EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

19 November 2023
EMA/CVMP/505842/2023
Committee for Veterinary Medicinal Products

European public MRL assessment report (EPMAR)

Ketoprofen (Poultry)

On 19 October 2023, the European Commission adopted a Regulation[1] establishing maximum residue limits for ketoprofen in poultry, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Veterinary Medicinal Products.

Ketoprofen is intended for use in chickens for the treatment of inflammatory and painful conditions at an oral dose of 10 mg/kg bw/day for 5 days, administered in drinking water.

Huvepharma NV submitted to the European Medicines Agency an application for the extension of maximum residue limits on 14 December 2020. The substance was already included in Table 1 of the Annex to Regulation (EU) No. 37/2010 with a "No MRL required" classification for bovine, porcine and *Equidae* tissues.

Based on the original and complementary data in the dossier, the Committee for Veterinary Medicinal Products recommended on 12 May 2022 the extension of maximum residue limits for ketoprofen to poultry with a "No MRL required" classification. Four CVMP members did not support this recommendation and signed a divergent position.

The CVMP opinion was considered by the Standing Committee on Veterinary Medicinal Products at its meeting on 31 January 2023. As a result of the concerns raised by CVMP members in the aforementioned divergent position the Standing Committee was unable to support the CVMP opinion.

On 1 March 2023 the European Commission requested the CVMP to reconsider its opinion with a view to further examining the issues identified in the divergent opinion and indicated that if the proposed "No MRL required" status for poultry could not be further substantiated, then the CVMP was requested to recommend numerical MRLs for ketoprofen in chicken tissues.

On 16 May 2023 the Committee adopted a revised opinion recommending the extension of maximum residue limits for ketoprofen to poultry.

Subsequently the Commission recommended on 4 September 2023 that maximum residue limits in poultry are established. This recommendation was confirmed on 19 September 2023 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 19 October 2023.

[1] Commission Implementing Regulation (EU) No 2023/2194, O.J., of 20 October 2023

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Summary of the scientific discussion for the establishment of MRLs

Substance name:	Ketoprofen
Therapeutic class:	Nonsteroidal anti-inflammatory agents
Procedure number:	EMEA/V/MRL/003652/EXTN/0004
Applicant:	Huvepharma NV
Target species applied for:	Chicken
Intended therapeutic indication:	Treatment of inflammatory and painful conditions
Route(s) of administration:	Oral

1. Introduction

Ketoprofen is a non-steroidal anti-inflammatory drug belonging to the arylpropionic acid group. It is used in human and veterinary medicine for its anti-inflammatory, analgesic and antipyretic activities. It is indicated for inflammatory and painful conditions of the bones, joints and muscular-skeletal systems in cattle, horses, dogs and cats, for alleviation of pain associated with colic in horses and cattle, for reducing pyrexia and respiratory rate in case of respiratory infections in pigs and as supportive treatment of mastitis-metritis-agalactia syndrome in the sow. The dose in food producing species is 2 to 3 mg/kg bw by intravenous or intramuscular route.

Ketoprofen was previously assessed by the CVMP and a toxicological ADI of 5 µg/kg bw, i.e. 0.3 mg/person was established.

Currently ketoprofen is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 of 22 December 2009 in accordance with the following table:

Pharmaco-logically active substance	**Marker residue**	**Animal species**	**MRLs**	**Target tissues**	**Other provisions**	**Therapeutic classification**
Ketoprofen	NOT APPLICABLE	Bovine, porcine, *Equidae*	No MRL required	NOT APPLICABLE	NO ENTRY	NO ENTRY

Huvepharma N.V. submitted an application for the extension of MRLs for ketoprofen to chicken on 14 December 2020. Ketoprofen is intended for use in chickens for the treatment of inflammatory and painful conditions at an oral dose of 10 mg/kg bw/day for 5 days, administered in drinking water.

Based on its evaluation of the data submitted by the Huvepharma N.V., the Committee adopted an opinion on 12 May 2022 recommending the extension of maximum residue limits for ketoprofen to chicken and considered that the establishment of numerical MRLs for ketoprofen in chicken tissues is not necessary for the protection of human health. Furthermore, with reference to Article 5 of Regulation (EC) No 470/2009 and in line with the criteria laid down in Commission Regulation (EU) 2017/880, the Committee agreed to extrapolate the conclusions to poultry. Four CVMP members did not support the recommendation for a "No MRL required" classification and signed a divergent position.

The CVMP opinion was considered by the Standing Committee on Veterinary Medicinal Products at its meeting on 31 January 2023. As a result of the concerns raised by CVMP members in the

aforementioned divergent position the Standing Committee was unable to support the CVMP opinion. On 1 March 2023 the European Commission requested the CVMP to reconsider its opinion with a view to further examining the issues identified in the divergent opinion and indicated that if the proposed "No MRL required" status for poultry could not be further substantiated, then the CVMP is requested to recommend numerical MRLs for ketoprofen in chicken tissues.

The specific issues highlighted in the Commission's request to reconsider the CVMP opinion were drawn from the divergent position and related to:

1. the presence of an unidentified new metabolite (metabolite B) of ketoprofen in chicken tissues that, as stated in the divergent opinion, could pose a concern as it seems to make up a significant part of the total residue value (in some cases >10% and more than 100 µg/kg).

2. residue concentrations in tissues from the target population, that may exceed the ADI when metabolite B is taken into account and assuming that the metabolite has (at least) the same pharmacological and toxicological activity as ketoprofen. Furthermore, the divergent opinion noted that the two other major metabolites A and C (supposedly hydroxylated metabolites of ketoprofen) have not been fully identified/characterised either.

The divergent position argued that these metabolites should also be considered for consumer exposure, i.e. assuming the same toxicological and pharmacological activity as ketoprofen. Furthermore, it was noted that, as highlighted in the divergent position, 'No MRL required' substances are normally not included in residue control programs and therefore no monitoring of the possible occurrence of residues and the associated risk for consumers is performed. Any other legal use or inappropriate use of the VMP potentially leading to higher residues would not or would only exceptionally come to light and could lead to public health concerns.

2. Scientific risk assessment

This scientific risk assessment is based on the previous safety assessment carried out by the CVMP, and on new data in relation to the residues of ketoprofen in chickens. The data in relation to the safety of ketoprofen (i.e. pharmacological and toxicological data) have not been re-assessed.

2.1. Safety assessment

The CVMP has previously assessed the consumer safety of ketoprofen and established an overall ADI of 0.005 mg/kg bw, i.e. 0.3 mg/person, based on the BMDL5 of 0.04 mg/kg bw/day for kidney lesions in rats and a chemical specific uncertainty factor of 7.6.

No further assessment regarding the consumer safety of the substance is required for the purpose of this extension application.

2.2. Residues assessment

Only residues data that are relevant to this MRL-extension application to chickens are presented here.

2.2.1. Pharmacokinetics in target species

In a published study on the pharmacokinetic properties of ketoprofen in broiler chickens using intravenous and oral administration, both enantiomers of ketoprofen showed a fast but incomplete

absorption from the gastrointestinal tract, with an absolute oral bioavailability of 31.5% and 52.6% for *R*(-) and *S*(+) ketoprofen, respectively. Ketoprofen showed a limited distribution and was rapidly eliminated.

In a radiolabel study, [^{14}C]-ketoprofen was given in gelatine capsules to male and female Ross broiler chickens at a dose of 10 mg/kg bw/day for 5 days. The animals were slaughtered in groups at 1, 3, 6, 24, 48, and 72 hours after cessation of treatment. The total radioactive residue (TRR) in edible tissues (liver, kidney, muscle and fat plus skin) was quantified, as well as the concentration of ketoprofen and its main metabolite 2-[3-(alphahydroxybenzyl) phenyl]propanoic acid (referred to as OH-ketoprofen or RP 69400). The highest TRR concentrations were found in kidney, followed by liver and fat plus skin. Total residues in muscle were very low and only detectable up to 6 hours, with approximate mean residues of TRR (as µg ketoprofen equivalents per kg) of 80 and 40 at 3 and 6 hours, respectively. The approximate mean residues of TRR (as µg ketoprofen equivalents per kg) at 1, 3, 6 and 72 hours were 15700, 8420, 4600 and 1300 in kidney, 3750, 1970, 1080 and 330 in liver, and 1100, 770, 730 and 480 in fat plus skin. The approximate mean marker to total residue ratios at 3 hours were 0.37, 0.11, 0.13 and 0.05 and at 6 hours were 0.04, 0.03, 0.02, 0.003 for muscle, fat plus skin, liver and kidney, respectively. Ketoprofen was rapidly and extensively metabolised into OH-ketoprofen, into two further hydroxylated ketoprofen forms, and into other unknown metabolites. Ketoprofen and its hydroxylated forms were only detected during the first 6 hours in all tissues, with the exception of fat plus skin, where low levels of ketoprofen were detected up to 48 hours. One unidentified metabolite ("metabolite B") persisted longer and represented more than 10% of the radioactivity in most samples at later time points. This metabolite was characterised as very hydrophilic, and probably consisted of several compounds. Its chromatographic behaviour was inconsistent with known glucuronides of ketoprofen. It was concluded that metabolite B consisted of a number of very polar downstream metabolites with no structural resemblance to parent ketoprofen, and therefore without pharmacological activity. In an additional study with unlabelled ketoprofen given in the drinking water at a dose of 20 mg/kg bw/day for 5 days to Ross and Sasso broilers, metabolite B was not found. In this latter study, the main residues were parent ketoprofen, OH-ketoprofen, and the two aforementioned other hydroxylated forms.

2.2.2. Residue depletion studies

Depletion in tissues

In a cold residue depletion study, nine male and nine female broiler chickens were given ketoprofen in the drinking water at a target dose of 10 mg/kg bw/day for 5 consecutive days. Groups of 3 males and 3 females were slaughtered at 3, 12, and 24 hours after the cessation of treatment. Samples of liver, kidney, muscle, and fat plus skin were taken and analysed for ketoprofen and OH-ketoprofen using a validated UHPLC-MS/MS method with an LOQ of 10 µg/kg in all tissue matrices. The metabolite OH-ketoprofen was not found in any tissue at any timepoint. At 3 hours, ketoprofen was found in all fat plus skin samples (11.6 – 45.9 µg/kg) and 1 out of 6 kidney samples (11.6 µg/kg). At 12 and 24 hours, ketoprofen was only found in fat plus skin (2/6 samples at both time points, range 10.3 – 21.5 µg/kg).

The levels of ketoprofen in this study were lower than in the ADME study (see 2.2.1), that used the same oral dose and duration, although the method of administration was different (gavage versus drinking water). In addition, at 3 hours, the levels in liver and kidney were respectively 3 and 5 times higher than in fat plus skin in the ADME study, whereas in this cold study the levels in fat plus

skin were highest at this timepoint. An additional study was performed to explain these differences. The results of this study show that parent ketoprofen concentrations in the target tissue fat plus skin are highest and also comparable for both administration methods (i.e. by gavage and in drinking water). However, in the other tissues, in liver and kidney in particular, the residue concentrations following gavage are 3-4 times higher than with drinking water administration, albeit that this is largely based on residue concentrations between LOD and LOQ. The difference is explained by the fact that continuous exposure via drinking water results in a steady state, whereas short peak concentrations occur at approximately 6 hours after each gavage administration. This is not the case for fat plus skin, because of the longer retention in this tissue which causes accumulation. It can be concluded that the data from animals treated via drinking water are more representative for the field situation and should be considered in the establishment of MRLs.

Depletion in eggs

No residue depletion data were provided for ketoprofen in eggs from treated layer chickens.

Selection of marker residue and ratio of marker to total residues

In its opinion of 12 May 2022, the Committee considered that no MRLs were required for ketoprofen in chicken tissues and there was no need to identify a marker residue. For the risk assessment, the parent substance ketoprofen was considered the relevant residue of concern, because it is the only substance that contributes significantly to the pharmacological activity of the incurred residues in chicken tissues.

Ketoprofen has a toxicological ADI of 0.005 mg/kg bw, i.e. 0.3 mg/person, based on the BMDL5 of 0.04 mg/kg bw/day for kidney lesions in rats and a chemical specific uncertainty factor of 7.6 (as described in European Public MRL Assessment Report EMA/CVMP/94885/2020). In case of a toxicological ADI, it is often assumed that the total residue is the relevant residue for the risk assessment, because it is usually not known whether metabolites could have contributed to the toxicological effect. However, in the case of ketoprofen, the kidney lesions can be explained by the pharmacological mode of action, i.e. its binding to the cyclooxygenase receptor: COX inhibition leads to lower prostaglandin levels; prostaglandins are important mediators of renal vascular tone and sodium balance resulting in natriuresis because of both vasodilatory action and inhibition of Na^+ reabsorption (Rios et al., 2012[2]). Therefore, in its opinion of 12 May 2022, the Committee considered that the relevant residue for consumer risk assessment was the residue with pharmacological activity, i.e. the parent compound.

Following the request from the European Commission dated 1 March 2023 and having reflected on the concerns raised in the request, the Committee confirmed its original position that ketoprofen, as the residue with pharmacological activity, is the appropriate marker residue for monitoring. However, in line with the concerns raised in the divergent position the calculation of consumer intake of residues will take account of total residues, i.e. it will include all metabolites and consequently a ratio of marker to total residues is established. From the radiolabel study data, where ketoprofen was administered in gelatine capsules, the following ratios of marker to total residues of 0.043, 0.031, 0.02, 0.003 for muscle, fat plus skin, liver and kidney observed 6 hours after treatment were retained. The ratios of marker to total residues at 6 hours are considered to be sufficiently conservative for the purpose of consumer safety.

[2] Rios, A., Vargas-Robles, H., Gámez-Méndez, A. M., & Escalante, B. (2012). Cyclooxygenase-2 and kidney failure. Prostaglandins & other lipid mediators, 98(3-4), 86-90.

2.2.3. Monitoring or exposure data

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

2.2.4. Analytical method for monitoring of residues

A UHPLC-MS/MS method has been developed and validated for the determination of ketoprofen and OH-ketoprofen in muscle, skin plus fat, liver and kidney of chickens. This method was used in the residue studies described above. The method is presented in an internationally recognized format and has been validated in line with the current requirements of VICH Guideline 49. The method was sufficiently validated in the range of 10 – 1500 µg/kg.

Following the request from the European Commission dated 1 March 2023 and having reflected on the issue, the Committee reconsidered the available analytical method in view of the proposal to establish numerical MRLs (see section 3.2 below). The Committee concluded that the method can be considered appropriately validated. The relevant EURL has reviewed the method and is in agreement with this conclusion.

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

As the substance is not expected to possess antimicrobial activity no effects on microorganisms used for industrial food processing are expected.

2.2.6. Findings of EU or international scientific bodies

Ketoprofen has not been evaluated for this purpose by EU or international scientific bodies.

3. Risk management recommendations

3.1. Availability of alternative medicines and other legitimate factors

Availability of alternative medicines

At present, no oral NSAIDs are available for chickens.

Conditions of use

In the absence of residue data for eggs the use of ketoprofen should be restricted to animals not producing eggs for human consumption.

3.2. Elaboration of MRLs

Calculation of the theoretical daily intake of residues

In its opinion of 12 May 2022 the CVMP considered the following.

The highest tissue concentrations of parent ketoprofen (the residue of concern) were found at 3

hours after cessation of dosing in the pivotal residue depletion study in chickens treated via drinking water at the recommended dose and duration. Residues in muscle and liver were below the LOQ (10 µg/kg). Taking the highest residue levels seen at this timepoint the theoretical maximum consumer intake of residues of concern is calculated using the standard food basket:

Tissue	**Maximum concentration of ketoprofen (µg/kg)**	**Daily consumption (kg)**	**Intake (µg/day)**
Muscle	-	0.3	-
Fat + skin	45.9	0.09	4.131
Liver	-	0.1	-
Kidney	11.6	0.01	0.116
Total			4.274

Based on the above intake calculation the maximum intake of toxicologically relevant residues represents only 1.4% of the ADI. Even if residue levels at the LOQ in muscle and liver are factored into the calculation the intake calculation remains below 3% of the ADI. Therefore, in line with Commission Regulation (EU) 2018/782, it was concluded that the establishment of numerical MRLs is not needed for the protection of human health.

In its letter of 1 March 2023, the European Commission requested a review of the CVMP opinion with a view to further examining the issues identified in the divergent opinion and, if the proposed "No MRL required" status for poultry cannot be further substantiated, to recommend numerical MRLs for ketoprofen in chicken tissues.

Having reflected on the specific request of the European Commission, and noting the absence of additional data to substantiate the proposed "No MRL required" status for poultry (that is, in the absence of additional characterisation of metabolites A, B and C, further justification as to why the parent compound ketoprofen is considered the only relevant residue with pharmacological activity cannot be provided), the Committee decided to recommend numerical MRLs. Based on the available residue depletion data, and the ratios of marker to total residues at 6 hours, the following MRLs can be proposed:

Muscle, liver and kidney: 10 µg/kg

Skin and fat in natural proportions: 30 µg/kg

The calculation of the theoretical daily intake of residues is as follows:

Edible tissue or products	**Daily consumption (kg)**	**MRL proposal (µg/kg)**	**Ratio of the marker/total residue**	**Amount per edible tissue or product**
Muscle	0.30	10	0.0426	70.4
Fat	0.09#	30	0.0313	86.3
Liver	0.10	10	0.0195	51.3
Kidney	0.01	10	0.0034	29.4

fat and skin in natural proportion

Based on the above figures the maximum theoretical consumer intake is 237.4 µg, representing 79.1% of the ADI (of 300 µg/person). This calculation takes account of all residues and assumes that all metabolites possess the same pharmacological/toxicological activity as the parent substance.

Exposure to residues may also take place as a result of consumption of milk. Previously evaluated data (reported in European Public MRL Assessment Report EMA/CVMP/94885/2020) indicate that

ketoprofen residues were not detected in milk. Using the limit of detection of 25 µg/L to estimate potential worst-case exposure to residues via milk results in an intake value of 37 µg per person. When added to the intake from poultry tissues this represents 91% of the ADI, which leaves limited room for potential future uses of the substance.

4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EC) No 470/2009, the CVMP considered the possibility of extrapolating the maximum residue limits recommended for ketoprofen to other food producing species and commodities. Taking into account the provisions laid down in Commission Regulation (EU) 2017/880, and current scientific knowledge, the recommendations on extrapolation are justified as follows:

Animal species/ food commodities	**Extrapolation possible (Yes/No)**	**Justification**
Poultry eggs	No	According to article 7(f) of Commission Regulation (EU) 2017/880, extrapolation of MRLs from poultry tissues to poultry eggs shall not be carried out.
Poultry species other than chickens	Yes	In accordance with Commission Regulation (EU) 2017/880, an MRL can be extrapolated from a major species to a related species where the parent substance is the marker residue in the reference species. As such, the MRL recommendation for chickens should be extrapolated to poultry.

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

Having considered that:

- the toxicological ADI of 0.005 mg/kg bw (i.e. 0.3 mg/person) was established as the overall ADI for ketoprofen,
- oral administration of ketoprofen resulted in a fast absorption, limited distribution, and fast depletion,
- ketoprofen is extensively metabolised in chickens following oral administration,
- the calculated consumer intake of residues of ketoprofen from chicken tissues always remains well below the ADI,
- extrapolation from chicken to all poultry species is appropriate,
- with a view to facilitating official residue control activities numerical MRLs are recommended,
- ketoprofen is retained as the marker residue,
- the ratios of marker to total residues at 6 hours were 0.043 in muscle, 0.031 in fat plus skin, 0.02 in liver and 0.003 in kidney,
- an appropriately validated analytical method for the determination of ketoprofen in chicken tissues is available,

the Committee recommends the extension of maximum residue limits for ketoprofen to chicken.

Furthermore, with reference to Article 5 of Regulation (EC) No 470/2009 and in line with the criteria laid down in Commission Regulation (EU) 2017/880, the Committee recommends the extrapolation of maximum residue limits for ketoprofen to poultry species and the amendment of the entry for ketoprofen in table 1 of the Annex to Commission Regulation (EU) No 37/2010 as follows:

Pharmaco-logically active substance	**Marker residue**	**Animal species**	**MRLs**	**Target tissues**	**Other provisions**	**Therapeutic classification**
Ketoprofen	NOT APPLICABLE	Bovine, porcine, *Equidae*	No MRL required	NOT APPLICABLE	NO ENTRY	NO ENTRY
	Ketoprofen	Poultry	10 µg/kg 30 µg/kg 10 µg/kg 10 µg/kg	Muscle Skin and fat in natural proportion Liver Kidney	Not for use in animals from which eggs are produced for human consumption	NO ENTRY

The theoretical intake of residues from chicken tissues represents less than 80% of the ADI.

6. Background information on the procedure

Submission of the dossier	14 December 2020
Steps taken for assessment of the substance	
Application validated:	12 January 2021
Clock started:	13 January 2021
List of questions adopted:	12 May 2021
Consolidated response to list of questions submitted:	19 November 2020
Clock restarted:	22 November 2020
List of outstanding issues adopted:	20 January 2022
Oral explanation provided by applicant:	11 April 2022
Consolidated response to the list of outstanding issues	12 April 2022
Clock restarted:	13 April 2022
CVMP opinion adopted:	12 May 2022
Commission request for reconsideration of opinion:	1 March 2023
Revised CVMP opinion adopted:	16 May 2023