

Toxicological evaluation of certain veterinary drug residues in food

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DERQUANTEL

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1. **EXPLANATION**

Derquantel (Chemical Abstracts Service No. 187865-22-1), also known as 2-desoxoparaherquamide, 2-deoxyparaherquamide, 2-DOPH, PF-520904 and PNU-141962, is a semi-synthetic spiroindole characterized by an indole or oxindole moiety fused with a cyclopentyl ring at position C-3 of the indole ([Figure 1](#)) (Lee, Clothier & Johnson, 2001; Lee et al., 2002; Little et al., 2011). Derquantel is a broad-spectrum anthelmintic agent with activity against the adult and larval stages of gastrointestinal nematodes in sheep.

Derquantel acts as a nicotinic cholinergic receptor antagonist. Its mode of action is blockage of cation channels in nematode muscle cell membranes.

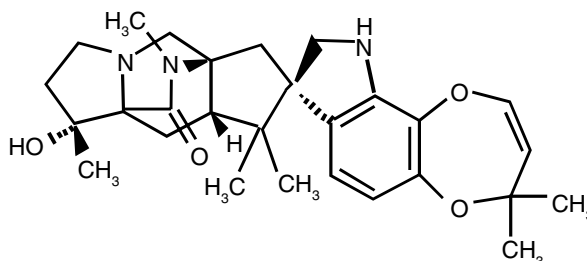
When used as an anthelmintic agent for sheep, derquantel is combined with abamectin to control infections by nematodes that are resistant to macrocyclic lactone-, levamisole-, benzimidazole- and closantel-based drenches (and combinations of these). The combination product is composed of derquantel at a concentration of 10 mg/ml and abamectin at a concentration of 1 mg/ml. It is administered to sheep and lambs 3–4 times per year as a single-dose oral drench at a dose rate of 2 mg/kg body weight (bw) for derquantel and 0.2 mg/kg bw for abamectin.

Abamectin was previously evaluated by the Committee at its forty-seventh meeting (Annex 1, reference [125](#)), at which maximum residue limits (MRLs) for cattle fat, liver and kidney were proposed. An acceptable daily intake (ADI) of 0–1 µg/kg bw was established by the 1995 Joint FAO/WHO Meeting on Pesticide Residues for abamectin used as a veterinary drug (FAO/WHO, 1996).

Derquantel has not previously been evaluated by the Committee. Derquantel was placed on the agenda of the current meeting at the request of the Nineteenth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (FAO/WHO, 2011). The Committee was asked to establish an ADI and recommend MRLs for derquantel in sheep tissues.

The Committee considered the results of studies on receptor pharmacology, pharmacokinetics, acute and short-term toxicity, in vitro, in vivo and in silico genotoxicity, reproductive toxicity and microbiological safety in humans. The majority of the studies were performed in accordance with good laboratory practice (GLP) standards. In addition, a literature search in Embase database was performed using the keywords derquantel, abamectin, Startect, toxicity, tolerance, mechanism, human, animal, mice, mouse, rat, dog, rabbit, monkey, guinea pig, sheep, in vivo, in

Figure 1. Chemical structure of derquantel, a spiroindole anthelmintic



vitro, toxic metabolites and paraherquamide. No additional information relevant to this evaluation was identified.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Rats

In a study that was conducted in compliance with GLP, male and female Sprague-Dawley rats (three of each sex per group) were dosed by oral gavage once daily with vehicle control (1% carboxymethylcellulose) or derquantel at 200 mg/kg bw per day for 4 days. Blood samples were collected from each animal at 0.5, 1, 2 and 24 hours post-dosing on day 4. The peak plasma concentrations (C_{max}), 15.7 and 22.3 µg/ml, occurred at 2 hours post-dosing (T_{max}) for both male and female rats, respectively (Coffing, 2008).

Male and female Sprague-Dawley rats (one of each sex per group) were given a single oral administration of [^{14} C]derquantel at a dose rate of 100 mg/kg bw (14.8 MBq/kg bw) in a non-GLP-compliant study. Urine and faeces were collected for 7 days post-dosing, and blood and tissues were sampled at 7 days post-dosing and analysed for total radioactivity content. Ninety-five per cent of the radioactive dose was recovered in urine and faeces. Approximately 87–90% of the dose was excreted in the faeces during the 7-day post-dosing period. Faecal excretion of radiolabel in the male rat was 37.5% of the administered dose at day 1 and 47.2% at day 2. The female rat had 0.005% and 67.0% of the administered dose in the day 1 and day 2 faecal samples, respectively. Urinary excretion of radiolabel was low, with total recoveries of 5.5% and 7.6% in the male and female rats, respectively. Negligible amounts of the dose were detected in the carcass and terminal blood samples. Overall, derquantel was excreted mainly in the faeces in 2 days after oral administration and may not be distributed in tissues (Marsh, 2006).

In a GLP-compliant study, Sprague-Dawley rats were dosed orally with [^{14} C]-derquantel at 50 mg/kg bw per day to identify metabolite profiles in urine, faeces,

bile and tissues. Four male and four female rats were dosed daily for 7 days with a single oral administration of [^{14}C]derquantel. Urine and faeces were collected 24 hours after the first dose and until 6 hours after the seventh dose, and liver, kidney, muscle and fat were sampled at 6 hours after the seventh dose. In a second group, bile duct-cannulated rats (one of each sex) were given a single oral administration of [^{14}C]derquantel at 50 mg/kg bw, and then bile, urine and faeces were collected up to 48 hours post-dosing, and liver, kidney, muscle and fat were obtained at 48 hours post-dosing. The majority of the radioactive dose (69–75%) was recovered in faeces, with small amounts, 3–4% and 1%, recovered in urine and tissues, respectively. In bile duct-cannulated rats, the majority of the radioactive dose was recovered in the bile (64–70%). This supports a high oral bioavailability of derquantel in rats and the high percentage of dose excreted in the faeces via biliary elimination (Wu & Liu, 2008).

In a 90-day dosing study performed under GLP conditions, derquantel was administered to Sprague-Dawley rats by oral gavage once daily at a dose of 0, 1, 5, 50 or 150 mg/kg bw per day for 3 months. Blood samples were collected from two males and two females 1 hour after dosing on days 0, 7, 14, 21, 29, 61 and 90. The mean concentrations of derquantel in plasma across the entire treatment period showed a dose-dependent increase: 118.3, 317.5, 2127 and 5272 ng/ml at 1, 5, 50 and 150 mg/kg bw per day, respectively. There was no accumulation of derquantel with time (Goldenthal, 2006).

Male and female Sprague-Dawley rats were given derquantel orally by gavage once daily at 0, 1, 5 or 50 mg/kg bw per day for 1 year under GLP conditions. Plasma samples were collected from male and female rats (three of each sex per time point per dose group) at 0.5, 1, 2, 4, 8 and 12 hours post-dosing on days 1, 178 and 365 and 2 hours post-dosing on days 90 and 270. The area under the plasma concentration–time curve ($\text{AUC}_{0-t(\text{last})}$) for both sexes ranged from 179 to 182 ng·h/ml at 1 mg/kg bw per day and increased to 2150–4000 ng·h/ml at 5 mg/kg bw per day and 33 300–50 000 ng·h/ml at 50 mg/kg bw per day. Mean peak plasma concentrations (C_{max}) ranged from 82.0 to 109 ng/ml at 1 mg/kg bw per day and increased dose dependently. The time to C_{max} (T_{max}) occurred at 0.5 hour at 1 and 5 mg/kg bw per day and ranged from 0.5 to 2 hours at 50 mg/kg bw per day. Females showed a tendency towards higher plasma concentrations of derquantel throughout the exposure period. There was no accumulation of derquantel with time (Yancey & Grover, 2008).

Derquantel was administered to male and female Sprague-Dawley rats (nine of each sex per group) by oral gavage at a dose of 0, 0.01, 0.03, 0.1 or 0.3 mg/kg bw per day for 1 year in a GLP-compliant study. Plasma samples were collected on days 1, 84 and 365 at 0, 1, 2 and 4 hours post-dosing from three animals of each sex per time point. $\text{AUC}_{0-t(\text{last})}$ and C_{max} increased with dose dependency, and derquantel appeared to accumulate at all doses, with a concentration on day 365 that was 2–3 times higher than on days 1 and 84 at the highest dose. The plasma T_{max} occurred at 1–2 hours post-dosing for all dose groups. Exposure as measured by AUC and C_{max} was consistently higher in females than in males (Bonnnette, 2009b).

In a two-generation reproduction study performed in compliance with GLP, male and female Sprague-Dawley rats were given derquantel by oral gavage once

daily at 0, 1, 5 or 25 mg/kg bw per day through two generations. Mean plasma derquantel concentrations on day 50, 2 hours post-dosing, in the P_1 generation (adult females) were 44.1, 525 and 2240 ng/ml at 1, 5 and 25 mg/kg bw per day, respectively. Mean plasma derquantel concentrations on day 76, 2 hours post-dosing, in the F_1 generation of adult females were 59.1, 414 and 2230 ng/ml at 1, 5 and 25 mg/kg bw per day, respectively. Mean plasma derquantel concentrations in the F_1 and F_2 generation pups ranged from 0.01 to 2.69 ng/ml on lactation day 4 and from less than 0.050 to 0.622 ng/ml on lactation day 21 across the dose ranges. In general, mean plasma derquantel concentrations were similar in the P_1 and F_1 generation adult female rats and in the F_1 and F_2 generation lactating pups (Barnett, 2009).

Ten male and 10 female Sprague-Dawley rats were dosed once daily by oral gavage for 7 days with the combination product containing a 10:1 ratio of derquantel and abamectin, at doses of 0/0, 1/0.1, 2/0.2, 10/1 and 20/2 mg/kg bw per day. Plasma samples were collected from four animals of each sex per time point per dose group at 0, 2, 6 and 24 hours post-dosing on days 1 and 7. Plasma concentrations of derquantel and abamectin increased with increasing doses of the combination product. Derquantel appeared not to accumulate, based on no difference in $AUC_{0-t(\text{last})}$ and C_{max} values on day 7 compared with day 1. However, abamectin showed accumulation, with increased $AUC_{0-t(\text{last})}$ and C_{max} values on day 7 compared with day 1. Greater derquantel concentrations in female rats were noted at all time points for all doses on both days 1 and 7 when compared with male rats. This trend was not noted for abamectin. T_{max} occurred mainly at 2 hours post-dosing for derquantel and 6 hours post-dosing for abamectin across the dose range (May, 2009d).

(b) Rabbits

In a GLP-compliant study, three female New Zealand White rabbits per treatment group were given a dose of derquantel by stomach tube once daily on days 7 through 19 of presumed gestation at 0.1, 1 and 10 mg/kg bw per day. Plasma samples were collected on days 7 and 19 of gestation at 0 hour (prior to dosing) and at 0.5, 2, 4, 8 and 24 hours post-dosing. $AUC_{0-t(\text{last})}$ and C_{max} increased with dose. T_{max} was observed at 0.5 hour, with the exception of the high-dose group on day 7, for which T_{max} occurred at 0.5–2 hours post-dosing (Lewis, 2007).

(c) Dogs

In a non-GLP-compliant study, derquantel was administered daily as a single oral dose via capsule to two male and two female Beagle dogs at 1, 5 or 10 mg/kg bw per day for 28 days. Plasma samples were collected at 0, 0.5, 1, 2, 4, 12 and 24 hours post-dosing on days 0 and 27, but only at 2 hours post-dosing on day 14. Plasma derquantel concentrations from the male and female dogs were not different. AUC_{0-24} and C_{max} increased at a rate greater than proportional with dose and indicated accumulation on day 27, only in the highest-dose group. T_{max} ranged from 0.5 to 2.25 hours, and the measured half-life was from 2.24 to 5.88 hours (Savides, 2006).

Derquantel was administered orally to Beagle dogs for 1 month in a GLP-compliant study. Four dogs of each sex per dose received derquantel once daily by oral gavage for 28 days at 0.01, 0.03 or 0.1 mg/kg bw per day. An additional group of two males and two females was dosed 3 times at 0.01 mg/kg bw for a total daily dose of 0.3 mg/kg bw per day. Plasma samples were collected at 0, 0.5, 1, 3, 8 and 24 hours post-dosing on days 3 and 26. There were no observable differences in plasma derquantel $AUC_{0-t(last)}$ or C_{max} values between male and female dogs. The mean plasma derquantel $AUC_{0-t(last)}$ increased from 0.0935 to 1.60 ng·h/ml on day 3 and from 0.0494 to 1.37 ng·h/ml on day 26 with doses ranging from 0.01 to 0.1 mg/kg bw per day. The mean plasma derquantel C_{max} showed a similar trend across dose groups between day 3 and day 26. There was no accumulation noted in any dose group for either AUC_{0-24} or C_{max} . T_{max} occurred primarily at 0.5 hour post-dosing in the majority of subjects (May, 2009a).

In a GLP-compliant study, derquantel was administered daily as a single oral dose via capsule to each of four male and four female Beagle dogs at a dose of 0.1, 0.5, 1 or 10 mg/kg bw per day for 3 months. Plasma samples were collected at 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours post-dosing on days 0 and 90, but only at 2 hours post-dosing on days 15, 30, 45, 60 and 75. The C_{max} values for derquantel indicated no accumulation over the duration for doses of 0.1, 0.5 and 1 mg/kg bw per day; at 10 mg/kg bw per day, however, the C_{max} on day 90 was approximately 10–80 times greater than on day 0. The AUC_{0-24} showed modest increases between days 0 and 90 in the three lowest-dose groups (1.4- to 1.8-fold increases), with an increase of 15-fold for the highest-dose group. T_{max} was generally between 0.5 and 2 hours in the three lowest-dose groups on days 0 and 90, but ranged from 2.5 to 8.9 hours in the highest-dose group. Half-lives generally increased with dose; however, all were relatively short, less than 7 hours (Savides, 2008).

Derquantel was administered daily as a single oral dose via oral gavage to male and female Beagle dogs (four of each sex per group) at a dose of 0.1, 0.5, 1 or 5 mg/kg bw per day for 3 months in a GLP-compliant study. Plasma samples were collected at 0, 0.5, 1, 3, 8 and 24 hours post-dosing on days 3, 30, 61 and 91. Mean plasma derquantel $AUC_{0-t(last)}$ and C_{max} tended to be higher in males than in females in all dose groups and on all study days. Mean plasma derquantel $AUC_{0-t(last)}$ on study day 3 in males ranged from 1.77 to 1750 ng·h/ml as the dose increased from 0.1 to 5 mg/kg bw per day. A similar trend was found in the $AUC_{0-t(last)}$ for females. On study day 3, the mean plasma derquantel C_{max} in males and females indicated a 194-fold and 269-fold increase over the dose range from 0.1 to 5 mg/kg bw per day. There was no trend in accumulation for the duration of the study. Plasma derquantel T_{max} was 0.5 hour most frequently at doses up to 1 mg/kg bw per day and 1–3 hours at 5 mg/kg bw per day (May, 2009b).

(d) *Horses*

One female and one castrated male horse were administered a single oral dose of derquantel at 2 mg/kg bw via nasogastric intubation as a 0.5% hydroxymethylcellulose suspension. Plasma samples were collected at 0, 1, 2, 4, 8, 24 and 48 hours post-dosing. Maximum concentrations of derquantel in plasma (C_{max}) of 0.157 and 0.075 µg/ml for the male and female horse, respectively, were

seen at 1 hour ($T_{\max}$). This corresponded with the onset of severe adverse reactions in both horses at 1–2 hours post-dosing. AUC values of 0.48 $\mu\text{g}\cdot\text{h}/\text{ml}$ and 0.27 $\mu\text{g}\cdot\text{h}/\text{ml}$ were calculated for the male and female horse, respectively. A mono-hydroxylated metabolite of derquantel, previously not seen in other species, was identified at higher apparent plasma concentrations than for the parent derquantel (Seaman, 2000b).

2.1.2 Biotransformation

One male and one female Sprague-Dawley rat were given a single oral administration of [^{14}C]derquantel at a dose of 100 mg/kg bw (14.8 MBq/kg bw) in a non-GLP-compliant study. Urine and faeces were collected for 7 days post-dosing and analysed for metabolism/degradation by high-performance liquid chromatography–mass spectrometry. Only 13% of the parent drug was found in urine and 3% in faecal samples at 24 hours post-dosing. The formation of approximately 11 metabolites or degradation products was observed during this time interval. Some of the molecular weights of the metabolites were indicative of normal metabolism products of glucuronidation and hydroxylation (Marsh, 2006).

In a GLP-compliant study, Sprague-Dawley rats were dosed orally with [^{14}C]derquantel at 50 mg/kg bw to identify metabolite profiles in urine, faeces, bile and tissues. Metabolic profiles found in urine, faeces and bile indicated a high degree of biotransformation to a large number of metabolites. Metabolite profiles in males and females were similar, although the metabolic rate was higher in males than in females. Small amounts of parent derquantel along with a large number of metabolites were found in faeces at the 144- to 150-hour collection interval, whereas parent drug was found mainly at the 0- to 24-hour collection interval. The metabolite profiles became increasingly complex between the 0- to 6-hour sample and the 24- to 48-hour samples. Parent derquantel and metabolites M1, M2, M4 and M5 were the most common components found in the tissues. In rat liver, derquantel comprised 6–8% of the total radioactivity at 6 hours following the final dose. The metabolite profiles were similar between males and females, except that metabolite M2 comprised 21% of the total profile in male liver and only 2% in female liver. Compared with liver, rat kidney contained higher percentages of derquantel, 21% in males and 40% in females. Metabolite M2 comprised a higher percentage of the profile in the kidney in males (11%) than in females (3%). In rat muscle and fat, derquantel comprised higher percentages of the total residues in tissues of females (21% and 53%, respectively) relative to the males (5% and 31%, respectively) (Wu & Liu, 2008).

In sheep, [^{14}C]derquantel was extensively metabolized. Parent derquantel and metabolites M1, M2, M4 and M5 were the most common components found in the tissues. The majority of metabolites found in tissues were less polar (longer retention times) than the parent (Wu & Liu, 2008).

A GLP-compliant in vitro metabolism study was conducted to determine the degree of metabolism and profiles of metabolites of derquantel in liver microsomes of rat, sheep, dog and human. [^{14}C]Derquantel (1 $\mu\text{mol}/\text{l}$) was incubated in triplicate with each of rat, sheep, dog and human liver microsomes at 37 °C for 1 hour in the presence of reduced nicotinamide adenine dinucleotide phosphate (1 mmol/l)

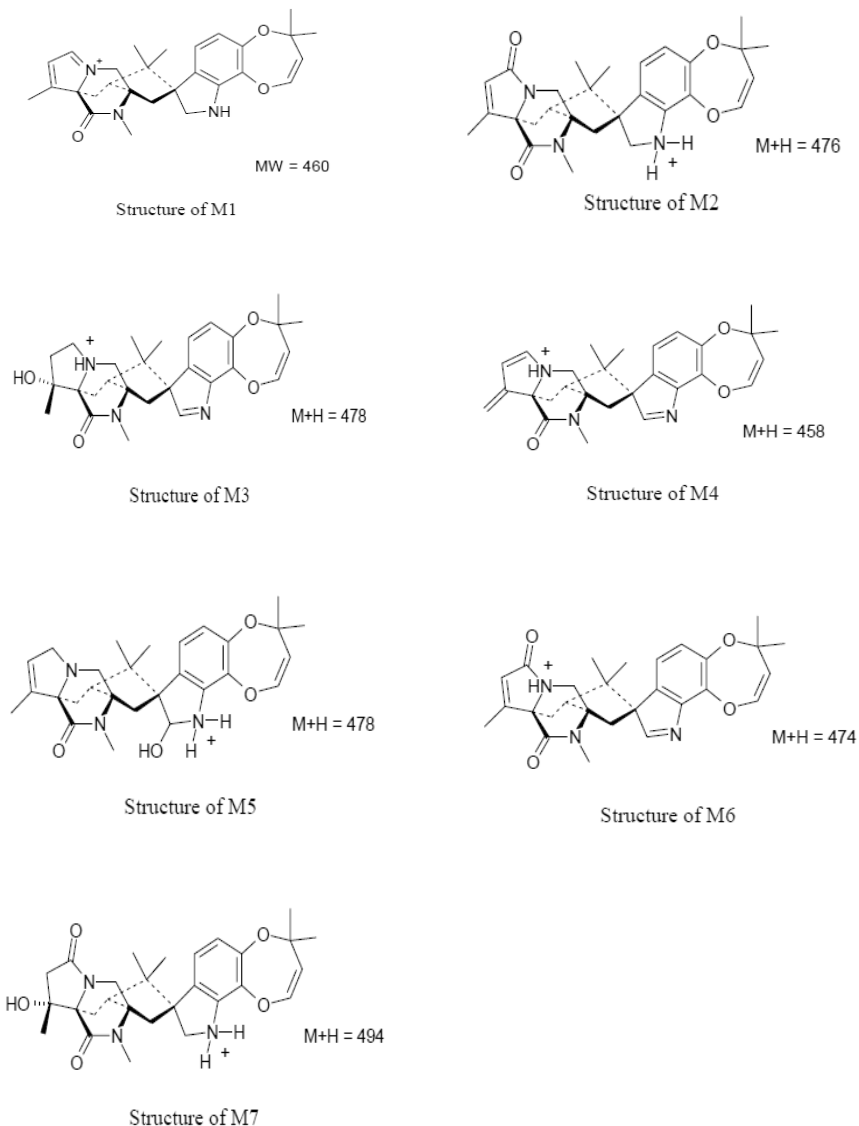
and magnesium dichloride (2 mmol/l). 7-Ethoxycoumarin (100 µmol/l) was also incubated with each of the liver microsomes as a positive control. Microsomal incubation samples were analysed by high-performance liquid chromatography to determine the ^{14}C metabolite profiles. All microsomal preparations used were determined to be enzymatically active, as 55.0%, 67.6%, 100.0% and 75.0% of 7-ethoxycoumarin were metabolized to mainly 7-hydroxycoumarin and other metabolites by rat, sheep, dog and human liver microsomes, respectively, after 1 hour. Derquantel was metabolized extensively in dog liver microsomes, as only 2.8% of the radioactive derquantel was detected after a 1-hour incubation. Derquantel was metabolized moderately by rat, sheep and human liver microsomes, as 45.2%, 39.8% and 55.0% of the radioactive derquantel were detected, respectively, after 1 hour. Unchanged [^{14}C]derquantel and 18 radioactive components were detected in rat, sheep, dog and/or human liver microsomal incubations. M3, M4, M5a and M5b were the predominant metabolites in dog liver microsomes, comprising 40.1%, 8.3%, 8.7% and 7.6% of the radioactivity. Rat, sheep and human liver microsomes presented a similar pattern of metabolites; M1, M9 and M10 were the major metabolites, accounting for 3.6–12.9% of the radioactivity, but M8 was also a major metabolite in rats, comprising 14.0% of the radioactivity (Ma, 2006a).

In another GLP-compliant study, the metabolism of derquantel was evaluated in hepatocytes of rat, sheep, dog and humans. [^{14}C]Derquantel (1 µmol/l) was incubated with cryopreserved hepatocytes from rats, dogs and humans and freshly prepared hepatocytes from sheep for 4 hours. Non-radiolabelled 7-ethoxycoumarin and 7-hydroxycoumarin were included as positive controls. The cellular viability was 36.4% for the rat hepatocytes and 70.0% for the sheep, dog and human hepatocytes at the 4-hour incubation. The prepared hepatocytes were determined to be enzymatically active, as 35.4%, 94.6%, 45.8% and 29.5% of 7-ethoxycoumarin and 18.5%, 100%, 96.3% and 90.4% of 7-hydroxycoumarin were metabolized by rat, sheep, dog and human hepatocytes, respectively. After a 4-hour incubation, unchanged derquantel comprised approximately 18.7%, 25.1%, 14.3% and 51.0% of the radioactivity in rat, sheep, dog and human hepatocytes, respectively. Unchanged [^{14}C]derquantel and 26 radioactive metabolites were detected in rat, sheep, dog and/or human hepatic incubations. M1, M8, M10, M12 and M19/20 were detected commonly as the major metabolites in rat, sheep, dog and/or human hepatic incubations, with varying compositions. M18 was one of the major metabolites, accounting for 7.6% and 7.7% of the radioactivity in rat and human hepatocytes, respectively. M16/17 was another major metabolite, detected at 7.8% in rat hepatocytes. M15 was a predominant metabolite, comprising 10.9% of the radioactivity only in dog hepatocytes, and M2 was a major metabolite in sheep hepatocytes, comprising 8.7% of the radioactivity (Ma, 2006b).

The chemical structures of some of the derquantel metabolites are shown in [Figure 2](#).

2.1.3 Effects on enzymes and other biochemical parameters

The antagonistic potency of derquantel on both $\alpha 7$ and $\alpha 3$ nicotinic acetylcholine receptors (nAChR) endogenously expressed in rats and canine dorsal root ganglion neuronal cells was evaluated using whole-cell patch clamp assay in

Figure 2. Chemical structures of selected derquantel metabolites

a GLP-compliant study. Mecamylamine, a known nAChR antagonist and a human antihypertensive agent, was used as a positive control. The antagonist properties of mecamylamine against acetylcholine at 1 mmol/l were similar in both rat and canine $\alpha 3$ nAChR, with median inhibitory concentrations (IC_{50} values) of 0.14 and 0.09 μ mol/l, respectively, and approximately 17–27 times greater in relative potency when compared with antagonist activity at rat $\alpha 7$ nAChR (IC_{50} value of 2.41

$\mu\text{mol/l}$). Derquantel displayed considerably more potent antagonist activity against acetylcholine at 1 mmol/l at canine $\alpha 3$ nAChR (IC_{50} value of 1.16 $\mu\text{mol/l}$) than at rat $\alpha 3$ nAChR (IC_{50} value of 31.51 $\mu\text{mol/l}$). The antagonistic potency of derquantel on $\alpha 7$ nAChR could not be assayed for the non-expression of the type of receptor in dog ganglion cells. Derquantel displayed approximately 2- to 3-fold more potent antagonist activity against acetylcholine at 1 mmol/l at rat $\alpha 3$ nAChR than at rat $\alpha 7$ nAChR (IC_{50} value of 70.14 $\mu\text{mol/l}$). Derquantel was approximately one log unit less potent than mecamylamine. These results suggested that derquantel blocks nAChR, acting more potently in dogs than in rats (May, 2009c).

Derquantel was evaluated for its binding affinity to subtypes of human nAChR using SH-Sy5Y cells (a human neuroblastoma cell line) that endogenously express $\alpha 3$ nAChR, $\alpha 7/5$ -HT3-SHEP cells (a human neuroblastoma cell line) that stably express a human $\alpha 7$ nAChR and TE-671 cells (a human medulloblastoma cell line) that endogenously express muscle-type nAChR α subunit. The cells were analysed on a fluorescent imaging plate reader to detect the change in calcium flux after the addition of derquantel at 30–100 $\mu\text{mol/l}$ (agonistic activity) and a second addition of nicotine at 100 $\mu\text{mol/l}$ (antagonistic activity). Derquantel had no agonist activity in any of the human nAChR models tested at concentrations of 30–100 $\mu\text{mol/l}$. Derquantel blocked nicotinic stimulation of cells expressing human $\alpha 3$ ganglionic (IC_{50} value of approximately 9 $\mu\text{mol/l}$) and muscle-type (IC_{50} value of approximately 10 $\mu\text{mol/l}$) nicotinic cholinergic receptors, but was inactive ($\text{IC}_{50} > 100 \mu\text{mol/l}$) on the $\alpha 7$ central nervous system subtype (Zinser et al., 2002).

2.2 Toxicological studies

2.2.1 Acute toxicity

(a) Single-dose toxicity studies

The results of single-dose toxicity studies with derquantel are summarized in [Table 1](#). Following oral exposures, abnormal clinical signs in mice were observed at and above 12.5 mg/kg bw and included ptosis, decreased activity, loss of righting reflex, rapid or laboured breathing, jerky movements or ataxia. There were no deaths, and clinical signs returned to normal 4 hours post-dosing.

In rats, a single oral derquantel dose of 500 mg/kg bw in the 60:40 propylene glycol:glycerol formal vehicle produced severe toxicity in both test article-treated and control rats; therefore, the toxicity was attributed to the vehicle. The vehicle was changed to 1% carboxymethylcellulose, and rats (one of each sex per dose) were administered single oral doses of 0 (carboxymethylcellulose vehicle), 200, 350, 500, 1000, 1500 or 2000 mg/kg bw and observed for 7 days. One rat at the highest dose of 2000 mg/kg bw died. Doses of 350 mg/kg bw and above produced jerky movements within 15 minutes of dose administration. Other clinical signs at greater than 1000 mg/kg bw included subdued behaviour, piloerection, tilted posture and reduced somatomotor activity. Clinical signs subsided at 6 hours in all animals and recurred on day 7 in the 1500 mg/kg bw group and the surviving 2000 mg/kg bw animal. Body weight loss occurred at and above 500 mg/kg bw (Brook, 2004a).

Table 1. Single-dose toxicity studies with derquantel

Species	No. of each sex per group	Route	Dose (mg/kg bw)	LD ₅₀ (mg/kg bw)	Reference
Mouse	3M, 3F	Oral ^a	0, 12.5, 25, 50	>50	Frank, Kechele & Palmer (1996a)
Rat	1M, 1F	Oral ^a	0 ^b , 0 ^c , 200 ^c , 350 ^c , 500 ^b , 500 ^c , 1000 ^c , 1500 ^c , 2000 ^c	≥ 2000	Brook (2004a)
Rat	5M, 5F	Oral ^a	0, 200, 500, 1200	>1200	Brook (2005e)
Rat	1M, 1F	Dermal ^d	500, 2000	>2000	Brook (2004c)
Rat	5M, 5F	Dermal ^d	2000	>2000	Brook (2005f)
Rat	5M, 5F	Inhalation	2.4 mg/l for 4 h	>2.4 mg/l for 4 h ^e	Kirkpatrick (2005)
Dog	1M, 1F	Oral ^f	5, 10, 20	>20	Frank, Kechele & Palmer (1996b)
Dog	2M, 2F	Oral ^{a,c}	1, 5	>5	May (2008)
Horse	1M, 1F	Oral ^{a,b}	0, 20	20	Seaman (2000a)
Horse	1M, 1F	Oral ^{a,c}	2	2	Seaman (2000b)

F, female; LD₅₀, median lethal dose; M, male

^a Via gavage.

^b 60:40 propylene glycol:glycerol formal vehicle.

^c 1% carboxymethylcellulose vehicle.

^d Neat test article applied.

^e LC₅₀.

^f Via capsule.

Single dermal applications of derquantel to rats at up to 2000 mg/kg bw resulted in no mortality and no signs of toxicity (Brook, 2004c, 2005f). Inhalation exposure of rats at 2.4 mg/l for 4 hours resulted in prostration, hypothermia, hypoactivity, rough haircoat and reduced faecal output, but no mortality. All animals were normal by study day 2 (Kirkpatrick, 2005).

Derquantel administered to young Beagle dogs at a dose level of 5, 10 or 20 mg/kg bw for 1 day was well tolerated. Clinical signs included scleral injection (bilateral) and mydriasis (bilateral) at and above 5 mg/kg bw, ptosis (bilateral), relaxed nictitating membranes and dry mouth at and above 10 mg/kg bw and dry conjunctiva at 20 mg/kg bw. All dogs appeared clinically normal after 24 hours post-dosing (Frank, Kechele & Palmer, 1996b). In an acute neurobehavioural study in dogs, oral administration of derquantel at 1 or 5 mg/kg bw for 1 day was without effect on mortality, did not produce qualitative or quantitative electrocardiogram abnormalities and was without effect on the functional observational battery measurements of activity/arousal, neuromuscular function and sensorimotor function. Doses of 1 and 5 mg/kg bw were associated with clinical signs of scleral

injection, partial/slow constriction of pupils and slightly drooping eyelids, as well as a dose-related increase in heart rate. Administration of derquantel at 5 mg/kg bw was associated with decreased direct and indirect pupillary light reflex on both eyes, mild to moderate photosensitivity, a prolapsed nictitating membrane, QTc prolongation, slight decreases in body temperature and single incidences of coughing, stereotypic chewing and an exaggerated patellar reflex response on both hindlimbs (May, 2008).

Abnormal clinical signs in horses included ataxia, falling, marked sweating, distended subcutaneous blood vessels, hypothermia, marked and unresponsive mydriasis, marked ptosis, paraphimosis, slow, laboured breathing, tremors, muscle fasciculations and body weight loss. Mortality by death or euthanasia occurred at 46 (2 mg/kg bw) to 58 (20 mg/kg bw) hours post-dosing. Postmortem findings included gaseous distension of the lower gastrointestinal tract and microscopic vacuolations and mild degeneration of centrilobular hepatocytes (Seaman, 2000a,b).

Clinical signs observed in these studies were consistent with effects associated with derquantel antagonism of nAChR. The greater sensitivity in dogs compared with rats or mice was attributed to a greater sensitivity of dog $\alpha 3$ nicotinic receptors in the dorsal root ganglion in *in vitro* studies. The antagonistic potency of derquantel in these studies was measured as IC_{50} values, which were 1.16 $\mu\text{mol/l}$ for the dog and 31.51 $\mu\text{mol/l}$ for the rat (Lowney, 2009a; May, 2009c). The severe toxicity observed in horses may have been related to a difference in drug metabolism, as evidenced by the presence of a hydroxylated metabolite of derquantel seen in plasma at a higher concentration than for the parent derquantel and not seen in other species tested, or due to an enhanced pharmacological sensitivity of this species to the mode of action of this class of compound (Seaman, 2000b).

(b) Dermal sensitization

The skin sensitization potential of derquantel (lot no. 5789-MAM-9901) in corn oil vehicle was tested using the Magnusson & Kligman guinea-pig maximization test in accordance with Organisation for Economic Co-operation and Development (OECD) guideline 406 and OECD principles of GLP. Thirty-two guinea-pigs (16 of each sex) were used for the test (2 pigs for the preliminary test and 30 pigs for the main test). Skin sensitization of the derquantel/abamectin combination was tested in a modified Buehler technique in guinea-pigs in accordance with United States Environmental Protection Agency (USEPA) GLP standards and OECD guideline 406. Seventy-four guinea-pigs (37 of each sex) were used for the test (2 pigs of each sex for the range-finding phase, 15 of each sex for the induction phase and 20 of each sex for the challenge phase). Derquantel and the derquantel/abamectin combination were non-sensitizing to the skin of guinea-pigs in these studies (Brook, 2005d; Lloyd, 2008a).

(c) Dermal irritation

The skin irritation potential of derquantel (lot no. 5789-MAM-9901) was tested in three adult female rabbits using a topical dose of 0.5 g of neat test article, which was applied to a shaved area of skin on each rabbit. The study was performed

in accordance with OECD guideline 404 and OECD principles of GLP. The skin irritation potential of the derquantel/abamectin combination was tested in three adult male rabbits in accordance with OECD guideline 404 and USEPA principles of GLP. A topical dose of 0.5 ml of a 10 mg/ml / 1 mg/ml derquantel/abamectin combination was applied to an area of shaved skin on each rabbit. Derquantel and the derquantel/abamectin combination were non-irritating to the skin of rabbits in these studies (Brook, 2004b; Lloyd, 2008b).

(d) *Ocular irritation*

In a GLP-compliant study, the ocular irritation potential of derquantel (lot no. E010002720) was tested in adult rabbits (one of each sex) by application of a single 100 mg dose of neat derquantel into the cupped lower conjunctival sac of the right eye of each animal. Positive conjunctival irritation was noted for both animals. All irritation subsided by study termination on day 4. The maximum average score for derquantel was 8 out of a total maximum score of 110 at 1 hour post-instillation. The ocular irritation potential of the derquantel/abamectin combination was tested in a GLP-compliant study in adult rabbits (three males). A single 0.1 ml dose of the 10 mg/ml / 1 mg/ml derquantel/abamectin combination was instilled into the cupped lower conjunctival sac of the right eye of each animal. Chemosis or discharge scores of 1 (minimal) were obtained in one animal each at the 1 hour post-dosing time point. The effects had cleared in less than 24 hours. The derquantel/abamectin combination was non-irritating to rabbit eyes in this study (Hurley, 2005; Lloyd, 2008c).

2.2.2 *Short-term studies of toxicity*

(a) *Rats*

In a non-GLP-compliant, 3-day escalating oral dose range-finding study, 3-month-old rats (two of each sex per group) received derquantel (PNU-141962, batch no. 31082-MAM-149A) via gastric intubation at a dose of 0, 25, 50, 100, 200, 300, 400 or 600 mg/kg bw per day. Dose volumes increased with dose up to a maximum volume of 12 ml/kg bw for the 600 mg/kg bw per day group. Owing to clinical signs of excessive toxicity, dosing of the 400 mg/kg bw per day group was interrupted, and the animals were allowed a 2-week washout period. Dosing then resumed at a dose level of 300 mg/kg bw per day for 2 days. The 600 mg/kg bw per day dose was given only once due to excessive toxicity. Control animals received the formulation vehicle (60% propylene glycol and 40% glycerol formal, volume per volume) at a dose volume of 12 ml/kg bw, the maximum dose volume used during the study. A 2-week observation period followed the dosing phase. Feed and water were provided ad libitum. The rats were inspected at least daily, and clinical observations were made at least 4 times per day during the dosing phase. Body weight was determined daily. All rats that died during the study or survived for 2 weeks post-dosing were necropsied, followed by a gross examination of the brain and thoracic and abdominal cavities.

There was one test article-related death. A female dosed once at 600 mg/kg bw per day died 2 days after showing severe toxicity soon after dosing. Clinical

signs prior to this animal's death included ataxia and hypoactivity for the first 3 hours post-dosing. The condition worsened at approximately 4 hours post-dosing, when the animal became prostrate with no righting reflex and laboured respiration. Opisthotonos (abnormal body position) was observed at approximately 5.5 hours post-dosing, and the animal never recovered.

For animals that survived the initial 600 mg/kg bw per day dose, the female became prostrate soon after dosing and exhibited loss of the righting reflex, jerky body movements and tremors when handled, but appeared normal approximately 5.5 hours post-dosing. Males had decreased activity and ataxia within 20 minutes post-dosing, lasting until approximately 4 hours post-dosing.

At 400 mg/kg bw per day, males showed no signs of toxicity until day 2 of dosing, when one male was ataxic and hypoactive within 10 minutes of dosing until approximately 3 hours post-dosing. The second male showed prostration, ataxia, loss of the righting reflex and opisthotonos within 11 minutes of dosing, but returned to normal by 6 hours post-dosing. Females showed signs of toxicity after the first dose. Both exhibited ataxia and hypoactivity within 20 minutes of dosing, and one lost its righting reflex. On day 2, within 20 minutes of dosing, both females exhibited ataxia, prostration, loss of the righting reflex and jerky movements, and one also had ptosis. They did not return to normal until the next day.

There were no clinical signs of toxicity at doses of 25, 50, 100, 200 and 300 mg/kg bw per day.

Decreases in mean body weight were observed during the dosing phase in all treated groups. Losses in males ranged from 2% to 8%, and losses in females ranged from 0% to 9%. Control males and females had 13% and 9% losses in mean body weight, respectively, during the dosing phase, which suggested a vehicle effect on body weight. All groups regained weight lost during the dosing phase by 4 days after the last dose administered, and mean values exceeded pre-dosing means by 10 days after the last dose administered.

There were no treatment-related findings in the postmortem data (Seaman, 1999). Based on the absence of test article-related clinical signs, the no-observed-adverse-effect level (NOAEL) for this study was 300 mg/kg bw per day.

In a GLP-compliant, 12-day oral dose range-finding study, 6- to 8-week-old rats (two of each sex per group) received derquantel (PF-520904, batch no. 5789-MAM-9901) via gastric intubation at a dose of 0, 30, 50, 100, 150, 200, 250, 400, 600 or 800 mg/kg bw per day. Controls were treated with the vehicle (1% carboxymethylcellulose). The dose volume given to all groups was approximately 10 ml/kg bw. Feed and water were provided ad libitum. Animals were observed at least once daily. Body weights were determined prior to the first dose, on day 8 and at study termination. At study termination, animals were euthanized and subjected to a gross necropsy. Liver, kidneys, adrenals, gonads and spleen from all groups were collected and weighed, and livers from animals in the 30 and 150 mg/kg bw per day groups were processed for histopathological examinations.

All animals in the 400, 600 and 800 mg/kg bw per day groups were either found dead 24 hours after dose administration on day 2 (one 400 mg/kg bw per

day female) or euthanized on day 2 because of clinical signs of severe toxicity. At 250 mg/kg bw per day, all animals were euthanized on day 3 as a result of severe clinical signs. At 600 and 800 mg/kg bw per day, clinical signs prior to death or euthanasia included hypoactivity, piloerection and jerky movements 15 minutes after dose administration on day 1. After dosing on day 2, the signs appeared with increased severity. At 400 mg/kg bw per day, clinical signs included hypoactivity and jerky movements after day 1 dose administration. After dosing on day 2, all animals were prostrate and had convulsions. Clinical signs in the 250 mg/kg bw per day group were seen after dosing on day 2 and included prostration and paralysis, with recovery 4 hours later. Following dosing on day 3, convulsions and excessive salivation were seen in addition to prostration and paralysis.

At 200 mg/kg bw per day, test article-related clinical signs were observed beginning on day 3 and included wobbly gait, porphyrin staining around the nose, piloerection, hunched or tilted posture and jerky movements. These clinical signs appeared 10–15 minutes after dose administration and resolved within 4 hours. The appearance of animals was assessed as normal prior to dosing on the following day. A significant reduction in body weight gain compared with controls was observed for both males and females in this group after 7 days of treatment. No difference was observed for the remainder of the dosing days.

No test article-related clinical signs or effects on body weight were seen at 30, 50, 100 or 150 mg/kg bw per day, and no abnormal clinical signs were seen in the control group.

Treatment-related gross pathology findings were limited to a dose-related increase in mean liver weight of males and females in all surviving test article-treated groups compared with controls. The greatest increases were seen in the 150 and 200 mg/kg bw per day groups. No organ weights were collected for animals in the 250 mg/kg bw per day dose groups and higher. Histopathological examinations of the liver were performed on animals in the 30 and 150 mg/kg bw per day groups only. At 150 mg/kg bw per day, two of four animals (one of each sex) had mild, periacinar hepatocyte swelling and cytoplasmic vacuolation with a few scattered necrotic hepatocytes. There were no findings at 30 mg/kg bw per day.

Based on a slight increase in mean liver weight with no associated histopathological findings, the NOAEL for this study was 30 mg/kg bw per day (Brook, 2005a).

In a GLP-compliant, 28-day oral toxicity study, 6- to 8-week-old rats (seven of each sex per group) received derquantel (PF-520904, batch no. 5789-MAM-9901) via gastric intubation at a dose of 0, 20, 100 or 200 mg/kg bw per day. Controls were treated with the vehicle (1% carboxymethylcellulose). The dose volume given to all groups was approximately 10 ml/kg bw. Feed and water were provided *ad libitum*. Feed consumption was determined weekly. The animals were observed daily approximately 1 and 3 hours after dosing. Detailed clinical examinations were performed weekly, and functional observations were performed in the last week of the experimental period. Body weights were determined prior to the first dose, weekly thereafter, at death or 1 day before study termination. Blood samples for haematology and biochemistry were collected on day 29 prior to euthanasia. Urine

was collected for urine analysis on day 29 by manual expression or cystocentesis at necropsy. Following gross necropsy examination, liver, kidneys, adrenals, brain, gonads and spleen were collected, weighed and preserved. Sections of these organs as well as sections from 27 additional tissues or organs were prepared for histological examination. Histological examinations were performed in the vehicle control and 200 mg/kg bw per day groups, and livers of 20 and 100 mg/kg bw per day animals were also examined.

Test article-related mortality was seen in females at 200 mg/kg bw per day. One female died on day 7. Abnormal clinical signs were first seen in this animal on day 5, 1–2 hours after dosing, and included wobbly gait, prostration and convulsions, all of which had resolved within 4 hours. On day 6, wobbly gait, prostration and convulsions were seen again 1 hour post-dosing, resolving 3 hours later. The animal was found dead on day 7. A second female died on day 10. Abnormal clinical signs were first seen in this animal on day 8, 30 minutes after dosing, and consisted of subdued behaviour lasting 3 hours. On day 9, the animal became prostrate immediately after dosing, had convulsions for 15 minutes and had no or subdued somatomotor activity for 4 hours. On day 10, the animal became prostrate again after dosing. Piloerection, laboured breathing, subdued behaviour and convulsions were also seen. The animal was found dead 2.5 hours after dosing.

Clinical signs of toxicity were seen in surviving 200 mg/kg bw per day males and females throughout the dosing phase, beginning early in week 1 for males and the middle of week 1 for females. The predominant clinical signs included subdued behaviour, prostration, convulsions, paralysis and tilted posture. Other clinical signs included wobbly gait, jerky movements, piloerection, laboured breathing, tremors and splayed hindlimbs. No signs of toxicity were seen in males during week 4. Females had no clinical signs of toxicity in week 3, but signs of toxicity were observed in week 4. Clinical signs of toxicity in the 100 mg/kg bw per day group include subdued behaviour seen in three males and three females during week 3 lasting approximately 2 hours, and porphyrin staining around the nose was seen in one male during week 2. Porphyrin staining was also seen transiently during weeks 2 and 3 in one control female and three males and one female in the 20 mg/kg bw per day group.

Treatment-related decreases in body weight gain were seen in males and females at 200 mg/kg bw per day. These decreases corresponded with decreases in feed consumption.

Results of functional observations performed on all animals in the last week of dosing, prior to test article administration, showed an increase in forelimb grip strength in both males and females in each group compared with controls. This finding is of uncertain toxicological relevance.

Effects on haematology parameters included a slight decrease in mean red blood cells, haemoglobin and haematocrit values relative to controls for 20, 100 and 200 mg/kg bw per day males and 100 and 200 mg/kg bw per day females. A dose-related decrease was observed in females, but not males. A slight, dose-related increase in mean platelet values relative to controls was seen in males and females at all dose levels. None of the differences were statistically significant. These findings are of uncertain toxicological relevance.

Effects on blood biochemistry analyses included slight decreases in mean glucose levels in 200 mg/kg bw per day males and 100 and 200 mg/kg bw per day females; slight increases in urea levels in 200 mg/kg bw per day males and females; lower albumin levels in 200 mg/kg bw per day females; lower albumin to globulin ratios (as a result of increased globulin levels) in females in all treatment groups and males at 100 and 200 mg/kg bw per day; elevated cholesterol levels in males and females at 200 mg/kg bw per day; and increased gamma-glutamyltransferase (GGT) levels at 200 mg/kg bw per day. An elevated serum GGT level is consistent with cholestasis. The other changes are of uncertain toxicological significance. None of the changes were statistically significant.

Relative mean liver weight was significantly increased compared with controls in males and females in the 100 and 200 mg/kg bw per day groups.

Treatment-related histopathology findings were limited to bile duct hyperplasia, which was of minimal severity in two males and three females at 20 mg/kg bw per day; minimal to mild severity in six males and three females at 100 mg/kg bw per day; and minimal to severe severity in five males and five females at 200 mg/kg bw per day.

Based on the significant increases in mean liver weights at 100 and 200 mg/kg bw with an associated dose-related increase in the incidence and severity of bile duct hyperplasia and elevated serum GGT levels and mortality at 200 mg/kg bw per day, the NOAEL for this study was 20 mg/kg bw per day (Rozinova, 2005).

In a GLP-compliant, 28-day oral toxicity study, 7- to 8-week-old rats (15 of each sex per group) received derquantel (batch no. 115883-tis-111) enriched with 4% *N*-oxide (batch no. 115883-tis-7) by gastric intubation at a dose level of 0, 0.5, 5, 25 or 175 mg/kg bw per day, at a dose volume of 5 ml/kg bw. Owing to excessive toxicity and deaths in females, the 175 mg/kg bw dose level was lowered to 150 mg/kg bw per day for females only beginning on day 9, by lowering the dose volume to 4.3 ml/kg bw. Controls were dosed with 1% (weight per volume [w/v]) carboxymethylcellulose. *N*-Oxide is a degradation product of derquantel seen during accelerated stability testing of the derquantel/abamectin drug product. Feed and water were provided ad libitum. Animals were observed for morbidity, mortality, injury and the availability of feed twice daily. Detailed clinical examinations were performed twice daily (pre-dosing and 1 and 4 hours post-dosing). Functional observational battery evaluations were performed once pretest, 1 hour post-dosing on day 3, pre-dosing on day 4, 2 hours post-dosing on day 11 and pre-dosing on day 28. Locomotor activity evaluations were performed once pretest, immediately post-dosing on day 3 and pre-dosing on day 28. Body weights were measured pretest and weekly thereafter. Feed consumption was measured weekly. Ophthalmoscopic examinations were performed pretest and prior to terminal necropsy. Clinical pathology evaluations were conducted on fasted animals prior to the terminal necropsy. At study termination, animals were humanely killed and a gross necropsy was performed. A standard assortment of organs was collected and weighed. Tissues from weighed organs along with a comprehensive assortment of other organs and tissues collected at necropsy were processed for microscopic examination. All tissues from the 0 and 175/150 mg/kg bw per day groups were examined, whereas only the epididymis, kidney, liver, testis and thyroid gland (with

parathyroid) were examined from animals at 0.5, 5 and 25 mg/kg bw per day. A peer review of the histopathological findings was performed.

Test article-related toxicity was the cause of death for four females given 175 mg/kg bw per day through day 8. The dose was lowered to 150 mg/kg bw per day on day 9, and two more females died, one each on days 10 and 14. Adverse clinical signs associated with mortality included decreased activity, ataxia, prostration, an impairment or loss of righting reflex, head tilt, salivation, stereotypy, thin appearance, coldness to the touch and laboured respiration. After the dose was lowered on day 9, these signs persisted through day 14. There were no test article-related clinical signs in males at this dose level or in males or females at the lower dose levels.

Test article-related effects in the functional observational battery observations included a significantly increased incidence, compared with controls, of rearing activity in 175 mg/kg bw per day males. This finding was considered incidental, as it was not seen in females, and there were no adverse test article-related effects on the test battery indices for neuromuscular function or sensorimotor measurements.

Test article-related effects on autonomic measurements included increased palpebral closure in 4 of 10, 6 of 10 and 4 of 6 females at 5, 25 and 175/150 mg/kg bw per day, respectively, on day 11. The incidence was significant at and above 25 mg/kg bw per day. These effects were not seen on day 28 and therefore were not considered adverse because of their transient nature. Similarly, decreased pupillary response was observed in three of nine 175/150 mg/kg bw per day females on day 3 and only in one of nine on day 4. Pupillary response was normal 2 hours post-dosing on day 11 and prior to administration on day 28. This effect was not considered adverse because of its transient nature. A decrease in mean body temperature in 175/150 mg/kg bw per day females was also observed on days 3, 4 and 11, but not on day 28. Decreased body temperature was associated with mortality in four animals.

Test article-related effects on locomotor activity included lower rearing counts (0–240 minutes) in males and females at and above 5 mg/kg bw per day on day 28. For females, the decrease was dose related, with significant differences ($P < 0.01$) at 5, 25 and 175/150 mg/kg bw per day. For males, the dose-related decrease was apparent at 5 mg/kg bw per day, and differences were significant ($P < 0.05$) at 25 and 175 mg/kg bw per day. This finding is considered adverse where the differences were statistically significant.

Mean body weights for 175 mg/kg bw per day males during weeks 2 through 4 and for 175/150 mg/kg bw per day females during week 2 were decreased by 5–8% compared with controls. Corresponding test article-related decreases in mean feed consumption were noted for males and females at this dose level during week 2. These test article-related effects on body weight and feed consumption were assessed as minor and not adverse.

The haematology data for 175 mg/kg bw per day males and 175/150 mg/kg bw per day females reflected a mild anaemia of inflammation characterized by mild decreases in red blood cell mass (erythrocytes, haemoglobin and haematocrit),

which was slightly more severe in females. This diagnosis was based on evidence of an inflammatory response in the clinical chemistry data (increases in globulin and fibrinogen; decreases in albumin), which can suppress erythroid function and cause the observed changes. A test article-related decrease in lymphocyte and eosinophil counts was also observed in males and females at this dose level.

The coagulation data showed a treatment-related but non-adverse increase in platelets, activated partial thromboplastin times and fibrinogen in males and females and non-adverse decreases in prothrombin times in females at the 175/150 mg/kg bw per day dose level. In 25 mg/kg bw per day females, a treatment-related but non-adverse decrease in prothrombin time and an increase in fibrinogen level were also noted.

Treatment-related effects in the clinical chemistry data included increases in GGT, alanine aminotransferase (ALT) and sorbitol dehydrogenase activities in males and females at 175/150 mg/kg bw per day. Urea nitrogen levels were also increased in both sexes in this group; the increases were attributable to mild dehydration resulting from reduced feed intake. Decreases in albumin levels with increases in globulin levels were observed in both sexes at 175/150 mg/kg bw per day. Minimal increases in globulin levels were seen in 25 mg/kg bw per day females. These changes resulted in decreased albumin to globulin ratios for 175/150 mg/kg bw per day males and females and 25 mg/kg bw per day females. Non-adverse treatment-related increases in cholesterol levels were observed in 25 mg/kg bw per day females and in both sexes at 175/150 mg/kg bw per day, and a mild decrease in triglyceride was seen in 175 mg/kg bw per day males.

There were no treatment-related macroscopic findings at necropsy. Significantly ($P < 0.05$ or $P \leq 0.01$) increased mean absolute and relative liver weights in 25 and 175/150 mg/kg bw per day males and females corresponded microscopically with panlobular hepatocellular hypertrophy at 175/150 mg/kg bw per day and was therefore considered adverse at this dose level. There was an increased presence of hepatocellular mitotic figures in two of six early-death females from the 175/150 mg/kg bw per day group, but no evidence of hepatocellular hypertrophy in early-death animals. Similarly, significantly ($P < 0.01$) increased absolute and relative mean thyroid weights in 175/150 mg/kg bw per day females were an adverse effect corresponding microscopically with follicular cell hypertrophy. Minimal to mild follicular cell hypertrophy was seen in the thyroid glands of males and females at 175/150 mg/kg bw per day and in females at 5 and 25 mg/kg bw per day.

Based on significantly lower rearing counts on day 28 in females at 5, 25 and 175/150 mg/kg bw per day, adverse treatment-related effects seen at the 175/150 mg/kg bw per day dose level, which included mortality and evidence of thyroid toxicity in females, and evidence of hepatotoxicity in males and females at 25 and 175/150 mg/kg bw per day, the NOAEL for this study was 0.5 mg/kg bw for females and 5 mg/kg bw per day for males (May, 2009f).

In a GLP-compliant, 90-day oral toxicity study, rats (10 of each sex per group) less than 9 weeks of age received derquantel (PF-520904, batch no. E010002418) by gastric intubation at a dose of 0, 1, 5, 50 or 150 mg/kg bw per day. Controls

were dosed with the vehicle, carboxymethylcellulose. The dose volume given to all groups was 3 ml/dose, except in the latter part of the study, when some animals heavier than 600 g required slightly more than 3 ml. Feed and water were provided ad libitum. Animals were observed twice daily for signs of morbidity, mortality or change in behaviour or appearance. Clinical examinations were performed weekly, approximately 1 hour after dose administration. Body weights were measured weekly. Ophthalmoscopic examinations were performed prior to dose initiation and on days 56/57 and 84/85. Neurological examinations consisting of a standard functional observational battery were performed on days 29 and 90. Blood samples for haematology and clinical chemistry evaluations were collected prior to necropsy on day 90. At study termination, animals were humanely killed with an overdose of anaesthetic agent followed by cardiac puncture. Gross necropsies were performed, and heart, kidneys, thymus, brain, liver, adrenals, testes and ovaries were collected and weighed and processed for histopathological evaluation, along with a comprehensive assortment of other tissues and organs. All tissues from controls and 150 mg/kg bw per day animals and livers from 1, 5 and 50 mg/kg bw per day animals were examined microscopically.

There were no test article-related deaths during the study. Treatment-related clinical signs included a dose-related increased incidence of palpebral closure and decreased alertness at and above 1 mg/kg bw per day, increased incidence of ataxia at and above 5 mg/kg bw per day and piloerection at and above 50 mg/kg bw per day. Test article-related decreases in mean body weight were seen in 150 mg/kg bw per day males and females. In males, the difference was significant ($P < 0.0001$) compared with all male groups and was associated with a lower mean weekly feed consumption compared with controls. Treatment-related neurological effects were observed at 50 and 150 mg/kg bw per day and included changes in behavioural response, piloerection at both dose levels and reduced response to stimuli, tremors and abnormal posture at 150 mg/kg bw per day.

Test article-related changes in the clinical chemistry data included significant increases in serum protein in females at and above 5 mg/kg bw per day ($P = 0.036$, $P = 0.003$ and $P < 0.0001$, respectively) and in males at and above 1 mg/kg bw per day ($P = 0.028$, $P = 0.039$, $P = 0.002$ and $P = 0.001$, respectively); significant ($P = 0.0009$) decreases in albumin in 150 mg/kg bw per day females; significant ($P < 0.0001$) increases in globulin levels in females at and above 5 mg/kg bw per day and in males at and above 50 mg/kg bw per day; significant decreases in the albumin to globulin ratio in females at and above 50 mg/kg bw per day ($P = 0.029$ and $P < 0.001$) and in 150 mg/kg bw per day males ($P < 0.0001$); significantly ($P \leq 0.0001$) increased urea values for females at and above 50 mg/kg bw per day ($P = 0.028$ and $P < 0.0001$) and males at 5 and 150 mg/kg bw per day ($P = 0.032$ and $P = 0.009$); and significant increases in triglycerides in males and females at 150 mg/kg bw ($P = 0.013$ and $P = 0.036$, respectively). ALT and aspartate aminotransferase (AST) levels were increased in 150 mg/kg bw per day males. There were no changes in the haematology data that were attributable to test article toxicity.

Gross necropsy findings associated with treatment included abnormal appearance of the liver, described as enlarged, pale or lobular, which occurred with a dose-related incidence at and above 5 mg/kg bw per day. There was a treatment-

related increase in mean liver weight in 50 and 150 mg/kg bw per day males and females. The differences compared with controls were significant ($P = 0.0003$ and $P < 0.0001$ for females and $P = 0.027$ and $P < 0.0001$ for males). Mean heart weight was significantly increased in 50 and 150 mg/kg bw per day males ($P = 0.049$ and $P = 0.013$, respectively). Microscopically, test article-related changes were characterized as increased incidence and severity of biliary cell degeneration and multifocal biliary cell proliferation. Focal, minimal biliary cell degeneration was seen in one male and one female control animal. Minimal to slight biliary degeneration was seen in three 1 mg/kg bw per day males, but no females. At 5 mg/kg bw per day, minimal to slight biliary cell degeneration was seen in six males. In five females with this finding, the severity was minimal to moderate. At 50 mg/kg bw per day, multifocal biliary cell degeneration was seen in six males (minimal to slight severity) and five females (minimal to moderate severity). At 150 mg/kg bw per day, biliary proliferation was seen in nine males (minimal to marked severity) and seven females (multifocal, minimal to slight severity). An additional two males had minimal to moderate biliary cell degeneration with portal inflammatory cell aggregates. An additional two females had moderate biliary cell degeneration. One female had no remarkable liver findings (Goldenthal, 2006).

A cover memo accompanying this study report reviewed reasons why the results of this study should be considered unreliable and not suitable for an assessment of the toxicity of derquantel. In particular, it was found that the liver slides evaluated by the study pathologist were of poor quality; as a result, some of the biliary degeneration reported by the study pathologist could have been due to tissue shrinkage and artefact. Other deficiencies noted included the limited histopathological evaluation of the 1, 5 and 50 mg/kg bw per day groups and post-dosing monitoring that was insufficient to adequately capture clinical signs (Lowney, 2009b).

Owing to the questionable quality or reliability of the clinical observations and histopathology data, a NOAEL could not be established in this study.

In a GLP-compliant, 1-year oral toxicity study, 8-week-old rats (27 of each sex per group) received derquantel (PF-520904, batch no. E010002940) by gastric intubation at a dose of 0, 1, 5 or 50 mg/kg bw per day. Controls were treated with the vehicle (1% carboxymethylcellulose). The dose volume given to all groups was 5 ml/kg bw. Feed and water were provided ad libitum. In addition to twice daily assessments for mortality or moribundity, detailed clinical observations were performed once prior to treatment and weekly prior to dosing. Cage-side observations were performed daily within 3 hours following dose administration. Body weights were determined prior to dose initiation and weekly thereafter. Feed consumption was measured weekly. Ophthalmological examinations were performed prior to dose initiation and during the last week of the treatment period. Blood samples for haematology, biochemistry and coagulation evaluations were collected at study termination (day 366). Samples for urine analysis were collected by cage pan drainage overnight at study termination. At necropsy, 17 organs were collected for organ weight determinations. Sections of these as well as sections from a comprehensive assortment of other tissues and organs were prepared for histological examination. All tissues from control and 50 mg/kg bw per day animals

were examined microscopically. The liver, eyes, testes, epididymis and kidneys were identified as target organs and were therefore processed and examined from the 1 and 5 mg/kg bw per day groups. The histopathology evaluation was peer reviewed.

There were no treatment-related mortalities. A treatment-related significant ($P < 0.05$) decrease in mean body weight occurred in 50 mg/kg bw per day males during the last 3 weeks of the study, although feed consumption was not affected.

There were no clear test article-related changes in haematology, coagulation or red blood cell morphology data.

Test article-related changes in the clinical chemistry data included elevated mean levels of GGT for 50 mg/kg bw per day males and females. Mean bilirubin levels were significantly ($P < 0.05$) elevated, as were mean cholesterol levels ($P < 0.01$), and mean triglyceride levels were increased in 50 mg/kg bw per day females. In 50 mg/kg bw per day males, a significant ($P < 0.01$) increase in mean globulin levels resulted in a significant ($P < 0.01$) increase in mean total protein levels and a lower albumin to globulin ratio compared with controls. In females, mean globulin levels were increased at all dose levels compared with controls. The difference was significant ($P < 0.01$) at 50 mg/kg bw per day, resulting in a significant ($P < 0.01$) decrease in the albumin to globulin ratio and significantly ($P < 0.01$) increased total protein levels at this dose level. In addition, mean total protein levels were significantly increased in all test article-treated groups ($P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively).

Ophthalmoscopic findings were limited to an increased incidence of cataracts in males at 50 mg/kg bw per day, although only 2 of 27 cataracts seen on ophthalmic examination were confirmed histologically. Cataracts in males at 50 mg/kg bw per day are likely a treatment-related effect in this study, given the relatively high incidence of cataracts observed by the board-certified ophthalmologist who performed the in-life examinations and the absence of this finding at lower dose levels.

Test article-related necropsy findings were limited to enlarged livers observed in 9 of 27 males and 8 of 27 females at 50 mg/kg bw per day. Significant changes in organ weights relative to controls were seen at 50 mg/kg bw per day and included increases in mean absolute and relative liver weights in males and females, decreased mean absolute and relative thyroid gland and thymus weights in males and increased mean absolute and relative thyroid weights in females.

Test article-related histopathology findings were observed in the liver, kidney, testis and epididymis. Liver findings were seen in males and females at all dose levels, including controls, and were characterized as spontaneous, non-adverse, age-related biliary hyperplasia. In many cases, bile duct proliferation was accompanied by periportal (and periductal) fibrosis or infiltration of mononuclear cells. The incidence or severity of the liver findings increased with increasing dose, starting at 1 mg/kg bw per day, indicating that the test article had an accelerating effect on the development of this lesion. Biliary hyperplasia and associated elevations in ALT and bilirubin levels and increased mean liver weight at 50 mg/kg bw per day were considered adverse effects. Kidney findings were seen in males

and females at all dose levels, including controls, and were characterized as age-related, spontaneous, chronic progressive nephropathy with a treatment-related increase in severity at 50 mg/kg bw per day. In the absence of clinical pathology changes, these kidney findings were not considered adverse. Adverse test article-related effects were noted in 50 mg/kg bw per day males and consisted of mild to marked degeneration of seminiferous tubules in 3 of 27 animals and mild to marked hypospermia in 4 of 27 animals in this dose group. Minimal degeneration of seminiferous tubules without corresponding hypospermia was considered a non-adverse finding seen in 2 of 25 males at 5 mg/kg bw per day. Marked degeneration of seminiferous tubules was also seen in one male in the 1 mg/kg bw per day group. This finding was not test article related because the lesion had moderate mononuclear cell infiltration and was therefore qualitatively different from the degeneration seen at 50 mg/kg bw per day. Marked hypospermia also seen in this animal was an incidental finding associated with degeneration of the testes. There were no abnormal findings in the testis or epididymis of control animals.

Based on adverse testicular effects in males and evidence of hepatotoxicity in males and females at 50 mg/kg bw per day, the NOAEL was 5 mg/kg bw per day (Bonnette, 2009a).

A second GLP-compliant, 1-year oral toxicity study in the rat was conducted with lower dose levels, which allowed for a better evaluation of the hepatobiliary effects of derquantel seen in the previous study. In this study, 8-week-old rats (15 of each sex per group) received derquantel (PF-520904, batch nos E010002940 and NO70041) by gastric intubation at a dose of 0, 0.01, 0.03, 0.1 or 0.3 mg/kg bw per day. Controls were treated with the vehicle (1% [w/v] carboxymethylcellulose). An additional 15 rats of each sex per group were assigned to the control and 0.01 mg/kg bw per day groups for interim termination on day 91. The dose volume given to all groups was 5 ml/kg bw. Feed and water were provided ad libitum. In addition to twice daily assessments for mortality or moribundity, detailed clinical observations were performed once prior to treatment and weekly prior to dosing. Cage-side observations were performed daily within 3 hours following dose administration. Body weights were determined prior to dose initiation and weekly thereafter. Feed consumption was measured weekly. Ophthalmological examinations were performed prior to dose initiation, for interim-phase animals on day 84 and during the last week of the treatment period. Blood samples for haematology, biochemistry and coagulation evaluations were collected from control and 0.01 mg/kg bw per day animals at interim sacrifice (day 91) and from all groups at study termination (day 366/367). Samples for urine analysis were collected by cage pan drainage overnight at both intervals. At necropsy, 17 organs were collected for organ weight determinations. Sections of these as well as sections from a comprehensive assortment of other tissues and organs were prepared for histological examination. All tissues and organs collected at necropsy from control and 0.3 mg/kg bw per day main-phase animals, the liver from control and 0.01 mg/kg bw per day interim-phase animals and all tissues from early-death animals in 0.01 mg/kg bw per day interim-phase and 0.01 and 0.03 mg/kg bw per day main-phase groups were processed and examined microscopically. Subsequently, the livers from 0.01, 0.03 and 0.1 mg/kg bw per day animals were processed to slides and examined microscopically.

There were no test article–related deaths in the study. Test article–related effects in this study were limited to histopathological findings of an increased incidence and severity of biliary hyperplasia and fibrosis in the liver in male main-study animals at and above 0.03 mg/kg bw per day, as compared with females or controls. These findings were assessed as non-adverse age-related spontaneous findings, the incidence or severity of which was accentuated by the test article, and there were no associated changes in the serum chemistry data.

The only changes of toxicological relevance in this 1-year study were the increased incidence and severity of biliary hyperplasia and fibrosis of the liver seen in males compared with controls or females at 0.03, 0.1 and 0.3 mg/kg bw per day. In the absence of corresponding elevations in hepatic enzymes indicative of hepatobiliary injury or increased liver weights, this finding was not considered to be adverse. Therefore, the NOAEL in this study was 0.3 mg/kg bw per day, the highest dose tested (Bonnette, 2009b).

Biliary hyperplasia occurs spontaneously in rats and has not been correlated with liver carcinogenicity or neoplasia. A statistical analysis of the biliary hyperplasia response in the two chronic rat studies with derquantel comparing any positive response in each dose group with the control response showed that the incidence of biliary hyperplasia was significantly elevated relative to controls only at the 50 mg/kg bw per day dose level. In addition, this dose level was associated with significant increases in liver weight and changes in GGT levels consistent with hepatotoxicity and is therefore a clear adverse effect level. There was no statistically detectable difference in the incidence of biliary hyperplasia seen at lower dose levels, nor were there significant increases in liver weights or changes in serum chemistry parameters indicative of hepatocellular injury.

Thus, 5 mg/kg bw per day would be considered the NOAEL on the basis of this analysis. This NOAEL is further supported by benchmark dose (BMD) modelling of the pooled data from both 1-year toxicity studies in the rat using various models. This modelling was performed by the sponsor and reviewed by the Committee. The lower bound of the one-sided 95% confidence interval for a benchmark dose representative of 10% extra risk compared with placebo (BMDL₁₀) derived from these models ranged from 2.0 to 7.6 mg/kg bw per day, which was in close agreement with the previously statistically verified NOAEL of 5 mg/kg bw per day (Lowney, 2011).

(b) Dogs

In a non-GLP-compliant, 28-day preliminary oral toxicity study, Beagle dogs 6–7 months of age (two of each sex per group) received derquantel (PF-520904, batch no. E01000242) as neat test article in gelatine capsules at a dose of 0, 1, 5 or 10 mg/kg bw per day. Control animals were dosed with an empty gelatine capsule. Feed was offered daily in the morning for approximately 2 hours, and water was provided ad libitum. Dose administration occurred after the feeding period. In addition to morning and afternoon checks for signs of morbidity or mortality, clinical observations were performed daily at 1, 2.5 and 4 hours following dosing. Detailed clinical evaluations were performed weekly. Body weights were measured prior to

treatment initiation, weekly thereafter and at study termination. Feed consumption was measured daily. Blood samples for haematology, biochemistry and coagulation evaluations were collected from fasted animals prior to dose initiation and on days 14 and 28. At study termination, animals were euthanized and necropsied, and adrenals, kidneys, heart and thyroids with parathyroids were collected, weighed, preserved and processed for microscopic evaluation.

All animals survived to study termination. Test article-related clinical signs were seen within 4 hours of dosing in all dogs during the first week of dosing, with a generally dose-related increase in incidence, and included dry conjunctiva, dry eyes, relaxed nictitating membrane, ptosis, red eyes, dilated pupils and dry mouth. The incidence of dry conjunctiva and dry eyes was lower in weeks 3 and week 4 (compared with the first 2 weeks of the study). These clinical signs were absent in 1 mg/kg bw per day males and females and 5 mg/kg bw per day females in week 4. Red eyes, dilated pupils and relaxed nictitating membrane were sporadically seen in males or females as a pre-dose observation weekly at all dose levels. Ptosis was seen sporadically as a pre-dose observation in 5 and 10 mg/kg bw per day females beginning in week 2 and in 10 mg/kg bw per day males during week 4. No other test article-related effects were observed.

The lowest-observed-adverse-effect level (LOAEL) for this study was 1 mg/kg bw per day, based on test article-related clinical observations seen at all dose levels (Savides, 2006).

In a GLP-compliant, 28-day oral toxicity study, 7.5- to 9-month-old Beagle dogs (four of each sex per group) received derquantel (PF-520904, batch no. 07VMDA010201A) by gavage at a dose of 0, 0.01, 0.03 (0.01 mg/kg bw per dose, 3 times per day), 0.03 or 0.1 mg/kg bw per day. The dose volume was 1 ml/kg bw. Control animals received the vehicle, 0.1% (w/v) carboxymethylcellulose. Owing to the absence of clinical signs at 0.03 mg/kg bw per day once per day and 3 times per day, dosing and data collection were suspended in the 3 times per day group after 7 days of dosing. Feed was provided *ad libitum* except during designated periods. Water was provided *ad libitum*. All animals were observed twice daily for signs of morbidity or mortality, and detailed clinical examinations were performed twice daily and following each functional observational battery evaluation. Functional observational battery evaluations were conducted prior to dose initiation, 1 hour post-dosing on day 1 and prior to dosing on days 2 and 28. Neurological examinations were scheduled at the same intervals, following the functional observational battery evaluation and 1 hour post-dosing on day 28. Body weights and feed consumption were measured prior to dose initiation and weekly thereafter. Ophthalmoscopic examinations were conducted at study termination. Electrocardiographic examinations were performed prior to dose initiation and pre-dosing and 1 hour post-dosing on days 2 and 27. Clinical pathology evaluations were performed on all animals pretest and on fasted animals at study termination. At study termination, animals were returned to stock to be used in a subsequent study with this test article.

All animals survived to study termination. There was no evidence of toxicity in any of the parameters evaluated.

Based on the absence of evidence of toxicity at all dose levels, the NOAEL was 0.1 mg/kg bw per day, the highest dose tested (May, 2009a).

In a GLP-compliant, 90-day oral toxicity study, Beagle dogs (four of each sex per group) that were approximately 6 months of age received neat derquantel (PF-520904, batch no. E010002421) by capsule at a dose of 0, 0.1, 0.5, 1 or 10 mg/kg bw per day. Control animals received an empty gelatine capsule. Feed was offered daily for approximately 1 hour in the morning. Dose administration occurred after the feeding period. Water was provided *ad libitum*. All animals were observed twice daily for signs of morbidity or mortality. Once dosing began, animals were observed for clinical signs daily pre-dosing, between 1 and 2 hours after dosing every day and 5 hours after dosing on Tuesdays and Fridays. Body weights were measured prior to dose initiation and weekly thereafter and at study termination. Feed consumption was measured daily. Ophthalmoscopic examinations were conducted prior to dose initiation, midway through the study and during the last week of dosing. Blood samples for evaluation of haematology, biochemistry and coagulation were collected from feed-fasted animals prior to dose initiation and on days 45 and 90. Samples for urine analysis were collected onto clean cage pans at the same intervals. At termination (day 92), animals were euthanized and necropsied, and adrenals, kidneys, liver, testes, thymus, heart, thyroid with parathyroids, brain, spleen and ovaries were collected and weighed. Sections of these as well as sections from a comprehensive assortment of other tissues and organs were prepared for histological examination. Microscopic examinations were performed on all animals and all tissues.

All animals survived to study termination. Test article-related clinical observations included dry mouth, dilated pupils, ptosis, dry conjunctiva, dry eyes, red eyes and relaxed nictitating membrane. Less commonly seen were vomit in the cage, salivation and abnormalities of faeces, and rarely seen were tremors and decreased activity. In general, these findings were first seen during the first week of dosing and occurred with a dose-related increase in numbers of animals affected, persistence of the finding post-dosing and number of days affected.

Dry mouth was observed in all test article-treated groups, with a dose-related increase in incidence. The incidence was highest 2 hours post-dosing; by 24 hours post-dosing, dry mouth was seen in only a few cases at 0.5 mg/kg bw per day and higher.

Dilated pupils were seen in all treated animals at both the 2- and 5-hour observations at one point or another in the study. The incidence increased with dose: at 0, 0.1, 0.5, 1 and 10 mg/kg bw per day, the average respective incidence was approximately 0%, 5%, 57%, 90% and 97% at 2 hours post-dosing and 8%, 10%, 37%, 65% and 98% at 5 hours post-dosing (on two occasions for each animal, mid-point and end of study, eyes were dilated with Mydriacil® for an ophthalmic examination; this accounts for some observations of dilated pupils). At 24 hours post-dosing, the pupils were still dilated, with incidences of 4%, 7% and 47% at 0.5, 1 and 10 mg/kg bw per day, respectively.

Ptosis was noted with a dose-related increase in incidence in all test article-treated groups at the 2- and 5-hour post-dosing observations. Ptosis was seen as

a single incident in one male and one female control animal. By 24 hours post-dosing, ptosis was present in all test article-treated groups (including the control group); however, the incidence in the 10 mg/kg bw per day group remained high, averaging about 20%.

Dry conjunctiva was seen occasionally 2 hours post-dosing in half of the 1 mg/kg bw per day animals and in all 10 mg/kg bw per day animals. The incidence of this finding decreased over time, with only one 10 mg/kg bw per day female seen with this finding 24 hours post-dosing.

Dry eyes were seen with increasing incidence at 0.5, 1 and 10 mg/kg bw per day. The highest incidence occurred at 10 mg/kg bw per day 2 and 5 hours post-dosing, then decreased over time, with one 0.5 mg/kg bw per day female and one male and one female in the 10 mg/kg bw per day group seen with this finding 24 hours post-dosing.

Red eyes were seen in one 0.1 mg/kg bw per day male, half of the 0.5 mg/kg bw per day animals and all 1 and 10 mg/kg bw per day animals 2 hours post-dosing. By 24 hours post-dosing, red eyes were seen in only two males and two females at 10 mg/kg bw per day.

Relaxed nictitating membrane was seen in all groups, including two males and one female in the control group. In test article-treated groups, this finding was seen at all dose levels at 2 and 5 hours post-dosing. Twenty-four hours post-dosing, the incidence of this finding at 1 and 10 mg/kg bw was still rather substantial (9% and 23%, respectively).

Tremors were noted sporadically in three of four 10 mg/kg bw per day females. Tremors were seen at 2 and 5 hours post-dosing and were not seen in any of the animals after week 8.

Test article-related decreases in mean body weight relative to controls occurred in males and females at 10 mg/kg bw per day. The decrease was associated with significantly ($P = 0.0023$) lower mean daily feed consumption.

Possible test article-related effects included significant decreases in kidney weight ($P = 0.0015$) and absolute and relative heart weights ($P < 0.002$) relative to controls at 10 mg/kg bw per day. There were no corresponding histopathological findings in these tissues.

On the basis of observed test article-related clinical signs at all dose levels, the LOAEL for this study is 0.1 mg/kg bw per day (Savides, 2008).

In a GLP-compliant, 90-day oral toxicity study, non-naïve Beagle dogs (four of each sex per group) that were 10.5–12 months old received derquantel (PF-520904, batch no. 07VMDA010201A) by gavage at a dose of 0, 0.1, 0.5, 1 or 5 mg/kg bw per day. Control animals were dosed with the vehicle, 1.0% (w/v) carboxymethylcellulose. The dose volume for all groups was 2 ml/kg bw. The animals used in this study were transferred from the May (2009a) study previously reported. The washout period was at least 6 weeks. Feed was available ad libitum except during designated periods, and water was available ad libitum. Animals were checked twice daily for signs of morbidity or mortality. Detailed clinical examinations

were performed twice daily during the dosing phase and at the end of each functional observational battery evaluation. Functional observational battery evaluations were performed on all animals once prior to dose initiation, 2 hours post-dosing on day 1, pre-dosing on days 2 and 28, again 2 hours post-dosing on day 59 and pre-dosing on day 90. A neurological examination was conducted on each animal following each functional observational battery evaluation. Ophthalmoscopic examinations were performed on all animals prior to dose initiation and prior to the terminal necropsy. Electrocardiographic examinations were performed prior to dose initiation and twice on days 2, 27, 58 and 89 (pre-dosing and 2 hours post-dosing). Indirect blood pressures were measured following each electrocardiographic examination. Blood samples for haematology, biochemistry and coagulation evaluations were collected from feed-fasted animals prior to dose initiation and immediately prior to terminal necropsy. Samples for urine analysis were collected via cystocentesis at terminal necropsy. Bone marrow smears were collected at necropsy and examined microscopically. At study termination, animals were euthanized and necropsied. Sections of organs that were collected and weighed as well as sections from a comprehensive assortment of other tissues and organs were prepared for histological examination. A complete list of tissues from control and 5 mg/kg bw per day animals was examined microscopically. Only liver and gross lesions collected from animals in the 0.1, 0.5 and 1 mg/kg bw per day groups were examined.

All animals survived to scheduled necropsy. Treatment-related clinical signs included protruding nictitating membrane, dilated pupils and red eyes.

Protruding nictitating membrane and dilated pupils were seen in all test article-treated groups, with a general dose-related increase in incidence and number of animals affected. Protruding nictitating membrane was seen in males and females (0/0, 1/1, 2/4, 4/3 and 4/4 males/females at 0, 0.1, 0.5, 1 and 5 mg/kg bw per day), which resolved prior to the next dose in all 0.1, 0.5 and 1 mg/kg bw per day males and females. Dilated pupils were seen in males and females (0/0, 1/1, 3/1, 4/4 and 4/4 males/females at 0, 0.1, 0.5, 1 and 5 mg/kg bw per day), which resolved prior to the next dose in all 0.1, 0.5 and 1 mg/kg bw per day females and in 0.1 and 1 mg/kg bw per day males.

Eye redness was seen in control animals, but the incidence and number of animals affected increased to levels suggesting a test article-related effect in males and females at 1 and 5 mg/kg bw per day. Eye redness resolved prior to the next dose in 5 mg/kg bw per day females.

In functional observational battery tests 2 hours post-dosing on days 1 and 59, decreased pupil response and increased pupil size were noted in males and females at 5 mg/kg bw per day, occasionally reaching statistical significance. Decreases in these same parameters were also noted in males at 1 mg/kg bw per day on day 1. Also at 2 hours post-dosing on days 1 and 59, ptosis scores were noted in one male and three or four females at 5 mg/kg bw per day. Scores for females were occasionally statistically significant. Ptosis was also noted in one female during the pre-dose functional observational battery on day 2.

Veterinary neurological examinations performed 2 hours post-dosing on days 1 and 59 found all males and two females at 5 mg/kg bw per day and one

male at 1 mg/kg bw per day with decreased or absent direct and indirect pupillary light reflex. Decreased indirect pupillary light reflex was also noted in one male at 0.1 mg/kg bw per day on day 59.

There were no test article-related findings in any of the other parameters evaluated. None of the test article-related findings were considered adverse, because there were no associated pathological changes observed.

Based on observations of protruding nictitating membranes, dilated pupils, eye redness and decreased indirect pupillary light response at all dose levels, the LOAEL for derquantel was 0.1 mg/kg bw per day (May, 2009b).

In an attempt to better define a point of departure for nictitating membrane protrusion in the dog, which was among the most sensitive effects seen in the dog, BMD modelling was performed. The BMDL₁₀ was calculated for a benchmark response of 10% incidence above the modelled background incidence. The USEPA's BMDS version 2.2 was used for modelling the dose-response relationship for this sensitive end-point in derquantel-treated male and female dogs using data from the two 90-day oral toxicity studies reviewed above.

The dose-response models were fitted to the dose-incidence data: gamma, logistic, logistic with background, log-logistic, multistage, multistage with background, probit, log-probit, Weibull, Weibull with background and quantal-linear models. The BMD and BMDL values for an extra 10% risk compared with the modelled background incidence (BMD₁₀ and BMDL₁₀) were estimated by performing 250 iterations.

The BMD₁₀ values from the accepted models ranged from 0.0112 to 0.0695 mg/kg bw per day, and the BMDL₁₀ values from 0.00015 to 0.0682 mg/kg bw per day. Most models presented BMD₁₀ and BMDL₁₀ values as 0.03 and 0.02 mg/kg bw per day, respectively, with good fitness (Akaike information criterion 62.543–62.787; *P*-value: 0.6026–0.6068).

However, considering the limited data available with which to model a dose-response relationship for elicitation of protrusion of the nictitating membrane in the dogs, the high degree of uncertainty at the low end of the dose-response curve and the fact that the two studies from which the data were derived differed in the age of dogs on study (juvenile versus adult) and mode of test article administration (capsule versus gavage), the Committee concluded that the BMDL₁₀ approach was not appropriate for the purpose of this evaluation.

2.2.3 Long-term studies of toxicity and carcinogenicity

No long-term studies of toxicity and carcinogenicity were performed.

2.2.4 Genotoxicity

Derquantel was tested for the potential to induce microbial gene mutations in six Ames *Salmonella typhimurium* tester strains and in *Escherichia coli* strain WP2uvrA. Derquantel was also tested in a chromosomal aberration assay in human lymphocytes. The in vitro microbial and mammalian cell gene assays were

Table 2. Results of genotoxicity assays with derquantel

Test system	Test object	Concentration	Results	References
In vitro				
Bacterial mutagen screen ^a	<i>Salmonella typhimurium</i> ^b TA97A, TA98, TA100, TA102	0–2500 µg/plate	Not mutagenic	Mayo (1998)
Ames reverse mutation assay ^{a,c}	<i>S. typhimurium</i> TA98, TA100, TA102, TA1535, TA1537	10–1000 ^d µg/plate	Not mutagenic	Brook (2005b)
Ames reverse mutation assay ^a	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 <i>Escherichia coli</i> WP2uvrA	1.6–5000 µg/plate	Not mutagenic	Farabaugh (2009)
Chromosomal aberration assay in human peripheral lymphocytes ^{a,e}	Adult human peripheral blood lymphocyte primary cell cultures	Fifteen concentrations from 25 to 1000 µg/ml ^f	Positive; chromosomal aberrations were induced without metabolic activation ^g	Mauthe (2005)
In vivo				
Micronuclei induction ^h	Mouse bone marrow	50, 100 and 200 mg/kg bw	Negative; not clastogenic	Brook (2005c)
Micronuclei induction ⁱ	Juvenile rat hepatocytes	25, 50, 100 and 200 mg/kg bw	Negative; no micronuclei formation	Coffing (2008)

^a With and without Aroclor 1254–induced rat liver S9 fraction.

^b Non-GLP Ames assay.

^c A positive control without metabolic activation was not included in the assay, and 2-aminoanthracene was used as the sole indicator of the efficacy of the S9 mix.

^d Maximum soluble dose.

^e Cyclophosphamide and mitomycin C were used as positive controls.

^f For 3 hours with or without metabolic activation and for 24 hours without metabolic activation.

^g Only seen at concentrations that produced at least 50% toxicity.

^h 9,10-Dimethylbenzanthracene was used as the positive control.

ⁱ Diethylnitrosamine was used as the positive control.

completed in the presence and absence of metabolic activation using S9 prepared from the livers of Aroclor 1254–induced rats to compare the direct genotoxic potential of derquantel and the genotoxic potential of any major derquantel metabolites. In vivo testing included screening for micronuclei induction in mouse bone marrow and juvenile rat hepatocytes.

The results of these genotoxicity assays are shown in Table 2.

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

(i) Rats

In a USEPA and OECD GLP-compliant, two-generation (one litter per generation) reproduction study, male and female Sprague-Dawley rats (30 of each sex per group) were dosed with derquantel (lot no. E010002940) by oral gavage at a dose of 0, 1, 5 or 25 mg/kg bw per day. Control animals received the vehicle, 1% aqueous carboxymethylcellulose. The dose volume was 10 ml/kg bw. Males were treated once daily beginning 70 days prior to cohabitation and continuing through the last day of cohabitation and then euthanized and necropsied. Sperm parameters were evaluated. Females were treated once daily beginning 70 days prior to cohabitation and continuing throughout gestation and 21 days postpartum and then euthanized and necropsied. Reproductive organs, including the pituitary gland, and gross lesions were also retained from all P₁ generation males and females for possible histological evaluation. Prior to the initiation of dose administration, 10 male and 10 female F₁ generation rats per dose group received a motor activity evaluation. The same rats received a functional observational battery and an additional motor evaluation on postpartum days 63–65. Feed and water were provided ad libitum. Rats were observed for signs of morbidity or mortality at least twice daily, and observations for treatment-related clinical signs were performed daily before dosing and approximately 2 hours post-dosing. Body weights were recorded at least weekly during gestation and on lactation days 1, 4, 7, 10, 14 and 21. Feed consumption was recorded weekly prior to cohabitation, on gestation days 0, 6, 10, 15, 18, 20 and 25 (non-pregnant rats) and on lactation days 1, 4, 7, 14 and 21. Clinical observations of pups were performed daily, and body weights were recorded on lactation days 1, 4, 7, 14 and 21.

Test article-related findings in P₁ males included significantly increased ($P \leq 0.05$ or $P \leq 0.01$) absolute and relative liver weights and thyroid follicular epithelial hypertrophy and hyperplasia at 25 mg/kg bw per day. Findings in P₁ females included a test article-related significant ($P \leq 0.05$) increase in the number of F₁ pups in the 25 mg/kg bw per day group observed with discoloration (purple, dark purple, green, grey and/or black) of the head, nose, whole body, both hindlimbs, both hindpaws, lower midline, tail or tip of tail. There were no other findings to verify that discoloration was treatment related or adverse. Postmortem findings in P₁ females included a significant ($P \leq 0.01$) increase in absolute and relative thyroid weights in all test article-treated groups compared with controls. This finding was considered adverse at 25 mg/kg bw per day, because it corresponded with microscopically observed thyroid follicular epithelial hypertrophy and hyperplasia. Terminal body weight of P₁ females was significantly ($P \leq 0.01$) increased at 25 mg/kg bw per day, as were absolute and relative mean liver weights and paired adrenals weight ($P \leq 0.05$ or $P \leq 0.01$). These findings were test article related. There were no test article-related macroscopic or microscopic changes observed in any of the reproductive tissues from P₁ males or females.

Test article–related findings in F_1 generation males included a significant increase in absolute and relative liver weights at 25 mg/kg bw per day. For F_1 females, test article–related findings were observed at 25 mg/kg bw per day and limited to significantly ($P \leq 0.01$) increased absolute and relative liver weights, significantly ($P \leq 0.05$ or $P \leq 0.01$) increased absolute terminal body weight with associated decrease in brain to body weight ratio, and increased absolute spleen weight. An increased incidence of thyroid follicular epithelial hyperplasia was observed in F_1 males and females at 25 mg/kg bw per day. Thyroid follicular hyperplasia was also observed in the 0, 1 and 5 mg/kg bw per day groups but was not test article related, based on lower incidence or severity compared with 25 mg/kg bw per day.

There were no test article–related findings in reproductive or developmental parameters.

A NOAEL of 5 mg/kg bw per day was established for males, based on histopathological findings of thyroid follicular epithelial hyperplasia at 25 mg/kg bw per day. For females, a NOAEL of 1 mg/kg bw per day was established, based on significant increases in thyroid weights of P_1 female rats. The NOAEL for reproductive effects was 25 mg/kg bw per day, the highest dose tested (Barnett, 2009).

(b) *Developmental toxicity*

(i) *Rats*

A GLP-compliant dose range–finding study for developmental toxicity was conducted to determine doses for the subsequent main study in rats. Derquantel (lot no. 5789-MAM-9901) suspended in 1% aqueous carboxymethylcellulose was administered orally by gavage to pregnant Sprague-Dawley rats (seven per group) at a dose of 0 (vehicle), 2, 10, 50 or 100 mg/kg bw per day on gestation days 5–20, at a dose volume of approximately 10 ml/kg bw. Feed and water were provided ad libitum. Body weights and feed consumption were determined every 3 days throughout the study. The animals were observed daily at approximately the same time each day between 1 and 4 hours post-dosing. On day 21, rats were euthanized and caesarean sectioned. A gross necropsy of the thoracic and abdominal viscera was performed. The uteri were removed to ascertain the pregnancy status. The number of corpora lutea on the ovaries was recorded. The fetuses were removed, weighed and examined for sex, live or dead status, and early or late resorptions.

No mortalities occurred during the study. Clinical signs observed only at 100 mg/kg bw per day included subdued behaviour, tilted posture and laboured breathing. Porphyrin staining around the nose was seen in all groups, including controls (with an incidence of 2/7, 1/7, 3/7, 2/7 and 7/7 animals per group, respectively). Decreased body weight gain and lower average total feed intake were observed at 100 mg/kg bw per day when compared with the vehicle control group. A lower mean number of corpora lutea in the 100 mg/kg bw per day group compared with the control group (11.4 versus 15.0) corresponded with the mean number of live fetuses found in the uteri of these two groups (10.7 versus 14.2). The mean number of female fetuses in the 100 mg/kg bw per day group was about half that of the vehicle control group. This difference between the 100 mg/kg bw per

day and the control groups was reflected in the mean litter weights. Based on the findings in this preliminary study, doses selected for the main study were 20, 70 and 120 mg/kg bw per day (Henderson, 2006).

In the definitive, GLP-compliant study, derquantel (lot no. 5789-MAM-9901) suspended in 1% aqueous carboxymethylcellulose was administered orally by gavage to pregnant Sprague-Dawley rats (23 per group) at a dose of 0 (vehicle), 20, 70 or 120 mg/kg bw per day on gestation days 5 through 20, at a dose volume of approximately 10 ml/kg bw. Feed and water were provided ad libitum. Body weights and feed consumption were determined every 3 days throughout the study. The animals were observed daily at approximately the same time each day between 1 and 4 hours post-dosing. On day 21, rats were euthanized and caesarean sectioned. A gross necropsy of the thoracic and abdominal viscera was performed. The uteri were removed to ascertain the pregnancy status. The number of corpora lutea on each ovary was recorded. The fetuses were removed, weighed and examined for sex, live or dead status, early or late resorptions, gross external alterations and fetus allocation. Soft tissue and skeletal examinations were performed.

Two mortalities occurred during the experimental period, but neither was attributable to treatment with the test article.

No signs of maternal toxicity were observed at the lowest dose of 20 mg/kg bw per day. Test article-related increased incidences of porphyrin discharge and piloerection were seen at 70 and 120 mg/kg bw per day. At the highest dose of 120 mg/kg bw per day, test article-related signs of toxicity included increased porphyrin, subdued behaviour, laboured breathing, thin appearance, piloerection and a significant ($P < 0.05$) reduction in body weight gain correlating with significantly ($P < 0.05$) reduced feed intake. There were no biological differences in the number of corpora lutea, number of implantation sites, number of live fetuses and percentage of resorptions between treated groups and the vehicle control group. A lower trend in uterus weight was observed in dams in the 120 mg/kg bw per day group. The number and distribution of fetuses were not affected by treatment. Differences in sex ratio were considered to be of no biological significance. Male fetus weight in the 120 mg/kg bw per day group was found to be significantly ($P < 0.05$) lower than that of vehicle control group animals. Ossification retardation was observed in fetuses in the 120 mg/kg bw per day group, which may be due to nonspecific inhibition or retardation of physiological growth due to the effects of maternal toxicity at this dose. Skeletal development was related to the reduced body weight of the fetuses. No soft tissue abnormalities were identified in any treatment group.

Based on the results of this study, derquantel was not teratogenic. The NOAEL for maternal toxicity was 20 mg/kg bw per day, and the NOAEL for developmental toxicity was 70 mg/kg bw per day (Rozinova, 2006).

(ii) Rabbits

A GLP-compliant dose range-finding study for developmental toxicity was conducted to determine doses for the subsequent main study in rabbits. Derquantel (lot no. E010002720) suspended in 1% aqueous carboxymethylcellulose was administered orally by gavage to pregnant New Zealand White rabbits (five per

group) at a dose of 0 (vehicle), 0.1, 1, 10 or 100 mg/kg bw per day on gestation days 7 through 19, at a dose volume of 5 ml/kg bw. Owing to severe signs of test article–related toxicity and mortality at 100 mg/kg bw per day, this group was terminated on gestation day 10. Approximately 150 g feed per rabbit per day was offered until the first day of dosing, at which time the daily feed allotment was increased to 180–185 g feed per rabbit per day. Water was available ad libitum. Animals were observed for clinical signs daily at approximately 1-hour intervals post-dosing for 4 consecutive hours and once at the end of the day. Daily observations were performed on non-dosing days. Body weight and feed consumption were measured daily. On gestation day 29, surviving rabbits were euthanized and caesarean sectioned. Examinations of the number and distribution of corpora lutea, implantation sites and uterine contents and a gross necropsy of the thoracic, abdominal and pelvic viscera were performed. Fetuses were weighed and examined for gross external alterations and sex.

There were no test article–related deaths at doses below 100 mg/kg bw per day.

A test article–related reduction in body weight gain was observed in the 1 mg/kg bw per day group during the post-dosing phase (gestation days 20 through 29), which corresponded with lower feed consumption compared with controls. At 10 mg/kg bw per day, body weight gains and feed consumption relative to the control group were reduced during the dosing period. During the post-dosing phase, body weight gains and feed consumption rebounded, but body weight gain remained reduced for the entire gestation period.

There were no abnormal findings in the caesarean section or fetal examination data. Based on the results of this study, doses of 0 (vehicle), 0.1, 1 and 10 mg/kg bw per day were recommended for the definitive study of developmental toxicity in rabbits (Lewis, 2006).

In the definitive GLP-compliant study, derquantel (lot no. E010002720) suspended in 1% aqueous carboxymethylcellulose was administered orally by gavage to pregnant New Zealand White rabbits (20 per group) at a dose of 0 (vehicle), 0.1, 1 or 10 mg/kg bw per day on gestation days 7 through 19 at a dose volume of 5 ml/kg bw. Approximately 150 g feed per rabbit per day was offered until the first day of dosing, at which time the daily feed allotment was increased to 180–185 g feed per rabbit per day. Water was available ad libitum. Animals were observed for clinical signs daily at approximately 1-hour intervals post-dosing for 4 consecutive hours and once at the end of the day. Daily observations were performed on non-dosing days. Body weight and feed consumption were measured daily. On gestation day 29, surviving rabbits were euthanized and caesarean sectioned. The number and distribution of corpora lutea were recorded. The uterus of each rabbit was excised and examined for pregnancy, number and distribution of implantation sites, live and dead fetuses and early and late resorptions. Placentae were examined for size, colour and shape. Fetuses were weighed and examined for gross external alterations and sex. Live fetuses were euthanized, and soft tissue and skeletal examinations were performed.

There were no abortions or premature deliveries. One rabbit in the 10 mg/kg bw per day group was found dead on day 10 of gestation. Although the cause of this death was not determined, it was presumed that it was test article related.

Test article-related clinical signs in does were limited to scant faeces at 10 mg/kg bw per day, the incidence of which was significantly ($P \leq 0.01$) increased relative to controls and correlated with low feed consumption in this dose group.

Test article-related effects on body weight of does were seen at 10 mg/kg bw per day and consisted of significant ($P \leq 0.01$) body weight loss relative to controls during the dosing phase and reduced to significantly reduced ($P \leq 0.05$) body weight gains between gestation days 13 and 20. Reduced body weight gain and body weight losses in the 10 mg/kg bw per day group were associated with decreases in absolute and relative feed consumption.

Increased post-implantation loss due to an increase in total resorptions was a test article-related effect seen at 10 mg/kg bw per day. One doe in this group had a litter that consisted of all resorbed conceptuses. Fetal body weights in this group were also reduced to significantly reduced ($P \leq 0.05$) relative to controls. These findings were secondary effects resulting from maternal toxicity.

Developmental toxicity was seen at 10 mg/kg bw per day and consisted of significant increases ($P \leq 0.01$) in the incidence of supernumerary thoracic ribs with associated significant increases and decreases ($P \leq 0.01$) in the numbers of thoracic and lumbar vertebrae, respectively. This variation is commonly seen at maternally toxic doses. Decreases in fetal ossification sites are indicative of a delay in development, which corresponds with the decreased fetal weights observed in this group.

Based on these findings, the NOAEL for developmental toxicity was 1 mg/kg bw per day (Lewis, 2007).

2.2.6 Special studies

(a) Structural toxicity alerts for derquantel and related impurities

An assessment of the chemical structure of derquantel and related impurities paraherquamide, *N*-methyl-2-desoxyparaherquamide, PF-03334456, PF-03198957, 2-desoxyparaherquamide E and *N*-oxide for potential structural alerts for genotoxicity or carcinogenicity was conducted using the computer program DEREK (Deductive Estimation of Risk from Existing Knowledge) (Vogel, 2005; Kenyon, 2009). No structural alerts were identified for derquantel or any of the related compounds tested (Kenyon, 2009).

(b) Impurities

2-Desoxyparaherquamide E is an impurity that can occur during the synthetic conversion of the paraherquamide E fermentation impurity in paraherquamide to the derquantel moiety equivalent. It has been present at variable concentrations up to 1.4% in development batches used in toxicity studies. An acceptance criterion of not more than 1% is proposed for the active pharmaceutical ingredient. 2-Desoxyparaherquamide E was tested for the potential to induce microbial gene

Table 3. Results of a genotoxicity assay with 2-desoxy paraherquamide E

Test system	Test object	Concentration	Results	Reference
In vitro				
Ames reverse mutation assay ^a	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537 <i>Escherichia coli</i> WP2uvrA	6.67–5000 µg/plate	Not mutagenic	Mecchi (2009)

^a With and without Aroclor 1254–induced rat liver S9 fraction.

mutations in four Ames tester strains and in *Escherichia coli* strain WP2uvrA. The results are shown in Table 3.

(c) Microbiological effects

A JECFA decision-tree approach that was adopted at the sixty-sixth meeting of the Committee (Annex 1, reference 181) and that complies with International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) Guideline 36 (GL36) was used by the Committee to determine the need to establish a microbiological ADI for derquantel. The decision-tree approach initially seeks to determine if there may be microbiologically active derquantel residues entering the human colon.

Derquantel is not classified as an antimicrobial agent and is not structurally related to antimicrobial agents used in animal or human medicine. The Committee considered an in vitro evaluation of derquantel against 62 strains representing a wide range of Gram-positive and Gram-negative bacteria of veterinary importance. Derquantel showed limited strain-dependent activity against the staphylococci (minimum inhibitory concentrations [MICs] ranging from 64 to 128 µg/ml for all strains). Against the streptococci, derquantel showed very limited activity (MICs ≥128 µg/ml). Against *Pasteurella* spp., derquantel showed limited activity (MICs ranging from 32 to >128 µg/ml). Derquantel was not active against the enteric organisms tested (Salmon, Case & Watts, 1998).

The Committee concluded that a microbiological ADI for derquantel was not necessary.

2.3 Toxicological studies with derquantel/abamectin (Startect)

A summary of these studies is included to assess the toxicity of derquantel when used in combination with abamectin as an anthelmintic drug combination product for veterinary use. The formulation consists of derquantel at 10 mg/ml and abamectin at 1 mg/ml.

2.3.1 Acute toxicity

Single-dose toxicity studies with the derquantel/abamectin combination are summarized in Table 4.

Table 4. Acute toxicity studies with derquantel/abamectin

Species	No. of each sex per group	Route	Dose	LD ₅₀	Reference
Rat	7F	Oral	55/5.5, 100/10, 175/17.5, 200/20 mg/kg bw	88.72/8.872 mg/kg bw	Lloyd (2008d)
Rat	5M, 6F	Dermal	30/3 mg/kg bw	>30/3 ^a mg/kg bw	Lloyd (2008e)
Rat	5M, 5F	Inhalation	5.04 mg/l ^b for 4 h	>5.04 mg/l ^c	Rodabaugh (2008)

F, female; LD₅₀, median lethal dose; M, male

^a No dermal toxicity observed.

^b Calculated dose from this exposure was 0.05 mg/l derquantel and 0.005 mg/l abamectin.

^c LC₅₀. No clinical signs of toxicity were observed.

2.3.2 Short-term studies of toxicity

(a) Rats

In a non-GLP-compliant, 7-day oral dose range-finding study, rats approximately 6 weeks of age (four of each sex per group) were given derquantel/abamectin (batch no. 071027-1) by gavage at a dose of 0/0, 1/0.1, 2/0.2, 10/1 or 20/2 mg/kg bw per day. Control animals were dosed with the vehicle, 0.05% (w/v) butylated hydroxytoluene, 40% (w/v) triacetin and Miglyol 840. The dose volume given to all groups was 5 ml/kg bw. Feed and water were provided ad libitum. Animals were checked twice daily for signs of morbidity or mortality. Detailed clinical examinations were performed twice daily pre-dosing and 2–3 hours post-dosing. Body weights were measured prior to dose initiation and at termination. Feed consumption was measured once (weekly). At study termination, animals were euthanized and necropsied. Fourteen organs were collected, weighed and preserved, and a comprehensive assortment of other tissues and organs was collected and preserved.

All animals survived to scheduled necropsy. Clinical signs of salivation were observed at 20/2 mg derquantel/abamectin per kilogram body weight per day in a single male and three of four females on days 6 and 7. This observation was treatment related but not considered adverse. No other clinical signs were attributable to treatment with the test article, and there were no effects on body weight or feed consumption.

Mean absolute and relative liver weights were increased in 20/2 mg derquantel/abamectin per kilogram body weight per day females relative to controls. The difference for the mean relative value was statistically significant. There were no associated histopathological findings. Cytoplasmic clearing of hepatocytes without vacuolation was noted in males and females in all groups, except control females. This finding is consistent with glycogen accumulation and was likely a vehicle effect.

On the basis of an increased incidence of salivation seen in males and females and increased liver weight without corresponding increases in biochemical markers associated with hepatocellular injury or microscopic evidence of toxicity in females, the NOAEL was 20/2 mg derquantel/abamectin per kilogram body weight per day, the highest dose tested (May, 2009d).

In a GLP-compliant, 28-day oral toxicity study, rats approximately 8 weeks of age (15 of each sex per group) were given derquantel/abamectin (batch no. 080523-1) by gavage at a dose of 0/0, 3/0.3, 20/2 or 30/3 mg/kg bw per day, at dose volumes of 3, 1, 2 and 3 ml/kg bw, respectively. Control animals received placebo 16957A, which consisted of 0.05% (w/v) butylated hydroxytoluene, 5% (w/v) glycerol formal, 40% (w/v) triacetin and approximately 54.95% (w/v) Miglyol 840. The 3/0.3 mg derquantel/abamectin per kilogram body weight per day dose was prepared by diluting a 10/1 stock solution with placebo 16957A. Undiluted test article was used for the higher dose levels. Owing to early deaths, the 20/2 mg/kg bw per day dose level was lowered to 10/1 mg/kg bw per day between days 1 and 3, and the 30/3 mg/kg bw per day dose was lowered to 20/2 mg/kg bw per day on day 4. Dose volumes were respectively lowered to 1 and 2 ml/kg bw. Feed was available ad libitum, except during designated periods. Water was available ad libitum. All animals were checked twice daily for signs of morbidity or mortality. Detailed clinical examinations were performed during the acclimation period and 4 times daily (pre-dosing and 1, 4 and 6 hours post-dosing). The 6-hour post-dosing observation was added on day 4. Functional observational battery evaluations were conducted prior to dose initiation, 2 hours post-dosing on day 1, prior to dosing on day 2, 4 hours post-dosing on day 21 and prior to dosing on day 28. Locomotor activity evaluations were conducted on five designated main-study animals of each sex post-dosing on day 1 and pre-dosing on day 28. Body weights were measured prior to dose initiation and weekly during the study. Feed consumption was recorded weekly. Ophthalmic examinations were conducted on all animals prior to dose initiation and just prior to terminal necropsy. Samples for clinical pathology evaluations were collected just prior to terminal necropsy. Blood was collected from feed-fasted animals, and urine was collected while animals were housed in metabolism cages. Bone marrow smears were collected at necropsy and examined microscopically. At study termination, animals were euthanized and necropsied. Fourteen organs were collected and weighed. Sections of these as well as sections from a comprehensive assortment of other tissues and organs were prepared for microscopic examination. All tissues from the control and 20/2 mg/kg bw per day groups and gross lesions or tissue masses from all groups were examined microscopically. In addition, the adrenals, liver, pituitary, thyroid gland and vagina were identified as target organs and were examined from the 3/0.3 and 10/1 mg/kg bw per day groups. A peer review of the histopathological findings was also conducted.

There were three deaths during the study. On day 3, a 30/3 mg/kg bw per day female was euthanized owing to severe signs of test article-related toxicity. On day 5, a 20/2 mg/kg bw per day female was euthanized owing to severe signs of test article-related toxicity after having received three 30/3 mg/kg bw and two 20/2 mg/kg bw dose administrations. On day 7, a 10/1 mg/kg bw per day female was euthanized as a result of a malocclusion of the incisors and associated loss of ability to eat. The malocclusion was assessed as being caused by the animal

biting the wire of the cage bottom during post-dosing tremors observed before the dose level was lowered from 20/2 to 10/1 mg/kg bw per day. Therefore, this animal's death was not directly related to treatment with the test article.

Test article–related clinical signs in animals given 30/3 and 20/2 mg/kg bw per day were decreased activity, ataxia, difficult and shallow breathing, head tilt, prostration, tremors, vocalization, hunched posture, partially/completely closed eyes, piloerection, salivation, and thin and unkempt appearance. The majority of these signs were associated with 30/3 mg/kg bw per day dose administrations. In animals given 10/1 mg/kg bw per day, clinical signs were tremors, piloerection, abnormal respiration, salivation, and thin and unkempt appearance, all of which were noted in a limited number of animals. Salivation was the only abnormal clinical sign at 3/0.3 mg/kg bw per day; however, the incidence was comparable to that in controls and therefore was not a test article–related effect. Salivation was noted as a test article–related effect in males and females at 20/2 mg/kg bw per day based on the markedly increased numbers of animals affected compared with lower-dose groups and controls.

Increases in mean forelimb and hindlimb grip strength were noted in 20/2 and 10/1 mg/kg bw per day males and females on days 21 and 28. The effect was test article related but not considered adverse, because the differences were of low magnitude and only sometimes statistically significant.

Tail pinch response was significantly decreased in 20/2 mg/kg bw per day females on day 28. This finding was test article related.

On day 28, rearing counts were decreased compared with controls for males and females at all dose levels. Differences (0–240 minutes) were significant ($P < 0.01$) for males in all test article–treated groups and females at 20/2 and 10/1 mg/kg bw per day. The majority of statistically significant decreases were noted at measurement intervals during the first 90 minutes of monitoring and were not associated with decreases in any other dimension of locomotor activity evaluated. Mean rearing counts decreased with a dose-related response in males, but not females. Based on the magnitude of the decreases noted, this effect was test article related at all dose levels, but not considered adverse, as the finding was not associated with adverse effects on the overall health or well-being of the animals.

Test article–related effects on body weight included significant decreases relative to controls in mean body weight for 20/2 mg/kg bw per day males at weeks 2 ($P < 0.01$), 3 ($P < 0.05$) and 5 ($P < 0.05$). Mean body weight values for females were slightly lower ($\leq 5\%$) compared with controls at these intervals. The lower value for week 2 was associated with significantly lower mean feed consumption for males and females in this group during week 1. Mean feed consumption during subsequent weekly intervals was comparable to that of controls, indicating a direct, adverse test article–related effect on body weight gain in males and females at 20/2 mg/kg bw per day.

There were no test article–related effects in the clinical pathology data.

Test article–related changes in organ weights included increased mean liver weights (absolute and relative) in males and females at 10/1 and 20/2 mg/kg bw per

day; increased adrenal weights (absolute and relative) in males at 10/1 and 20/2 mg/kg bw per day; and increased thyroid weights (absolute and relative) in females at 10/1 and 20/2 mg/kg bw per day. In addition, there was a significant increase in relative pituitary weight in males at 20/2 mg/kg bw per day.

Test article-related histopathology findings included minimal centrilobular hepatocellular hypertrophy and minimal follicular cell hypertrophy in the thyroid gland in females at 20/2 mg/kg bw per day. Both of these findings were adverse because they correlated with increases in respective organ weights. Minimal diffuse cortical hypertrophy was observed in adrenal glands of males and females at 10/1 and 20/2 mg/kg bw per day and corresponded with significant adrenal weight changes in males. Based on the absence of a dose-response relationship for adrenal weight changes in males and females relative to controls, the adrenal gland cortical hypertrophy observed is of uncertain toxicological relevance. Minimal to mild cellular hypertrophy of cells in the pars distalis of the pituitary gland in 10/1 and 20/2 mg/kg bw per day males was an adverse finding that correlated with increased relative pituitary weights.

The NOAEL for this study was 3/0.3 mg derquantel/abamectin per kilogram body weight per day. This is based on signs of neurotoxicity and adverse effects on body weight of males and females and hepatotoxicity, thyroid toxicity and mortality observed in females at the higher dose levels (May, 2009e).

2.4 Observations in humans

No data were available, as derquantel is not a drug used in human medicine, and there were no reports of accidental human exposures submitted or identified in the literature search.

3. COMMENTS

The Committee considered results of studies on receptor pharmacology, pharmacokinetics, acute and short-term toxicity, in vitro, in vivo and in silico genotoxicity, reproductive toxicity and microbiological safety. The majority of the studies were performed in accordance with GLP standards. In addition, a literature search in Embase database was performed. No additional information relevant to this evaluation was identified.

3.1 Biochemical data

In rats dosed orally with derquantel at 200 mg/kg bw per day for 4 days, peak plasma concentrations (C_{max}) of approximately 20 µg/ml occurred at 2 hours post-dosing (T_{max}). When rats were administered daily oral doses of derquantel ranging from 0.01 to 150 mg/kg bw per day for a longer duration (90 days, 1 year or multigeneration), the mean concentration of derquantel in plasma showed a dose-dependent increase, with no accumulation. T_{max} was mainly 2 hours (ranging from 0.5 to 2 hours), according to the dose administered. Exposure, as measured by AUC and C_{max} , was consistently higher in females than in males.

In rats dosed once daily by oral gavage for 7 days with the combination product containing a 10:1 ratio of derquantel and abamectin at doses up to

20/2 mg/kg bw per day, the plasma concentration of derquantel and abamectin increased with increasing doses of the combination product. Derquantel appeared not to accumulate, whereas abamectin showed accumulation. $T_{\max}$ was 2 hours post-dosing for derquantel and 6 hours post-dosing for abamectin.

When pregnant rabbits were given derquantel orally by stomach tube once daily on days 7 through 19 of gestation at doses up to 10 mg/kg bw per day, the $AUC_{0-t(\text{last})}$ and $C_{\max}$ values increased with increasing dose, and $T_{\max}$ was consistently 0.5 hour post-dosing.

When dogs were given daily oral doses of derquantel of up to 10 mg/kg bw per day for 28 days, the AUC_{0-24} and $C_{\max}$ values increased at a rate greater than proportional to increasing dose. Accumulation was found on day 27 only at 10 mg/kg bw per day. $T_{\max}$ ranged from 0.5 to 2 hours, and the elimination half-life was 2–6 hours.

In two studies, derquantel was given to dogs as a single oral dose via capsule at dose levels up to 10 mg/kg bw per day or by oral gavage at dose levels up to 5 mg/kg bw per day for 3 months. The $C_{\max}$ values for derquantel indicated no accumulation over time for doses of 0.1, 0.5, 1 and 5 mg/kg bw per day. Accumulation was seen at 10 mg/kg bw per day. $T_{\max}$ was generally between 0.5 and 2 hours in the three lowest dose groups, but ranged from 1 to 9 hours at 5 and 10 mg/kg bw per day. Half-lives generally increased with dose; however, all were relatively short, less than 7 hours.

One male and one female horse were given a single oral dose of derquantel at 2 mg/kg bw. Maximum plasma concentrations occurred at 1 hour post-dosing, with $C_{\max}$ values of 0.157 µg/ml and 0.075 µg/ml and AUC values of 0.48 µg·h/ml and 0.27 µg·h/ml for the male and female, respectively. Additionally, a mono-hydroxylated metabolite of derquantel, previously not seen in other species, was identified at higher apparent plasma concentrations than for the parent derquantel.

Derquantel (1 µmol/l) was metabolized extensively in dog liver microsomes, with only 2.8% of parent detected after a 1-hour incubation. In rat, sheep and human liver microsomes, derquantel was metabolized moderately, with approximately half of the derquantel unchanged. Among 18 metabolites occurring in microsomal cultures, M3, M4, M5a and M5b were predominant in dog liver microsomes, whereas M1, M9 and M10 were found as predominant metabolites in rat, sheep and human liver microsomes. The M8 metabolite was another predominant metabolite in rats.

Hepatocytes from rat, sheep, dog and human metabolized 49–81% of derquantel (1 µmol/l) over a 4-hour incubation. Unchanged derquantel and 26 radioactive metabolites were detected in rat, sheep, dog and/or human hepatic incubations. Hepatocytes from rats, dogs, sheep and humans exhibited similar patterns of metabolites, as M1, M8, M10, M12 and M19/20 commonly occurred as predominant metabolites, with variations in the relative levels of each. Also, M18 was a prominent metabolite, accounting for 8% of the total in rat and human hepatocytes. M15 was found as a predominant metabolite only in dogs, comprising 11% of the total.

Rats (one of each sex) were given a single oral dose of [^{14}C]derquantel at 100 mg/kg bw (14.8 MBq/kg bw). Most of the radiolabel (85% and 67% for the male and

female, respectively) was recovered in the faeces in the first 2 days. Approximately 95% of the radioactive dose was recovered in urine and faeces during the 7-day post-dosing period, with 87–90% of the dose in the faeces and 5.5–7.6% in the urine. Negligible amounts of the dose were detected in the carcass and terminal blood samples. The formation of approximately 11 metabolites was observed during 7 days post-dosing. The molecular weights of some of the metabolites indicated metabolism by glucuronidation and hydroxylation.

When cannulated rats were dosed orally with [^{14}C]derquantel at 50 mg/kg bw to identify metabolite profiles in urine, faeces, bile and tissues, the majority (69–75%) of the radioactive dose was recovered in faeces, and small amounts (3–4% and 1%, respectively) were recovered in urine and tissues. The majority of the radioactive dose (64–70%) was excreted in bile. The results showed that derquantel has high oral bioavailability. Large numbers of metabolites were found in urine, faeces and bile, indicating a high degree of biotransformation in the rat. Metabolic profiles in males and females were similar, although the rate of metabolism was more rapid in males than in females. Faeces contained mainly the parent drug at the 0- to 24-hour collection interval, whereas only small amounts of parent derquantel along with a large number of metabolites were found at the 144- to 150-hour collection interval. Parent derquantel and metabolites M1, M2, M4 and M5 are the most common components found in the tissues. In rat liver, derquantel comprised 6–8% of the total radioactivity. In rat muscle and fat, derquantel comprised higher percentages of the total residues in tissues of females (21% and 53%, respectively) relative to the males (5% and 31%, respectively).

3.2 Toxicological data

The acute oral toxicity of derquantel varies between species. Derquantel was extremely toxic to horses, but showed low toxicity in rats ($\text{LD}_{50} > 2000$ mg/kg bw). Clinical signs of acute toxicity in mice, rats and dogs were attributable to the pharmacological activity of derquantel as an antagonist of nAChR. Whether higher sensitivity in horses was due to a toxic metabolite or greater sensitivity to the pharmacological activity of derquantel could not be determined from the data. The primary clinical signs of acute toxicity included various effects on the nervous system, such as prostration, seizures, hypoactivity and ataxia, and various ocular effects, including mydriasis, ptosis and relaxation of the nictitating membrane. Derquantel was not a skin sensitizer in guinea-pigs or a skin or ocular irritant in rabbits.

In a non-GLP-compliant, 3-day escalating oral dose range-finding study, 3-month-old rats (two of each sex per group) received derquantel via gastric intubation at a dose of 0, 25, 50, 100, 200, 300, 400 or 600 mg/kg bw per day. Dose volumes increased with dose up to a maximum volume of 12 ml/kg bw for the 600 mg/kg bw per day group. Because of clinical signs of excessive toxicity, dosing of the 400 mg/kg bw per day group was interrupted, and the animals were allowed a 2-week washout period. Dosing then resumed at a dose level of 300 mg/kg bw per day for 2 days. The 600 mg/kg bw per day dose was given only once due to excessive toxicity. Clinical signs of toxicity included death, hypoactivity, loss of righting reflex, ataxia, prostration, jerky movements, abnormal posture, laboured respiration, tremors and ptosis. There were no clinical signs of toxicity at doses up

to and including 300 mg/kg bw per day. There was no microscopic examination of tissues. The NOAEL for this study was 300 mg/kg bw per day.

In a 12-day oral dose range-finding study, 6- to 8-week-old rats (two of each sex per group) received derquantel via gastric intubation at a dose of 0, 30, 50, 100, 150, 200, 250, 400, 600 or 800 mg/kg bw per day. Clinical signs of toxicity included death (≥ 250 mg/kg bw per day), prostration, paralysis, convulsions, jerky movements, ataxia, hypoactivity, hunched or tilted posture, piloerection, porphyrin staining around the nose and excessive salivation. No test article-related clinical signs or effects on body weight were seen up to and including 150 mg/kg bw per day. Test article-related macroscopic findings included a dose-related increase in mean liver weight of males and females in all test article-treated groups. Microscopic examinations were limited to livers of animals in the 30 and 150 mg/kg bw per day groups. Test article-related microscopic findings included periportal hepatocyte swelling and hepatic cytoplasmic vacuolation with a few scattered necrotic hepatocytes at 150 mg/kg bw per day. There were no microscopic findings in the liver to correspond with increased liver weight at 30 mg/kg bw per day. Based on a slight increase in mean liver weight with no associated histopathological findings, the NOAEL for this study was 30 mg/kg bw per day.

In a 28-day oral toxicity study, 6- to 8-week-old rats received derquantel via gastric intubation at a dose of 0, 20, 100 or 200 mg/kg bw per day. Clinical signs of toxicity included death (200 mg/kg bw per day), ataxia, prostration, convulsions, hypoactivity, piloerection, laboured breathing, paralysis, tilted posture, jerky movements, tremors, splayed hindlimbs and porphyrin staining around the nose. Decreases in body weight gain and feed consumption occurred in males and females at 200 mg/kg bw per day. Mean relative liver weights were significantly increased in males and females in the 100 and 200 mg/kg bw per day groups. Elevated serum GGT levels were seen in males and females at 200 mg/kg bw per day. Microscopically, bile duct hyperplasia of minimal severity was seen in males and females at 20 mg/kg bw per day. The severity of this finding increased to minimal to mild in males and females at 100 mg/kg bw per day and to minimal to severe in males and females at 200 mg/kg bw per day. Based on the significant increases in mean liver weights at 100 and 200 mg/kg bw per day with an associated dose-related increase in the incidence and severity of bile duct hyperplasia and elevated serum GGT levels and mortality at 200 mg/kg bw, the NOAEL for this study was 20 mg/kg bw per day.

In a 28-day oral toxicity study, 7- to 8-week-old rats received derquantel enriched with 4% *N*-oxide, a degradation product of derquantel seen during accelerated stability testing of the derquantel/abamectin drug product. Treatment was given by gastric intubation at dose levels of 0, 0.5, 5, 25 and 175 mg/kg bw per day. As a result of excessive toxicity and deaths in females, the 175 mg/kg bw per day dose level was lowered to 150 mg/kg bw per day in females only beginning on day 9. Significantly lower rearing counts were seen in females at 5 mg/kg bw per day and in males and females at 25 and 175/150 mg/kg bw per day. Additional treatment-related effects included significantly increased liver weight and hepatocellular hypertrophy at 25 mg/kg bw per day and elevations of serum GGT, ALT and sorbitol dehydrogenase enzymes in males and females and significantly

increased thyroid weight and follicular cell hypertrophy in females at 175/150 mg/kg bw per day. Based on lower rearing counts in females at 5 mg/kg bw per day and higher, the NOAEL for this study was 0.5 mg/kg bw per day.

In a 90-day oral toxicity study, rats less than 9 weeks of age received derquantel by gastric intubation at a dose of 0, 1, 5, 50 or 150 mg/kg bw per day. The quality and reliability of the histopathology data were limited due to the poor quality of the slides. There were also concerns about whether the timing of the clinical observations was adequate to capture the potential neurobehavioural effects of derquantel. As a result, the Committee could not determine a NOAEL for this study.

In a 1-year oral toxicity study, 8-week-old rats received derquantel by gastric intubation at a dose of 0, 1, 5 or 50 mg/kg bw per day. There were no test article-related deaths and no clinical signs of toxicity. Evidence of toxicity in the serum chemistry data included elevated mean levels of GGT at 50 mg/kg bw per day in both males and females. In 50 mg/kg bw per day females, mean bilirubin level was significantly elevated, and mean cholesterol and triglyceride levels were increased. In males at 50 mg/kg bw per day, a significant increase in mean globulin level resulted in a significant increase in mean total protein level and a lower albumin to globulin ratio compared with controls. In females at 50 mg/kg bw per day, mean globulin levels were increased at all dose levels compared with controls. The difference was significant, resulting in a significant ($P < 0.01$) decrease in the albumin to globulin ratio and significantly increased total protein at this dose level. In addition, mean total protein levels were significantly increased in all test article-treated groups. Cataracts seen in males at 50 mg/kg bw per day were treatment related. Treatment-related effects on the liver included enlarged livers and increased mean liver weight. Increased incidence and severity of age-related biliary hyperplasia were also seen starting at 1 mg/kg bw per day. Macroscopic and microscopic liver effects were considered adverse at 50 mg/kg bw per day in males and females due to associated elevations in serum chemistry parameters indicative of hepatocellular injury. The severity of spontaneous, chronic progressive nephropathy was increased in males and females at 50 mg/kg bw per day. Mild to marked hypospermia and degeneration of the seminiferous tubules were adverse effects seen in males at 50 mg/kg bw per day. Minimal degeneration of the seminiferous tubules was a non-adverse test article-related finding in males at 5 mg/kg bw per day.

A second 1-year oral toxicity study in the rat was conducted with lower dose levels, which allowed for a better evaluation of the hepatobiliary effects of derquantel seen in the previous study. In this study, 8-week-old rats received derquantel by gastric intubation at a dose of 0, 0.01, 0.03, 0.1 or 0.3 mg/kg bw per day. There were no test article-related deaths or clinical signs of toxicity. Test article-related effects in this study were limited to histopathological findings of an increased incidence and severity of biliary hyperplasia and fibrosis in the liver in males at and above 0.03 mg/kg bw per day. These were considered to be non-adverse and age-related spontaneous findings, the incidence or severity of which was accentuated by the test article. There were no associated changes in liver weights or in the serum chemistry data.

Biliary hyperplasia occurs spontaneously in rats and has not been correlated with liver carcinogenicity or neoplasia (Allen et al., 2004). A statistical analysis of the biliary hyperplasia response in the two chronic rat studies with derquantel comparing any positive response in each dose group with the control response showed that the incidence of biliary hyperplasia was significantly elevated relative to controls only at 50 mg/kg bw per day. In addition, this dose level was associated with significant increases in liver weight and changes in GGT levels consistent with hepatotoxicity and is therefore a clear adverse effect level. There was no statistically detectable difference in the incidence of biliary hyperplasia seen at lower dose levels, nor were there significant increases in liver weights or changes in serum chemistry parameters indicative of hepatocellular injury. Thus, 5 mg/kg bw per day would be considered the NOAEL on the basis of this analysis. This NOAEL is further supported by BMD modelling of the pooled data from both 1-year toxicity studies in the rat using various models. This modelling was performed by the sponsor and reviewed by the Committee. The BMDL₁₀ derived from these models ranged from 2.0 to 7.6 mg/kg bw per day, which was in close agreement with the previously statistically verified NOAEL of 5 mg/kg bw per day. On the basis of these two analyses, the Committee concluded that the NOAEL for biliary hyperplasia in the rat was 5 mg/kg bw per day. The Committee further noted that this NOAEL is consistent with the overall NOAEL that the Committee determined for both 1-year studies, which was also 5 mg/kg bw per day based on testicular effects.

In a non-GLP-compliant 28-day preliminary oral toxicity study, dogs received derquantel as neat test article in gelatine capsules at a dose of 0, 1, 5 or 10 mg/kg bw per day. Control animals were dosed with an empty gelatine capsule. Clinical signs of dry conjunctiva, dry eyes, relaxed nictitating membrane, ptosis, red eyes or mydriasis were seen in every dog dosed with derquantel during the first week of dosing, but the incidence of dry conjunctiva and dry eyes was lower in weeks 3 and 4 (compared with the first 2 weeks of the study). There was no associated pathology. The LOAEL for this study was 1 mg/kg bw per day, based on test article-related clinical observations seen at all dose levels.

In a 28-day oral toxicity study, dogs received derquantel by gavage at a dose of 0, 0.01, 0.03 (0.01 mg/kg bw per dose, 3 times per day), 0.03 or 0.1 mg/kg bw per day. Owing to the absence of clinical signs at 0.03 mg/kg bw per day given once daily or at 0.01 mg/kg bw given 3 times daily, dosing and data collection were suspended in the 3 times daily group after 7 days of dosing. There were no test article-related effects at any dose tested. Therefore, the NOAEL for this study was 0.1 mg/kg bw per day, the highest dose tested.

In a 90-day oral toxicity study, adolescent dogs received neat derquantel by capsule at a dose of 0, 0.1, 0.5, 1 or 10 mg/kg bw per day. Clinical signs seen in all animals in all treatment groups included dry mouth, mydriasis and relaxed nictitating membranes. Dilated pupils were seen in all treated animals at both 2 and 5 hours post-dosing at one point or another in the study. The incidence increased with dose. At 24 hours post-dosing, the pupils were still dilated, with incidences of 4%, 7% and 47% at 0.5, 1 and 10 mg/kg bw per day, respectively. Ptosis was also seen in all treatment groups, with a dose-related increase in the number of animals affected and the incidence of observations in individual animals. Dry conjunctiva,

dry eyes, red eyes and tremors were also treatment related in some groups based on increased numbers of animals affected and increased incidence of observations in individual animals relative to controls. Test article-related decreases in mean body weight and feed consumption of dogs at 10 mg/kg bw per day were also noted. On the basis of observed test article-related clinical signs at all dose levels, the LOAEL for this study was 0.1 mg/kg bw per day.

In a second 90-day oral toxicity study, mature dogs received derquantel by gavage at a dose of 0, 0.1, 0.5, 1 or 5 mg/kg bw per day. Test article-related clinical signs included relaxed nictitating membrane (in 0/0, 1/1, 2/4, 4/3 and 4/4 males/females at 0, 0.1, 0.5, 1 and 5 mg/kg bw per day) and mydriasis (in 0/0, 1/1, 3/1, 4/4 and 4/4 males/females at 0, 0.1, 0.5, 1 and 5 mg/kg bw per day). In affected animals, the incidence of observations in individual animals increased with increasing dose. An increased incidence of eye redness was seen at 1 and 5 mg/kg bw per day for males and females. There was no pathology associated with the clinical signs observed. Based on treatment-related observations consistent with the known pharmacological activity of the test article at all dose levels, the LOAEL for derquantel was 0.1 mg/kg bw per day.

The Committee performed and considered the results of BMD modelling to better define a point of departure for nictitating membrane protrusion, which was among the most sensitive effects seen in the dog. The Committee concluded that the BMDL₁₀ approach was not appropriate for the purpose of this evaluation. This conclusion was based on the high degree of uncertainty at the low end of the dose-response curve, the limited data available with which to model a dose-response relationship for elicitation of protrusion of the nictitating membrane in the dog and the fact that the two studies from which the data were derived differed in the age of dogs on study (juvenile versus adult) and mode of test article administration (capsule versus gavage).

In an adequate range of in vitro and in vivo genotoxicity studies, derquantel produced positive results in an in vitro chromosomal aberration assay in human peripheral lymphocytes, without metabolic activation, at dose levels that produced at least 50% cytotoxicity. Derquantel was not genotoxic in in vitro tests for bacterial mutagenicity or in in vivo studies in mouse bone marrow or rat hepatocytes. The Committee concluded that derquantel was weakly clastogenic in vitro, but was unlikely to be genotoxic in vivo. Derquantel had no structural alerts for mutagenicity, carcinogenicity or clastogenicity as determined by in silico analysis using the computer program DEREK.

No carcinogenicity studies were performed. Based on the absence of genotoxicity in vivo, the absence of structural alerts for carcinogenic, mutagenic or clastogenic potential by in silico analysis and lack of evidence of carcinogenicity or preneoplastic changes in rats dosed up to 1 year, the Committee concluded that derquantel is unlikely to pose a carcinogenic risk to humans at exposure levels likely to occur in food.

In a two-generation (one litter per generation) reproduction study, male and female rats were dosed with derquantel by oral gavage at dose levels of 0, 1, 5 or 25 mg/kg bw per day. Derquantel was well tolerated, with no evidence of reproductive

impairment or adverse effects on progeny at 0, 1, 5 or 25 mg/kg bw per day. At 25 mg/kg bw per day, an increase in liver weight was seen in P₁ males, and P₁ females had increased thyroid weight and increased incidence of thyroid follicular epithelial cell hypertrophy and hyperplasia. Terminal body weight, liver weight and adrenal weight were also increased in 25 mg/kg bw per day P₁ females. At 25 mg/kg bw per day, increased liver weights were also seen in F₁ males and females, and terminal body weight was increased in F₁ females. Increased incidence and severity of thyroid follicular epithelial hyperplasia were seen in F₁ males and females at 25 mg/kg bw per day. The NOAEL for reproductive effects was 25 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study, derquantel was administered orally by gavage to pregnant rats at doses of 0 (vehicle), 20, 70 or 120 mg/kg bw per day on gestation days 5 through 20. The dose levels were established in a previously conducted pilot study in pregnant rats using dose levels of 0, 2, 10, 50 and 100 mg/kg bw per day. No signs of maternal toxicity were observed at the lowest dose of 20 mg/kg bw per day. Test article-related increased incidences of porphyrin discharge and piloerection were seen at 70 and 120 mg/kg bw per day. At the highest dose of 120 mg/kg bw per day, test article-related signs of toxicity included increased porphyria, subdued behaviour, laboured breathing, thin appearance, piloerection and a significant reduction in body weight gain correlating with significantly reduced feed intake. There were no biological differences in the number of corpora lutea, number of implantation sites, number of live fetuses and percentage of resorptions between treated groups and the vehicle control group. A lower trend in uterus weight was observed in dams in the 120 mg/kg bw per day group. Male fetus weight was significantly lower in the 120 mg/kg bw per day group than in vehicle control-treated animals. Ossification retardation was observed in fetuses in the 120 mg/kg bw per day group and was attributed to nonspecific inhibition or retardation of physiological growth due to the effects of maternal toxicity at this dose. Skeletal development was related to the reduced body weight of the fetuses. No soft tissue abnormalities were identified in any treatment group. Based on the results of this study, derquantel was not teratogenic. The NOAEL for maternal toxicity was 20 mg/kg bw per day, and the NOAEL for developmental toxicity was 70 mg/kg bw per day.

In another developmental toxicity study, derquantel was administered orally by gavage to pregnant rabbits at doses of 0 (vehicle), 0.1, 1 or 10 mg/kg bw per day on gestation days 7 through 19. Dose levels were determined by a previously conducted pilot study in pregnant rabbits given derquantel at dose levels of 0, 0.1, 1, 10 or 100 mg/kg bw per day. There was one death attributable to test article toxicity at 10 mg/kg bw per day. Other clinical findings at 10 mg/kg bw per day included low feed consumption, resulting in scant faeces, significant body weight loss and reduced body weight gains. At 10 mg/kg bw per day, an increase in total resorptions was seen, and fetal body weights were significantly reduced. Developmental toxicity characterized by an increased incidence of supernumerary thoracic ribs with associated increases and decreases in the numbers of thoracic and lumbar vertebrae, respectively, at 10 mg/kg bw per day was attributable to maternal toxicity and the decreased fetal weights observed in this group. Based on the findings at 10 mg/kg bw per day, the NOAEL for developmental toxicity was 1 mg/kg bw per day.

Table 5. Toxicity comparison of derquantel/abamectin combination product

Study type	NOAEL		
	Derquantel/abamectin	Derquantel	Abamectin ^a
Acute oral, rat	88.72/8.872 mg/kg bw ^b	>2000 mg/kg bw ^b	8–2.8 mg/kg bw ^b
Acute dermal, rat	No dermal toxicity	No dermal toxicity	>330 mg/kg bw ^b
Acute (4 h) inhalation, rat	>5.04 mg/l ^c	>2.4 mg/l ^c	ND
Short-term oral, rat	3/0.3 mg/kg bw per day ^d 20/2 mg/kg bw per day ^e	0.5 mg/kg bw per day ^d 30 mg/kg bw per day ^f	1.5 mg/kg bw per day ^g

ND, no data

^a WHO (1992).

^b Estimated LD₅₀.

^c LC₅₀.

^d Twenty-eight-day study.

^e Seven-day study.

^f Twelve-day study.

^g Eight-week study.

The Committee observed that adverse effects on developmental parameters occurred only at dose levels that were maternally toxic. Therefore, the Committee concluded that derquantel had no direct reproductive or developmental toxicity potential.

For comparative toxicity purposes, results of toxicity studies with the 10 mg/ml derquantel and 1 mg/ml abamectin combination product, derquantel and abamectin are shown in Table 5.

The Committee concluded that there was no indication that the derquantel/abamectin combination showed any greater toxicity than the more toxic component, abamectin. It was further noted that the pharmacokinetic profile for derquantel when given in the combination product was similar to the pharmacokinetic profile of derquantel given alone in the laboratory species tested.

When the functional antagonistic potency of derquantel on $\alpha 3$ nAChR endogenously expressed in rat and canine dorsal root ganglion neuron cell cultures was evaluated using whole-cell patch clamp assays, derquantel displayed more potent antagonist action against acetylcholine at canine $\alpha 3$ nAChR (IC₅₀ of 1 μ mol/l) than at rat $\alpha 3$ nicotinic acetylcholine receptors (IC₅₀ of 30 μ mol/l).

In an in vitro study of functional agonistic and antagonistic potency using a fluorescent imaging plate reader assay to measure calcium flux, derquantel showed no agonist activity in human $\alpha 3$, muscle-type or $\alpha 7$ nAChR models. Antagonist activity of derquantel was demonstrated by inhibiting the activity of nicotine at $\alpha 3$ nAChR of humans with an IC₅₀ of 9 μ mol/l. In addition, in vitro antagonistic potency of derquantel at human muscle-type nAChRs was reported with an IC₅₀ value of 10 μ mol/l, but derquantel was inactive (with an IC₅₀ value of >100 μ mol/l) at the central

nervous system $\alpha 7$ nAChR subtype. Direct comparison of the results of this study with those from studies in dogs and rats was not possible because of differences in study design.

3.3 Microbiological data

A JECFA decision-tree approach that was adopted at the sixty-sixth meeting of the Committee (Annex 1, reference 181) and complies with VICH GL36 was used by the Committee to determine the need to establish a microbiological ADI for derquantel. The decision-tree approach initially seeks to determine if there may be microbiologically active derquantel residues entering the human colon.

Derquantel is not classified as an antimicrobial agent and is not structurally related to antimicrobial agents used in animal or human medicine.

The Committee considered an in vitro evaluation of derquantel against 62 strains representing a wide range of Gram-positive and Gram-negative bacteria of veterinary importance. Derquantel showed limited strain-dependent activity against the staphylococci (MIC values ranging from 64 to 128 $\mu\text{g/ml}$ for all strains). Against the streptococci, derquantel showed very limited activity (MIC values ≥ 128 $\mu\text{g/ml}$). Against *Pasteurella* species, derquantel showed limited activity (MIC values ranging from 32 to >128 $\mu\text{g/ml}$). Derquantel was not active against the enteric organisms tested.

The Committee concluded that a microbiological ADI for derquantel is not necessary.

4. EVALUATION

The Committee considered the acute clinical observations in dogs, which were consistent with the antagonistic activity of derquantel on nAChR, as the most relevant toxicological effect for the establishment of an ADI for derquantel. The LOAEL for this effect was 0.1 mg/kg bw per day. The Committee established an ADI of 0–0.3 $\mu\text{g/kg}$ bw by applying an uncertainty factor of 300, using the default uncertainty factor of 100 and an additional uncertainty factor of 3 to account for setting the ADI on the basis of a LOAEL instead of a NOAEL. The Committee noted that the dog is appreciably more sensitive than the rat to the anti-nicotinergic effects of derquantel, but had no information to allow a relative comparison with humans. The Committee further noted that it may be possible to refine the ADI with additional studies, in particular on the comparative sensitivity of the nicotinic receptors to derquantel.

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