# Derquantel

First draft prepared by

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## Identity

**International Non-proprietary names (INN):** Derquantel

**Synonyms:** PF-00520904, PNU-141962, 2-DOPH, 2-desoxyparaherquamide, 2-deoxyparaherquamide, Startect® (derquantel + abamectin)

**IUPAC Name:** (1R,3S,5R,7S,12R)-12-Hydroxy-4,4,4',4',12,14-hexamethyl-9',10'-dihydro-4'Hspiro[9,14-diazatetracyclo [5.5.2.0$^{1,9}$.0$^{3,7}$]tetradecane-5,8'-[1,4]dioxepino[2,3-g]indol]-13-one

**Chemical Abstract Service Number:** 187865-22-1

**Structural formula of main components:**



Derquantel



Chiral
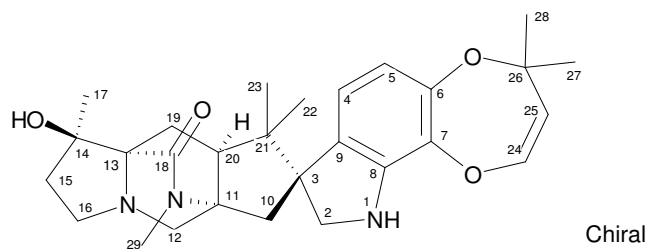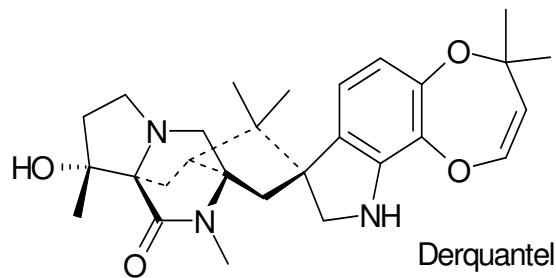
**Molecular formula:** C$_{28}$H$_{37}$N$_3$O$_4$

**Molecular weight:** 479.6

## Other information on identity and properties

**Appearance:** White to tan coloured powder, essentially free from foreign matter

**Melting point:** 196.5–197.5 °C

**Solubility:**

| From Gottschall, 2010a | From MSDS, 2009 |
|---|---|
| 125 mg/L in water at 25°C | 0.1–1 mg/L in water |
|  | 0.1–1 mg/ml in n-heptane |
| 21.6 mg/ml in isopropanol at 25°C | 10–33 mg/ml in propanol |
| 29.1 mg/ml in tert-amyl alcohol at 25°C | 33–100 mg/ml |
| 10.0 mg/ml in tert-butylmethylether at 25°C | 1-10 mg/ml |
| 55.9 mg/ml in ethyl acetate at 25°C |  |
| 54.8 mg/ml in methanol at 25°C | 100–1000 mg/ml |
| 39.6 mg/ml in acetonitrile at 25°C |  |
| 0.1 mg/ml in isooctane at 25°C |  |
| 79.4 mg/ml in toluene at 25°C |  |
|  | 33–100 mg/ml in acetone |

# Residues in food and their evaluation

## Conditions of use

Derquantel, a spiroindole, is an oral anthelmintic for use in sheep. It is registered only in combination with abamectin. The combination product contains 10 mg/ml derquantel and 1 mg/ml abamectin. Derquantel, in combination with abamectin, is used to treat and control a broad range of adult and immature (L4) gastrointestinal nematodes of sheep, including parasites resistant to macrocyclic lactone-, levamisole-, benzimidazole- and closantel-based drenches (and combinations of these) (Kaminsky *et al.*, 2011; Little *et al.*, 2010, 2011).

## Dosage

The dose for sheep and lambs is 2 mg derquantel and 0.2 mg abamectin/kg bw.

# Pharmacokinetics and metabolism

## Pharmacokinetics in laboratory animals and humans

No *in vivo* pharmacokinetic studies are provided for derquantel in laboratory animals or humans. A report from the published literature (Aloysius *et al.*, 2008) describes the comparative pharmacokinetics of paraherquamide, a closely related compound, in dogs, gerbils and sheep (Tables 3.1 and 3.2). The GLP status of this study cannot be determined from the literature report.

**Table 3.1.** Pharmacokinetics of paraherquamide and total drug related material (DRM) in sheep and dogs following a single 0.5 mg/kg oral dose of $^3$H-paraherquamide

| Pharmacokinetic parameter | Sheep | | Dog | |
|---|---|---|---|---|
|  | PHQ | Total DRM | PHQ | Total DRM |
| $C_{max}$ (ng/ml) | 38 | 46 | 73 | 167 |
| $T_{max}$ (h) | 0.5 | 8 | 0.5 | 1 |
| $AUC_{0-last}$ (ng-h/ml) | 547 | 1192 | 136 | 1387 |
| $t_{1/2}$ (h) | 8.5 | 13 | 1.5 | 23 |

**Table 3.2.** Excretion of radioactivity in sheep, dogs and gerbils following a single oral dose of $^3$H-paraherquamide

|  | Sheep | Dog | Gerbil |
|---|---|---|---|
| Dose (mg/kg) | 0.5 | 0.5 | 20 |
| Collection time (h) | 0–96 | 0–72 | 0–72 |
| Urine | 9% | 23% | 17% |
| Faeces | 81% | 58% | 67% |
| Total | 90% | 81% | 84% |

## *Pharmacokinetics in food-producing animals*

### Sheep

Two GLP-compliant pharmacokinetic studies were conducted in sheep, the target species for the commercial combination product (derquantel plus abamectin).

A GLP-compliant pharmacokinetic study (Walker, 2009b) was conducted in Texel and Texel-cross sheep to compare pharmacokinetic parameters after oral or intravenous dosing. Twelve sheep were assigned (3 castrated males, 3 intact males and 6 females) per dose group. Group 1 received the final commercial formulation (2 mg derquantel/kg bw and 0.2 mg abamectin/kg bw) by drench. Group 2 received an i.v. injection of derquantel alone at a dose of 1.0 mg/kg bw, equivalent to half the dose of derquantel in the commercial combination product formulation. Group 3 received an i.v. injection of abamectin at a dose level of 0.1 mg/kg bw, again equivalent to half the dose of abamectin in the commercial combination product formulation. Plasma samples were collected at pre-dose and for 168 hours post-dose. A validated LC-MS/MS method, with an LOQ of 0.5 ng/ml for each analyte, was used to quantify derquantel and abamectin in plasma. The pharmacokinetic parameter estimates for derquantel and abamectin are presented in Tables 3.3 and 3.4, respectively.

**Table 3.3**. Pharmacokinetic parameters of derquantel in plasma of sheep (males+females) following i.v. (derquantel alone) and drench (commercial combination product) administration

| Parameter | Oral commercial combination product | | | i.v. Derquantel | | |
|---|---|---|---|---|---|---|
| | Mean | 95% Confidence Interval | | Mean | 95% Confidence Interval | |
| | | Lower | Upper | | Lower | Upper |
| $C_{max}$ (ng/ml) | 108 | 80.8 | 145 | 959 | 716 | 1280 |
| $T_{max}$ (hours) | 4.17 | 3.25 | 5.10 | 0.182 | -0.741 | 1.11 |
| $t_{1/2}$ (hours) | 9.3 | 6.1 | 19.3 | 5.7 | 4.0 | 9.7 |
| AUC $_{0\text{-last}}$ (ng-h/ml) | 1760 | 1330 | 2340 | 1570 | 1390 | 1780 |
| AUC $_{0\text{-}\infty}$ (ng-h/ml) | 1790 | 1360 | 2360 | 1590 | 1410 | 1810 |
| VD$_{SS}$ (ml/kg) | NC | NC | NC | 3220 | 2470 | 3970 |
| Clearance (ml/kg/min) | NC | NC | NC | 11.1 | 9.49 | 12.8 |
| Bio-availability (%) | 56.3 | 44.3 | 71.4 | NC | NC | NC |

NOTES: NC = not calculated.

**Table 3.4.** Pharmacokinetic parameters of abamectin in plasma of sheep (males+females) following oral administration of abamectin alone or in the commercial combination formulation

| Parameter | Oral commercial combination product | | | i.v. Abamectin | | |
| --- | --- | --- | --- | --- | --- | --- |
| | Mean | 95% Confidence Interval | | Mean | 95% Confidence Interval | |
| | | Lower | Upper | | Lower | Upper |
| $C_{max}$ (ng/ml) | 31.1 | 22.5 | 42.9 | 109 | 79.0 | 150 |
| $T_{max}$ (hours) | 23.8 | 17.8 | 29.8 | 0.122 | 0.029 | 0.216 |
| $t_{1/2}$ (hours) | 27.7 | 18.0 | 59.9 | 27.6 | 18.0 | 59.6 |
| AUC $_{0\text{-last}}$ (ng-h/ml) | 1660 | 1220 | 2260 | 1170 | 865 | 1590 |
| AUC $_{0-\infty}$ (ng-h/ml) | 1730 | 1250 | 2390 | 1240 | 898 | 1710 |

There was no statistically significant ($P$>0.05) sex-related differences in any pharmacokinetic estimates between castrated males, intact males and females for derquantel or abamectin, and all data represent combined male+female results. The derquantel in the combination product had a $T_{max}$ of 4.17 h (range 3.25–5.10 h) and a $C_{max}$ of 108 ng/ml (range 80.8-145 ng/ml). The oral bio-availability of derquantel in the combination product was approximately 56% (range 44.3-71.3%). Following i.v. administration, derquantel was well distributed in the body with a volume of distribution at steady state ($VD_{SS}$) of 3220 ml/kg (range 2470-3970 ml/kg) and a moderate clearance of 11.1 ml/kg/min (9.5-12.8 ml/kg/min). Derquantel, in the combination product, had a half-life of 9.3 h (range 6.1–19.3 h). The maximum plasma concentration of abamectin was reached in about 24 h after a single oral dose of the combination product. The oral bio-availability was about 70%. Abamectin was well distributed in the body with a $VD_{SS}$ of 3530 ml/kg. Clearance of abamectin was low, at 1.5 ml/kg/min. The half-life was 27.6 h.

In a second GLP-compliant plasma pharmacokinetic study (Walker, 2009a), the potential for interaction between derquantel and abamectin, when co-administered by oral drench as the commercial combination product, was evaluated in Texel and Texel-cross sheep. Three treatment groups were evaluated: derquantel alone, abamectin alone, and the commercial combination product formulation containing 2 mg/kg bw derquantel and 0.2 mg/kg bw abamectin. Each treatment group contained 6 males and 6 females. Plasma samples were collected at pre-dose and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, 72, 96, 120, 144 and 168 h after dosing. Samples were analysed using a validated LCMS/MS method. The method has an LOQ of 0.5 ng/ml for each analyte in plasma.

With the exception of $T_{max}$ derquantel, there were no statistically significant ($P$>0.05) sex differences in any pharmacokinetic parameters for either derquantel or abamectin. The $T_{max}$ for derquantel for males was 3.96 (2.84–5.07) h and for females was 2.65 (1.53–3.76) h. Although there was a statistically significant difference in derquantel plasma $T_{max}$ between males and females, $T_{max}$ was considered similar and did not result in a sex difference in overall exposure when defined by the $AUC_{0\text{-t(last)}}$, $AUC_{0-\infty}$, and $C_{max}$.

All other comparisons were made using combined male and female data. There were no statistically significant differences ($P$>0.05) for any pharmacokinetic parameter between dose groups for either derquantel or abamectin with the exception of $AUC_{0\text{-t(last)}}$ for derquantel ($P$=0.044). The $T_{max}$ (combined) was 2.60 h (range 1.05–4.16 h) for derquantel in the combination product versus 4.00 for derquantel alone. For the commercial combination, the half-life for derquantel was 13.1 h (range 9.82–19.6 h) versus 10.4 h for derquantel alone. The $AUC_{0-\infty}$ was 1250 ng-h/ml (range 1000–1570 ng-h/ml) for the combination product versus 1710 ng-h/ml for derquantel alone. The $C_{max}$ for derquantel in the combination product was 92.8 ng/ml (range 68.2–126 ng/ml) in the second study versus 105 ng/ml for the derquantel alone.

The half-life for abamectin in the combination product (27.7 h in the first study and 25.4 h in the second study) is consistent with that found during the evaluation of abamectin at the 45th Meeting of the Committee (1.2 ± 0.3 days) (FAO, 1995; 1996) and is generally consistent with the somewhat more variable half-life results reported in the scientific literature (Irish Medicines Board, 2007; Hongbo *et al.,* 1998). See Table 3.4A.

**Table 3.4A.** Half-life values for abamectin reported in the literature

| Parameter | Source of data | | |
|---|---|---|---|
| | Walker, 2009b. | Hongbo *et al.,* 1998. | Irish Medicines Board, 2007 |
| Dose (mg/kg) | 0.2 | 0.2 | 0.2 |
| $C_{max}$ (ng/ml) | 31.1 | 21.2 | 44 |
| $T_{max}$ (h) | 24 | 14 | 24 |
| $t_{1/2}$ (h) | 28 (17.9-56.5)* | 69.9 | 61.8 (12-192) |

NOTES: * = mean (range)

**Table 3.5.** Pharmacokinetic parameters of derquantel in plasma of sheep (males+females) following oral administration of derquantel alone or in the commercial combination product formulation

| Parameter | Oral commercial combination product | | | Oral Derquantel | | |
|---|---|---|---|---|---|---|
| | Mean | 95% Confidence Interval | | Mean | 95% Confidence Interval | |
| | | Lower | Upper | | Lower | Upper |
| $C_{max}$ (ng/ml) | 92.8 | 68.2 | 126 | 105 | 76.9 | 143 |
| $T_{max}$ (hours) | 2.60 | 1.05 | 4.16 | 4.00 | 2.45 | 5.55 |
| $t_{1/2}$ (hours) | 13.1 | 9.82 | 19.6 | 10.4 | 9.25 | 12.0 |
| AUC $_{0-last}$ (ng-h/ml) | 1210 | 974 | 1500 | 1680 | 1310 | 2140 |
| AUC $_{0-\infty}$ (ng-h/ml) | 1250 | 1000 | 1570 | 1710 | 1340 | 2180 |

**Table 3.6.** Pharmacokinetic parameters of abamectin in plasma of sheep (males+females) following oral administration of abamectin alone or in the commercial combination product formulation

| Parameter | Oral commercial combination product | | | Oral Abamectin | | |
|---|---|---|---|---|---|---|
| | Mean | 95% Confidence Interval | | Mean | 95% Confidence Interval | |
| | | Lower | Upper | | Lower | Upper |
| $C_{max}$ (ng/ml) | 26.5 | 21.9 | 32.1 | 26.1 | 21.5 | 31.6 |
| $T_{max}$ (hours) | 23.5 | 17.1 | 29.9 | 16.8 | 7.8 | 25.9 |
| $t_{1/2}$ (hours) | 25.4 | 19.1 | 38.1 | 22.1 | 17.2 | 31.2 |
| AUC $_{0-last}$ (ng-h/ml) | 1670 | 1170 | 2400 | 1190 | 831 | 1700 |
| AUC $_{0-\infty}$ (ng-h/ml) | 1730 | 1190 | 2520 | 1240 | 849 | 1800 |

Taken together, the overall evidence indicates no significant interaction between derquantel and abamectin when dosed in combination. Pharmacokinetic parameters for derquantel are summarized in Table 3.5 (see also EMA, 2010). Pharmacokinetic parameters for abamectin are summarized in Table 3.6.

Two disposition studies using radioisotopically labelled derquantel (one non-GLP-compliant pilot study and one GLP-compliant study) in sheep were evaluated.

In the non-GLP pilot disposition study (Byrd and Liu, 2006), sheep were treated orally by capsule with $^{14}$C-derquantel to achieve a target dose of 2 mg derquantel/kg bw. In Phase 1 of the study (one female), 6 capsules were needed to achieve the target dose due to the low specific activity of the test article. In Phase 2 (four males, one per timepoint), material with a higher specific activity was used and only one capsule per sheep was needed. The Phase 1 animal was slaughtered 3 h after dosing. The Phase 2 animals were slaughtered 6, 12, 24 and 48 h after dosing.

Faeces, urine and cage rinse samples were collected beginning on day 1 (both phases) and continuing until slaughter. Blood was collected immediately prior to slaughter. Whole blood was combusted and analysed by liquid scintillation counting (LSC). Plasma, urine and cage rinse samples were analysed directly by LSC. Faeces were homogenized, combusted and analysed by LSC.

The majority of the radioactivity was eliminated in the faeces at each sacrifice interval after 12 h. In the first 24 h, approximately 50% of the radioactivity was recovered in the excreta: 42% in the faeces and 8% in the urine. By 48 h, approximately 85% of the radio-activity was recovered in the excreta: 55% in the faeces and 30% in the urine (Table 3.7).

**Table 3.7.** $^{14}$C-derquantel equivalent residues (μg/kg) from sheep treated with a single oral dose of $^{14}$C-derquantel at a dose of 2 mg/kg bw

| Group | Slaughter interval after dosing | Urine | Faeces | Bile | Whole blood | Plasma | Cage rinse |
|---|---|---|---|---|---|---|---|
| 1 | 3 h | — | 20 | NA | 147.1 | 178.9 | 1.8 |
| 2 | 6 h | 5 405 | 875.1 | — | 337.7 | 349.1 | 665.9 |
| 3 | 12 h | 13 125 | 11 968 | 34 572 | 222.2 | 220.3 | 1 015 |
| 4 | 24 h | 6 160 | 47 975 | 26 682 | 58.3 | 49.1 | 925.6 |
| 5 | 48 h | 2 128 | 28 584 | 8 323 | 17.9 | 14.0 | 427.6 |

NOTES: NA = not analysed.

In the GLP-compliant disposition study (Byrd and Liu, 2008), 20 crossbred sheep (n=3 per group) were treated orally by gastric tube with $^{14}$C-derquantel. The specific activity of the test article was adjusted so that animals in the later slaughter groups received derquantel with the higher specific activity. All animals received a single oral dose of approximately 2.10 mg derquantel/kg bw. Animals were slaughtered approximately 6, 12, 24, 48, 96 and 144 h after dosing. Samples were stored at -20°C until analysed.

Urine, faeces, plasma, blood, bile and cage rinses were collected from each sheep daily, beginning on day (-1) and continuing through slaughter. Urine, plasma, bile and cage rinses were analysed directly by LSC. Whole-blood samples were analysed directly by combustion and LSC. Faeces were homogenized and submitted for combustion and LSC analysis.

The average total recovery of the administered dose in Groups 1, 2, 3, 4, 5 and 6 were 7.0%, 24.1%, 49.2%, 88.3%, 89.5% and 91.8%, respectively. Most of the administered radio-activity was recovered in the faeces, followed by urine and liver (for full tissue disposition, see Figure 3.1 and Table 3.8). The total radiolabelled residue (TRR) in urine (plus cage rinse)

and faeces accounted for approximately 10% and 85% of the dose administered, respectively (see Figure 3.1 and Table 3.8).
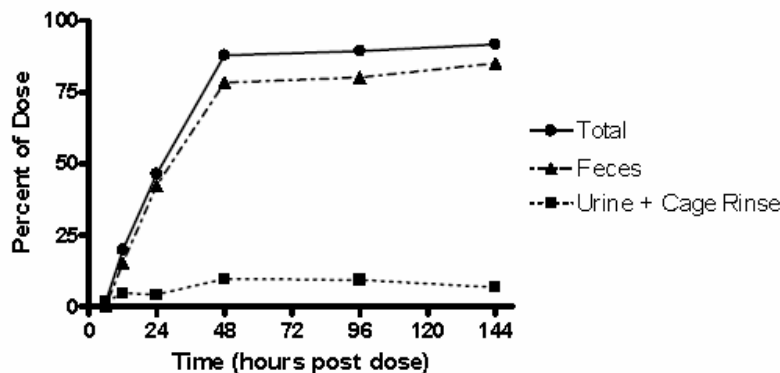


**Figure 3.1.** Cumulative recovery of ^{14}C-Derquantel equivalents (µg/kg)

No disposition data are provided for abamectin following use of the combination product. Although plasma and biliary data for abamectin were not included with the earlier Committee evaluation (FAO, 1995), it was concluded that, with 90% dose recovery, the majority (70%) was recovered in faeces. Both Committee evaluations (FAO, 1995, 1996) noted that abamectin, at the time it was reviewed, was registered only for use in beef cattle.

**Table 3.8.** Average total ^{14}C-derquantel equivalents*(µg/kg) in urine, faeces, bile, whole blood, plasma and cage rinse in sheep given a single oral dose of 2.10 mg ^{14}C-derquantel/kg bw

| Group | Slaughter interval post-dose | Urine | Faeces | Bile | Whole blood | Plasma | Cage rinse |
|-------|------------------------------|-------|--------|------|-------------|--------|------------|
| 1 | 6 h | 827.4 | 490.7 | NA | 176 | 217 | 474.2 |
| 2 | 12 h | 3 161 | 28 339 | 34 572 | 106 | 119 | 348.3 |
| 3 | 24 h | 1 996 | 45 128 | 26 682 | 84 | 89 | 705.3 |
| 4 | 48 h | 2 701 | 43 513 | 8 323 | 11 | 10 | 840 |
| 5 | 96 h | 20.7 | 1 606 | 56 | 5 | 2 | 16 |
| 6 | 144 h | 6.7 | 169 | 14 | 6 | 2 | 4.7 |

NOTES: * = Average is for the specific slaughter time only

### *Metabolism in rats, sheep, dogs and humans*

Two GLP-compliant studies were conducted to evaluate the metabolism of derquantel in the rat, sheep, humans and dogs.

In a GLP-compliant metabolism study (Ma, 2006a), radiolabelled desoxoparaherquamide (derquantel) was incubated with rat, sheep, dog and human liver microsomes. Non-radio-labelled 7-ethoxycoumarin (7-EC) was included as a positive control to test the metabolic integrity of the enzymatic preparations. After a 1-hour incubation, 55.0, 67.6, 100.0, and 75.0% of 7-EC was metabolized by rat, sheep, dog and human liver microsomes, respectively. A significant amount of 7-hydroxycoumarin (7-HC), a metabolite of 7-EC, was observed in 1-hour incubation samples and, consequently, all microsomal preparations were determined to be enzymatically active.

Incubation mixtures of ^{14}C-2-desoxoparaherquamide were extracted with methanol and analysed for metabolites using radio-HPLC. HPLC eluants were collected at 0.25 minute intervals using a fraction collector and were analysed for radioactivity using LSC. Radio-

activity peaks were integrated to determine the percent distribution of individual metabolites in each sample.

The data show that $^{14}$C-2-desoxoparaherquamide (derquantel) was extensively metabolized by dog liver microsomes and moderately metabolized by rat, sheep and human liver microsomes (Tables 3.9 and 3.10). After a 1-hour incubation, approximately 45.2, 39.8, 2.8 and 55.0% of the radioactivity was attributable to unchanged drug in rat, sheep, dog and human liver microsomes, respectively. In addition to unchanged $^{14}$C-2-desoxoparaherquamide, 18 radioactive components were detected in rat, sheep, dog or human liver microsomal incubations. The predominant metabolites after 1-hour incubation included M1, M9, M10 and M12 and accounted for about 9.5, 3.6, 7.3 and 3.4% of the radioactivity in the rat; 12.9, 5.3, 6.1 and 4.0% in sheep; 2.6, 2.1, 5.5 and 2.9% in the dog; and 12.4, 7.1, 8.9, and 3.6% in humans, respectively. M2, which accounted for approximately 2.1, 3.9 and 1.7% of the radioactivity in 1-hour rat, sheep and human liver microsomal incubations, respectively, was not detected in 1-hour dog liver microsomal incubations. Similarly, M3, a predominant metabolite accounting for approximately 40.1% of radioactivity in 1-hour dog liver microsomal incubations, accounted for only 2.4 and 7.6% of radioactivity in 1-hour rat and sheep liver microsomal incubations. Recovery of radioactivity was high at all sampling times, 0.25–1.0 h, and in all species: greater than 98% in the rat; nearly 95% in sheep; greater than 95% in dogs; and greater than 97% in humans.

**Table 3.9.** Relative percentage distribution of radioactivity (%HPLC)[1] in methanol extracts from rat, sheep, human and dog liver microsomes incubated for 0.25 hours at 37°C with $^{14}$C-derquantel[2]

| Metabolite | Species | | | |
|---|---|---|---|---|
| R*t* (min) | Rat | Sheep | Human | Dog |
| 2.6–3.6 | ND | 1.40 | ND | 2.28 |
| 11.4–12.4 | ND | ND | ND | 2.05 |
| 13.4–14.5 | 0.73 | 2.28 | ND | 20.85 |
| 14.0–15.6 | ND | 1.08 | ND | 6.54 |
| 15.4–17.1 | ND | ND | ND | 4.52 |
| 17.9–19.6 | ND | ND | ND | 7.16 |
| 19.9–21.6 | ND | ND | ND | 1.44 |
| 21.9–24.6 | 0.94 | ND | ND | ND |
| 24.1–26.4 | 0.86 | ND | ND | ND |
| 26.9–28.4 | 8.37 | 1.94 | ND | 10.22 |
| 27.9–29.9 | 1.99 | 1.24 | 3.71 | 1.93 |
| 29.4–31.9 | 3.59 | 2.87 | 3.96 | 3.32 |
| 31.9–33.1 | ND | ND | ND | ND |
| 36.1–38.1 | 3.05 | 2.95 | 3.21 | 3.28 |
| 39.4–41.1 | 1.62 | 1.08 | 1.92 | 1.14 |
| 41.1–43.1 | 69.59 | 73.52 | 79.99 | 30.13 |
| 45.1–47.1 | 7.06 | 7.67 | 5.17 | 3.73 |
| 52.5–54.6 | 1.10 | 0.99 | 0.88 | ND |
| 62.9–64.9 | ND | ND | ND | ND |
| Total (%) | 98.90 | 97.02 | 98.84 | 98.59 |

Notes: (1) Average of triplicate samples. (2) $^{14}$C-derquantel at 1 µM. ND = not detectable.

In a similarly designed GLP-compliant study (Ma, 2006b), $^{14}$C-2-desoxoparaherquamide (derquantel) was incubated with cryopreserved hepatocytes from rats, dogs and humans, and freshly prepared hepatocytes from sheep. Non-radiolabelled 7-ethoxycoumarin (7-EC) and 7-hydroxycoumarin (7-HC) were included as positive controls to test the metabolic integrity of the hepatocyte preparations. Derquantel incubated in the absence of hepatocytes

served as a negative control. The viability of the control hepatocytes was checked after 0, 2 and 4 h of incubations. At the completion of the 4-hour incubation, 36.4% of the rat hepatocytes and 70.0% of the sheep, dog and human hepatocytes remained viable.

The stability of ^{14}C-derquantel (1 µM and 25 µM concentrations) was assessed in incubations with media for 0 h and 4 h. At the 1 µM concentration, only 69.5% and 62.9% of unchanged parent remained in the 0 and 4 h incubations, respectively. At the 25 µM concentration, 90.4% and 90.1% of unchanged parent remained in the 0 and 4 h incubations, respectively.

The positive controls were used to determine the enzymatic activity of the hepatocyte preparations. After a 4-hr incubation, 35.4, 94.6, 45.8, and 29.5% of 7-EC and 18.5, 100, 96.3, and 90.4% of 7-HC were metabolized by rat, sheep, dog, and human hepatocytes, respectively. Sulphate and glucuronide conjugates also were identified. The hepatocyte preparations were deemed enzymatically active.

**Table 3.10.** Relative percentage distribution of radioactivity (%HPLC)[1] in methanol extracts from rat, sheep, human and dog liver microsomes incubated for 1 hour at 37°C with ^{14}C-derquantel[2]

| Metabolite | Species | | | |
|---|---|---|---|---|
| R*t* (min) | Rat | Sheep | Human | Dog |
| 2.6–3.6 | 0.98 | 4.43 | 0.98 | 5.55 |
| 11.4–12.4 | ND | ND | ND | 2.99 |
| 13.4–14.5 | 2.39 | 7.62 | ND | 40.09 |
| 14.0–15.6 | ND | 2.10 | ND | 8.34 |
| 15.4–17.1 | ND | 1.02 | ND | 8.68 |
| 17.9–19.6 | ND | ND | ND | 7.58 |
| 19.9–21.6 | ND | ND | ND | 2.22 |
| 21.9–24.1 | 2.47 | ND | ND | ND |
| 24.1–26.4 | 1.44 | 1.44 | ND | ND |
| 26.9–28.4 | 13.96 | 2.67 | ND | 4.21 |
| 27.9–29.9 | 3.57 | 5.25 | 7.05 | 2.14 |
| 29.4–31.9 | 7.33 | 6.06 | 8.89 | 5.47 |
| 31.9–33.1 | 1.85 | ND | 1.35 | ND |
| 36.1–38.1 | 3.44 | 3.96 | 3.58 | 2.94 |
| 39.4–41.1 | 3.18 | 2.47 | 4.87 | ND |
| 41.1–43.1 | 45.23 | 39.82 | 55.04 | 2.78 |
| 45.1–47.1 | 9.46 | 12.86 | 12.43 | 2.60 |
| 52.5–54.6 | 1.27 | 1.31 | 1.54 | ND |
| 62.9–64.9 | 2.09 | 3.88 | 1.69 | ND |
| Total (%) | 98.66 | 94.89 | 97.42 | 95.59 |

NOTES: (1) Average of triplicate samples. (2) ^{14}C-derquantel at 1 µM. ND = not detectable.

Incubation mixtures of $^{14}$C-2-desoxoparaherquamide were extracted with methanol and analysed for metabolites using radio-HPLC. HPLC eluants were collected at 0.25 minute intervals using a fraction collector and were analysed for radioactivity using LSC. Radio-activity peaks were integrated to determine the percent distribution of individual metabolites in each sample.

**Table 3.11.** Relative percentage distribution of radioactivity (%HPLC)[1] in methanol extracts from rat, sheep, human and dog hepatocytes (1 µM) incubated for 4 h at 37°C with $^{14}$C-derquantel[2]

| Metabolite | Species | | | |
|:---:|:---:|:---:|:---:|:---:|
| R$t$ (min) | Rat | Sheep | Human | Dog |
| ~2.9 | 6.32 | 5.52 | 6.23 | 17.20 |
| ~11.6 | ND | ND | 2.68 | ND |
| 12.1-13.1 | ND | ND | ND | 1.94 |
| ~14.1 | ND | ND | ND | 10.85 |
| ~14.9 | ND | ND | ND | 2.31 |
| 15.4-16.0 | ND | ND | ND | 4.51 |
| ~17.0 | ND | ND | ND | 1.91 |
| 19.9-20.9 | ND | ND | ND | 2.91 |
| ~21.5 | ND | ND | ND | 1.10 |
| 22.6-23.3 | ND | 1.73 | ND | ND |
| 23.4-24.5 | 7.76 | 4.23 | 1.00 | ND |
| 23.9-25.6 | 7.60 | 1.96 | 7.72 | ND |
| 25.6-27.1 | 20.86 | 17.41 | 5.02 | 4.07 |
| 27.4-28.8 | 2.04 | 3.43 | 1.69 | 2.36 |
| 30.5-30.8 | 6.18 | 2.21 | 3.36 | 9.35 |
| 32.6-32.9 | 1.83 | ND | ND | 1.61 |
| 33.1-34.1 | 1.77 | ND | ND | 0.92 |
| 36.9-37.1 | 5.31 | 4.07 | 9.06 | 4.94 |
| ~38.1 | ND | 1.31 | ND | ND |
| ~39.9 | ND | 3.31 | ND | 2.93 |
| 41.6-42.1 | 18.65 | 25.09 | 50.97 | 14.29 |
| 45.6-45.9 | 3.13 | 8.59 | 1.77 | 5.17 |
| 47.4-47.5 | 1.49 | ND | ND | ND |
| 51.4-51.8 | 4.33 | 2.53 | 3.54 | 3.08 |
| 53.5-53.9 | 3.41 | 1.52 | 4.32 | 1.58 |
| 53.4-56.6 | ND | 2.70 | ND | ND |
| ~64.1 | 1.36 | 8.73 | ND | ND |
| Total (%) | 92.04 | 94.34 | 94.68 | 95.71 |

NOTES: (1) Average of triplicate samples (Hennessy, 2006). (2) $^{14}$C-2-Desoxoparaherquamide, at 1 µM, was incubated with each lot of hepatocytes at 37°C for 4 h. ND = not detectable.

As with the microsome study, the hepatocyte preparations produced several metabolites of derquantel (see Table 3.11). The profiling data show that $^{14}$C-2-desoxoparaherquamide was extensively metabolized by hepatocytes from rats, sheep and dogs. Derquantel was moderately metabolized by human hepatocytes. In addition to unchanged $^{14}$C-derquantel, 26 radioactive components were detected in rat, sheep, dog or human hepatic incubations. After a 4-hour incubation, unchanged derquantel accounted for approximately 18.7, 25.1, 14.3 and 51.0% of radioactivity in rat, sheep, dog and human hepatic incubations, respectively. The major metabolites of derquantel, M1, M19/M20, M10 and M12, accounted for approximately 3.1, 20.9, 6.2 and 5.3% of the radioactivity for the rat hepatocyte preparations; 8.6, 17.4, 2.2

and 4.1% of the radioactivity for the sheep hepatocytes; 5.2, 4.1, 9.4 and 4.9% of the radio-activity for the dog hepatocytes; and 1.8, 5.0, 3.4 and 9.1% of the radioactivity for the hepatocyte preparations from humans. M2 accounted for approximately 1.4 and 8.7% of the radioactivity in 4-hour rat and sheep hepatic incubations, respectively. M2 was not detected in the 4-hour hepatocyte incubation samples from dogs and humans. Conversely, M15 was detected in only the 4-hour dog hepatic incubations and accounted for approximately 10.9% of the radioactivity. Recovery of radioactivity was high for all species: >92% in the rat; >94% in sheep; >95% in dogs; and >94% in humans.

Paraherquamide (PHQ) was metabolized rapidly by microsomes from sheep, dogs, and gerbils. In sheep, 20% of the paraherquamide remained after a 1-hour incubation. Microsomal metabolism was even faster in dogs (10% remained after 30 minutes) and was fastest in gerbils (7% remained after just 15 minutes). PHQ and 8 metabolites were found in microsomal incubations from sheep and gerbils. In dogs, PHQ and 12 metabolites were found in microsomal incubations. M5 was absent from the radiochromatogram of dog microsomal incubations. The various metabolites were structurally identified in dogs, gerbils and sheep (Figure 2).

The *in vivo* metabolism of PHQ was also evaluated in sheep, dogs and gerbils (Aloysius *et al.*, 2008). Radiochromatograms show PHQ and 5 metabolites in sheep faeces, 5 metabolites but no PHQ in dog faeces, and PHQ and 6 metabolites in gerbil faeces. In urine, radio-chromatograms reveal PHQ and 3 metabolites for sheep, 6 metabolites but no PHQ in dogs, and 4 metabolites and no PHQ in gerbils. In plasma, there were 5 metabolites and PHQ for sheep, 2 metabolites and PHQ for dogs, and 6 metabolites and PHQ for gerbils. The proposed metabolism of PHQ and formation of its major metabolites is provided in Figure 3.
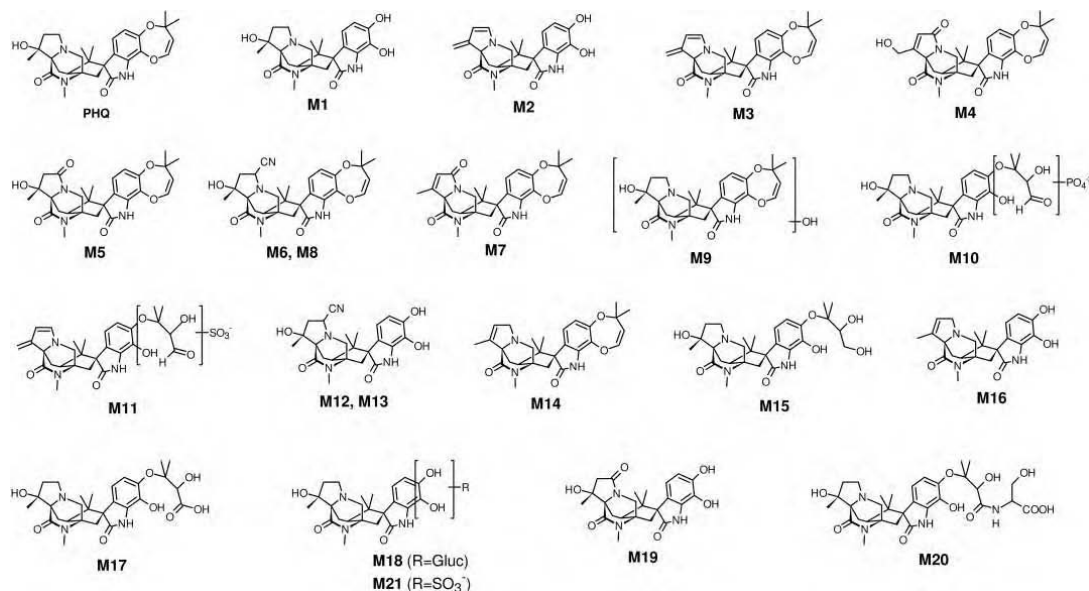


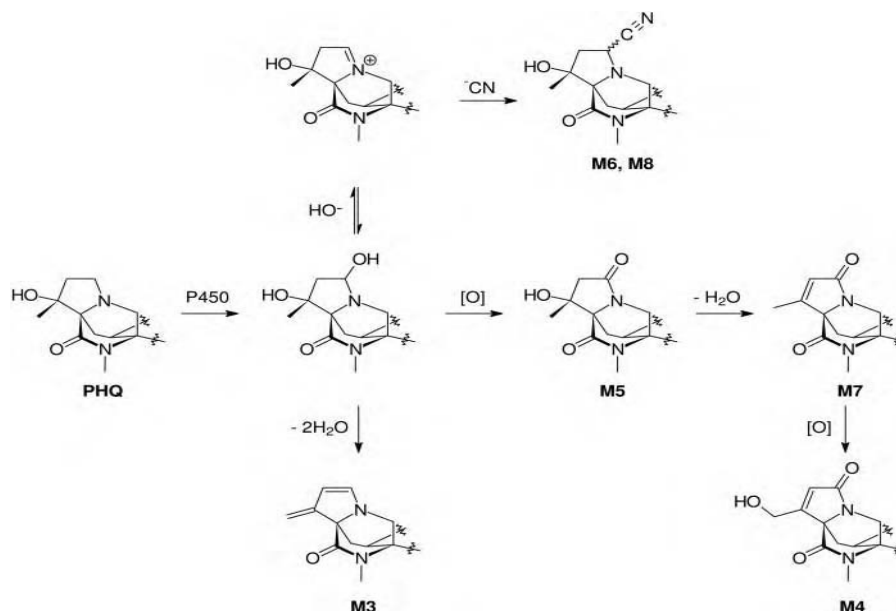**Figure 3.2.** Identified metabolites in dogs, gerbils and sheep

**Figure 3.3.** Proposed scheme for the metabolism of PHQ and formation of its major metabolites in gerbils, dogs and sheep

## *Metabolism in food-producing animals*

### Sheep

In a non-GLP-compliant study (Liu, 2009), tissues, urine, faeces and bile from sheep treated in the total residue study (Byrd and Liu, 2008) were evaluated for derquantel-related metabolites using LC-MS/MS. Although metabolism in sheep was the primary focus of the study, additional analyses were conducted using bile-cannulated rats to better identify metabolites in sheep. Tissue samples were extracted with saline/acetonitrile. Faecal samples were extracted with saline/acetonitrile or methanol/water. Urine and bile samples were centrifuged prior to analysis. Tissue and faecal extracts and urine and bile samples were separated using HPLC radiochromatography and chromatography effluents were collect at 0.25 minute intervals. Fractions were analysed using 96-well plates and the radioactivity in each fraction was determined with microscintillation and luminescence counting. Selected urine and bile samples and tissue and faecal extracts were analysed by LC-RAM/ESI-MS and MS/MS to obtain structural information on derquantel and its metabolites. Metabolite identification focused on compounds present in sheep liver. To facilitate metabolite identification, other sheep tissue extracts, urine, faeces and bile from both sheep and rats treated with derquantel were analysed. Parent derquantel was detected in sheep liver, muscle, fat, urine, faeces and bile.

Metabolite 1 was a major radioactive peak in the radiochromatograms of sheep liver eluting at approximately 44 minutes. It also was detected in sheep kidney, muscle, fat, urine, faeces and bile. LC-MS/MS spectral data of M1 in urine, liver and faeces yielded a [M+H]$^+$ at $m/z$ 460. M1 was also found as a major metabolite in pooled rat bile collected from bile-cannulated rats treated orally with $^{14}$C-derquantel. A degradation product, D1, resulting from the instability of M1 during isolation, was also detected. Further analysis resulted in the identification of D1 as reference standard KXN-869. M1 and D1 had been detected previously in *in vitro* studies using rat, sheep, dog and human liver microsomes and hepatocytes (Ma, 2006a, b).
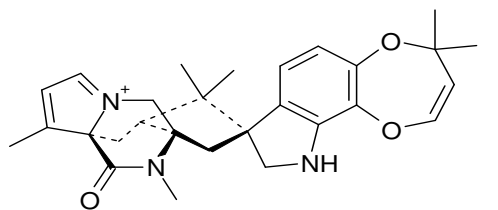
MW = 460
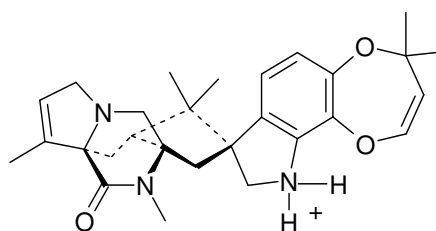
M+H = 462

Structure of M1
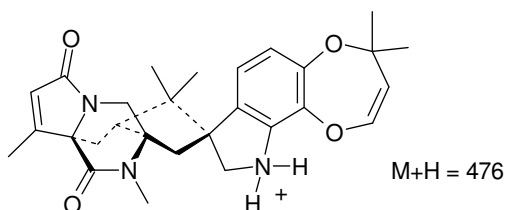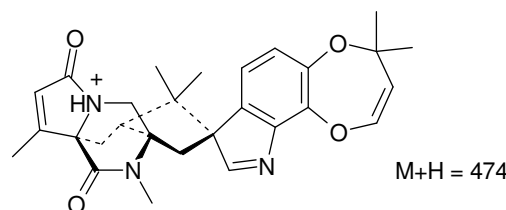
Structure of D1

A second major radioactive peak, eluting at approximately 62 minutes, was found in sheep liver, kidney, muscle and fat. This metabolite, designated as M2, yielded a $[M+H]^+$ at $m/z$ 476. M2 yielded $[M-d+D]^+$ at $m/z$ 478 after H/D exchange, suggesting that M2 contains one exchangeable proton. M2 previously had been detected in *in vitro* studies using rat, sheep, dog and human liver microsomes and hepatocytes (Ma, 2006a, b).

Another radioactive peak, eluting at approximately 63–64 minutes, was found in sheep liver, kidney, muscle and fat. This metabolite, designated M6, yielded a $[M+H]^+$at $m/z$ 474. M6 yielded a $[M-d+D]^+$ at $m/z$ 476 after H/D exchange, suggesting that M6 contains two exchangeable protons. Metabolite M2 was partially converted to M6 during the isolation and purification process (Ma, 2006a, b).
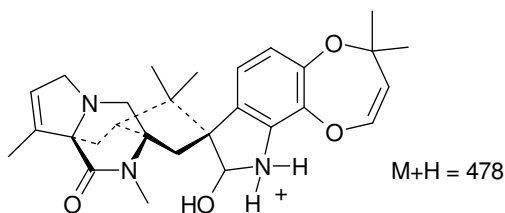


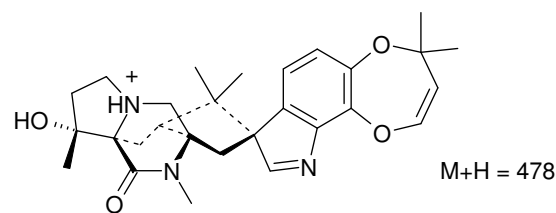M+H = 476

M+H = 474

Structure of M2

Structure of M6

A major radioactive peak in muscle and fat, eluting at approximately 55 minutes, was also detected in liver and kidney. This metabolite, designated M5, yielded a $[M+H]^+$ at $m/z$ 478. M5 previously had been detected in *in vitro* studies using rat, sheep, dog and human liver microsomes and hepatocytes but, in those studies, it was labelled as M14 (Ma, 2006a, b).

Another radioactive peak, eluting at approximately 37 minutes, was detected in sheep fat. This metabolite, designated M3, yielded a $[M+H]^+$ at $m/z$ 478 and was similar to M5. M3 was also detected in *in vitro* metabolism studies using rat, sheep, dog and human liver microsomes and hepatocytes, but in those studies was designated M12 (Ma, 2006a, b).



M+H = 478

M+H = 478

Structure of M5

Structure of M3

A radioactive peak, eluting at approximately 52.0 minutes, was detected in sheep fat, muscle, liver and kidney. This metabolite, designated M4, yielded a $[M+H]^+$ at $m/z$ 458. M13, detected in *in vitro* metabolism studies using rat, sheep, dog and human liver microsomes and hepatocytes had the same $[M+H]^+$ at $m/z$ 458 (Ma, 2006a, b).

A final radioactive peak, eluting at approximately 30.0 minutes, was detected in sheep faeces. This metabolite, designated M7, yielded a $[M+H]^+$ at $m/z$ 494. M7 was also detected in *in vitro* metabolism studies using rat, sheep, dog and human liver microsomes and hepatocytes, but in those studies it was designated as M10 (Ma, 2006a, b).

M+H = 458          M+H = 494

Structure of M4                    Structure of M7
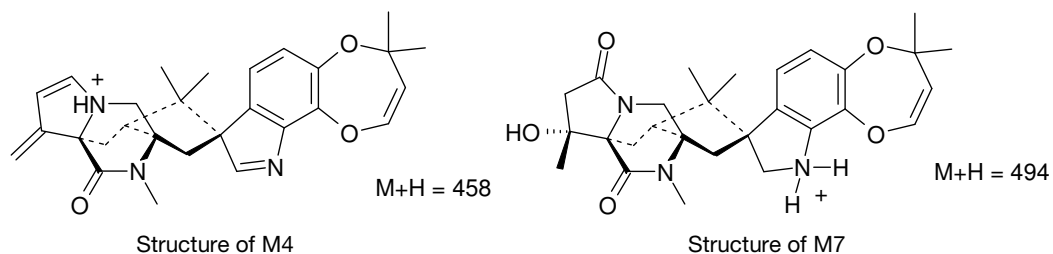
A summary of the mass spectrometry (*m/z*) transitions (M + H) used to characterize derquantel and its metabolites is presented in Table 3.12.

**Table 3.12.** Derquantel metabolites identified in sheep tissue, bile, urine and faeces

| Metabolite | [M+H] | Metabolite | [M+H] |
|---|---|---|---|
| M1 | m/z 460 | M5 | m/z 478 |
| M2 | m/z 476 | M6 | m/z 474 |
| M3 | m/z 478 | M7 | m/z 494 |
| M4 | m/z 458 | D1(KXN-869) | m/z 462 |

M1 and D1 were isolated for NMR and D1 was derived from M1 during isolation of M1.

The proposed metabolism scheme is shown in Figure 4. The bracketed structures indicate proposed intermediates.
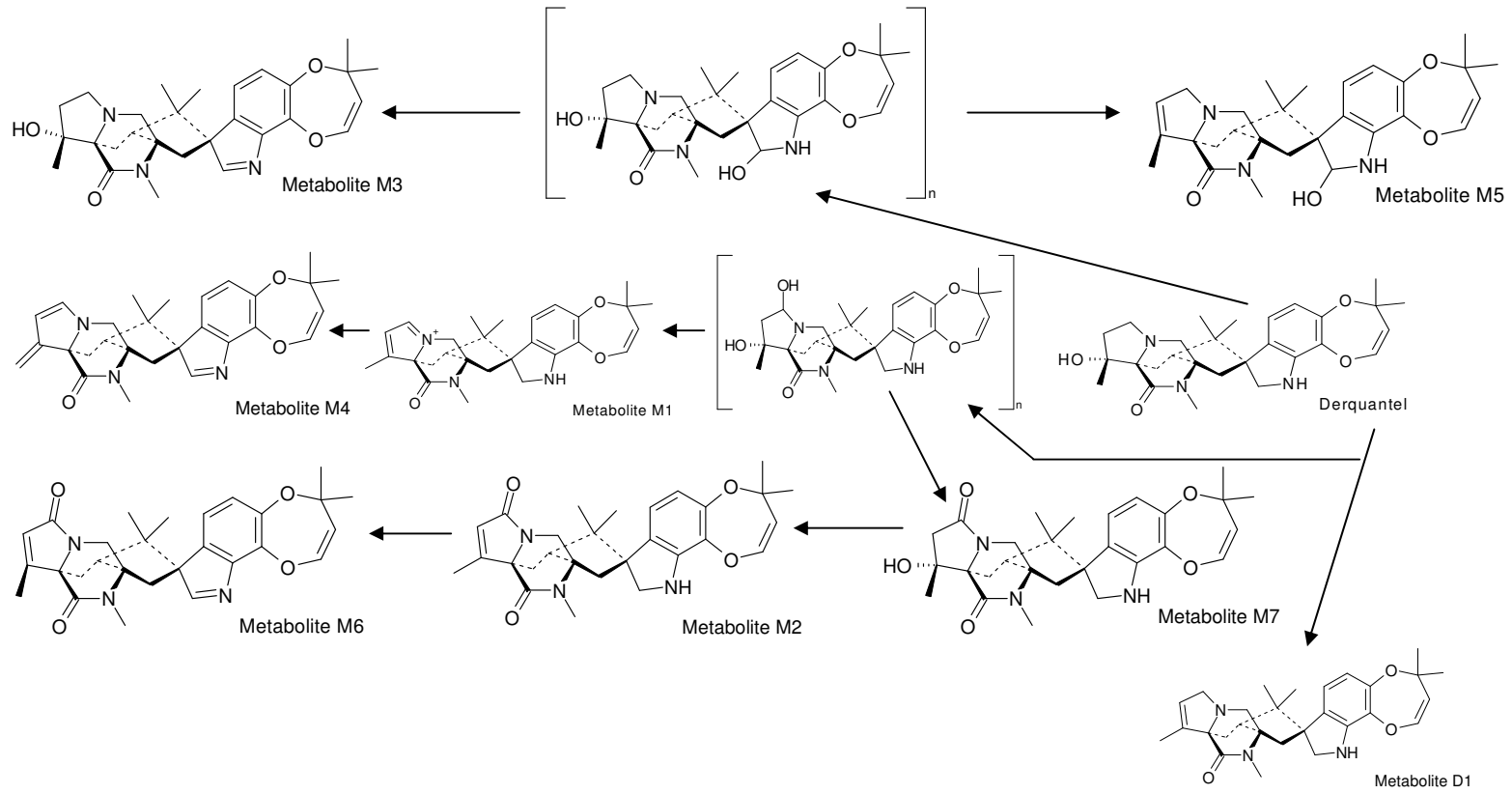
**Figure 3.4.** Proposed metabolism scheme in sheep. Bracketed structures indicate proposed intermediates.

# Tissue residue depletion studies

## *Radiolabelled residue depletion studies*

### Sheep

One non-GLP- and two GLP-compliant studies were evaluated to assess total ^{14}C-derquantel residues (total radiolabelled residues - TRR) in sheep tissues.

In a non-GLP-compliant pilot disposition study (Byrd and Liu, 2006), sheep were treated orally *via* capsule with ^{14}C-derquantel to achieve a target dose of 2 mg derquantel/kg bw. The study was conducted in two phases. Phase 1 of the study (one female) required the use of 6 capsules to achieve the target dose due to the low specific activity of the test article. The radiopurity of the derquantel used in Phase 1 was approximately 90%. Phase 2 (four males; one per time point) used material with a higher specific activity and required only one capsule per sheep. The radiopurity of the derquantel used in Phase 2 was approximately 99%. The Phase 1 animal was slaughtered 3 h after dosing. The Phase 2 animals were slaughtered 6, 12, 24 and 48 h after dosing.

Although samples were available from one animal at 3 h withdrawal, data indicates that residues in tissues continue to increase until at least 6 h after dosing. The highest residues in each of the tissues were seen at the 6-hour sampling point. At all sampling times, total radiolabelled tissue residues were highest in liver. Residues also were high in fat and kidney, but declined more rapidly than did those in liver. Residues were consistently low in muscle. Results are summarized in Table 3.13.

**Table 3.13.** Total ^{14}C-derquantel equivalent residues (µg/kg) measured in tissues from sheep that received a single oral dose of 2 mg/kg bw

| Group No. | Slaughter interval post-dosing | Liver | Kidney | Muscle | Fat |
|---|---|---|---|---|---|
| 1 | 3 h | 5133 | 611.4 | 122.7 | 351.8 |
| 2 | 6 h | 6339 | 1461 | 420.5 | 1881 |
| 3 | 12 h | 4549 | 983.6 | 267.1 | 838.0 |
| 4 | 24 h | 2481 | 274.5 | 48.4 | 156.1 |
| 5 | 48 h | 780.1 | 127.7 | 9.5 | 32.4 |

NOTES: The average value is reported for each specific time and tissue

The marker residue:total ratios were not specifically determined in this pilot study. In acetonitrile extracts of liver, derquantel represented 26.19%, 9.21% and 1.83% of the total radioactivity at 3, 12 and 24 h post-dosing. The acetonitrile extract from the 3-hour, 12-hour and 24-hour liver samples accounted for 76.0%, 79.5% and 58.6% of their TRR, respectively. In an acetonitrile extract of a 6-hour kidney sample, derquantel represented 26.29% of the total radioactivity. The acetonitrile extract from the 6-hour kidney sample accounted for 86.3% of the TRR.

In a GLP-compliant disposition study (Byrd and Liu, 2008), 20 crossbred sheep (3 per time point) were treated orally by gastric tube with ^{14}C-derquantel. Radiopurity was greater than 98.5% for all batches used. Animals received a single oral dose of approximately 2.10 mg derquantel/kg bw. Animals were slaughtered approximately 6, 12, 24, 48, 96 and 144 h after dosing. Samples were stored at below -10°C until analysed.

As in the pilot study, total radiolabelled residues were highest in liver and lowest in muscle. Residues in kidney and fat were similar at the early sampling times. At later sampling times, residues were generally higher in kidney than in fat. There was little change

in concentrations between the 96- and 144-hour sampling times (See Table 3.14 and Figure 3.5).

The study also provided the data needed to assess the ratio of marker residue to total radioactivity (See Table 3.15).

**Table 3.14.** Total $^{14}$C-derquantel equivalent residues (µg/kg) measured in tissues from sheep that received a single oral dose of 2 mg/kg bw

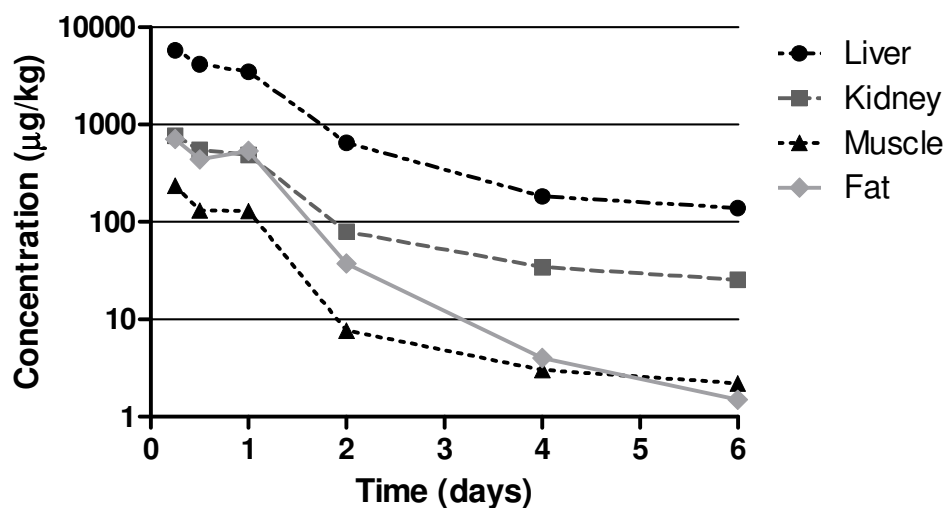| Group No. | Slaughter interval post-dosing) | Liver | Kidney | Muscle | Fat |
|---|---|---|---|---|---|
| 1 | 6 h | 5769 | 770 | 236 | 709 |
| 2 | 12 h | 4155 | 549 | 131 | 438 |
| 3 | 24 h | 3490 | 486 | 129 | 536 |
| 4 | 48 h | 650 | 79 | 8 | 37 |
| 5 | 96 h | 183 | 34 | 3 | 4 |
| 6 | 144 h | 138 | 25 | 2 | 2 |



**Figure 3.5.** $^{14}$C-derquantel equivalent residues (µg/kg) in sheep tissues

**Table 3.15.** Percentage of derquantel to total radiolabelled residues measured in tissues from sheep that received a single oral dose of 2 mg/kg bw

| Group No. | Slaughter interval post-dosing | Liver (%) | Kidney (%) | Muscle (%) | Fat (%) |
|---|---|---|---|---|---|
| 1 | 6 h | 5.86 | 1.54 | 7.80 | 0.78 |
| 2 | 12 h | 2.09 | 0.897 | 8.54 | 0.83 |
| 3 | 24 h | 3.25 | 8.11 | 0.19 | 31.1 |
| 4 | 48 h | 4.34 | 10.50 | 0 | 11.94 |
| 5 | 96 h | NA | | | |
| 6 | 144 h | 0 | | | |

NOTES: NA = not applicable.

In a second GLP-compliant study (Byrd, 2008), 6 sheep (one per time point) were treated once with 2 mg $^{14}$C-2-deoxyparaherquamide/kg bw orally by gastric tube. The study was

designed to confirm the marker to total ratio for derquantel in sheep. The test material had a radiopurity of approximately 92.5%. Animals were slaughtered 12, 24, 48, 96 and 672 h (0.5, 1, 2, 4 and 28 days) post-dose. Based on information in both the study protocol and study report, the samples were stored at below -70°C, significantly colder than in the previous GLP-compliant study. As in the pilot and previous GLP studies, radiolabelled total residues were highest in liver and lowest in muscle. Residues in kidney were greater than residues in fat at all sampling times. Results are shown in Table 3.16 and Figure 3.6.

The study also was used to assess the ratio of marker residue to total radioactivity (See Table 3.17).

**Table 3.16.** Total $^{14}$C-derquantel equivalent residues (µg/kg) measured in tissues from sheep that received a single oral dose of 2 mg/kg bw

| Group No. | Slaughter interval post-dosing | Liver | Kidney | Muscle | Fat |
|---|---|---|---|---|---|
| 1 | 12 h | 2546 | 339 | 49.8 | 186 |
| 2 | 24 h | 1254 | 145 | 17.8 | 114 |
| 3 | 48 h | 551 | 66.0 | 6.7 | 23.7 |
| 4 | 96 h | 229 | 53.7 | 3.4 | 13.3 |
| 5 | 672 h | 17.4 | 6.2 | 0.7 | 2.2 |



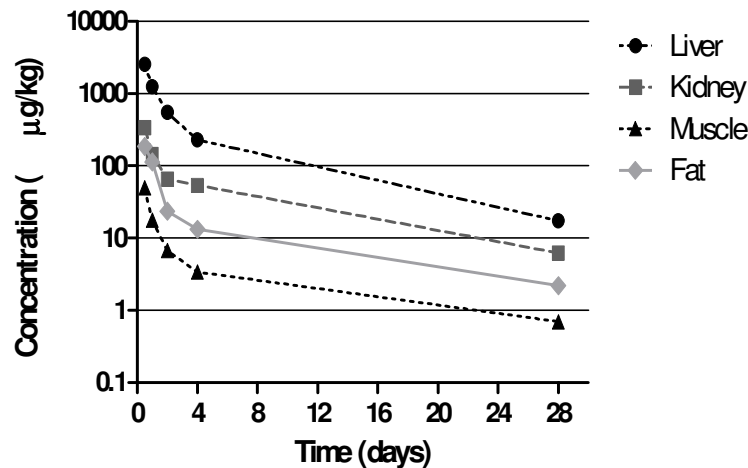**Figure 3.6.** $^{14}$C-derquantel equivalent residues (µg/kg) in sheep tissues

**Table 3.17.** Percentage of derquantel to total radiolabelled residues measured in tissues from sheep that received a single oral dose of 2 mg/kg bw

| Group No. | Slaughter interval post-dosing | Liver (%) | Kidney (%) | Muscle (%) | Fat (%) |
|---|---|---|---|---|---|
| 1 | 12 h | 4.36 | 14.16 | 8.00 | 43.55 |
| 2 | 24 h | 2.23 | 18.62 | 16.67 | 36.84 |
| 3 | 48 h | 0.73 | 12.12 | NA | 16.67 |
| 4 | 96 h | 0.44 | 0 | NA | 7.69 |
| 5 | 672 h | NA | NA | NA | NA |

NOTES: NA = not analysed because of insufficient radioactivity to radioprofile

In samples collected from sheep, the TRR values are comparable between the two GLP-compliant studies, at time-points likely to be relevant to recommending an MRL, even though the samples in the two studies were stored at different temperatures (See Table 3.18).

**Table 3.18.** Total ^{14}C-derquantel equivalent residues (µg/kg) in tissues from two different GLP-compliant total residue studies where tissues were stored at different temperatures

| Slaughter interval post-dosing | Liver | | Kidney | | Muscle | | Fat | |
|---|---|---|---|---|---|---|---|---|
| | Byrd and Liu, 2008 < -10°C | Byrd, 2008 < -70°C | Byrd and Liu, 2008 < -10°C | Byrd, 2008 < -70°C | Byrd and Liu, 2008 < -10°C | Byrd, 2008 < -70°C | Byrd and Liu, 2008 < -10°C | Byrd, 2008 < -70°C |
| 6 h | 5769 | | 770 | | 236 | | 709 | |
| 12 h | 4155 | 2546 | 549 | 339 | 131 | 49.8 | 438 | 186 |
| 24 h | 3490 | 1254 | 486 | 145 | 129 | 17.8 | 536 | 114 |
| 48 h | 650 | 551 | 79 | 66 | 8 | 6.7 | 37 | 23.7 |
| 96 h | 183 | 229 | 34 | 53.7 | 3 | 3.4 | 4 | 13.3 |
| 144 h | 138 | | 25 | | 2 | | 2 | |
| 672 h | | 17.4 | | 6.2 | | 0.7 | | 2.2 |

The marker residue:total residue ratios from the two radiolabelled residue studies are variable. The combined results for the two GLP-compliant studies are summarized in Table 3.19 (Gottschall, 2010b). Based on the significantly lower sample storage temperature in the Byrd. (2008) study, the marker residue:total residue ratios from this second GLP-compliant study, where samples were stored at < -70°C, may be a more accurate reflection of the marker:total ratio. However, the samples stored at < -10°C may represent a marker residue:total residue ratio more applicable to samples collected under normal use and monitoring conditions.

**Table 3.19.** Summary of marker:total (M/T) residue ratios (%) from the GLP-compliant total residue studies in sheep

| Withdrawal time (hours) | Study ID | Derquantel Marker Residue:Total Residue (%) | | | |
|---|---|---|---|---|---|
| | | Liver | Kidney | Muscle | Fat |
| 6 | 1545N-60-05-171* | 5.86 | 1.54 | 7.80 | 0.78 |
| 12 | 1545N-60-05-171 | 2.09 | 0.897 | 8.54 | 0.83 |
| 12 | 1545N-60-07-186** | 4.36 | 14.16 | 8.00 | 43.55 |
| 24 | 1545N-60-05-171 | 3.25 | 8.11 | 0.19 | 31.1 |
| 24 | 1545N-60-07-186 | 2.23 | 18.62 | 16.67 | 36.84 |
| 48 | 1545N-60-05-171 | 4.34 | 10.50 | 0.0 | 11.94 |
| 48 | 1545N-60-07-186 | 0.73 | 12.12 | NA | 16.67 |
| 96 | 1545N-60-05-171 | NA | NA | NA | NA |
| 96 | 1545N-60-07-186 | 0.44 | 0.0 | NA | 7.69 |
| 144 | 1545N-60-05-171 | 0.0 | NA | NA | NA |
| Mean M/T | | 2.5 | 6.8 | 5.8 | 15.0 |

NOTES: * = Storage at between -10°C and -20°C; 3 animals per time point (Byrd and Liu, 2008). ** = Storage at between -70°C and -80°C; 1 animal per time point (Byrd, 2008). NA = not analysed.

To evaluate storage stability, fortified tissue samples containing 1 or 500 µg derquantel/kg were analysed after storage at below -20°C for one month (Carnevale, 2008). Significant changes from the theoretical concentrations were evident in all tissues, indicating that derquantel fortified into tissues is not stable when stored at below -20°C (Table 3.20).

**Table 3.20.** Stability of derquantel in fortified tissues stored at below -20°C

| Tissue | Theoretical concentration (µg/kg) | Concentration of Derquantel | % Change from theoretical | Mean % change from theoretical |
|--------|-----------------------|-----------------------|-----------------|-----------------|
| Liver | 1.00 | 0.47 ± 18.4 | -53.0 | -49.8 |
| | 500.0 | 266.9 ± 7.6 | -46.6 | |
| Kidney | 1.00 | 0.27 ± 12.8 | -73.0 | -64.5 |
| | 500.0 | 219.8 ± 8.0 | -56.0 | |
| Muscle | 1.00 | 0.2 ± 49.6 | -80.0 | -76.1 |
| | 500.0 | 139.7 ± 26.9 | -72.1 | |
| Fat | 1.00 | 0.7 ± 7.7 | -30.0 | -37.4 |
| | 500.0 | 276.1 ± 10.3 | -44.8 | |

As part of the validation for an ultraperformance liquid chromatographic (UPLC-MS/MS) procedure, an extended storage stability study was conducted using samples from the residue depletion study. Samples originally collected and analysed in Australia were shipped frozen to the method development laboratory. Samples were shipped, received and stored at below -70°C. The time from initial analysis to second analysis (re-analysis) was from 4 to 5 months, depending on the tissue. Results for the two analyses on the single overlapping analysis day (Sample day 4; 96 h withdrawal) compared favourably (Chambers, 2009). Results are shown in Table 3.21.

**Table 3.21.** Residues of derquantel (µg/kg) in sheep treated with 3 mg derquantel/kg bw and 0.3 mg abamectin/kg bw analysed at two different laboratories

| Laboratory | Liver | Kidney | Muscle | Subcutaneous fat | Perirenal fat |
|-----------|-------|--------|--------|-----------------|---------------|
| Original* | 2.98 ± 2.42 | 1.80 ± 1.54 | <LOQ† | 4.75 ± 8.47 | 3.99 ± 5.25 |
| Re-analysis** | 2.64 ± 1.65 | 1.27 ± 1.46 | 0.45 ± 0.52 | 4.46 ± 4.45 | 3.50 ± 4.12 |

NOTES: * LOQ = 1.0 µg/kg. ** LOQ = 0.1 µg/kg. † = Reported by laboratory as 0.51 ± 0.58 µg/kg.

When samples fortified with derquantel are stored at below -20°C, there is a loss of approximately 50% from theoretical after 1 month of storage. When samples containing incurred derquantel residues are stored at below -70°C, the nominal concentrations are consistent after 4 to 5 months of storage, even when analysed at two different facilities. Taken together, these two studies indicate that derquantel requires storage at significantly lower temperatures than those usually employed in residue studies or for monitoring samples.

### *Residue depletion studies with unlabelled drug*

#### Sheep

In the only residue depletion study (Chambers, 2009) provided, equal numbers of females and male castrate Merino sheep (38) and $2_{nd}$ Cross Prime lambs (38), were randomly allocated to 12 slaughter groups (6 animals per group, with 4 controls). Animals in groups 2–13 were treated orally with the test formulation containing derquantel and abamectin. The dose delivered was 3 mg derquantel/kg bw and 0.3 mg abamectin/kg bw (150% of the label dose). Group 1 animals served as controls and received tap water orally.

Two control animals were slaughtered 6 h post treatment. At slaughter, the entire liver, both whole kidneys, gluteal muscle, perirenal and subcutaneous fat samples were collected. Tissues were weighed and diced, and frozen at approximately -70 to -80°C for 24 h. Further processing consisted of homogenization with dry ice to produce a fine powder. Additional gluteal muscle samples were collected, diced, and homogenized with a 0.9% saline solution. Samples were stored at -70 to -80°C pending analysis.

Analyses were conducted at two locations. Site 1 (original method; Table 3.22) analysed tissues from all animals for derquantel and abamectin in group order. Site 2 (UPLC-MS/MS; Table 3.23) re-analysed tissues for derquantel only, in reverse group order, and only for Groups 5 to 13 (96–840 h post-dosing). The LOQ for all tissues is 1.0 µg/kg at Analytical Site 1 and 0.1 µg/kg at Analytical Site 2. The study results are summarized in Tables 3.22 and 3.23.

No tissue residue depletion information was provided for chickens, turkeys, rabbits or residues in milk and eggs.

**Table 3.22.** Residues of derquantel (µg/kg) in tissues of sheep treated with the combination product to deliver 3 mg derquantel/kg bw and 0.3 mg abamectin/kg bw

| Slaughter time post-dose | Liver | Kidney | Muscle | SC Fat | PR Fat |
|---|---|---|---|---|---|
| 12 h | 421.5 ± 131.7 | 211.3 ± 55.8 | 43.1 ± 29.0 | 720.9 ± 362.7 | 829.9 ± 340.2 |
| 24 h | 247.9 ± 122.4 | 121.3 ± 49.1 | 24.7 ± 11.9 | 594.5 ± 297.2 | 461.8 ± 173.0 |
| 48 h | 67.6 ± 60.8 | 22.3 ± 17.5 | 4.36 ± 4.86 | 125.5 ± 154.8 | 90.5 ± 61.2 |
| 96 h | 2.98 ± 2.42 | 1.80 ± 1.54 | <LOQ | 4.75 ± 8.47 | 3.99 ± 5.25 |
| 144 h | <LOQ | <LOQ | <LOQ | 1.93 | <LOQ |
| 192 h | <LOQ | <LOQ | <LOQ | 1.08 | <LOQ |
| 240 h | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| 336 h | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| 408 h | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| 504 h | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| 672 h | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| 840 h | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |

NOTES: LOQ = 1µg/kg (Analytical Site 1). SC Fat = subcutaneous fat; PR Fat = perirenal fat.

**Table 3.23.** Residues of derquantel (µg/kg) in tissues of sheep treated with the combination product to deliver 3 mg derquantel/kg bw and 0.3 mg abamectin/kg bw

| Slaughter time post-dose | Liver | Kidney | Muscle | SC Fat | PR Fat |
|---|---|---|---|---|---|
| 12 h | NM | NM | NM | NM | NM |
| 24 h | NM | NM | NM | NM | NM |
| 48 h | NM | NM | NM | NM | NM |
| 96 h | 2.64 ± 1.65 | 1.27 ± 1.46 | 0.45 ± 0.52 | 4.46 ± 4.45 | 3.50 ± 4.12 |
| 144 h | 0.90 ± 0.38 | 0.12 | <LOQ | 0.37 ± 0.43 | 0.31 ± 0.16 |
| 192 h | 0.36 ± 0.16 | 0.14 | <LOQ | 0.39 ± 0.36 | 0.29 ± 0.21 |
| 240 | 0.67 ± 0.33 | <LOQ | <LOQ | <LOQ | 0.34 ± 0.40 |
| 336 | 0.36 ± 0.06 | <LOQ | <LOQ | 0.114 | 0.16 ± 0.03 |
| 408 | 0.31 ± 0.14 | <LOQ | <LOQ | <LOQ | 0.12 ± 0.02 |
| 504 | 0.20 ± 0.10 | <LOQ | <LOQ | <LOQ | 0.11 ± 0.01 |
| 672 h | 0.32 ± 0.19 | <LOQ | <LOQ | <LOQ | 0.16 ± 0.03 |
| 840 h | 0.19 ± 0.11 | <LOQ | <LOQ | <LOQ | 0.13 |

NOTES: LOQ = 0.1 µg/kg (Analytical Site 2). SC Fat = subcutaneous fat; PR Fat = perirenal fat; NM = not measured.

# Methods of analysis for residues in tissues
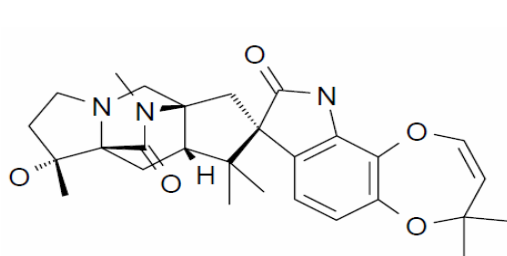


**Paraherquamide**/PF-03198957
*Role*: internal standard
*Chemical Name*: Paraherquamide
CAS Registry Number: 77392-58-6
Chemical Formula: $C_{28}H_{35}N_3O_5$
Molecular Weight: 493.6
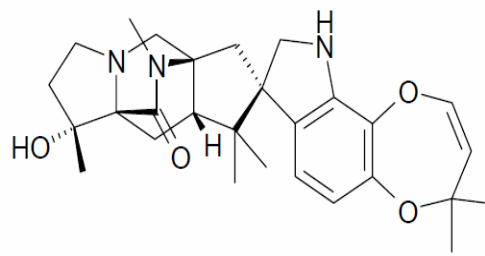
2-DOPH(**Derquantel**)/PF-00520904
*Role*: active pharmacological compound
*Chemical Name*: 2-deoxoparaherquamide
CAS Registry Number: 187865-22-1
Chemical Formula: $C_{28}H_{37}N_3O_4$
Molecular Weight: 479.6

Two different analytical methods have been developed for the detection of derquantel; validation was performed against OECD GLP (EU/VICH and Aus/New Zealand/SA) regulations. These methods comply with the requirements of Australian Veterinary Medicines Authority (APVMA) Residue Guideline No. 26 – Veterinary Drug Residue Analytical Method, and "The Rules Governing Medicinal Products in the European Community – Volume VIII October 2005– Established by the European Community of Maximum Residue Limits (MRLs) for Residues of Veterinary Medicinal Products in Foodstuffs of Animal Origin" as well as with applicable Pfizer Animal Health Standard Operating Procedures.

The two analytical methods are based on a liquid extraction of homogenized ovine tissue followed by a solid-phase extraction (SPE) prior to reconstitution and quantitation by Liquid Chromatography/Mass Spectrometry (LC/MS). The most significant differences between the two methods are the treatment of muscle samples, the use (or not) of high-resolution chromatography for separation, and MS/MS (versus MS) for detection. The original method muscle assay used a saline homogenate and a matrix standard curve, whereas the revised method muscle assay used cryogenically processed muscle and a neat (matrix free) solution standard curve. All other tissues in both assays used cryogenically processed samples and neat solution standard curves. The original muscle method required these modifications because of significant ion enhancement during the electrospray ionization (ESI) process observed during method development. The original method is based on a conventional chromatographic separation followed by a single dimensional mass spectrometry characterization ($MS^1$ on a single quadrupole), whereas the revised method used fast and high resolution chromatography coupled to multi-dimensional mass spectrometry ($MS^2$ on a triple quadrupole) (See Guyton, 2009). The LC-MS system used by the original method is quantitative to 1 µg/kg whereas the UPLC-MS/MS method used by the revised method is quantitative to 0.1 µg/kg.

## *Original method*

Blank liver, kidney and fat samples are prepared cryogenically as a powder homogenate. To prepare a homogenate, intact frozen tissue is sliced into thin sections and stored at -15°C prior to homogenization. An approximately equivalent amount of dry ice is added to the tissue (1:1 v/v) and the mixture is transferred to a blending homogenizer and processed to a fine powder. Blank muscle is prepared as a saline homogenate. To prepare a homogenate, each intact tissue is sliced into wafer thin shavings and chilled physiological saline is added to the muscle tissue sections at a ratio of 2 parts tissue to 1 part saline. The sample is contained in an ice bath and homogenized to a smooth consistency using a homogenizer. All sample processing is performed in small batches to avoid unnecessary time that samples are exposed to temperatures above -80°C. Post processing, muscle homogenate is immediately transferred to -80°C storage. The tissue sample is fortified with the internal standard solution. The sample is left in ambient air for 15 min prior to the extraction with acetonitrile. After the addition of phosphate buffer, the extract is applied onto an MCX SPE cartridge. The column is eluted with acetonitrile/purified water (80/20, 6% $NH_4OH$) solution. After evaporation, and reconstitution with acetonitrile and purified water, 5.0 µl of the sample is injected onto the column (Xterra® MS C18 5 µm 2.1 × 150 mm stainless steel column) for detection and analysis mass spectrometry (single quadrupole mass spectrometer detector). The mobile phase consists of methanol (0.075% formic acid) : purified water (0.075% formic acid) [36:64]. The analytes elutes under isocratic conditions at a flow rate of 0.25 ml/min. The chromatographic run time is 10 min with the target analyte eluting at approximately 6 min and the internal standard eluting at approximately 7.5 min. Positive electrospray (+ESI) is used to ionize target analytes. Signals are recorded using the selected ion monitoring (SIM) mode; *m/z* 480 and *m/z* 494 are monitored to characterize 2-DOPH and its internal standard, respectively. The quantity of 2-DOPH in each sample is calculated from standard curves based on linear least squares of the respective peak area response ratios of 2-DOPH and its internal standard. There is no weighting applied and calibration curves are not forced through zero. Curve 'fit' is accepted where the coefficient of determination ($r^2$) is >0.995.

## *Revised method*

Tissue samples are extracted with acetonitrile, followed by sample clean-up with cation exchange solid phase extraction (MCX-SPE). The eluant from the SPE column is evaporated to dryness and reconstituted with 20% acetonitrile in 10 mM ammonium acetate prior to UPLC-MS/MS analysis. The analytes are eluted from the UPLC column by increasing the

mobile phase B composition (0.1% ammonium hydroxide in methanol) linearly from 20% to 80% over 0.5 min (mobile phase A being 0.1% ammonium hydroxide in water), with a return to initial conditions at 1.75 min and holding at 20% Mobile Phase B until the end of the 2 min run. Injection volume is set at 5.0 µl, sample temperature is kept at 4°C, and column (UPLC BEH C18, 1.7 µm, 2.1 × 50 mm) is maintained at 40°C. Electrospray ionization is preferred to other atmospheric pressure ionization techniques, and detection is conducted in the negative ion mode using acquisition of ions in the selected reaction monitoring (SRM) mode. 2-DOPH and paraherquamide transitions are $m/z$ 480→405 and $m/z$ 494→419, respectively. An internal standard calibration method is used for quantitation with paraherquamide as the internal standard. The plot of peak area ratio vs 2-DOPH concentration produces a line of best fit according to the chosen regression and weighting factor. For this validation the standard curves are fitted to a linear equation (y = mx+b), where $m$ is the slope of the line and $b$ is the y-intercept. The weighting factor used is 1/x. The concentrations are determined by back calculating against the fitted line from the peak area ratio. If back-calculated standards are more than ±15% of their nominal concentration, that standard is dropped from the regression.

## Specificity and selectivity

The specificity is assessed by monitoring a large set on blank tissues; no response is detected in the retention time region of interest.
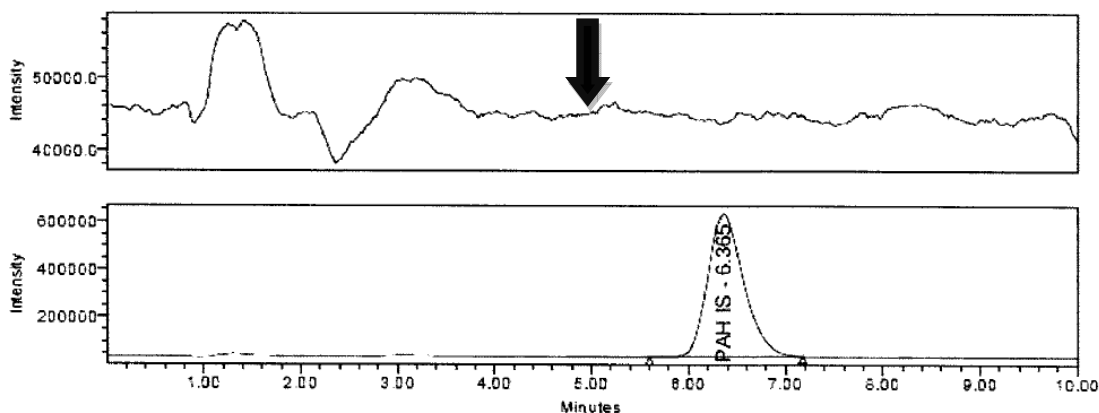
### *Original method*



**Figure 3.7.** Example of an LC-MS trace of blank liver sample (DOPH at RT 4.98 min, internal standard at 6.37 min)

**Figure 3.8.** Example of an LC-MS trace of quality control (QC) liver sample fortified with 5 µg/kg with 2-DOPH (top trace) and the internal standard (bottom trace)
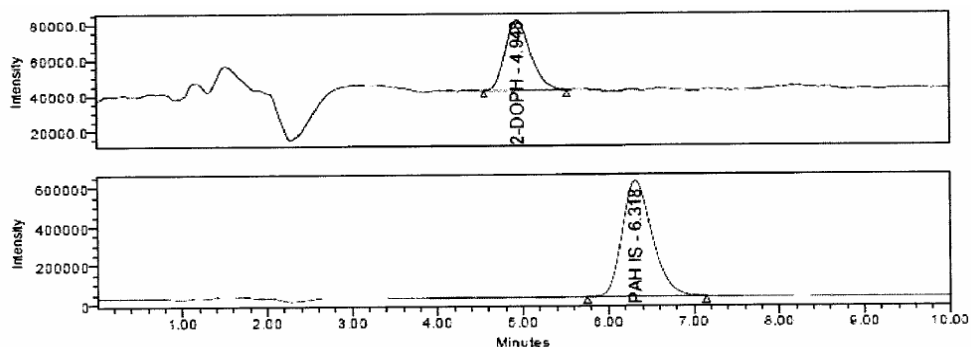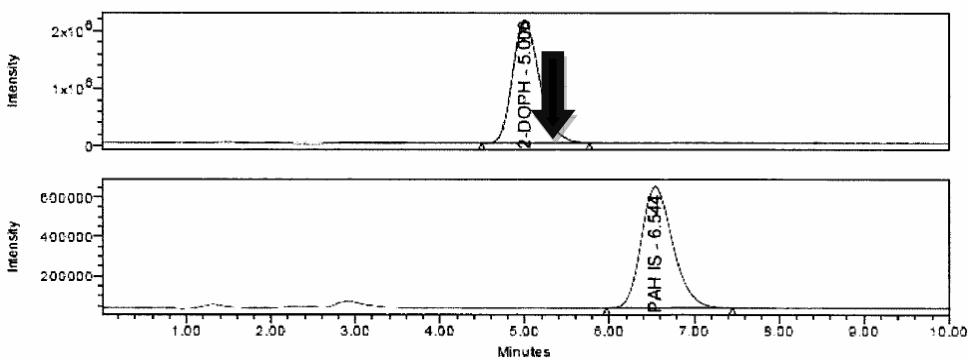


**Figure 3.9.** Example of an LC-MS trace of incurred residues in liver from animal 1336 (assay 1, 24 June 2008); 2-DOPH (top trace) and the internal standard (bottom trace)



## Revised method

2-DOPH transitions *m/z* 480 →405 and *m/z* 494→419 for paraherquamide are not interfered with by abamectin under the chromatographic conditions employed and the mobile phase used to elute the target analyte and its internal standard, respectively.
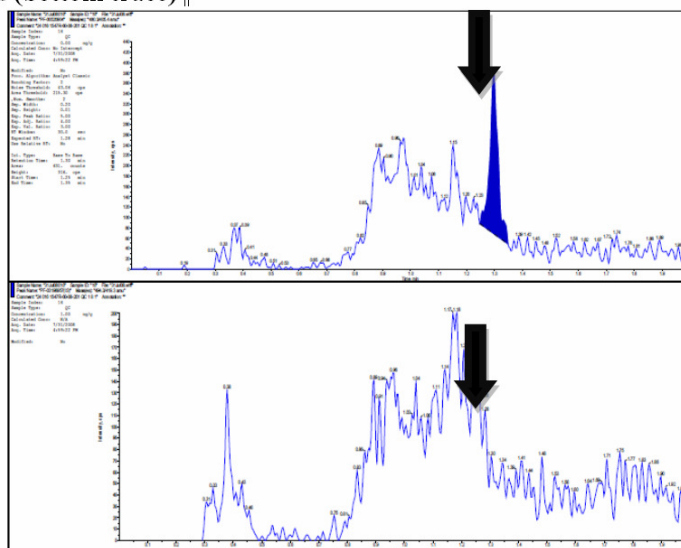


**Figure 3.10.** Example of an LC-MS/MS trace of blank liver sample; 2-DOPH (top trace) and the internal standard (bottom trace)
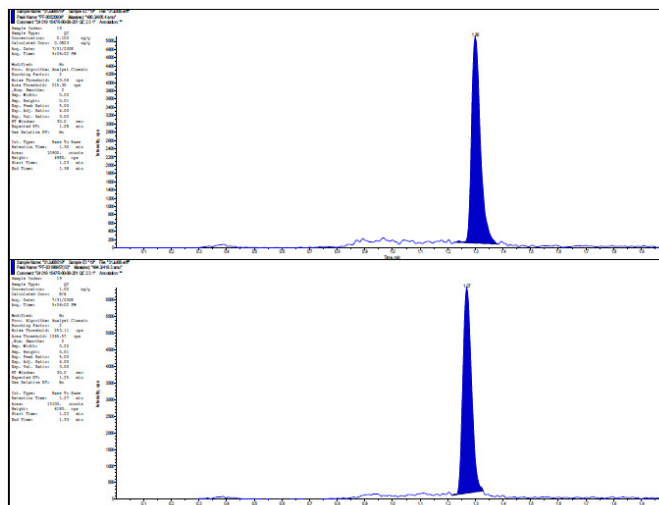
**Figure 3.11.** Example of an LC-MS/MS trace of quality control (QC) liver sample fortified with 0.1 µg/kg with 2-DOPH (top trace) and the internal standard (bottom trace)

## Accuracy and precision

### Original method

The data in the validation study provided a fit for purpose accuracy and precision over the concentrations tested (1–500 µg/kg) for 2-DOPH quantitation in ovine tissue. An ad hoc linearity ($r^2$ >0.998) is demonstrated; intra-day accuracy of the validation samples is in the ranges 84.3–99.7% (liver), 88.6-103.6% (kidney), 72.3-117.7% (muscle) and 88.4-111.2% (fat). The imprecision (%CV) for each tissue is in the ranges of 2.4-6.9% (liver), 3.2-11.5% (kidney), 3.2-24.0% (muscle) and 2.3-29.3% (fat). The inter-day accuracy is 86.1-108.6% across all tissue types and validation concentrations. The inter-day imprecision is 3.6-25.0% (but generally <15%) across all tissue types and validation concentrations.

### Revised method

The analysis of the provided validation data confirms very good accuracy and precision for 2-DOPH quantitation over the concentrations tested (0.1 to 1 µg/kg) in ovine tissue. An ad hoc linearity ($r^2$ >0.990) is shown; intra-day accuracy of the validation samples was in the ranges 52.0-139.0% (liver), 48.4-113.6% (kidney), 58.4-92.9% (muscle) and 73.8-123.0% (fat). The imprecision (CV%) for each tissue was in the ranges 8.5-16.9% (liver), 4.6-19.9% (kidney), 4.3-11.3% (muscle) and 3.5-6.8% (fat). The inter-day accuracy is 68.3-102.3% across all tissue types and validation concentrations. The inter-day imprecision is 5.5-23.6% (but generally, <20%) across all tissue types and validation concentrations.

### LODs and LOQs

Determinations of LODs and LOQs for 2-DOPH in ovine tissue are made from concentration responses from 20 ovine tissue blanks per matrix and from 6 different sources per matrix. The LOD is calculated considering the mean concentration response (at the retention time of 2-DOPH) in the 20 blank samples plus three standard deviation of the mean value. The original method LOD is 0.04-0.07 µg/kg. The revised method LOD is 0.007-0.022 µg/kg. The limits of quantitation are set at the lowest concentration used for the validation runs. For the original method, the LOQ is 1.0 µg/kg, whereas, for the revised method, the LOQ is 0.1 µg/kg.

**Table 3.24.** Summary of performance for 2-DOPH in different matrices (Original method)

| Assay parameter | Liver | Kidney | Muscle | Fat |
|---|---|---|---|---|
| Linearity $r^2$ | >0.998 | >0.999 | >0.999 | >0.999 |
| Estimated LOD (µg/kg) | 0.07 | 0.05 | 0.04 | 0.05 |
| LOQ (µg/kg) | 1.0 | 1.0 | 1.0 | 1.0 |
| Interday Accuracy ± Imprecision (%) | | | | |
| 1.0 µg/kg | 89.6 ± 5.1 | 98.7 ± 8.5 | 108.6 ± 15.6 | 100.3 ± 17.7 |
| 5.0 µg/kg | 91.8 ± 4.9 | 91.9 ± 8.6 | 87.9 ± 25.0 | 91.1 ± 3.6 |
| 50 µg/kg | 94.4 ± 6.5 | 93.3 ± 7.8 | 89.5 ± 11.8 | 98.3 ± 6.5 |
| 500 µg/kg | 86.1 ± 3.9 | 88.7 ± 6.3 | 88.6 ± 6.4 | 91.4 ± 4.0 |
| Absolute Recovery (%) | | | | |
| 1.0 µg/kg | 57.1 | 73.3 | 186.7 | 81.0 |
| 5.0 µg/kg | 57.8 | 60.7 | 70.7 | 90.0 |
| 50 µg/kg | 64.9 | 100.0 | 53.6 | 89.2 |
| 500 µg/kg | 68.6 | 64.8 | 58.4 | 93.3 |
| Mean Abs Recovery (%) | 62.1 | 74.7 | 92.4 | 88.4 |
| Int. Std Recovery | 56.1-85.6 | 66.3-108.2 | 41.0-94.6 | 72.0-104.7 |

**Table 3.25.** Summary of performance for 2-DOPH in different matrices (Revised method)

| Assay Parameter | Liver | Kidney | Muscle | Fat |
|---|---|---|---|---|
| Linearity $r^2$ | >0.996 | >0.993 | >0.990 | >0.995 |
| Estimated LOD (µg/kg) | 0.007 | 0.009 | 0.022 | 0.020 |
| LOQ (µg/kg) | 0.1 | 0.1 | 0.1 | 0.1 |
| Interday Accuracy ± Imprecision (%) | | | | |
| 0.1 µg/kg | 91.9 ± 19.1 | 80.2 ± 18.9 | 75.5 ± 11.5 | 101.8 ± 17.2 |
| 0.5 µg/kg | 102.3 ± 16.7 | 78.4 ± 23.6 | 72.0 ± 11.2 | 97.0 ± 6.4 |
| 1.0 µg/kg | 82.2 ± 19.0 | 74.7 ± 17.5 | 68.3 ± 9.8 | 92.0 ± 5.5 |

## Stability

The stability of the fortified tissue samples at -20°C was assessed and the results indicate that significant degradation of 2-DOPH residues (compared with freshly prepared samples) occurs upon extended frozen storage. An additional stability assessment concluded that 2-DOPH in ovine tissues is stable over the typical duration of an assay (including all steps of the analytical method, from sample preparation to injection into the autosampler).

The consequence of freeze-thaw cycles was investigated and assessed regarding the degradation of tissue samples. Results indicate that increasing the number of freeze-thaw cycles for incurred tissue samples or storing incurred samples at ≥ -20°C leads to a significant degradation of analyte in the matrix. 2-DOPH residues in the ovine matrix are most stable when stored at approximately -80°C and subjected to minimal freeze-thaw cycles. However, the mean 2-DOPH concentrations (µg/kg) in ovine muscle homogenate following extended storage stability at approximately -80°C over 3 and 6 months was outside the specified ±15% limits for most samples.

# Appraisal

Derquantel has not been previously reviewed by the Committee. Derquantel, a spiroindole, is an oral anthelmintic for use in sheep. It is registered only as a combination product with abamectin. The combination product contains 10 mg/ml derquantel and 1 mg/ml abamectin. Derquantel, in combination with abamectin, is used to treat and control a broad range of adult and immature (L4) gastrointestinal nematodes of sheep. In the registered combination product, the withdrawal period is determined by the depletion of residues of the abamectin component. The 14-day withdrawal period identified on the registered labelling from New Zealand and the Republic of South Africa is consistent with the withdrawal period registered for abamectin alone in these countries and in Australia.

Derquantel is metabolized extensively; there are 8 principle metabolites in sheep. The metabolic profiles are qualitatively similar across the tested species. In studies using preparations of hepatocytes and microsomes, more than 20 metabolites were identified in test animals and sheep. Derquantel is eliminated relatively rapidly, primarily in the faeces. The terminal plasma half-life is approximately 10 h.

Depletion studies using radiolabelled derquantel were conducted in the sheep, the only species for which derquantel is registered. Radiolabelled total residues in muscle were uniformly low. At 6 h withdrawal, radiolabelled residues were highest in liver, followed by kidney and fat. Residues decline rapidly over the next 24–48 h, but were still detectable in one study in all tissues at 28 days withdrawal. There are no residue data available for sheep milk.

Data from two total residue studies are available for determining the marker to total ratio for derquantel in sheep tissues. The first study used a large number of animals, but samples were stored only at < -10°C, a temperature subsequently determined to be insufficient for maintaining derquantel stability during storage. The second study, intended as a confirmatory study, utilized a much small number of animals but samples were stored at < -70°C, a temperature demonstrated to maintain derquantel stability in tissues. Effectively, neither study is ideal because the marker to total residue ratios are variable. As such, the marker to total residue ratios from both studies are used when estimating exposures.

Derquantel, although constituting a small percentage of the total residues, is an appropriate marker residue for derquantel residues in tissues.

A single residue depletion study using unlabelled derquantel was evaluated (as the commercial combination product formulation) in the target species, sheep. The dose was 150% of the labelled dose for sheep (3 mg/kg bw derquantel and 0.3 mg abamectin /kg bw). As in the radiolabelled derquantel studies, residues in muscle were uniformly low. In this residue depletion study, the highest concentrations of derquantel residues at early sampling times were found in fat, followed by liver and kidney. At later sampling times, the highest residues were found in liver. Using an analytical method with an LOQ of 1 µg/kg, derquantel residues were below the LOQ at 8 days post-treatment in all tissues except subcutaneous fat. When the same samples were re-analysed using an analytical method with an LOQ of 0.1 µg/kg, derquantel residues were still quantifiable in liver and perirenal fat at 35 days withdrawal, the final time point in the study. Because derquantel is not registered for use in lactating sheep, there are no residue data for sheep milk.

The MRLs recommended for sheep liver and fat are based on the upper limit of the one-sided 95% confidence interval over the 95th percentile ("95/95 tolerance limit") for the 8-day post-treatment data from the unlabelled residue depletion study. The MRLs recommended for sheep muscle and kidney are twice the LOQ of the UPLC-MS/MS method. No MRL is recommended for sheep milk as no residue data were provided for milk.

# Maximum residue limits

In recommending MRLs for derquantel, the Committee considered the following factors:

- An ADI of 0–0.3 µg/kg of body weight was established by the Committee based on an acute toxicological endpoint. This ADI is equivalent to up to 18 µg for a 60 kg person.

- Derquantel is extensively metabolized; derquantel represents, conservatively, 6% of total residues in muscle, 3% in liver, 7% in kidney and 15% in fat. Derquantel, although constituting a small percentage of total residues, is suitable as the marker residue in tissues. No data are provided for sheep milk.

- Liver contains the highest concentration of total radiolabelled residues at all sampling times. Fat contains the highest concentrations of derquantel residues in the unlabelled residue depletion studies at early sampling points. At times beyond the 4-day sampling time, residues are highest in liver. The highest concentration of the proposed marker residue, derquantel, at time points relevant to recommending MRLs is found in liver, followed by fat, then kidney and then muscle. Liver and fat can serve as the target tissues.

- A validated analytical procedure for the determination of derquantel in edible sheep tissues (liver, kidney, muscle and fat) is available and may be used for monitoring purposes.

- The MRLs recommended for sheep liver and fat are based on the upper limit of the one-sided 95% confidence interval over the 95th percentile ("95/95 tolerance limit") for the 8-day post-treatment data from the unlabelled residue depletion study. The MRLs recommended for sheep muscle and kidney are twice the LOQ of the UPLC-MS/MS method.

- No MRLs were recommended for sheep milk as no residue data were provided for milk.

The Committee recommended MRLs for derquantel in sheep tissues of 0.2 µg/kg in muscle, 2.0 µg/kg in liver, 0.2 µg/kg in kidney and 0.7 µg/kg in fat, determined as derquantel. No MRLs are recommended for sheep milk.

Using the model diet and the ratio of the concentration of the marker residue to the concentration of the total residues noted above, these MRLs result in an intake of 8 µg/person, which represents 45% of the upper bound of the ADI.

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