestradiol-17 $\beta$ -induced growth of the uterus of ovariectomized rats.

Unlike oestradiol-17 $\beta$ , estriol only binds very weakly to plasma proteins, and can therefore easily be metabolized (conjugated). Oestradiol-17 $\beta$  is, due to its good plasma protein binding, much slower metabolized. The consequence of this difference is that plasma levels of oestradiol-17 $\beta$  will be maintained much longer than those of estriol. This explains partly the short-acting character of estriol.

With respect to the dynamics of receptor interaction there are marked differences between oestradiol-17 $\beta$  and estriol. To exert oestrogenic activity, oestrogens combine with the receptor after entering the cell and this oestrogen-receptor complex is then transported into the cell nucleus where it elicits a response which depends on the length of time it remains functionally bound within the nucleus: the longer the nuclear effect the stronger the biological effect. In vitro, the rate of the formation of the estriol receptor complex is similar to that of oestradiol-17 $\beta$  receptor complex, but the former dissociates far more rapidly. In vivo, in ovariectomized rats, the nuclear oestrogen receptor complex, resulting from a single administration of oestradiol-17 $\beta$  (0.1  $\mu$ g) is still detectable after 12 hours, whereas the complex formed after administration of estriol (1  $\mu$ g) is only detectable for 6 hours. This is too short to permit long term oestrogenic effects such as proliferation of endometrial cells.

These pharmacodynamic and pharmacokinetics findings, explain why the biological action of estriol is shorter than that of oestradiol-17 $\beta$ . Estriol also competes with oestradiol-17 $\beta$  for the oestrogen receptor, and thus prevents oestradiol-17 $\beta$  from manifesting its full biological potential. In other words estriol can under certain circumstances even act as an oestrogen antagonist.

Lemon (1980) reviewed literature in which the estriol/oestradiol-17 $\beta$  + oestrone ratios were linked to the risk of breast cancer. The conclusion is that there is an inverse relationship between this ratio and the risk: the higher the ratio the lower the risk. Lemon (1980) also describes studies in which carcinogenic promoting potential of estriol in rats is investigated and compared with that of other oestrogens among which oestradiol-17 $\beta$ . In life-time studies intact female rats were fed dimethylbenzanthracene (DMBA), a compound which is known to induce breast carcinoma. The animals received every 1-2 months an implant with an oestrogen.

Estriol showed a clear and significant reduction of the frequency and incidence rate of the DMBA-induced breast carcinoma. Oestradiol-17 $\beta$  and oestrone showed no effect. Similar effects were observed with procarbazine as tumor inducer.

These studies show that estriol has an “anti-mammary carcinogenic activity” in rats treated with a carcinoma-inducing agent. This is probably due to the fact that, due to its short acting character, estriol does not stimulate breast cell proliferation and competes for the oestrogen receptor with the endogenous oestrogens like oestradiol-17 $\beta$ , showing its anti-oestrogenic effect.

The results presented above allow the conclusion that estriol has no or only very low carcinoma-promoting potential compared to oestradiol-17 $\beta$ .

### **3.4 Studies of other effects**

It is well known that oestrogens may affect the immune system, however, since estriol may be considered a short acting oestrogen, this effect should not be relevant. In fact, during the 13 week safety study in dogs, no myelotoxic effects and lymph node, thymus and spleen alterations were recorded.

### **3.5 Ecotoxicity**

A Phase I assessment has been presented. It was concluded that environmental exposure would be insignificant as the product is indicated for use in companion animals only and, in accordance with CVMP guidelines (EMEA/CVMP/055/96 FINAL), no further testing is required.

### **3.6 User Safety**

No particular precautions have to be taken in the handling of the product.

## **III.B RESIDUES**

The application is for a non-food producing species, and therefore residue documentation is not applicable.

## **4. OVERVIEW OF PART IV OF THE DOSSIER: CLINICAL ASPECTS**

### **4.1- Pre-clinical studies:**

#### **4.1.1. Pharmacology – see Part III for**

##### **4.1.1.1 Pharmacodynamics: - see Part III**

##### **4.1.1.2 Pharmacokinetics – see Part III**

#### **4.1.2 Tolerance in the target species**

A 13 week target animal safety study was performed using Incurin at the dosages of 2, 6, and 10 mg estriol/dog/day, which is equivalent with 1, 3 and 5 fold the maximum intended clinical dose. Dose dependent oestrogenic effects (swollen vulva and vaginal discharge), associated with gross and histologic alterations in the uterus, ovaries and mammary glands, were observed.

Special attention has been paid to possible haematological alteration and bone marrow suppression. The only modification observed was a transient increase of leukocyte number in the group of dogs treated with 10 mg of estriol/day. The effect was most pronounced during the first 3 weeks of exposure. Histologic examination of bone marrow (performed after 13 weeks of treatment) revealed the absence of pathologic changes. Thrombocytes and erythrocytes were normal throughout the experimental period.

Also in the clinical trials safety aspects have been checked and no effects of bone marrow suppression were recorded.

Safety studies demonstrated that the prolonged administration (13 weeks) of daily doses up to 5 fold higher than that claimed are well tolerated in the dog. Clinical evaluation, gross pathology, and histological examination revealed minor alterations ascribable to endocrine effects. No case of pyometra was observed. No clinical signs or histological alterations indicating bone marrow suppression were recorded. Reproductive toxicity tests, performed in laboratory animals, indicated that estriol is less embryotoxic than other oestrogens. Moreover, Incurin is claimed for use in ovariectomised bitches and is contraindicated during pregnancy. Estriol did not display any mutagenic activity.

No information is available about the influence of liver/kidney dysfunctions on the biotransformation/elimination of estriol, possibly leading to significant modifications in the pharmacokinetics of the molecule and consequent over-effect. As individual dosing is required for this product, it is possible to compensate for any over-effects.

#### **4.1.3 Resistance**

Not applicable.

### **4.2 Clinical studies**

In order to evaluate the efficacy of Incurin in the treatment of incontinence due to sphincter mechanism incompetence in the bitch, the Applicant has performed 3 clinical trials under field conditions. The first trial has been considered a pilot trial, whereas the other 2 trials have been performed according to the EU note for guidance III/3767/92. In all trials no control animals, negative (placebo) or positive (reference product) have been used. The approach has been justified on the basis of clinical and ethical reasons and by the absence of an appropriate reference product. In the clinical trials particular attention has been paid to safety aspects.

## **Field Trials**

1. The first clinical trial was performed in Australia using 22 bitches affected by urinary incontinence. Eleven bitches out of 22 were ovariectomised, while for the remaining 11 no indications were presented. Most dogs were treated with 0.5 or 1 mg estriol/day or on alternating days. One bitch was occasionally administered with 2 mg estriol/day. Seventeen bitches responded positively to estriol treatment. Three of the non-responsive dogs became seriously ill (liver tumour, heart failure, renal failure). This trial was not well conducted and considered as a pilot experiment. However, the efficacy of estriol in the therapy of urinary incontinence has been shown.

2. The second clinical trial was performed in The Netherlands using 20 ovariectomised bitches and was set up as a dose-schedule finding study. The treatment was started with a dose of 0.5 mg estriol/dog/day and if inadequate response was obtained the dose was augmented to 1 or 2 mg. When adequate clinical response was obtained the dose was administered on alternating days or every third day. In 13 cases a complete recovery was obtained, whereas in 2 cases a marked improvement was observed. The final effective dose was in most positive cases of 1 or 2 mg. In 6 cases the bitches were treated daily, in 2 cases on alternating days and in 7 cases every third day. No relationship between dose and body weight resulted from the experiment, by contrast it should be noted that the dosage must be adjusted individually. If initially low doses are administered, in many cases failures or poor results are obtained and only after increasing the dose an effective clinical response may be obtained.

3. The third trial, performed in The Netherlands, Belgium, France and Germany, was started using the dosage of 2 mg estriol/dog/day. This experiment had the advantage that it was possible to observe if the dog responded to the treatment already after 1 week of exposure. Of the 133 bitches used in the trial 96% had been ovariectomised. Restoration of continence or improvements were observed in 83.5% of the dogs and 82% of the owners were satisfied by the treatment. At the end of the experiment the therapeutic dose was 0.5 mg (35% of the cases), 1 mg (35%) and 2 mg (27%); 65% of the bitches were treated daily and 35% on alternate days. No relationship between dose and body weight was observed.

During the trial 2 and 3 safety aspects were accurately checked. The clinical status of the animals has been monitored and blood samples were collected in order to evaluate possible signs of bone marrow suppression.

Two types of clinical side effects have been observed:

- effects associated with the hormonal activity of estriol (swollen vulva and mammary gland, changed behavior, attractiveness to males), which mostly appeared at the higher dose (2 mg) and disappeared after the reduction of the dose.
- occasionally, in a limited number of cases, gastrointestinal disturbances have been observed.

No signs of bone marrow suppression were observed.

Results obtained in the clinical trials demonstrated that the product is effective and safe when used against urinary incontinence due to sphincter mechanism incompetence. The majority of the bitches responded positively to estriol treatment. In the 2 most important trials 121 out of 153 treated bitches showed a positive response that could be classified as excellent, good or sufficient. In the third trial, 82% of the owner were satisfied with the treatment.

Clinical investigation shows that there is no apparent relationship between the final effective dosage and body weight. Consequently, it should be concluded that it is inappropriate to use a fixed dose per kg of body weight. The effective clinical dose should be titrated individually for each animal. On the basis of the responses recorded in the clinical trials it seems appropriate to start the therapeutic approach with a mean dose of 1 mg/dog/day, when positive response is obtained the dose may be reduced to 0.5 mg/dog/day, whereas if the response is negative or insufficient the dose may be increased up to

2 mg/dog/day. Whenever an effective dosage is obtained, estriol may be administered on alternate days.

Although this therapeutic regimen had not been used in the clinical trials, it may be assumed that this approach may give the best results in the presence of minimal side effects.

As regards untoward effects 2 types of response were recorded:

- side effects related to physiological oestrogen activity (sexual and behavioural effects); they are shown by the 9% of the bitches treated with the highest dose of estriol (2 mg). They are reversible after the reduction of the dose.
- gastrointestinal effects were occasionally observed in 9% of the dogs. These effects were more evident in the bitches initially treated with 2 mg/day.

The hematological parameter examination did not reveal any sign of bone marrow suppression. No cases of pyometra were recorded.

In a 13-week oral dose safety study in intact Beagle dogs, bleeding from the vagina occurred in rare cases.

Data reported in the present dossier indicate that the product is safe and effective for the treatment of urinary incontinence in ovariectomised dogs.

A report was also presented of dogs from the last field trial which have been kept on the Incurin treatment after the end of the official trial period. Forty-eight dogs have been treated after cessation of the trial. The duration of treatment ranged from 14 days up to 1198 days. In most cases the duration was between 1 to 3 years.

At the time Intervet sent out a questionnaire to the veterinarians involved in the field trial, twenty-one dogs were still being treated. All dogs were still continent. Twenty-seven dogs had ended the treatment at the time the questionnaire was sent out. The cessation of treatment was for various reasons in most cases not related to the treatment. In 3 cases the dogs became incontinent again. These data show that the efficacy of Incurin in the long term is good.

## **5. RISK-BENEFIT ASSESSMENT AND CONCLUSION**

Urinary incontinence is known to occur in bitches and the urethral sphincter mechanism incompetence is commonly and usually associated with ovariectomy. Several studies have demonstrated the relationship between the incidence of urinary incontinence and oestrogen depletion. Repeated administration (three months) of 1 mg estriol per dog produced beneficial effects in bitches showing involuntary leakage of urine. Clinical studies showed the sensitivity of incontinent dogs to estriol to be variable and therefore a dose has to be assessed on an individual basis and dosing should not be per kg body weight but per animal.

A minority of 2 Members of the Committee considered these data to be inconclusive, because in their opinion there was a lack of proven efficacy and safety data in the documentation. Therefore, these Members expressed divergent positions which are appended to this report.

Based on the original and complementary data presented, the majority of Members of the Committee for Veterinary Medicinal Products concluded that the quality, the safety and the efficacy of the product were considered to be in accordance with the requirements of Council Directive 81/852/EEC and supported the claims agreed with the Applicant.

Consequently, the majority of Members of the Committee recommended on 8 December 1999 that the product could be recommended for the granting of a Community marketing authorisation.