Food and Agriculture Organization of the United Nations

World Health Organization

Residue Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 98th Meeting 2024

Clopidol

This monograph was also published in: Residue evaluation of certain veterinary drugs. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 98th meeting 2024. FAO JECFA Monographs 33

Clopidol

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Identity

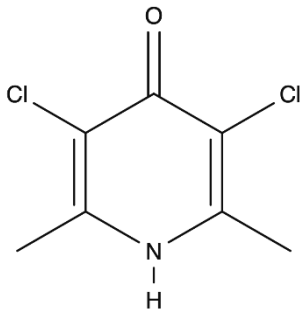
International Non-proprietary Names (INN): Clopidol

Synonyms: clopindol; 3,5-dichloro-2,6-dimethyl-1*H*-pyridin-4-ol; meticlorpindol; coyden

IUPAC name: 3,5-dichloro-2,6-dimethyl-1*H*-pyridin-4-one

Chemical abstract service No.: 2971-90-6

Structural formula:



Molecular formula: $C_7H_7Cl_2NO$

Molecular weight or molecular weight of the salt form: 192.04 g/mol

Other information on identity and properties

Appearance: White to light-brown, crystalline solid; powder

Melting point: >320 °C

Solubility: in water, 10 mg/L

Log P: 2.6 (estimated value)

Residues in food and their evaluation

Conditions of use

Clopidol is a pyridone-derivative structurally related to the quinolones. Pyridones are the carbonyl tautomeric forms of hydroxypyridines. The tautomeric equilibrium of 3,5-dichloro-2,6-dimethyl-1*H*-pyridin-4-one and 3,5-dichloro-2,6-dimethyl-1*H*-pyridin-4-ol is a common phenomenon with aromatic compounds (Monastyrskyi, Kyle and Manetsch, 2014).

Clopidol is a coccidiostat approved for use as an aid in the prevention of coccidiosis in broiler chickens and replacement layers (pullets) caused by certain *Eimeria* species. Clopidol inhibits the development of sporozoites and trophozoites of certain *Eimeria* species (Noack, Chapman and Selzer, 2019). Available products are recommended not to be fed to laying hens or to pullets after 16 weeks of age. Clopidol is also approved as an aid in the prevention of leucocytozoonosis caused by *Leucocytozoon smithi* in turkeys grown for meat purposes only. In the United States of America, clopidol is approved for use as a Type A medicated article to manufacture a Type C medicated feed in the concentration range of 0.0125 percent w/w (weight by weight) to 0.025 percent w/w clopidol. When feeding 0.025 percent w/w clopidol, the dosage should be reduced to 0.0125 percent w/w or the medication entirely withdrawn five days prior to slaughter. In turkeys, regardless of whether the sole ration of clopidol is fed at 0.0125 percent w/w (weight by weight) or 0.025 percent w/w, clopidol should be withdrawn five days before slaughter. In New Zealand, clopidol is registered for use in broiler chickens at a concentration of 125 mg/kg (0.0125 percent w/w) with a 2-day withdrawal period. In the case of severe infection, the dosage can be increased to 250 mg/kg (0.025 percent w/w). In Canada, clopidol is registered for use in broiler chickens at a rate of 125 mg/kg (0.0125 percent w/w) with a 0-day withdrawal period. In the Republic of Korea, clopidol is registered for use in broiler chickens at a rate of 80 to 250 mg/kg with a withdrawal period of 5 or 7 days, depending on usage. Clopidol is not currently registered for use as a pesticide.

Dosage

Clopidol is administered in feed at a dose rate of 0.008 percent (80 mg/kg feed) to 0.025 percent (250 mg/kg feed), which can be fed to the birds continuously until slaughter (broilers), or until 16 weeks of age (replacement pullets). Some product labels recommend that the dose should be reduced from 250 mg/kg feed to 125 mg/kg feed five days prior to slaughter for broilers.

Pharmacokinetics and metabolism

Pharmacokinetics in laboratory animals

Literature references for pharmacokinetic and metabolism studies conducted in rats and rabbits were provided by the sponsor and are summarized below. The sponsor did not provide additional pharmacokinetic or metabolism studies in laboratory animals.

Twelve white rats were each administered an oral capsule containing 10 mg of [^{36}Cl]-clopidol (approximately 50 mg ^{36}Cl-clopidol/kg bw) under anaesthesia and were euthanized at 4, 24, 48, 72, 168 or 264 h post-dosing (Smith and Watson, 1969). Urine, faeces, blood and tissues (heart, liver, lungs, spleen, kidney, testes, muscle, fat, bone, skin and stomach) were collected at each timepoint. At 4 h post-dosing, urine and faecal samples did not contain a significant amount of radioactivity. Approximately 58 percent of the dose was excreted in the urine, and approximately 42 percent was excreted in the faeces, from 24 to 168 h post-dosing, with the levels recovered dropping quickly from 24 h post-dosing onwards, indicating the rapid elimination of clopidol. Mean distribution of

radioactivity in whole blood at 4 h post-dosing was approximately 18 mg equiv/kg, indicating that the radioactive compound was being circulated throughout the body. At 4 h post-dosing, mean radioactivity was approximately 28 mg equiv/kg in plasma and 8 mg equiv/kg in red blood cells, suggesting that the radioactive compound was not bound to proteins. This was confirmed by the authors by demonstrating that the radioactivity was easily extracted from the plasma and cells with methanol. Radioactivity in the blood rapidly decreased, with concentrations below 1 mg equiv/kg by 24 h and at 0.05 mg equiv/kg at 264 h post-dosing. The limit of quantification (LOQ) of the method used to quantify radioactivity was 0.02 mg equiv/kg.

The pharmacokinetics of clopidol were also studied in rabbits. Eight adult New Zealand white rabbits were administered either a single oral dose (16 mg/kg) or multiple (16 mg/kg per day) oral doses up to five days of [^{14}C]-clopidol and slaughtered at various timepoints after dosing (Cameron, Chasseaud and Hawkins, 1975). Urine and faeces were collected from each rabbit at each timepoint. Radioactivity was measured by combustion and liquid scintillation counting (LSC). Results indicated that most of the oral dose of [^{14}C]-clopidol was rapidly absorbed and excreted in the urine, with 99 percent of a single dose and 97 percent of two daily doses being present in the urine 24 h after the last dose. Additional rabbits were slaughtered at different times after receiving the last of five daily doses. In rabbits slaughtered at 16 and 32 h after the last of five daily doses, less than 1 percent of the total dose remained in the total carcass. Concentrations of radioactivity expressed as clopidol equivalents were greater in the plasma than in the tissues. Based on the carcass and plasma levels, the authors calculated the biological half-life of clopidol and its metabolites to be approximately 3 h in rabbits.

Pharmacokinetics in food-producing animals

Chickens

No pharmacokinetics studies in chickens were provided by the sponsor.

Metabolism in laboratory animals

While no traditional *in vivo* or *in vitro* metabolism studies in laboratory species were provided by the sponsor, the literature referenced by the sponsor for rats and rabbits does include metabolism data. Those metabolism data from the above-referenced literature are summarized below.

In the Smith and Watson study (1969), tissues from rats administered an oral capsule containing 10 mg of [^{36}Cl]-clopidol (approximately 50 mg [^{36}Cl]-clopidol/kg bw) were collected, including heart, liver, lungs, spleen, kidney, testes, muscle, fat, bone, skin and stomach. Tissues were analysed for radioactivity by a combustion procedure and LSC. Results indicated that, at 4 h post-dosing, approximately 10 percent of the radioactivity was present in the stomach tissues. At 4 h post-dosing, kidney tissues had the highest level of radioactivity (24 mg equiv/kg), followed by liver (~13 mg equiv/kg), suggesting rapid elimination from these tissues. Radioactivity rapidly decreased over time, with concentrations of [^{36}Cl]-clopidol dropping to below 1 mg equiv/kg in all tissues by 48 h (Table 1).

The authors plotted the rates of elimination for rat kidney, liver, muscle and fat tissues. and calculated a biological half-life of 10 h for [^{36}Cl]-clopidol. The authors noted that preliminary identification of the radioactive compound in the tissues by paper chromatography indicated the presence of unchanged clopidol only.

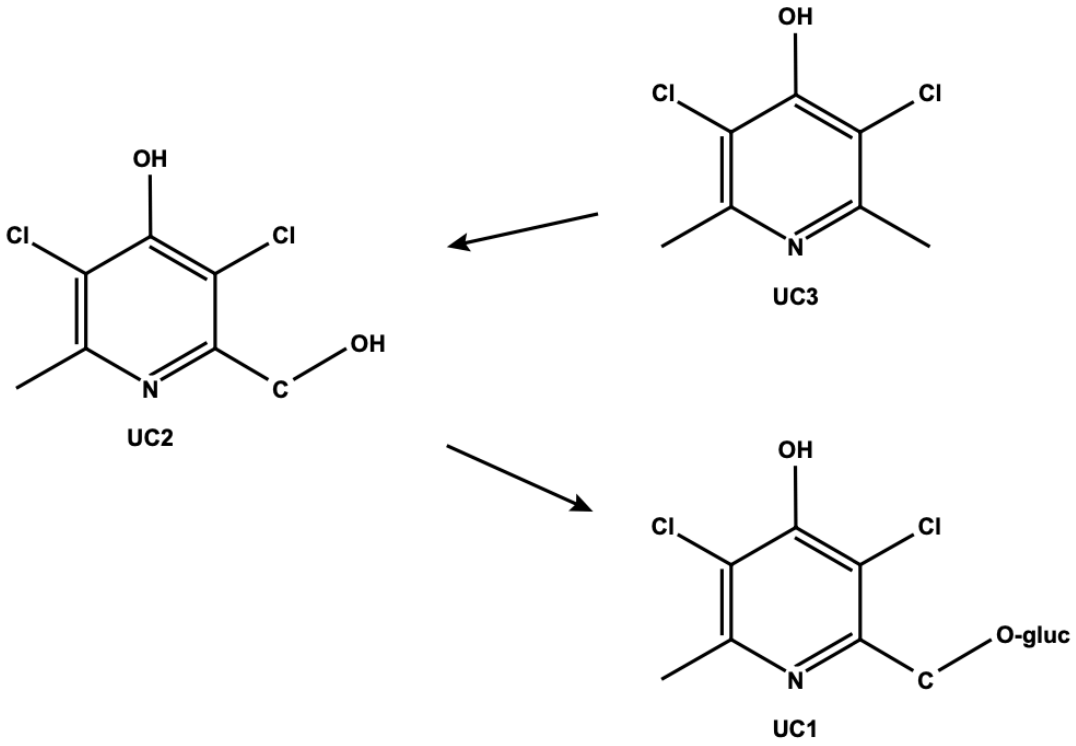
In the study reported by Cameron, Chasseaud and Hawkins (1975), rabbit liver, kidney, bladder, gastrointestinal tract and muscle tissues were collected and analysed by combustion and liquid scintillation counting. The limits of detection (LOD) for clopidol in the tissues were from 0.01 to 0.04 mg equiv/kg.

Table 1. Distribution of radioactivity in various tissues of rats fed [^{36}Cl]-clopidol

Tissue	Results in ppm (mg equiv/kg) at timepoint (h)						
	4	24	48	72	96	168	264
Heart	10.72	1.08	0.05	<0.02	0.12	0.02	0.03
Liver	12.68	1.80	0.08	<0.02	0.07	0.05	<0.02
Lungs	11.01	1.14	0.07	0.03	0.25	<0.02	<0.02
Spleen	6.45	0.54	0.08	<0.02	0.06	<0.02	<0.02
Kidney	24.09	2.58	0.09	0.04	0.14	0.06	<0.02
Testes	6.32	6.17	0.05	0.03	0.30	0.03	<0.02
Muscle	6.03	0.56	<0.02	0.05	0.07	<0.02	<0.02
Fat	1.22	0.17	0.04	<0.02	0.57	<0.02	<0.02
Bone	3.47	0.48	0.04	0.08	0.03	<0.02	0.02
Skin	7.03	0.97	0.48	0.89	0.41	0.42	0.15
Stomach	1 203.59	0.74	0.09	0.22	0.11	0.10	0.05
Whole blood	17.92	1.80	0.10	0.14	0.06	0.10	0.05
Plasma	27.87	-	0.09	0.03	0.08	0.08	<0.02
Red cells	8.13	0.91	0.05	0.04	0.04	0.05	<0.02

Source: adapted from Smith, G. N. & Watson, B. L. 1969. The metabolism of ^{36}Cl-Clopidol (3,5-Dichloro- 2,6-Dimethyl-4-Pyridonol) in rats. *Poultry Science*, 48: 437–443.

Figure 1. Proposed metabolic pathway of clopidol (UC3), involving hydroxylation (UC2) and glucuronidation (UC1)



Source: Cameron, B. D., Chasseaud, L. F. & Hawkins, D. R. 1975. Metabolic fate of clopidol after repeated oral administration to rabbits. *Journal of Agricultural and Food Chemistry*, 23: 269–274.

Concentrations of radioactivity were low in all tissues, with no accumulation of radioactivity apparent in those animals that had received five daily doses of clopidol. Three major radioactive components in rabbit urine were separated by thin layer chromatography (TLC), and designated as UC1, UC2 and UC3 (Figure 1). UC2, accounting for 32 percent of the urine radioactivity, was characterized by mass spectrometry to be 3,5-dichloro-2-hydroxymethyl-6-methylpyridin-4-ol. UC3, accounting for 47 percent of urinary radioactivity, was determined to be unchanged clopidol. UC1, accounting for 21 percent of the urinary radioactivity, was suggested to be a glucuronide conjugate of UC2. The authors provided the following proposed metabolic pathway of clopidol, involving hydroxylation and then glucuronidation of the resulting alcohol.

Metabolism in food-producing animals

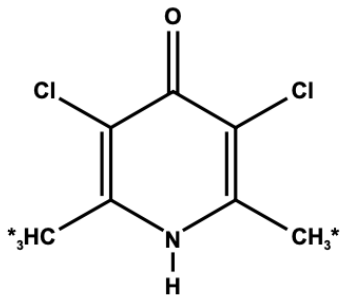
Chicken

A search of the literature conducted by the Committee provided additional information regarding the metabolism of clopidol in the target species, chickens, in addition to the sponsor-provided metabolism and residue kinetics report.

In the Smith (1969) study, six-week-old chickens were continuously fed a feed containing 0.0125 percent [^{36}Cl]-clopidol (125 mg/kg feed) for seven days. The specific activity of the compound was 0.154 µCi/g. Chickens were euthanized while still on medicated feed and blood, muscle and liver samples were collected. Blood, muscle and liver samples were subjected to combustion to measure total radioactivity and then isolated and identified by infrared, X-ray diffraction and nuclear magnetic resonance spectroscopy (NMR) analyses. The identity was also further verified by paper chromatography and crystallography. Most of the radioactivity was associated with unchanged clopidol and a minor component of radioactivity was determined to be traces of inorganic [^{36}Cl]-chloride.

In a sponsor-provided metabolism study (Kim, 2023) reported to be Good Laboratory Practice (GLP)-compliant, twenty 21-day old Arbor Acre broilers weighing approximately 1 kg, and acclimated for seven days, were dosed with a single oral dose of 25 mg [^{3}H]-clopidol/ kg bw, with an aqueous solution of 5 000 mg/L. Broilers were subjected to a 12 h light/12 h dark cycle during the acclimation and testing phases. To make the dosing solution, [^{3}H]-clopidol (0.1 mg; Figure 2) and non-radiolabelled clopidol (749.9 mg) were added to 150 mL of 0.5 percent CMC solution (carboxymethylcellulose sodium salt in sterilized water) and were homogenized. The content and homogeneity of clopidol in the dose solution was confirmed by LSC analysis. The specific activity of [^{3}H]-clopidol was 4.8 Ci/mmol with a purity reported to be 100 percent.

Figure 2. Radiolabelled [^{3}H]-clopidol



Source: adapted from Kim, J-H. 2023. *Metabolism and residue kinetics of [^{3}H]Clopidol in Broiler.* Gyeongnam Department of Environmental Toxicology and Chemistry, Korea Institute of Toxicology. Sponsor submitted.

To assess the extent of tritium exchange with water, the radioactivity values of both wet and dry samples were measured. Dry samples were prepared by drying tissues at 50 °C for 24 to 30 h and adding 5 mL of Soluene-350 solution (a tissue solubilizer). Samples were shaken at 180 rpm and 50 ± 1 °C for 24 h until completely dissolved. An aliquot of each sample was taken and mixed with a scintillation cocktail and radioactivity was measured by LSC. The wet samples were solubilized without drying and radioactivity was measured by LSC. The sponsor reported that radioactivity in the samples was very low after day 3 and was not measured further. Only the 6-h and 1-day post-dosing samples were reported. Numerous samples at both timepoints exceeded the VICH-recommended (Veterinary International Committee for Harmonisation) acceptance criterion of <5 percent (from -40.2 percent to +28.8 percent), suggesting that the tritium label was not stable.

Broilers were euthanized using xylazine and succinylcholine, followed by exsanguination at 6 h, and 1, 3, 5 and 10 days post-dosing. Liver, skin and fat in natural proportions (skin/fat), muscle and kidney tissues were collected, ground and homogenized on dry ice, and stored at -20 °C until analysis. The tissue extracts were subjected to radio-HPLC (high performance liquid chromatography) to separate clopidol and its metabolite(s).

Kidney samples were extracted twice using a mixture of acetonitrile and water at 1 000 rpm for ten minutes. Samples were centrifuged and all extracts were combined and subjected to clean-up using $MgSO_4$, C18E and graphitized carbon black (GCB). After shaking and centrifugation, the resulting supernatant was evaporated using nitrogen gas, and brought up to 0.9 mL with dimethylsulfoxide (DMSO). The sample was filtered (0.22 µm) prior to analysis and an aliquot of filtrate was mixed with the scintillation cocktail for analysis by radio-HPLC to separate clopidol and its metabolites.

Liver, muscle and skin/fat samples were extracted through agitation (1 000 rpm, 10 min), twice, using a mixture of acetonitrile:water (8:2 v/v). After centrifugation, all extracts were combined and subjected to clean-up. Muscle and liver extracts underwent clean-up using $MgSO_4$, C18E and GCB, while skin/fat extracts were cleaned up using $MgSO_4$ and primary secondary amine (PSA). After shaking and centrifugation, the resulting supernatant was evaporated using nitrogen gas and brought up to 1 mL with DMSO. The sample was filtered (0.22 µm) prior to analysis and an aliquot of filtrate was mixed with the scintillation cocktail for radioactivity quantitation by LSC. Simultaneously, analysis by radio-HPLC to separate clopidol and its metabolites was performed.

The remaining tissue after extraction was dried at 30–35 °C and thoroughly mixed. The total weight was measured and 0.1 g of dried sample was combusted with cellulose in a sample oxidizer and subjected to LSC analysis, to determine the amount of radioactivity associated with the non-extractable residue. Table 2 illustrates the recovery of [^{3}H]-clopidol in broiler tissues. Total recovery was greater than 90 percent in all tissues. Table 3 shows the distribution of radioactivity, as %TRR (total radioactive residue), in the broiler tissues following oral administration of [^{3}H]-clopidol. Overall, extractability of radioactivity was almost complete, with all tissues at all timepoints (except liver at 6 h) exceeding 90 percent extractability.

Table 2. Recovery of [^{3}H]-clopidol in liver, fat, muscle and kidney tissues of broilers

Tissue	Level[a]	Recovery[b] (extract, %) (mean ± SD)	CV[c] (%)	Unextractable (%)[d] (mean ± SD)	Total recovery[e] (%)
Liver	1	94.3 ± 0.4	0.4	6.3 ± 0.0	100.6
	2	100.0 ± 1.6	1.6	5.8 ± 0.1	105.8
	3	101.3 ± 0.8	0.8	5.7 ± 0.4	107.0
Skin/Fat	1	95.6 ± 1.0	1.0	1.7 ± 0.1	97.3
	2	98.1 ± 0.1	0.1	1.5 ± 0.0	99.6
	3	95.2 ± 1.6	1.6	2.7 ± 0.4	97.9
Muscle	1	97.0 ± 0.1	0.1	11.6 ± 0.1	108.6
	2	91.6 ± 0.8	0.8	9.2 ± 0.1	100.87
	3	91.7 ± 0.4	0.4	7.5 ± 0.1	99.2
Kidney	1	91.1 ± 0.0	0.0	2.3 ± 0.0	93.4
	2	91.4 ± 0.1	0.1	2.4 ± 0.0	93.8
	3	92.2 ± 0.7	0.7	2.3 ± 0.1	94.5

Notes: [a]Level 1: 10% level of total radioactivity administered, Level 2: 1% level of total radioactivity administered, Level 3: 0.1% level of total radioactivity administered; [b](Detected dpm/fortified dpm) × 100; [c]Coefficient of variation = SD/mean × 100%; [d]Remaining residue after extraction; [e]Extract (%) + Unextractable (%); SD: standard deviation

Source: adapted from Kim, J-H. 2023. *Metabolism and residue kinetics of [^{3}H]Clopidol in Broiler.* Gyeongnam Department of Environmental Toxicology and Chemistry, Korea Institute of Toxicology. Sponsor submitted.

Table 3. Distribution of radioactivity, expressed as %TRR (total radioactive residue), in broiler tissues following oral administration of [^{3}H]-clopidol at a dose of 25 mg/kg bw

Tissue	Time post-dose (days)	Fraction	%TRR (mean ± SD)
Liver	6 h	Extract	88.5 ± 2.2
		Unextractable	11.6 ± 2.2
		Total	100.0 ± 0.0
	1	Extract	91.0 ± 2.3
		Unextractable	10.8 ± 2.8
		Total	100.0 ± 0.0
	3	Extract	100.0 ± 0.0
		Unextractable	-
		Total	100.0 ± 0.0
	5	Extract	-
		Unextractable	-
		Total	-
	10	Extract	-
		Unextractable	-
		Total	-
Skin/Fat	6 h	Extract	100.0 ± 0.0
		Unextractable	-
		Total	100.0 ± 0.0

Tissue	Time post-dose (days)	Fraction	%TRR (mean ± SD)
Skin/Fat	1	Extract	100.0 ± 0.0
		Unextractable	-
		Total	100.0 ± 0.0
	3	Extract	100.0 ± 0.0
		Unextractable	-
		Total	100.0 ± 0.0
	5	Extract	-
		Unextractable	-
		Total	-
	10	Extract	-
		Unextractable	-
		Total	-
Muscle	6 h	Extract	90.2 ± 3.9
		Unextractable	9.9 ± 3.9
		Total	100.1 ± 0.0
	1	Extract	100.0 ± 0.0
		Unextractable	-
		Total	100.0 ± 0.0
	3	Extract	-
		Unextractable	-
		Total	-
	5	Extract	100.0 ± 0.0
		Unextractable	-
		Total	-
	10	Extract	-
		Unextractable	-
		Total	-
Kidney	6 h	Extract	97.4 ± 0.7
		Unextractable	3.1 ± 0.3
		Total	100.0 ± 0.0
	1	Extract	100.0 ± 0.0
		Unextractable	-
		Total	100.0 ± 0.0
	3	Extract	100.0 ± 0.0
		Unextractable	-
		Total	100.0
	5	Extract	-
		Unextractable	-
		Total	-
	10	Extract	-
		Unextractable	-
		Total	-

Source: adapted from Kim, J-H. 2023. *Metabolism and residue kinetics of [^{3}H]Clopidol in Broiler.* Gyeongnam Department of Environmental Toxicology and Chemistry, Korea Institute of Toxicology. Sponsor submitted.

Radio-HPLC analysis of the tissue extracts showed the presence of two radiolabelled components: unchanged [^{3}H]-clopidol and a metabolite. These two radiolabelled components were isolated by eluting from the HPLC column at the specific retention time and the eluates from each tissue were extracted and identified by liquid chromatography-mass spectrometry (LC-MS). Unchanged [^{3}H]-clopidol was confirmed by comparison with an authentic standard. Through mass measurement and fragmentation patterns, the metabolite was identified as 3,5-dichloro-2-hydroxymethyl-6-methylpyridin-4-ol (exact mass: 206.99 g/mol), a hydroxylated metabolite. Table 4 illustrates the distribution of radioactivity amongst unchanged [^{3}H]-clopidol and the hydroxylated metabolite. [^{3}H]-clopidol comprised more than 80 percent of the TRR in all broiler tissues. The hydroxylated metabolite was found in all tissues, but only at the 6-h timepoint and was never above 10 percent of the TRR. The sponsor did not propose a metabolic pathway for clopidol in chickens.

Table 4. Radio-HPLC analysis of tissue extracts, expressed as %TRR (total radioactive residue), following oral administration of [^{3}H]-clopidol at a dose of 25 mg/kg bw

Tissue	Time post-dose (days)	Fraction	%TRR (mean ± SD)
Liver	6 h	Clopidol	84.4 ± 2.5
		Hydroxylated metabolite	4.1 ± 0.7
		Total fractioned	88.5 ± 2.2
	1	Clopidol	91.0 ± 2.3
		Hydroxylated metabolite	-
		Total fractioned	91.0 ± 2.3
	3	Clopidol	100.0
		Hydroxylated metabolite	-
		Total fractioned	100.0
	5	Clopidol	-
		Hydroxylated metabolite	-
		Total fractioned	-
	10	Clopidol	-
		Hydroxylated metabolite	-
		Total fractioned	-
Skin/Fat	6 h	Clopidol	93.1 ± 2.1
		Hydroxylated metabolite	7.0 ± 2.1
		Total fractioned	100.1 ± 0.0
	1	Clopidol	100.0 ± 0.0
		Hydroxylated metabolite	-
		Total fractioned	100.0 ± 0.0
	3	Clopidol	100.0 ± 0.0
		Hydroxylated metabolite	-
		Total fractioned	100.0 ± 0.0
	5	Clopidol	-
		Hydroxylated metabolite	-
		Total fractioned	-
	10	Clopidol	-
		Hydroxylated metabolite	-
		Total fractioned	-

Tissue	Time post-dose (days)	Fraction	%TRR (mean ± SD)
Muscle	6 h	Clopidol	85.1 ± 4.2
		Hydroxylated metabolite	5.1 ± 0.8
		Total fractioned	90.2 ± 3.9
	1	Clopidol	100.0 ± 0.0
		Hydroxylated metabolite	-
		Total fractioned	100.0 ± 0.0
	3	Clopidol	-
		Hydroxylated metabolite	-
		Total fractioned	-
	5	Clopidol	-
		Hydroxylated metabolite	-
		Total fractioned	-
	10	Clopidol	-
		Hydroxylated metabolite	-
		Total fractioned	-
Kidney	6 h	Clopidol	87.8 ± 3.2
		Hydroxylated metabolite	9.7 ± 4.3
		Total fractioned	97.5 ± 0.7
	1	Clopidol	100.0 ± 0.0
		Hydroxylated metabolite	-
		Total fractioned	100.0 ± 0.0
	3	Clopidol	-
		Hydroxylated metabolite	-
		Total fractioned	-
	5	Clopidol	-
		Hydroxylated metabolite	-
		Total fractioned	-
	10	Clopidol	-
		Hydroxylated metabolite	-
		Total fractioned	-

Source: adapted from Kim, J-H. 2023. *Metabolism and residue kinetics of [^{3}H]Clopidol in Broiler.* Gyeongnam Department of Environmental Toxicology and Chemistry, Korea Institute of Toxicology. Sponsor submitted.

Comparative metabolism

The sponsor did not provide comparative metabolism studies. The literature provides limited references on the metabolites present in laboratory animals (rats and rabbits), although the data do suggest that clopidol is the only major compound present in rat tissues. In rabbit, the data suggest that clopidol undergoes hydroxylation to 3,5-dichloro-2-hydroxymethyl-6-methylpyridin-4-ol which then undergoes glucuronidation of the resulting alcohol. The literature also suggests rapid absorption and excretion of clopidol. Data provided from the literature for chickens (Smith, 1969) and from the sponsor (Kim, 2023) also suggest the major residue is clopidol which undergoes hydroxylation to 3,5-dichloro-2-hydroxymethyl-6-methylpyridin-4-ol (Kim, 2023), similar to the proposed metabolic pathway in rabbits.

Tissue residue depletion studies

Radiolabelled residue depletion study

Chickens

In a sponsor-provided radiolabelled residue depletion study, reported to be GLP-compliant (Kim, 2023), twenty 21-day old Arbor Acre broilers weighing approximately 1 kg, and acclimated for seven days, were dosed with a single oral dose of 25 mg [^{3}H]-clopidol per kg bw. Broilers were subjected to a 12 h light/12 h dark cycle during the acclimation and testing phases. The dose solution (5 000 mg/L) was prepared to allow dosing at *ca.* 25 mg [^{3}H]-clopidol/kg bw. Radiolabelled [^{3}H]-clopidol (Figure 2; 0.1 mg) and non--radiolabelled clopidol (749.9 mg) were added to 150 mL of 0.5 percent CMC solution (carboxymethylcellulose sodium salt in sterilized water) and were homogenized. The content and homogeneity of clopidol in the dose solution was confirmed by LSC analysis. The specific activity of [^{3}H]-clopidol was 4.8 Ci/mmol with a reported purity of 100 percent.

Broilers were euthanized using xylazine and succinylcholine, followed by exsanguination, at 6 h, and 1, 3, 5 and 10 days post-dosing. Liver, skin and fat in natural proportions (skin/fat), muscle and kidney tissues were collected, homogenized on dry ice, and stored at -20 °C until analysis. The sponsor indicated that [^{3}H]-clopidol in tissues was stable for 14 days. Total radioactivity was determined by combustion, followed by LSC, and all radio assays were performed in duplicate. Concentrations of the marker residue, clopidol, were determined by radio-HPLC analysis. Total residues were highest in kidney tissues (29.2 mg equiv/kg), followed closely by liver (28.8 mg equiv/kg), muscle (14.3 mg equiv/kg) and skin/fat (7.3 mg equiv/kg). Total residue concentrations decreased rapidly in all tissues, with TRR concentrations below the LOQ in all tissues except muscle by day 5 post-dosing (Table 5).

Clopidol residue concentrations, comprising more than 80 percent of the TRR, depleted most slowly from liver, followed by kidney, muscle and skin/fat. Clopidol residue concentrations were below the LOQ in all tissues except liver, by day 3 post-dosing (Table 6). The data confirm that clopidol is a suitable marker residue. Ratios of marker to total radioactive residues are presented in Table 7, with ratios able to be calculated only at the 6 -h and 1 -day timepoints because of the rapid elimination of clopidol. The sponsor-reported marker to total ratios remained generally stable amongst the tissues and timepoints. However, because of the uncertainty about the stability of the tritium radiolabel, the Committee considered it appropriate to evaluate dietary exposure with a range of marker residue to total residue (MR:TR) values.

Table 5. Mean total radiolabelled residues (mg equiv/kg) in tissues of broilers orally administered 25 mg [^{3}H]-clopidol/kg bw, once

Time post-dose (days)	Kidney	Liver	Muscle	Skin/Fat
6 h	29.222	28.811	14.334	7.257
1	4.934	4.762	1.333	0.849
3	0.148	0.357	<LOQ	0.087
5	<LOQ	<LOQ	0.086	<LOQ
10	<LOQ	<LOQ	<LOQ	<LOQ

Notes: LOQ for kidney is 0.00882 mg equiv/kg; LOQ for liver is 0.00151 mg equiv/kg; LOQ for muscle is 0.00042 mg equiv/kg; LOQ for skin/fat is 0.00166 mg equiv/kg

Source: adapted from Kim, J-H. 2023. *Metabolism and residue kinetics of [^{3}H]Clopidol in Broiler.* Gyeongnam Department of Environmental Toxicology and Chemistry, Korea Institute of Toxicology. Sponsor submitted.

Table 6. Mean clopidol residues (mg equiv/kg) in tissues of broilers orally administered 25 mg [^{3}H]-clopidol/kg bw, once

Time post-dose (days)	Kidney	Liver	Muscle	Skin/Fat
6 h	25.703	24.288	12.176	6.758
1	4.934	4.330	1.333	0.849
3	<LOQ	0.357	<LOQ	<LOQ
5	<LOQ	<LOQ	<LOQ	<LOQ
10	<LOQ	<LOQ	<LOQ	<LOQ

Notes: LOQ for kidney is 0.342 mg equiv/kg; LOQ for liver is 0.183 mg equiv/kg; LOQ for muscle is 0.114 mg equiv/kg; LOQ for skin/fat is 0.183 mg equiv/kg

Source: adapted from Kim, J-H. 2023. *Metabolism and residue kinetics of [^{3}H]Clopidol in Broiler.* Gyeongnam Department of Environmental Toxicology and Chemistry, Korea Institute of Toxicology. Sponsor submitted.

Table 7. Marker to total residue ratios (MR:TR) in broiler tissues

Time post-dose (days)	Kidney	Liver	Muscle	Skin/Fat
6 h	0.88	0.84	0.85	0.93
1	1.0	0.91	1.0	1.0
3	NA	1.0*	NA	<LOQ
5	NA	NA	NA	<LOQ
10	NA	NA	NA	<LOQ

Notes: *MR:TR is based on a liver sample from one animal. All other values were <LOQ.

Source: adapted from Kim, J-H. 2023. *Metabolism and residue kinetics of [^{3}H]Clopidol in Broiler.* Gyeongnam Department of Environmental Toxicology and Chemistry, Korea Institute of Toxicology. Sponsor submitted.

Residue depletion study with non-radiolabelled drug

Chicken

In a sponsor-provided residue depletion study, reported to be GLP-compliant, sixty-six 21-day-old Ross broilers were fed 125 mg clopidol/kg feed or 250 mg clopidol/kg feed as the sole ration for 14 days. Six chickens per dose per timepoint were euthanized using xylazine and succinylcholine, followed by exsanguination at 1, 3, 5, 7 and 10 days post-dosing. Broilers were subjected to a 20 h light/4 h dark cycle. Six broilers were used as control animals and were euthanized on the first day after the 14-day dosing period. The remaining broilers were split into two treatment groups, with one group receiving 125 mg clopidol/kg feed and the second group receiving 250 mg clopidol/kg feed. At each withdrawal timepoint, muscle, skin and fat in natural proportions (skin/fat), liver and kidney samples were collected from each broiler and stored at -20 °C prior to shipment to the analytical laboratory. Tissue samples were analysed in triplicate by a validated liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) method. The tissues showed similar depletion profiles of clopidol, regardless of dose level (125 mg/kg or 250 mg/kg feed). Clopidol concentrations in all tissues were highest at 1-day post-final dosing, with all residues below the LOQ (50 µg/kg) by day 5 (kidney, liver, muscle) and day 7/10 (skin/fat: 125 mg/kg/250 mg/kg feed) (Table 8 and Table 9).

Table 8. Clopidol residue concentrations (µg/kg) in tissues of broilers administered 250 mg clopidol/kg feed

Slaughter timepoint (days)	Animal ID	Results (µg/kg)			
		Muscle	Liver	Kidney	Skin/Fat
1	15	<LOQ	<LOQ	<LOQ	887
	38	1 139	2 508	2 716	1 033
	39	1 516	4 781	3 803	1 032
	40	2 032	3 099	2 622	1 075
	41	1 349	4 601	4 029	794.9
	42	<LOQ	<LOQ	<LOQ	494.2
3	55	122	358.2	358.9	199.2
	56	<LOQ	111.9	100.9	95.3
	57	<LOQ	128.9	68	84.6
	58	79.4	201.9	128.1	84.7
	59	98	194.6	111.3	150.6
	60	309.6	478.3	412.3	173.4
5	13	<LOQ	<LOQ	<LOQ	<LOQ
	14	<LOQ	<LOQ	<LOQ	62.3
	16	<LOQ	<LOQ	<LOQ	48.7
	17	<LOQ	<LOQ	<LOQ	58.4
	18	<LOQ	<LOQ	<LOQ	62.6
	37	<LOQ	<LOQ	<LOQ	93.9
7	31	<LOQ	<LOQ	<LOQ	<LOQ
	32	<LOQ	<LOQ	<LOQ	<LOQ
	33	<LOQ	<LOQ	<LOQ	<LOQ
	34	<LOQ	<LOQ	<LOQ	54.6
	35	<LOQ	<LOQ	<LOQ	<LOQ
	36	<LOQ	<LOQ	<LOQ	68.7
10	19	<LOQ	<LOQ	<LOQ	<LOQ
	20	<LOQ	<LOQ	<LOQ	<LOQ
	21	<LOQ	<LOQ	<LOQ	<LOQ
	22	<LOQ	<LOQ	<LOQ	<LOQ
	23	<LOQ	<LOQ	<LOQ	<LOQ
	24	<LOQ	<LOQ	<LOQ	<LOQ

Note: <LOQ: below the limit of quantitation (50 µg/kg)

Source: adapted from Jeong, S., Jong-hwan, K., Jeong-Ran, M., Chang-hun, L. & Min-cheol, S. 2023. *A study on residue depletion of clopidol in edible tissues of chickens. Report Number RED22018*. Hoseo Biomedical Science Research Center, Hoseo University. Sponsor submitted.

Table 9. Mean (± SD) clopidol residue concentrations (µg/kg) in tissues of broilers administered either 125 mg clopidol/kg feed or 250 mg clopidol/kg feed

Dose level (mg/kg in feed)	Withdrawal (days)	Kidney	Liver	Muscle	Skin/Fat
125	1	3 016 ± 442.1	3 420 ± 732	1 537.3 ± 224	619.5 ± 279.3
	3	121.2 ± 68.7	160.1 ± 115.3	128.3	87.8 ± 15.4
	5	111.2	<LOQ	<LOQ	73.2 ± 31.7
	7	<LOQ	<LOQ	<LOQ	<LOQ
	10	<LOQ	<LOQ	<LOQ	<LOQ
250	1	3 293 ± 726.9	3 747 ± 1 118.6	1 509 ± 381.3	886.1 ± 219.3
	3	196.6 ± 148.7	245.6 ± 143.4	152.3 ± 106.3	131.3 ± 49.8
	5	<LOQ	<LOQ	<LOQ	69.3 ± 16.5
	7	<LOQ	<LOQ	<LOQ	61.7 ± 10.0
	10	<LOQ	<LOQ	<LOQ	<LOQ

Note: <LOQ: below the limit of quantitation (50 µg/kg)

Source: adapted from Jeong, S., Jong-hwan, K., Jeong-Ran, M., Chang-hun, L. & Min-cheol, S. 2023. *A study on residue depletion of clopidol in edible tissues of chickens. Report Number RED22018*. Hoseo Biomedical Science Research Center, Hoseo University. Sponsor submitted.

Review of published literature – residue depletion data

Drug residues in the organs of broiler chickens after flock treatment with Rigecoccin R (clopidol)

The study conducted by Czegledi-Jankó, Balla and Tóth (1976), was not conducted in line with VICH Guidelines or the principles of GLP. Three hundred broiler chickens (strain Tetra B) were administered clopidol in feed at an inclusion rate of 125 mg/kg feed. After 10 weeks of treatment, 25 birds were slaughtered daily on day 0, 1, 2 and 3 after removal of clopidol from the feed. The same was done after 16 weeks of treatment (Table 10).

Samples of liver, kidney, thigh and breast muscle, intestinal contents and blood were taken, homogenized and analysed using gas chromatography (LOD/LOQ not mentioned); the samples were extracted using three different methods, which were roughly equivalent in terms of recovery from spiked samples. The samples that were analysed were homogenates from all 25 birds per timepoint studied; it is not stated whether or how many duplicate samples were analysed.

Table 10. Clopidol residues in broiler chickens after long-term preventative treatment with Rigecoccin R at 125 mg/kg dietary dose leveled

Duration of treatment (weeks)	Slaughter timepoint (days)	Concentration of clopidol detected (µg/kg) in tissues				
		Muscle	Liver	Kidney	Blood	Intestinal contents
10	0	870	3 600	1 290	1 590	1 260
	1	440	1 300	590	720	800
	2	270	330	260	320	610
	3	100	120	180	200	-
16	0	620	1 370	430	680	2 020
	1	310	610	470	360	700
	2	380	320	200	280	-
	3	70	380	240	120	420

Source: adapted from Czeglédi-Jankó, G., Balla, J., & Tóth, L. 1976. Drug residues in the organs of broiler chickens after flock treatment with Rigecoccin R (clopidol). *Acta Veterinaria Academiae Scientiarum Hungaricae*, 26(4): 445–53. PMID: 1052659.

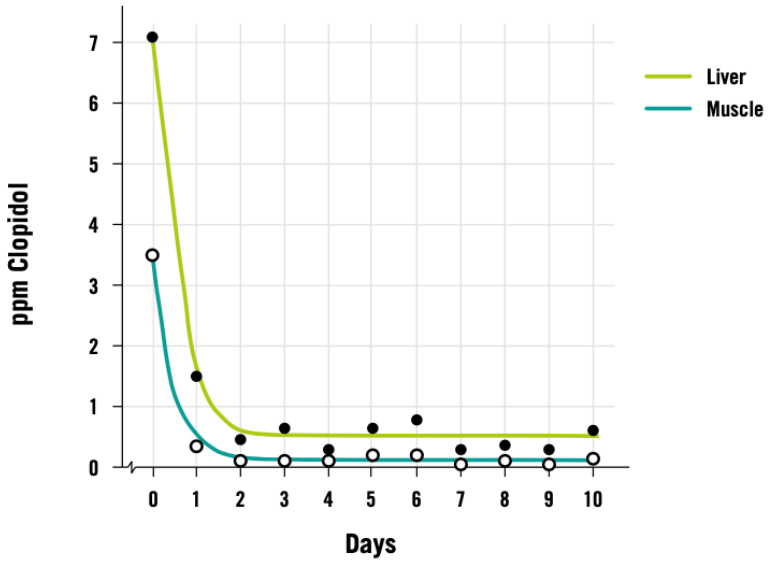
Tissue residues of clopidol (3,5-dichloro-2,6-dimethyl-4-pyridinol) in chickens in relation to withdrawal times

The study, conducted by Ekström, Slanina and Dahlström-King, (1984) was not conducted in line with VICH guidance or with GLP. Broilers (males and females; strain Hybro) were fed clopidol at a rate of 125 mg/kg in feed for 34 days, starting when they were one day old. Four birds per day were slaughtered for 10 days after withdrawal of the medicated feed. The birds weighed between 1.2 and 1.4 kg at slaughter. Samples of thigh muscle and liver were taken from each bird, pooled and stored at -20 °C, for a maximum of one month, until analysis (in duplicate) using a gas chromatographic method. The method is described in Ekström and Kuivinen, 1984.

The results of the study showed residues of about 7 000 µg/kg in liver and 3 000 µg/kg in muscle at zero days (8 h after the last medicated feed was consumed). Once clopidol was no longer being added to the feed, there was a rapid decrease in the tissue concentrations over the first two days. This was followed by a levelling out of the residue concentration in samples of both liver (200–800 µg/kg) and muscle (50–180 µg/kg) between days 2 and 10 post treatment. No individual data were provided in this paper, so only the trends can be seen in Figure 3. It was also noted that there was a correlation between the levels found in the liver and muscle of treated birds (Figure 4).

The authors note that, at the time of writing, the withdrawal periods authorized for the inclusion rate of 125 g/tonne feed was 0 days in the United States of America, 3 days in Sweden and 7 days in Japan.

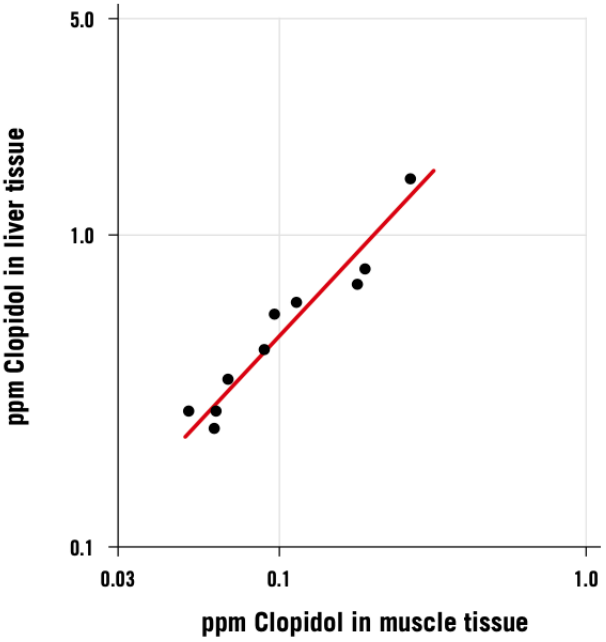
Figure 3. Clopidol levels in liver and muscle samples from broiler chickens slaughtered daily after withdrawal of clopidol-containing feed



Notes: Each point shows the results for pooled tissue samples from four chickens; each sample was analysed in duplicate; ppm = mg/kg; black circles: liver; clear circles: muscle

Source: reproduced from Ekström, L. G., Slanina, P. & Dahlström-King, L. 1984. Tissue residues of clopidol (3,5-dichloro-2,6-dimethyl-4-pyridinol) in chickens in relation to withdrawal times. *Food Additives & Contaminants*, 1(1): 17–22.

Figure 4. Relationship between clopidol levels in liver and muscle samples from broiler chickens slaughtered daily after withdrawal of clopidol-containing feed



Note: R^2: 0.92 (day 0 values not included)

Source: reproduced from Ekström, L. G., Slanina, P. & Dahlström-King, L. 1984. Tissue residues of clopidol (3,5-dichloro-2,6-dimethyl-4-pyridinol) in chickens in relation to withdrawal times. *Food Additives & Contaminants*, 1(1): 17–22.

Determination of clopidol residues in chicken tissues by liquid chromatography: part II

Distribution and depletion of clopidol in chicken tissues

The study, conducted by Pang *et al*. (2001a) was not claimed to be compliant with VICH guidance or with GLP. One hundred chickens (22–42 days old) were administered feed with 125 mg/kg clopidol mixed into it for 12 days. Ten chickens per day were slaughtered randomly, starting 8 h after withdrawal of the clopidol from the feed, until no clopidol was detected in the samples taken from the chickens. Samples of liver (whole), kidney (whole), upper breast (100 g), lower breast (100 g) and leg muscle (100 g) were taken for analysis. Samples were blended on arrival at the laboratory.

Samples were analysed using a validated LC-MS method (Pang *et al*., 2001b) with an LOD of clopidol of 5 µg/kg. Additionally, frozen storage stability was demonstrated for clopidol in chicken muscle samples for three months at -18 °C using incurred samples at three fortification levels: 15, 55 and 1 019 µg/kg. Stability data for other tissues were not reported.

Although the feeding conditions were the same, the intake of clopidol for each chicken was different, which may be due to the inhomogeneous mixture of the feed, or the intake and absorption of each chicken, leading to relatively large variability of individual residue levels (Table 11 and Table 12).

Table 11. Clopidol residues in chicken samples

Timepoint (day)	Animal no.	Clopidol (µg/kg)				
		Upper breast	Lower breast	Leg muscle	Liver	Kidney
1	1	1 500	1 486	1 222	3 956	3 610
	2	1 529	1 412	1 382	3 733	4 195
	3	2 294	2 147	1 824	5 600	3 610
	4	889	861	765	3 289	2 146
	5	2 059	2 118	1 910	6 311	5 073
	6	1 882	1 971	1 882	4 267	3 512
	7	2 176	1 824	2 029	6 133	4 293
	8	1 676	1 412	1 147	3 022	1 951
	9	2 029	2 000	1 735	5 067	4 780
	10	1 382	1 176	1 353	4 622	3 024
	Mean	1 742	1 641	1 525	4 600	3 619
	SD	430	436	412	1 154	1 031
	CV%	24.7	26.6	27	25.1	28.5
2	1	1 429	1 400	1 114	4 000	4 444
	2	314	314	257	511	1 067
	3	429	443	343	1 447	1 600
	4	943	1 057	914	4 085	3 200
	5	714	686	657	1 872	2 578
	6	1 971	1 914	1 686	3 319	2 043
	7	471	486	414	2 043	1 956
	8	1 714	1 771	1 486	1 277	4 178
	9	800	814	743	1 277	2 311
	10	457	514	500	2 936	1 956
	Mean	924	940	811	2 277	2 978
	SD	585	575	487	1 238	1 640
	CV%	63.3	61.1	60	54.4	55.1

Timepoint (day)	Animal no.	Clopidol (µg/kg)				
		Upper breast	Lower breast	Leg muscle	Liver	Kidney
3	1	82	82	85	268	225
	2	115	94	88	585	200
	3	92	82	82	439	275
	4	209*	238*	194*	2 293*	974*
	5	76	71	70	171	128
	6	106	115	112	683	275
	7	108	85	85	610	317
	8	82	94	88	439	275
	9	65	33*	70	390	226
	10	90	100	78	122	341
	Mean*	91*	90*	84*	412*	251*
	SD*	16	13	12	196	64
4	1	26	27	25	107	109
	2	20	24	18	74	62
	3	22	18	17	85	52
	4	8	8	9	38	26
	5	18	26	21	126	76
	6	22	20	21	56	60
	7	68*	58*	66	350*	171
	8	33	32	34	170	93
	9	29	33	30	84	114
	10	26	25	41	136	114
	Mean*	23*	24*	28	97*	88
	SD*	7*	8*	16	42*	41
5	1	16*	13	17	**	**
	2	8	4	7	**	**
	3	9	10	9	16	14
	4	6*	6	9	16	14
	5	8	6	9	12	12
	6	9	9	13	28	20
	7	10	10	10	16	18
	8	8	9	9	22	20
	9	9	Nd	6	Nd	Nd
	10	9	7	11	17	16
	Mean*	9*	7	11	17	16
	SD*	1	4	4	9	8
	CV%	8.1	60.6	40.9	51	49.6
6	1	5	5	5	Nd	Nd
	2	8	7	14	Nd	Nd
	3	Nd	8	12	10	12
	4	7	8	5	8	6
	5	5	5	5	10	6
	6	7	7	7	Nd	Nd

Timepoint (day)	Animal no.	Clopidol (µg/kg)				
		Upper breast	Lower breast	Leg muscle	Liver	Kidney
	7	7	8	12	24*	22
	8	Nd	Nd	7	Nd	Nd
	9	Nd	Nd	Nd	Nd	Nd
	10	5	Nd	7	10	10
	Mean	4	5	7	4*	6
	SD	3	3	4	5*	7
	CV%	72.8	72.7	56.7	119.5	131.3
7	All samples had undetectable levels (<5 µg/kg) of clopidol from day 7.					

Notes: *Values were omitted from statistical calculations; using 'Dixon testing1', they were considered outliers. **Results not reported

Source: adapted from Pang, G. F., Cao, Y. Z., Fan, C. L., Zhang, J. J. & Li, X. M. 2001a. Determination of clopidol residues in chicken tissues by liquid chromatography: part II. Distribution and depletion of clopidol in chicken tissues. *Journal of AOAC INTERNATIONAL*, 84(5): 1343–1346. PMID: 11601451.

Table 12. Clopidol mean concentrations

Timepoint (day)	Mean clopidol concentration (µg/kg)				
	Upper breast	Lower breast	Leg muscle	Liver	Kidney
1	1 742	1 641	1 525	4 600	3 619
2	924	940	811	2 277	2 978
3	91*	90*	84*	412*	251*
4	23*	24*	28	97*	88
5	9*	7	11	17	16
6	4	5	7	4*	6

Notes: *Values were omitted from statistical calculations; using 'Dixon testing1', they were considered outliers.

Source: adapted from Pang, G. F., Cao, Y. Z., Fan, C. L., Zhang, J. J. & Li, X. M. 2001a. Determination of clopidol residues in chicken tissues by liquid chromatography: part II. Distribution and depletion of clopidol in chicken tissues. *Journal of AOAC INTERNATIONAL*, 84(5): 1343–1346. PMID: 11601451.

From the above data, the authors calculated the elimination half-lives ($t_{½el}$) for each tissue (Table 13).

Table 13. Elimination half-lives ($t_{½el}$) for various chicken tissues

Tissue	Elimination half-life (*h*)
Liver	11.8
Kidney	13.3
Upper breast	13.7
Lower breast	14.3
Leg muscle	15.5

Source: adapted from Pang, G. F., Cao, Y. Z., Fan, C. L., Zhang, J. J. & Li, X. M. 2001a. Determination of clopidol residues in chicken tissues by liquid chromatography: part II. Distribution and depletion of clopidol in chicken tissues. *Journal of AOAC INTERNATIONAL*, 84(5): 1343–1346. PMID: 11601451.

Method for the determination of clopidol in muscle, skin/fat, liver and kidney of chickens (sponsor)

In summary, sample preparation involves the addition of 2 mL of water to 2 g of ground and homogenized tissue. This mixture is agitated for 5 min, after which 10 mL of acetonitrile is added. Following agitation, the mixture undergoes centrifugation at 4 ºC and 4 500 *g* for 10 min. The resulting supernatant is collected and extracted by adding 10 mL of hexane. After agitation and centrifugation at 4 ºC and 4 500 *g* for 10 min, the upper layer (hexane) is removed. The remaining extract is evaporated and the resulting residue is resuspended in 1 mL of 50 percent aqueous acetonitrile. The mixture is agitated, followed by centrifugation at 4 °C, 4 500 *g* for 5 min. The supernatant is filtered (0.2 µm) and subjected to analysis by LC-MS/MS.

The separation of clopidol is performed on a Poroshell 120-EC-C18 column (2.1 × 100 mm, 2.7 µm), at 40 ºC, utilizing a mobile phase containing aqueous 0.1 percent v/v formic acid (solvent A) and acetonitrile containing 0.1 percent v/v formic acid (solvent B). The gradient started at 95% solvent A. At 1 min solvent A linear decreased from 95% to 5% in 8 min. At 11 min, the gradient is returned to its initial condition of 95% solvent A within 1 min and the column is allowed to equilibrate for 5 min, resulting in a total run of 17 min. The flow rate is 0.35 mL/min and the injection volume is 1 µL.

Detection is carried out by tandem mass spectrometry, or MS/MS, using an API4000 (Sciex) mass spectrometer and the electrospray ionization source operated in positive mode.

Quantitation is performed using acquisition of ions in the multiple reaction mode (MRM). The precursor ion for clopidol is *m/z* 192 and the product ions are *m/z* 101 and *m/z* 87.1, with *m/z* 192 to 101 being the transition for quantitation.

The method underwent a validation procedure and the following parameters were determined: specificity, linearity, LOD, LOQ, accuracy and precision (intra- and inter-day).

Specificity: In order to determine the specificity, blank samples and samples fortified with clopidol were analysed without addition of clopidol. Specificity was assessed by checking the signal of the blank extracts for interferences at the retention time corresponding to clopidol.

Linearity: On three different days, a matrix matched calibration curve was prepared at 0, 0.2, 0.5, 1, 2, 5, 10 and 50 ng/mL by adding clopidol to the extract of blank samples. Calibration curves were constructed by plotting the area versus the added concentration and carrying out least squares linear regression. The linearity was considered acceptable if the linear coefficient of correlation was at least 0.99 and if the maximal relative error for each concentration was <±15 percent.

LOD and LOQ: The LOD and LOQ for clopidol were set based on signal-to-noise (S/N) ratios.

Accuracy and precision: On one day, three muscle samples were fortified at four levels, specifically: 1, 2.5, 5 and 10 µg/g. These samples were used to determine the accuracy and intra-day precision. The same procedure was carried out on three different days to assess the inter-day precision.

As part of method validation, the sponsor did not determine the stability of clopidol in chicken tissues. However, the sponsor noted that, as part of the radiolabelled residue depletion study, clopidol was stable in chicken tissues for 14 days. As part of the non-radiolabelled residue depletion study, the sponsor noted that all samples were stored at -20 °C and were analysed within 2–3 days of collection. Therefore, the Committee determined that because chicken tissue samples had been analysed shortly after collection, it was not necessary to conduct further stability testing.

The validation parameters are shown in Table 14.

Table 14. Validation parameters of the LC-MS/MS method for the determination of clopidol in chicken

Validation parameter	Fortification level	Muscle	Skin/Fat	Liver	Kidney
Accuracy (%)	1.0 µg/g	86.57	89.54	88.28	85.46
	2.5 µg/g	84.24	86.42	84.89	84.38
	5.0 µg/g	86.45	88.41	89.24	86.13
	10.0 µg/g	87.05	90.77	84.04	85.16
Inter-day precision (%)	1.0 µg/g	2.5	6.5	12.6	3.8
	2.5 µg/g	2.6	6.2	11.0	3.7
	5.0 µg/g	2.0	7.9	8.1	3.7
	10.0 µg/g	1.8	4.1	6.4	3.1
LOD (µg/kg)		25	25	25	25
LOQ (µg/kg)		50	50	50	50
Range (ng/mL)		0.2–50	0.2–50	0.2–50	0.2–50
Linearity (r)		>0.99	>0.99	>0.99	>0.99
Specificity		No interference observed	No interference observed	No interference observed	No interference observed

Source: adapted from Jeong, S., Jong-hwan, K., Jeong-Ran, M., Chang-hun, L. & Min-cheol, S. 2023. *A study on residue depletion of clopidol in edible tissues of chickens. Report Number RED22018*. Hoseo Biomedical Science Research Center, Hoseo University. Sponsor submitted.

Overall comment on validation of the method

According to VICH GL49 (Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: validation of analytical methods used in residue depletion studies), linearity, accuracy, precision, LOD, LOQ, selectivity, stability in matrix, processed sample stability and robustness should be determined. The validation of the analytical method was based on parameters outlined in Codex or OECD guidelines that don't include stability in matrix, processed sample stability and robustness. The performance characteristics determined meet the acceptance criteria. However, stability in matrix, processed sample stability and robustness were not determined.

Limited information on the stability of clopidol in matrices is available in the public literature. Matus and Boison (2016) developed an LC-MS method for the determination of coccidiostats in poultry liver and as part of the validation study fortified poultry liver tissues were stored at 20 °C and checked weekly for eight weeks. No significant loss in recovery was reported for clopidol, indicating that it is supposedly stable in poultry liver for at least two months when frozen. However, only limited information was provided. Furthermore, multiple RASFF notifications (Rapid Alert System for Food and Feed – European system for rapid sharing of food safety related issues) for clopidol in poultry muscle have been reported (Banach *et al*., 2017), which further confirm that clopidol residues in matrices are relatively stable since it has been regularly detected and reported.

The Committee concluded that, because the sponsor noted the stability of clopidol in chicken tissues to be 14 days as part of the radiolabelled residue depletion study and because samples collected as part of the non-radiolabelled residue depletion study were stored at -20 °C and analysed within 2 to 3 days of collection, further stability testing was not necessary.

Methods of analysis for clopidol residues in tissues

Analytical methods for the detection of residual clopidol in a variety of matrices using a variety of techniques have been published over the years. In recent years, LC-MS/MS or liquid chromatography coupled to high-resolution mass spectrometry (LC--HRMS) using positive mode electrospray ionization (ESI+) appears to be the preferred analytical technique (Dasenaki and Thomaidis, 2019; Moloney *et al*., 2012; Pietruk *et al*., 2015; Pietruk, Olejnik and Posyniak, 2018; Rusko *et al*., 2019; Rydchuk *et al*., 2023; Valese *et al*., 2017; Wang *et al*., 2022), although older examples of gas chromatography (GC)-based methods are also available (Ekström and Kuivinen, 1984; Fang *et al*., 2009; Pang *et al*., 2001b). Tandem mass spectrometry is the preferred detection technique due to its combination of high selectivity and high sensitivity. All methods reported in the literature focus on the determination of the parent compound, unmetabolized clopidol. Clopidol is often included in multi-residue methods used for the simultaneous detection of multiple coccidiostats or veterinary drugs. Additionally, multiple screening approaches using different techniques have been published (Chao *et al*., 2020; Jiang *et al*., 2014; Li *et al*., 2020; Radi, El-Naggar and Nassef, 2014).

Modern confirmatory methods for clopidol generally include reversed phase liquid chromatography for separation (i.e. Barreto *et al*., 2017; Pietruk, Olejnik and Posyniak, 2018; Rydchuk *et al*., 2023; Valese *et al*., 2017), although an example of a hydrophilic interaction liquid chromatography (HILIC) based method is also available (Dasenaki and Thomaidis, 2019). Generally, C18 columns are used in combination with a gradient elution and a mobile phase consisting of water and acetonitrile including formic acid and ammonium acetate or ammonium acetate as modifiers. After separation, detection and quantitation is generally performed using tandem mass spectrometry in multiple reaction monitoring (MRM) mode, monitoring two ion-transitions which use the protonated molecule as the precursor ion at *m/z* 192, with *m/z* 101 and *m/z* 87 as the quantifying and qualifying ions, respectively (Barreto *et al*., 2017; Dasenaki and Thomaidis, 2019; Rydchuk *et al*., 2023). Multiple sample preparation approaches, such as liquid-liquid extractions and dispersive solid phase extraction (dSPE), have been presented and they all seem viable based on the method performance characteristics (Dasenaki and Thomaidis, 2019; Rydchuk *et al*., 2023). Clopidol is often included in official residue control plans, which means that the published analytical methods are subjected to rigorous validation procedures, such as (EU) 2002/657/EC and (EU) 2021/808, which can help verify their analytical performance. Several validated methods for determining clopidol in muscle and organ tissue deemed representative and most relevant have been described below in more detail.

Screening methods

Chao *et al*. (2020) developed a lateral-flow colloidal gold immunoassay (LCGA) for the detection of clopidol in poultry muscle. Samples are extracted using methanol, defatted using n-hexane and then reconstituted using a methanol phosphate buffer solution. The method was validated in terms of selectivity, sensitivity, linearity and recovery. Low cross-reactivity with other coccidiostats and an LOD of 0.14 µg/kg in chicken muscle was reported.

Radi, El-Naggar and Nassef (2014) developed an electropolymerized molecularly imprinted polypyrrole polymer modified screen printed carbon electrode (MIP-SPCE) for the detection of clopidol in poultry muscle. Samples were hydrolysed using an ethanol:HCl 1 mol/L mixture (1:1, v/v) and the final mixture was neutralized before analysis. The method was validated in terms of recovery and reproducibility.

Jiang *et al*. (2014) developed a one-step enzyme-linked immunosorbent assay (ELISA) for the detection of clopidol in poultry muscle. Samples are extracted using 0.01 mol/L NaOH:acetonitrile (1:10 v/v) and further diluted using 0.01 mol/L phosphate buffer. The method was validated in terms of matrix effects, selectivity and sensitivity. An LOD of 0.3 µg/kg in poultry muscle was reported.

Confirmatory methods

Rydchuk *et al*. (2023) developed an LC-MS/MS method for the determination of 14 coccidiostats, including clopidol, in poultry liver and muscle tissues. Both liver and muscle samples are prepared using a combined acetonitrile and 0.2 mol/L phosphate-citrate buffer extraction of the ground and homogenized material. After pH neutralization, a liquid-liquid extraction is performed using ethyl acetate and dichloromethane. Next, the upper layer is evaporated and reconstituted using 0.5 percent ammonium acetate in 40 percent methanol which is then defatted using n-hexane. Finally, the extract is cleaned up further using a microcentrifuge at 21 000 *g*. Reversed-phase liquid chromatography is performed using a Waters™ ACQUITY UPLC BEH C18 analytical column (50 × 2.1 mm, 1.7 µm) using a gradient elution with 0.1 percent formic acid in water as mobile phase A and 0.1 percent formic acid in methanol as mobile phase B. Finally, detection and quantitation are performed using tandem mass spectrometry in MRM mode. Multiple labelled internal standards were included in the method, although it is not specified which one was used to correct for clopidol. The method was validated as a quantitative confirmatory method according to (EU) 2021/808 guidelines, including identification and confirmation criteria, selectivity, linearity, matrix effects, recovery, repeatability and reproducibility. For chicken muscle, an LOD of 0.9 µg/kg and an LOQ of 3.6 µg/kg were reported, and for chicken liver, an LOD of 1.2 µg/kg and LOQ of 4.1 µg/kg were reported. The method has been used by the national reference laboratory of veterinary drug residues controls in Ukraine.

Dasenaki and Thomaidis (2019) report a hydrophilic interaction LC-MS/MS based method for the determination of 16 coccidiostats in poultry, bovine, porcine, ovine and rabbit tissues. Homogenized samples are extracted using acetonitrile and dispersive solid phase extraction (dSPE) clean-up is performed using $MgSO_4$ and C_{18} sorbent. The extract is evaporated and reconstituted using 1 mmol/L ammonium formate with 0.1 percent formic acid in acetonitrile (20:80, v/v) and filtered (0.22 µm). Chromatography is performed using a Waters™ ACQUITY UPLC BEH HILIC (100 × 2.1 mm, 1.7 µm) column under isocratic elution using acetonitrile as mobile phase A and aqueous ammonium formate 1 mmol/L with 0.1 percent formic acid (v/v) as mobile phase B. Detection and quantitation is performed using tandem mass spectrometry in MRM mode. The electrospray ionization source is operated in positive mode (ESI+). The method was fully validated as a quantitative confirmatory method according to (EU) 2002/657/EC in all aforementioned matrices and the validated parameters include identification and confirmation criteria, selectivity, linearity, matrix effects, recovery, repeatability, reproducibility and sensitivity. Furthermore, proficiency test materials were analysed for further method performance verification. LODs for clopidol between 0.263 and 0.513 µg/kg were reported for the investigated matrices. The method has been used in the Greek National Residue Control Plan.

Barreto *et al*. (2017) developed an LC-MS/MS method for the determination of 14 coccidiostats, including clopidol, in poultry muscle. Homogenized samples were extracted with acetonitrile using an Ultra-Turrax® system for tissue disruption. A low--temperature clean-up was performed and after centrifugation the supernatant was evaporated. Next, the extract was simultaneously reconstituted and defatted using n-hexane and water:acetonitrile (1:1, v/v). Finally, the aqueous phase was used for analysis. Chromatography was performed with a Agilent Poroshell 120 ECC18 column (50 × 3.0 mm, 2.7 µm) using a gradient elution consisting of water as mobile phase A and acetonitrile as mobile phase B, both containing 5 mmol/L formic acid and 5 mmol/L ammonium acetate. Detection and quantitation were performed using a linear ion trap-tandem mass spectrometer in MRM mode. The electrospray ionization source was operated in positive mode (ESI+). Robenidine-d8 was used as an internal standard. The method was validated as a quantitative confirmatory method according to (EU) 2002/657/EC and Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) guidelines, including linearity, sensitivity, repeatability, reproducibility, recovery, matrix effects and selectivity for poultry muscle. A

calculated LOD of 2.5 µg/kg and LOQ of 6.25 µg/kg for poultry muscle were reported. The method has been used in the Brazilian National Residue Control Plan.

Appraisal

Clopidol is a coccidiostat registered for use in several Member States at inclusion rates of 80–250 mg/kg feed in broiler chickens and pullets up to 16 weeks of age. The withdrawal periods range from 0–7 days. Clopidol is not authorized for use in laying hens.

Metabolism

Limited literature exists on the pharmacokinetics and metabolism of clopidol in laboratory animals and most studies were conducted over 50 years ago. The literature suggests that, in the laboratory animal (rats and rabbits), clopidol is rapidly absorbed and eliminated. However, no studies were provided for assessing the pharmacokinetics in chickens or for comparing the metabolism of clopidol in laboratory animals to that in chickens. Nevertheless, similarities in the metabolism reported in published papers on laboratory animals and in the sponsor-provided radiolabelled metabolism study in chickens (Kim, 2023), in which clopidol was identified as the major residue in tissues, were noted. In the study in chickens, the metabolite 3,5-dichloro-2-hydroxymethyl-6-methylpyridin-4-ol was identified. However, this metabolite constituted <10 percent of the TRR and was detected only at 6 h post-dose. While the same metabolite was detected in rabbits, it was not reported in rats, the laboratory species used in the toxicology studies used to determine the acceptable daily intake (ADI).

Radiolabelled residue depletion

Results from the radiolabelled residue depletion study (Kim, 2023) indicate that clopidol rapidly depletes from chicken tissues, with most of the total radioactivity and clopidol residue concentrations below the LOQ by days 5 and 3 post-dose, respectively. Clopidol constituted >80 percent of the TRR, confirming its suitability as the marker residue. MR:TR could be calculated only at 6 h and 1 day after dosing in all tissues, because of the rapid elimination of clopidol. The sponsor reported MR:TRs of 1 in kidney, muscle, and skin/fat and 0.91 in liver, 1 day after dosing.

Limitations with the study were noted. Numerous samples at the 6 h and 1 day timepoints exceeded the VICH-recommended acceptance criterion of ≤5 percent (from -40.2 percent to +28.8 percent) for tritium exchange with water, suggesting that the tritium label was not stable. Because of this uncertainty, the Committee considered it appropriate to use a conservative MR:TR value of 0.5 in assessing dietary exposure. In addition, clopidol is intended as a continuous use product *via* feed. Radiolabelled clopidol was administered once via an oral aqueous solution. Although VICH GL46 allows for the employment of gavage or bolus dosing for drugs intended for oral administration, it also is recommended that the dose be divided and given in the morning and afternoon to better approximate actual use conditions. In addition, for continuously administered drugs, a steady state should be achieved. The sponsor did not provide data regarding steady state in the edible tissues of chickens. Clopidol is registered for use at an inclusion rate of 80 mg clopidol/kg feed to 250 mg clopidol/kg feed. In the radiolabelled residue depletion study, chickens were dosed based on their bodyweight. However, based on the calculated mg/kg bodyweight doses for both the 125 and 250 mg feed inclusion rates from the non-radiolabelled residue depletion study of 11 and 22 mg/kg bw per day, respectively, the Committee concluded that the use of a 25 mg/kg bodyweight dose rate was acceptable. The Committee noted that animals were euthanized with xylazine and succinylcholine. However, because the LC-MS/MS was operated in selected reaction monitoring (SRM) mode, interference with chemical analysis due to use of chemical euthanasia was not expected. Lastly, the Committee noted that the numerical values for

those residue concentrations determined to be below the LOQ were not provided, so the Committee could not verify that the concentrations were indeed below the LOQ.

Non-radiolabelled residue depletion

A non-radiolabelled clopidol residue depletion study (Jeong *et al.*, 2023) conducted in chickens continuously fed either the approved label dose of 125 or 250 mg clopidol/kg feed for 14 days, further corroborated previous findings that clopidol rapidly depleted from the edible tissues of broilers. Clopidol concentrations depleted most slowly from skin/fat in both treatment groups. By days 7 and 10 after the final dose, clopidol concentrations in chickens treated with either inclusion rate were below the LOQ (50 µg/kg) in all tissues. No explanation was provided for the similar residue concentrations observed despite the difference in inclusion rates.

The Committee noted similar limitations with the sponsor-provided non-radiolabelled residue depletion study as were found with the radiolabelled residue depletion study. Specifically, animals were euthanized with xylazine and succinylcholine and numerical values for those residue concentrations below the LOQ were not provided.

Non-radiolabelled residue depletion data found in the published literature are all from studies where the dose rate was 125 mg clopidol/kg feed, which is around half the maximum dose authorized in Member States in accordance with Good Veterinary Practice (GVP).

In all three studies (Czeglédi-Jankó, Balla and Tóth, 1976; Ekström, Slanina and Dahlström-King, 1984; Pang *et al*., 2001a), samples were analysed for parent clopidol only, which is in line with the pharmacokinetic data which demonstrate only limited metabolism.

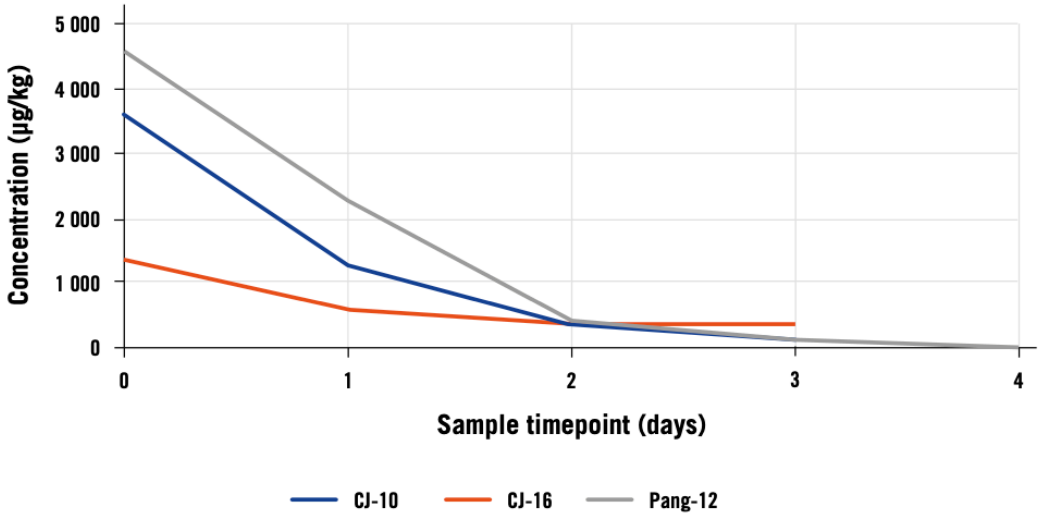
The duration of treatment differed between the studies. In one study (Pang *et al*., 2001b), birds were treated for 12 days, in the second (Ekström, Slanina and Dahlström-King, 1984), the birds were treated for 5 weeks (34 days) and in another (Czeglédi-Jankó, Balla and Tóth, 1976), they were treated for either 10 or 16 weeks before withdrawal of the medicated feed and slaughter for sampling.

In all studies, liver and kidney had the highest levels of residues at each timepoint studied, followed by muscle tissues. None of the studies investigated residues in skin or fat, which is a requirement of VICH GL48.

From the studies where the data were presented, there is a trend for those birds that were treated for a longer duration to have lower concentrations of clopidol in their tissues (Figure 5). This may be due to larger birds consuming less feed on a w/w basis, and thus less clopidol, or it may be that elimination becomes more efficient when constantly exposed (increased metabolism due to induction of liver enzymes is less likely to be a factor, as there appears to be no reduction in MR:TRR over time (Kim, 2023)).

Other sources of difference between the studies include the strain of chicken used, with faster growing birds becoming more predominant over time, and the analytical methodology used, which is not always described in great detail.

Figure 5. Comparison of residue levels in chicken livers from published studies



Notes: CJ-10: residue levels after treatment with 125 mg/kg feed for 10 weeks, as reported in Czeglédi-Jankó, Balla and Tóth (1976); CJ-16: residue levels after treatment with 125 mg/kg feed for 16 weeks, as reported in Czeglédi-Jankó, Balla and Tóth (1976); Pang-12: residue levels after treatment with 125mg/kg feed for 12 days, as reported in Pang *et al*. (2001a); bw: body weight

Source: based on Czeglédi-Jankó, G., Balla, J., & Tóth, L. 1976. Drug residues in the organs of broiler chickens after flock treatment with Rigecoccin R (clopidol). *Acta Veterinaria Academiae Scientiarum Hungaricae*, 26(4): 445–53. PMID: 1052659; Pang, G. F., Cao, Y. Z., Fan, C. L., Zhang, J. J. & Li, X. M. 2001a. Determination of clopidol residues in chicken tissues by liquid chromatography: part II. Distribution and depletion of clopidol in chicken tissues. *Journal of AOAC INTERNATIONAL*, 84(5): 1343–1346.

The sponsor-provided non-radiolabelled residue depletion study was sufficient to calculate percentile concentrations and corresponding one-sided 95 percent confidence interval over the 95th percentile of residue concentrations (95/95 upper tolerance limit, UTL) in chicken skin/fat at 1-day withdrawal. Because quantifiable residues in chicken kidney, liver and muscle were found at only two sampling timepoints (day 1 and 3), regression analysis could not be used to determine UTLs in those tissues. Therefore, a 95/95 UTL was calculated at a single timepoint (1-day withdrawal) for these tissues, using the results of the 250 mg/kg feed inclusion rate.

Analytical methodology

The Committee assessed the validation data against the requirements for analytical methods published in Codex Guideline CAC/GL 71-2009.

A sponsor-provided validated LC-MS/MS method was considered suitable for routine monitoring of clopidol as the marker residue in chicken liver, kidney, muscle and skin/fat. The LOQ of the method is 50 µg/kg.

In addition, public literature provides information on the development of analytical methods, including extraction, clean-up where required, and analysis of clopidol residues in poultry muscle, liver and kidney tissues. The methods described are single analyte methods, or multi-residue methods, which can detect or confirm multiple coccidiostats in tissue samples. These methods can be used as part of a residues control programme. Recent publications present validation data and confirm the reliability of the methods described. Overall, it is considered that there is enough information in the public domain to

allow a Member State or regional competent authority to set up a valid method to analyse chicken tissue samples for residues of clopidol.

Dietary exposure assessment

Chronic dietary exposure assessment

When used as a veterinary drug, dietary exposure to clopidol was estimated based on the potential occurrence of clopidol residues in chicken tissues. Residue concentrations were taken from measurements made at 24-h withdrawal (day 1) for an inclusion rate of 250 mg/kg feed. The studies reported residue concentrations in terms of clopidol (the marker residue).

The available studies provide residue data for both chicken liver and kidney. However, the available food consumption information on consumption of chicken kidney includes only a single individual. Given that chicken liver is much more commonly consumed and contained higher residue concentrations than chicken kidney, only chicken liver was included in the assessment of chronic dietary exposure to clopidol.

Given uncertainty around the MR:TR, sensitivity of dietary exposure estimates to this parameter was assessed by deriving dietary exposure estimates based on three MR:TRs for all tissue types: 1, 0.9 and 0.5. Clopidol residue values used to estimate dietary exposure were derived from the regression analysis of depletion for skin with fat and from the median residue determined at 24-h for chicken muscle and chicken liver.

Based on incurred clopidol residues at 24-h withdrawal time in chicken muscle, chicken liver and skin with fat (250 mg/kg feed) and a MR:TR of 1, the global estimates of chronic dietary exposure (GECDE) for the adults and the elderly, children and adolescents, and for infants and toddlers were 16.5, 16.8 and 14.3 µg/kg bw per day, respectively, which represent 41, 42 and 36 percent, respectively, of the upper bound of the ADI of 40 µg/kg bw (Table 15).

Based on incurred clopidol residues at 24-h withdrawal time in chicken muscle, chicken liver and skin with fat (250 mg/kg feed) and a MR:TR of 0.9, the GECDE for adults and the elderly, children and adolescents, and for infants and toddlers were 18.3, 18.6 and 15.9 µg/kg bw per day, respectively, which represent 46, 47 and 40 percent, respectively, of the upper bound of the ADI of 40 µg/kg bw.

Based on incurred clopidol residues at 24-h withdrawal time in chicken muscle, chicken liver and skin with fat (250 mg/kg feed) and a MR:TR of 0.5, the GECDE for adults and the elderly, children and adolescents, and for infants and toddlers were 32.9, 33.5 and 28.6 µg/kg bw per day, respectively, which represent 82, 84 and 71 percent, respectively, of the upper bound of the ADI of 40 µg/kg bw.

As part of the GECDE methodology, further estimates of chronic dietary exposure were carried out. Instead of using the highest mean and the highest reliable percentile consumption across surveys, the calculations were carried out using the mean and the highest reliable percentile for each individual national survey from available datasets (FAO/WHO Chronic individual food consumption database, CIFOCOss). The highest GECDE for each age class for each country was determined.

For the inclusion rate of clopidol at 250 mg/kg feed and the most conservative MR:TR of 0.5, the mean (range) of 35 country-specific estimates for clopidol dietary exposure for adults and the elderly at 24-h withdrawal was 8.5 (1–27.9) µg/kg bw per day, or 21 percent (2–70 percent) of the upper bound of the ADI. The mean (range) of 26 country-specific estimates of clopidol dietary exposure for children and adolescents at 24-h withdrawal was 13.8 (0.6–33) µg/kg bw per day, or 35 percent (1–83 percent) of

the upper bound of the ADI. The mean (range) of 18 country-specific estimates of clopidol dietary exposure for infants and toddlers at 24-h withdrawal was 16 (2.7–27.9) µg/kg bw per day or 40 percent (7–70 percent) of the upper bound of the ADI.

As no acute reference dose (ARfD) was necessary, acute dietary exposure (global estimate of acute dietary exposure, GEADE) was not assessed for clopidol.

Maximum residue limits

In recommending maximum residue limits (MRLs) for clopidol in chicken liver, kidney, muscle and skin/fat, the Committee considered the following factors:

- The Committee established an ADI of 0–0.04 mg/kg bw for clopidol.
- The Committee concluded that establishment of an ARfD for clopidol was unnecessary.
- Clopidol is registered for use in several Member States. The withdrawal periods range from 0–7 days for use of clopidol at inclusion rates of 80–250 mg/kg feed in broilers and replacement layers (pullets) up to 16 weeks of age.
- Clopidol is not authorized for use in laying hens.
- Clopidol is rapidly absorbed and excreted after oral administration.
- Clopidol is a suitable marker residue in all edible tissues of chickens.
- In the radiolabel study, numerous samples at both times exceeded the VICH-recommended acceptance criterion for exchange of tritium with water, suggesting that the tritium label was unstable. Because of this uncertainty, the Committee considered it appropriate to use a conservative MR:TR value of 0.5 in assessing dietary exposure.
- The study of non-radiolabelled residue depletion at the highest inclusion rate (250 mg/kg feed) was sufficient to determine the mean marker residue and 95/95 UTL concentrations in chicken skin/fat at 1-day withdrawal.
- Quantifiable residues in chicken kidney, liver and muscle were found at only two sampling times. Therefore, regression analysis could not be used to determine UTLs in those tissues. A 95/95 UTL was calculated at a single timepoint for these tissues, with the results of the 250 mg/kg feed inclusion rate at 1-day withdrawal.
- A validated LC-MS/MS method was considered suitable for routine monitoring of clopidol as the marker residue in chicken liver, kidney, muscle and skin/fat.

Table 15. Global estimate of chronic dietary exposure (GECDE) for clopidol in chicken tissues

Category	Type	Median concentration[1] (µg/kg)	Mean consumption, whole population[2] (g/kg bw per day)	HRP consumption, consumers only[3] (g/kg bw per day)	MR:TR ratio	Exposure (µg/kg bw per day)		GECDE[4]	
						Mean	HRP	µg/kg bw per day	%ADI
250 mg/kg clopidol: 24 h post-withdrawal, MR:TR = 1.0									
Adults and the elderly									
Poultry muscle	Chicken muscle	1 244	1.25	11.2	1.00	1.6	14.0	14.0	
Poultry offal	Chicken liver	2 804	0.88	1.59	1.00	2.5	4.4	2.5	
Poultry trimmed fat	Chicken fat	725	0.06	0.35	1.00	0.04	0.25	0.04	
TOTAL								16.5	41
Children and adolescents									
Poultry muscle	Chicken muscle	1 430	2.75	13.3	1.00	3.4	16.5	16.5	
Poultry offal	Chicken offal	3 850	0.06	2.04	1.00	0.17	5.7	0.17	
Poultry trimmed fat	Chicken fat	960	0.12	0.65	1.00	0.09	0.47	0.09	42
TOTAL								16.8	
Infants and toddlers									
Poultry muscle	Chicken muscle	1 430	3.96	11.2	1.00	4.9	14.0	14.0	
Poultry offal	Chicken offal	3 850	0.06	1.08	1.00	0.25	3.0	0.25	
Poultry trimmed fat	Chicken fat	960	0.10	0.63	1.00	0.07	0.45	0.07	
TOTAL								14.3	36
250 mg/kg clopidol: 24 h post-withdrawal, MR:TR = 0.9									
Adults and the elderly									
Poultry muscle	Chicken muscle	1 430	1.25	11.2	0.90	1.7	15.5	15.5	
Poultry offal	Chicken liver	3 850	0.88	1.59	0.90	2.7	4.9	2.7	
Poultry trimmed fat	Chicken fat	725	0.06	0.35	0.90	0.05	0.28	0.05	
TOTAL								18.3	46
Children and adolescents									
Poultry muscle	Chicken muscle	1 430	2.75	13.3	0.90	3.8	18.3	18.3	
Poultry offal	Chicken offal	3 850	0.06	2.04	0.90	0.19	6.4	0.19	
Poultry trimmed fat	Chicken fat	960	0.12	0.65	0.90	0.10	0.52	0.10	
TOTAL								18.6	47
Infants and toddlers									

Category	Type	Median concentration[1] (µg/kg)	Mean consumption, whole population[2] (g/kg bw per day)	HRP consumption, consumers only[3] (g/kg bw per day)	MR:TR ratio	Exposure (µg/kg bw per day)		GECDE[4]	
						Mean	HRP	µg/kg bw per day	%ADI
Poultry muscle	Chicken muscle	1 430	3.96	11.2	0.90	5.5	15.5	15.5	
Poultry offal	Chicken offal	3 850	0.06	1.08	0.90	0.27	3.4	0.27	
Poultry trimmed fat	Chicken fat	960	0.10	0.63	0.90	0.08	0.51	0.08	
TOTAL								15.9	40
250 mg/kg clopidol: 24 h post-withdrawal, MR:TR = 0.5									
Adults and the elderly									
Poultry muscle	Chicken muscle	1 430	1.25	11.2	0.50	3.1	27.9	27.9	
Poultry offal	Chicken liver	3 850	0.88	1.59	0.50	4.9	8.9	4.9	
Poultry trimmed fat	Chicken fat	725	0.06	0.35	0.50	0.09	0.51	0.09	
TOTAL								32.9	82
Children and adolescents									
Poultry muscle	Chicken muscle	1 430	2.75	13.3	0.50	6.8	33.0	33.0	
Poultry offal	Chicken offal	3 850	0.06	2.04	0.50	0.34	11.4	0.34	
Poultry trimmed fat	Chicken fat	960	0.12	0.65	0.50	0.18	0.94	0.18	
TOTAL								33.5	84
Infants and toddlers									
Poultry muscle	Chicken muscle	1 430	3.96	11.2	0.50	10.0	27.9	27.9	
Poultry offal	Chicken offal	3 850	0.06	1.08	0.50	0.49	6.1	0.49	
Poultry trimmed fat	Chicken fat	960	0.10	0.63	0.50	0.14	0.91	0.14	
TOTAL								28.6	71

Notes: MR: marker residue; TR: total residue; HRP: highest reliable percentile; GECDE: global estimates of chronic dietary exposure; [1]Median concentration of the marker residue at the specified times after the end of treatment expressed as clopidol; [2]Highest mean consumption figures based on whole population considered from the available dataset; [3]Highest reliable percentile food consumption figures based on consumers only considered from the available dataset; [4]GECDE is the sum of the highest exposure at the highest reliable percentile of consumption for a food and the mean dietary exposures of the other food.

Source: Authors' own elaboration.

The Committee recommended MRLs of 10 400 µg/kg (liver), 8 800 µg/kg (kidney), 4 100 µg/kg (muscle) and 2 600 µg/kg (skin/fat) in chickens.

For calculation of the UTL at a single timepoint, the Committee followed the approach described at https://www.itl.nist.gov/div898/handbook/prc/section2/prc263.htm. The general formula for any UTL is:

UTL = mean residue concentration + (k × standard deviation),

where k is a factor chosen to ensure the specified coverage and confidence (95/95).

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