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Journal of Pharmacological and Toxicological Methods xx (2006) xxx–xxx

**Journal of  
Pharmacological  
and  
Toxicological  
Methods**

[www.elsevier.com/locate/jpharmtox](http://www.elsevier.com/locate/jpharmtox)

Original article

## A novel approach to data processing of the QT interval response in the conscious telemetered beagle dog

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Received 14 November 2005; accepted 16 February 2006

### Abstract

**Introduction:** Drug-induced QT interval prolongation may lead to ventricular arrhythmias. The aim of the study was to optimize QT interval data processing to quantify drug-induced QT interval prolongation in the telemetry instrumented conscious dog model. **Methods:** The test substances cisapride, dofetilide, haloperidol, and terfenadine and corresponding vehicles were given to male and female beagle dogs during two consecutive 90-min intravenous infusions. Cardiovascular parameters were recorded for 24 h and exposure to the drugs was measured. The delayed response in the QT interval after an abrupt change in heart rate was investigated. Eight mathematical models to describe the QT interval–heart rate relationship were compared and different sets of covariates were used to quantify the drug-induced effect on the QT interval. **Results:** After an abrupt decrease in heart rate, a 75% adaptation of the QT interval was reached after  $54 \pm 9$  s. A linear model was preferred to correct the drug-induced effect on the QT interval for heart rate, vehicle effect, serial correlation, plasma concentration and time of day. All test substances significantly prolonged the QT interval. **Discussion:** To optimize the processing of QT interval data, the delay in QT interval response after an abrupt change in heart rate should be considered. The QT interval–heart rate relationship and vehicle response were individual-specific and corrections were therefore made individually. When estimating the drug-induced effect on the QT interval it is considered advantageous to use plasma concentration as a covariate, as well as adjusting for vehicle effect and serial correlation in measurements. The conscious dog model detected significant increases in the QT interval for all test substances investigated.

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**Keywords:** Cisapride; Dofetilide; Dog; Haloperidol; Methods; Plasma concentrations; QT interval; Telemetry; Terfenadine

### 1. Introduction

Cardiac ventricular depolarization and repolarization are represented by the QT interval of an electrocardiogram (ECG) recording. Assessment of the duration of the QT interval is important since drug-induced prolongation of ventricular repolarization may cause a ventricular arrhythmia called torsades de pointes (TdP) and sudden death (Ahneve, 1991; Peters, Byington, Barker, & Yusuf, 1990). The investigation of drug effect on the QT interval in vivo is one of the three primary assays of assessing ventricular repolarization risk (ICH S7A 2000; S7B 2005).

One of the challenges in assessing the drug-induced changes in the QT interval is the inverse relationship between the QT interval and the heart rate (Batchvarov & Malik, 2002). Correction of the QT interval for changes in heart rate is required in order to reliably detect drug-induced changes in the QT interval, irrespective of physiological and/or pharmacological modulation of heart rate, especially in dogs that exhibit a large variation in heart rate. The correction of the QT interval has been discussed and numerous approaches have been proposed to normalize, or correct, the QT interval for variations in heart rate. Some of the most commonly used correction formulas are the square root formula of Bazett (1920) or the cubic root formula of Fridericia (1920). These and others (e.g. Van de Water, Verheyen, Xhonneux, & Reneman, 1989) are examples of formulas that correct the QT interval in a fixed manner

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without taking into account existing interindividual variations. In addition, the utility of these correction formulas is limited in the dog due to the pronounced presence of respiratory sinus arrhythmia and the occurrence of large and rapid variations in heart rate in response to the emotional and physical state of the animal, advocating corrections in an individual manner.

Other factors such as the autonomic nervous system, gender, age, circadian rhythm, and potassium level have been suggested as affecting the duration of the QT interval (Can, Aytamer, Kose, & Oto, 2002; De Ponti, Poluzzi, Cavalli, Recanatini, & Montanaro, 2002). The autonomic nervous system is an important modulator of cardiovascular function, e.g., through circadian rhythm variations in the QT interval, which could affect the relationship between the QT interval and heart rate in dogs. Another complicating factor in the interpretation of QT interval data is the time delay in the response of the QT interval to abrupt changes in heart rate. The presence of the QT interval delayed response has been reported previously in both humans and dogs (Franz, Swerdlow, Liem, & Schaefer, 1988; Lau et al., 1988; Oguchi & Hamlin, 1994; Pladys et al., 2001).

In the present study the effect of four test substances (cisapride, dofetilide, haloperidol and terfenadine) on the QT interval were studied in conscious telemetered dogs. All the test substances chosen have known  $I_{Kr}$  channel-blocking effects (Mohammad, Zhou, Gong, & January, 1997; Rasmussen, Allen, Blackburn, Butrous, & Dalrymple, 1992; Suessbrich et al., 1997; Suessbrich, Waldegger, Lang, & Busch, 1996) and were selected on the basis of their diverse chemical classes and their pharmacodynamic and pharmacokinetic profiles. Cisapride is a prokinetic agent that facilitates or restores motility throughout the length of the gastrointestinal tract and dofetilide is a potent antiarrhythmic selective class III drug. Haloperidol is an antipsychotic drug, possessing dopamine ( $D_2$ ) receptor-blocking properties and terfenadine is a non-sedating histamine ( $H_1$ ) receptor antagonist. Marketing of cisapride and terfenadine has been discontinued in the USA due to proarrhythmic effects.

The aim of the present study was to optimize the data processing utilized when assessing the QT interval prolongation in telemetered conscious dogs. The time delay in the QT interval response after an abrupt decrease in heart rate under sinus rhythm and the QT interval–heart rate relationship in conscious beagle dogs were investigated. Different approaches to model the QT interval and heart rate relationships were compared and different sets of covariates were used to quantify the net drug-induced effect on the QT interval.

## 2. Methods

### 2.1. Animals

Four male and three female beagle dogs (weight range 13–19 kg; Rååhöjden, Örkelljunga, Sweden) were used in this study. All experiments were conducted in accordance with an application approved by the Southern Stockholm Ethical Committee.

Dogs were chronically instrumented with radiotelemetry probes. Acepromazine (Plegicil; Pharmacia and Upjohn Animal Health AB, Helsingborg, Sweden) was used as premedication

before the operation. Instrumentation was conducted under anesthesia, which was induced with Diprivan and maintained with isoflurane (Baxter Medical AB, Kista, Sweden) in oxygen. Temgesic (buprenorphine) was used for analgesia, given after induction of anesthesia. The chest was opened and a radio-telemetry probe measuring arterial blood pressure, ECG, intrathoracic pressure, and body temperature (T27F-10A, Konigsberg Instruments, Inc., Pasadena, USA) was implanted. The aortic transducer (which also served as one electrode of the ECG) was inserted into the thoracic aorta just below the aortic arch. The second ECG electrode was placed under the skin and muscles close to the sternum, in the 6th intercostal giving a lead II ECG recording. The body of the telemetry device and the battery were separately placed in two skin pouches on the abdominal wall. After surgery Rimadyl (carprofen) was used for analgesia for 3–4 days. Surgery was performed at least two months prior to experiments.

For a minimum of one week before the start of the experiment, each dog was familiarized with the personnel and the experimental conditions. On the day of treatment the dogs were placed on a laboratory table and an intravenous cannula (Viggo Venflon 22G, Viggo-Spectramed, Helsingborg, Sweden) was introduced into the cephalic vein for infusion of vehicle or test substances. Venous blood samples (2 ml), for determination of total plasma concentrations, were taken according to the blood sampling regime in Section 2.4. The blood samples were withdrawn from a jugular vein using superficial venepuncture into heparinized (sodium-heparin) tubes (Venoject<sup>®</sup>, Terumo, Belgium; dofetilide) or into tubes with spray-dried EDTA (BD Vacutainer<sup>™</sup>, Beckton, Dickson and Company, UK; cisapride, haloperidol, and terfenadine). The blood samples were placed on wet ice until centrifugation (within 30 min) at +4 °C for 10 min at 1500 g. The separated plasma was transferred into Nunc Cryo tubes (1.8 ml) using Eppendorf pipettes. The plasma samples were immediately frozen at –20 °C and then stored at –70 °C until analysis.

### 2.2. Drugs and formulations

In this study four different test substances (dofetilide, cisapride, haloperidol, and terfenadine) were investigated. All the test substances were given as two consecutive 90-min intravenous infusions at a rate of 11  $\mu\text{l}/\text{min}/\text{kg}$ . Vehicle experiments were performed in all dogs corresponding to each solvent for the test substances in an equivalent manner. The total volume administered was 2 ml/kg body weight in all experiments. Test formulations for intravenous administration were prepared at AstraZeneca R&D Södertälje on the day before or on the day of the experiments. All chemicals were purchased from Sigma Aldrich, St. Louis, Mo., USA, unless stated otherwise.

**Cisapride HCl** (Apin Chemicals Limited, Abingdon, UK; MW: 484.0) was given in doses of 0.2 and 1.0  $\mu\text{mol}/\text{kg}$  (97 and 484  $\mu\text{g}/\text{kg}$ ). Cisapride was prepared in 1.2 mM tartaric acid with 5w/v% mannitol (pH was adjusted to 5 with 0.1 M NaOH).

**Dofetilide** (synthesized by the Department of Medicinal Chemistry, AstraZeneca R&D Mölndal, Sweden; MW: 441.6) was given in doses of 0.05 and 0.20  $\mu\text{mol}/\text{kg}$  (22 and 88  $\mu\text{g}/\text{kg}$ ).

Dofetilide was dissolved in 0.5 mM tartaric acid in saline (9 mg/ml; Pharmacia and Upjohn, Helsingborg, Sweden), which served as a vehicle for dofetilide.

**Haloperidol** (ICN Biomedicals Inc., Escwege, Germany; MW: 375.9) was given in doses of 1.6 and 6.4  $\mu\text{mol/kg}$  (601 and 2406  $\mu\text{g/kg}$ ). The same vehicle as for the preparation of cisapride was used to dissolve haloperidol.

**Terfenadine** (ICN Biomedicals Inc., Escwege, Germany; MW: 471.7) was given in doses of 1.7 and 6.8  $\mu\text{mol/kg}$  (terfenadine, high dose; 802 and 3208  $\mu\text{g/kg}$ ). Terfenadine was prepared in 25% PEG-400 (AstraZeneca, Södertälje, Sweden) and 1.25% 1 M HCl, pH-adjusted to 4–5 with 0.1 M NaOH. Due to problems with the vehicle, lower doses were prepared in another vehicle. The new formulation consisted of 5% cyclodextrin (kleptose HPB; Roquette Frères SA, Lestrem, France), 2.15% glycerol, and redistilled water. 1 M HCl (1 vol. %) was used to dissolve the substance and 0.1 M NaOH (7 vol. %) was used to adjust the pH to 7. Terfenadine in doses of 0.1 and 3  $\mu\text{mol/kg}$  (47 and 1415  $\mu\text{g/kg}$ ) was administered to the dogs in this vehicle (terfenadine, low dose).

### 2.3. Determinations of total drug concentrations in plasma

**Cisapride** concentrations were determined after 96-well ultrafiltration of 150  $\mu\text{l}$  plasma and 150  $\mu\text{l}$  internal standard solution in 0.05 M formic acid, the molecular weight cutoff of the filter being 10 kD. Twenty microliters of the ultrafiltrate was directly injected into the LC-MS/MS system consisting of a cooled autosampler, a  $2 \times 50$  mm C18 LC column, a dual pump high-pressure gradient setup, and tandem mass spectrometry with positive electrospray ionization. The responses were measured using multiple reaction monitoring for the transitions  $m/z$  466  $\rightarrow$  184 for cisapride and  $m/z$  421  $\rightarrow$  254 for the internal standard. Concentrations in the range 5–2000 nM were determined with a typical precision of about 5% (CV).

**Dofetilide** concentrations were determined after 96-well ultrafiltration of 120  $\mu\text{l}$  plasma and 120  $\mu\text{l}$  internal standard solution in 1% formic acid, the molecular weight cutoff of the filter being 10 kD. 50  $\mu\text{l}$  of the ultrafiltrate was directly injected into the LC-MS/MS system consisting of a cooled autosampler, a  $2 \times 50$  mm C18 LC column, a dual pump high-pressure gradient setup, and tandem mass spectrometry with positive electrospray ionization. The responses were measured using multiple reaction monitoring for the transitions  $m/z$  442 to 198 for dofetilide and  $m/z$  319  $\rightarrow$  194 for the internal standard. Concentrations in the range 1–512 nM were determined with a typical precision of about 5% (CV).

**Haloperidol** concentrations were determined after liquid–liquid extraction of 250  $\mu\text{l}$  plasma made alkaline with 200  $\mu\text{l}$  solution of internal standard (NAD-299) (Johansson et al., 1997) in 0.5 M NaOH with 2 ml of ethyl acetate and heptane (50 / 50, v/v). Samples were extracted for 15 s and then centrifuged. The aqueous layer was frozen in a carbon dioxide/ethanol bath and the upper, organic layer was decanted to a new glass tube and evaporated. The residue was dissolved in 200  $\mu\text{l}$  of 0.025 M formic acid and 20  $\mu\text{l}$  was injected into the LC-MS/MS system consisting of a cooled autosampler, a  $2 \times 50$  mm C18 LC column,

and a dual pump high-pressure gradient setup, and tandem mass spectrometry with positive electrospray ionization. The responses were measured using multiple reaction monitoring for the transitions  $m/z$  376  $\rightarrow$  165 for haloperidol and  $m/z$  322  $\rightarrow$  197 for the internal standard. Concentrations in the range 10–3000 nM were determined with a typical precision of about 10% (CV).

**Terfenadine** concentrations were determined in 100  $\mu\text{l}$  plasma after precipitation of the plasma proteins using 200  $\mu\text{l}$  acetonitrile, and adding 10  $\mu\text{l}$  internal standard solution in 1 M formic acid. After centrifugation the liquid layer was transferred to a new glass tube and evaporated to dryness. The residue was dissolved in 400  $\mu\text{l}$  of 0.025 M formic acid and 20  $\mu\text{l}$  was injected into the LC-MS/MS system consisting of a cooled autosampler, a  $2 \times 50$  mm C18 LC column, a dual pump high-pressure gradient setup, and tandem mass spectrometry with positive electrospray ionization. The responses were measured using multiple reaction monitoring for the transitions  $m/z$  472  $\rightarrow$  436 for terfenadine and  $m/z$  376  $\rightarrow$  165 for the internal standard (haloperidol). Glassware was used as far as possible as terfenadine is prone to nonspecific adsorption and the samples were analyzed immediately after workup as terfenadine is not very stable. Concentrations in the range 1–2000 nM were determined with a typical precision of about 5% (CV).

Estimates of unbound plasma concentrations of test substances were made in accordance with published data (54% (Walker, Beumont, Stopher, & Smith, 1996), 95% (Veereman-Wauters et al., 1991), and 98% (Webster et al., 2001) for dofetilide, cisapride, and terfenadine, respectively). For haloperidol the plasma binding used to calculate the unbound plasma concentration is that reported in human plasma, 92% (Forsman & Ohman, 1977).

### 2.4. Data collection

The ECG waveforms were sampled at 500 Hz and saved to disk with a PC-based data acquisition system (CA recorder™ system) using Data Integrated Scientific Systems software CA Recorder Series II version 2.0 software (Pinckney, Mich., USA). Mean, systolic and diastolic blood pressure, heart rate, ECG, and body temperature were measured continuously in 20-s segments by radiotelemetry for approx. 45 min before the start of administration of vehicle or test substance and during the two 90-min intravenous infusions of vehicle or test substance and for 30 min following the end of infusion. The animals were then placed in a laboratory cage and held there for 180 min for blood sampling and data acquisition, after which they were moved to their home pens, where data collection continued overnight. One hour of the data corresponding to the period immediately following the move from the laboratory to the pens was excluded due to high variability in the relationship between the QT interval and heart rate.

Plasma concentrations of the test substances were determined from venous blood samples taken at 15 min prior to administration, 10, 30, 60, 89, 100, 120, 150, and 180 min during infusion of test substance, 10, 30, 100 min and 3.5 and 21 h after the end of infusion. Sham blood sampling was performed in the vehicle experiments to replicate the disturbance in the measured parameters. The ECG waveforms were replayed and reviewed using CA Recorder Series II version 2.0

and VR<sup>2</sup> version 1.0 software (Data Integrated Scientific Systems, Pinckney, Mich., USA).

### 2.5. Delay in QT interval response

The time delay of the QT interval response after an abrupt decrease in heart rate was investigated using data from each dog from the dofetilide vehicle experiment ( $n=7$ ). In performing this analysis the ECG complexes were averaged in 10-s segments to improve the resolution of the adaptation analysis. An abrupt decrease in heart rate was defined as a spontaneously occurring drop in heart rate of  $\geq 15$  beats/min (bpm), after a period of at least 30 s with stable heart rate (not changing more than 15 bpm), and followed by at least two minutes of heart rate changing less than 20 bpm (Fig. 1).

The time to reach full adaptation of the QT interval after an abrupt change in heart rate was modeled using WinNonlin 4.0

(Pharsight Corporation, Mountain View, CA, USA) according to an exponential model:

$$QT = QT_{SS} \cdot e^{-K \cdot t}$$

where  $QT_{SS}$  is the QT interval at steady state and  $K$  is a constant. The half-life ( $t_{1/2}$ ) of the QT interval adaptation response is estimated as:

$$t_{1/2} = \frac{\ln 2}{K}$$

An automated routine, based on the following algorithm, was used to find abrupt changes in heart rate: Using the notation from Fig. 2, QT\* was excluded from the dataset:

1. if  $HR^*$  and  $HR_{+1}$  differed more than 10 bpm,
2. else if  $HR^*$  and  $HR_{-1}$  differed more than 10 bpm,

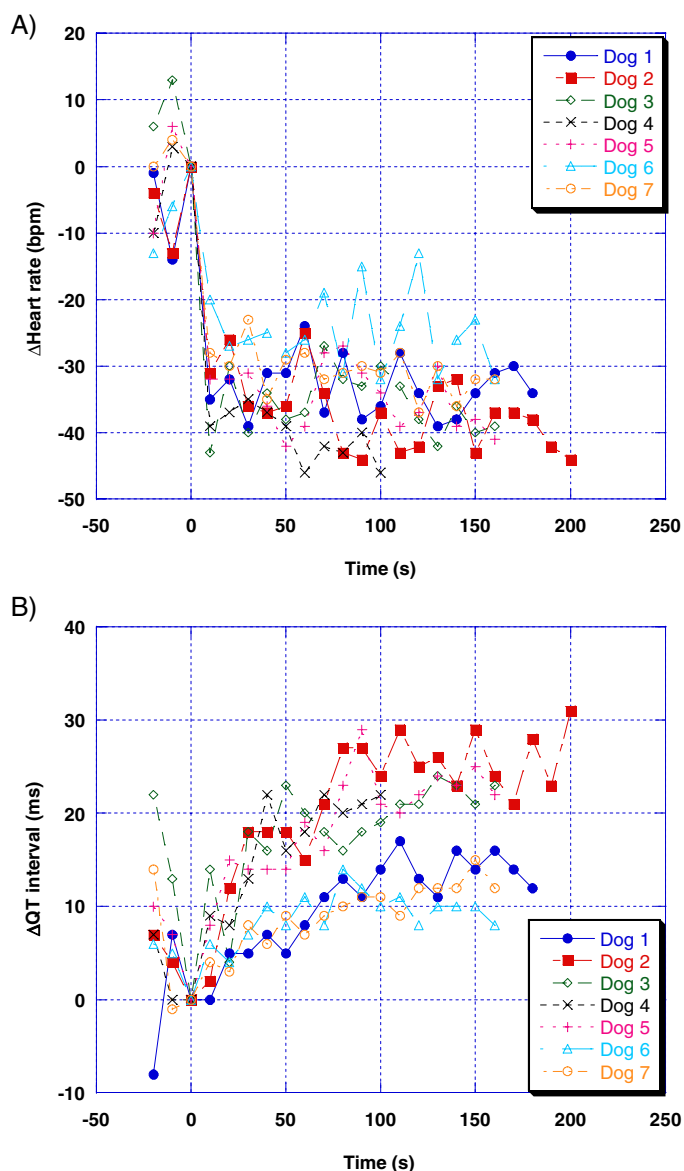


Fig. 1. The delayed response of the QT interval after an abrupt decrease in heart rate. Panel A shows the heart rate in beats/min (bpm) and panel B the corresponding change in the QT interval for selected intervals based on criteria described in Section 2.5.

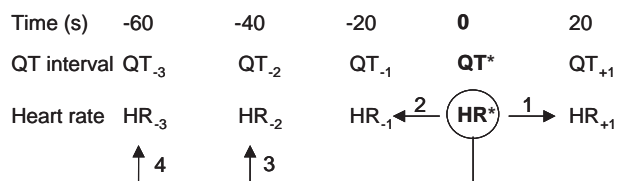


Fig. 2. Schematic representation of checks of the QT interval data for variations in heart rate. The figure shows five 20-s segments of QT interval (QT<sub>-3</sub> to +1) and corresponding averaged heart rate (HR<sub>-3</sub> to +1) data. The HR\* is the heart rate under comparison and the QT\* is the QT interval that is being kept or excluded based on the results of the heart rate comparisons. The arrows with figures describe in what order the comparisons are made. See Section 2.5 for further description.

3. else if HR\* and HR<sub>-2</sub> differed more than 10 bpm,
4. else if HR\* and HR<sub>-3</sub> differed more than 20 bpm.
5. Steps 1 to 4 were repeated for all averaged heart rates.

Note that, since averaged QT intervals were used, if for example HR<sub>+1</sub> differed > 10 bpm from HR\*, the change in HR could have occurred within the 20-s segment over which HR\* is averaged, hence QT\* is removed. Exclusion of data was performed by filtering in Microsoft Excel. All QT interval data presented in this study have been checked and approved as described above.

## 2.6. Models for normalizing the QT interval for heart rate dependence

Different models for describing the relationship between QT interval and heart rate were investigated using data from all vehicle experiments: three traditional correction models (Bazett, Fridericia, and Van de Water, models 1–3) and five models described as individual correction models (models 4–8). The traditional models use a standard formula when used for correcting the QT interval, while models 4–8 rely on individually estimated parameters. The models were:

model 1. $QT = aRR^{1/2}$	(Bazett, 1920)
model 2. $QT = aRR^{1/3}$	(Fridericia, 1920)
model 3. $QT = a + 0.087RR$	(Van de Water et al., 1989)
model 4. $QT = aRR^b$	(Individual exponent)
model 5. $QT = a + bHR$	(Linear model)
model 6. $QT = a + b \log_e RR$	(Davis & Middleton, 1999)
model 7. $QT = a + b \exp(-cRR/1000)$	(Raunig, Depasquale, Huang, Winslow, & Fossa, 2001)
model 8. $QT = a \exp(-\exp(b - cRR/1000))$	(Gompertz, 1825; Thörneborn, 2002)

Data from a total of 21 vehicle experiments were used when evaluating the models. Additionally, the models were fitted to the data originating from the last 30 min of the second dose from the test substance experiment. This was done to compare the models at high exposure of the drugs. The accuracy of fit of the models was compared using the Akaike Information Criterion (AIC) and the calculations were performed using the software SAS (SAS institute Inc., NC, USA). AIC is commonly used as a measure for comparing the fit of different models to a data set.

## 2.7. Calculating drug-induced changes in QT interval using the linear model

The drug-induced effect on the QT interval was defined as the difference in change in QT interval from pretreatment between test substance and vehicle experiments. Changes in the QT interval from pretreatment were calculated separately for each experiment, i.e., each dog in both vehicle and test substance experiment. All calculations were performed using SAS (SAS institute Inc., NC, USA).

An exploratory analysis was carried out to investigate possible influences of the experimental conditions and/or time of day on the slope of the QT interval and heart rate relationship. The time course of each experiment was divided into different time periods based on the placement of the dog and the time of day: lab table (approx. 0800–1200 h), lab cage (approx. 1200–1500 h), pen (approx. 1600–1900 h, 1900–2400 h, and 2400–0600 h). Linear regression models were fitted to data from different time periods and the resulting slope estimates were studied.

For each dog and experiment (vehicle and test substance), a linear model was fitted to data:

$$QT_{j(k)l} = \alpha_{j(k)} + \beta \times HR + \varepsilon_{j(k)l} \quad (1)$$

Here  $j$  denotes the event in the experiment and placement of the animals, e.g., home cage, lab cage, etc., and  $l$  denotes the time. For the active treatment, each event  $j$  was partitioned into time periods of 5 min,  $j(k)$ , to capture short-term effects. The parameter  $\alpha_{j(k)}$  describes the length of the QT interval at time  $j(k)$  following administration of vehicle or test substance. The random error term,  $\varepsilon_{j(k)l}$ , was assumed to follow an autoregressive structure of order one within each  $j(k)$  combination to correct for serial correlation. The regression coefficient  $\beta$  corresponded to one or more parameters indicating which variables were taken into account when modeling the data. A total of six different models were used and are described by replacing  $\beta$  in Eq. (1) with  $\beta_A - \beta_F$  below.

$$\begin{aligned} \beta_A &= \beta_{\text{vehicle}} \\ \beta_B &= \beta_{\text{vehicle}} + \beta_p I_p \\ \beta_C &= \beta_t \\ \beta_D &= \beta_t + \beta_{tp} I_p \\ \beta_E &= \beta_t + \beta_{tc} C \\ \beta_F &= \beta_t + \beta_{tp} I_p + \beta_{tc} C \end{aligned}$$

Here  $I_p$  is equal to 1 during pen placement (approx. 1600 to 0600 h) and 0 otherwise and  $C$  is the plasma concentration of the test substance. Also,  $\beta_t$  is the regression coefficient for the studied treatment  $t$  (vehicle or test substance). Hence,  $\beta_{tp}$  is the regression coefficient indicating the difference in slopes between laboratory and pen placement for treatment  $t$ , and  $\beta_{tc}$  is the change in slope caused by one unit (nM) increase of the plasma concentration of the test substance. Note that since the plasma concentration of the test substance is always zero in the vehicle experiments,  $\beta_{tc}C$  give no addition to  $\beta$  when data from the vehicle experiments were investigated. Also, substituting  $\beta_A$  or  $\beta_B$  in Eq. (1) implies that the

slope of the QT interval–heart rate relationship is estimated using data from the vehicle experiment, and that this slope then is used when modeling the data originating from active treatment.

Finally, the drug-induced QT effect was estimated for each time interval,  $j(k)$ , as

$$\hat{Q}T_{j(k)} = \Delta\hat{Q}T_{\text{active},j(k)} - \Delta\hat{Q}T_{\text{vehicle},j(k)}, \quad (2)$$

where

$$\Delta\hat{Q}T_{i,j(k)} = \hat{\alpha}_{i,j(k)} + \hat{\beta}_i^i \text{HR} - (\hat{\alpha}_{i,0} + \hat{\beta}_i^i \text{HR}) \quad (3)$$

Here  $j(k)=0$  is pretreatment and  $\text{HR}=60$  is used in the calculations. The index  $i$  indicates the chosen approach for estimating  $\beta$ . Note that the terms with heart rate cancel each other out when the slope is estimated as described by slopes A and B.

The average drug-induced effects on the QT interval taken over the last 30 min of the first and second dose were calculated for all dogs. To test whether this drug-induced effect on the QT interval differed from zero, a two-tailed Student's  $t$  test was utilized. The test was performed separately for each substance and dose and no correction for multiplicity was made. Throughout the paper, a  $p$ -value of less than 0.05 is considered statistically significant.

## 2.8. Pharmacokinetic analysis

Pharmacokinetic analysis was performed by fitting a standard two-compartment model to the concentration–time profiles of the substances tested. The concentration–time courses were modeled according to the following equations:

$$\begin{cases} dC_p/dt = (\text{Input} - \text{CL}_D \cdot C_p + \text{CL}_D \cdot C_t - \text{CL} \cdot C_p)/V_1 \\ dC_t/dt = (\text{CL}_D \cdot C_p - \text{CL}_D \cdot C_t)/V_2 \end{cases} \quad (4)$$

where  $C_p$  and  $C_t$  represent the concentration of test substance in the central and peripheral compartments,  $\text{CL}$  denotes clearance, and  $\text{CL}_D$  the intercompartmental distribution parameter.  $V_1$  and  $V_2$  are the volumes of the central and peripheral compartments, respectively. The drug input was defined as a constant rate during the first infusion (0–90 min) and another constant rate during the

Table 1

Individual and mean values of maximal delay in response of QT interval after spontaneously occurring abrupt decrease in heart rate (Max response),  $t_{1/2}$ , and time to reach a steady state QT interval ( $\text{QT}_{\text{SS}}$ ) at 75% and 90% after an abrupt decrease in heart rate in seven dogs

Dog	Max response (ms)	$t_{1/2}$ (s)	$\text{QT}_{\text{SS}}$ 75% (s)	$\text{QT}_{\text{SS}}$ 90% (s)
1	16	48	96	158
2	27	26	53	87
3	21	17	34	55
4	22	20	40	66
5	23	24	47	78
6	10	14	28	47
7	13	39	78	129
Mean	19	27	54	89
SE	2	5	9	15

Data obtained from vehicle experiments.

Table 2

The Akaike Information Criterion (AIC) calculated for eight different models, all fitted to the vehicle data

Model	Name of formula	Formula	AIC
<i>Traditional correction models</i>			
1	Bazett	$\text{QT} = a\text{RR}^{1/2}$	17,995
2	Fridericia	$\text{QT} = a\text{RR}^{1/3}$	15,906
3	Van de Water	$\text{QT} = a + 0.087\text{RR}$	15,848
<i>Individual correction models</i>			
4	Individual exponent	$\text{QT} = a\text{RR}^b$	14,735
5	Linear regression	$\text{QT} = a + b\text{HR}$	14,628
6	Davis and Middleton	$\text{QT} = a + b \log_e \text{RR}$	14,691
7	Raunig	$\text{QT} = a + b \exp(-c\text{RR}/1000)$	14,450
8	Gompertz	$\text{QT} = a \exp(-\exp(b - c\text{RR}/1000))$	14,451

A lower value of AIC indicates better fit of the model.

Abbreviations and definitions:  $a$ ,  $b$  and  $c$ , variables obtained from data set, see (Bazett, 1920; Davis & Middleton, 1999; Fridericia, 1920; Gompertz, 1825; Raunig et al., 2001; Thörneborn, 2002; Van de Water et al., 1989); RR, interval between two R peaks of the ECG; HR, heart rate.

second infusion between 90 and 180 min or set to zero. Individual parameter estimates were used to calculate individual substance plasma concentrations at the times of the substance-induced change in QT interval using Eq. (4). All pharmacokinetic modeling was performed using the nonlinear mixed-effect modeling software package NONMEM (Version V, Globomax, Md., USA).

## 2.9. Analysis of QRS complex, heart rate and blood pressure

The QRS complex data reported represent the means of three collection periods separated by 1 min from each other from the end of each of the following periods: pretreatment, dose 1, dose 2, and 3 h 30 min after dosing during confinement in the laboratory. These results were averaged together for all the dogs, since no differences were found between the male and female dogs.

The change from pretreatment in heart rate and blood pressure was analyzed using a linear mixed-effect model with time,

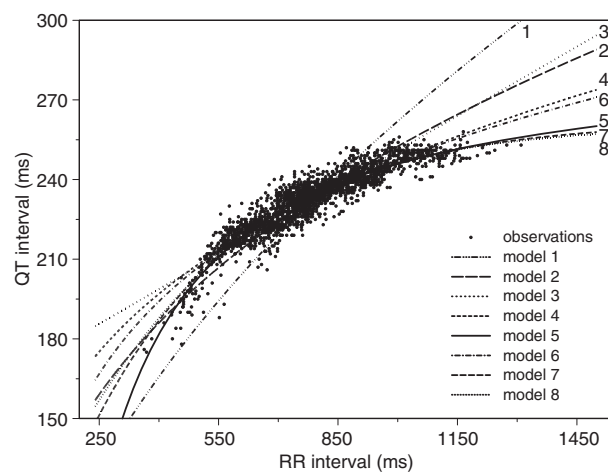


Fig. 3. Illustration of the fit of eight models (1. Bazett; 2. Fridericia; 3. Van de Water; 4. Individual exponent; 5. Linear regression; 6. Davis and Middleton; 7. Raunig; 8. Gompertz) to QT/RR interval data from one typical vehicle experiment.

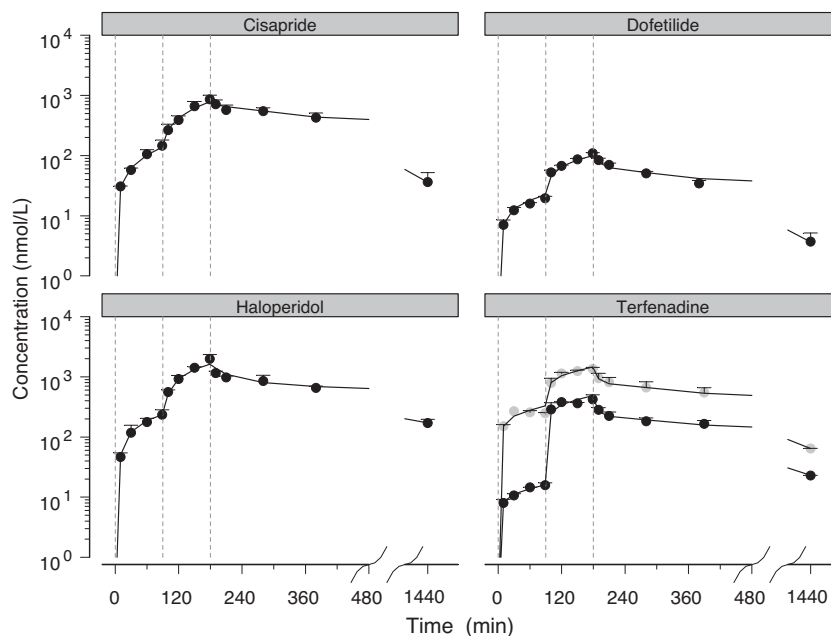


Fig. 4. Mean  $\pm$  SE concentration–time profiles (nM) for cisapride ( $n=7$ ), dofetilide ( $n=7$ ), haloperidol ( $n=7$ ), and terfenadine ( $n=5$ , black symbols corresponds to terfenadine low dose and gray symbols to terfenadine high dose). Lines indicate pharmacokinetic fit following parameters listed in Table 4. First dotted vertical reference line indicate start of infusion 1, the second indicate end of infusion 1 and start of infusion 2. The third line indicates end of infusion 2. See Methods section for information on doses.

treatment (test substance or vehicle), and the interaction between time and treatment as fixed effects and animal as random effect. Serial correlation was assumed to follow compound symmetry structure within each experiment.

### 3. Results

#### 3.1. Delay in QT interval response after an abrupt decrease in heart rate

In the investigated experiments the mean drop in heart rate was  $32 \pm 3$  bpm. The individual results and the mean QT interval maximal change and change half-life ( $t_{1/2}$ ) after an abrupt decrease in heart rate are presented in Table 1. After approximately 1 min 75% of the adaptation of the QT interval was achieved. After the process to exclude QT interval data collected 20 s prior to and 60 s following a highly variable heart rate, on average  $49 \pm 1\%$  of the vehicle data and  $51 \pm 2\%$  of the test substance data remained. These remaining data were

distributed over the entire collection period and the amount of data was large enough to allow a reliable assessment of the effects of the test substances on the QT interval. This was based on visual inspection of the complete data set vs. remaining data after exclusion.

#### 3.2. Estimation of the drug-induced change in QT intervals

In Table 2 the AIC obtained when fitting the eight models in Section 2.6 is shown. In a data set with many data points, as the present one, a difference in AIC of 58, such as can be seen when comparing Friedricia and Van de Water, is negligible. The fitting of the models deploys into three groups (Table 2); Bazett in one, Van der Water and Friedricia in a second, and finally the individual models in a third group.

The estimated relations are plotted in Fig. 3, where the data from one typical vehicle experiment and the fits of the eight models are presented. The models diverged mostly outside the RR interval range of 400 to 1300 ms, where there are very few

Table 3  
Mean  $\pm$  SE pharmacokinetic parameter estimates for cisapride, dofetilide, haloperidol, and terfenadine following two subsequent infusions of 90 min

Parameter		Cisapride	Dofetilide	Haloperidol	Terfenadine
CL	ml/min/kg	$3.8 \pm 0.6$ (42%)	$8.1 \pm 1.3$ (39%)	$10 \pm 1$ (18%)	$18 \pm 1$ (14%)
$CL_d$	ml/min/kg	$76 \pm 15$ (<1%)	$44 \pm 15$ (33%)	$38 \pm 2$ (<1%)	$100 \pm 13$ (40%)
$V_1$	l/kg	$0.33 \pm 0.10$ (32%)	$0.45 \pm 0.13$ (37%)	$2.5 \pm 0.3$ (22%)	$0.51 \pm 0.05$ (<1%)
$V_2$	l/kg	$1.1 \pm 0.2$ (32%)	$1.9 \pm 0.1$ (<1%)	$4.6 \pm 0.6$ (28%)	$7.3 \pm 0.5$ (21%)
Intraindividual variation	%	18	19	21	18
$t_{1/2, \text{ initial}}$	min	$2.4 \pm 0.3$	$5.3 \pm 0.3$	$27 \pm 2$	$3.0 \pm 0.3$
$t_{1/2, \text{ terminal}}$	min	$278 \pm 20$	$244 \pm 28$	$542 \pm 33$	$351 \pm 12$

Interindividual variability is shown in brackets.

For dose details, see Methods section. Abbreviations: CL, Clearance;  $CL_d$ , Intercompartmental distribution parameter;  $V_1$ , volume of the central compartment;  $V_2$ , volume of the peripheral compartment;  $t_{1/2}$ , half life.

Table 4  
Drug-induced effect on the QT interval (ms; mean±SE) the last 30 min of infusion of doses 1 and 2, calculated using the six models introduced and corrected for heart rate, pretreatment level, vehicle effect, and serial correlation

Formula	Cisapride		Dofetilide		Haloperidol		Terfenadine high		Terfenadine low	
	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2
Mean plasma concentration (nM)	125±3	761±71	18±1	97±5	207±21	1701±126	252±17	1295±95	15±1	389±21
$\beta_A = \beta_{vehicle}$	7.5±1.3	11.8±2.9	27.9±3.6	47.9±5.5	13.8±2.3	30.5±4.4	6.9±3.3	3.9±5.1	2.1±0.9	3.3±2.1
$\beta_B = \beta_{vehicle} + \beta_p I_p$	7.3±1.2	12.3±3.1	27.7±3.4	48.2±5.6	14.0±2.3	32.9±4.7	6.6±3.4	5.3±4.5	2.2±1.0	3.8±1.8
$\beta_C = \beta_t$	8.0±1.0	12.3±3.0	28.6±3.4	48.3±5.4	15.3±2.3	29.9±4.3	8.1±3.3	4.7±5.7	2.2±1.2	3.2±2.2
$\beta_D = \beta_t + \beta_{tp} I_p$	8.6±1.1	13.4±3.2	27.4±2.8	48.1±5.2	15.7±2.4	32.3±4.6	8.2±3.4	5.6±5.2	2.7±1.4	4.1±1.8
$\beta_E = \beta_t + \beta_{tc} C$	8.3±1.1	12.3±3.3	30.9±4.1	54.0±7.4	15.6±2.7	30.7±5.5	8.2±3.5	4.6±6.0	1.8±1.1	4.5±1.3
$\beta_F = \beta_t + \beta_{tp} I_p + \beta_{tc} C$	8.2±1.2	12.2±3.8	28.9±3.0	51.2±6.1	15.4±2.8	30.8±5.5	7.2±3.9	2.8±6.4	1.8±1.1	4.9±0.5

Mean±SE plasma concentrations (nM) are estimated for similar intervals based on observed data.

For dose details see the Methods section. Abbreviations:  $\beta_{vehicle}$ , regression coefficient for the corresponding vehicle treatment;  $I_p$ , an indicator variable equal to 1 during placement in pen (approximately 1600 to 0600 h) and 0 otherwise;  $C$ , plasma concentration of the test substance;  $\beta_t$ , regression coefficient for the studied treatment  $t$ ;  $\beta_{tp}$ , regression coefficient indicating the difference in slopes between laboratory and pen for treatment  $t$ ;  $\beta_{tc}$ , change in slope caused by one unit increase of the plasma concentration of the test substance.

observations (RR interval 400 to 1300 ms corresponds to a heart rate of about 40 to 150 bpm, Fig. 3). The two models with three parameters (models 7–8) showed the smallest AIC, indicating the best fit of the models to the data. However, both models are complex and for one animal the models did not converge. The linear model (model 5) was chosen because of its good performance and appealing simplicity in correcting the QT interval for heart rate.

When fitting the models to the data obtained over the last 30 min of the infusion of the second dose of test substance administration, the goodness of fits were similar to those obtained when data originating from the vehicle experiments were examined (results not shown).

The exploratory investigation of the possible effects of the placement of the animal and/or time of day on the slope of the linear relationship between QT interval and heart rate showed that the estimated slope while the dogs were in the pen was about 30% lower than the slope of the relation when the dogs were in the laboratory.

### 3.3. Plasma concentrations of test substances

A standard two-compartment model best described all the concentration–time profiles. For each substance, the mean plasma concentrations and the fit of the model are shown in Fig. 4. No significant differences between female and male dogs

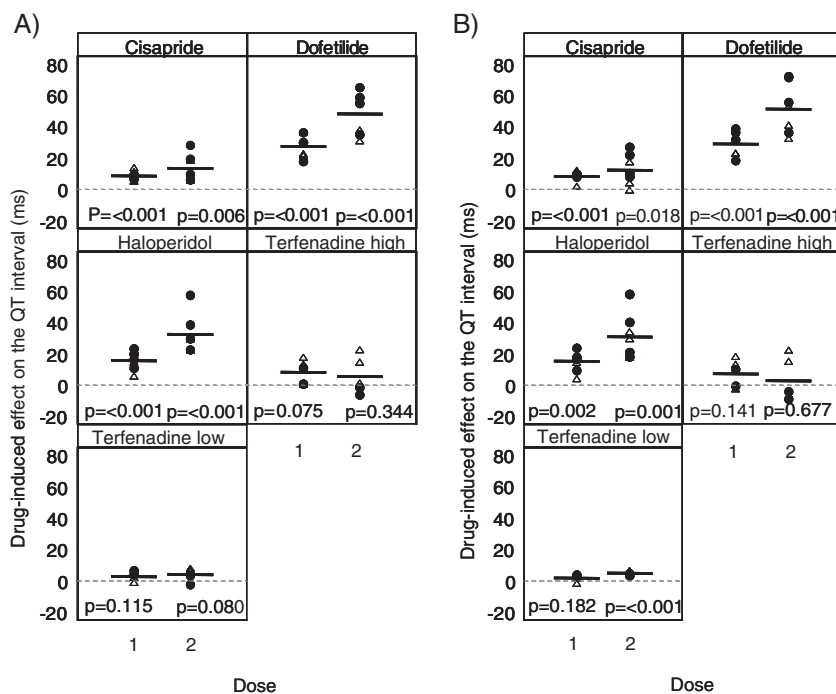


Fig. 5. Drug-induced effects on the QT interval (ms) estimated for the last 30 min of infusions 1 and 2, respectively. For dose details, see Methods section. Circles denote males, triangles denote females, and solid lines denote averages. Panels A and B describe results obtained when using slopes D and F ( $\beta_D$  and  $\beta_F$ ) in the correction of the QT interval, respectively. Dotted line indicates zero effect.  $P$ -values given in the figure corresponds to the mean effect of both male and female dogs compared to zero effect.

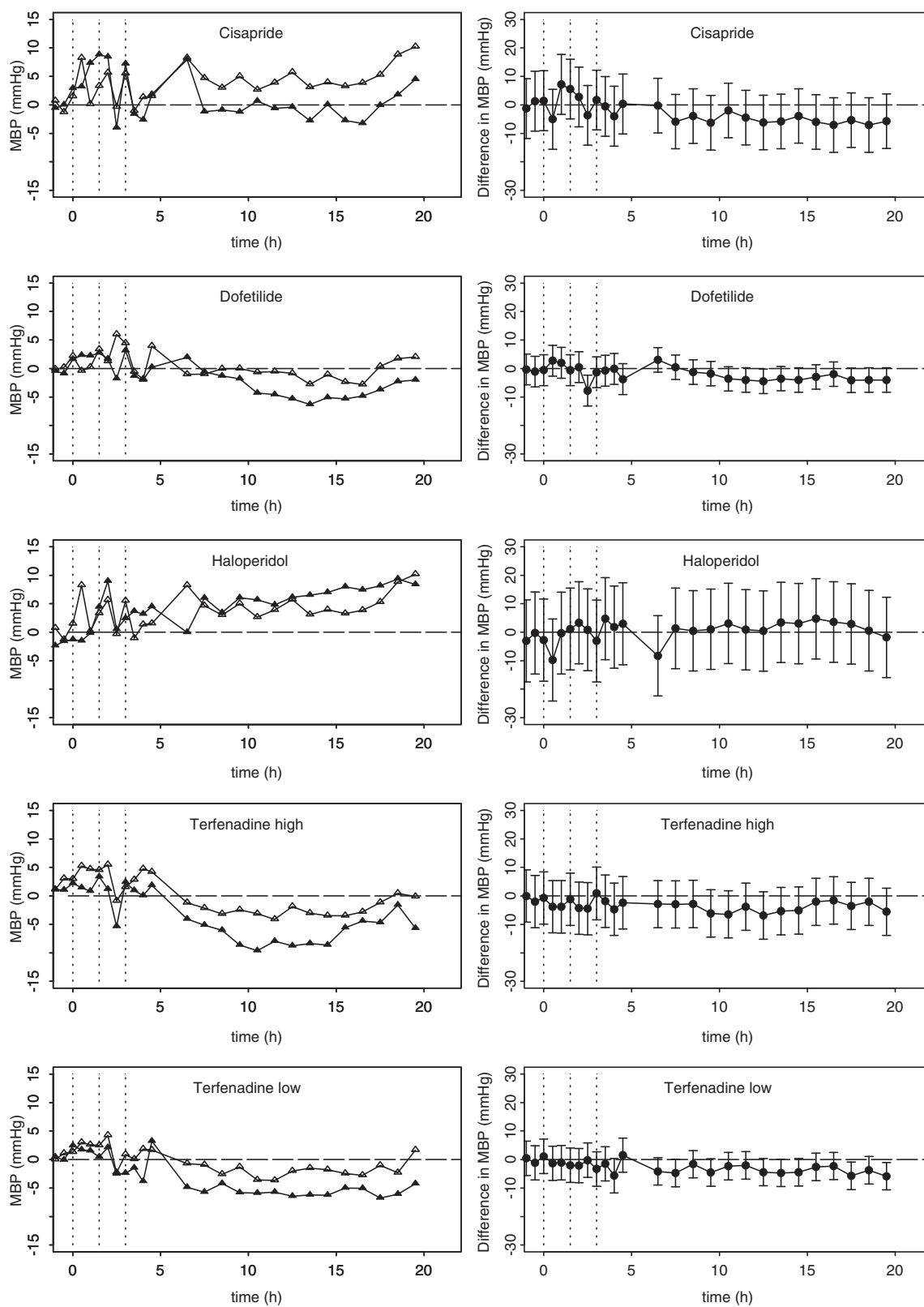


Fig. 6. Change (mm Hg) from pretreatment in mean blood pressure (MBP). Panels on the left show averages for the dogs with vehicle (hollow triangles) and test substance (solid triangles). The panels on the right show the treatment effect as the differences at comparable time points between test substance and vehicle and 95% confidence intervals. Averages were calculated over 30-min periods for the first six hours and 60-min periods afterwards. Dotted lines indicate start of infusion 1 (0 min), end of infusion 1 and start of infusion 2 (90 min), and end of infusion 2 (180 min). See Methods section for information on doses.

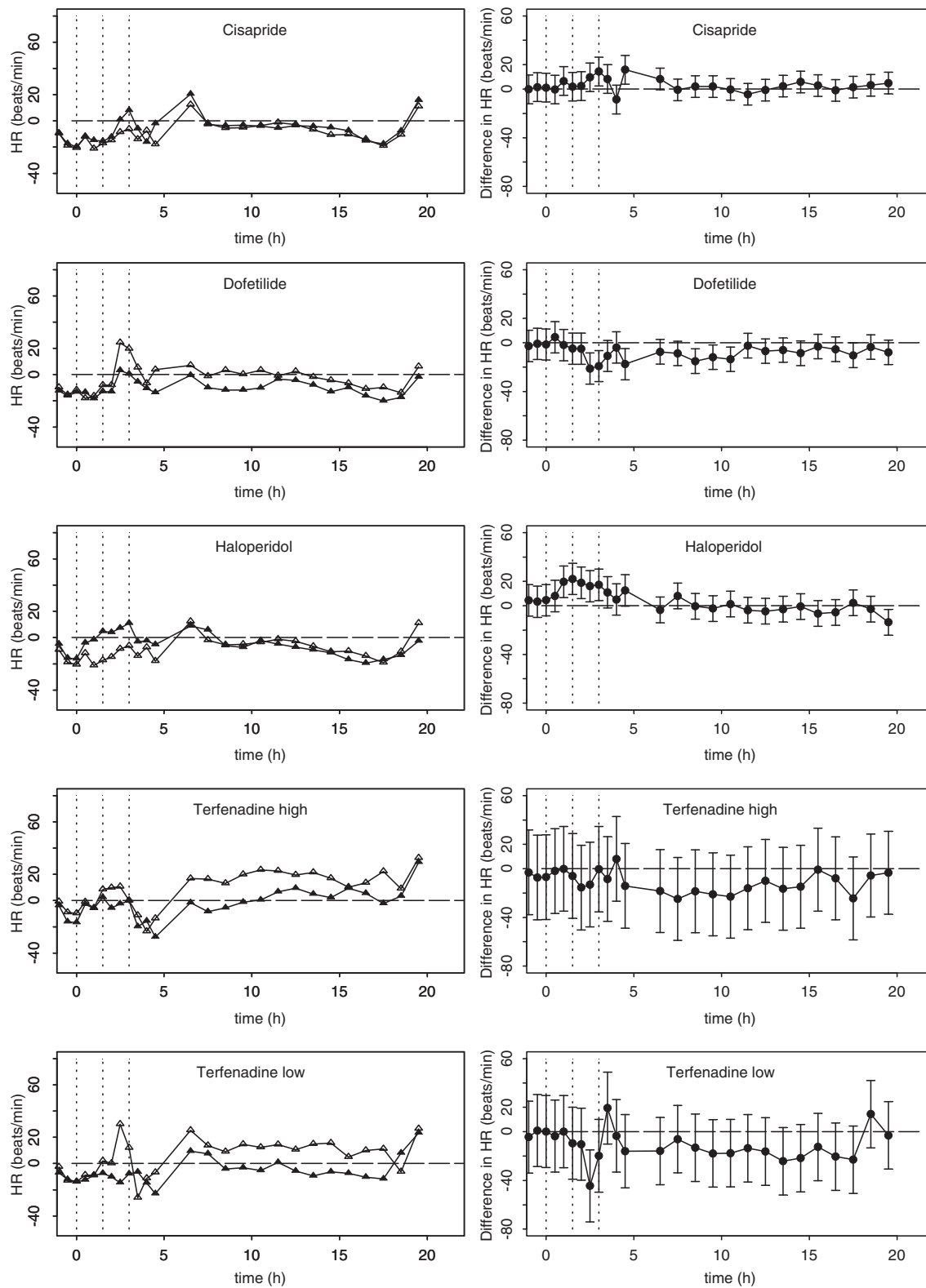


Fig. 7. Change from pretreatment in heart rate (HR). Panels on the left show averages for the dogs with vehicle (hollow triangles) and test substance (solid triangles). The panels on the right show the treatment effect as the differences between test substance and vehicle and 95% confidence intervals. Averages were calculated over 30-min periods for the first 6 h and 60-min periods afterward. Dotted lines indicate start of infusion 1 (0 min), end of infusion 1 and start of infusion 2 (90 min), and end of infusion 2 (180 min). See Methods section for information on doses.

were observed, for which reason the mean parameter estimates for all dogs for each substance are listed in Table 3. In Table 4 the mean plasma concentration for the last 30 min of infusions of doses 1 and 2 for all test substances are given. The average total  $C_{\max}$  values (mean  $\pm$  SE; at end of infusion two) for the cisapride, dofetilide, haloperidol, and terfenadine high- and low-dose experiments were  $859 \pm 104$ ,  $109 \pm 7$ ,  $1987 \pm 198$ ,  $1346 \pm 149$ , and  $423 \pm 33$  nM, respectively. The unbound  $C_{\max}$  values (mean; at end of infusion two) for the cisapride, dofetilide, haloperidol, and terfenadine high- and low-dose experiments were calculated to approx. 43, 50, 159, 27 and 8 nM, respectively.

### 3.4. Drug-induced effect on the QT interval

The pretreatment level of the QT interval for all experiments was  $239 \pm 1$  ms (mean  $\pm$  SE) and did not differ substantially between the experiments. As can be seen, the slopes yield similar estimates (Table 4) and there is no obvious pattern with regard to which slope produces the highest and lowest estimates of the drug-induced effect on the QT interval. For certain drug experiments all slopes yield similar results (e.g. Cisapride, Dose 2) while for other experiments the estimated effect is quite different depending on the slope used (e.g. Terfenadine High, Dose 2). Fig. 5 shows the individual data of the drug-induced effect on the QT interval using slope  $\beta_D$  or  $\beta_F$ . There were no differences between the male and female dogs in the QT interval response after cisapride, dofetilide, and haloperidol. After terfenadine, there was a tendency for a smaller effect in the female dogs, though this was not statistically significant.

Cisapride, dofetilide, and haloperidol administration resulted in a significant and sustained increase in the drug-induced QT interval during the last 30 min of the first and second infusions. Dofetilide gave the most pronounced effect, with an increase in the mean QT interval of about 50 ms at unbound plasma concentration of about 45 nM (Table 4). Terfenadine did not induce significant changes in the QT interval during the last 30 min of infusion one in either the high- or low-dose experiments. However, if the information about the plasma concentration of terfenadine was utilized when estimating the slope used in the individual linear model employed to calculate the drug-induced QT interval in the last 30 min of infusion two in the low-dose terfenadine experiments, a small, significant effect on the QT interval was observed (Table 4 and Fig. 5). Terfenadine also induced a delayed response of 29 ms in the low-dose experiment and a 39 ms increase in the QT interval in the high-dose experiment. The maximum increase in the QT interval caused by terfenadine occurred approximately 6 h after the end of drug infusion at estimated plasma concentrations of 130 and 360 nM in the terfenadine low- and high-dose experiments, respectively.

### 3.5. The QRS complex, blood pressure and heart rate

The duration of the QRS complexes varied between  $46 \pm 1$  and  $48 \pm 1$  ms for all test substance and vehicle experiments at all periods investigated. There was no effect on the duration of the QRS complex after any of the drugs tested.

The mean ( $\pm$ SE) pretreatment level of heart rate for all experiments was  $81 \pm 2$  bpm. The effect of test substances on blood pressure and heart rate are presented in Figs. 6 and 7, respectively, as point estimates and 95% confidence intervals. For 5–8 h following administration of test substances there was a tendency towards a decreased blood pressure and heart rate compared to vehicle for all test substances except for haloperidol (Figs. 6 and 7) and for cisapride as regards heart rate (Fig. 7).

## 4. Discussion

This study presents a novel approach to data processing of the QT interval response obtained from the conscious telemetered dog model commonly used in preclinical safety pharmacology studies. QT interval data after abrupt changes in heart rate were excluded from the analysis due to the delay in QT interval response. In this study an individually fitted linear relationship between QT interval and heart rate was found to be suitable to correct the QT interval for heart rate dependence.

There is an ongoing debate concerning the best practice to fulfill the ICH guidelines S7A and S7B (2000, 2005) in regards to experimental protocol and species used in the assessment of drug-induced QT interval prolongation (Bass, Tomaselli, Bullingham, & Kinter, 2005; Friedrichs, Patmore, & Bass, 2005; Valentin, Bass, Atrakchi, Olejniczak, & Kannosuke, 2005). In a survey distributed to pharmaceutical companies worldwide showed that the majority of the in vivo assessment of QT interval prolongation is conducted in dogs (Friedrichs et al., 2005). The present study focus on the analysis of the QT interval data to enhance the quality in the data reported through use of individual correction and exclusion of QT interval data after abrupt changes in heart rate.

In this study, a slow intravenous administration regime is employed to gradually obtain the appropriate plasma exposure, followed by a washout period, mimicking the gradual increase in plasma levels after oral administration. In addition, the slow intravenous infusion gives rise to less variation in exposure compared to oral dosing and also grants the possibility to terminate the infusion in case of serious adverse effects.

It is important to have a stable heart rate when assessing drug effects on the QT interval. A delay in QT interval response after abrupt changes in heart rate has been reported previously (Franz et al., 1988; Lau et al., 1988). In the present study a 19 ms difference was found between the first reported QT interval immediately after an abrupt decrease in heart rate and that after full adaptation. There is a risk of under/overestimating the QT interval response after substance administration if the abrupt changes of the heart rate are not taken into consideration, especially if a limited amount of data is available. In humans, Lau et al. (1988) found that the time lag to reach a new steady state QT interval after an increase or a decrease in heart rate was independent of the magnitude of the rate change and the baseline heart rate from which the change occurred. Consequently, the effect of the different magnitude of heart rate change (Fig. 1) and individual variation in baseline from which it occurred probably has a limited impact on the results of the present study.

In humans the QT interval requires 2–3 min to adapt to changes in heart rate (Franz et al., 1988; Lau et al., 1988). In the conscious paced dog complete adaptation was reached in 2–3 min after an increase in the RR interval (Pladys et al., 2001). In the present study 90% adaptation was reached after 1.5 min in the conscious dogs with sinus rhythm. The availability of an automated technique for recording the ECG variables allows simultaneous and long-term monitoring of the QT interval and heart rate over several hours. The resulting quantitative data made it possible to exclude the data retrieved during the adaptation of the QT interval to changes in heart rate without compromising the quality of the data set. In this study, the delay in the response of the QT interval following an abrupt decrease in heart rate was investigated since it has been shown that it takes longer time for the QT interval to adapt to the new level after a decrease in heart rate than after an increase (Lau et al., 1988).

Numerous approaches have been proposed to normalize the QT interval for variations in heart rate. The QT interval correction formulas proposed by Bazett (1920) and Fridericia (1920) are widely used in preclinical and clinical studies. However, these standard correction formulas have limitations in comparison with newer approaches (e.g., Davis & Middleton, 1999; Fossa, DePasquale, Raunig, Avery, & Leishman, 2002; Hanton, Nahas, Priou, Rabemampianina, & Baneux, 2001; Malik, 2001; Malik, Farbom, Batchvarov, Hnatkova, & Camm, 2002; Raunig et al., 2001; Watanabe & Miyazaki, in press). Recent studies suggest different optimal relations between the QT and RR intervals or between the QT interval and heart rate. The common feature of most of the recent approaches is finding an individual relationship between QT interval and heart rate, in contrast to the standard correction formulas of, for example Bazett, Fridericia, and Van de Water. In this study, models of the QT interval/heart rate relationship that uses individual correction described the QT interval/heart rate relationship much better than the traditional correction formulas.

Parameters as e.g. the animal model and/or the individual animal might influence the observed data. Thus, an estimated relationship that fits the data from an experiment might not represent the best fit for data obtained using another experimental protocol or individual. This may explain why the majority of published studies suggest slightly different relationships. The choice made in this study was to use a simple and individually estimated linear relationship between the QT interval and heart rate and to use this to correct the QT interval for changes in heart rate.

In addition to changes in heart rate, factors such as placement of the dog, the time of day, and serial correlation in data should be taken into consideration when correcting the QT intervals and estimating the drug-induced effect on the QT interval. As in this study, the serial correlation should be modeled in a way that suits the animal model, where, for example, the frequency of data sampling and stability of the measured parameter are considered (Fitzmaurice, Laird, & Ware, 2004).

It was noted that the QT–heart rate relationship changed during the experiment in response to alteration in the level of exposure to the test substance, indicating a frequency-dependent

effect of the substances. A larger drug-induced effect on the QT interval at lower heart rates has been observed in several studies and is a well-known phenomenon called reverse frequency dependence. This effect has been shown to be pronounced after administration of agents that block the  $I_{Kr}$  channel (Okada, Ogawa, Sadanaga, & Mitamura, 1996) and is supposed to contribute to the proarrhythmic effects seen for such compounds (Drolet, Khalifa, Daleau, Hamelin, & Turgeon, 1998; Hondeghem, Carlsson, & Duker, 2001; Usui et al., 1998). Six individual linear models for estimating the QT interval–heart rate relationship were employed when calculating the drug-induced effect on the QT interval data from test substance experiments. The models incorporating the concentration in the correction are intuitively preferable to the others since they include more information. The period where the plasma concentration of the test substances is high and the major effect on the QT/RR interval relationship is present is relatively short in relation to the total time of the experiment. Consequently, measurements made when the plasma concentration is relatively low have a larger weight since they are more numerous and the true drug-induced effect on the QT interval can be underestimated. When knowledge of the plasma concentration is available, this information should, if possible, be used in the correction of the QT interval for changes in heart rate.

In humans cisapride causes a dose-related QT interval prolongation at therapeutic concentrations (Van Haarst et al., 1998). In this study cisapride prolonged the QT interval by 8 and 12 ms at plasma concentrations of about 1.5- and 9-fold the unbound therapeutic plasma concentration (3–5 nM), respectively. As expected, the selective and potent  $I_{Kr}$  inhibitor dofetilide caused the largest increases in QT interval (29 and 51 ms at about 4- and 30-fold the unbound therapeutic concentration (2 nM), respectively). At exposures of approximately 4- and 38-fold the total therapeutic concentration (45 nM), haloperidol caused prolongations of the QT interval by 15 and 31 ms, respectively, which is in accordance with earlier studies (e.g. Satoh, Sugiyama, Tamura, & Hashimoto, 2000).

When information about plasma concentration of terfenadine was utilized to estimate the slope in the individual linear model employed to calculate the drug-induced QT interval, the second dose in the low-dose terfenadine experiments gave a significant mean increase of 4–5 ms. However, delayed and larger increases in the QT interval (29 and 39 ms for the low- and high-dose terfenadine experiments, respectively) were found about 6 h after the end of drug administrations (at exposures of about 10- and 25-fold the unbound therapeutic concentration). This is in accordance with earlier observations in dogs. In a study from Laine, Perez, Dubreuil, and Gillet (1998) a delayed response was observed after high terfenadine exposure, where the increase in the QT interval was evident after 5–6 h. The delay in QT interval prolongation found in the present study could be due to the high exposure level of terfenadine during and immediately after the infusion, whereas after 6 h the decreasing exposure affected primarily the  $I_{Kr}$  channel and not inhibition of counteracting ion channels such as  $I_{Na}$  and  $I_{Ca}$  ( $IC_{50}$  approx. 0.9 and 0.15  $\mu$ M respectively; Ming & Nordin, 1995; Lu & Wang, 1999; Liu, Melchert, & Kennedy, 1997). This assumption was not

supported by the present study, where a lower dose of terfenadine (terfenadine low dose) was used to obtain lower plasma concentrations also during the infusion periods. In these experiments the same delayed effect in QT interval prolongation was observed. Other factors that may contribute to this significant delay in QT interval response such as a delay in tissue distribution or metabolism cannot be excluded.

The knowledge of the plasma concentration (exposure) could be used to further interpret the QT interval data through pharmacokinetic–pharmacodynamic analysis. The assessment of the individual concentration–effect relationship and confounding factors such as delayed response (i.e., hysteresis) to drugs will provide a better prediction of the safety profiles of new drug candidates (Nolan et al., 2006; Ollerstam et al., 2006). Application of this analysis to the data from this study would be relevant.

In conclusion, this study presents a novel approach to the data processing of the QT interval response obtained from the conscious telemetered dog model commonly used in preclinical safety pharmacology studies. QT interval data after abrupt changes in heart rate should be excluded from the analysis due to delay in the QT interval response. QT interval–heart rate relationship and vehicle response were individual-specific and corrections including covariates should therefore be made individually using a linear model. In addition, it is considered advantageous to include plasma concentration data in obtaining the slope in the linear model to estimate the drug-induced change in the QT interval. Significant drug-induced increases in the QT interval were detected for all test substances investigated.

## Acknowledgements

We would like to thank Jenny Söderström, Annica Hägg, Caroline Sundqvist, Linnea Sjödin, Nina Ahlström, and Malin Antonsson for skilled technical assistance and animal procedures.

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