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Halquinol

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Halquinol

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Identity

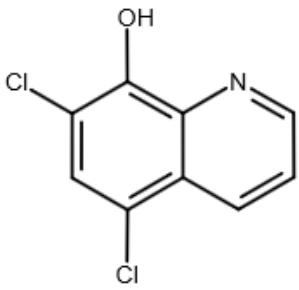
International Non-proprietary Names (INN): Halquinol

Synonyms: Halquinol BP 80, Chloroquinol, CHQ

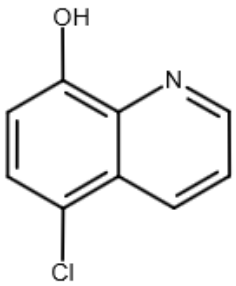
IUPAC name: 5,7-dichloro-8-hydroxy quinoline; 5-chloro-8-hydroxy quinoline; 7-chloro-8-hydroxy quinoline

Chemical abstract service N°: 8067-69-4; 130-16-5; 876-86-8

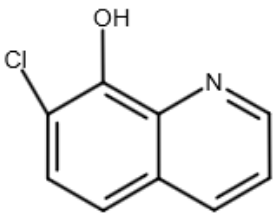
Structural formula: Structures of 5,7-dichloro-8-hydroxy quinoline (5,7-DCL), 5-chloro-8-hydroxy quinoline (5-CL) and 7-chloro-8-hydroxy quinoline (7-CL), are shown below:



5.7-DCL



5-CL



7-CL

Molecular formula: The molecular formulas of the three constituent molecules of halquinol are:

5,7-dichloro-8-hydroxy quinoline: $C_9H_5Cl_2NO$

5-chloro-8-hydroxy quinoline and 7-chloro-8-hydroxy quinoline: C_9H_6ClNO

Molecular weight: The molecular weights of the three constituent molecules of halquinol are:

5,7-dichloro-8-hydroxy quinoline: 214.1

5-chloro-8-hydroxy quinoline and 7-chloro-8-hydroxy quinoline: 179.6

Other information on identity and properties

Pure active ingredient: Halquinol

Appearance: Cream to pale green, fine powder

Melting point: 145-165 °C

Solubility: Insoluble in water; soluble in 250 parts ethanol (96 %), in 130 parts ether and in 50 parts chloroform

Residues in food and their evaluation

Conditions of use

Halquinol (trade name Quixalud®) is an antimicrobial used as a feed additive for poultry and as a growth promotant in pigs. Halquinol also is used in swine for the control, treatment and prevention of scours caused or complicated by *E. coli* and *Salmonella spp*. Halquinol for swine is approved in multiple Asian and Latin American countries and feed concentrates range from 12-60 % halquinol, with varying dose regimens and withdrawal periods (Table 1).

Table 1. Halquinol Product Approvals and Withdrawal Periods

Country	Dose (ppm)	Indication	Withdrawal Period
Quixalud 60 %			
Philippines	100-200	Treatment and prevention of chronic swine scour and diarrhea. Treatment of mold infection.	Standard of 5-7 days as required by local authority
Taiwan	500-600	Treatment and prevention of chronic swine scour and diarrhea. Treatment of mold infection.	2 days
Thailand	120	Prevention and treatment of scours in pigs associated with *Salmonella* and *E. coli*.	7 days
Brazil	60	Prevention and treatment of scours in pigs associated with *Salmonella spp.* and *E. coli.*	4 days
Argentina, Bolivia, Paraguay	100-200	Treatment of diarrhea, specifically due to *E. coli* and *Salmonella spp.*, but also diarrhea caused by diet, fungi or protozoa. Antidiarrheal agent with active improvement of performance.	No withdrawal period
Colombia	60-120	Growth promoter and feed efficiency improver.	No withdrawal period
Quixalud 60 NF			
Colombia, Ecuador, Peru	60-120	Control and treatment of diarrhea due to or complicated by *E. coli* and *Salmonella spp.*, but also unspecific	4 days

		diarrhea caused by fungi (*Candida albicans, Aspergillus spp.*) and protozoa (*Entamoeba spp., Trichomonas spp.*)	
Quixalud 12 %			
Peru, Panama, Ecuador, Venezuela, El Salvador, Nicaragua, Honduras, Costa Rica, Colombia, Dominican Republic, Guatemala	60-120	Growth promoter and feed efficiency	No withdrawal period
Peru, Panama, Ecuador, Venezuela, El Salvador, Nicaragua, Honduras, Costa Rica, Colombia, Dominican Republic, Guatemala	600	Treatment of chronic diarrhea	No withdrawal period
Other Product Names			
India	Feed Supplement	Feed supplement	No withdrawal period
Vietnam	120	Prevention and treatment of scours in pigs associated with *Salmonella* and *E. coli*.	7 days

The sponsor reported that halquinol has been approved for over a decade in Thailand, Vietnam, Indonesia and Taiwan and 6 years in the Philippines. During the initial registration of halquinol in these countries, human food safety data (including ADME, residue depletion, etc.) were not required. Withdrawal time was set by default by the respective authorities.

Australia completed a special review of halquinol in 1996 but did not publish a final report, as the registrant had withdrawn all products before the completion of the review. The Australian Pesticides and Veterinary Medicines Authority (APVMA) concluded that there were insufficient toxicological data to support the continued registration of halquinol or its associated MRLs. The APVMA recommended cancelling the active constituent approval for halquinol, cancelling the MRLs for halquinol and cancelling the registrations of all products containing halquinol, effective March 31, 1996. There are no veterinary products containing halquinol currently registered in Australia for the treatment of food-producing animals.

Dosage

Halquinol is composed of a mixture of chlorinated products of quinolin-8-ol. Chlorinating quinolin-8-ol yields a mixture, generically called halquinol, which contains 5,7-dichloroquinolin-8-ol (5,7-DCL; 57-74 % w/w), 5-chloroquinolin-8-ol (5-CL; 23-40 % w/w) and 7-chlorquinolin-8-ol (7-CL; 0-4 % w/w). The product, available under the trade name Quixalud, is composed of chlorohydroxyquinoline (halquinol; 60 % w/v), silicon dioxide (1.2 % w/v) and chalk (calcium carbonate; to 100 % w/v). Halquinol is administered to swine orally in the feed at a dose inclusion rate varying from 60 to 600 mg halquinol/kg feed (ppm), for up to 10 consecutive days. Based on a typical daily feed intake of approximately 4 % body weight/day for pigs, this results in a dose of approximately 2.4 to 24 mg halquinol/kg bw.

Pharmacokinetics and metabolism

Test material used in all toxicokinetic/pharmacokinetic studies

The toxicokinetic data in laboratory animals (rats, dogs, and minipigs) were derived from studies conducted using the same batch of Halquinol BP 80 (HLA 4067).

Purity: 98.82 %, comprised of 5-chloroquinol-8-ol (5-CL; 26.29 %) and 5,7-dichloroquinol-8-ol (5,7-DCL; 72.53 %). The amount of 7-chloro-8-hydroxy quinoline (7-CL) in this batch was not assessed, but could not account for more than 1.18 % of the total halquinol. Because plasma and/or tissue concentrations of 7-CL were expected to be negligible relative to the other halquinol components and their metabolites, and because 7-CL represents only 0-4 % of the approved formulation, this component was not analysed in any pharmacokinetic or residue depletion study.

Pharmacokinetics in laboratory animals

Studies examining the pharmacokinetics of halquinol in laboratory animals were conducted as part of the toxicology program. Halquinol was administered *via* the oral route, though the doses used were not in the therapeutic dose range as per the label indications for swine. Overall, the pharmacokinetics of halquinol in laboratory animals is similar to swine in that parent halquinol is absorbed quickly (t_{max} of 1 – 6 h) and rapidly converted to conjugate forms (glucuronides and sulfates).

Rats

As part of a GLP-compliant 13-week toxicity study (Bentz, 2015), three groups of 8 Sprague-Dawley rats (4 male and 4 female)/group each received the test item, Halquinol BP 80 by daily oral gavage. Halquinol was administered at doses of 50, 150 and 450 mg/kg/day. Doses were administered as a suspension in the vehicle [methylcellulose at 0.5 % (w/w) in drinking water], with a constant gavage volume of 5 mL/kg/day.

Blood samples were collected on Day 1 at 0, 1, 3, 6 and 24 h after gavage; and at the end of the treatment period (Day 91) at 0 and 1 hour after gavage. The concentrations of parent halquinol (5-CL and 5,7-DCL) and glucuronided and sulfated metabolites were determined in plasma

using a validated LC-MS/MS method (Decorde, 2016). Noncompartmental kinetic analysis was performed to derive the PK parameters.

Mean pharmacokinetic parameters after halquinol administration in rats is presented in Table 2. Following a single oral administration of halquinol (Day 1), 5-chloroquinolin-8-ol (5-CL) was not quantifiable in plasma samples in any dose group. After repeated oral administration (13 weeks), 5-CL was still not quantifiable in plasma in all animals for dose-levels 50 and 150 mg/kg/day, but was quantifiable in two males and two females at 450 mg/kg/day (1 h post-dose). However, glucuronided and sulfated metabolites of 5-CL (5-CLG, 5-CLS) were rapidly produced, with time to peak concentrations (t_{max}) observed at the first sample collection (1 hour post-dose) for all rats in the 50 and 150 mg/kg/day dose groups. At the highest dose (450 mg/kg/day), maximum concentrations of 5-CL metabolites were produced between 1-3 h post-dose. After initial halquinol dosing, the mean peak concentrations (C_{max}) of 5-CLG were 2913, 4304, and 11203 ng/mL for the 50, 150, and 450 mg/kg/day dose groups, respectively. Peak concentrations of 5-CLS were approximately 28 – 40 % of the peak 5-CLG values, at 818, 1616, and 4494 ng/mL for the respective dose groups.

5,7-dichloro-8-hydroxyquinoline (5,7-DCL) and its glucuronide (5,7-DCLG) and sulfate (5,7-DCLS) metabolites were quantifiable in plasma for all dose groups. The t_{max} for 5,7-DCL and its metabolites increased with increasing dose. For 5,7-DCL and its metabolites, the t_{max} was observed at the first sampling time (1 hr) for all rats in the 50 mg/kg/day group. In the 150 mg/kg/day dose group, maximum concentrations were observed between 1 – 3 h post-dose, and in the 450 mg/kg/day group between 1–6 h post-dose. After initial halquinol dosing, the mean peak concentrations (C_{max}) of 5,7-DCL were 245, 800, and 1710 ng/mL for the 50 ,150, and 450 mg/kg/day dose groups. Mean C_{max} values for the metabolites were approximately 5-10x higher than parent: 2607, 4187, and 9013 ng/mL for 5,7-DCLG, and 3009, 4849, and 11552 ng/mL for 5,7-DCLS at the respective dose groups. Unlike 5-CL metabolites, where the peak concentrations of the glucuronide form exceeded the sulfated form, the peak concentrations of 5,7-DCLS exceeded 5,7-DCLG by ~ 23 %.

For all halquinol components, plasma concentrations depleted rapidly and were typically only detectable at 1, 3, and 6 h after the first dose. The exception was 5,7-DCLG, which was detectable at 24-h post-dose in all 8 rats.

After chronic halquinol administration (13 weeks), 5-CLG peak concentrations (C_{max}) were comparable to those observed after a single dose. Conversely, the peak concentration of 5-CLS after 13 weeks increased dramatically at all doses (3.1x, 3.1x, and 2.2x the initial C_{max}, for the 50, 150, and 450 mg/kg/day dose groups respectively), suggesting an accumulation of the sulfate form. Similar findings were observed for 5,7-DCL. Peak concentrations were comparable between day 1 and week 13 samples for both 5,7-DCL and its glucuronide metabolite. However, the C_{max} of 5,7-DCLS was 2.2x, 1.6x, and 1.1x the C_{max} observed on Day 1 for the respective dose groups.

Table 2. Summary of mean pharmacokinetic parameters after halquinol oral gavage in rats (Bentz, 2015)

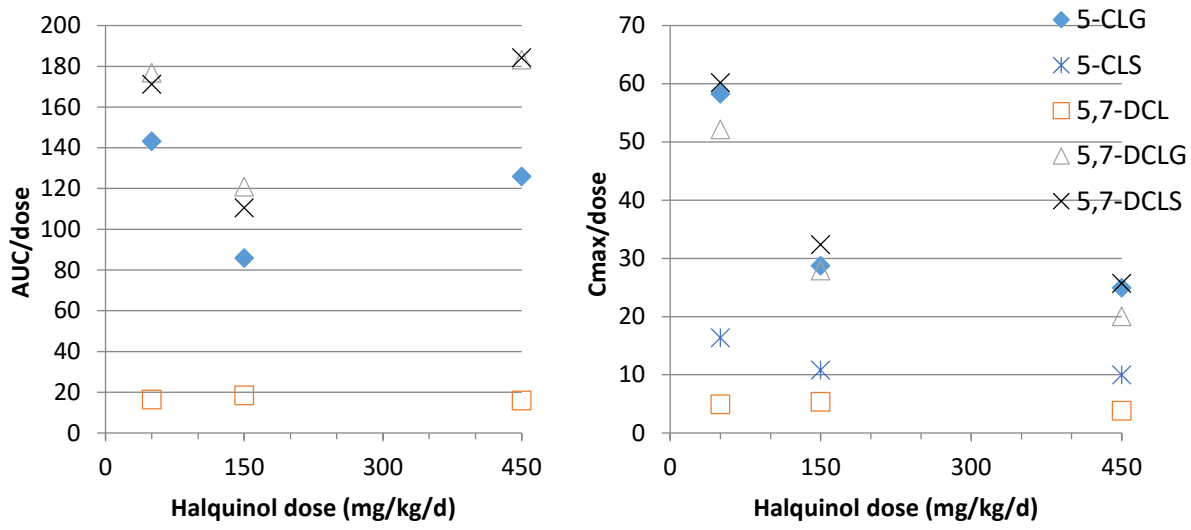
Dose (mg/kg/day)	Period	Parameter	Unit	5-CL	5-CLG	5-CLS	5,7-DCL	5,7-DCLG	5,7-DCLS
50	Day 1	t_{max}	h	ND	1.0	1.0	1.0	1.0	1.0
		C_{max}	ng/mL	ND	2913	818	245	2607	3009
		AUC_{0-t}	ng*h/mL	ND	7158	ND	817	8837	8559
	Week 13	C_{max}	ng/mL	ND	2794	2521	96	2878	6502
150	Day 1	t_{max}	h	ND	1.2	1.2	1.2	1.6	1.2
		C_{max}	ng/mL	ND	4304	1616	800	4187	4849
		AUC_{0-t}	ng*h/mL	ND	12879	ND	2770	18128	16572
	Week 13	C_{max}	ng/mL	ND	4614	5055	518	3933	7889
450	Day 1	t_{max}	h	ND	1.6	1.2	2.6	3.0	2.5
		C_{max}	ng/mL	ND	11203	4494	1710	9013	11552
		AUC_{0-t}	ng*h/mL	ND	56630	13647	7175	82465	82872
	Week 13	C_{max}	ng/mL	13.1	11787	9757	1108	6993	12247

ND = not determined

In general, no significant gender difference was observed in plasma exposure for either 5-CL, 5,7-DCL, or any metabolites. Female rats typically had higher concentrations of 5-CLG after administration of the two lower doses, but not at the 450 mg/kg/day dose.

Given the small sample size and moderate pharmacokinetic variance, no conclusion can be drawn regarding the linearity of halquinol pharmacokinetics in rats in the 50 – 450 mg/kg/day dose range. For 5,7-DCL, dose-normalized drug exposure (AUC) and C_{max} were proportional within the dose range administered (Figure 1). However, for dose-normalized AUC there was deviation from linearity for specific metabolites at the 150 mg/kg/day dose. A trend towards decreasing dose-normalized C_{max} with increasing dose was also noted for some metabolites.

Figure 1. Dose proportionality of halquinol in rats. (Bentz, 2015)



Dogs

As part of a GLP-compliant 13-week toxicity study (El Amrani-Callens, 2016), four groups of 8 Beagle dogs (4 male and 4 female)/group each received daily administration of the test item, Halquinol BP 80. The halquinol was administered as oral capsules at doses of 3, 10, 50, and 150 mg/kg bw/day. Dogs were fasted prior to dosing, with feed offered no earlier than one hour post-dose.

Blood samples were collected at 0, 1, 2, 4, 6 and 24 h following the first dose, and at the same time points following the last dose at the end of the 13-week treatment period. The concentrations of parent halquinol (5-CL and 5,7-DCL) and glucuronide and sulfate metabolites were determined in plasma using a validated LC-MS/MS method (Decorde, 2017). Noncompartmental kinetic analysis was performed to derive the PK parameters.

5-CL and its metabolites were only intermittently detected in canine plasma samples. 5-CL was not detected in any plasma samples in the 3, 10, and 60 mg/kg/day groups, and in only one plasma sample from one animal in the 150 mg/kg/day dose group. For the 5-CL metabolites, plasma concentrations were only detectable in the 1h sample at 3 mg/kg/day, but were detectable for increasing durations as the dose increased (detected up to 24 h post-dose for the 150 mg/kg/day group). Median time to maximum plasma concentrations (t_{max}) was achieved 1–2 h post-administration for both 5-CLG and 5-CLS at all dose levels (Table 3). Combining both the Day 0 and Week 13 PK results, the mean peak concentrations (C_{max}) of 5-CLG were approximately 500, 2700, and 6000 ng/mL (10, 60, and 150 mg/kg/day, respectively). Unlike the rat toxicokinetic results, peak 5-CLS sulfate concentrations were higher than the corresponding glucuronide metabolite by a factor of 1–2x. Combined (D1-Wk13) peak concentrations of 5-CLS were approximately 350, 950, 4340, and 5800 ng/mL for the 3, 10, 60, and 150 mg/kg/day dose groups, respectively.

Table 3. Summary of mean* pharmacokinetic parameters after halquinol oral administration in dogs (El Amrani-Callens, 2016)

Dose (mg/kg/ d)	Period	Parameter	Unit	5-CL	5-CLG	5-CLS	5,7-DCL	5,7-DCLG	5,7-DCLS
3	Day 1	t_{max}	h	NA	NA	1	1	NA	2
		C_{max}	ng/mL	NA	NA	337 (7)	196 (8)	NA	863 (7)
		AUC_{0-t}	ng*h/mL	NA	NA	NA	541 (2)	NA	2892 (1)
	Wk 13	t_{max}	h	NA	NA	1 (4)	2	NA	2
		C_{max}	ng/mL	NA	NA	363 (4)	134 (8)	NA	862 (8)
		AUC_{0-t}	ng*h/mL	NA	NA	NA	NA	NA	2052 (2)
10	Day 1	t_{max}	h	NA	1	1	2	1.5	2
		C_{max}	ng/mL	NA	447 (7)	887 (8)	454 (8)	350 (2)	1566 (8)
		AUC_{0-t}	ng*h/mL	NA	NA	2430 (3)	1159 (7)	NA	3926 (6)
	Wk 13	t_{max}	h	NA	1	1	2	2	2
		C_{max}	ng/mL	NA	565 (8)	1003 (8)	363 (8)	302 (5)	2168 (8)
		AUC_{0-t}	ng*h/mL	NA	1188 (1)	2344 (5)	1081 (7)	NA	6820 (7)
60	Day 1	t_{max}	h	NA	2	1	2	2	2
		C_{max}	ng/mL	NA	2457 (8)	4352 (8)	1886 (8)	769 (8)	4604 (8)
		AUC_{0-t}	ng*h/mL	NA	5383 (7)	13316 (8)	5385 (8)	2162 (4)	16024 (8)
	Wk 13	t_{max}	h	NA	1.5	1	2	3	3
		C_{max}	ng/mL	NA	3024 (8)	4324 (8)	1815 (8)	1296 (8)	7413 (8)
		AUC_{0-t}	ng*h/mL	NA	6147 (8)	10752 (8)	5438 (8)	4077 (5)	23845 (8)
150	Day 1	t_{max}	h	2	2	2	3	4	2
		C_{max}	ng/mL	36 (1)	4255 (8)	5733 (8)	1959 (8)	2682 (7)	6055 (8)
		AUC_{0-t}	ng*h/mL	NA	10410 (8)	13858 (8)	6428 (8)	10337 (4)	31061 (8)
	Wk 13	t_{max}	h	1	2	2	2	4	2
		C_{max}	ng/mL	27.3 (1)	7744 (7)	5874 (7)	1511 (7)	2573 (7)	6674 (7)
		AUC_{0-t}	ng*h/mL	NA	23314 (7)	22753 (7)	5230 (7)	14282 (5)	46318 (7)

NA = not available

Parentheses indicate the number of dogs with quantifiable values (n=8 for all groups, except n=7 for 150 mg/kg/day group at Week 13 due to one removal).

*Median (not mean) t_{max} values reported

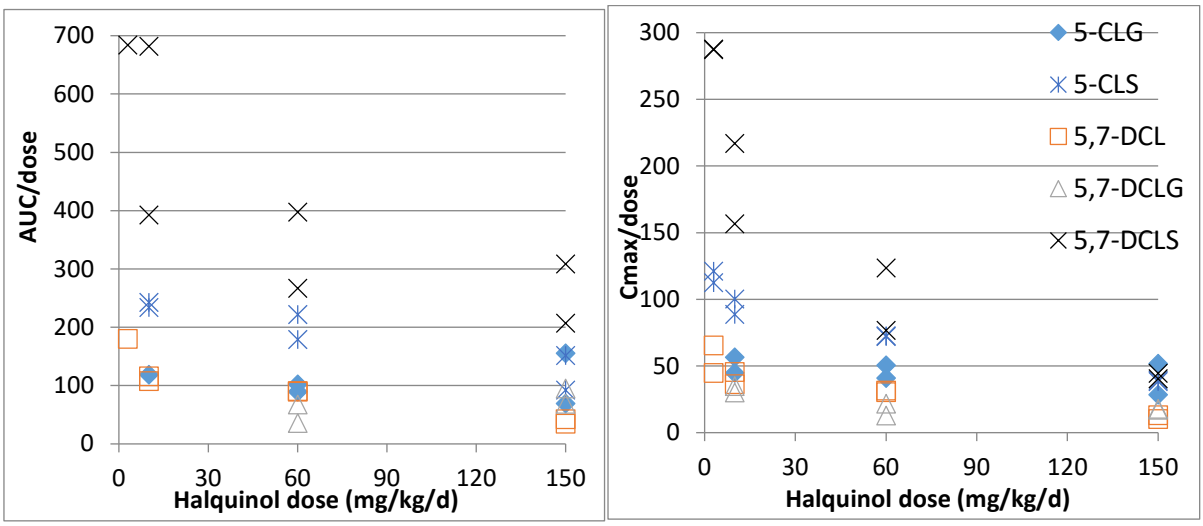
5,7-DCL was intermittently detected in canine plasma samples, but was quantified in at least one sample from all animals (including the lowest 3 mg/kg/day dose group). The glucuronide metabolite was not detected in any samples from the lowest dose group, but was quantifiable in at least some plasma samples from doses 10 mg/kg/day and greater. As with the 5-CL metabolites, plasma concentrations for 5,7-DCL metabolites were detectable for longer durations with increasing dose. Median time to maximum plasma concentrations (t_{max}) was achieved 2–4 h post-administration for both 5,7-DCLG and 5,7-DCLS at all dose levels (Table 3). Combining both the Day 0 and Week 13 results, the mean peak concentrations (C_{max}) of 5,7-DCL were approximately 165, 400, 1850, and 1735 ng/mL for the 3, 10, 60, and 150 mg/kg/day groups, respectively. Unlike the rat toxicokinetic study results, peak concentrations of 5,7-DCLG were typically lower than parent 5,7-DCL, except for the highest dose group. But similar to the rat study, concentrations of 5,7-DCLS predominated and were higher than 5,7-DCL and 5,7-DCLG at all time points.

Insufficient data were available to statistically evaluate the potential effect of gender on halquinol pharmacokinetics in dogs. No gender effect was consistently apparent when comparing concentrations of 5-CL, 5,7-DCL, or their metabolites in dogs.

No significant pharmacokinetic pattern was noted after multiple dosing (daily for 13 weeks) compared to the initial PK parameters derived after the first dose. Accumulation of halquinol parent compounds or metabolites in plasma was not readily apparent after repeated dosing.

The pharmacokinetics of halquinol in dogs were generally linear at the lower 3–10 mg/kg/day dose range (Figure 2). However, evidence of nonlinear kinetics appeared at higher doses (60 and 150 mg/kg/day). At these doses, the exposure (AUC) and peak amounts (C_{max}) of 5,7-DCL, 5,7-DCLS, and 5-CLS decreased relative to the dose administered. Conversely, the limited data displayed a trend of increased 5,7-DCLG exposure relative to the dose in the highest dose group. This potentially indicates saturation of the 5,7-DCLS metabolic pathway in dogs after large dose administration, with subsequent shunting to the glucuronide pathway. However, no definite conclusions can be drawn given the limited sample size and moderate inter-animal PK variance.

Figure 2. Dose proportionality of halquinol in dogs (El-Amrani-Collins, 2016)



Mini-pigs

In a GLP-compliant study (Chevalier, 2014), three groups of Göttingen minipigs (n=3 male and 3 female per group) received Halquinol BP 80 by oral gavage at 25, 75 or 225 mg/kg bw/day for 4 weeks. Plasma was drawn at 2 h post-treatment on day 26; levels of 5-CL, 5,7-DCL, and their sulphate and glucuronide metabolites were determined using a non-validated LC-MS/MS method.

PK parameters were not determined due to the limited plasma sampling. 5-CL was not quantifiable in plasma samples from any dose group. 5-CLG was the primary metabolite produced from 5-CL, with 5-CLS only detectable in the 225 mg/kg dose group. 5,7-DCL was detected in plasma of all animals. The 5,7-DCLG concentration was typically 2 – 5x higher than parent 5,7-DCL, with 5,7-DCLS concentrations generally lower (25-125 %) than parent 5,7-DCL. Due to the small number of minipigs in the study and plasma samples collected, no conclusions regarding gender effect or dose linearity could be made.

Pharmacokinetics in Food-producing Animals

Pigs

In a non-GLP-compliant study (Swan 2016), four male (castrated) and four female (gilt) pigs received oral halquinol by one of two dose regimens: a single oral dose of 12 mg/kg administered as a 100 mg/mL halquinol suspension in 0.5 % methylcellulose, or a 10-day dosage regimen comprised of 6 mg/kg bid (12 mg/kg/day) using the same halquinol suspension. This dose regimen is midway between the approved lower and upper dose regimens for Quixalud® in pigs (2.4 – 24 mg/kg/day). The same commercial-bred pigs were used in both studies, with a washout period of 14 days between them. The total volume of all oral doses was < 5 mL, and was orally gavaged at the back of the throat followed by gavage of 5-10 mL of water. Pigs were fed a non-medicated diet a*d libitum.* All pigs weighed approximately 8 kg upon arrival.

Blood was collected at the following time points during the study:

- Single-dose portion: 0, 0.5, 1, 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, and 72 hr post-dosing;
- Multiple dose portion (blood collected from only 3 male and 3 female pigs):
    - 0, 0.5, 1, 2, 4, 6, 8, 12, 16, and 24 hr post-dosing on Day 0;
    - A single blood sample prior to the first dose on days 2, 3, 4, 5, 7 and 10;
    - 0, 0.5, 1, 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, and 72 h post final dose on day 10.

The concentrations of parent halquinol components (5-CL and 5,7-DCL) and their glucuronide and sulfate metabolites were to be determined in plasma using a validated LC-MS/MS method (Scheele, 2015). However, the transferred method was not validated; and the revised method with a lower LLOQ was not fully developed. The sponsor indicated that this deviation had a minor impact on the study.

Noncompartmental kinetic analysis was performed to derive the PK parameters. In the single 12 mg/kg oral dose study, no plasma samples had 5-CL concentrations above the LLOQ of 9.0

ng/mL. The other parent component, 5,7-DCLG, was quantifiable in plasma (LLOQ 45.0 ng/mL).

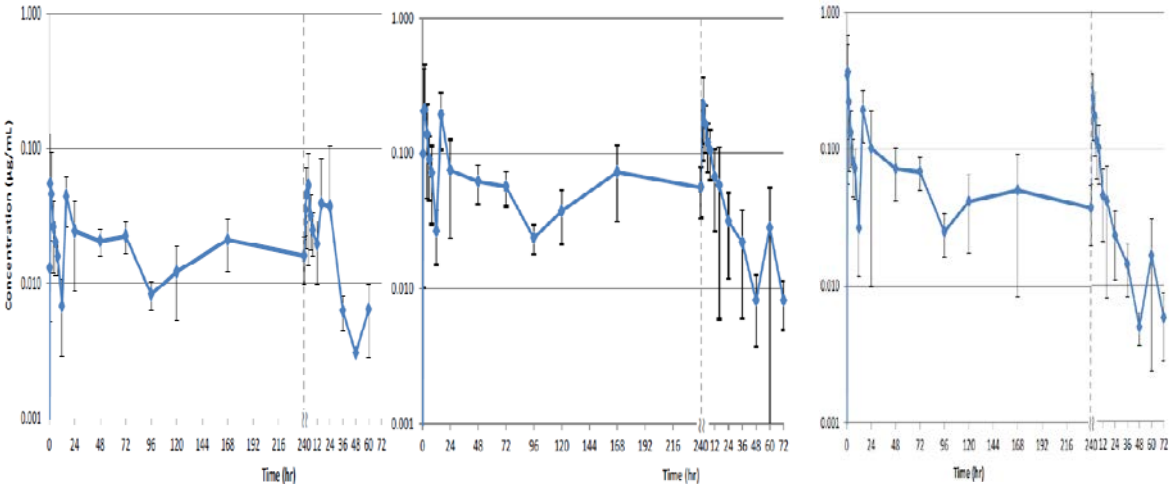
The glucuronide forms (5-CLG and 5,7-DCLG) were the primary halquinol metabolites after single-dose administration with quantifiable results for most pigs. Although the 5-CL isomer could not be detected in systemic circulation, the 5-CLG metabolite (as well as corresponding 5,7-DCLG) were rapidly produced (t_{max} 0.5–6 h). Detection of the glucuronide form of both compounds indicates that absorption of both the 5-CL and the 5,7-DCL components occurred, with rapid metabolism to the glucuronide form.

Limited quantifiable concentrations of the sulfate metabolites (5-CLS and 5,7-DCLS) were observed. For 5-CLS, only 3 pigs had plasma concentrations above the LLOQ of 225 ng/mL (at a single time point, 0.5 h post-dose). For 5,7-DCLS, only 1 pig (at 2 time points) had plasma concentrations above the LLOQ of 225 ng/mL.

Limited pharmacokinetic analysis could be performed for 5-CL, 5-CLS, and 5,7-DCLS due to lack of quantifiable plasma results. For the 5-CLG metabolite, t_{max} occurred between 0.5–1 h with a C_{max} range of 552 – 2960 ng/mL. The t_{max} range of 5,7-DCL was 1–6 h post-dose, with a C_{max} range of 67–494 ng/mL. For 5,7-DCLG, the t_{max} occurred between 0.5–6 h post dose, with plasma concentrations comparable to 5-CLG. The C_{max} for 5,7-DCLG was approximately 4x higher than parent 5,7-DCL (range 242-1770 ng/mL).

In the multiple dose portion of the study, again only the 5,7-DCL component and both glucuronide metabolites (5-CLG and 5,7-DCLG) were quantifiable in any plasma samples. The LLOQs for the non-quantifiable analytes were 9.00 ng/mL (5-CL) and 225 ng/mL (both sulfate metabolites). Because only a small number of 5,7-DCL, 5-CLG, and 5,7-DCLG samples were quantifiable using the initial method (LLOQ 45 ng/mL), a revised analytical method (LLOQ 3.0 ng/mL) was developed and the samples re-analysed. However, the QC samples that were part of the reanalysis showed considerable variability from nominal. Therefore, the sample results were not considered quantitatively reliable and thus were only considered to be qualitative. No pharmacokinetic analyses were conducted on the Cycle 2 data, but plasma concentrations over time are shown in Figure 3.

Figure 3. Plasma concentrations of 5,7-DCL, 5,7-DCLG, and 5-CLG in pigs receiving halquinol at 12 mg/kg/day. (Swan, 2016)



5,7-DCL 5,7-DCLG 5-CLG

Summary of PK data – all species

Direct comparisons of halquinol pharmacokinetic parameters (C_{max} and AUC) cannot be made between species based on the data provided. This is due to significant differences in halquinol dose ranges used, methods of oral administration, plasma sampling schedules, and assay characteristics (LLOQ) for each study. However, despite these challenges, a number of observations were common to all studies:

- 5-CL concentrations were non-detectable in all but one plasma sample from all studies combined, even after repeated and/or supra-therapeutic (> 25 mg/kg/day) doses of halquinol. As the glucuronide and/or sulfate metabolites were rapidly detected in plasma after halquinol administration in all species studied, it is presumed that 5-CL is absorbed from the GI tract and undergoes extensive first-pass metabolism.
- 5,7-DCL was detectable after oral halquinol administration in all species tested, even at < 25 mg/kg/day (therapeutic dose range in pigs). Peak plasma concentrations were typically reached a few hours after halquinol administration, and then declined rapidly (typically quantifiable for 4–12 h post-dose).
- Significant bioaccumulation after repeated dosing was not observed in any species. Plasma concentrations of parent halquinol or the glucuronide/sulfate metabolites were not increased after prolonged daily dosing regimens.
- There appeared to be no significant gender effects on halquinol pharmacokinetics in any species, though the sample sizes for all studies were too small to draw definite conclusions.

- The linearity of halquinol pharmacokinetics cannot be conclusively determined based on dose ranges used in the dog and rat studies. A trend towards decreasing halquinol exposure with increasing dose was observed in both rats and dogs, but only at supra-therapeutic doses (beyond 50 mg/kg/d). At the therapeutic dose range in pigs (2.4 – 24 mg/kg/d), halquinol pharmacokinetics were linear in dogs.
- Note that the methods of oral administration in all studies differ from the approved halquinol formulation (mixed in feed). The PK studies administered halquinol via oral gavage, either as a methylcellulose gel or in capsule form. It is not known if concurrent feed intake alters halquinol kinetics in the target species.
- Oral bioavailability of halquinol was not evaluated in any species.

Differences in halquinol metabolism between species are apparent. Overall metabolite exposure (AUC) data is not available for all metabolites in all species, due to differences in dose levels, plasma sampling times, and assay sensitivity between studies. However, using maximum plasma concentrations (C_{max}) of the various halquinol metabolites as a rough estimate of metabolite production, and normalizing the results based on dose, certain trends are observed (Table 4).

- For 5-CL, the glucuronide metabolism pathway predominates in the rat and pig, whereas the sulfate pathway predominates in the dog (at least until very high doses of halquinol are administered, at which point increasing ratios of glucuronide: sulfate metabolites were observed).
- For 5,7-DCL, only the glucuronide pathway produced quantifiable metabolites in the pig. However, the sulfate pathway predominates for 5,7-DCL in the dog, and both pathways produce roughly comparable metabolite concentrations in the rat.

Table 4. Comparison of mean halquinol C_{max} and dose-adjusted C_{max} by species and dose.

Species	Dose (mg/kg/d)	Period	5-CL		5-CLG		5-CLS		5,7-DCL		5,7-DCLG		5,7-DCLS	
			C_{max}	Dose-adj.	C_{max}	Dose-adj.	C_{max}	Dose-adj.	C_{max}	Dose-adj.	C_{max}	Dose-adj.	C_{max}	Dose-adj.
Dog	3	Day 0	ND	NA	NA	NA	337	112.3	196	65.3	NA	NA	863	287.7
		Wk 13	ND	NA	NA	NA	363	121.0	134	44.7	NA	NA	862	287.3
	10	Day 0	ND	NA	447	44.7	887	88.7	454	45.4	350	35.0	1566	156.6
		Wk 13	ND	NA	565	56.5	1003	100.3	363	36.3	302	30.2	2168	216.8
	60	Day 0	ND	NA	2457	41.0	4352	72.5	1886	31.4	769	12.8	4604	76.7
		Wk 13	ND	NA	3024	50.4	4324	72.1	1815	30.3	1296	21.6	7413	123.6
	150	Day 0	36	0.2	4255	28.4	5733	38.2	1959	13.1	2682	17.9	6055	40.4
		Wk 13	27.3	0.2	7744	51.6	5874	39.2	1511	10.1	2573	17.2	6674	44.5
Rat	50	Day 0	ND	NA	2913	58.3	818	16.4	245	4.9	2607	52.1	3009	60.2
		Wk13	ND	NA	2794	55.9	2521	50.4	96	1.9	2878	57.6	6502	130.0
	150	Day 0	ND	NA	4304	28.7	1616	10.8	800	5.3	4187	27.9	4849	32.3
		Wk 13	ND	NA	4614	30.8	5055	33.7	518	3.5	3933	26.2	7889	52.6

Minipig	25	Day 26	ND	NA	2711	108.4	ND	NA	1308	52.3	4498	179.9	867	34.7
	75	Day 26	ND	NA	6588	87.8	ND	NA	2534	33.8	9467	126.2	1232	16.4
	225	Day 26	ND	NA	29633	131.7	321	1.4	8086	35.9	28769	127.9	3276	14.6
Pig*	12	Single	ND	NA	963	80.2	ND	NA	177	14.8	648	54.0	ND	NA
		Day 0	ND	NA	705	58.8	ND	NA	132	11.0	428	35.7	ND	NA
		Day 10	ND	NA	326	27.2	ND	NA	63	5.3	345	28.8	ND	NA

*Note: For pig data, original PK results from the less sensitive (LOQ 225 ng/mL) but validated assay were used, as the revised sample results were considered unreliable.

Metabolism in laboratory animals

While no traditional *in vivo* metabolism studies have been conducted in laboratory animals by the Sponsor, there are examples of halquinol and same class molecule metabolism in laboratory animals referenced in the literature.

In a balance-excretion study in male Wistar rats (Bories & Tulliez, 1972), a mixture of 5,7-DCL and 5-CL, labeled with [^{36}Cl] (dose = 3.75 mg, ~15 mg/kg bw) was dissolved in peanut oil and administered orally by stomach intubation. In a second experiment, rats were administered labeled drug orally in the feed at the same dose (equivalent to 250 ppm in the feed). Approximately 90 % of the radioactivity in rats had been excreted in the urine or feces within 48 h, feces being the predominant route of elimination. The authors reported elimination as the unmetabolized parent, conjugate forms (undistinguished between sulfate and glucuronide), and limited dechlorinated form.

In Hayashi, *et.al*., (1976), urinary and biliary excretion was examined in rats, guinea-pigs and rabbits. Male albino rats were administered iodochlorohydroxyquin by oral gavage, as a 1.5 mL 0.32 % w/v sodium carboxymethyl cellulose (CMC-Na) suspension (dose = 15 mg, ~ 50 mg/kg bw). In rats, iodochlorohydroxyquin sulfate was the primary metabolite excreted in the urine with smaller amounts of glucuronide and trace amounts of unmetabolized parent detected in the urine. The same suspension of iodochlorohydroxyquin was administered orally in the rat with bile fistula; glucuronide conjugate was almost exclusively present in the bile, with little sulfate conjugate or unmetabolized parent detected. In plasma, the sulfate conjugate was detected at 1.5–2 times higher concentrations than the glucuronide metabolite; only trace amounts of the unmetabolized parent were present. In male guinea-pigs, iodochlorohydroxyquin was administered by oral gavage as the same CMC-Na suspension as per the rat experiment (dose = 24 mg/animal, ~ 60 mg/kg bw). The glucuronide metabolite was the primary metabolite detected in urine. Small amounts of the sulfate conjugate were detected in urine, and only trace amounts of the unmetabolized parent were detected. In plasma, the glucuronide conjugate was detected at higher concentrations than the sulfate metabolite; and only trace amounts of the unmetabolized parent were present.

The more recent toxicokinetic and pharmacokinetic data in laboratory animals and pigs demonstrates rapid metabolism of parent halquinol components to their glucuronide and sulfate conjugate forms. The relative proportions of glucuronide versus sulfate metabolites are not uniform, but vary depending on species, dose, and parent halquinol component. A simplistic

summary of metabolite formation (based on maximum plasma concentrations) is shown in Table 5.

Table 5. Relative prevalence of halquinol conjugates in plasma by species.

Species	5-CL	5,7-DCL
Rat	G > S	S > G
Dog	S > G	S > G
Minipig	G > S	G > S
Pig	G > S	G > S

Metabolism in Food-producing Animals

Pigs

In a GLP compliant study (McLean, 2016), four groups of 2 male and 2 female 8-week old swine, weighing 17.2 to 23.2 kg received 12 mg [^{14}C]-halquinol/kg bw/d for 7 consecutive days. The dose was administered in gelatin capsules twice daily, approximately every 12 h, such that half of the daily dose (6 mg [^{14}C]-Halquinol/kg body weight/dose) was administered in each capsule. The dose was estimated to be approximately one-half of the maximum label dose, assuming a feed consumption rate for pigs of this age at 4 % of body weight. The benzene ring was uniformly labelled for both halquinol components. The radiochemical purity of the test article was 99.3 % with a specific activity of 2.7 µCi/mg. Animals in group 4 were individually housed in metabolism crates and urine and feces were collected every 24 h until slaughter. A final cage wash also was collected following removal of animals from the metabolism crates. Animals in treatment groups 1-4 were slaughtered at 3, 6, 12 and 48 h, respectively, post-final dose. Liver, kidneys, loin muscle, skin with fat, bile, heart and small intestine were collected. All samples were analysed by combustion, LSC and HPLC. Table 6 summarizes the total radioactive recovery (as % total dose administered) in the urine, feces, and cage wash from each animal.

Table 6. Total Radioactive Recovery of [^{14}C]-halquinol (as % total dose administered) in the urine, feces and cage wash of Group 4 Animals.

Sample	Total Radioactive Recovery (as % total dose)			
	13M	**14M**	**15F**	**16F**
Feces	34.6	35.6	36.2	37.8
Urine	53.5	56.0	31.2	25.1
Cage Wash	0.9	2.2	3.1	2.9
Total	89.1	93.85	70.4	65.8

The highest radioactive recovery was found in males, compared to females. The dose was rapidly excreted with a mean 80 % of the dose recovered within 48 h of the last dose. The cumulative excretion of radioactivity in feces was similar in all animals, with a mean excretion of 36 % of the total dose administered. The cumulative excretion of radioactivity in urine was higher in males than in females, with a mean recovery of 55 % in males compared to a mean

recovery of 28 % in females. The total radioactive recovery of [^{14}C]-Halquinol in the cage wash was low, with a mean recovery of 2.3 %.

The recovery of radioactivity in tissues was very low. Liver contained the highest recovery, ranging between 0.008 to 0.107 %. Kidneys ranged between 0.002 to 0.044 %. The lowest radioactive recovery was found in the loin muscle and skin with fat samples which both had 0 % recovery of the total dose administered across all animals. Selected edible tissues (liver, kidney, muscle and skin with fat) were subject to serial solvent extraction and subsequent quantification of the extractable residues. Table 7 provides a summary of the distribution of extractable and non-extractable radioactivity in tissues, expressed as a percent of the TRR.

Table 7. Distribution of extractable and non-extractable radioactivity in tissues, expressed as % of the total radioactive residues (TRR).

Tissue	Animal ID	Withdrawal Period (h)	Extractable Radioactivity (% TRR)	Non-extractable Radioactivity (% TRR)
Liver	1M	3	50.7	49.3
	4F	3	26.8	73.2
	6M	6	37.4	62.6
	8F	6	37.0	63.0
	10M	12	24.0	76.0
	11F	12	18.3	81.7
Kidney	1M	3	87.6	12.4
	4F	3	62.2	37.8
	6M	6	81.0	19.1
	8F	6	73.4	26.6
	10M	12	43.2	56.8
	11F	12	32.5	67.5
	13M	48	15.9	84.1
	16F	48	25.2	74.8
Muscle	1M	3	81.6	18.4
Skin with Fat	1M	3	85.6	14.5

Extractability was higher in muscle and skin with fat (81.6 % and 85.6 % TRR, respectively) at the 3-hour time point. Extractability was lower in the liver and kidney (18.3 % to 50.7 % and 15.9 % to 87.6 %, respectively). By 12 h, extractability in the kidney decreased. Although the extractability in kidney and liver decreased at later times, the concentration of TRR continued to decline over time, indicating that the product was continuing to be eliminated.

The quantitative distribution of radioactive residues was assessed by HPLC in urine, feces, 6 liver, 4 kidney, one muscle, and one skin with fat samples. Glucuronide metabolites accounted for 83 % of the urine TRR and unchanged parent accounted for 13 %. Unchanged parent accounted for 12 % of the TRR and 100 % of the extractable residues in feces. 5-CL was not detected in liver, kidney, muscle or skin with fat. 5,7-DCL was detected and quantified in liver, kidney, muscle and skin with fat. The metabolite 5-CLG was confirmed in liver, kidney, muscle

and skin with fat. 5,7-DCLG was confirmed in kidney, muscle and skin with fat. An additional component was identified in liver; however, it could not be identified and was a suspected source fragment from the mass spectrometer.

Table 8 lists the quantitative distribution of radiolabelled residues present in the tissues analysed. In liver, the radiolabeled halquinol and its glucuronide metabolites accounted for only 1 – 5 % of the total radioactivity present. Other uncharacterized metabolites were detected, but the sum of all extracted radiolabeled components in liver accounted for only 18 – 42 % of the total tissue radioactivity. In kidney, halquinol and its glucuronide metabolites accounted for 25 – 52 % of the total tissue radioactivity. Including other uncharacterized metabolites, the sum of all extracted radiolabeled components in kidney accounted for ≤ 70 % of the total radioactive residues. The identity of the uncharacterized residues in liver and kidney could not be ascertained based solely on the chromatograms. In muscle and skin with fat, halquinol and its glucuronide metabolites were the only radiolabelled components detected, and accounted for 60 and 73 % of total tissue radioactivity, respectively. However, only one sample was analysed for each of muscle and skin with fat, as the total tissue radioactivity was extremely low in all other muscle and skin with fat samples.

Table 8. Quantitative distribution of radiolabelled halquinol components in various swine tissue expressed as percentage of total and extractable radioactive residues (TRR/ERR).

Tissue	Animal	WP	5-CLG % TRR (% ERR[1])	5,7-DCLG % TRR (% ERR)	5-CL % TRR (% ERR)	5,7-DCL % TRR (% ERR)	Total MR as % TRR[2]	Total individual components as % TRR[3]
Liver	1M	3	3.6 (8.7)	ND	ND	1.4 (3.5)	5.0	41.7
	4M	3	2.3 (8.8)	ND	ND	2.5 (9.6)	4.8	25.8
	6M	6	3.3 (10.1)	ND	ND	0.8 (2.6)	3.9	33.1
	8F	6	2.1 (7.7)	ND	ND	2.2 (8.1)	4.3	27.2
	10M	12	1.3 (5.4)	ND	ND	0.7 (2.9)	2.0	24.0
	11F	12	ND	ND	ND	1.4 (7.4)	1.4	18.3
Kidney	1M	3	20.8 (29.7)	ND	ND	6 (8.5)	26.8	70.4
	4F	3	15.3 (28.7)	ND	ND	9.9 (18.6)	25.2	53.1
	6M	6	25 (35.4)	22.6 (31.9)	0.6 (0.8)	5 (7.1)	53.2	70.7
	8F	6	22.5	ND	ND	5.7	28.2	58.5
Muscle	1M	3	18.8 (31.1)	37.2 (61.4)	ND	4.5 (7.5)	60.5	60.5
Skin/fat	1M	3	17.3 (23.4)	23.8 (32.2)	ND	32.7 (44.4)	73.8	73.8

[1]% ERR based on extractability of the final extractions used for the HPLC analysis, and differs slightly from the extractability listed in Table 7.

[2]Proposed marker residue = sum of 5-CL, 5,7-DCL, 5-CLG, and 5,7-DCLG

[3]Sum of all individual components (marker residue and other uncharacterized metabolites on chromatogram) as a percentage of TRR

ND = not detected

Because of the quick rate of metabolism and limited presence of parent halquinol, the marker residue is proposed by the sponsor to be the sum of 5-CL, 5,7-DCL, 5-CLG (expressed as 5-CL-equivalents), and 5,7-DCLG (expressed as 5,7-DCL-equivalents).

Comparative metabolism

In a GLP compliant study (Novo, 2015), cryopreserved hepatocytes and hepatic microsomes prepared from male and female Sprague Dawley rats, beagle dogs, Goettingen minipigs, Landrace pigs and humans were incubated with 5 and 20 µM [^{14}C]-5,7-DCL. The rate of metabolism was determined by subjecting samples to LC-MS analysis with online radiodetection. The viability of each hepatocyte preparation was assessed using a Trypan blue exclusion assay and determined to be between 67 and 97 %.

[^{14}C]-5,7-DCL was extensively metabolized in all species and genders. Complete metabolism was observed in hepatocytes from male and female minipigs and pigs. Complete metabolism also was observed in microsomes from male and female dogs and pigs and female minipigs where no [^{14}C]-5,7-DCL was detected at the end of the incubation period. The lowest rates of metabolism were observed in rat male and female microsomes, which showed 19.6 and 26.8 % total metabolism, respectively. However, the rates of metabolism in rat hepatocytes was high (92.6 and 86.7 % in male and female, respectively), suggesting a preference for Phase II metabolism of [^{14}C]-5,7-DCL in this species.

LC-MS analysis was used to determine the structural identity of the metabolites formed in the high dose (20 µM) hepatocyte and microsome samples (see Table 9). In hepatocytes of all species, one metabolite of [^{14}C]-5,7-DCL was detected: a glucuronide conjugate (M2). A glucose conjugate (M1) was detected in hepatocytes of all species, except humans. Dechloro hydroxyl conjugates of [^{14}C]-5,7-DCL (2 isomers; M4) also were detected in hepatocytes of minipigs and pigs. In hepatic microsomes, one metabolite, oxidized parent compound (M3) was identified in all species, except rats. One additional peak at approximately 14 minutes was present in some hepatic microsome samples (dogs and minipigs), but could not be identified. The results of the study demonstrate that the metabolic profiles of 5,7-DCL in Sprague Dawley rats, dogs, minipigs, pigs, and humans are qualitatively similar.

Table 9. Metabolites identified by LC-MS from incubation of hepatocytes and microsomes from rats, dogs, minipigs, pigs and humans with 20 µM [^{14}C]-5,7-dichloroquinolin-8-ol.

	Glucose Conugate (M1)	**Glucuronide Conjugate (M2)**	**Oxidized Parent (M3)**	**Dechloro hydroxyl conjugates (2 isomers; M4)**	**Unidentified Peak at ~ 14 min**
Hepatocytes					
Sprague-Dawley Rat	+	+	-	-	-
Beagle Dog	+	+	-	-	-
Goettingen Minipig	+	+	-	+	-
Landrace Pig	+	+	+ (male)	+	-
Human	-	+	-	-	-
Microsomes					
SD Rat	-	-	-	-	-
Beagle Dog	-	-	+ (male)	-	+

Goettingen Minipig	-	-	+ (male)	-	+
Landrace Pig	-	-	+	-	-
Human	-	-	+	-	-

Of note, no sulfate conjugates of 5,7-DCL were observed in this *in vitro* study. 5,7-DCLS was not observed in pharmacokinetic studies in swine, though it was the predominant 5,7-DCL metabolite identified in dog and rat pharmacokinetic studies. The authors hypothesized that lack of observed sulfate conjugates may be the results of 5,7-DCL not being a substrate of sulfoltransferease enzymes *in vitro,* or the incubation conditions strongly favoring glucuronidation as a conjugation pathway.

On the basis of the experimental observations, the following biotransformation pathways are proposed for 5,7-DCL and 5-CL (Figures 4 and 5, respectively). The biotransformation pathway for 5,7-DCL represents any metabolite that was detected *in vitro,* as well as any known metabolites (e.g. sulfate) detected in the toxicology and pharmacokinetic studies performed in pigs, rats, dogs and minipigs. The biotransformation pathway for 5-CL only represents the known metabolites detected in the toxicology and pharmacokinetic studies conducted in pigs, rats, dogs and minipigs (5-CL was not included in the *in vitro* testing).

Figure 4. Biotransformation pathway for 5,7-DCL.

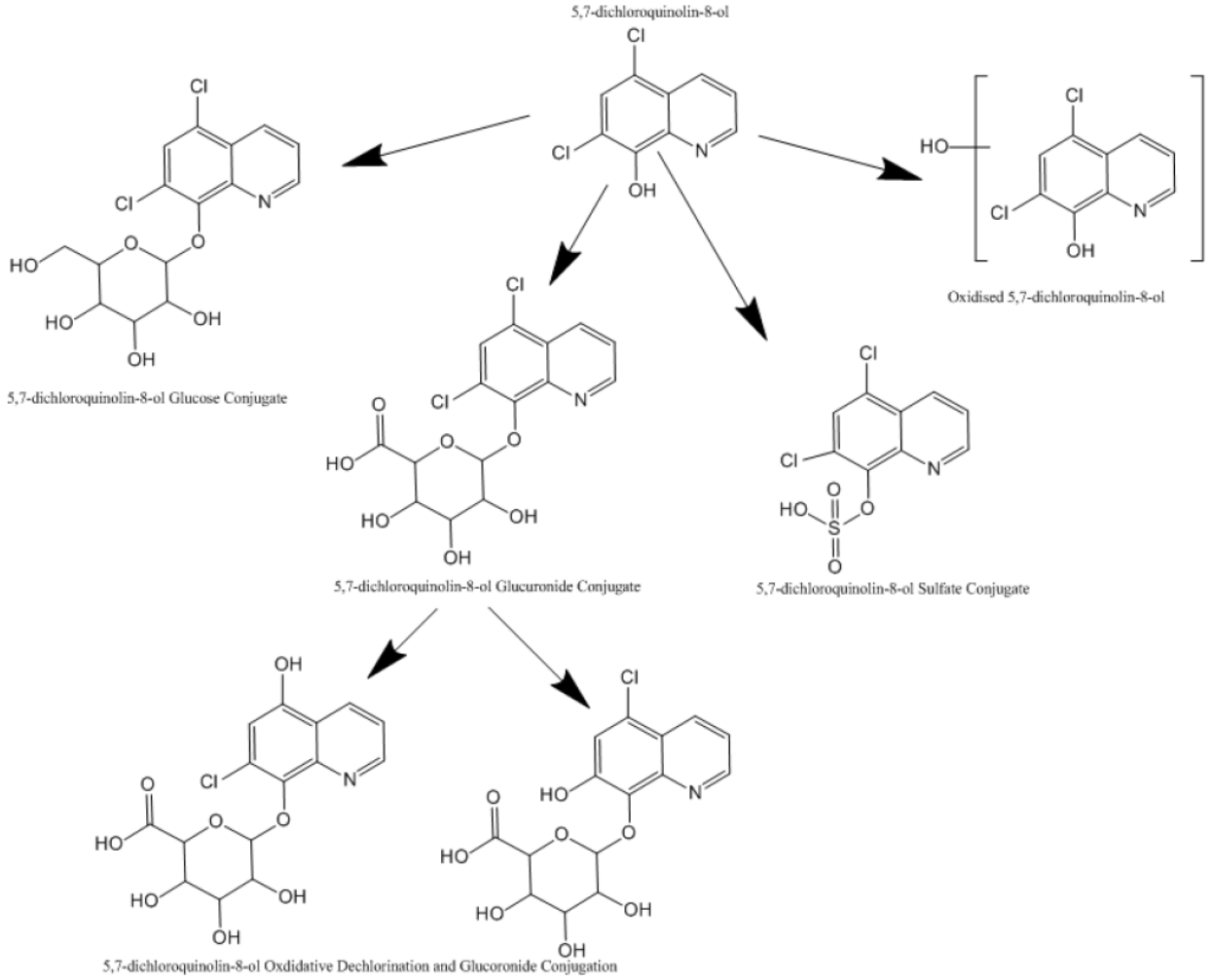
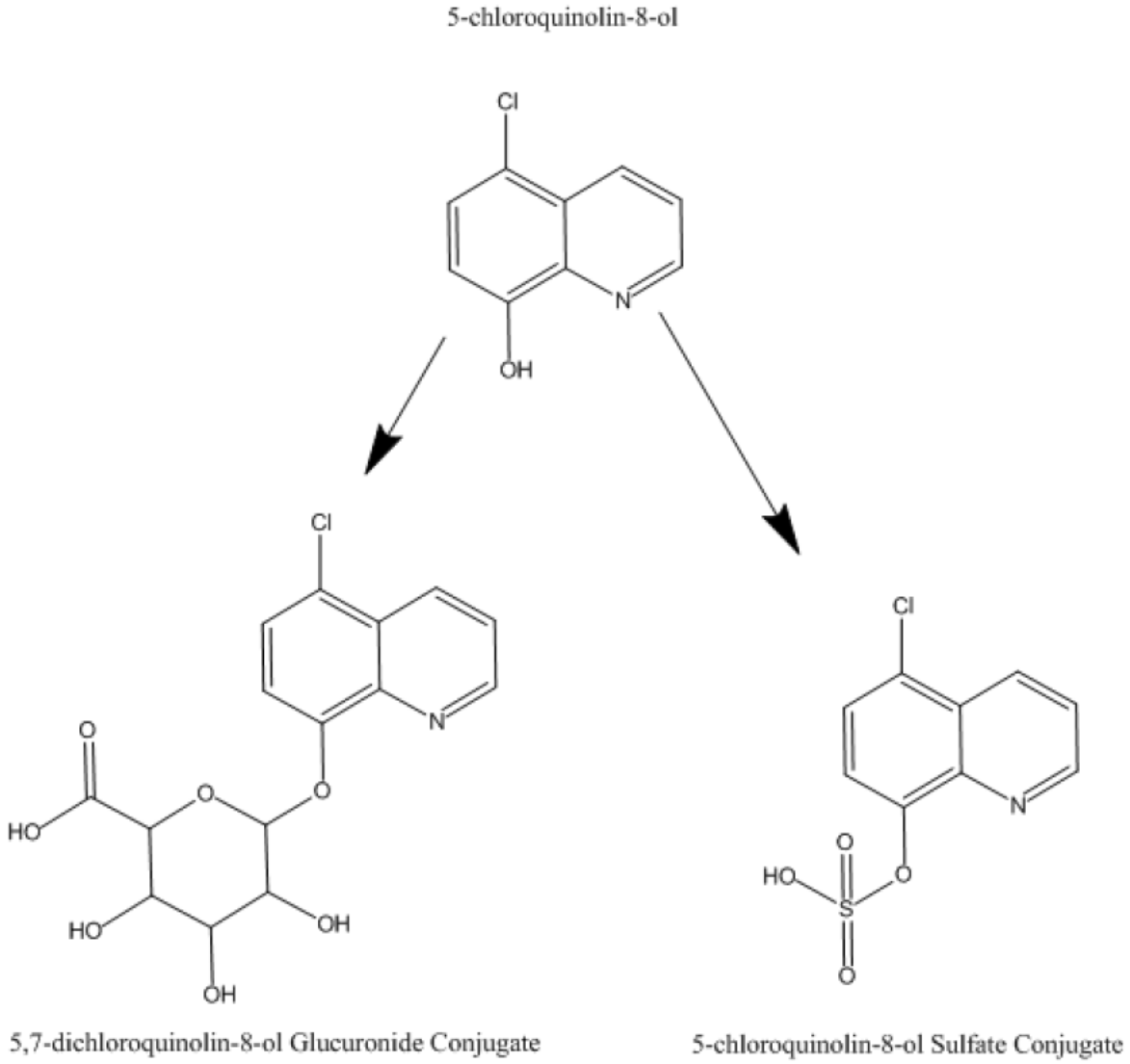


Figure 5. Biotransformation pathway for 5-chloroquinolin-8-ol.



Tissue residue depletion studies

Radiolabelled residue depletion studies

Pigs

In a GLP-compliant study (McLean, 2016), four groups of 2 male and 2 female 8-week old swine, weighing 14.3 to 19.4 kg received 12 mg [^{14}C]-Halquinol/kg body weight/day for 7 consecutive days. The dose was administered in gelatin capsules twice daily, approximately every 12 h, such that half of the daily dose (6 mg [^{14}C]-Halquinol/kg body weight/dose) was administered in each capsule. Capsules were administered along with bread, and animals had access to food at the time of capsule administration in order to mimic the fed state (as per label dosing instructions in feed). The dose was estimated to be approximately one-half of the maximum label dose, assuming a feed consumption rate for pigs of this age at 4 % of body weight. The benzene ring was uniformly radiolabelled for both components. Animals were slaughtered at 3, 6, 12 and 48 h, respectively, post-final dose. Liver, kidneys, loin muscle, skin

with fat, bile, heart and small intestine were collected and analysed for total radioactive residues using liquid scintillation and combustion. Mean TRR in edible tissues across the groups were highest in kidney, followed by liver, skin with fat and muscle (Table 10).

Table 10. Mean concentrations (µg/kg) of total radioactive residues (TRR) in tissues of swine dosed with [^{14}C]-halquinol at 12 mg/kg for 7 days.

Time Post-Dose (h)	Kidney TRR (µg/kg)	Liver TRR (µg/kg)	Muscle TRR (µg/kg)	Skin with Fat TRR (µg/kg)
3	3379	1605	81	286
6	3677	1063	53	155
12	1800	770	33	105
48	494	247	10	27

Because the proposed marker residue method was not fully developed or validated at the time of the radiolabelled study, the samples incurred from this 2016 study were transferred to GLP study NAH-016-077 and analysed using the validated LC-MS/MS method for the sum of halquinol residues (Ward, 2017a, 2017b). Two tissue samples (one male and one female) from each treatment group were analysed 440 days after collection for the components comprising the proposed halquinol marker residue (5-CL, 5,7-DCL, 5-CLG [expressed as 5-CL equivalents] and 5,7-DCLG [expressed as 5,7-DCL equivalents]). As these tissue samples had been frozen for 440 days between collection and analysis, the stability of halquinol-fortified frozen (-80 °C) tissue samples was assessed in 2017 (unpublished data). After 191 – 239 days of storage (-80 °C), the stability of halquinol in kidney was consistently below the acceptance range of 70 – 100 % of initial concentration. Stability of halquinol residues in frozen muscle samples was borderline (~ 70 % of initial concentration). Note that the frozen stability of all incurred (as opposed to fortified) halquinol tissue residues from Hall 2017 did meet acceptance criteria. Due to the instability of frozen halquinol residues in kidney (and possibly muscle) tissues at 440 days, the halquinol concentrations in pig tissues from the 2016 McLean study, but derived using the 2017 Ward assay, are not considered valid for determining a marker residue: total residue. For comparison with the total radioactive residue concentrations determined in McLean 2016, the unvalidated halquinol marker residue concentrations of the same sample (analysed 440 days later using the Ward LC-MS/MS method) are listed in Table 11.

Table 11. Proposed halquinol marker residue concentrations (µg/kg) in select tissues of swine dosed with [^{14}C]-halquinol at 12 mg/kg for 7 days.

Tissue	Animal	WP	TRR (McLean)	MR (McLean)	MR* (Ward)
Muscle	1M	3	163	99	89
Skin/fat	1M	3	552	406	393
Kidney	1M	3	5935	1592	3080
Kidney	4F	3	3657	922	1130
Kidney	6M	6	4022	2140	2130
Kidney	8F	6	4135	1165	1710
Liver	1M	3	2344	119	622
Liver	4F	3	2222	105	230
Liver	6M	6	671	28	146
Liver	8F	6	1575	68	299
Liver	10M	12	870	17	72
Liver	11F	12	985	13	28

*Concentrations not validated

The lack of frozen sample stability should have resulted in lower proposed marker residue concentrations as determined by the Ward LC-MS/MS assay. However, the liver (and some kidney) LC-MS/MS results were considerably higher. The sponsor proposed that halquinol residue is conjugated to macromolecules (potentially contributing to the low extractability of radioactive residues in liver and kidney), and that degradation of these conjugates upon long-term storage and/or sample processing for LC-MS/MS resulted in an apparent increase in marker residue concentrations.

Residue depletion studies with non-radiolabelled drug

Pigs

In a GLP compliant study, four groups of 2 male and 2 female 8-week old swine, weighing 19.8 to 28 kg were offered medicated feed containing 700 ppm halquinol *ad libitum* to achieve a targeted inclusion rate of 12 mg/kg for 10 consecutive days (Hall, 2017). Flavor enhancer and sweetener were added to the treated feed at 300 ppm. Two control animals (1 male and 1 female) were allowed *ad libitum* access to a non-medicated feed. Feed analysis results indicated the halquinol concentration ranged from 619 – 654 ppm in feed (nominal concentration 700 ppm) and was homogenous in all feed samples tested. The stability of the medicated feed was acceptable (> 80 % potency for the duration of the study) for 2/3 batches tested, and nearly acceptable (76 % potency) for the remaining batch.

Control animals were slaughtered at day -2. Treated animals were slaughtered at 8, 24, 48 and 120 h, respectively, post-final dose. Liver, kidney, loin muscle, skin with fat and small intestine were collected. All tissue samples except for small intestine were analysed for the proposed halquinol marker residue (sum of 5-CL, 5,7-DCL, and their glucuronide metabolites expressed as parent equivalents) using a validated LC-MS/MS method (Ward 2017a, 2017b).

The individual animal and mean concentrations of proposed halquinol marker residue are listed in Table 12. Halquinol marker residues depleted most slowly from the kidney. By 120 h withdrawal, residues only were detected in the kidney (4/4 samples) and skin with fat (1/4 samples).

Table 12. Individual animal and mean concentrations (µg/kg) of proposed halquinol marker residue (± s.d.) in tissues of swine dosed with 700 ppm halquinol for 10 days.

Withdrawal Period (h)	Muscle	Liver	Kidney	Skin with fat
8	29	210	1482	96
	33	299	918	118
	23	206	2569	96
	27	286	1780	90
Mean ± s.d.	28 ± 4	250 ± 49	1687 ± 688	100 ± 12
24	<LOQ	66	230	48
	<LOQ	38	219	25
	<LOQ	45	1195	31
	<LOQ	26	192	21
Mean ± s.d.		44 ± 17	459 ± 491	31 ± 12
48	<LOQ	<LOQ	65	<LOQ
	<LOQ	<LOQ	42	<LOQ
	<LOQ	<LOQ	20	<LOQ
	<LOQ	<LOQ	52	19
Mean ± s.d.			45 ±19	
120	<LOQ	<LOQ	21	<LOQ
	<LOQ	<LOQ	23	<LOQ
	<LOQ	<LOQ	108	16
	<LOQ	<LOQ	<LOQ	<LOQ
Mean ± s.d.			51 ±50	

An exponential regression on the log transformation of the tissue residues showed that the depletion half-lives for liver and kidney were approximately 9 and 21 h, respectively. The depletion half-life for skin with fat was approximately 11 h and the depletion half-life for muscle was approximately 7 h.

Determination of the Marker: Total (MR:TRR) Ratio

A dosing error was noted as being written into the protocol of the radiolabelled halquinol residue depletion study (McLean, 2016). In that study, animals should have received two daily doses of halquinol at 12 mg/kg, for a total of 24 mg/kg/day; rather than 6 mg/kg per dose, for a total of 12 mg/kg/day. The actual dose administered in the radiolabelled study was determined to be 12.4 mg/kg bw/piglet. In the non-radiolabelled residue depletion study (Hall, 2017), the total daily dose was 40.1 mg/kg due to higher than expected *ad libitum* medicated feed intake in study animals. To account for the discrepancy between the doses in the total residue and residue depletion studies, the sponsor applied a correction factor of 3.2 ([40.1 mg/kg]/[12.4 mg/kg]) to all total radioactive residue concentrations from Table 10. This correction factor assumes pharmacokinetic linearity over this dose range in pigs, which has not been confirmed but is extrapolated from the results of the toxicokinetic study in dogs (El Amrani-Callens, 2016). The sponsor then derived MR:TRR ratios by combining the marker results of the non-radiolabelled study (Hall, 2017) with the dose-corrected radiolabelled total residue results from McLean, 2016. A second approach for calculating the MR:TRR ratio was considered by the sponsor, using only the marker residue concentrations from McLean, 2016 as assessed by the LC-MS/MS method (Ward, 2017a). However, this approach was considered inappropriate because only two samples per time point were analysed, and some tissue samples were deemed unstable after 440 d storage.

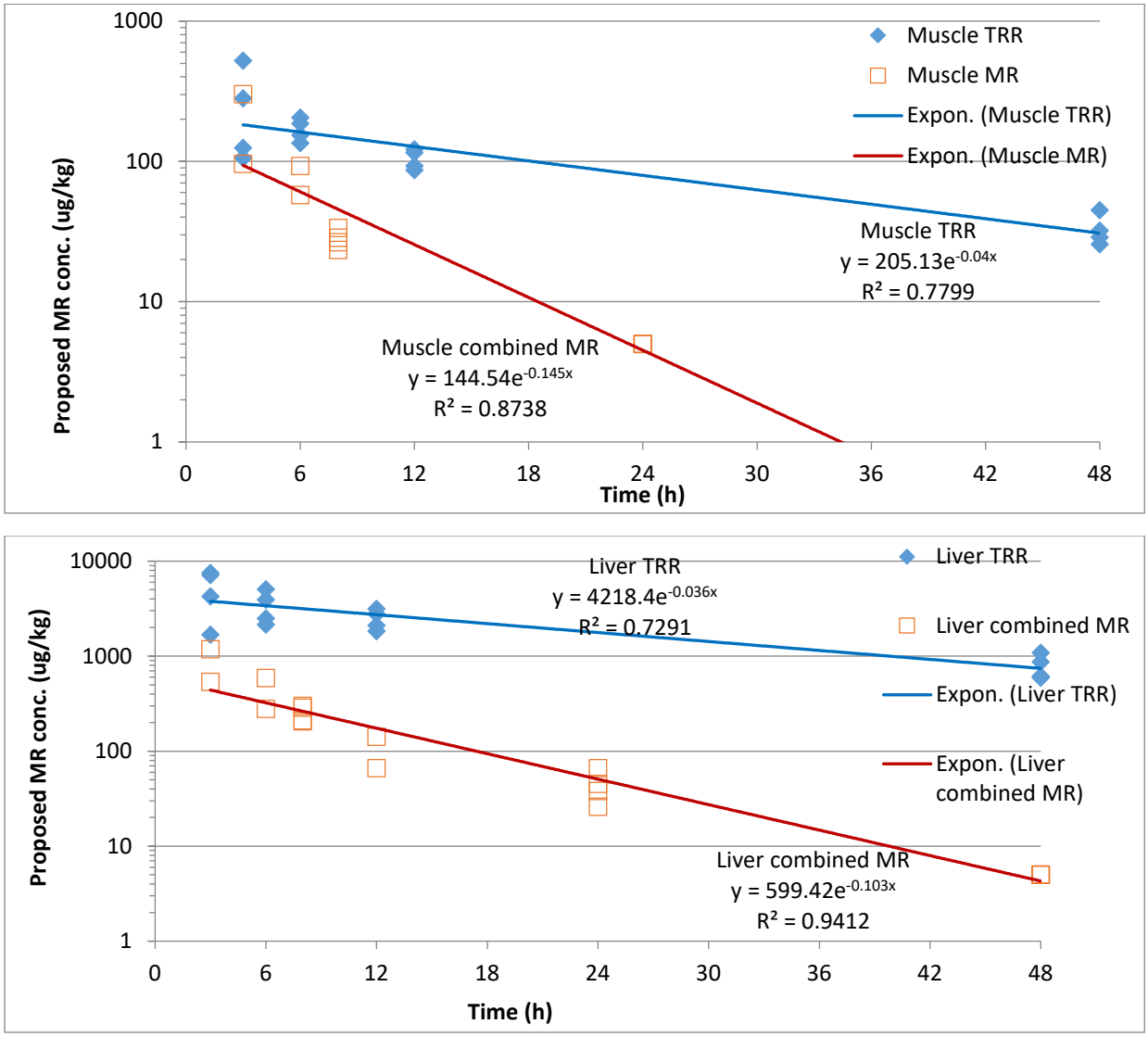
The Committee independently examined the validity of the sponsor's proposed approaches. Preliminary halquinol MR:TRR ratios were further derived using modifications of the proposed approaches, including combining marker residue values at various withdrawal times from both non-radiolabelled (Hall) and radiolabelled (McLean) studies. Note that for purposes of residue depletion modelling, the Committee applied the following criteria to the (non-radiolabelled) marker residue data:

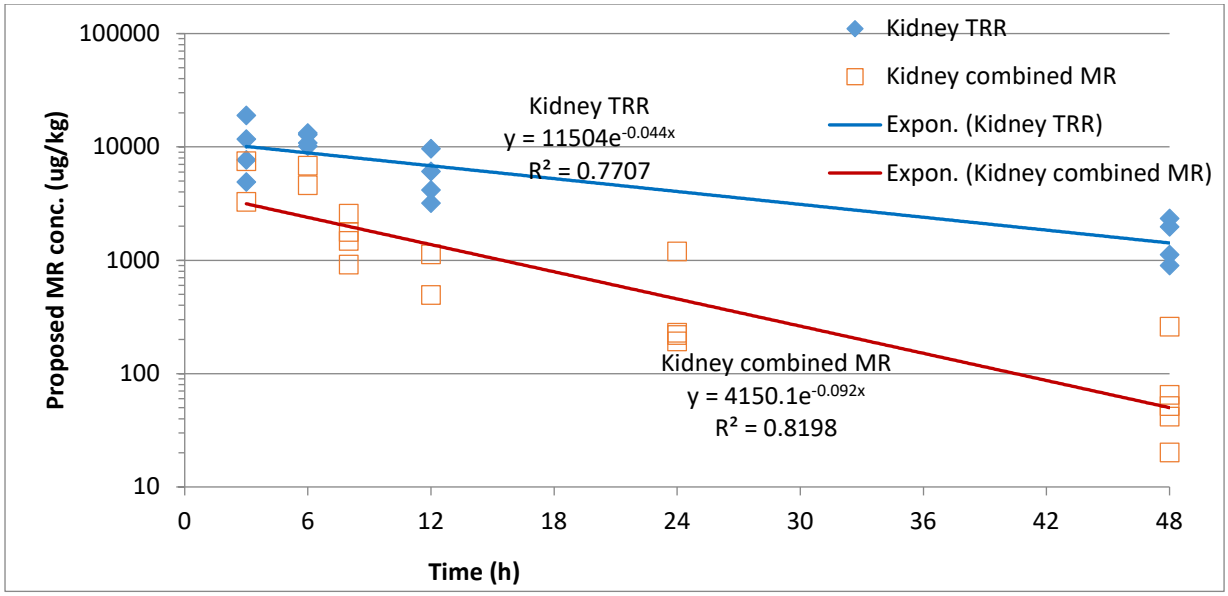
- Numerous tissue samples had one halquinol component (typically 5-CL) that was detectable (>0.4 – 4 μg/kg, depending on component and tissue) but <LOQ (10 μg/kg), while the concentration of the other component in the same sample was > LOQ. In this case a value of ½ LOQ (5 μg/kg) was applied for the <LOQ component. This was deemed to be more appropriate than excluding the <LOQ component entirely (assigning a zero value), or using the numeric (but <LOQ) value as reported.
- For tissue samples in which both halquinol components were less than their individual LOQs, the total sample was also considered <LOQ.
- For each tissue, the first withdrawal time with all MR concentrations <LOQ was included in the residue depletion model and assigned values of ½ LOQ. However, subsequent time points with all concentrations <LOQ were not included, so as not to skew the regression.

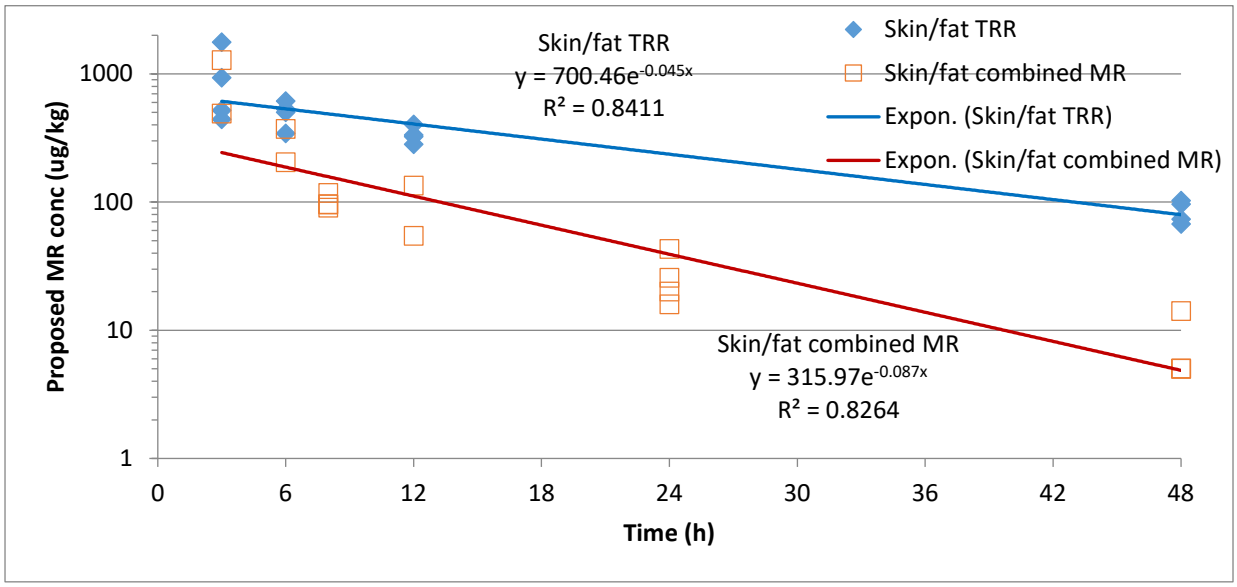
The log-transformed marker residue concentrations (combined from results of McLean 2016 & Hall 2017 studies) and dose-corrected total radioactive residue concentrations (McLean,

2016) were plotted for each tissue and an exponential regression was performed. From this regression, a marker to total ratio was initially determined at 12, 24, 48 and 120 h withdrawal. However, it was readily apparent to the Committee that any regression beyond 48 h was inappropriate, primarily due to the limited numbers of quantifiable MR and TRR concentrations in most tissues beyond 24 – 48 h. Figure 6 shows the MR:TRR regressions for each tissue.

Figure 6. Preliminary MR:TRR regression analysis in swine tissues.

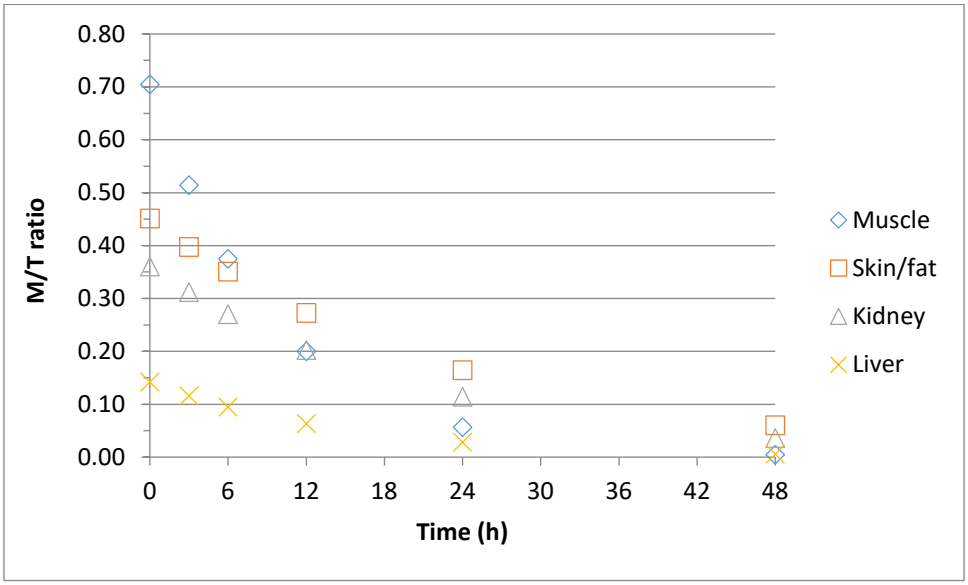






Based on the regression equations dervied from Figure 6, preliminary halquinol MR:TRR ratios at various times from 0 – 48 h withdrawal were predicted for each swine tissue (Figure 7).

Figure 7. Predicted halquinol MR:TRR ratios in swine tissue over time.



Methods of analysis for residues in tissues

A validated regulatory method suitable for the routine analysis of halquinol residues in edible swine tissues according to the criteria established by the CCRVDF, as contained in CAC/GL 71-2009, was available. Briefly, the method for the extraction and analysis of halquinol marker residue is as follows: To a ground 1.0 g tissue sample, > 2000 IU glucuronidase/mL in 25 mM sodium acetate, 9 % w/v EDTA, and 100 mM quinidine and diclofenac in acetonitrile is added together with the internal standards for the parent 5,7-DCL, 5-CL, and their sulphate and glucoronide metabolites, and the sample is homogenized in a Genogrinder for 3 minutes. Samples are centrifuged and then incubated in a water bath at 37 ºC for between 2 and 2.5 h (Note: skin with fat samples should be re-homogenized and centrifuged after approximately 1 h incubation, and then returned to the incubator for the remaining time). Samples are then extracted with acidified acetone, homogenized again for 3 mins, and additional EDTA is added to the sample and centrifuged for 5 minutes. The supernatant is decanted and retained; the pellet is extracted a second time with acidified acetone. The pelleted sample is further homogenized for 3 mins and then mixed vigorously for 30 minutes. EDTA is added to the extract, centrifuged, and the supernatant is decanted and combined with the retained supernatant from the first extraction. All samples are made up to 10 mL with 20 mM EDTA and centrifuged for 5 minutes. A 500 µL aliquot of the resulting sample is transferred to a 96 well plate or vial and 1 mL of Milli-Q water is added to all samples. The extract is injected onto an equilibrated LC-MS/MS, which is calibrated with injections of varying concentrations of pure standards (1 to 100 µg/mL, equivalent to 10 to 1000 µg/kg, for extracts not requiring further dilution) and analysed for the proposed halquinol marker residue which is defined as the sum of 5-CL, 5,7-DCL, 5-CLG, and 5,7-DCLG using the validated analytical method NAH-16-032 v1.3 (Ward, 2017a) with a LOQ of 10 µg/kg.

A gradient elution is employed on a reverse-phase column, followed by electrospray ionization in positive mode. Detection is by tandem MS, with an analyte specific quantifying transition and one qualifying transition for confirmation, all of which are monitored simultaneously. Quantification in unknown samples was done by comparing the analyte:IS peak area ratios to those of the calibration curve (except for 5-CL in skin with fat, which was based on peak area) generated by weighted nonlinear (quadratic) regression ($1/x^2$ except 1/x for kidney; origin excluded).

Validation of method for residues for bovine tissues

Analytical Procedure NAH-16-032 was validated under GLP compliance (Ward, 2017b). However, the validation was not conducted using the traditional procedure of validating at 0.5, 1 and 2 times the proposed kidney and liver MRLs. No rationale was provided for the non-traditional approach used.

Selectivity instead of Specificity

The detection method (MS/MS) is highly specific for the target analytes. For each tissue type, six sources were tested for interference at LOQ.

In kidney and skin with fat, the interference calculated as the percent peak area of the lowest calibration standard equivalent to the assay LOQ, was less than 2 % for all sources against the analytes (5-CL and 5,7-DCL) as well as the internal standard. In liver, no interference was detected for 5-CL or the internal standard; a single liver source produced an interference equivalent to 5.72 % of the lowest calibration standard for 5,7-DCL. In muscle, one tissue source showed higher interference for both the 5-CL and 5,7-DCL analytes at 9.46 % and 8.51 %, respectively; the internal standard in muscle showed interference of less than 2 %. In all sources, for all matrices and analytes the percent interference did no exceed 10 % of the lowest calibration standard or 5 % of the mean IS peak in a blank sample.

Additionally, several commonly used swine drugs were tested for interference with the chromatographic method, to ensure no chromatographic interference with 5-CL or 5,7-DCL in the presence of other drugs. The compounds tested were fenbendazole, tylosin, tilmicosin, ivermectin, tetracycline, and enrofloxacin. None of the compounds interfered with the performance of the assay.

Accuracy

For a range of fortifications, the mean accuracy was acceptable, falling within -30 to +10 % for samples ≥ 10 to <100 µg/kg and -20 to +10 % for samples ≥ 100 µg/kg. In kidney, an additional concentration, at 5000 µg/kg was fortified to demonstrate accuracy following dilution. The results of fortified QC samples generated during the validation are shown in Table 13 and Table 14.

The accuracy results for each tissue (and each analyte) at the 100 µg/kg fortification include one batch (n=6) of samples that were hydrolyzed from a fortified sample of glucuronide conjugate.

Precision

Precision under repeatability conditions for the method was acceptable, with inter-day precision of any given matrix and analyte ≥ 10 to <100 µg/kg not exceeding 16.8 % (VICH acceptance criteria is ≤23 %) and any given matrix and analyte ≥ 100 µg/kg not exceeding 12.2 % (VICH acceptance criteria is ≤16 %).

Intra-day precision for muscle and skin with fat met VICH guidelines for all matrixes, analytes and concentrations (for ≥ 10 to <100 µg/kg % RSD ≤15 %; for ≥ 100 µg/kg % RSD ≤10 %;). The intra-day precision at 500 µg/kg 5,7-DCL in liver and kidney each had one occasion in which the intra-day precision exceeded 10 %. The intra-day precision for 5-CL in liver and kidney each had several occasions and concentrations in which the intra-day precision exceeded VICH recommendation; to account for this, the method recommends that incurred samples should be assayed in duplicate.

Table 13: Accuracy and precision of 5-chlorquinolin-8-ol (5-CL) recoveries in tissues

5-Chloroquinolin-8-ol												
Fortification	**Liver**			**Kidney**			**Muscle**			**Skin w/Fat**		
µg/kg	n	Mean % Accuracy	%CV	n	Mean % Accuracy	%CV	n	Mean % Accuracy	%CV	n	Mean % Accuracy	%CV
10	18	89.2	10	18	89.4	8.05	18	95.1	9.54	18	98.8	9.76
30	18	98.5	16.4	18	92.8	6.19	18	96.9	5.33	18	93.1	15.9
100	23	98.1	10.3	24	90.3	9.84	22	96.3	6.77	24	97.1	6.55
500	17	100	12.2	18	94.4	11.0	18	98.9	4.55	18	102	5.10
5000 (1:10 dilution)	--	--	--	6	104	5.74	--	--	--	--	--	--

Table 14: Accuracy and precision of 5,7-dichlorquinolin-8-ol (5,7-DCL) recoveries in tissues

5,7-Dichloroquinolin-8-ol												
Fortification	**Liver**			**Kidney**			**Muscle**			**Skin w/Fat**		
µg/kg	n	Mean % Accuracy	%CV	n	Mean % Accuracy	%CV	n	Mean % Accuracy	%CV	n	Mean % Accuracy	%CV
10	18	95.4	11.5	18	99.3	9.43	18	106	6.75	18	96.0	12.6
30	18	106	11.1	18	102	6.69	18	107	4.82	18	98.6	11.8
100	23	103	7.67	24	98	8.62	22	107	5.08	24	101	8.65
500	17	106	9.71	18	102	10.7	18	108	7.11	18	103	6.90
5000 (1:10 dilution)	--	--	--	6	104	5.37	--	--	--	--	--	--

Limit of detection and quantitation

The calculated LODs, based on analyses of control samples (n = 4 replicates in 6 independent sources), are shown in Table 15. Calculated LOQs, based off the analysis of 6 independent tissues sources, are shown in Table 15. However, accuracy and precision were demonstrated at the nominal LOQ of 10.0 µg/kg in all edible tissues and these were acceptable, therefore the validated LOQ is 10.0 µg/kg.

Table 15: Calculated LOD and LOQ

Tissue	n	LOD (µg/kg)		Calculated LOQ (µg/kg)	
		5-CL	5,7-DCL	5-CL	5,7-DCL
Liver	23	0.557	1.14	1.15	2.51
Kidney	24	0.261	2.03	0.535	3.93
Muscle	24	0.211	0.292	0.427	0.577
Skin with Fat	24	0.250	0.373	0.471	0.764

Practicability and applicability under normal laboratory conditions

The method executes in a highly robust manner, but is time consuming due to the hydrolysis and incubation steps. Additionally, to account for the occasional higher variation in precision of the method, it is recommended that incurred samples are assayed in duplicate, to confirm assay results. The method does not utilize any hazardous steps. Up to 20 samples can be processed in a working day and automated injections can be performed overnight.

Table 16: Validated parameters for the LC-MS/MS method for the analysis of the marker residue in edible swine tissues

	5-CL in Liver Tissue	**5-CL in Muscle Tissue**
	Fortified samples	**Fortified samples**
Intraday accuracy (% bias)	84.8 - 110	88.8 - 104
Intraday precision (% CV)	2.62 - 19.2	2.17 – 8.74
Interday accuracy	89.2 - 100	95.1 – 98.9
Interday precision	10.0 – 16.3	4.55 – 9.54
LOQ/LOD µg/kg	0.557/1.15	0.211/0.427
Analytical range	10 - 1000	10 - 1000
Specificity/selectivity	No interference observed	No interference observed
Ruggedness testing	Acceptable	Acceptable
Extraction recovery	43.5 – 50.7 %	74.8 – 77.9 %
Stability:		
• **Freeze-thaw**	3 cycles (-80C)	3 cycles
• **Stock solution**	78 days at < 20 ºC	
Confirmatory analysis:		
• **Incurred samples**	< 10 %	<10 % with 2 exceptions

	5-CL in Kidney Tissue	**5-CL in Skin with Fat Tissue**
	Fortified samples	**Fortified samples**

Intraday accuracy (% bias)	83.3 – 99.0	74.9 - 106
Intraday precision (% CV)	2.93 – 11.8	1.43 – 10.4
Interday accuracy	89.4 – 94.4	93.1 - 102
Interday precision	6.19 – 11.0	5.10 – 15.9
LOQ/LOD µg/kg	0.261/0.535	0.250/0.471
Analytical range	10 - 1000	10 - 1000
Specificity/selectivity	No interference observed	No interference observed
Ruggedness testing	Acceptable	Acceptable
Extraction recovery	87.8 – 90.0 %	85.4 – 87.7 %
Stability:		
• **Freeze-thaw**	3 cycles	3 cycles
• **Stock solution**	78 days at < 20 ºC	
Confirmatory analysis:		
• **Incurred samples**	< 10 %	<10 % with 2 exceptions

	5,7-DCL in Liver Tissue	**5,7-DCL in Muscle Tissue**
	Fortified samples	**Fortified samples**
Intraday accuracy (% bias)	89.9 - 116	88.8 - 104
Intraday precision (% CV)	4.02 – 13.7	2.17 – 8.74
Interday accuracy	95.4 - 106	95.1 – 98.9
Interday precision	7.67 – 11.5	4.55 – 9.54
LOQ/LOD µg/kg	1.14/2.51	0.292/0.577
Analytical range	10 - 1000	10 - 1000
Specificity/selectivity	No interference observed	No interference observed
Ruggedness testing	Acceptable	Acceptable
Extraction recovery	55.0 – 59.8 %	74.2 – 75.9 %
Stability:		
• **Freeze-thaw**	3 cycles	3 cycles
• **Stock solution**	78 days at < 20 ºC	
Confirmatory analysis:		
• **Incurred samples**	< 10 %	<10 % with 2 exceptions

	5,7-DCL in Kidney Tissue	5,7-DCL in Skin with Fat Tissue
	Fortified samples	**Fortified samples**
Intraday accuracy (% bias)	91.2 – 106	89.8 - 106
Intraday precision (% CV)	3.65 – 15.7	3.33 – 12.8
Interday accuracy	98.0 – 102	96.0 - 103
Interday precision	6.69 – 10.7	6.90 – 12.6
LOQ/LOD µg/kg	2.03/3.93	0.373/0.764
Analytical range	10 - 1000	10 - 1000
Specificity/selectivity	No interference observed	No interference observed
Ruggedness testing	Acceptable	Acceptable
Extraction recovery	85.8 – 86.8 %	68.5 – 69.6 %
Stability:		
• **Freeze-thaw**	3 cycles	3 cycles
• **Stock solution**	78 days at < 20 ºC	
Confirmatory analysis:		
• **Incurred samples**	< 10 %	<10 % with 2 exceptions

Stability of residues

Stock and working solutions of parent analytes (5-CL and 5,7-DCL) are prepared in ACN and should be stored at <20˚C in polypropylene storage containers. Stock solutions of parent analytes and the internal standard are stable for at least 78 days and working solutions of the parent analytes are stable for at least 24 days. Internal standard working solution is prepared fresh daily.

Stock and working solutions of glucuronide standards were tested for stability at 4˚C and -20˚C. Stock and working solutions are prepared in methanol. Stock and working solutions of glucuronide standards have been shown to have < -8 % difference from freshly prepared standards at 4˚C after 63 days. It is recommended that solutions be stored at -20˚C.

Incurred sample stability was evaluated in the residue depletion study (Hall, 2017). Incurred samples in muscle, liver, kidney and skin and fat were evaluated for stability at 34, 36, 35 and 35 days respectively. All samples analyzed for stability (except for the 36 day liver stability time point from a single animal) were within 88-103 % of the initial value. The liver stability time point was 64.8 % of the initial value; no assignable cause could be determined for the low result. Fortified sample frozen storage stability at -80˚C has been confirmed for 5-CL and 5,7-DCL up to the following durations. Additional stability storage stability analysis were conducted for 31 days in kidney, 22 days in liver, 33 days in muscle and 61 days in skin with fat.

All matrices and analytes confirmed bench top stability for at least 2 h at ambient temperatures; the percent difference from baseline following 2 h storage did not exceed more than 17.7 % in any tissue or analyte (liver, 30 µg/kg, 5-CL).

All matrices and analytes confirmed freeze/thaw stability up to three cycles; the percent difference from the freshly prepared QCs and the baseline samples following 3 freeze/thaw cycles did not exceed -16.0 % in any tissue or analyte (skin with fat, 500 µg/kg, 5,7-DCL).

Therefore, stability of samples was adequately demonstrated for normal conditions of laboratory handling.

Appraisal

Because of the significant differences in halquinol dose ranges used, methods of oral administration, plasma sampling, schedules and assay characteristics (LLOQ) for each pharmacokinetic study, it is difficult to compare halquinol pharmacokinetics between species. Direct comparison of C_{max} and AUC is not appropriate. 5-CL concentrations were non-detectable in all but one plasma sample from all PK studies combined. 5,7-DCL was detectable after oral halquinol administration in all species tested, with rapidly declining plasma concentrations. Significant bioaccumulation after repeated dosing was not observed in any species, and no significant gender effect was noted on halquinol pharmacokinetics in any species. For 5-CL, the glucuronide metabolism predominates in the rat and pig, whereas the sulfate pathway predominates in the dog. For 5,7-DCL, only the glucuronide pathway produced quantifiable metabolites in the pig. However, the sulfate pathway predominates for 5,7-DCL in the dog, and both pathways produce comparable concentrations in the rat. A comparative metabolism study demonstrated qualitatively similar metabolic profiles of 5,7-DCL in Sprague Dawley rats, dogs, minipigs, pigs and humans, though it is noted that this *in vitro* study did not identify sulfate metabolites of 5,7-DCL in dogs or rat hepatocytes or microsomes (though 5,7-DCLG was produced by these species *in vivo*).

Radiolabelled halquinol residue depletion studies conducted in swine show that halquinol is rapidly metabolized and excreted in the urine and feces within 48 h of the last dose administered. Extractability of radiolabelled halquinol residues was much greater in muscle and skin with fat than in liver and kidney. The extractability was lowest in liver (≤ 50 % for all samples assessed) and highly variable in kidney. One potential explanation for the poor extractability in these samples may be protein binding of residues. The lack of extractability of radiolabelled residues in liver and kidney decreases the confidence of the derived total radioactive residues (TRR) in these tissues. Furthermore, the lack of characterization of these non-extractable residues, as well as many of the extractable (but not defined) metabolites present on the liver and kidney chromatograms, is problematic.

The halquinol marker residue was proposed by the sponsor to be the sum of 5-CL, 5,7-DCL, 5-CLG (expressed as 5-CL equivalents), and 5,7-DCLG (expressed as 5,7-DCL equivalents).

Multiple approaches were used by the Committee to determine the halquinol marker residue (based on the sponsor's proposed MR) to total residue ratios for each edible tissue. In an effort to characterize the changing MR:TRR over time, a combination of two separate study results (MR results from Hall 2017, TRR results from McLean 2016) was considered. This was based on a preliminary proposal by the sponsor using the lower bound of estimated MR:TRR ratios, which was acknowledged to be more conservative (leading to higher predicted total residues).

However, the Committee considers it inappropriate to predict total residues based on potentially unsound MR:TRR estimates, and these approaches used to derive MR:TRR ratios were ultimately deemed unsuitable due to the following reasons:

- A greater than 3-fold difference in halquinol doses used between the studies (acknowledging that while the pharmacokinetics of halquinol may be linear over this dose range in other species, this has not been demonstrated conclusively in pigs);
- The discordance between the halquinol MR:TRR ratios derived from the radiolabelled study alone, compared to the regression approach derived from the combination of radiolabelled and non-radiolabelled data;
- The generally low amount of total and/or extractable radioactivity observed in swine tissues may cause unacceptable uncertainty in the MR and TRR counts;

Furthermore, due to insufficient characterization of the total metabolite profile in edible tissues (particularly liver and kidney), the Committee cannot confirm the suitability of the sponsor-proposed marker residue at this time. Without further characterisation of the unknown non-extractable halquinol residues, and the undefined extractable metabolites, the proposed halquinol marker residue may not be appropriate. Therefore any MR:TRR ratio determined at this time could only be a preliminary estimate, subject to further assessment once an appropriate marker residue has been confirmed.

The non-radiolabelled halquinol residue depletion data (Hall, 2017) were sufficient to determine median sponsor-proposed marker residues and subsequent 95/95 UTLs in muscle, liver, kidney, and skin with fat for time periods up to 120 h post-withdrawal (data not shown). However, the total residue of concern cannot be estimated with confidence from these (proposed) marker residue concentrations due to insufficient MR:TRR data. As the residue of concern may be based on total halquinol residues, and not any specific residue components (e.g. 5-CL, 5,7-DCLG and their glucuronide metabolites), accurate prediction of total halquinol residues from robust MR:TRR ratios is essential.

An LC-MS/MS method has been developed and validated for the radiolabelled and proposed marker residue depletion studies in swine. The LOQ of the method is 10 µg/kg. The stability of samples was adequately demonstrated for normal conditions of laboratory handling.

MRLs could not be recommended for halquinol due to the lack of an established HBGV, incomplete characterization of residues in tissues (particularly liver and kidney), and a lack of data necessary to establish reliable MR: TRR ratios over time for calculation of total residues in tissues. The suitability of the proposed marker residue for halquinol cannot be confirmed without further characterisation of the residues.

Dietary Exposure Assessment

Dietary exposure to halquinol may occur only through its use as a veterinary drug. There is no registered use for halquinol as a pesticide. The Committee has not previously considered halquinol as a veterinary drug.

No dietary exposure assessments were performed for halquinol in swine tissues due to the lack of residue characterization, total residue concentrations, and established health-based guidance value.

Maximum Residue Limits

In considering MRLs for halquinol in swine, the committee considered the following factors:

- A microbiological ADI of 0–0.3 mg/kg bw and a microbiological ARfD of 0.9 mg/kg bw were established by the Committee. A toxicological ADI could not be established due to the lack of information required to assess the *in vivo* mutagenicity and carcinogenicity potential of halquinol. It was not possible to establish an ADI for halquinol in the absence of a toxicological ADI.
- Withdrawal periods range from 0 to 7 days for approved veterinary uses in swine.
- Halquinol is extensively metabolized in swine, primarily to glucuronide metabolites.
- The Committee cannot confirm the suitability of the sponsor-proposed marker residue at this time due to insufficient characterization of the total metabolite profile in edible tissues.
- In the absence of an acceptable marker residue, the Committee cannot comment on the suitability of any analytical method for halquinol residue monitoring purposes.
- The non-radiolabelled halquinol residue depletion data were sufficient to determine median sponsor-proposed marker residues and 95/95 UTLs in muscle, liver, kidney, and skin with fat for time periods up to 120 h post-withdrawal. However, the total residue of concern cannot be estimated with confidence from these proposed marker residue concentrations due to insufficient MR:TRR data. The residue of concern is likely to be total halquinol residues and not any specific residue components.

MRLs could not be recommended for halquinol due to the lack of an established HBGV, incomplete characterization of residues in tissues (particularly liver and kidney), and a lack of data necessary to establish reliable MR:TRR ratios over time for calculation of total residues in tissues.

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