



The European Agency for the Evaluation of Medicinal Products  
*Evaluation of Medicines for Human Use*

London, 1 March 2001  
CPMP/SWP/2145/00

**COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS  
(CPMP)**

**NOTE FOR GUIDANCE ON NON-CLINICAL LOCAL TOLERANCE  
TESTING OF MEDICINAL PRODUCTS**

<b>DISCUSSION IN THE SAFETY WORKING PARTY (SWP)</b>	November 1999/ June 2000
<b>TRANSMISSION TO THE CPMP</b>	September 2000
<b>RELEASE FOR CONSULTATION</b>	September 2000
<b>DEADLINE FOR COMMENTS</b>	December 2000
<b>DISCUSSION IN THE SWP</b>	February 2001
<b>ADOPTION BY CPMP</b>	February 2001
<b>DATE FOR COMING INTO OPERATION</b>	February 2001

This Note for guidance (3BS12a), first adopted in 1990, has been revised. The testing of sensitising potential has been updated. Under dermal tolerance testing, reference is made to Chapter B.40. of the Annex V to Directive 67/548/EEC, which has recently been updated with Commission Directive 2000/33/EC of 25 April 2000. Guidance on phototoxicity and photosensitisation has been separated to form a *Note for guidance on Photosafety testing*, which is under preparation by the CPMP Safety Working Party.

# NOTE FOR GUIDANCE ON NON-CLINICAL LOCAL TOLERANCE TESTING OF MEDICINAL PRODUCTS

## 1. INTRODUCTION

The evaluation of local tolerance should be performed in laboratory experiments prior to human exposure to the product. The purpose of these studies is to ascertain whether medicinal products (both active substances and excipients) are tolerated at sites in the body, which may come into contact with the product as a result of its administration in clinical use. The testing strategy should be such that any mechanical effects of administration or purely physico-chemical actions of the product can be distinguished from toxicological or pharmacodynamic ones.

General considerations as described in the Council Directive on Animal Welfare 86/609/EEC and Council Decision on the European Convention on the Protection of Vertebrate Animals apply. Studies on animals can be substituted by validated *in vitro* tests as described in the CPMP position paper "Replacement of animal studies by *in vitro* models" CPMP/SWP/728/95. Consideration should be given to developments in alternative testing methods.

## 2. GENERAL CONSIDERATIONS WITH REGARD TO LOCAL TOLERANCE TESTING

### 2.1 Tolerance testing at the site of administration

Tolerance should be determined at those sites which come into contact with the medicinal product as a result of the method of administration, and also at those sites which might come into contact through accidental or unavoidable exposure to the product. The site of administration can be the same organ or tissue which is intended to be the therapeutic target (e.g. the skin for externally administered dermatological products, the eye for ophthalmic medicinal products), or the site of administration can be remote from the intended therapeutic target (e.g. transdermal patches, intravenously administered medicinal products).

In planning local tolerance testing the following information should be taken into consideration:

- a) the physico-chemical properties of the product in its intended formulation. For example, to avoid unnecessary suffering, products which are corrosive by nature of their pH value should be excluded from studies in live animals;
- b) the pharmacodynamic, toxicological and pharmacokinetic data for the active substance, or combination of active substances, and the excipients.

It should be noted that local tolerance testing may be part of other toxicity studies provided the medicinal product is administered under adequate conditions. Local tolerance testing should be conducted according to state of the art guidelines.

### 2.2 Systemic toxicity testing

In cases where the medicinal product has not been tested previously, studies for the evaluation of general toxicity will usually have to be conducted (according to the appropriate notes for guidance). These studies should employ a route of administration which results in adequate systemic exposure. The testing should include the necessary investigations to reveal signs of toxicity at target organs. Generally, no further studies for the evaluation of systemic toxicity will be required in circumstances where:

- a) absorption of the product can be demonstrated to be so low that the possibility of

systemic effects can effectively be ruled out;

- b) the product is absorbed, but its systemic toxicity has previously been adequately investigated.

### **3. POINTS TO CONSIDER IN THE DESIGN OF LOCAL TOLERANCE TESTS**

#### **3.1 Choice of species**

The choice of species will depend upon the problem to be investigated and the test model considered appropriate. Usually, only one species should be evaluated for each type of test. Where two or more toxicological endpoints are being investigated (e.g. ocular tolerance and skin sensitisation), species appropriate to the test will need to be used.

#### **3.2 Frequency and duration of administration**

The frequency and duration of administration to animals should be determined by the proposed conditions of administration in clinical use. However, the application period should generally not exceed four weeks. Investigation of local tolerance after accidental administration may be performed by single dose studies.

#### **3.3 Reversibility**

An evaluation of the reversibility of local lesions should be included where relevant.

#### **3.4 Preparation to be tested**

Local tolerance testing should be conducted with the preparation being developed for use in man, using the vehicle and/or excipients in treating the control group(s). Positive controls/reference substances may be included where necessary.

#### **3.5 Choice of dose**

As a rule, the actual concentration of active substances to be used in humans should be tested. The dose may then be adjusted by varying the frequency of administration. Other regimens are discussed in the sections pertaining to the individual routes of administration.

#### **3.6 Animal welfare**

In case of strong irritancy in less sensitive testing models, the progress to more sensitive models should be carefully considered. Care should also be taken to minimise painful exposure of animals to irritants by terminating the experiments at the point where severe adverse reactions are seen and the continuation is not expected to provide results essential for risk assessment.

#### **3.7 Route of administration**

The route of administration in the test model has to be selected according to the envisaged administration route for humans (see section 2.1). The anatomy and physiology of the application site in the selected test model have to be taken into consideration while selecting dose levels (section 3.5) and frequency of administration (section 3.2). Testing different routes of administration in the same animal is permissible as long as the tests do not influence each other, and inasmuch as systemic tolerance permits. Contralateral administration of the control preparation is acceptable when the study is not compromised by so doing.

#### **3.8 Evaluation of results**

Global evaluation of results should include a discussion on the adequacy of the design of the local tolerance test and on the significance of the findings for the clinical use of the product.

## **4. TESTING PROCEDURES FOR PARTICULAR ROUTES OF ADMINISTRATION**

Guidance on testing procedures by common routes of administration such as ocular and dermal routes is given in sections 4.1 and 4.2. Guidance for routes of administration specific to medicinal products, such as parenteral, rectal and vaginal administration, is given in sections 4.3, 4.4 and 4.5. For all those routes not yet mentioned (e.g. ear, nose, mouth, respiratory tract, joints) the General Consideration (section 2) and the Points to Consider (section 3) should be adequately applied.

### **4.1 Ocular tolerance testing**

#### **4.1.1 Testing strategy**

The type and extent of ocular tolerance testing will be determined by the context in which the eyes are exposed to the product (e.g. products which are intended to be repeatedly administered to the eye will obviously require more extensive testing than those for which accidental exposure may occur).

The evaluation of ocular tolerance for medicinal products intended for administration to the eye requires:

- a) an ocular tolerance test by a single administration (usually conducted in rabbits). Tests for the detection of anaesthetising properties of the administration compound should be included.
- b) a repeated dose ocular tolerance test (usually conducted in rabbits with daily administration for four weeks).

In both types of experiments, investigations of the different tissues in contact with the product as well as of the lens, the vitreous body and the ocular fundus should be included. The evaluation of ocular tolerance is also necessary for products which are not intended to be administered to the eye, but which might reasonably be expected to result in exposure during the course of their normal clinical use (e.g. lotions or gels used for the treatment of the skin of the face, medicinal shampoos, etc.). In these cases an ocular tolerance test using a single administration should be performed.

#### **4.1.2 Single dose ocular tolerance test**

One eye serves as the target to be treated, the other as control. The areas surrounding the eyes, including the lids, conjunctiva, nictitating membrane, cornea and iris, should also be examined during the test. The eyes should be examined for at least 72 hours after administration. The investigator should conclude the test with a suitable evaluation of the reactions observed. The benefits of flushing out the eye with physiological saline after administration should be included in the evaluation where relevant. The type and extent of investigations performed should be made on an individual case by case basis.

#### **4.1.3 Repeated dose ocular tolerance test**

The design of this test should take into account the results from the single dose ocular tolerance test. Histological examination should be considered on a case by case basis.

### **4.2 Dermal tolerance testing**

#### **4.2.1 Testing strategy**

The evaluation of dermal tolerance for products intended for administration to the skin requires a dermal tolerance test by a single administration, a repeated dose dermal tolerance test, an evaluation of sensitising potential and, in some cases, an evaluation of the phototoxicity and photosensitisation potential. Unintentional application of the product to

other sites of the body in clinical use (e.g. eyes) should also be considered. Medicinal products applied to the skin in order to obtain systemic effects and new vehicles should be tested in a similar manner to the above. As a general rule, the formulation to be used clinically should be used in all tests. If the dose is to be varied (e.g. determination of systemic toxicity by dermal administration), this should be achieved by altering the amount of the product applied and/or by changing the area of administration, since modifications in the concentration or vehicle may lead to non-proportional changes in absorption and local tolerance. Whether or not occlusive dressings are employed depends on the intended clinical use of the product.

#### **4.2.2 Single dose dermal tolerance test**

Skin corrosion can be tested in vitro as described in Chapter B.40. of the Annex V to Directive 67/548/EEC. Irritancy tests are generally performed in the rabbit, on shaved intact skin and on an equivalent area of shaved and abraded skin. It should, however, be noted that abrasion can lead to an oversensitive model, and that the need to use it should be evaluated on a case by case basis. Vehicle controls should be included. The skin is examined to evaluate the degree of erythema, oedema, desquamation, scab formation and any other lesions. The duration of the study will depend on the changes observed at 24, 48 and 72 hours after administration. If the changes persist, observation may in some cases be necessary for up to 8 days after administration.

#### **4.2.3 Repeated dose dermal tolerance test**

This test is usually conducted in rabbits on shaved intact and abraded skin, if possible for periods of up to four weeks. Controls are treated with the vehicle alone. Other animal species may be used if adequately validated. The skin should be evaluated as in section 4.2.2. Histological examination should be considered on a case by case basis.

Consideration should be given to the type and amount of any decomposition products produced. Where appropriate these products should be characterised and evaluated separately.

### **4.3 Parenteral tolerance testing**

Parenteral tolerance testing includes intravenous, intra-arterial, intramuscular, intrathecal, subcutaneous and paravenous routes.

#### **4.3.1 Testing animals**

The selection of the species should be justified.

#### **4.3.2 Application sites**

According to the route

- a) suitable veins of the ear, the tail or the front of hind limbs;
- b) central artery of the ear in rabbits, femoral arteries or other suitable arteries in other species;
- c) dorsal or femoral muscles;
- d) subcutaneous tissue of the lateral chest wall or other suitable application sites;
- e) paravenous tissue near one of the suitable veins mentioned under item 4.3.2 (a).

#### **4.3.3 Injection frequency**

Single administration. In some cases, repeated administration (7 days) can be of interest. Comparison with controls (vehicle) where necessary.

#### **4.3.4 Injection volume and rate**

The injection volume and rate of administration should be correctly adjusted to the animal species.

#### **4.3.5 Observations**

##### ***4.3.5.1 Single administration***

Repeated observations of the animals and the injection sites should be performed during 48-96 hours after administration. After this delay, a careful macroscopic examination of the injection sites and surrounding tissues is carried out. A histological examination should be carried out, if necessary for diagnosis. Reversibility studies and additional investigations may be required case by case.

##### ***4.3.5.2 Repeated administration***

Repeated observations of the injection sites and animals should be performed. After the last administration the procedure should be followed as outlined under 4.3.5.1.

#### **4.4 Rectal tolerance testing**

##### **4.4.1 Testing animals**

The selection of the species should be justified; most commonly used are rabbits or dogs.

##### **4.4.2 Application volume**

The envisaged human therapeutic dose volume of the galenic formulation or the maximum applicable volume for the animal species.

##### **4.4.3 Administration frequency**

Usually once or twice per day for at least 7 days, according to the clinical use.

##### **4.4.4 Observations**

Observation of the anal region and anal sphincter, clinical signs (e.g. sliding on the back-side, signs of pain) and faeces (e.g. blood, mucus). Sacrifice of animals after administration, necropsy and macroscopic examination of the rectal mucosa. Histological examination if required for diagnosis.

#### **4.5. Vaginal tolerance testing**

##### **4.5.1 Experimental animals**

The selection of species should be justified; most commonly used are rats, rabbits or dogs.

##### **4.5.2 Application volume**

The envisaged therapeutic dose volume of the galenic formulation or the maximum applicable volume for the animal species.

##### **4.5.3 Administration frequency**

Usually once or twice per day for at least 7 days, according to the clinical use.

##### **4.5.4 Observations**

Observation of the vaginal region, clinical signs (e.g. sliding on the back-side, signs of pain) and vaginal secretion (e.g. blood, mucus). If animals are sacrificed after administration, necropsy and macroscopic examination of the vaginal tract and of the reproduction system should be performed. Histological examination if required for diagnosis. Additional investigations (e.g. effect on cervical mucus, spermicidal action) should be considered case by case.

## **5. SENSITISING POTENTIAL**

For chemicals applied to skin (dermal, rectal or vaginal) the sensitising potential should be evaluated. Currently two test systems are available for the evaluation of sensitising potential, i.e. the guinea pig assay and the local lymph node assay. Evaluation of sensitising potential should be carried out in at least one of these test systems. (The physical chemical properties of a compound should be the rationale for the choice of the assay, for example: hydrophilic compounds, metal salts and metals should preferably be tested in the guinea pig assay.)

### **5.1 Guinea pig assay**

Guidance on the test protocols for the guinea pig assay can be found in the EEC/OECD guideline for testing of chemicals no 406.

### **5.2 Local lymph node assay**

The Local Lymph Node Assay should be conducted in female mice (8-12 weeks old) of the CBA/Ca or CBA/J strain. The test substance should be dissolved in an appropriate solvent or vehicle. The maximum concentration tested should be the highest achievable level avoiding overt systemic toxicity and excessive local irritation. Positive and negative controls are to be included in each test. Lymph nodes should be collected and pooled per animal. The results for each treatment group are expressed as the mean stimulation index (SI). In addition to an assessment of the magnitude of the SI, a statistical analysis should be conducted which includes an assessment of the dose-response relationship as well as pairwise dosed group versus concurrent solvent/vehicle concurrent control comparisons.

For detailed guidance on the test protocol requirements for the LLNA, see note 1.

#### **Note 1.**

The test protocol requirements for LLNA assay are described in the ICCVAM/NICEATM report: "The Murine Local Lymph node assay: A test method for assessing the allergic contact dermatitis potential of chemicals/compounds" (NIH Publication No: 99-4494). An example of a test protocol is included in the pages J-1 to J-10". The use of non-radioisotope endpoint assays assessing cell proliferation may be acceptable provided they give reproducible and reliable results. The addition of functional parameters, which may provide valuable mechanistic information, is optional.