The Interim Director General

Maisons-Alfort, 31 March 2016

OPINION

of the French Agency for Food, Environmental and Occupational Health & Safety

on the "Request concerning extension of the list of substances with additional clinical benefit to other substances of interest in equine medicine"

ANSES undertakes independent and pluralistic scientific expert assessments.

ANSES's public health mission involves ensuring environmental, occupational and food safety as well as assessing the potential health risks they may entail.

It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with the necessary information concerning these risks as well as the requisite expertise and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are made public.

This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 31 March 2016 shall prevail.

ANSES received a request on 12 May 2015 from the Directorate General for Food to carry out an expert appraisal on the extension of the list of substances with additional clinical benefit to other substances of interest in equine medicine.

1. BACKGROUND AND PURPOSE OF THE REQUEST

The background of this request concerns the aim of increasing the number of compounds included in the list of essential substances and with additional clinical benefit as defined in Commission Regulation (EC) No 1950/2006[1] in order to widen the therapeutic armamentarium to treat Equidae producing meat and other products intended for consumption by humans. This regulation enables Equidae producing meat and other products intended for human consumption to be treated with certain essential substances for the treatment of Equidae and with additional clinical benefit, even when maximum residue limits have not been determined. A withdrawal period of 6 months must then be observed before the foodstuffs produced from the treated animals can be made available for human consumption.

The Directorate General for Food sent a request asking ANSES to evaluate whether complying with a withdrawal period of 6 months after the last administration of one of the substances listed in

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[1] Commission Regulation (EC) No 1950/2006 of 13 December 2006 establishing, in accordance with Directive 2001/82/EC of the European Parliament and of the Council on the Community code relating to veterinary medicinal products, a list of substances essential for the treatment of Equidae

French Agency for Food, Environmental and Occupational Health & Safety,
14 rue Pierre et Marie Curie, 94701 Maisons-Alfort Cedex
Telephone: + 33 (0)1 49 77 13 50 - Fax: + 33 (0)1 49 77 26 26 - www.anses.fr

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the annex to the request, for the given therapeutic indications and dosages, constitutes a satisfactory measure guaranteeing the absence of health risk for consumers. The request lists 17 substances that are to be assessed.

Since the withdrawal period of 6 months is incompatible with withdrawal periods for milk, the response to the request will only concern withdrawal periods for 'meat and offal'.
In addition, the response to the request will not deal with the clinical benefit of these substances, nor with the usefulness of adding new antibiotics to the list of essential substances for the treatment of Equidae.

2. EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)" by experts from ANSES-ANMV.
The collective expert appraisal was carried out by the Expert Committee (CES) on Veterinary medicinal products.
ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal.
The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS

Commission Regulation (EC) No 1950/2006 of 13 December 2006 establishing, in accordance with Directive 2001/82/EC of the European Parliament and of the Council on the Community code relating to veterinary medicinal products, a list of substances essential and with additional clinical benefit for the treatment of Equidae, enables treatment of Equidae intended for human consumption with substances for which no maximum residue limits (MRLs) have been determined. Treatment of horses with these agents is possible when no veterinary medicinal product containing the active substance is authorised for Equidae, or when no medicinal product used as part of the "therapeutic cascade" (Article L. 5143-4 of the Public Health Code) would provide equally satisfactory clinical results in terms of treatment.

Article 4 of this Regulation excludes from its scope any substance included in the lists in Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Any substance for which an MRL has been established and that is included in Table 1 of Regulation (EU) No 37/2010, or any substance that is prohibited and included in Table 2, is removed from the list of essential substances for the treatment of Equidae and with additional clinical benefit.

Among the substances listed in the request, eight are already included in Table 1 of Regulation (EU) No 37/2010:

- **Lidocaine,**
- **Prednisolone,**
- **Atropine,**
- **Erythromycin,**

- **Acetyl cysteine,**
- **Gentamicin,**
- **Framycetin / Neomycin.**

An assessment of the risks for consumers related to potential residues of these substances in foodstuffs from treated animals has already been carried out and will not be addressed again as part of this expert appraisal. When these substances are used to treat Equidae, either a veterinary medicinal product has been authorised for the equine species with a specific withdrawal period defined in the marketing authorisation, or the veterinarian applies a standard withdrawal period as provided for in Article L. 5143-4 (known as the "therapeutic cascade").

Moreover, three other substances are used in medicinal products for horses not intended for human consumption in application of the exemption provided for in Article 6(3) of Directive 2001/82/EC on the Community code relating to veterinary medicinal products. Pergolide, thenoic acid and phenylbutazone are contained in veterinary medicinal products approved for Equidae not intended for human consumption. These three substances are not included in the tables of Regulation (EU) No 37/2010.

The assessment carried out as part of this request deals with substances that do not have an MRL status, i.e. the following nine substances: phenylbutazone, thenoic acid as a lithium salt, pergolide, croton oil, tetracaine, tetryzoline, rifamycin, synephrine and polymyxin B. For the last five substances (tetracaine, tetryzoline, rifamycin, synephrine and polymyxin B), the assessment will focus only on the risks for consumers related to their administration in animals via the ocular route. This is because arguments in favour of additional clinical benefit are associated with ocular use. Regulation (EC) No 1950/2006 can restrict the conditions of use of substances, specifically the routes of administration.

■ **METHODOLOGY**

The aim is not to establish MRL but rather to characterise consumer exposure 6 months after veterinary treatment and compare exposure with a toxicity reference value (TRV).

Exposure of consumers is evaluated based on available concentrations observed in tissue for consumption or, if these data are not available, on the basis of plasma concentrations. In some cases, when there are no data for tissues or plasma, an overall approach to exposure is adopted whereby we considered that the entire amount administered is found in the animal.

Regulation (EC) No 854/2004 (Annex I, Section II, Chapter V) excludes the liver and kidneys of Equidae over 2 years of age from human consumption due to accumulation of toxins in these two tissues. Consumption of Equidae under 2 years of age is infrequent in France.

If there are no ADME data available (absorption, distribution, metabolism, elimination) and/or no bioavailability data on the substance of interest in humans, it is assumed that the drug is distributed to all parts of the animal after absorption.

For these calculations, we considered consumption of 300 g of muscle (see daily food basket in the MRL note for guidance) and estimated the weight of the horse to be 600 kg.

When available, NOAEL, LOAEL, and DNEL[2] values from experimental studies were retained as the TRV. The approach known as the threshold of toxicological concern (TTC) was adopted to define an exposure dose for substances that have not been studied that will not lead to a significant toxic or carcinogenic effect.

The TTC value was established as 1.5 µg/person/day, in particular for people whose exposure to this substance is related to an expected therapeutic benefit (ICH Guideline M7 establishing threshold values for genotoxic impurities). To assess the risk to consumers for whom no benefit is expected in relation to exposure to this residue, the value of 0.15 µg/person/day was considered appropriate (addition of a factor of 10 to take into account this absence of expected benefit). This approach was followed in particular by the EMA's Committee for Veterinary Medicinal Products (CVMP) in a recent opinion concerning the potential risk to the consumer related to lidocaine residues in foodstuffs of animal origin (CVMP Assessment report regarding the request for an opinion under Article 30(3) of Regulation (EC) No 726/2004).

In the absence of TRVs defined in studies, the principle underlying this assessment was therefore to use the value of 0.15 µg/person/day associated with a scientific rationale on the risk related to the amount of potential residue remaining 6 months after treatment.

If the exposure value obtained after treatment was higher than the TRV or the TTC, the time needed to reach this value (TRV or TTC) was estimated using the elimination half-life ($t\frac{1}{2}$) and the equation $t = (t\frac{1}{2} / \ln 2)(\ln X0 - \ln X)$, where $X0$ is the initial amount and X the amount to reach.

When the elimination half-life had not been clearly established in the equine species, calculations were carried out using the hypothesis of elimination half-lives of 10 days and 24 hours, assuming a long half-life and a short half-life, respectively.

- **ASSESSMENT OF SUBSTANCES**

  - **PHENYLBUTAZONE**

Phenylbutazone is a non-steroidal anti-inflammatory belonging to the pyrazole family and also has analgesic and antipyretic properties.
It acts by inhibiting the production of prostaglandins which are involved in pain, inflammation, and fever.
Its main metabolite, oxyphenbutazone, has similar pharmacological properties.

  o Toxicology

Phenylbutazone is very commonly used in equine medicine. It is indicated for the treatment of locomotor disorders, joint pain, muscle pain, bone pain, and limping. Administration pre-operatively (4.4 mg/kg IV) and post-operatively (2 mg/kg/day IV for 2.5 days) can alleviate post-operative pain. Phenylbutazone has a relatively small margin of safety. Toxic effects may occur at a dose twice the therapeutic dose. The incidence of these effects increases with the dose, treatment duration, and degree of dehydration of the treated animal. The toxic effects of phenylbutazone are not always related to the serum concentration and many individual variations are observed.

In terms of veterinary medicinal products with MAs in France, phenylbutazone is approved for use in horses (not intended for human consumption) via the oral route to treat chronic laminitis at a

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[2] NOAEL = No-observed-adverse-effect level
LOAEL = Lowest-observed-adverse-affect level
DNEL = Derived-no-effect level

dose of 4 mg/kg (morning and evening of the first day) followed by 2 mg/kg (from day 2 to day 5, morning and evening), then 1 mg/kg (from day 6 to day 9 as a single dose).

In humans, from a plasma concentration of between 50 and 150 µg/mL, signs of toxicity including hypersensitivity reactions, diarrhoea, vomiting, sweating, mucosal ulcerations, hepatitis, nephritis, and anaemia may occur.

In a joint statement dated 15 April 2013, the EMA and EFSA presented a conclusion on the risks related to the presence of phenylbutazone residues in horse meat in a context of fraudulent practices, i.e. for consumption with no withdrawal period after administration of the substance.
The statement emphasised that given the identified risks for consumers concerning idiosyncratic blood dyscrasia and the genotoxic and carcinogenic potential, for which no thresholds have been established, a maximum residue limit could not be set by the CVMP, and as a result, the substance cannot be used in animals intended for human consumption.
In addition, the opinion issued by the two agencies based on exposure data collected in national control plans indicated that the carcinogenic risk for the consumer was very low and that the risk for an individual of developing aplastic anaemia and being exposed to phenylbutazone was less than 1 in 1 million.

Phenylbutazone cannot be classified in terms of its carcinogenic potential in humans (Group 3 in the IARC classification (International Agency for Research on Cancer) (IPCS INCHEM, 1987).

   o Plasma pharmacokinetics (Lees & Toutain, 2013) (Smith et al, 1987) (Mills et al, 1996) (Tobin et al, 1986) (Toutain et al, 1994)

In horses, phenylbutazone is generally well absorbed following oral administration and has bioavailability of about 69-91%. However, absorption can be decreased and delayed by food intake. A single Tmax[3] is observed at around 4-6 hours when phenylbutazone is administered in fasting conditions. When free access to food is provided, the first plasma peak is observed at around 1-2 hours, then a second at around 10-24 hours. The bioavailability is lower when the animal is not fasting (longer Tmax and lower Cmax).
The plasma protein binding of phenylbutazone is very high at 98%, corresponding to a low volume of distribution (0.1-0.3 L/kg).
29.4% of phenylbutazone in the body is located in the plasma, 45.5% in other extra-cellular fluids, and the remaining 25% in the rest of the body. It is therefore unlikely that high tissue concentrations will be found in the horse.
Two metabolites have non-negligible pharmacological effects: oxyphenbutazone and γ-hydroxyphenylbutazone.

Given the plasma elimination half-life value of 4-6 hours in the horse, accumulation in plasma is unlikely. However, accumulation has been observed in horses following oral administration of a 5 mg/kg live weight (kg LW) dose every 12 hours.
The elimination half-life is much longer in cattle (65 hours).

   o Depletion (Lees & Toutain, 2013) (Mills et al 1996)

Given these pharmacokinetic parameters, it unlikely that high phenylbutazone concentrations would be observed in tissue. The plasma:tissue ratio is very high. It appear to be approx. 10:1 between plasma and muscle. Tissue concentrations of phenylbutazone are observed mainly in the kidneys and the liver, then the muscle.

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[3] Tmax: time at which maximum concentration (Cmax) is observed

The various studies show that plasma concentrations are proportional to tissue concentrations. Monitoring plasma concentrations is a good way of predicting tissue concentrations taking account of the proportionality.

Following administration of phenylbutazone by the <u>intravenous route</u> at a high dose of 8.8 mg/kg LW in six horses, plasma concentrations found at 24, 48, 72 and 96 hours were respectively 1.00 to 4.00, 0.10 to 0.30, 0.02 to 0.09, and 0.005 to 0.03 µg/mL.
Considering a plasma:muscle ratio of 10:1 and an ingested amount of muscle of 300 g, the maximum amount of phenylbutazone ingested would be 120, 9, 2.7, and 0.9 µg at each time-point (See graph "PBZ plasma concentration (IV)" below with concentration in ng/mL on the y-axis and time in days on the x-axis).
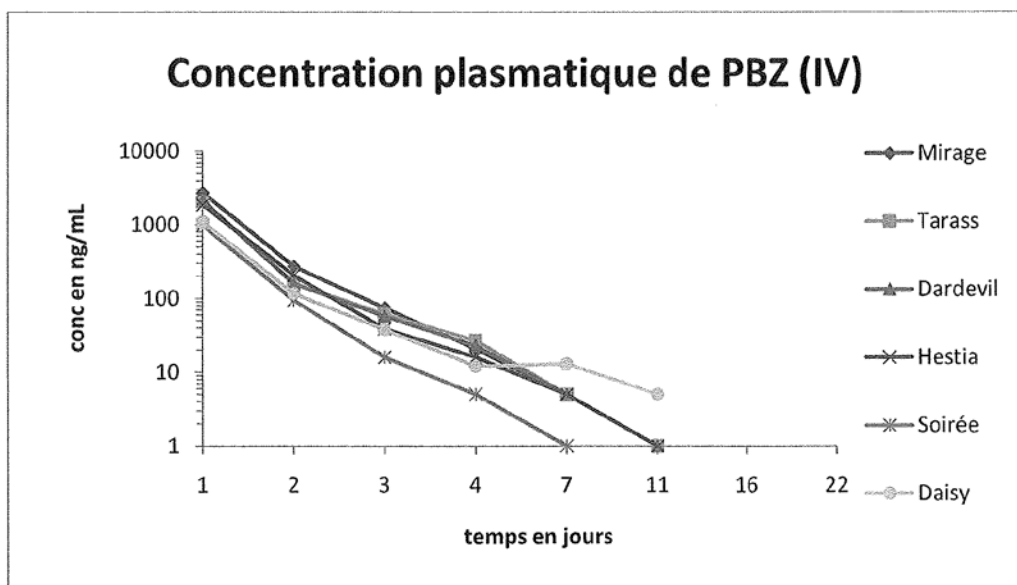
After <u>oral</u> administration of the medicinal product Equipalazone at doses of 4 g on the first day of treatment, then 2 g for the next 10 days, plasma concentrations observed in the six horses 10 days after the end of treatment ranged from 0.001 to 0.005 µg/mL. Considering the same ratio as before, this represents a maximum of **0.15 µg** of phenylbutazone if a person ingests 300 g of horse muscle 10 days after oral administration of phenylbutazone/kg LW at a dose of 4 g the first day and then 2 g the next 10 days (Orszag, 2008).
(See graph "PBZ plasma concentration (OR)" below with concentration in ng/mL on the y-axis and time in days on the x-axis).

Concentration plasmatique de PBZ (VO)
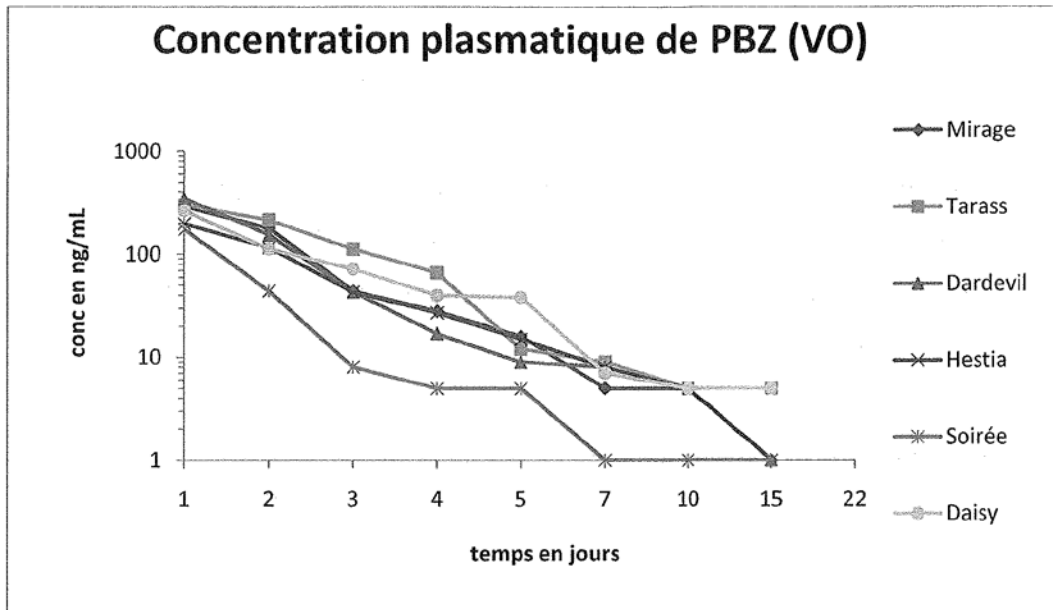
Summary table of concentrations observed in various fluids/tissues in horses receiving phenylbutazone at a dose of 4.4 mg/kg LW (Lees and Taylor, 1987):

Horse	Route	Time after treatment	Matrix	Phenylbutazone		Oxyphenbutazone	
				Concentration (µg/mL or µg/g)	Amount*	Concentration (µg/mL or µg/g)	Amount*
No 1	IV	6 h	Plasma	12.3	405 µg	2.6	150 µg
			Biceps muscle	0.5		0.1	
			Gluteal muscle	0.3		0.1	
			Liver	1.5		0.3	
			Kidneys (cortex)	2.1		1.8	
No 2	Oral	6 h	Plasma	3.3	135 µg	1.1	105 µg
			Biceps muscle	0.1		0.1	
			Gluteal muscle	0.1		0.1	
			Liver	0.7		0.2	
			Kidneys (cortex)	0.7		1.1	
No 3	Oral	12 h	Plasma	6.4	200 µg	1.1	90 µg
			Biceps muscle	0.2		0.1	
			Gluteal muscle	0.1		0.1	
			Liver	0.2		< 0.1	
			Kidneys (cortex)	2.4		1.0	

* The amounts ingested are calculated based on the daily food basket: 300 g of muscle, 100 g of liver and 50 g of kidney. The maximum concentration observed in the muscle tissue is used in the calculation.

The data are mainly for the muscle, the foodstuff in which the assays were performed. No data are available for adipose tissue. The concentrations are for single observations and are not mean values.

o Exposure calculation

The results of the depletion study in which a dose of 4 g phenylbutazone was administered orally to the horses the first day, then 2 g for the next 10 days, demonstrated that the maximum amount of phenylbutazone ingested per individual (i.e. in 300 g of muscle) would be 0.15 µg, 10 days after the end of treatment.

To assess the risk for the consumer based on the threshold of toxicological concern (TTC) approach, bearing in mind that no benefit is expected, the TTC value of 0.15 µg/person/day was compared with the exposure value of 0.15 µg/person. The result shows an acceptable risk from 10 days after administration. The risk is also considered acceptable 6 months after phenylbutazone treatment given the elimination of the active substance (plasma elimination half-life of 4-6 hours in horses).

In view of the available data, the risk of phenylbutazone for the consumer with a 6-month withdrawal period after administration in horses is considered acceptable, given the level reached which is well below the threshold of toxicological concern in humans.

- **THENOIC ACID (AS LITHIUM SALT)**

Lithium thenoate is a derivative of thiophene.
The substance has a stimulant effect on the cells of the pulmonary lining, producing mucus, and on the cells of the nasal and bronchial mucous membranes.
It has an antiseptic effect through direct antimicrobial activity and through mechanical clearing with an increase in natural secretions.

o Toxicology

No toxicological data are available for thenoic acid.

Concerning lithium, an INRS toxicology data sheet has been issued for lithium and its mineral components. In terms of experimental toxicity, the LD50 levels (median lethal doses) by the oral route are not significantly different between the studied salts (531-1198 mg/kg). Two studies have demonstrated a teratogenic effect after daily intraperitoneal injection of 50 mg lithium chloride. Ocular and outer ear malformations as well as cleft palates have been observed. Concerning chronic human toxicity, lithium treatment can lead to various symptoms, including tremor, speech disorders, electrocardiogram anomalies, dermatoses, and thyroid conditions, and above all chronic renal failure. Concerning teratogenicity, a specific cardiac abnormality was found more frequently in children born to mothers treated with lithium.

In the registration dossier submitted to the European Chemicals Agency (ECHA), a chronic oral toxicity study enabled calculation of a NOAEL of 1.2 mg/kg/day, on the basis of data in humans obtained as part of long-term treatment for psychiatric disorders. This value was then used to determine a DNEL (derived-no-effect level) of 0.25 mg/kg/day for long-term oral exposure of the general population. Of note, no genotoxicity was found in a battery of *in vitro* and *in vivo* tests, or in humans following a read-across approach, a technique used to predict the toxicological effects of a chemical substance based on data already available for other similar substances.

Concerning reprotoxicity and foetal toxicity after oral exposure, the NOAEL retained was 45 mg/kg/day, in the absence of changes in reproduction. Using a read-across approach, the NOAEL levels calculated for lithium were 3 mg/kg/day for systemic parental toxicity and 8 mg/kg/day for an effect on reproduction. In the area of developmental toxicity, no embryotoxic properties were observed (NOAEL = 16.91 mg lithium/kg/day, highest dose used), while an effect on weight and food intake was found in females (NOAEL = 5.64 mg lithium/kg/day).
In terms of the consumer risk assessment, it is recommended to use the **DNEL value of 0.25 mg/kg/day** for long-term oral exposure of the general population.

o Plasma pharmacokinetics

Thenoic acid (2-thiophene carboxylic acid) is a carboxylic acid derived from thiophene. No pharmacokinetics data are available.
In mice, thiophene shows rapid oral absorption and very rapid elimination (almost complete within 24 hours) and undergoes significant metabolism (Chanal et al, 1973).

Oral absorption of lithium is rapid in mice. Lithium is not bound to plasma proteins. It is rapidly distributed to tissues. Its elimination is rather slow. This is due to its slow release from tissues.

o Depletion

No depletion data are available for thenoic acid.

o Exposure calculation

Given the dosage of 20 mL on day 1 and 15 mL on days 2 and 3 (solution containing 5.15 mg lithium per mL and 95.5 mg thenoic acid per mL), and considering that the entire dose administered is distributed uniformly in the horse after absorption, humans ingesting 300 g of muscle would be exposed to:

- 0.129 mg of <u>lithium</u>, i.e. 0.002 mg/kg/day. This single exposure should be compared with the DNEL of 0.25 mg/kg/day, based on a study in humans as part of long-term treatment.
- 2.38 mg of <u>thenoic acid</u>. This exposure should be compared with the TTC of 0.15 µg/person/day.

If the exposure value obtained after treatment is higher than the TTC, the time needed to reach this TTC is estimated using the equation $t = (t_{1/2} / \ln 2)(\ln X_0 - \ln X)$, where $t_{1/2}$ is the elimination half-life, X_0 the initial amount, and X the amount to reach, i.e. the TTC.

Considering the amount administered at the time of treatment (X_0, here on day 3) of 4,775,000 µg thenoic acid, and based on hypothetical values of the elimination half-life of 10 days or 24 hours, the time to reach the TTC is approx. 250 days or 25 days after the end of treatment, respectively.

The elimination half-life needed to be below the TTC at 6 months after treatment is at most 7 days. With an elimination half-life of 24 hours, exposure of the consumer is acceptable from 25 days post-treatment in the horse.
Through a literature search, we were not able to identify an elimination half-life for thenoic acid, but it is a derivative of thiophene for which very rapid elimination has been observed in mice.

In view of the available data, the risk of thenoic acid for the consumer (as lithium) with a 6-month withdrawal period post-administration in horses is considered acceptable.

- **PERGOLIDE MESYLATE**

Pergolide is a synthetic derivative of ergot.

It is a potent dopamine receptor agonist with a long duration of action. *In vitro* and *in vivo* pharmacology studies have shown that pergolide acts as a selective dopaminergic agonist with little to no effect on the noradrenergic, adrenergic or serotonergic pathways at therapeutic doses. Like other dopaminergic agonists, pergolide inhibits prolactin secretion.

In horses with *pituitary pars intermedia* dysfunction (PPID), also known as equine Cushing's disease, the mean dose of pergolide used is 2 µg/kg (dose range: 1.3 to 2.4 µg/kg). Pergolide acts therapeutically by stimulation of dopamine receptors. In addition, pergolide has been found to decrease plasma levels of ACTH (adrenocorticotropic hormone), MSH (melanocyte-stimulating hormone) and other peptide precursors of polypeptide hormones (pro-opiomelanocortin).

o Toxicology

According to the Public Assessment Report for Prascend®, literature data show a high toxic potential following single administration of pergolide mesylate. However, the safety factor for the oral route in horses was at least 3000.

The most significant effects were observed during chronic and subchronic studies in female rats. Changes in locomotor activity in laboratory animals and vomiting in dogs may also be related to the pharmacological activity of the compound.

No teratogenic effects were observed in mice and rabbits. In the rabbit, no effect on reproduction or foetal development was found. The effects observed in mice, including the impacts on fertility, foetal weight, and survival rate of the litter, were attributed to inhibition of prolactin secretion, resulting in failure of lactation.

Pergolide mesylate has no genotoxic activity.

In carcinogenicity studies, an increased incidence of uterine tumours related to the pharmacological action of pergolide was considered to be non-significant in horses and in humans.

In humans, the LOAEL of pergolide is 25 µg/day (0.42 µg/kg) (Berezin, 1991). It is therefore recommended to use the LOAEL value of 0.42 µg/kg for assessment of the risk to consumers. A safety factor of 100 is added, 10 for inter-individual variability and 10 because the reference toxicological dose used is a LOAEL and not a NOAEL.

o Plasma pharmacokinetics

In horses, absorption following oral administration of 2 and 10 µg pergolide/kg body weight is rapid (Tmax of approx. 0.4 hours). The maximum concentration is low and highly variable. Plasma protein binding is significant: 90%. The plasma elimination half-life is approx. 6 hours.

o Depletion

No depletion data are available for pergolide.

Stopping treatment for 6 months is not compatible with the well-being of a horse with Cushing's disease because this disorder is chronic and requires life-long treatment. The health status of a horse with Cushing's disease does not appear to be compatible with slaughter for human consumption.
Given the available scientific data and the disease involved, Equidae treated with pergolide mesylate should not be intended for human consumption.

- **CROTON OIL**

Croton eluteria is a shrub or small tree species belonging to the Euphorbiaceae family originating in the Americas. It is used as an essential oil to treat respiratory disorders.

o   Toxicology

In vitro mutagenesis studies (chromosomal aberrations) have shown negative results. However, a tumour-promoting effect of croton oil has been observed for the skin and also for the stomach, following oral exposure associated with n-methyl-n'-nitro-n-nitrosoguanidine in the rat. Various studies have confirmed this tumour-promoting effect of croton oil (Subramanian, 2014).
Croton oil has also been used in cancer research as a co-carcinogen (Merck Index). It is an irritant (moderate to severe) and induces irritant and inflammatory effects in mice in acute studies via the dermal route.
Although it is possible to define a threshold dose for tumour promoters, currently available data do not make it possible to define this dose.
In addition, croton is included in List B of medicinal plants used traditionally as-is or as a preparation with potential adverse effects that are greater than the expected therapeutic benefit[4].

o   Plasma pharmacokinetics and depletion

No data on the pharmacokinetics or depletion of croton oil are available.

No pharmaceutical or pharmacological data are available to characterise consumer exposure, and toxicology data are unfavourable.
In the absence of data to quantify and evaluate exposure (administered dose and absorption), it is therefore not possible to conclude on the risk of croton oil for the consumer with a 6-month withdrawal period post-administration in horses.

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[4] List published in Chapter IV.7.B of the French Pharmacopoeia, mentioned in Article D. 4211-12 of the Public Health Code

■ **ASSESSMENT OF SUBSTANCES USED IN EYE DROPS**

● **SYSTEMIC ABSORPTION FOLLOWING OCULAR ADMINISTRATION OF A SUBSTANCE**

(JHA G & Kumar A, 2011) (Beyssac E, 1996) (Maitani et al, 1995) (Leeming, 1999)

Traditionally, ocular administration of an active substance was intended to have a local effect. The active substance is diluted in lachrymal secretions, then, depending on its physicochemical properties (liposolubility, for example), diffuses through the semi-permeable membranes.
About 95% of the substance contained in ophthalmic drops is lost via absorption through the conjunctiva or via drainage in tears (Jarvinen et al, 1995). The formulation of the medicinal product and the physiological state of the eye also have an impact on systemic absorption of the substance.

Systemic absorption following ocular administration of an active substance is low. A study carried out in rabbits showed absorption 25,000 times lower via the ocular route than the intravenous route for a tumour necrosis factor (TNF) inhibitor (Furrer et al, 2009). However, absolute bioavailability of 16% was observed for thiamphenicol in rabbits following ocular administration (Aldana et al, 1992). Plasma concentrations are often around the limits of quantification of analytical methods (Hsu et al, 2015) (Levy et al, 2015). Some publications nonetheless describe systemic toxicological effects following administration of eye drops, suggesting that there is some systemic absorption (Abdelhalim et al, 2012) (Nieminen et al, 2007) (Gunaydin et al, 2011). A literature review (Urtti & Salminen, 1993) indicated that absolute bioavailabilities vary from 0.1% to 100% in the rabbit for a certain number of active substances administered ophthalmically.
Concerning the active substances of interest for this request, no absolute bioavailabilities are known following ocular administration. Exposure of 100% will therefore be retained.

For all the active substances intended for use as eye drops, the approach involves estimating the amount of substance potentially absorbed and comparing it with the TTC value, which is an exposure dose for substances that have not been studied that does not lead to significant toxic or carcinogenic effects. Regarding the risk to the consumer, the value of 0.15 µg/person/day is considered appropriate, bearing in mind that no benefit is expected.
This value is compared to the exposure amount, which is estimated as 100% of the total amount administered in the eye.
The hypothesis of a dosage with 2 drops 4 times daily dose is adopted. This reflects high treatment compliance.

Since the above hypotheses are absolute maximums (no loss through tears, complete absorption of the substances, and treatment four times a day), repeated treatment for several days is not taken into account. The hypothesis of non-accumulation is therefore adopted.

- **TETRACAINE (eye drops)**

Like other local anaesthetics, tetracaine blocks nerve conductivity by reducing significant temporary increases in the membrane permeability of nerve fibres to sodium ions (Na+), which under normal circumstances results from slight depolarisation of the membrane. Nerve conduction is first blocked in the autonomic fibres, then in sensory fibres, and lastly in motor fibres.

o Toxicology

The only data currently available are negative results for an Ames test (Waskell, 1978).
Within the consumer risk assessment, it is not possible to draw conclusions on the potential risk given the currently available data. The solution put forward is to compare the potential exposure value with the TTC.

o Plasma pharmacokinetics

In humans, systemic exposure after administration of eye drops is unknown. Plasma protein binding of tetracaine is about 75%.

Tetracaine is rapidly hydrolysed by non-specific cholinesterases (butyrylcholinesterase: plasma cholinesterase), mainly in plasma and in the liver. The main metabolite of tetracaine is para-(butylamino)benzoic acid. Tetracaine is eliminated in the urine as para-(butylamino)benzoic acid.

o Depletion

No depletion data are available for tetracaine.

o Exposure calculation

The dosage of tetracaine eye drops is 2 drops 4 times daily as a solution containing 8.79 mg/mL. If 2 drops are equivalent to 0.1 mL, the corresponding amount is 0.88 mg, 4 times daily, i.e. 3.52 mg/600 kg/day, or 5.87 µg/kg/day. If we consider that 100% is absorbed in the eye, we obtain a systemic concentration of 5.9 µg/kg. If this concentration were the same in meat, with consumption of 300 g, the amount of tetracaine ingested would be 1.77 µg/person immediately after treatment of the animal. If we compare this exposure value with the TTC of 0.15 µg/person/day, there is a risk immediately after treatment of the animal.

Considering the amount administered at the time of treatment (X0) of 3,520 µg, and based on a hypothetical elimination half-life of 10 days or 24 hours, the time to reach the TTC is approx. 146 days or 15 days after the end of treatment, respectively.

With an elimination half-life of 10 days, exposure of the consumer is acceptable 5 months after the horse was treated.
With an elimination half-life of 24 hours, exposure of the consumer is acceptable 15 days after the horse was treated.

Given that the plasma half-life of tetracaine, according to Weber C, 2006 and Venkataran M, 2012, is approx. 2 hours in humans (injection route), the risk for the consumer after a 6-month withdrawal period after ocular administration of tetracaine in the horse is considered acceptable.

In view of the available data, the risk of tetracaine for the consumer after the 6-month withdrawal period post-administration in horses is considered acceptable in the context of use as a local anaesthetic in the eye.

- **TETRYZOLINE (eye drops) (Synonym of tetrahydrozoline)**

Tetryzoline is a sympathomimetic agent used locally as a vasoconstrictor in the ENT area.

  o Toxicology

In dogs, various signs of intoxication have been reported. However, it is not possible to define a no-effect-dose with the currently available data.
Within the consumer risk assessment, it is not possible to draw conclusions on the potential risk given the currently available data. The solution put forward is to compare the potential exposure value to the TTC (Kahn, 2005).

  o Plasma pharmacokinetics and depletion

No data are available on pharmacokinetics and depletion.

  o Exposure calculation

The dosage of tetryzoline as eye drops is 2 drops 4 times daily as a solution containing 0.42 mg/mL. If 2 drops are equivalent to 0.1 mL, the corresponding amount is 0.042 mg, 4 times daily, i.e. 0.168 mg/600kg/day, or 0.28 µg/kg. If we consider that 100% is absorbed in the eye, we obtain a systemic concentration of 0.28 µg/kg. If this concentration were the same in meat, with consumption of 300 g, the amount of tetryzoline ingested would be 0.084 µg/person immediately after treatment of the animal. If we compare this exposure value with the TTC of 0.15 µg/person, the risk is acceptable.

In view of the available data, the risk of tetryzoline for the consumer with a 6-month withdrawal period post-administration in horses is considered acceptable in the context of use as a **local treatment of eye disorders.**

- **RIFAMYCIN (eye drops)**

Rifamycin is an antibacterial antibiotic active via the local route on most Gram+ and Gram- infectious agents. Rifamycin activity is related to DNA-dependent RNA-polymerase by formation of a stable complex leading to inhibition of bacterial growth.
Rifamycin and rifampicin belong to the same antibiotic class. Rifampicin is a major anti-tuberculosis drug.

o Toxicology

Established oral LD50 values for rifamycin in mice, rats and chickens vary from 1,951 to 5,700 mg/kg. After administration via the sub-cutaneous route in mice, the LD50 was 815.9 mg/kg. Within the consumer risk assessment, it is not possible to draw conclusions on the potential risk given the currently available data. The solution put forward is to compare the potential exposure value with the TTC (Pashov, 1987).

o Plasma pharmacokinetics

The plasma half-life of rifamycin is 23h in mice and 14h in humans (Lounis N, 1997).

o Depletion

No depletion data are available.

o Exposure calculation

The dosage of rifamycin as eye drops is 2 drops 4 times daily as a solution containing 100,000 IU/10mL, i.e. 11.27 mg/mL. If 2 drops are equivalent to 0.1 mL, the corresponding amount is 1.13 mg, 4 times daily, i.e. 4.52 mg/600kg/day, or 7.5 µg/kg. If we consider that 100% of the dose is absorbed in the eye, we obtain a systemic concentration of 7.5 µg/kg. If this concentration were the same in meat, with consumption of 300 g, the amount of rifamycin ingested would be 2.25 µg/person/day. If we compare this exposure value with the TTC of 0.15 µg/person, there is a risk.

Considering the elimination half-life of 23 hours observed in mice (the longest known half-life) and the amount administered at the time of treatment (X0) of 4,520 µg, the time needed to reach the TTC is approx. 15 days after the end of treatment.

In view of the available data, the risk of rifamycin for the consumer with a 6-month withdrawal period post-administration in horses is considered acceptable in the context of use as a local treatment of eye infections.

- **SYNEPHRINE (eye drops)**

Synephrine is a synthetic alpha-sympathomimetic with vasoconstrictor and decongestant properties on ocular conjunctiva.

o Toxicology

Toxicology data demonstrate development of effects such as respiratory distress and hypertension from a dose of 150 mg/kg of p-synephrine in acute administration via the oral route. Chronic toxicity studies have not enabled establishment of a NOEL due to numerous methodological deficiencies. It is not possible to conclude that there is no genotoxicity. Exploitable data are limited to the absence of a teratogenic potential and various adverse effects observed in humans. However, within the consumer risk assessment, it is not possible to draw conclusions on the potential risk given the currently available data. The solution put forward is to compare the potential exposure value with the TTC (Hansen, 2005; Grazziotin, 2011).

o Plasma pharmacokinetics and depletion

No data on pharmacokinetics and depletion are available.

o Exposure calculation

The dosage of m-synephrine as eye drops is 2 drops 4 times daily as a solution containing 0.826 mg/mL. If 2 drops are equivalent to 0.1 mL, the corresponding amount is 0.083 mg, 4 times daily, i.e. 0.332 mg/600 kg/day, or 0.55 µg/kg. If we consider that 100% of the dose is absorbed in the eye, we obtain a systemic concentration of 0.55 µg/kg. It this concentration were the same in meat, with consumption of 300 g, the amount of synephrine ingested would be 0.165 µg/person/day immediately after treatment of the animal. If we compare this exposure value with the TTC of 0.15 µg/person, there is a risk.

However, this value must also be compared with the dose of 20 mg/day corresponding to the dose ingested by high-level consumers of citrus fruit and which can serve as a reference for intakes of p-synephrine not to exceed for food supplements (but which is not a safety limit in the strict sense of the term) (ANSES, 2014).

Considering the amount administered at the time of treatment (X0) of 332 µg, and based on hypothetical values of the elimination half-life of 10 days or 24 hours, the time needed to reach the TTC is approx. 112 or 12 days after the end of treatment, respectively.

With an elimination half-life of 10 days, exposure of consumers is acceptable 4 months after treatment of the horse;
With an elimination half-life of 24 hours, exposure of consumers is acceptable 12 days after treatment of the horse.
Bearing in mind that the plasma half-life of synephrine is about 2 hours in humans (intravenous route) according to Hengstmann & Aulepp (1978), the risk to consumers after a 6-month withdrawal period after ocular administration of synephrine in the horse is considered acceptable.

In view of the available data, the risk of synephrine for the consumer with a 6-month withdrawal period post-administration to horses is considered acceptable in the context of use as a **local treatment for ocular disorders.**

- **POLYMYXINE B (AS SULPHATE) (eye drops)**

Polymyxin B belongs to the group of polypeptide antibiotics isolated from bacteria. It acts solely against Gram-negative bacteria.
It acts mainly against Gram-negative bacilli bacteria, e.g. infections with enterobacteria, and *Pseudomonas*. It acts by binding to the phospholipid membrane and rupturing the bacterial cytoplasmic membrane.

o Toxicology

Given the low absorption following topical application, polymyxin B is considered to have low toxicity in infants. However, within the consumer risk assessment, it is not possible to draw conclusions on the potential risk given the currently available data. The solution put forward is to compare the potential exposure value with the TTC.

o Plasma pharmacokinetics

Local application of polymyxin B results in practically no absorption of the substance through the skin or intact mucous membranes, but high absorption is found through wounds.

o Depletion

No depletion data are available.

o Exposure calculation

The dosage of polymyxin B as eye drops is 2 drops 4 times daily with a solution containing 10,000 IU/mL, i.e. 1.19 mg/mL. If 2 drops are equivalent to 0.1 mL, the corresponding amount is 0.12 mg, 4 times daily, i.e. 0.48 mg/600 kg/day, or 0.8 µg/kg. If we consider that 100% of the dose is absorbed in the eye, we obtain a systemic concentration of 0.8 µg/kg. If this concentration were the same in meat, with consumption of 300 g, the amount of polymyxin B ingested would be 0.24 µg/person/day immediately after treatment of the animal. If we compare this exposure value with the TTC of 0.15 µg/person, there is a risk.

Considering the amount administered at the time of treatment (X0) of 480 µg, and based on hypothetical values of the elimination half-life of 10 days or 24 hours, the time needed to reach the TTC is approx. 117 or 12 days after the end of treatment, respectively.

With an elimination half-life of 10 days, exposure of consumers is acceptable 4 months after treatment of the horse.
With an elimination half-life of 24 hours, exposure of consumers is acceptable 12 days after treatment of the horse.

Bearing in mind that the plasma half-life of polymyxin B is about 6 hours in humans (intramuscular route) (VIDAL Compendium), and that the elimination half-life of an antibiotic belonging to the same class, colistin, is 4 to 6 hours (intravenous and intramuscular routes in the calf (Renard, Sanders, Laurentie, 1991), the risk to consumers after a 6-month withdrawal period after ocular administration of polymyxin B in horses is considered acceptable.

In view of the available data, the risk of polymyxin B for the consumer with a 6-month withdrawal period post-administration in horses is considered acceptable in the context of use as a **local treatment of eye infections**.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety concludes that:

Either on the basis of data or using various hypotheses, the potential level of consumer exposure has been established. The exposure level was compared to toxicity reference values (TRVs), or to the threshold of toxicological concern (TTC) when reference values were not available. The results of the assessment show that the risk to the consumer appears acceptable 6 months after treatment with **phenylbutazone** and with **thenoic acid**.

However, it is not possible to draw conclusions on the risk for the consumer of use of **croton oil**. Concerning **pergolide mesylate**, it is recommended that treated Equidae not be intended for human consumption mainly because of the associated disease and the need to maintain treatment in affected animals.

With regard to substances used in **eye drops**, again based on available data and calculations used to define the time needed to reach the TTC, the risk is considered acceptable for **tetracaine** in the context of use as a local anaesthetic in the eye, and for **tetryzoline**, **rifamycin**, **synephrine** and **polymyxin B** in the context of use as a local treatment of eye disorders.

The Interim Director General

Caroline GARDETTE

KEY WORDS

Medicinal product, active substance, Equidae, consumer risk

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A literature survey was carried out on the various substances of interest (MEDLINE, SCOPUS, PUBMED, CINAHL, opengrey.eu). Public data available in MA dossiers for veterinary medicinal products containing these active substances (SPCs) were also used to produce this summary of pharmacokinetic data. The sources used are listed below.

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ECHA: Registration dossier for thenoic acid, 30 June 2014

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EMA/EFSA 1987: Joint Statement of EMA and EFSA on the presence of residues of phenylbutazone in horse meat

EMA: CVMP assessment report regarding the request for an opinion under Article 30(3) of Regulation (EC) No 726/2004

EMA: ICH guideline M7 on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk

EPA (http://www2.epa.gov/iris)

European Medicine Agency (http://www.ema.europa.eu/ema/)

Extoxnet (http://extoxnet.orst.edu/), Furetox (http://www.furetox.fr/)

INRS, thenoic acid: Toxicology data sheet No 183 (2000)

IPCS INCHEM 1987, phenylbutazone:
http://inchemsearch.ccohs.ca/inchem/jsp/search/search.jsp?inchemcasreg=1&Coll=inchemall&serverSpec=charlie.ccohs.ca%3A9900&QueryText1=&QueryText2=phenylbutazone&Search.x=0&Search.y=0

Index of Veterinary Medicinal Products authorised in France, ANSES (http://www.ircp.anmv.anses.fr/): PAR PRASCEND®

IRIS, EPA (http://www2.epa.gov/iris)

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Portal for chemical substances, INERIS (http://www.ineris.fr/substances/fr/)

ANSES website (http://www.anses.fr): 2014, Opinion of the French Agency for Food, Environmental and Occupational Health & Safety on the risks associated with the presence in food supplements of p-synephrine or ingredients obtained from Citrus spp. fruits containing this substance

French thesis database (http://www.theses.fr/)

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Toxic Substances Portal, ATSDR (http://www.atsdr.cdc.gov/substances/indexAZ.asp#T)

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