Biocidal Products Committee (BPC)

Opinion on a request according to Article 38 of Regulation (EU) No 528/2012 on

On the questions of unresolved objections during the mutual recognition procedure of a PT 3 biocidal product intended for disinfection of livestock animal housings and equipment, and animal transportation vehicles

ECHA/BPC/373/2023

Adopted

01 March 2023

Opinion of the Biocidal Products Committee

On the questions of unresolved objections during the mutual recognition procedure of a PT 3 biocidal product intended for disinfection of livestock animal housings and equipment, and animal transportation vehicles

In accordance with Article 38 of Regulation (EU) No 528/2012 of the European Parliament and of the Council 22 May 2012 concerning the making available on the market and use of biocidal products, the Biocidal Products Committee (BPC) has adopted this opinion on a question concerning an unresolved objection during a mutual recognition of a PT3 chlorocresol and L-(+)-lactic acid containing biocidal product intended for the disinfection of livestock animal housings and equipment, and animal transportation vehicles..

This document presents the opinion adopted by the BPC.

Process for the adoption of the opinion

ECHA received a request from the Commission on 24 November 2022. ECHA acts as the rapporteur in this type of procedures as agreed at BPC-3. The rapporteur presented the draft opinion to the BPC-46 meeting of 1 March 2023. Following the adoption of the opinion at BPC-46, the opinion was amended according to the outcome of the discussion.

Adoption of the opinion

Rapporteur: European Chemicals Agency (ECHA)

The BPC opinion was reached on 1 March 2023.

The BPC opinion was adopted by consensus.

The opinion is published on the ECHA website at: https://echa.europa.eu/bpc-opinions-on-article-38

Further details of the opinion and background

1. Request for the opinion

Article 38 of Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products (the "BPR") establishes that, if so requested by the Commission, pursuant to Article 36(2) or Article 37(2) of the BPR, the Agency shall issue an opinion within 120 days from the date on which the question was referred to it.

On 24 November 2022, ECHA received a request for a BPC opinion from the Commission to address the questions relative to unresolved objections during a mutual recognition procedure of a PT 3 biocidal product PHENOGEN intended for disinfection of livestock animal housings and equipment, and animal transportation vehicles.

The Commission has requested ECHA to formulate an opinion via the BPC on the following questions in order to decide on the authorisation of the product:

1. ECHA is requested to determine whether the experimental data from residue studies in pigs and poultry can be used for the refinement of livestock exposure assessment to PHENOGEN. ECHA is requested to assess what would be the conclusions as regards the risks for the consumers and whether the use of the product will lead to an exceedance of the default MRL of 0.01 mg/kg.

2. ECHA is requested to determine whether the dermal absorption value of 50% (EFSA guidance on dermal absorption (2017)) should be used for the livestock exposure assessment, and what would be the conclusions when using that value as regards the risks for the consumers and whether the use of the product will lead to an exceedance of the MRL of 0.01 mg/kg. If the dermal absorption value of 50% cannot be used, which dermal absorption value would be appropriate for the exposure assessment and what would be the conclusions as regards the residue levels in edible tissues from using that value and the potential risk to consumers.

3. Regarding the refinements proposed by Germany below, ECHA is requested to determine what would be the conclusions as regards the residue levels in edible tissues and the potential risk to consumers:

    a) The application of a default transfer coefficient for dislodgeable residues transferred from treated surfaces (ECHA Human Health Exposure Methodology, 2015). France did not agree with that option as this transfer coefficient is only applicable for human dermal exposure and not for livestock since these transfer coefficients represent transfer from treated surfaces to human hand skin.

    b) The application of empirical transfer factors from Leeman et al. study (2007) (EMA guideline on risk characterisation and assessment of maximum residue limits (MRL) for biocides, EMA/CVMP/90250/2010, 2015)) to estimate the maximum transfer of an external oral dose to livestock edible tissues. France agreed that is possible to apply this refinement.

The Commission further indicated that, when addressing the above-mentioned questions, the following elements should be taken into account by the BPC:

(a) The product assessment report (PAR) of the biocidal product PHENOGEN;

(b) The assessment report of the active substance (Chlorocresol);

(c) The discussions on the assessment of PHENOGEN that took place during the Coordination Group and discussions on a similar product containing chlorecoresol held in the Biocidal Products Committee on Union authorisations.

2. Background

Biocidal product PHENOGEN is a soluble concentrate disinfectant that was authorised by MSCA FR (reference Member State (rMS)) under the National authorisation procedure in accordance with Article 30 and 33(4) of the BPR. It is a PT3 product containing Chlorocresol and L-(+)-lactic acid.

It is used for the disinfection of equipment and livestock animal housings (cattle, pigs, poultry), including poultry hatcheries, by spraying on surfaces. It is also used for the disinfection of animal transportation vehicles by spraying. It contains 3 authorised uses.

The product is reported to act by reducing the number of the relevant target organisms under defined conditions. In detail, its action targets:

- viable bacterial cells (bactericidal activity);
- Yeast cells (yeasticidal activity);
- moulds spores (fungicidal activity);
- oocyste cells (oocydal activity);
- Cryptosporidium parvum oocyste cells (oocydal activity);
- Viruses (virucidal activity).

The referral of the disagreement on the evaluation of PHENOGEN was submitted on 25 November 2021 by the initiating concerned Member State (icMS) DE to the Coordination Group (CG), in accordance with Article 35(2) of the BPR. The referral was discussed during two additional CG meetings on 12 and 25 January 2022. During the discussions, both points of disagreement remained unresolved. The unresolved disagreement points are related to the refinement of livestock exposure based on experimental data and on default dermal absorption value. A third point of disagreement was whether to consider in the assessment the exceedance of the default MRL value. This is a regulatory issue and therefore, it was not part of the request of the Commission to the Agency in line with Article 36(2) of the BPR, according to which, the Commission may ask the Agency for an opinion on scientific or technical questions, and not on regulatory issues which are outside the remit of the BPC.

The two points of disagreements were as follows:

1) The experimental data from residue studies in pigs and poultry are not suitable for refinement of livestock exposure assessment due to the fact that no metabolites of chlorocresol were measured and only 1 of the 3 studies covers the application rate of PHENOGEN. As estimation of residues in edible tissues exceeds the default MRL of 0.01 mg/kg established for chlorocresol in Art. 18(1)(b) of Regulation (EC) No 396/2005, alternative refinements (i.e. RMMs, or the application of default transfer coefficient for dislodgeable residues transferred from treated surfaces together with the application

of empirical transfer factors from Leeman et al. study (2007)) should be considered in order to reduce the estimated livestock exposure below the default MRL.

2) As the default dermal absorption value of 50% only applies to human skin and there is no scientific proof that this value can be used for animals, it is not suitable for the refinement of the livestock exposure assessment. Thus, alternative refinements (i.e. RMMs, or the application of default transfer coefficient for dislodgeable residues transferred from treated surfaces together with the application of empirical transfer factors from Leeman et al. study (2007)) should be considered in order to reduce the estimated livestock exposure below the default MRL.

It is stressed that the use of the experimental data from residue studies is needed to refine the exposure at levels below the default MRL of 0.01 mg/kg, which is exceeded even if all of the alternative refinements are applied.

During the referral discussions, the rMS FR argued that:

- regarding the 1st point of disagreement: Considering the properties of the active substance it is unlikely that metabolites will be produced. In the CAR, the experimental residue studies were used for the assessment of the active substance to conclude that no residues were expected in livestock edible tissue after use of chlorocresol at a rate of 2000 mg/m2 (CAR). As application rate intended in PHENOGEN dossier is lower than the one assessed at EU level for the active substance approval (maximum of 1200 mg/m2), FR CA followed the approach detailed in the CAR.

- regarding the 2nd point of disagreement: Considering the default dermal absorption value for water-based dilutions of 50 % (EFSA guidance on dermal absorption (2017)), this factor has also been used in the CAR for both DRA and animal health. Therefore, FR CA was of opinion that it is applicable to refine livestock exposure calculation. Moreover, in absence of data about livestock dermal absorption, FR CA was of the opinion that this default factor could apply and is worst case, taken into account skin thickness and animal fur.

During the referral, the Member States agreed that the empirical transfer factors from Leeman et al. study (2007)) should be applied for the refinement of the livestock exposure. However, no agreement was reached on whether:

- the experimental data from the 3 residue studies in pigs and poultry are suitable for refinement of livestock exposure assessment;

- the default dermal absorption value of 50% is suitable for the refinement of the livestock exposure assessment;

- the MRL of 0.01 mg/kg established for chlorocresol in Art. 18(1)(b) of Regulation (EC) No 396/2005 should be considered.

As the CG did not reach a consensus agreement for the above mentioned two disagreement points, the rMS referred the unresolved objection to the Commission in accordance with Article 36(1) of the BPR.

3. Answers to the questions from the Commission

The opinion of the BPC has considered:

- the background information provided by the Commission in the opinion request;
- the Product Assessment Report (PAR) of the product in question;
- the discussion that took place on 08/12/2022 during the HH WGIV2022 meeting;
- the comments received by DE, NL, FR, SI, AT, DK and the applicant during the e-consultation with the members of the Human Health Working Group on the Article 38 mandate for PHENOGEN.

Question 1

ECHA is requested to determine whether the experimental data from residue studies in pigs and poultry can be used for the refinement of livestock exposure assessment to PHENOGEN. ECHA is requested to assess what would be the conclusions as regards the risks for the consumers and whether the use of the product will lead to an exceedance of the default MRL of 0.01 mg/kg.

ECHA considers that the experimental data from residue studies in pigs and poultry **can be used** for the refinement of livestock exposure assessment to PHENOGEN **under the condition that a rinsing step is introduced before letting the animals enter the facilities** in the instructions of use. This step is needed in order to reduce the uncertainty from using residue studies with highest application rate than PHENOGEN and from the missing data on livestock metabolism.

The rinsing step is considered to have 90% efficiency for water soluble compounds such as chlorocresol (solubility 3.4 g/L at 20°C pH 7) and therefore, it is assumed that 10% of the applied chlorocresol will remain in the disinfected facilities. This reduction of the levels of chlorocresol enables using the residue studies.

It is noted that the maximum application rate of PHENOGEN is:

- 1200 mg CMK/m2 (disinfection of animal housing);
- 500 mg CMK/m2 (disinfection of transport vehicle).

This rate is covered only by one of the residue studies, ie the Anonymous, 2011 (appl. rate 2.900 mg CMK/m2), whereas it is not covered by Anonymous, 2012a, b (200 mg CMK/m2 and 290 mg CMK/m2). With the introduction of the rinsing step, it is assumed that 90% of chlorocresol will be washed off and therefore, the amount of chlorocresol left will be at levels that are covered by all three residue studies.

ECHA is of the opinion, that although a study measuring the efficiency of the rinsing step is missing, it is reasonable to assume a 90% reduction in the case of chlorocresol.

Notably, the BPC opinion for chlorocresol (PT3, 2016) notes: "*an updated assessment of the risk in food and feed areas may be required at product authorisation where use of the product may lead to contamination of food and feeding stuffs*". The request of these studies along with a study on the rinsing efficiency can also be considered at the renewal of approval for chlorocresol.

The applicant indicated during the WG-IV-2022 discussion and in its comments in the e-consultation that, although they share the rMS FR view that the experimental residue studies can be used without inclusion of rinsing step, if rinsing is considered necessary by BPC, they agree with its use.

Detailed assessment of the question is provided in Annex I.

Question 2

ECHA is requested to determine whether the dermal absorption value of 50 % (EFSA guidance on dermal absorption (2017)) should be used for the livestock exposure assessment, and what would be the conclusions when using that value as regards the risks for the consumers and whether the use of the product will lead to an exceedance of the MRL of 0.01 mg/kg. If the dermal absorption value of 50 % cannot be used, which dermal absorption value would be appropriate for the exposure assessment and what would be the conclusions as regards the residue levels in edible tissues from using that value and the potential risk to consumers.

ECHA suggests that a protection factor for furs and feathers of 50 % can be used, whereas the livestock dermal absorption should be considered 100%. The protection factor for furs and feathers of 50% (i.e. the 'system availability' value) is the (worst-case) sum of the extent to which dried residues are dislodged from treated surfaces by feathers or fur (or skin; domesticated pigs are generally 'furless') plus the extent to which the dislodged material passes through the layer of feathers or fur to reach the skin.

This proposal is in line with the one made by icMS DE and with the conclusion on the same question of HH WG-I-2022 for a Union Authorisation PAR of a chlorocresol BPF with uses in housing disinfection and transport vehicles. The WG concluded on dermal absorption value of 100%, and systemic availability of 50% of the material applied on the animal. It should be noted that the rMS FR has applied 50% as dermal absportion value in the PAR of PHENOGEN (June, 2022). This has to be corrected.

ECHA also notes that agreement is needed at HH WG level on the dermal absorption of different livestock animals and the material provided by AT at the present e-consultation will be used to this purpose.

Question 3

Regarding the refinements proposed by icMS DE below, ECHA is requested to determine what would be the conclusions as regards the residue levels in edible tissues and the potential risk to consumers:

a) The application of a default transfer coefficient for dislodgeable residues transferred from treated surfaces (ECHA Human Health Exposure Methodology, 2015). France did not agree with that option as this transfer coefficient is only applicable for human dermal exposure and not for livestock since these transfer coefficients represent transfer from treated surfaces to human hand skin.

b) The application of empirical transfer factors from Leeman et al. study (2007) (EMA guideline on risk characterisation and assessment of maximum residue limits (MRL) for

biocides, EMA/CVMP/90250/2010, 2015)) to estimate the maximum transfer of an external oral dose to livestock edible tissues. France agreed that is possible to apply this refinement.

ECHA notes that the question on the transfer coefficient for the residues on animal skin has been addressed in the reply to the 2nd question. The value agreed at WG-I-2022 is 50% (the agreed term was “systemic availability”).

During the e-consultation, icMS DE asked for a clear position on whether the default transfer coefficients are applicable for livestock, since they have been used for the refinement of livestock exposure calculations for various biocidal product applications. ECHA notes that the outcome of the exposure assessment with the 60% value proposed by DE remains the same. ECHA proposes to use the value of 50% agreed recently at HH WG-I-2022 in the context of the Union Authorisation of a chlorocresol BPF, and to have a discussion at Human Health WG level in order to agree on which value is the more appropriate.

Regarding the application of empirical transfer factors based on the Octanol–Water Partition Coefficient from Leeman et al. 2007 in order to estimate the maximum transfer of an external oral dose to livestock edible tissues, there was agreement at the Coordination Group on their use and these factors have already been used in the PHENOGEN PAR.

Overall conclusion

ECHA would like to point out the following:

- without any refinement, the consumer exposure does not exceed the ADI (adult 24 % ADI, child 80 % ADI);
- with all the above refinements, including rinsing step, but without the use of experimental residue studies, the livestock exposure will still exceed the default MRL value of 0.01 mg/kg for most animals.

Therefore, even without the use of residue studies, there is no concern for human health, but there is exceedance of the default MRL established in Art. 18(1)(b) of Regulation (EC) No 396/2005.

Overall, the use of the following data, measures and parameters is proposed in the dietary risk assessment of PHENOGEN in order not to exceed the default MRL of 0.01 mg/kg:

- rinsing step before the entry of livestock in the disinfected facilities;
- use of experimental data from the residue studies in livestock;
- 50% systemic availability (and residues transfer coefficient) of chlorocresol and 100% dermal absorption for livestock animals.

o0o

Annex I

ECHA Assessment on Question 1

The assessment of the residue studies[1] is included in Doc IIB of chlorocresol and is provided in Annex II.

ECHA summarises in Table 1 below the limitations in using the residue studies for refinement of livestock exposure and the arguments raised by rMS FR, icMS DE, other MSs and the applicant. A proposal on how to address the concern is also provided.

Table 1: Assessment of limitations in the use of livestock residue studies for refinement of chlorocresol (CMK) residue levels from the use of PHENOGEN.

Limitation	Counterargument to address limitation	ECHA remarks	Concern	Proposal to address concern
Max. application rate of PHENOGEN is: - 1200 mg CMK/m2 (disinfection of animal housing) - 500 mg CMK/m2 (disinfection of transport vehicle) Covered only by the Anonymous, 2011 (appl. rate 2.900 mg CMK/m^2). Not covered by Anonymous, 2012a, b (200 mg CMK/m2 and 290 mg CMK/m^2)	These residue studies were used for the refinement of the representative product in chlorocresol CAR (2017) with application rate of 2000 mg CMK/m^2.	The BPC opinion for chlorocresol (PT3, 2016) notes: "*an updated assessment of the risk in food and feed areas may be required at product authorisation where use of the product may lead to contamination of food and feeding stuffs*". Changes from the assessment in the CAR are expected, if scientifically justified.	High	Use rinsing step with default 90% efficiency for water soluble compounds such as chlorocresol (solubility 3.4 g/L at 20°C pH 7), before letting the animals enter the facilities. This reduces the levels of chlorocresol and enables using the residue studies.
Absence of data on livestock metabolism. Metabolism studies in rats show that chlorocresol is intensively metabolised (see Annex III).	The recovery of a.s. is ≥100% in the residue studies. Chlorocresol has no potential for accumulation in vivo	≥100% recovery of a.s. could have been overestimated. The quote from Doc IIB in Annex II, notes that the validation of the method is insufficient compared to requirements.	High	Use the rinsing step as Risk Mitigation Measure to result in 90% decrease in CMK residues.

[1] The applicant of PHENOGEN has access to data on the active substance chlorocresol with a Letter of Access from one applicant of CMK.

Limitation	Counterargument to address limitation	ECHA remarks	Concern	Proposal to address concern
No metabolites were analysed in the residue studies (Annex II).	(see Annex II). The phenolic OH group makes chlorocresol a ready target for conjugation and subsequent excretion.	The metabolic profile of CMK in livestock and the levels of its metabolites are unknown.		
No residue studies in transport vehicles, it is questionable if studies in animal housing can be used since: Residues measured > 35 days after exposure, ie weeks after exposure and before slaughter. For disinfection of vehicles, farm animals are usually transported only to the slaughterhouse and exposed to PHENOGEN only hours before the slaughter. Excretion rate of ~85% after 24hrs and 99% after 7 days (Annex III), thus, some metabolites could still be in the tissues.	The application rate of PHENOGEN in transport vehicles is low (500 mg CMK/m2)	It is possible that CMK or its metabolites are in the animal tissues before slaughter.	Medium	Use the rinsing step as Risk Mitigation Measure to result in 90% decrease in CMK residues. Due to the already low application rate of CMK in transport vehicles, the amount left after rinsing is expected to be very low.
No analysis of residues in eggs and milk	-	The residue study in poultry shows no residues at LOQ. As no residues were detected in the animals themselves, the concern of having CMK residues in eggs is low. Regarding milk and residues in cattle, the Table in Annex IIII taken from PHENOGEN PAR shows that in accordance with the	Low	A rinsing step would cover the concern by decreasing the livestock exposure to levels below the residue studies.

Limitation	Counterargument to address limitation	ECHA remarks	Concern	Proposal to address concern
		parameters from BPR guidance[2], the estimated CMK residues in muscle, fat, liver and kidney of fattening pig are higher than the residues in the same tissues in cattle and chicken and higher than in eggs and milk.		
No residue studies in cattle	Pigs constitute a worst-case species when estimating livestock exposure due to their assumed behaviours and subsequent exposure to surface residues	The counterargument assumes that the dermal exposure of pigs to chlorocresol residues would be higher than other livestock, because of pigs behaviours like rubbing and scratching on surfaces, but no data have been submitted to support this. Regarding the residue levels in different organs/tissues in fattening pig and cattle, see row above.	Low	A rinsing step would cover the concern by decreasing the livestock exposure to levels below the residue studies.

[2] Guidance on the Biocidal Products Regulation -Volume III Human Health - Assessment & Evaluation (Parts B+C) -6. Guidance on Estimating Livestock Exposure to Active Substances used in Biocidal Products

Annex II Residue studies (Quote from Doc IIB)

"*The experimental studies measure the level of CMK in pig (anonymous, 2012a; anonymous, 2011) and broiler (anonymous, 2012b) tissues after rearing on an area treated with a disinfectant containing CMK alone or CMK and 2-benzyl-4 chlorophenol.*

The objective of these 3 studies was to investigate the magnitude of CMK residue in the edible parts of fattening pigs (meat, fat, liver, kidney, skin) and broiler chickens (meat, liver, skin and fat) after one single application in the shed (except for the third study in which a disinfection occurred before each pen transfer, ie 4 disinfections). In all the studies, the shed was disinfected with a ready-to-use solution containing CMK. After drying, pigs or chickens were introduced and fed. Details are presented in the table below.

Table: Summary of 3 residue studies

Study	***Anonymous, 2012a***	***Anonymous, 2012b***	***Anonymous, 2011***
GLP	*No*	*No*	*No*
Number of animals slaughtered for analysis	*5 fattening pigs*	*15 broiler chicken*	*1 pig*
Total number of animals in the shed	*620 pigs introduced* *605 pigs at slaughter stage*	*23300 chicken introduced* *22055 chicken at slaughter stage*	*700*
Area treated (m²)	*983*	*3000*	*20.4*
Product applied	*275 L ready-to-use solution*	*800 L ready-to-use solution*	*240 g of concentrate*
Product concentration in CMK	*0.72 g/L CMK*	*1 %*	*250 g/L*
Application rate (g CMK/m²)	*0.2*	*0.27*	*2.9*
Other substance applied	*0.1 g/m² 2 benzyl 4 chlorophenol*	*0.13 g/m² 2 benzyl 4 chlorophenol*	*-*
Drying period	*16 hours*	*4 days*	*6 hours*
Age of animals introduced	*28 days*	*1 day*	*0 day (disinfection before each pen*

Study	***Anonymous, 2012a***	***Anonymous, 2012b***	***Anonymous, 2011***
			transfer – farrowing crate, flat deck, pre-fattening and fattening pen)
Feeding and rearing period	*42 days*	*35 days*	*221 days*
Age and average weight of animal at slaughter	*70 days, 50 kg*	*35 days, 1857 g*	*219 days, 98 kg*
Tissues analyzed	*Meat, fat, liver, kidney, skin (from 5 animals)*	*Meat, skin and fat, liver (from 15 animals)*	*Meat, fat, liver, kidney, skin*
Analytical method	*GC/MS/MS*	*GC/MS/MS*	*GC/MS/MS*
Limit of quantification (LOQ)	*0.01 mg/kg*	*0.01 mg/kg*	*0.01 mg/kg (meat, fat, skin)* *0.1 mg/kg (kidney and liver)*
Residue levels	*<LOQ for each tissue*	*<LOQ for each tissue*	*<LOQ for each tissue*

In the two first studies, recoveries were above 100% (130%). Therefore, even if validation of the method is insufficient compared to requirements, as no residue were observed, it can be concluded that it is really a no residue situation because the method seems to overestimate the residue level. However, these 2 studies have been performed with an application rate 10 times lower than the intended one. Even though the application rate in animal houses is 10 times higher, it can be assumed that there could be 10 times more residues in animal tissues than levels measured in the two first studies. Therefore, a residue level of 0.1 mg/kg could be expected in animal tissues at the intended application rate. This value of 0.1 mg/kg has been taken into account for the risk calculation (see doc IIC). In the third study performed at 1.5 N, all residue levels were below LOQ of 0.01 or 0.1 mg/kg depending on the tissue considered. The value of 0.1 mg/kg has been considered as a worst case.

Milk and eggs were not considered in these residue studies.

An additional study on chicken has been submitted late and has not been evaluated (see doc IIIA 6 15 4). However, results do not seem to be different than those from the other study performed on chicken. This study does not question the assessment performed in doc I, IIB and IIC.

CMK has no potential for accumulation in vivo as was shown in an ADME study in rats (see tox). The phenolic OH group makes CMK a ready target for conjugation and subsequent excretion. This general metabolic fate of CMK is going to be conserved among mammalian species, including pigs and ruminants".

Annex III TOXICOKINETICS, METABOLISM AND DISTRIBUTION Quote from Doc IIA (2016)

(cf. Doc. III-A Section 6.2)

Table: Toxicokinetic and metabolism studies with CMK

Route	**Method** **Guideline**	**Species** **Strain** **Sex** **No/group**	**Label**	**Dose levels**	**Reference** (Doc. IIIA)
Oral gavage	ADME study OECD 417	Rat, Wistar ♂+♀, 4 per sex	4-Chloro-3-methyl[U-^{14}C]phenol	Single dose: 300 mg/kg bw	**KEY STUDY** **6.2/04** **RI - 1**
Oral gavage	Excretion study No Guideline, Non-GLP	Rat, Wistar II ♂, 5	Unlabelled CMK	Single dose: 300 mg/kg bw	**6.2/01** **RI - 3**
Oral, in diet	Tissue study No guideline Non-GLP	Rat, Wistar TNO / W74 ♂, Tissue study: 6/sex/timepoint	Unlabelled CMK	150, 500,1500 ppm in diet	**6.2/02** **RI - 3**

Absorption, distribution, metabolism and excretion of CMK were investigated in rats in a recent study, required by RMS (doc IIIA6.2/04). This study was considered as the key study as it was a recent study and well conducted.

[^{14}C]-CMK was administered to male and female Wistar rats by *oral* gavage at a dose level of 300 mg/kg bw (doc IIIA6.2/04). Radioactivity was rapidly excreted in urine and faeces. Within 24 hours after administration, 85.21% and 84.30% of the administered dose was excreted in urine of male and female rats, respectively. During the same period of time 3.70% and 1.44% was excreted in faeces in male and female rats, respectively. The radioactivity excreted in the expired air was low (< 1%). After 7 days almost the entire administered dose (99%) was excreted and only a very low amount of radioactivity (< 1%) was found in the remaining carcass and GI-tract. The investigation of the metabolite pattern in urine and faeces revealed that the test item was extensively metabolized. In total, about 10-14% of the radioactivity was found as unchanged parent in urine and faeces. The majority of radiolabelled metabolites were excreted with the urine. The urinary metabolite pattern consisted of at least 5 metabolite fractions. It was dominated by two major fractions, i.e. U4 (37-39% of the dose) and U5 (41-46% of the dose). U5 is tentatively identified as CMK sulphate, whereas U4 is likely to represent the glucuronide of CMK. U3 fraction corresponds to the parent compound since it co-elutes with CMK standard. In the faecal metabolite pattern the major fraction was found as unchanged parent, F3, i.e. 3-5% of the dose and corresponds to 93.3% of faecal residues collected between 0-24h. Other faecal metabolites were only prominent in the second pool of faeces specimens collected between 24 and 48 h. The retention time of F5 (12.1 min) comes

closest to the one determined for U4 (11.5 min). Based on this similarity and on the fact that glucuronides are also excreted via bile, F5 is likely to be CMK glucuronide.

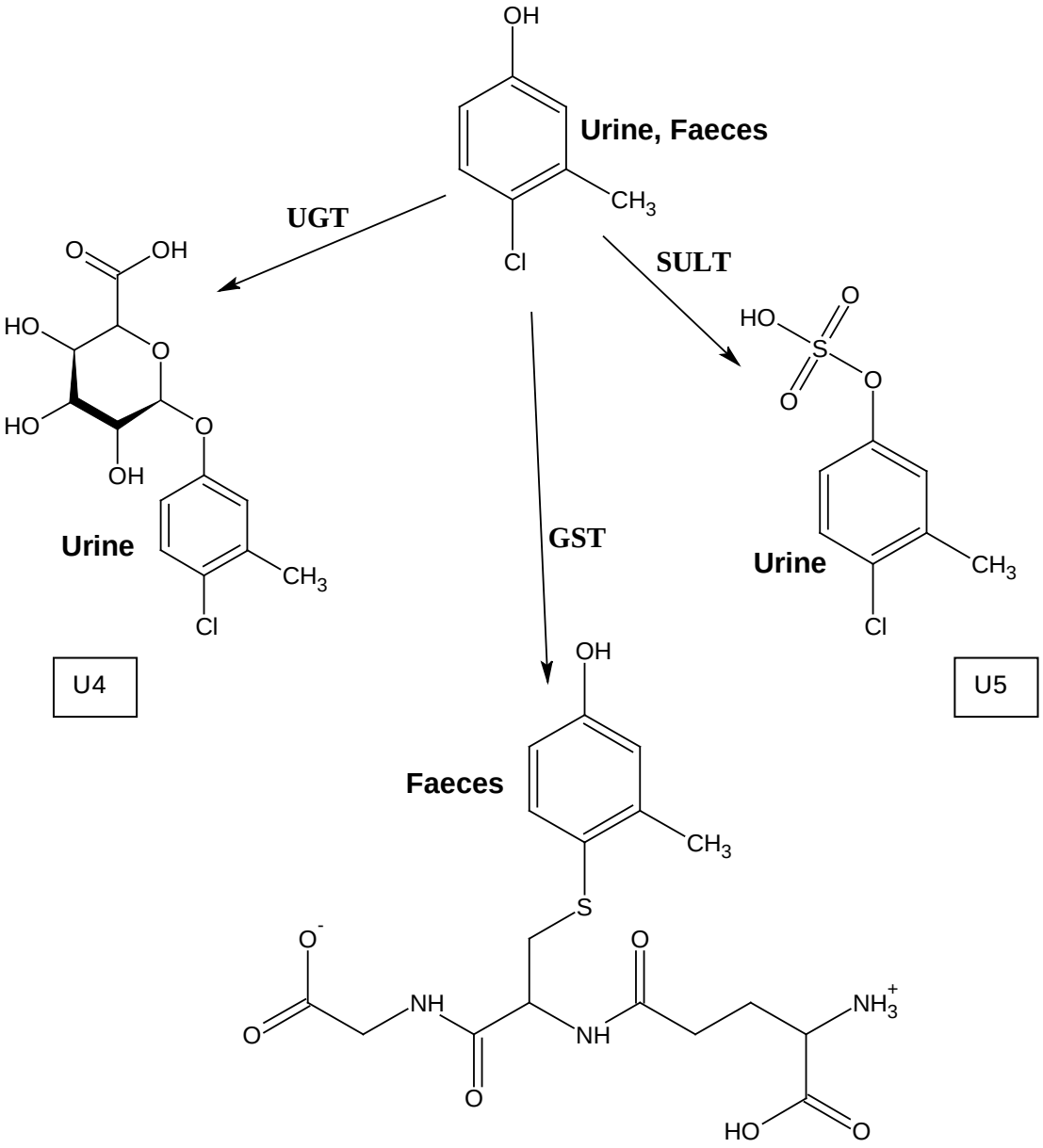
From this study, an oral absorption percentage of 100% has been chosen to set the systemic NOAEL as 85% of the administered dose was recovered in urines 24h after administration and as it was decided by the TM that from this percentage, an oral absorption of 100% could be considered.

Two other oral studies are presented as supportive information because of their reliability of 3.

Previously, excretion was investigated in another study (doc IIIA6.2/01). After a single oral dose of unlabelled test substance to male Wistar rats, 62.7% of the total applied dose was excreted via the urine within 24 hours after application. Small quantities were detected up to 72 hours after application. Two polar metabolites were also detected in the urine. The faeces represented the minor excretion route with a mean value of only 0.4% of the applied dose excreted within 24 hours after application. These results suggest that there is no accumulation of CMK in fatty tissues. This excretion study is not considered sufficiently reliable for evaluation of the toxicokinetic profile of the tested substance as more than 30 % of the absorbed substance is not clearly recovered and identified within the excretion process.

The absence of accumulation of CMK is confirmed in a 13-week feeding study, with doses of 150, 500 and 1500 ppm CMK in diet, performed on male Wistar rats. Indeed, analysis of liver and fatty tissues after 1, 4, 8 and 13 weeks showed no accumulation of the test substance in theses tissues (doc IIIA6.2/02).

Proposed metabolic pathways for CMK


OH
Urine, Faeces
CH3
Cl
UGT
SULT
GST
O
OH
HO
O
HO
O
OH
Urine
CH3
Cl
U4
HO
O
S
O
O
Urine
CH3
Cl
U5
OH
Faeces
CH3
S
O-
O
NH
NH
NH3+
O
O
HO
O

Annex IIII Residues in livestock tissues (Quote from page 84 PHENOGEN PAR June 2022)

Scenario [DRA-1] – animal housing disinfection – residues in livestock tissues (mg/kg)

				Residues in livestock tissues					
Animal Species		**Sum Oral Exposure**	**Sum Dermal and Inhalative Exposure**	**Eggs**	**Milk**	**Muscle**	**Fat**	**Liver**	**Kidney**
Beef cattle	-	0.348	1.476			1.48	1.48	1.49	1.49
Dairy cattle	-	0.714	1.325		1.33	1.34	1.34	1.35	1.35
Calf	-	0.737	2.227			2.24	2.24	2.26	2.26
Fattening pig	-	1.343	2.304			2.33	2.33	2.36	2.36
Breeding pig	-	0.314	1.656			1.66	1.66	1.67	1.67
Breeding pig	individual housing	1.934	0.000			0.04	0.04	0.08	0.08
Breeding pig	group housing	2.286	0.000			0.05	0.05	0.09	0.09
Sheep	-	1.087	0.013		0.02	0.03	0.03	0.06	0.06
Lamb	-	2.038	0.014			0.06	0.06	0.10	0.10
Slaughter goat	-	6.271	5.898			6.02	6.02	6.15	6.15
Lactating goat	-	1.165	3.288		3.30	3.31	3.31	3.33	3.33
Broilers	-	0.000	0.010			0.01	0.01	0.01	0.01
Broilers	free range, litter								

				Residues in livestock tissues					
Animal Species		**Sum Oral Exposure**	**Sum Dermal and Inhalative Exposure**	**Eggs**	**Milk**	**Muscle**	**Fat**	**Liver**	**Kidney**
	floor								
Broilers	parent broilers, free range (grating floor)								
Broilers	parent broilers in rearing, free range (grating floor)								
Laying hen	-	0.694	0.009	0.099		0.02	0.02	0.04	0.04
Laying hen	battery			0					
Laying hen	free range (litter floor)			0					
Laying hen	free range (grating floor)			0					
Turkey	-	0.000	0.007			0.01	0.01	0.01	0.01
Horse	-	0.000	2.072			2.07	2.07	2.07	2.07
Rabbit	-	0.000	0.030			0.03	0.03	0.03	0.03
			Maximum	**0.099**	**3.30**	**6.02**	**6.02**	**6.15**	**6.15**