8 September 2015
EMA/CVMP/632934/2014
Committee for Medicinal Products for Veterinary Use

European Public MRL assessment report (EPMAR)

Propyl 4-hydroxybenzoate and its sodium salt (all food producing species)

On 3 July 2015 the European Commission adopted a Regulation[1] establishing maximum residue limits for propyl 4-hydroxybenzoate and its sodium salt in all food producing species, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Propyl 4-hydroxybenzoate and its sodium salt are antimicrobial preservatives used in veterinary medicinal products.

The International Federation for Animal Health (Europe) submitted the application for the establishment of maximum residue limits to the European Medicines Agency, on 20 May 2014.

Based on the data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended on 6 November 2014 the establishment of maximum residue limits for propyl 4-hydroxybenzoate and its sodium salt in all food producing species.

Subsequently the Commission recommended on 23 May 2015 that maximum residue limits in all food producing species are established. This recommendation was confirmed on 13 June 2015 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 3 July 2015.

[1] Commission Implementing Regulation (EU) No 2015/1080, O.J. L 175, of 03 July 2015

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Summary of the scientific discussion for the establishment of MRLs

Substance name:	Propyl 4-hydroxybenzoate and its sodium salt
Therapeutic class:	Antimicrobial preservative
Procedure number:	EMEA/V/MRL/004039/FULL/0001
Applicant:	IFAH Europe on behalf of a consortium of 30 marketing authorisation holders
Target species:	All food producing species
Intended therapeutic indication:	Not applicable (for use as an excipient)
Route(s) of administration:	Oral, dermal, parenteral, intramuscular, intramammary

1. Introduction

Propyl 4-hydroxybenzoate (also known as propyl parahydroxybenzoate and propyl paraben) and its sodium salt (sodium propyl 4-hydroxybenzoate, also known as propyl parahydroxybenzoate and sodium propyl paraben) (CAS No 94-13-3) are antimicrobial preservatives used in veterinary medicinal products. Both substances were previously classed as food additives with E numbers (E 216 and E 217, respectively), and consequently both had a "No MRL required" classification in line with the entry for food additives (substances with a valid E number approved as additives in foodstuffs for human consumption), in Table 1 of the Annex to Regulation 37/2010.

However, these two substances are no longer covered by the entry for food additives in the Annex to Regulation 37/2010, as a result of EFSA's re-evaluation (2004) of parabens[2] with E numbers E214-E219, and the subsequent suspension of the E-numbers for these two substances. EFSA's conclusion on propyl 4-hydroxybenzoate and its sodium salt was based on data indicating that administration of propyl 4-hydroxybenzoate to male rats resulted in adverse effects on the hormonal system and male reproductive functions (Oishi, 2002[3]).

Given that these two substances are no longer covered by any entry in the Annex to Regulation 37/2010, the International Federation for Animal Health (Europe) (IFAH Europe), on behalf of a consortium of 30 marketing authorisation holders, submitted an application for the establishment of maximum residue limits in all food producing species to the European Medicines Agency, on 20 May 2014. In all food producing species, propyl 4-hydroxybenzoate and its sodium salt are intended to be used as antimicrobial preservatives in products intended for all routes of administration, with various durations of treatment, and with doses ranging from 1 to 6000 µg/kg bw depending on the route of administration, and product type.

[2] Parabens are a group of substances of parahydroxybenzoates or esters of parahydroxybenzoic acid.
[3] Oishi, S. Effects of propyl paraben on the male reproductive system. *Food Chemical Toxicology,* 2002, 40, 1807-1813

2. Scientific risk assessment

2.1. Safety assessment

2.1.1. Overview of pharmacological properties

No specific studies investigating the primary pharmacodynamics were available. Propyl 4-hydroxybenzoate and its sodium salt are well-known antimicrobial preservatives that act by inducing DNA double-strand breaks and oxidative damage, leading to a decrease on the percentage of mitotic cells, mainly due to cell-cycle arrest at the G0/G1 phase. However given the concentrations used in veterinary medicines and their rapid metabolism and elimination, they are not expected to have any antimicrobial or other pharmacological effects in the target animal or consumer.

Propyl 4-hydroxybenzoate is very rapidly absorbed and metabolized, shown by the negligible levels of the parent compound that are detected in the blood within minutes after oral administration, and the paraben metabolites detected in the urine within an hour after administration. Irrespective of the species studied, the metabolism of propyl 4-hydroxybenzoate resulted in hydrolysis to the principal metabolite 4-hydroxybenzoic acid. 4-hydroxybenzoic acid may be conjugated with glycine, glucuronic acid and sulfate. Excretion is principally urinary and rapid, with more than 90% of the dose excreted within 24 hours of administration.

The rapid conversion to 4-hydroxybenzoic acid in mammalian species is reassuring from a consumer safety perspective, as this substance is a natural constituent of food of vegetable origin and is not considered to possess pharmacological activity.

2.1.2. Calculation of pharmacological ADI, if relevant

Relevant pharmacological effects are considered to have been adequately represented in the toxicity studies provided, and no additional pharmacological effects that would need further characterisation have been identified. Consequently, in line with the CVMP Guideline on the approach to establish a pharmacological ADI (EMA/CVMP/SWP/355689/2006), no pharmacological ADI is considered necessary.

2.1.3. Overview of toxicology

Single-dose toxicity and short term studies including reproductive toxicity

The acute toxicity of propyl 4-hydroxybenzoate was evaluated in mice, rats, rabbits and dogs, and was low after oral and dermal administration, but greater after subcutaneous and intraperitoneal administration. Lethal doses in the mouse produced ataxia, deep central nervous system depression and rapid death. Intravenous injection of the sodium salt of propyl 4-hydroxybenzoate resulted in a transient fall in blood pressure in mice resulting in death due to hypotension. The acute toxicity is considered to be of low relevance for determining an ADI for propyl 4-hydroxybenzoate and its sodium salt.

In the Oishi study from 2002[2], the administration of propyl 4-hydroxybenzoate to male rats resulted in adverse effects on the hormonal system and the male reproductive functions. Effects were seen in all groups with a LOEL at 10 mg/kg. The reliability of this study has been discussed and questioned. The main arguments have been the lack of raw data, no data on systemic exposure and signs of lack of appropriateness for evaluation of relevant parameters, indicated by standard deviations which were far

less than expected based on normal biological variability, and mean values for some parameters far outside accepted historical control ranges. Recently, a well-designed and well-conducted GLP-compliant toxicity study in juvenile male Wistar rats was completed[4], where rats were dosed daily by oral gavage with propyl paraben at 0, 3, 10, 100 or 1000 mg/kg bw/day for 8 weeks, with or without a 26 week recovery period. Propyl paraben was rapidly absorbed, metabolised and cleared from plasma. The results demonstrated the absence of adverse effects of the compound on the male reproductive system, on serum testosterone, follicle stimulating hormone (FSH) and luteinising hormone (LH) concentrations, and on sperm counts and sperm motility. This study is considered to be of much better quality and thus more reliable than the Oishi study from 2002. In other supportive studies performed using methyl or butyl paraben absence of adverse effects were noted in male Wistar rats treated at doses of up to 1141 mg/kg bw/day of methyl paraben, or 1087.6 mg/kg bw/day of butyl paraben for 8 weeks. The overall results indicate that oral methyl, propyl and butyl parabens do not result in adverse effects on the male reproductive system, or produce adverse effects on sperm counts and sperm motility. The 8 week repeat dose toxicity NOAEL in male rats for propyl paraben is considered to be 1000 mg/kg bw/day.

In a study in prepubertal female rats, 21 day old animals were treated with parabens, including propyl 4-hydroxybenzoate by gavage at doses of 0, 62.5, 250 or 1000 mg/kg bw/day for 19 days, from post-natal day 21 to 40. Propyl 4-hydroxybenzoate produced no adverse effects on body weight, vaginal opening or on the oestrous cycle. There were no adverse effects on the weight of the uterus, pituitary, ovary, thyroid, kidney or liver but there was an increase in adrenal weight. There was a slight increase in uterine thickening in high dose animals, but no effects on the numbers of *corpora lutea* or cystic follicles. The NOEL from this 19-day repeat dose toxicity study in prepubertal female rats for propyl 4-hydroxybenzoate is 250 mg/kg bw/day.

No conventional reproductive toxicology studies are available for propyl 4-hydroxybenzoate. Literature data on closely related parabens indicated no reproductive toxicity effects following oral exposure. A well-conducted oral teratology study in rats with the closely related butyl paraben, produced no evidence of teratogenic activity with doses of up to 1000 mg/kg bw/day. There were no differences from controls in any of the developmental parameters measured including embryo/foetal viability, foetal weights, malformations or variations. In a study where 100 or 200 mg/kg bw/day butyl paraben was given subcutaneously to pregnant rats, both doses led to reductions in sperm count in male offspring. There were no effects on anogenital distance or vaginal opening times, and no effects on sex ratio of offspring or total pups per litter. However, the numbers of pups born alive and surviving to weaning were reduced at 200 mg/kg bw/day. In a study in rats using subcutaneous slow release of approximately 4.6 mg/day isobutyl paraben no major oestrogenic effects or other adverse effects were seen in offspring except for reductions in cortisone levels in female pups. In a separate study, administration of isobutyl paraben in the same manner led to male offspring spending less time in the open arms of the elevated maze test suggesting that in utero exposure and exposure to maternal milk may affect their anxiety and learning abilities when adults. Isobutyl paraben had no effects on other parameters studied.

On the basis of the literature available and similarities on the length of the alkyl chain, propyl 4-hydroxybenzoate is unlikely to affect fertility, embryo/foetal development or to be teratogenic following oral administration.

[4] Aubert N., Ameller T., Legrand JJ. Systemic exposure to parabens: Pharmacokinetics, tissue distribution, excretion balance and plasma metabolites of [14C]-methyl-, propyl- and butylparaben in rats after oral, topical or subcutaneous administration. *Food Chemical Toxicology*. 2012. **50**:445-454

Genotoxicity

Only brief reviews of old genotoxicity studies are available for propyl 4-hydroxybenzoate. Equivocal results exist for the Ames test, with two studies showing no mutagenicity in the presence and absence of metabolic activation, and one modified study showing a positive result in one strain (TA100) in the presence of metabolic activation. A weak positive result was reported in the Comet assay but an indirect measure of DNA damage was negative. It is noted that EFSA previously concluded that propyl 4-hydroxybenzoate is not mutagenic *in vitro* based on the same dataset. The CVMP supports the EFSA conclusion.

No *in vivo* studies are available. (Q)SAR analyses suggest that propyl 4-hydroxybenzoate is unlikely to bind to DNA.

Overall, the weight of evidence indicates that following metabolic activation propyl 4-hydroxybenzoate is unlikely to be genotoxic *in vivo* and there are no concerns of genotoxic effects following its use as an antimicrobial preservative in veterinary pharmaceutical products.

Carcinogenicity

Carcinogenicity studies with propyl 4-hydroxybenzoate and its sodium salt are not available, and this is acceptable. Propyl 4-hydroxybenzoate and its sodium salt are not structurally related to known carcinogens and lack structures associated with carcinogenicity. None of the toxicity studies conducted with propyl 4-hydroxybenzoate revealed any activity associated with a carcinogenic process (e.g., the production of preneoplastic lesions). It can be concluded that the weight of evidence indicates that propyl 4-hydroxybenzoate is not likely to be carcinogenic.

Studies of other effects including immunotoxicity and neurotoxicity

Weak oestrogenic effects have been observed *in vitro* and following parenteral administration of propyl 4-hydroxybenzoate to rats and fish. Oestrogenic effects are not expected following oral dosing as propyl 4-hydroxybenzoate and its sodium salt are subject to hydrolytic metabolism to yield 4-hydroxybenzoic acid, a substance shown to be devoid of oestrogenic activity. The use of data from animal studies with parenteral routes of administration is of limited relevance for risk assessment of human exposure from residues of veterinary medicinal products present in food products, as consumers are exposed orally.

2.1.4. Calculation of the toxicological ADI or alternative limit

The most suitable NOEL from which to derive an ADI is the NOEL of 250 mg/kg bw/day, based on effects on the female rat reproductive system, seen in a 19 day study.

There are no data to indicate a particular concern in relation to intra- or inter-species variation and consequently the standard uncertainty factor of 100 is considered adequate. Furthermore, the NOEL values for effects on the male and female reproductive system have been derived from adequately designed and well-conducted studies. An extra factor of 2 is added since the NOAEL is taken from a short-term study (19 days) and no standard repeat dose studies are available.

A toxicological ADI of 1.25 mg/kg bw (i.e. 75 mg/person) is therefore established.

2.1.5. Overview of microbiological properties of residues

Propyl 4-hydroxybenzoate and its sodium salt have antimicrobial properties *in vitro* and this forms the basis for their use as preservative agents. However, due to rapid metabolism and excretion in the target animals, no antimicrobial effects are expected in the consumer.

2.1.6. Calculation of microbiological ADI

Propyl 4-hydroxybenzoate and its sodium salt are not expected to possess antimicrobial activity at the doses administered to target animals following their use as excipients in veterinary medicinal products, and consequently the residues to which consumers may be exposed are not expected to possess antimicrobial activity. Therefore, the derivation of a microbiological ADI is not considered necessary.

2.1.7. Observations in humans

Propyl 4-hydroxybenzoate and its sodium salt are also used as an antimicrobial preservative in orally administered medicinal products for human use. The use in medicinal products for human use was recently reviewed by the Committee for Medicinal Products for Human Use (CHMP) with a focus on possible endocrine disrupting effects[5] (A NOEL of 250 mg/kg was determined for propyl 4-hydroxybenzoate based on the published effects on the female rat reproductive system. A permitted daily exposure (PDE) of 5 mg/kg/day was established for adults and children older than 2 years with mature metabolic capacity.

2.1.8. Findings of EU or international scientific bodies

The European Commission Scientific Committee on Food evaluated the parabens in 1994 and established a temporary ADI of 0-10 mg/kg bw, as the sum of ethyl, methyl and propyl p-hydroxybenzoic acid esters and their sodium salts. In 2004 the EFSA Panel on food additives undertook a review of parabens with E numbers, as a result of which propyl 4-hydroxybenzoate and its sodium salt were excluded from this group ADI, based on the effects on sex hormones and on male reproductive organs in juvenile rats, and the lack of a clear NOAEL based on the 2002 study by Oishi. If the new (2012) GLP-study in which a NOEL of 1000 mg/kg bw/day was established had been available at that time, propyl 4-hydroxybenzoate and its sodium salt would quite possibly have been included in that group ADI.

2.1.9. Overall conclusions on the ADI

As pharmacological and microbiological ADIs are not considered relevant for propyl 4-hydroxybenzoate and its sodium salt, the toxicological ADI of 1.25 mg /kg bw, i.e. 75 mg/person for a 60 kg person, is established as the overall ADI.

[5] Reflection paper on the use of methyl- and propylparaben as excipients in human medicinal products for oral use. (EMA/CHMP/SWP/272921/2012).

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

In all species examined propyl 4-hydroxybenzoate appears to be very rapidly metabolised since only negligible levels of the parent compound are detected in the blood minutes after oral administration and paraben metabolites can be detected in the urine within an hour post-dosing. Irrespective of the species studied, the metabolism of propyl 4-hydroxybenzoate resulted in hydrolysis to the principal metabolite 4-hydroxybenzoic acid. 4-hydroxybenzoic acid may be conjugated with glycine, glucuronic acid and sulfate. Excretion is principally urinary and rapid, with more than 90% of the dose excreted within 24 hours of administration.

2.2.2. Residue depletion studies

There are no data available on the depletion of residues in target animals.

The available pharmacokinetic data suggest that in mammals, absorption of propyl 4-hydroxybenzoate and other parabens after oral administration is rapid. After and during absorption following oral administration, metabolism is rapid to yield 4-hydroxybenzoic acid and propanol. 4-hydroxybenzoic acid is rapidly conjugated and excreted in urine, while propanol is extensively metabolised to yield ultimately carbon dioxide and water. It is highly unlikely that anything other than negligible amounts of propyl 4-hydroxybenzoate will remain in food producing animals after administration of propyl 4-hydroxybenzoate-containing veterinary medicinal products.

No marker residue or ratio of marker to total residues is proposed. This is consistent with the proposal for a ‘No MRL required’ classification.

2.2.3. Monitoring or exposure data

No monitoring or exposure data other than that described elsewhere in this report are available.

2.2.4. Analytical method for monitoring residues

No analytical method for monitoring of residues was provided for this substance. This can be accepted if a ‘No MRL required’ status is recommended.

2.2.5. Findings of EU or other international scientific bodies

No relevant reports relating to residues of propyl 4-hydroxybenzoate in food producing species were identified.

3. Risk management considerations

3.1. Potential effects on the microorganisms used for industrial food processing

Although propyl 4-hydroxybenzoate and its sodium salt have antimicrobial activity, the levels of residues occurring following use of these substances as excipients in veterinary medicinal products are expected to be too low to impact on industrial food processing.

3.2. Other relevant risk management considerations for the establishment of maximum residue limits

No marker residue has been identified which could be used for residue control purposes, which is appropriate as residue control is not considered necessary. In addition, as the principle metabolite of propyl 4-hydroxybenzoate is 4-hydroxybenzoic acid, a natural constituent of food of vegetable origin, this would not be useful for residue control purposes.

The current assessment relates to the use of propyl 4-hydroxybenzoate as an antimicrobial preservative. As detailed elsewhere in this report, the amount required for this purpose is not considered sufficient to exert antimicrobial effects in the target animal. In order to ensure that the use of propyl 4-hydroxybenzoate remains consistent with the purpose for which it was evaluated a restriction to “for use as preservative only” is considered appropriate.

No additional relevant factors were identified for consideration of the risk management recommendations.

3.3. Elaboration of MRLs

The establishment of maximum residue limits for propyl 4-hydroxybenzoate and its sodium salt is not scientifically justified, as the data indicate that these substances will undergo rapid metabolism and elimination after administration to animals. Consumers are not expected to be exposed to significant levels of pharmacologically active residues. Consequently, a ‘No MRL required’ classification is recommended.

3.4. Considerations on possible extrapolation of MRLs

Since the “No MRL required” recommendation is for all food producing species no further extrapolation is possible.

3.5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- the toxicological ADI of 1.25 mg/kg bw (i.e. 75 mg/person) was established as the overall ADI for propyl 4-hydroxybenzoate and its sodium salt,

- propyl 4-hydroxybenzoate and its sodium salt are rapidly metabolised to 4-hydroxybenzoic acid and propanol in all species examined, which is further conjugated and rapidly eliminated, mostly in urine,
- 4-hydroxybenzoic acid and propanol are considered natural constituents in food of vegetable origin,

the Committee recommends the establishment of maximum residue limits for propyl 4-hydroxybenzoate and its sodium salt in accordance with the following table:

Pharmaco-logically active substance	**Marker residue**	**Animal species**	**MRLs**	**Target tissues**	**Other provisions**	**Therapeutic classification**
Propyl 4-hydroxy-benzoate and its sodium salt	NOT APPLICABLE	All food producing species	No MRL required	NOT APPLICABLE	For use as preservative only	NO ENTRY

4. Background information on the procedure

Submission of the dossier	20 May 2014
Steps taken for assessment of the substance	
Application validated:	11 June 2014
Clock started:	12 June 2014
CVMP opinion adopted:	6 November 2014