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ZILPATEROL HYDROCHLORIDE page 157-213

Toxicological evaluation of certain veterinary drug residues in food

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ZILPATEROL HYDROCHLORIDE

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1. EXPLANATION

Zilpaterol hydrochloride (zilpaterol HCl, Chemical Abstracts Service No. 119520-06-8) is a β_2 -adrenoceptor agonist used for promoting body weight gain, feed efficiency and carcass muscle ratio in cattle fed in confinement before slaughter. There are four enantiomers of zilpaterol HCl: (6*R*,7*R*), (6*R*,7*S*), (6*S*,7*R*) and (6*S*,7*S*). The product in use, which has the code name RU 42173, is racemic *trans* zilpaterol HCl, a mixture of the (6*R*,7*R*) and (6*S*,7*S*) enantiomers; it will be referred to as zilpaterol HCl in this report.

Zilpaterol HCl exhibits the characteristics of a β_2 -adrenergic agonist, inducing relaxation of the muscles of the bronchi, an increase in heart rate and a decrease in diastolic blood pressure (Fichelle & Plassard, 1984; Plassard et al., 1985; Filloux, 1994; Corbier & Petit, 1999a). Zilpaterol HCl also produces the biochemical effects of stimulation of the β -adrenergic receptors, including increased lipolysis and reduced lipogenesis in adipose cells and increased protein synthesis and reduced proteolysis in striated muscle fibres (Kern et al., 2009).

The only use of zilpaterol HCl is as a veterinary drug in cattle. The recommended dose added to cattle feed is 7.5 mg/kg (on a 90% dry matter basis) of the total daily ration during the last 20–40 days of the feeding period before slaughter. This level in the feed is equivalent to approximately 0.15 mg/kg body weight (bw) per day or 60–90 mg/animal per day. Where use is authorized, a withdrawal period ranging from 2 to 4 days is applied.

Zilpaterol HCl has not previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Committee evaluated zilpaterol HCl at the current meeting at the request of the Twentieth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (FAO/WHO, 2012). The Committee was asked to establish an acceptable daily intake (ADI) and recommend maximum residue limits (MRLs) for zilpaterol HCl in cattle tissue.

The Committee considered the results of studies on blood protein binding in vitro, pharmacokinetics, pharmacological effects in vitro and in vivo, acute, short-term and long-term toxicity, genotoxicity, reproductive and developmental toxicity, and relay pharmacology, as well as observations in humans. The majority of the studies were performed in accordance with good laboratory practice (GLP).

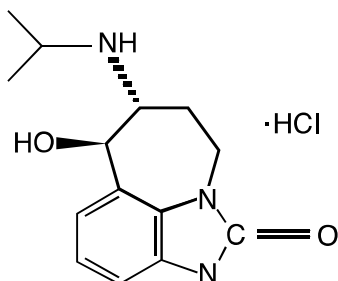
The structure of zilpaterol HCl is shown in [Fig. 1](#).

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Blood protein binding in vitro

The binding of zilpaterol HCl to blood proteins was investigated with blood serum collected from rats, dogs and humans. Blood serum from fasted male Sprague-Dawley rats (pooled sample from 30 rats weighing about 200 g) or from fasted male Beagle dogs (pooled sample from four dogs weighing about 10.7 kg) was loaded with [^{14} C]zilpaterol HCl (radiopurity > 97%) at final concentrations of

Fig. 1. Structure of zilpaterol HCl

$C_{14}H_{19}N_2O_2 \cdot HCl$ (molecular weight: 297.783 g/mol)

Synonyms: RU 42173 corresponds to racemic *trans* zilpaterol HCl, a mixture of the (6*R*,7*R*) and (6*S*,7*S*) enantiomers

0.005, 0.05, 0.5, 10 and 100 µg/mL. After dialysis to the equilibrium point, the binding percentage was calculated from the concentrations measured by counting the radioactivity (liquid scintillation counting). The average binding to serum proteins of rats and dogs was 14% and 15%, respectively, at 37 °C (Tremblay, Biechler & Cousty, 1990a, 1990b).

The characteristics of the binding of [^{14}C]zilpaterol HCl (radiopurity 95%; tested concentrations 7.4–29.8 µg/mL) to the various serum proteins were assessed in vitro using human blood elements. Pooled human serum from healthy volunteers was used, and the protein binding was assessed by equilibrium dialysis using either the serum or isolated proteins (human serum albumin, α_1 -glycoprotein, lipoproteins – very low density lipoprotein, low-density lipoprotein or high-density lipoprotein – and γ -globulins). Binding to erythrocytes was also determined using either erythrocytes in buffer or whole blood. In human serum, the binding percentage was constant at $14.9 \pm 0.8\%$, and binding was non-saturable, with a binding coefficient of 0.158 ± 0.005 . The test item did not bind to α_1 -glycoprotein, lipoproteins or γ -globulins. The non-saturable binding to human serum albumin in phosphate buffer (pH 7.4) was $14.1 \pm 1.5\%$, with a binding coefficient of 0.152 ± 0.002 . Binding of [^{14}C]zilpaterol HCl to erythrocytes was the same, in the range 7.4–29.8 µg/mL, regardless of the concentration tested, with a total erythrocyte fraction of $55.1 \pm 3.3\%$ and a binding constant of 1.57 ± 0.02 . In the presence of plasma, the total erythrocyte fraction was $55.2 \pm 3.6\%$, demonstrating that zilpaterol HCl is not retained by the plasma compartment. The results show that zilpaterol HCl binds to human serum protein with only a weak affinity and in a non-saturable way, and its affinity for erythrocytes is moderate (Bree, N'Guyen & Tillement, 1987).

2.1.2 Absorption, distribution, metabolism and excretion

(a) Rats

In a non-GLP study, the absolute oral bioavailability of zilpaterol HCl was determined in 12 male Sprague-Dawley rats weighing about 202 g and administered a single dose of [^{14}C]zilpaterol HCl (radiopurity > 98%). Each of six animals per

group was administered a dose of [^{14}C]zilpaterol HCl in saline at 1 mg/kg bw by either the intravenous or oral (gavage) route. Animals were fasted 21 hours before and 6 hours after dosing. Combined urine samples were collected from two rats per cage from 0 to 24 hours post-dosing, and then unchanged zilpaterol was determined by thin-layer chromatography following solid/liquid-phase microcolumn extraction. The amount of unchanged parent compound eliminated in urine over 24 hours was $27.7 \pm 0.6\%$ of the dose after intravenous dosing and $27.5 \pm 0.6\%$ of the dose after oral administration. Therefore, the absolute oral bioavailability calculated on the basis of urinary excretion was 99.3% (Tremblay et al., 1990a).

A GLP-compliant study was conducted to compare the pharmacokinetics of zilpaterol HCl in plasma after a single administration or administration for 2 weeks by the oral route. Zilpaterol HCl was administered at two different dose levels (0.055 and 1.10 mg/kg bw) by admixture in the diet or by gavage to Sprague-Dawley rats about 8 weeks of age and with an average body weight of 272 g (males) or 213 g (females). Two groups, each with 15 animals of each sex per dose, received the test item via dietary admixture, and two other groups, each with 20 animals of each sex per dose, received the test item by gavage (in water), at the respective dose levels. The purity of the test item was 100.6%. Blood samples were collected at various times at the beginning and end of the single or 2-week administration period. Animals were observed at least once daily for clinical signs or mortality/morbidity, and feed consumption and body weight were recorded twice per week. Plasma free zilpaterol was assayed using a validated radioimmunoassay with a limit of quantification of 0.02 ng/mL. No deaths or clinical signs were observed at any time throughout the study, and treatment had no impact on either feed consumption or body weight. Area under the plasma concentration–time curve over 24 hours ($\text{AUC}_{24\text{ h}}$) and peak concentrations in plasma (C_{max}) were generally 2–4 times higher in females than in males, but were 8-fold higher in females than in males in the low-dose group administered the compound in the diet for 2 days. Treatment via single gavage resulted in shorter time to reach C_{max} (T_{max}) (0.25–0.75 hour versus 3–23 hours) and higher $\text{AUC}_{24\text{ h}}$ (1.5–4 times) and C_{max} (3–13 times) compared with the dietary admixture. Repeated administration for 2 weeks had no change in these pharmacokinetic parameters, other than a reduction in T_{max} (3–7 hours) in animals receiving the compound in their diet. The mean C_{max} values were approximately proportional to the dose in males. In females, a higher variation was observed for C_{max} , and the C_{max} /dose ratio was lower at 1.10 mg/kg bw per day than at 0.055 mg/kg bw per day. The mean $\text{AUC}_{24\text{ h}}$ /dose ratio was higher (approximately 1.8–3.9 times) via gavage than via dietary admixture (Sauvez, 1995).

In a non-GLP experiment, groups of 10 fasted Sprague-Dawley rats (five animals of each sex per group) weighing about 203 g were administered [^{14}C]zilpaterol HCl dissolved in saline at 1 mg/kg bw once orally by gavage (radiopurity 93.6–98.9%). The organs of the treated animals were collected 0.5 and 24 hours after dosing to measure the distribution of radioactivity as a function of time. Concentrations of radioactive material in all tissues were substantially lower at 24 hours than at 0.5 hour, demonstrating that the radioactivity was in the elimination phase at 24 hours, and there was no tissue retention of radioactivity. Tissue/plasma concentration ratios (males: skin, eyes, sex organs, erythrocytes, adrenals and liver; females: subcutaneous fat and ovaries) at 24 hours increased

significantly compared with those at 0.5 hour, reflecting incomplete distribution at 0.5 hour for these organs. There was no sex difference except for tissue/plasma concentration ratio in subcutaneous fat at 0.5 hour, which was higher in females than in males, and tissue/plasma concentration ratios in muscle and kidneys at 24 hours, which were higher in males than in females (Tremblay et al., 1989).

Sprague-Dawley (CrI CS (SD) BR) rats about 6 weeks of age at study initiation (six animals of each sex per group) were administered zilpaterol HCl (purity 99.2%) dissolved in water orally by gavage at a dose of either 0.05 or 1 mg/kg bw per day for 13 consecutive weeks. Toxicokinetic evaluation was performed on days 1 and 92. Toxicokinetic analyses demonstrated the test item in all sampled animals, proving intestinal absorption of the compound. A mean apparent $T_{\max}$ of 1 hour was found in both treated groups. Mean $C_{\max}$ values were 1.14 and 3.72 ng/mL in males (days 1 and 92, respectively) and 2.73 and 4.48 ng/mL in females (days 1 and 92, respectively) treated with 0.05 mg/kg bw per day. These values were 28.9 and 39.6 ng/mL in males and 32.3 and 85.4 ng/mL in females given 1 mg/kg bw per day, respectively. For both tested doses, the half-life ranged from 2.4 to 5.5 hours, and zilpaterol levels were below the limit of quantification at 24 hours after dose administration. Total AUC values on days 1 and 92 were, respectively, 6.21 and 10.36 ng·h/mL in males and 9.75 and 14.59 ng·h/mL in females treated with 0.05 mg/kg bw per day. In the high-dose group, these values were, respectively, 93 and 187 ng·h/mL in males and 296 and 433 ng·h/mL in females, reflecting a higher systemic exposure in females than in males, together with a slight accumulation of the test item, with $C_{\max}$ being 1.5–3.5 times higher on day 92 than on day 1, and $T_{\max}$ being 1.5–2 times greater. Total AUC values were approximately proportional to dose in male rats. In females, the AUC value was higher in the high-dose group than would be expected from a strictly linear relationship (Sauvez, 1994b).

In a study performed in compliance with GLP, six adult Wistar rats weighing 200–250 g (three of each sex) were administered a single dose of [^{14}C]zilpaterol HCl (radiochemical purity > 98%) at 0.2 mg/kg bw by the oral route in a semi-synthetic diet. The metabolite profile in urine and faeces was determined using high-performance liquid chromatography (HPLC) with radiometric detection, and structural determination of metabolites was performed by mass spectrometry. In 8 days, rats eliminated an average of 48.9% of the dose in the urine and 42.3% in the faeces, with no sex-related differences, although males eliminated 37% of the dose in urine over the first 24 hours, compared with 20% for females. The metabolite profile in the urine collected during the first 2 days after dosing showed the parent compound and five metabolite peaks. In urine samples from days 1 and 2, respectively, unchanged zilpaterol represented 60.3% and 46.7% of the total radioactivity. The other metabolites in urine samples collected on days 1 and 2, respectively, were deisopropyl zilpaterol, 19.5% and 14.2%, acetylated deisopropyl zilpaterol, 4.4% and 4.2%, the glucuronide conjugate of hydroxy-zilpaterol, 9.3% and 22.3%, and two unidentified metabolites (B and C), each accounting for less than 2.3% of the radioactivity. In the liver and carcass, residue levels were less than 0.1% and 1% of the dose, respectively (Tulliez, 2000a).

In a GLP study, six adult Sprague-Dawley rats (three of each sex) weighing 200–250 g were administered a single dose of [^{14}C]zilpaterol HCl (radiochemical purity > 98%) at 0.2 mg/kg bw by the oral route in a semi-synthetic diet. The metabolite

profile of zilpaterol HCl in urine and faeces was established by HPLC with online radiometric detection, and structural determination of metabolites was performed by mass spectrometry. Over 8 days, male rats eliminated 55.6% and 40.9% of the administered dose in urine and faeces, respectively. In females, the values were 46.0% and 46.5%, respectively. Parent compound and six metabolites were found in urine, unchanged zilpaterol being the predominant component; the other metabolites were deisopropyl zilpaterol, acetylated deisopropyl zilpaterol, hydroxy-zilpaterol, a glucuronide conjugate of hydroxy-zilpaterol and two unidentified peaks. No single metabolite accounted for more than 8.4% of the radioactivity present in the urine. In faeces, parent compound accounted for less than 10% of radioactivity. The major component present was hydroxy-zilpaterol, which accounted for 60–80% of the radioactivity detected. In the liver, residue levels were less than 0.1% of the administered dose after 8 days (Tulliez, 2000b).

In a non-GLP study aiming to determine the metabolism of zilpaterol HCl, 10 adult Wistar rats (five of each sex) were administered a single oral dose of [^{14}C]zilpaterol HCl (radiochemical purity 98.5%) at 0.2 mg/kg bw by gavage. One male and one female were terminated at each of 12 hours and 48 hours for determination of radioactive concentration in tissues. The remaining six animals were kept in metabolism cages for measurement of elimination rates in urine and faeces (in one male and one female, for which daily collections of urine and faeces were made), as well as for measurement of the radioactivity concentration in tissues (three males and three females), 8 days after dosing. About 91% of the dose was eliminated in urine (49%) and faeces (42%) over 8 days, with the greatest elimination occurring during the first 48 hours (63% of the dose). The highest residue concentrations were about 35 and 6 $\mu\text{g/kg}$ in liver and kidney (mean of males and females), respectively, from rats killed 12 hours after dosing. Radioactivity was below the limit of detection in perirenal fat and muscle on day 8. Less than 1% of the radioactivity administered remained in the carcass after 8 days. In the liver collected 12 hours after dosing, bound radioactivity represented about 85% of the total hepatic radioactivity. Four metabolites and unchanged zilpaterol were found in urine samples by radio-HPLC. Metabolite identities were determined in a separate study by mass spectrometry. The main metabolites in urine were deisopropyl zilpaterol, hydroxy-zilpaterol and unchanged zilpaterol, accounting, respectively, for 1.9%, 8.8% and 81.3% of the radioactivity present. Unchanged zilpaterol (44.2% of the radioactivity present), deisopropyl zilpaterol (7.4%) and hydroxylated zilpaterol (40.4%) were the only compounds detected in faecal samples (Zalko, 1993).

An intraspecies (rat) comparison of metabolites, based on the above-described studies, is provided in [Table 1](#).

Six groups of 36 Wistar rats (3 males and 3 females per dose group) were fed for 3 weeks with feed supplemented with zilpaterol HCl at 0, 1 or 10 mg/kg. Animals were then killed on day 28, and microsomes were prepared from the livers. The microsomes were used to assess hepatic microsomal metabolism of zilpaterol HCl and testosterone. Livers from further groups of three male Wistar rats treated with various liver mono-oxygenase enzyme inducers (dexamethasone, phenobarbital, 3-methylcholanthrene and clofibrate) or with corn oil were also

Table 1. Metabolites in urine collected for 24 hours after single oral dosing of rats

Metabolite	Mean (males and females) percentages of the extractable radioactivity		
	Rat (Wistar)	Rat (Sprague-Dawley) ^a	
(A) Acetylated deisopropyl zilpaterol	Not detected ^b	4.35 ^c	Not detected
(B) Unidentified	Not detected ^b	2.24 ^c	Not detected
(C) Hydroxy-zilpaterol glucuronide	Not detected ^b	9.26 ^c	Not detected
(D) Unidentified	Not detected ^b	6.50 ^c	Not detected
(E1) Deisopropyl zilpaterol	1.9 ^b	19.48 ^{c,d}	4.3
(E2) Hydroxy-zilpaterol	8.8 ^b		1.6
(F) Zilpaterol	81.3 ^b	60.28 ^c	89.6

^a Data from Tulliez (2000b).^b Data from Zalko (1993).^c Data from Tulliez (2000a).^d Metabolites E1 and E2 were not resolved.

used for preparation of microsomes. Following incubation of zilpaterol HCl with the hepatic microsomal fraction, unchanged zilpaterol, deisopropyl zilpaterol and hydroxy-zilpaterol were identified. The metabolites were shown to be the products largely, if not entirely, of cytochromes P450 (CYP); deisopropyl zilpaterol seemed to be formed preferentially by members of the CYP1A subfamily, whereas hydroxy-zilpaterol was formed less specifically, possibly involving members of the CYP2B subfamily. Treatment with zilpaterol HCl for 3 weeks had no effect on the hepatic microsomal metabolism of this compound in either males or females. Similarly, hepatic microsomal metabolism of testosterone remained unaffected by 3 weeks of treatment with zilpaterol HCl, and there was no effect on total microsomal P450 content (Zalko, 1993).

(b) Dogs

In a non-GLP study, the absolute oral bioavailability of zilpaterol HCl was determined in four fasted male Beagle dogs with an average body weight of 10.0 kg. [¹⁴C]Zilpaterol HCl (radiopurity > 98%) dissolved in saline was administered by either the intravenous or the oral (gavage) route at a dose of 1 mg/kg bw. Urine samples were then collected from 0 to 48 hours post-dosing. Unchanged zilpaterol was determined by coincidence of the ultraviolet and radioactive traces by HPLC or thin-layer chromatography and then quantified by liquid scintillation counting. The amount of unchanged parent compound eliminated in urine in 48 hours after intravenous dosing was 22.8 ± 2.1% of the administered dose. After oral administration, the amount eliminated in urine was 23.9 ± 2.4%. Hence, the absolute oral bioavailability calculated on the basis of urinary excretion was 100% (Tremblay et al., 1990b).

The absorption, elimination and bioavailability of zilpaterol HCl in rats and dogs are presented in [Table 2](#).

Table 2. Estimated bioavailability^a and elimination after a single oral dose administered to rats and dogs

Parameters	Rat	Dog
Dose (mg/kg bw)	0.055 ^b , 0.2 ^{c,d} , 1 ^e , 1.10 ^b	1 ^f
Absolute oral bioavailability	99.3% ^e	100% ^f
$C_{\max}$ (ng _{eq} /mL)	2.35–115 ^b	No data
$T_{\max}$ (h)	0.25–0.75 ^b	No data
% of the dose eliminated in 8 days: urine	48.9% ^c , 49% ^d	No data
% of the dose eliminated in 8 days: faeces	42.3% ^c , 42% ^d	No data

$C_{\max}$: peak plasma concentration; eq: equivalent; $T_{\max}$: time to reach $C_{\max}$

^a Relative to an intravenous dose.

^b Sprague-Dawley rat (data from Sauvez, 1995).

^c Wistar rat (data from Tulliez, 2000a).

^d Wistar rat (data from Zalko, 1993).

^e Sprague-Dawley rat (data from Tremblay et al., 1990a).

^f Beagle dog (data from Tremblay et al., 1990b).

(c) Humans

In a clinical study, nine healthy male volunteers aged 28–55 years and weighing 56–76 kg were orally administered zilpaterol HCl (1 mg/mL in 2 mL ampoule, added into drinking-water) at single doses of 0.25, 0.5, 1.0 or 2.0 mg/adult to assess tolerance of zilpaterol HCl. Adults of each group were fasted and then received either the test item (increasing doses) or placebo, according to a single-blind protocol using a four-way cross-over design. Four subjects received 0.25 mg and five subjects received 0.5 mg; then, after a 7-day wash-out period, the next higher doses were administered. Blood samples were collected at 15 minutes, then at 1, 2, 3, 4, 5, 6, 8 and 24 hours after dosing. Plasma samples were assayed for zilpaterol using a radioimmunoassay method. The $T_{\max}$ was 1 hour, and the half-life was 4–5 hours at all doses. $C_{\max}$ and AUC values increased linearly with dose. The pharmacokinetic parameters are presented in Table 3 (Sutton & Budhram, 1987; Tremblay & Mouren, 1988).

Table 3. Plasma pharmacokinetic parameters of zilpaterol HCl in human healthy volunteers after a single oral dose

Parameters	Dose of zilpaterol HCl administered			
	0.25 mg/adult	0.5 mg/adult	1.0 mg/adult	2.0 mg/adult
$C_{\max}$ (ng/mL)	0.98 ± 0.16	2.28 ± 0.23	4.47 ± 0.33	8.56 ± 0.89
AUC _{0–∞} (ng·h/mL)	6.93 ± 0.20	12.3 ± 1.6	26.1 ± 1.7	51.7 ± 5.6
$T_{\max}$ (h)	1	1	1	1
$t_{1/2}$ (h)	4.81 ± 0.97	3.69 ± 0.34	4.15 ± 0.37	4.14 ± 0.26
Regression between $C_{\max}$ and the dose	$C_{\max} = 4.26 \times \text{dose} + 0.11$			

AUC: area under the plasma concentration–time curve; $C_{\max}$: peak plasma concentration;

$t_{1/2}$: half-life; $T_{\max}$: time to reach $C_{\max}$

Source: Sutton & Budhram (1987); Tremblay & Mouren (1988)

2.1.3 Effects on enzymes and other biochemical parameters

Many *in vitro*, *ex vivo* and *in vivo* studies have clearly demonstrated the β_2 -agonist effect of zilpaterol HCl. Those effects are manifested as contraction of the musculature of the cardiovascular system, as well as relaxation of the smooth muscles of the vasculature and the bronchi. The pharmacological properties and characterization of zilpaterol HCl and its main metabolite, deisopropyl zilpaterol (free base or hydrochloride form), were assessed in a series of *in vitro*, *ex vivo* and *in vivo* assays.

In a GLP study, the affinity of zilpaterol HCl and its main metabolite, deisopropyl zilpaterol (as free base or hydrochloride form), was determined for rat cortex β_1 -adrenoceptor and rat lung β_2 -adrenoceptor using *in vitro* receptor binding assays. The tested concentrations ranged from 10^{-10} to 10^{-4} mol/L. The reference agonist compounds were alprenolol and propranolol, respectively, for β_1 - and β_2 -adrenoceptors. Ligands were [^3H]CGP-26505 (0.6 nmol/L) for β_1 -adrenoceptor and [^{125}I]iodocyanopindolol (0.05 nmol/L) for β_2 -adrenoceptor. The purity of the test items was 100.6%, 100.3% and 97.5%, respectively, for zilpaterol HCl, deisopropyl zilpaterol HCl and deisopropyl zilpaterol free base. Zilpaterol HCl and its main metabolite displayed low affinity for β_1 -adrenoceptors (median inhibitory concentration [IC_{50}]: $2.1\text{--}2.8 \times 10^{-5}$ mol/L, compared with the IC_{50} of alprenolol: 2.0×10^{-8} mol/L). Regarding the β_2 -adrenoceptor, the affinity of the parent compound (IC_{50} : 6.9×10^{-6} mol/L) was about 1.5-fold higher than that of its main metabolite (Table 4) (Filloux, 1994).

The agonist activity of zilpaterol HCl, deisopropyl zilpaterol HCl and deisopropyl zilpaterol free base on β -adrenoceptor-coupled adenylyl cyclase was investigated using guinea-pig lung membranes, by measuring the amount of ^{32}P -labelled cyclic adenosine monophosphate synthesized. Tested concentrations ranged from 10^{-7} to 10^{-3} mol/L for each compound, and the positive control was isoproterenol. Antagonist activity was similarly determined, against isoproterenol-induced adenylyl cyclase activation (concentration 10^{-6} mol/L). Propranolol was used as control. The results demonstrated that zilpaterol and deisopropyl zilpaterol are only partial agonists at the β -adrenoceptor in terms of adenylyl cyclase activation (7–41% of basal level compared with 90–95% induced by isoproterenol; 8–22% basal activity remaining when used as an antagonist compared with 0–2% with propranolol) (Filloux, 1994).

Table 4. Affinity of zilpaterol HCl and deisopropyl zilpaterol (as free base or hydrochloride form) for rat cortex β_1 -adrenoceptors and lung β_2 -adrenoceptors

	IC_{50} (mol/L)			Reference agonist
	Zilpaterol HCl	Deisopropyl zilpaterol HCl	Deisopropyl zilpaterol free base	
β_1 -adrenoceptor	2.8×10^{-5}	1.8×10^{-5}	2.1×10^{-5}	2.0×10^{-8} (alprenolol)
β_2 -adrenoceptor	6.9×10^{-6}	1.0×10^{-5}	1.0×10^{-5}	1.7×10^{-9} (propranolol)

Source: Filloux (1994)

In a GLP study, the binding of zilpaterol HCl, deisopropyl zilpaterol HCl and deisopropyl zilpaterol free base to rat adrenoceptors (α_1 and α_2) and dopaminergic receptors (D_1 and D_2) was investigated. The affinity of the compounds for all of the receptors was very low ($IC_{50} \geq 50 \mu\text{mol/L}$), suggesting that there would be no pharmacological effects of the compounds via these receptors (Arbogast, Gillis & Church, 1995a, 1995b).

In a non-GLP study, the β_2 -adrenergic activity of zilpaterol HCl was investigated ex vivo using isolated guinea-pig trachea. In this assay, widely used for β_2 -adrenoceptor agonist activity testing, trachea were pretreated with propranolol at concentrations ranging from 10^{-9} to 10^{-7} mol/L for 30 minutes, then contracted with carbachol at a concentration of 3×10^{-7} mol/L. After contractions reached a plateau, zilpaterol HCl was tested by cumulative addition. The agonistic effects of zilpaterol HCl were inhibited competitively by propranolol, when the pA_2 (a measure of the affinity of the antagonist for its receptor) of propranolol determined with zilpaterol HCl was 9.07. The pA_2 of propranolol determined with zilpaterol HCl was comparable to that determined in the laboratory under the same conditions with isoprenaline. The pA_2 of propranolol determined with isoprenaline was 9.27. The results demonstrate the β_2 -adrenergic agonist activity of zilpaterol HCl (Advenier, 1987).

In a set of three in vivo non-GLP studies, anaesthetized, pithed Sprague-Dawley rats (males, 12–15 weeks old, 6–11 per group) were administered zilpaterol HCl or its main metabolite, deisopropyl zilpaterol (free base or hydrochloride form), intravenously (avoiding any potential low intestinal resorption and potential first-pass effect) at cumulative doses of 0, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 (deisopropyl zilpaterol + antagonists only) mg/kg bw. Compounds were administered alone or a few minutes after administration of well known and well characterized adrenoceptor antagonists, propranolol (β -adrenoceptor antagonist) at 1 mg/kg bw, atenolol (β_1 -adrenoceptor antagonist) at 2.5 mg/kg bw or 5 mg/kg bw, ICI 118,551 (β_2 -adrenoceptor antagonist) at 1 mg/kg bw, phentolamine (α -adrenoceptor antagonist) at 1 mg/kg bw or domperidone (peripheral dopamine receptor antagonist, studies with zilpaterol HCl only) at 0.1 mg/kg bw. Zilpaterol HCl induced a dose-dependent decrease in diastolic blood pressure from 0.01 mg/kg bw, with a maximum effect observed at a dose of 0.1 mg/kg bw. Tachycardia was observed from 0.3 mg/kg bw, with a maximum effect at a dose of 3 mg/kg bw. Both the vascular and cardiac effects induced by zilpaterol HCl could be abolished with propranolol. The vascular effects were blocked by ICI 118,551, but not by atenolol. Atenolol had little or no effect on the blood pressure response to zilpaterol HCl, but antagonized the effects on heart rate at high doses. Phentolamine and domperidone had no effect on either the vascular or cardiac response to zilpaterol HCl. Deisopropyl zilpaterol (as either its free base or hydrochloride form) dose-dependently decreased the blood pressure from 0.1 mg/kg bw, with a steady-state maximum effect observed at 0.3 mg/kg bw. In contrast, deisopropyl zilpaterol had no effect on the heart rate. The vascular effects of deisopropyl zilpaterol were antagonized by propranolol and by ICI 118,551, but not by atenolol or by phentolamine.

The in vivo results demonstrate that deisopropyl zilpaterol displays 10-fold lower β_2 -agonist activity on the cardiovascular system, compared with the parent compound. Moreover, the use of the specific β_2 -adrenoceptor antagonist

Table 5. In vivo activity of zilpaterol HCl and deisopropyl zilpaterol (as the free base or hydrochloride form) in pithed rat

Parameter	Compound	ED _x (mg/kg bw) ^a	Dose at which effect reaches maximum (mg/kg bw) (effect size)
Decreased diastolic blood pressure	Zilpaterol HCl	0.01	0.1 (–20.6 ± 1.9 mmHg ^b)
	Deisopropyl zilpaterol HCl	0.12	1 (–20.0 ± 0.8 mmHg)
	Deisopropyl zilpaterol free base	0.12	1 (–23.0 ± 1.8 mmHg)
Increased heart rate	Zilpaterol HCl	0.3	3 (+87.2 ± 4.5 bpm)
	Deisopropyl zilpaterol HCl	No effect at 10 mg/kg bw	None observed
	Deisopropyl zilpaterol free base	No effect at 1 mg/kg bw	10 (+22.8 ± 13.1 bpm)

bpm: beats per minute

^a ED_x: ED_{DBP}10 = dose that will decrease diastolic blood pressure (DBP) by 10 mmHg (~20% of the initial basal values); ED_{HR}50 = dose that will decrease heart rate (HR) by 50 bpm (~20% of the initial basal values).

^b 1 mmHg = 133.3 Pa.

Source: Corbier & Petit (1999a, 1999b, 1999c)

ICI 118,551 demonstrated that effects on the vascular system are β_2 -adrenoceptor mediated, whereas activity on the heart is associated with the β_1 -adrenoceptor (Corbier & Petit, 1999a, 1999b, 1999c).

The in vivo effects are summarized in [Table 5](#).

In conclusion, the main pharmacological activity of zilpaterol HCl is mediated by the β_2 -adrenoceptor. Its main metabolite, deisopropyl zilpaterol, is also a β_2 -specific agonist, but with a potency in vivo about an order of magnitude less than that of the parent compound. Zilpaterol HCl binds very poorly, if at all, to α_1 - or α_2 -adrenoceptors or dopaminergic receptors (D_1 and D_2), with high inhibitory constants, reflecting its lack of significant pharmacological effect via these receptors.

2.2 Toxicological studies

2.2.1 Acute toxicity

(a) Single-dose toxicity studies

The results of single-dose toxicity studies performed with zilpaterol HCl, its main metabolite, deisopropyl zilpaterol (free base form), and the final product (premix ZILMAX 4.8%) are summarized in [Table 6](#). Most of the studies were GLP compliant, and the purity of zilpaterol HCl was greater than 98%, whereas that of deisopropyl zilpaterol was greater than 97.5%. When ZILMAX was tested, the batches were compliant with the release specifications. The results show rather low toxicity of zilpaterol HCl.

Table 6. Single-dose toxicity of zilpaterol HCl, deisopropyl zilpaterol and zilpaterol HCl 4.8% premix

Species (strain)	Sex (no. of animals)	Route	Test article	Dose (mg/kg bw)	LD ₅₀ (mg/kg bw) ^a	Reference
Mouse (Swiss CD-1)	M (10) F (10)	Oral	Zilpaterol HCl	0, 200, 350, 500, 650	M: 430 (360–490) F: 580 (495–800)	Fournex, Collas & Audegond (1985a)
Mouse (Swiss OF1)	M (5) F (5)	Oral	Zilpaterol HCl	0, 385, 500, 650, 845, 1 100	M: 430 (170–530) F: 680 (520–785)	Catez et al. (1994b)
Mouse (Swiss CD-1)	M (10) F (10)	Intraperitoneal	Zilpaterol HCl	M: 0, 145, 170, 195 F: 0, 110, 145, 195, 265	M: 155 (150–165) F: 170 (150–190)	Fournex, Collas & Audegond (1985d)
Mouse (Swiss OF1)	M (5) F (5)	Oral	Deisopropyl zilpaterol	0, 650, 845, 965, 1 100	M: 1 065 F: 965–1 100 M & F: 1 030 (975–1 100)	Catez et al. (1994a)
Mouse (Swiss OF1)	M (5) F (5)	Oral	Zilpaterol HCl 4.8% premix	2 000	M & F: > 2 000	Catez et al. (1996a)
Rat (SD)	M (10) F (10)	Oral	Zilpaterol HCl	0, 312, 625, 1 250, 2 500, 5 000	M: 1 325 (880–2 020) F: 890 (600–1 275)	Fournex, Collas & Audegond (1985b)
Rat (SD)	M (10) F (10)	Intraperitoneal	Zilpaterol HCl	M: 0, 150, 250, 350, 450 F: 0, 150, 200, 250	M: 280 (225–330) F: 225 (205–240)	Fournex, Collas & Audegond (1985c)
Rat (SD)	M (5) F (5)	Inhalation	Zilpaterol HCl	5.04 mg/L of air	M & F: LC ₅₀ (4 h) > 5.04 mg/L	Coombs (1998)
Rat (SD)	M (5) F (5)	Dermal	Zilpaterol HCl	2 000	M & F: > 2 000	Manciaux (1998)
Rat (SD)	M (5) F (5)	Oral	Zilpaterol HCl 4.8% premix	2 000	M & F: > 2 000	Catez et al. (1996b)

bw: body weight; F: female; LC₅₀: median lethal concentration; LD₅₀: median lethal dose; M: male; SD: Sprague-Dawley

^a 95% confidence intervals provided in parentheses.

In Swiss CD-1 mice, a single oral administration of zilpaterol HCl at 200, 350, 500 or 650 mg/kg bw in 0.5% methyl cellulose via oral gavage induced apathy, hypomotility, hunched back, breathing or respiratory difficulties, slight locomotor difficulties and piloerection. The clinical signs persisted on the day following dosing. Mortality occurred within 0.25–4 hours of dosing. Animals found dead exhibited moderate congestion of the lungs, stomach and intestines. No abnormalities were observed in animals necropsied on day 14. The median lethal dose (LD_{50}) values were 430 (95% confidence interval [CI]: 360–490) and 580 (95% CI: 495–800) mg/kg bw for males and females, respectively (Fournex, Collas & Audegond, 1985a).

In Swiss OF1 mice, clinical signs of toxicity were observed during the 36- to 48-hour period following treatment with a single oral dose of zilpaterol HCl at 385, 500, 650, 850 or 1100 mg/kg bw, administered by gavage in 0.5% methyl cellulose. The clinical signs included dyspnoea, moderate to marked difficulties in walking, hypomotility and prostration. In addition, tremors and convulsions preceding death were recorded in the first 30 minutes following gavage. Mortality occurred within 0.25–3 hours of dosing. Animals found dead exhibited moderate congestion of the lungs, stomach and intestines. The LD_{50} values were 430 (95% CI: 170–530) and 630 (95% CI: 520–785) mg/kg bw for males and females, respectively (Catez et al., 1994b).

In Swiss CD-1 mice, a single intraperitoneal injection of zilpaterol HCl at 145, 170 or 195 mg/kg bw (males) or 110, 145, 195 or 265 mg/kg bw (females) in 0.5% methyl cellulose induced hypotonia or apathy, hypomotility, hunched back, respiratory difficulties, slight locomotor difficulties and tremor during the 24-hour period following treatment. Mortality occurred immediately after injection in animals receiving the highest dose. The LD_{50} values were 155 (95% CI: 150–165) and 170 (95% CI: 150–190) mg/kg bw for males and females, respectively (Fournex, Collas & Audegond, 1985d).

Deisopropyl zilpaterol, a major metabolite of zilpaterol, administered orally once to Swiss OF1 mice by gavage in 0.5% methyl cellulose at 650, 845, 965 or 1100 mg/kg bw caused hypomotility, moderate to marked difficulties in walking, dyspnoea (from 845 mg/kg bw), apathy or lethargy (1100 mg/kg bw) during the 24-hour period following treatment. Mortality occurred within 3 hours after administration of 1100 mg/kg bw in females and on day 2 for other animals. No abnormalities were observed in animals necropsied on day 14 except for congested appearance of the lung (1100 mg/kg bw). The LD_{50} s were 1065 (confidence interval could not be calculated) and 965–1100 (confidence interval could not be calculated) mg/kg bw for males and females, respectively, with a median for males and females combined of 1030 (95% CI: 975–1100) mg/kg bw, indicating that the main metabolite, deisopropyl zilpaterol, displays an acute toxicity of about half that of the parent compound (Catez et al., 1994a).

A single oral dose of zilpaterol HCl administered to Sprague-Dawley rats by gavage in 0.5% methyl cellulose at 312, 625, 1250, 2500 or 5000 mg/kg bw induced hypomotility, hunched back, breathing or respiratory difficulties, slight locomotor difficulties and piloerection during the 24 hours following treatment. Mortality occurred within 0.75–5 hours of dosing. Animals found dead exhibited moderate

congestion of the lungs, profuse saliva around the snout and a large quantity of water in the stomach. No abnormalities were observed in animals necropsied on day 14. The LD₅₀ values were 1325 (95% CI: 880–2020) and 890 (95% CI: 600–1275) mg/kg bw for males and females, respectively (Fournex, Collas & Audegond, 1985b).

Clinical signs observed after a single intraperitoneal injection of zilpaterol HCl in 0.5% methyl cellulose to Sprague-Dawley rats at 150, 250, 350 or 450 mg/kg bw (males) or 150, 200 or 250 mg/kg bw (females) included hypotonia, hunched back and slight locomotor difficulties during the 24 hours following injection. Mortality occurred within 0.3–2 hours of dosing. Animals found dead exhibited moderate congestion of the lungs and liver. No abnormalities were observed in animals necropsied on day 14. The LD₅₀ values were 280 (95% CI: 225–330) and 225 (95% CI: 205–240) mg/kg bw for males and females, respectively (Fournex, Collas & Audegond, 1985c).

Sprague-Dawley rats were exposed to zilpaterol HCl by inhalation at an aerosol concentration of 5.04 ± 0.2 mg/L via nose only for 4 hours. The mass median aerodynamic diameter was 3.8 μ m, and the percentage of breathable particles ($< 7 \mu$ m) was 73%. There were no unscheduled deaths during the study. During exposure, clinical signs included exaggerated respiratory movements, gasping and wet fur. Immediately following exposure, clinical signs included exaggerated respiratory movements, partial closing of the eyes, whole body tremors and wet fur. In males, staggering, gasping and lethargy were reported. Matted fur and brown staining were observed in some of the animals for 1–5 days following treatment. Body weights and feed consumption were reduced in males only in the day following treatment. At necropsy, lung weight to body weight ratios were comparable between treated and control animals. One animal exhibited a slightly congested lung, and the majority exhibited discoloration of the tail. The 4-hour median lethal concentration (LC₅₀) for zilpaterol HCl was greater than 5.04 mg/L of air (Coombs, 1998).

The shaved dorsal area of Sprague-Dawley rats was treated with zilpaterol HCl at a dose of 2000 mg/kg bw. The test site was covered by a semi-occlusive dressing for 24 hours, after which the site was washed with water. Ocular secretion was observed in one female from day 9 to day 15. No other clinical signs and no cutaneous reactions were noted during the study. Body weight gain of the animals was reduced when compared with historical control animals (there were no concurrent controls). Three males lost weight between days 1 and 5, and two females lost weight between days 5 and 8. Macroscopic examination of the main organs of the animals revealed no apparent abnormalities. The dermal LD₅₀ of zilpaterol HCl was greater than 2000 mg/kg bw in rats (Manciaux, 1998).

Zilpaterol HCl 4.8% premix was administered once to Swiss OF1 mice and Sprague-Dawley rats orally by gavage. Because of the granularity of the preparation, it had to be ground before administration and was delivered as an aqueous suspension in 0.5% methyl cellulose of 50 mg/mL in four batches of 10 mL/kg bw (mice) or 100 mg/mL in two batches of 10 mL/kg bw (rats) over 2 hours. No deaths and no clinical signs were observed. The LD₅₀ was greater than 2000 mg/kg bw for both mice and rats (Catez et al., 1996a, 1996b).

(b) *Dermal irritation*

In a GLP-compliant study, the skin irritation potential of zilpaterol HCl was tested in three adult male hybrid New Zealand White rabbits using 0.5 g of pure material administered as a thin layer to 6 cm² of shaved dorsal skin. The material was covered with a semi-occlusive dressing for 4 hours, after which the site was washed with water. No signs of cutaneous irritation were observed over 72 hours. There were no clinical signs noted or abnormal changes in body weight. Zilpaterol HCl was non-irritating to the skin (Catez et al., 1996c).

(c) *Ocular irritation*

In a GLP-compliant study, the ocular irritation potential of zilpaterol HCl was tested in three adult male hybrid New Zealand White rabbits using 0.1 g of pure material administered once to the right eye via the base of the conjunctival sac. Unrinsed cornea showed moderate opacity in 2/3 animals and slight opacity in 1/3 animals by 24 hours, with a recovery in 3/3 animals by 96 hours. The area involved was slight in 3/3 rabbits at 24 hours and 1/3 rabbits at 48 hours and had disappeared at 72 hours. The iris presented slight congestion at 1 hour in 1/3 animals. No reaction was observed at 24–72 hours. Conjunctiva showed slight redness and discharge at 1 hour and 24 hours after dosing in 3/3 animals. Zilpaterol HCl is slightly irritating to the eye (Catez et al., 1996d).

(d) *Dermal sensitization*

A GLP-compliant study was performed in the guinea-pig, using the maximization test of Magnusson and Kligman, to assess the potential of zilpaterol HCl to induce delayed-contact hypersensitivity. Male and female guinea-pigs (5 animals of each sex for the control group, 10 animals of each sex for the treated group) were used. On day 1, they were intradermally injected with Freund's complete adjuvant containing zilpaterol HCl (10% weight per weight [w/w]) or sterile isotonic saline solution. The purity of zilpaterol HCl was 100.1%. On day 7, lauryl sulfate was topically applied on the same site to induce skin irritation. The day after, the test item (10% w/w in saline) or control vehicle (saline) was topically applied under occlusion for 48 hours. Twelve days later, animals were challenged by topical application of zilpaterol HCl (10% w/w in saline) on the right flank. The left flank served as control and received the vehicle only. Application sites were maintained under occlusive condition for 24 hours, and skin reactions were evaluated and scored about 24 and 48 hours later. Animals were then terminated.

For the whole study duration, there were no clinical signs or deaths observed. There was no well defined cutaneous reaction observed after the challenge test, and zilpaterol HCl displayed no cutaneous reaction attributable to sensitization potential. Zilpaterol HCl was not a skin sensitizer in guinea-pigs (de Jouffrey, 1996).

2.2.2 *Short-term studies of toxicity*

A comprehensive set of short-term toxicity studies was performed with zilpaterol HCl in laboratory species, including rodents (rats and mice), Beagle dogs, Cynomolgus monkeys, Yucatan microswine and cattle (the target species). The

pivotal studies were performed in compliance with GLP, with appropriate standards for protocols and conduct.

(a) *Mice*

Zilpaterol HCl (purity 100.1%) was administered to Swiss CD-1 mice (10 animals of each sex per group) by the oral route (dietary admixture) at 0, 0.2, 2, 4 or 40 mg/kg feed (equal to 0, 46, 460, 895 and 8867 µg/kg bw per day for males and 0, 47, 483, 986 and 9601 µg/kg bw per day for females, respectively) for 4 weeks. In addition, two satellite groups of 32 animals of each sex treated orally with zilpaterol HCl via feed containing either 0.2 or 40 mg/kg were included for toxicokinetic purposes. The concentration of the test substance in the diet was modified weekly to maintain the nominal dose levels. Animals were observed daily for clinical signs and mortality. Feed consumption, body weights and achieved doses were recorded weekly. Haematology and blood biochemistry analyses were performed just before necropsy. Levels of zilpaterol in plasma collected from satellite groups were analysed on days 2–3 and 24–25 during administration. At the end of the treatment period, animals were killed and subjected to complete macroscopic examination. Organs were weighed and preserved for any further microscopic examinations.

No mortality or clinical signs of toxicity were observed at any time throughout the study. Mean feed consumption was slightly, although not statistically significantly, increased (by 8–9%) in females in the 4 and 40 mg/kg feed groups over the last 3 weeks of treatment. Body weight gain in females was increased slightly, but statistically significantly, on weeks 2 and 3 of treatment at the highest dose, but returned to the control range by week 4 of treatment. Toxicokinetics revealed a $T_{\max}$ of about 23 hours in both males and females. Plasma levels of the test substance were 2.3- to 3.3-fold higher (0.2 mg/kg feed per day) and 1.3-fold higher (40 mg/kg feed) in males than in females. Mean $AUC_{0-24\text{ h}}$ values were 2.68–3.54 ng·h/mL and 1.07–1.16 ng·h/mL, respectively, in males and females of the 0.2 mg/kg feed group. In the 40 mg/kg feed dose group, $AUC_{0-24\text{ h}}$ values were 674–765 ng·h/mL and 518–601 ng·h/mL in males and females, respectively. The ratio $AUC/dose$ was similar at both doses in males, but was lower in females in the low-dose group. Treatment duration had no impact on toxicokinetic parameters. There was no effect of the treatment on haematology parameters or blood biochemistry. At necropsy, there were no macroscopic findings, and organ weights were unaffected.

There were no treatment-related findings. The no-observed-adverse-effect level (NOAEL) was 4 mg/kg feed (equal to 986 µg/kg bw per day), based on the transient increase in body weight observed in females at 40 mg/kg feed (equal to 9601 µg/kg bw per day) (Sauvez, 1996).

(b) *Rats*

Sprague-Dawley rats (five animals of each sex per group) about 6 weeks of age with weights ranging from 175 g to 204 g for males and from 154 g to 174 g for females were administered zilpaterol HCl daily by gavage in oral solution in

distilled water for 10 days at a dose of 0 or 50 mg/kg bw per day in a preliminary short-term toxicity study. Animals were observed daily for clinical signs or mortality. Feed consumption and body weight were monitored throughout the study. Haematology, blood biochemistry and urine analysis parameters were also monitored. At study termination, organs were collected, weighed and subjected to histopathological examination.

No mortality or clinical signs of toxicity were observed. Body weight gain following treatment of animals was higher than in control rats. This was more marked and statistically significant in females, although feed consumption was unaffected. Haematology revealed a significant decrease in platelet count (females, by 20%), mainly associated with 2/5 animals. Significantly decreased blood glucose (males and females) and a more marked triglyceride decrease (males only) were observed in treated animals. Alkaline phosphatase was significantly increased by approximately 50% in treated females, although other liver function tests were normal. Blood urea level was significantly increased in female rats, but remained within the normal physiological range. At necropsy, macroscopic examination revealed no abnormal findings. Absolute organ weights were not affected by treatment, although a number of relative organ weights were lower in treated animals, particularly in females. This was associated with the higher body weight of treated animals when compared with the control group. Histopathological examination revealed no abnormal findings. The results of this study were used to set the doses for the 30-day toxicity study in rats (Collas & Fournex, 1984).

Sprague-Dawley (CrI CS (SD) BR) rats about 8 weeks of age at study initiation (four animals of each sex per group) were administered zilpaterol HCl (purity 100.6%) orally via dietary admixture at a dose of 0 or 1 mg/kg bw per day for 15 consecutive days. Achieved doses were 0.9 and 1.1 mg/kg bw per day for females and males, respectively. Animals were observed daily for clinical signs of toxicity and mortality. Feed consumption and body weights were monitored twice a week; thereafter, achieved doses were calculated. At the end of the treatment period, animals were sacrificed. No macroscopic examination was performed.

No mortality or clinical signs of toxicity were observed throughout the study duration. Feed consumption was lower in treated animals; on a weekly basis, the mean difference (compared with untreated animals) ranged from -6% to -10%. There was no effect of the treatment on mean body weight. Zilpaterol HCl administered at a dose of 1 mg/kg bw per day (dietary admixture) induced slightly decreased feed consumption (Sauvez, 1994c).

Sprague-Dawley rats about 6 weeks of age at study initiation (10 animals of each sex per group) were administered zilpaterol HCl (purity > 98%) daily by oral gavage in distilled water at a dose of 0, 1, 10 or 100 mg/kg bw per day for 30 consecutive days. Animals were observed daily for clinical signs of toxicity and mortality. Feed consumption and water consumption were monitored, as well as body weight. Haematology, blood biochemistry and urine analysis parameters were also

monitored, together with ophthalmological examinations and electrocardiograms (ECGs). At termination, following necropsy, organs were collected, weighed and subjected to histopathological examination.

All animals survived, and no clinical signs were observed. Body weight gain of males treated with 10 and 100 mg/kg bw per day and of females receiving 100 mg/kg bw per day was significantly increased throughout the study, although feed consumption remained unaffected. Water consumption was slightly increased in females in the high-dose group on week 1 of treatment. There was no treatment-related effect on blood pressure, and the ECG revealed no major differences. A fall in heart rate was observed in a dose-dependent manner in all treated females (–10%, –11% and –20% at 1, 10 and 100 mg/kg bw per day, respectively). In males, heart rate was reduced (–7%) only in the high-dose group, and the difference was not statistically significant (the raw data on heart rate were not provided in the study report). Haematology revealed a slight, statistically significant increase in total leukocyte count (males, 100 mg/kg bw per day), a significant, dose-dependent, slight decrease in activated partial thromboplastin time in males only (all treated groups) and a minor, dose-dependent, slightly decreased prothrombin time in females (10 and 100 mg/kg bw per day). Some significant variations were observed for blood biochemical parameters (sodium, calcium, phosphorus, chloride, cholesterol, glucose and creatinine kinase), but these remained within normal limits, were not consistent between sexes and were not dose dependent. Proteinograms were normal for females. Males showed significant variations in some proteins, but there was no dose dependency. Males treated with 100 mg/kg bw per day showed significant decreases in α_2 - and β_1 -globulins. Liver function tests were normal in males. In females, liver function tests were also unaffected, other than a 2-fold increase in lactate dehydrogenase in the high-dose group. However, this increase in lactate dehydrogenase is attributed to haemolysed samples rather than the treatment with zilpaterol HCl. Blood urea level was slightly, although significantly, raised (by approximately 50%) in females treated with 100 mg/kg bw per day, but remained within the normal physiological range. Urine analysis revealed no abnormal findings, and the test for occult blood in faeces was negative.

There were no ocular lesions or any macroscopic findings at necropsy. Absolute weight of heart was increased in males (10 and 100 mg/kg bw per day, with no dose–response relationship) and females (all dose groups, with no relationship with dose at 1 mg/kg bw). Ovarian weight in the high-dose group was significantly higher than in controls. These differences were generally not observed for relative organ weights (other than for heart in low- and mid-dose females), but a statistically significant, non-dose-dependent decrease in liver weight was observed in all treated males. Relative brain weight was significantly decreased in high-dose males and females. Microscopic examination revealed no treatment-induced lesions. The few abnormalities observed were naturally occurring in rats.

The lowest-observed-adverse-effect level (LOAEL) was 1 mg/kg bw per day, the lowest dose tested, based on decreased heart rate in females and decreased activated partial thromboplastin time in males at all dose levels (Fournex et al., 1985).

Sprague-Dawley (CrI CD (SD) BR) rats about 6 weeks of age at study initiation (20 animals of each sex per group) were administered zilpaterol HCl (purity > 98%) in water orally via gavage at a dose of 0, 1, 10 or 100 mg/kg bw per day for 90 days. At completion of the treatment period, eight animals of each sex per group were kept without treatment for a 32-day reversibility period. Animals were observed daily for clinical signs of toxicity and mortality. Feed consumption and body weights were monitored weekly. Ophthalmological examination, ECG and blood pressure recording (1–3 hours after dosing) were performed before the first treatment, on weeks 4 and 12, and then at the end of the reversibility period. Haematology, blood biochemistry analysis and urine analysis were performed on weeks 5, 13 and 18 (end of reversibility period). At the end of the treatment and reversibility periods, animals were terminated and subjected to complete macroscopic examination. Selected organs were weighed, and microscopic examinations were performed on several organs and on all organs displaying macroscopic lesions.

One male (1 mg/kg bw per day) and one female (100 mg/kg bw per day) were found dead on days 79 and 78, respectively. No clinical signs other than hypersalivation (female) were observed prior to these deaths. One female of the control group died under anaesthesia (blood sampling, day 32), and another female of the high-dose group was found dead on day 77, previously displaying piloerection, emaciation, pallor of the extremities, dyspnoea and decreased activity. The causes of these deaths were not established with certainty. In surviving animals, dose- and treatment-related hypersalivation was observed in 9/20 males and 12/20 females from the 10 mg/kg bw per day dose group and in all animals from the high-dose group. Salivation was first observed between day 29 and day 37 in most affected animals receiving 10 mg/kg bw per day and between day 2 and day 13 in most high-dose animals. There was no other clinical sign associated with the test item. A dose-dependent, reversible, slightly increased body weight gain ($\leq 10\%$ in the high-dose group) was observed in all treated animals. This was statistically significant at 10 and 100 mg/kg bw per day in females and at 100 mg/kg bw per day in males. Feed consumption was significantly increased in males at 10 and 100 mg/kg bw per day. Feed efficiency remained comparable between treated and control animals. No ophthalmic or blood pressure abnormalities related to treatment were observed. A slight dose-dependent reduction of heart rate, associated with increased PQ and QaT conduction times, which was statistically significant at 12 weeks in the high-dose group only (-14% in males, -7% in females), was observed. Values remained, however, within commonly accepted limits for rats. Haematology revealed no variation of any toxicological significance. Blood urea levels were significantly increased in mid- and high-dose males and in all treated groups of females at week 5 and in all treated groups of males and females at week 13. Plasma creatinine levels were significantly increased at 5 weeks in mid- and high-dose males and females and at 13 weeks in high-dose males and in mid-dose females. Although plasma creatinine levels were increased in high-dose females, changes were not significant. Urine analysis revealed significant increases in urine volume in males at 5 and 13 weeks and significant increases in pH, specific gravity and urine volume at 5 and 13 weeks. Some of these changes were apparent in all dose groups. They were due to variations in a few animals per dose group.

However, no significant differences were observed in any of these parameters following the period of reversibility. There was no treatment-related abnormality on either macroscopic or microscopic examinations. Absolute and relative weights of the kidneys were unaffected by treatment, other than an increase in absolute weights in high-dose females. Relative weights of some organs were slightly, but significantly, decreased in high-dose males (thyroid, thymus, brain) and in mid- and high-dose females (liver, uterus, heart). All variations regressed at the end of the reversibility period.

The LOAEL was 1 mg/kg bw per day, the lowest dose tested, based on increased blood urea in females at all doses (Moysan, 1989).

In a follow-up study, Sprague-Dawley (CrI CD (SD) BR) rats about 6 weeks of age at study initiation (six animals of each sex per group) were administered zilpaterol HCl (purity 99.2%) in water orally by gavage at a dose of 0, 0.05, 0.5 or 1 mg/kg bw per day for 4 consecutive weeks. The doses were based on the results from the previous 90-day study. Animals were observed daily for clinical signs of toxicity and mortality. Feed consumption and body weight were monitored weekly. ECG and blood pressure were monitored before and at the end of the treatment period. Blood biochemistry was performed about 24 hours after the last treatment. At termination, macroscopic examination was performed on all animals, with recording of any gross observations. Organs were weighed, and microscopy of the spleen was performed.

All animals survived the treatment period, and no clinical signs were observed. Feed consumption was slightly higher in all treated animals (from +5% to +15%), although this was not statistically significant and was less than 10%, other than in high-dose females. Body weights were increased in all dose groups, being statistically significant from week 2 in females from the high-dose group. There was no dose dependency in males or in females in the low- and mid-dose groups. The difference in final body weight was less than or equal to 10%, other than in high-dose females (+13%). There was no effect of the treatment on ECG, heart rate or blood pressure recorded at the end of the treatment period. Biochemical analysis revealed a decreased blood glucose level in males and females from the 0.5 and 1 mg/kg bw per day dose groups, although this was significant only in females, and values remained within the background range. Blood urea and creatinine levels were unaffected by treatment. Absolute heart weight was significantly increased (+17%) in females from the high-dose group, and testes weight was statistically decreased in males from the 1 mg/kg bw per day dose group. This was not treatment related. Males from the 0.5 and 1 mg/kg bw per day dose groups displayed significant decreases in adrenal weight (0.5 mg/kg bw per day: relative weight, 1/6 animals; 1 mg/kg bw per day: relative plus absolute weights, 2/6 animals). Absolute weight of testes was also significantly decreased in males from the high-dose group, mainly associated with one male that had small testes with soft consistency. These changes were not considered to be compound related. Macroscopic examination of the spleen revealed an irregular surface (one male, intermediate-dose group) and several raised greyish/whitish foci or areas (0.5 mg/kg bw: two males and one

female; 1 mg/kg bw: four males and one female), correlated with minimal to marked inflammatory cell infiltration. This was considered not to be compound related, as it is observed spontaneously in this strain of rats.

The NOAEL was 0.5 mg/kg bw per day, based on the significant increase in body weight of females at 1 mg/kg bw per day (Sauvez, 1993a).

Sprague-Dawley (CrI CS (SD) BR) rats about 6 weeks of age at study initiation (20 animals of each sex per group) were administered zilpaterol HCl (purity 99.2%) dissolved in water orally by gavage at a dose of 0, 0.05, 0.5 or 1 mg/kg bw per day for 13 consecutive weeks. These doses were based on the results from the preliminary 4-week and 90-day studies. Animals were observed daily for clinical signs of toxicity and mortality. Feed consumption and body weights were monitored weekly, and feed efficiency was estimated. Ophthalmological examinations were performed before the first treatment and on week 13. Systolic blood pressure and ECG were performed on 10 animals of each sex per group before treatment and then at 1–3 hours after dosing on weeks 3, 4, 8 and 13. Haematology, blood biochemistry analysis and urine analysis were performed on week 13 for 10 animals of each sex per group. At the end of the treatment period, animals were terminated and subjected to complete macroscopic examination. Organs were weighed (adrenals, heart, kidneys, liver, ovaries/testes, spleen and thymus), and microscopic examination was performed on several organs and on all organs displaying macroscopic lesions.

No mortality or clinical signs of toxicity were observed at any time throughout the study. A treatment-related increase in feed consumption and body weight was observed in the intermediate- and high-dose groups. Lower mean heart rates were observed during week 8 in females given 0.05 or 0.5 mg/kg bw per day and during week 3 in females given 1 mg/kg bw per day. This was associated with a longer PQ interval, which was also noted in males treated with 0.5 or 1 mg/kg bw per day. Lower systemic blood pressure, sometimes statistically significant, was observed in males at 0.5 and 1 mg/kg bw per day in week 3 or 4. These observations were considered treatment related.

There was no effect of treatment on ophthalmology, haematology, blood biochemistry or urine analysis. Slightly dose-related, statistically significant, higher absolute and relative heart weights were observed in females at 0.5 mg/kg bw per day and in males and females at 1 mg/kg bw per day. There were no major macroscopic or microscopic findings.

The LOAEL was 0.05 mg/kg bw per day, the lowest dose tested, based on decreased mean heart rate observed at all doses in females (Attia, 1994; Sauvez, 1994b).

(c) *Dogs*

One male and one female Beagle dog 9 months of age were given an oral dose of zilpaterol HCl (purity > 98%) at 100 mg/kg bw per day in capsules (gavage) for 7 consecutive days. Animals were observed daily for clinical signs of toxicity and mortality. Feed consumption and body weights were monitored. Haematology

and blood biochemistry were analysed before the first dosing and again at the end of the study. At termination, following necropsy, organs were subjected to macroscopic examination and then to histopathological examination.

Neither animal died. Frequent vomiting, reduced motor activity, apathy, lacrimation, vasodilatation of the abdomen and ears, as well as diarrhoea were observed. Between days 0 and 4, weight loss in the male, associated with partial loss of appetite, was observed, with recovery thereafter. Reductions of erythrocyte number (moderate) and haemoglobin/haematocrit values (marked) were observed in both animals. Blood biochemistry revealed decreases in sodium, chloride and total proteins and increases in transaminases and alkaline phosphatase (male and female). Increases in cholesterol and ornithine carbamyl transferase (male) and in phospholipids and phosphorus (female) were also observed. Macroscopic examination revealed putty-coloured liver of heterogeneous appearance with clear lobular structure, associated at the microscopic level with excess glycogen and lipids in the perilobular hepatocytes.

Overall, numerous clinical signs associated with the pharmacological activity of the test item, together with hepatocellular injury, were observed at the only dose used, 100 mg/kg bw per day (Fournex et al., 1984).

Beagle dogs (three animals of each sex per group) about 8 months of age at study initiation were administered zilpaterol HCl (purity 99.5%) orally in capsules daily at a dose of 0, 0.5, 5 or 50 mg/kg bw per day for 30 consecutive days. These doses were based on the results from the preliminary 7-day study. Animals were observed daily for clinical signs of toxicity and mortality. Feed consumption and water consumption were monitored daily, and body weights were monitored on a weekly basis. Ophthalmological examinations were performed before the first treatment and on week 13. Systolic blood pressure and ECG were recorded on several occasions. Haematology, blood biochemistry, urine analysis and faecal examination (occult blood) were performed at the end of the treatment period. Intestinal absorption of the test item was determined by blood-level analysis performed after the first and last administrations. At the end of the treatment period, animals were terminated and subjected to complete macroscopic examination. Organs were then subjected to microscopic examination.

One male (0.5 mg/kg bw per day) and one female (5 mg/kg bw per day) died during the experimental phase of the study. The deaths were not related to the test item. A dose-dependent peripheral vasodilatation was observed in all treated animals, occurring about 30 minutes after dosing, persisting for some hours to the whole day in the 50 mg/kg bw per day dose group. Irregular vomiting was observed in two males at 5 mg/kg bw per day and in all animals at 50 mg/kg bw per day. Motor activity was reduced in the 5 and 50 mg/kg bw per day dose groups. Body weight change was unaffected by the treatment in surviving animals. Feed consumption and water consumption were unaffected in the 0.5 and 5 mg/kg bw per day dose groups. In the 50 mg/kg bw per day group, one female had transient and partial loss of appetite, and two females had slightly increased water intake. Blood pressure was reduced in all treated animals 1 hour after dosing and returned to normal values 24 hours after dosing. Increased heart rate was observed in all

treated animals 1 hour after dosing and then returned to a normal beat 24 hours after administration.

Reduced red cell count, haemoglobin level and haematocrit value were observed in one male and one female, as well as a fall in platelet count in one male and two females, at 50 mg/kg bw per day. Blood biochemistry revealed no abnormal findings. Urine analysis revealed glycosuria, which was not correlated with any increase in blood glucose, in one male at 50 mg/kg bw per day and a high bilirubin level, which was not correlated with high blood bilirubin level, in two males at 50 mg/kg bw per day. No occult blood was found in faecal samples.

Ophthalmic examinations revealed no abnormal findings, and there were no macroscopic lesions observed at necropsy. No treatment-related change was observed in organ weights. Abnormal spermatogenesis, not related to the test item, was observed in two, two and one male of the 0.5, 5 and 50 mg/kg bw per day groups, respectively.

Peripheral vasodilatation, rise in heart rate and fall in blood pressure related to the treatment of zilpaterol HCl were observed at all doses. Hence, the LOAEL was 0.5 mg/kg bw per day, the lowest dose tested, based on the results observed (Fournex et al., 1986).

(d) *Swine*

One male and one female Yucatan microswine 7–8 months of age were given increasing oral doses (via gavage) of zilpaterol HCl (purity 99.2%) in distilled water in a dose range-finding study. The doses were 0, 0.05, 0.5, 1, 5 or 10 mg/kg bw per day over periods of 4 consecutive days, with a 3-day withdrawal period, to assess both the potential toxic and pharmacological effects of zilpaterol HCl. Animals were observed daily for clinical signs of toxicity and mortality. Feed consumption and body weights were monitored throughout the study. ECG and blood pressure were recorded before the first administration of each new dose and 1–3 hours after the last administration of each new dose. Haematology and blood biochemistry were monitored on a weekly basis. On completion of the last treatment period, animals were killed and subjected to complete macroscopic examination. Selected organs were weighed and subjected to microscopic examination.

No animal died, and there were no clinical signs of toxicity. Feed consumption and body weight were unaffected by treatment. Although there was no impact of the test item on blood pressure, a slightly increased heart rate associated with a decreased QT interval was observed at the end of the treatment periods (0.5, 1, 5 and 10 mg/kg bw per day in male and 10 mg/kg bw per day in female). The only effects observed were increased heart rate together with a decreased QT interval, which is related to the pharmacological effects of zilpaterol HCl (Sauvez, 1993c).

Groups of two male and two female Yucatan microswine weighing about 12 kg were given a daily oral dose of zilpaterol HCl (purity 99.2%) dissolved in water at 0, 5, 10, 50 or 5000 µg/kg bw per day for 4 weeks. The choice of the doses was based on the previous dose range-finding toxicity study (Sauvez, 1993c). Animals

were observed daily for clinical signs of toxicity and mortality. Feed consumption was monitored, as well as body weight. Haematology, blood biochemistry and urine analysis were also monitored, together with ophthalmological examinations and ECG. At termination, following necropsy, selected organs were collected, weighed and subjected to histopathological examination.

No mortality or clinical signs were observed. Treatment had no impact on body weight, and feed consumption was similar between treated and control animals. Treatment had no effect on either ECG or blood pressure, and ophthalmological examination revealed no differences between control and treated animals. Urine analysis revealed no qualitative or quantitative treatment-related abnormality. Haematology and blood biochemistry revealed no findings of importance. At necropsy, there were no macroscopic or microscopic findings.

A NOAEL of 5000 µg/kg bw per day was identified, the highest dose tested (Sauvez, 1994a).

Yucatan microswine (four animals of each sex per group) about 4–7 months of age at study initiation were administered zilpaterol HCl (purity 100.6%) dissolved in water orally by gavage at a dose of 0, 1, 50, 1000 or 10 000 µg/kg bw per day for 13 consecutive weeks. These doses were based on the results from the previous 4-week toxicity study. Plasma levels of the test item were determined on the day of first dosing, then once on week 5 and at the end of the treatment period. Animals were observed daily for clinical signs of toxicity and mortality. Feed consumption and body weight were monitored throughout the study. ECG examination and blood pressure recording were performed twice on both weeks 1 and 2, then once on weeks 4, 8 and 12. Ophthalmological examinations were performed before the first treatment and on week 13. Haematology, blood biochemistry analysis and urine analysis were performed on week 13. At the end of the treatment period, animals were terminated and subjected to complete macroscopic examination. Selected organs were weighed, and microscopic examinations were performed on several organs and on all organs displaying macroscopic lesions.

No clinical signs of toxicity were observed during the study. One male and one female of the 1 µg/kg bw per day dose group were found dead on days 70 and 20, respectively. Deaths were associated with severe bronchopneumonia and abscesses and were not related to test item administration. There was no treatment-related effect on feed consumption. Body weight was slightly increased, although statistically non-significantly, in males from the 1000 and 10 000 µg/kg bw per day dose groups. ECG and blood pressure, ophthalmological examination, haematology, blood biochemistry and urine analysis revealed no effect of the treatment. Macroscopic and microscopic examinations as well as organ weights revealed no effect of treatment.

The NOAEL was 10 000 µg/kg bw per day, the highest dose tested (Sauvez, 1994d).

(e) *Monkeys*

In a study aiming to evaluate the potential toxic and pharmacological effects of zilpaterol HCl, one male and one female *Cynomolgus* monkey weighing about 3 kg were given increasing oral (gavage) doses of zilpaterol HCl (purity 99.2%) in distilled water in a dose range-finding study. The doses were 0.05, 0.5, 1, 5 and 10 mg/kg bw per day over a period of 4 consecutive days, with a 3-day withdrawal period. Animals were observed daily for clinical signs of toxicity and mortality. Feed consumption and body weights were monitored throughout the study. ECG and blood pressure were recorded before the first administration of each new dose and 1–3 hours after the last administration of each new dose. Haematology and blood biochemistry were monitored on a weekly basis. On completion of the last treatment period, animals were terminated and subjected to complete macroscopic examination. Selected organs were weighed and subjected to microscopic examination.

No animal died, and there were no clinical signs of toxicity. Feed consumption and body weight were unaffected by treatment. Although there was no impact of the test item on blood pressure, a slight to moderate increase in heart rate associated with a decrease in QT interval was observed at the end of the treatment period (0.05, 0.5, 1, 5 and 10 mg/kg bw per day). There was no dose–response relationship in increased heart rate with a decreased QT interval, but association with the test item cannot be excluded (Sauvez, 1993b).

Groups of two male and two female *Cynomolgus* monkeys weighing about 2.5–2.9 kg were given a dose of zilpaterol HCl (purity 99.2%) dissolved in water orally (by gavage) at 0, 5, 10, 50 or 5000 µg/kg bw per day for 4 weeks. The choice of doses was based on the previous dose range-finding toxicity study. Animals were observed daily for clinical signs of toxicity and mortality, and feed consumption was monitored daily. Body weights were recorded weekly. ECG and blood pressure were monitored throughout the study 1–3 hours after dosing, and ophthalmological examinations were performed at the end of the dosing period. Haematology, blood biochemistry and urine analysis were also monitored. At termination, following necropsy, a complete macroscopic examination was performed, and selected organs were collected, weighed and subjected to histopathological examination.

No mortality or clinical signs were observed. Treatment had no impact on body weight, and feed consumption was similar between treated and control animals. Treatment had no effect on either ECG or blood pressure at 5 and 10 µg/kg bw per day. Blood pressure was slightly decreased in the two highest dose groups, and a slightly increased heart rate with an associated decrease in QT interval was observed from week 2 of treatment, mainly in males. Ophthalmological examination revealed no difference between control and treated animals. Urine analysis revealed no qualitative or quantitative treatment-related abnormalities. Haematology and blood biochemistry revealed no findings of importance, and all individual data remained within the range of the background values. At necropsy, even if some differences in organ weights were observed, there was no difference of major order that was considered to be treatment related. There were no macroscopic or microscopic findings.

The NOAEL was 10 µg/kg bw per day, based on reduced blood pressure and the increased heart rate with a decreased QT interval at 50 µg/kg bw per day (Sauvez, 1993d).

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) Mice

In a GLP-compliant study to evaluate the potential tumorigenic activity of zilpaterol HCl, five groups of 65 nulliparous and non-pregnant female CD-1 mice weighing about 23 g and 8 weeks of age at initiation of dosing were given a dose of zilpaterol HCl (purity 99.4%) at 0, 10, 20, 50 or 250 µg/kg bw per day via oral gavage for 18 consecutive months. Ten satellite animals per group were used for determination of zilpaterol concentration in plasma, with animals sampled 1 month after the first dosing. Animals were observed daily for clinical signs of toxicity and mortality. Feed consumption and body weight were recorded on a weekly basis until week 13, then once every 4 weeks. Haematology was monitored at the end of the administration period. At termination, following necropsy, a complete macroscopic examination was performed, and selected organs were collected and subjected to histopathological examination. All masses and macroscopic lesions were also subjected to microscopic examinations.

The incidence, nature and onset of clinical signs did not show any indication of a relationship with treatment or dose. Distribution of mortality and factors contributing to mortality were similar between control and treated animals, and the survival rate was in the range 66–75%. Plasma analysis for zilpaterol revealed systemic exposure in all treated groups, increasing with dose, except in the 20 µg/kg bw per day dose group, with average plasma concentrations of 0.204 ng/mL at 10 µg/kg bw per day, 0.112 ng/mL at 20 µg/kg bw per day, 0.390 ng/mL at 50 µg/kg bw per day and 2.240 ng/mL at 250 µg/kg bw per day. There was no systemic toxicity or tumorigenicity associated with zilpaterol HCl. Feed consumption, body weight and haematological parameters were similar between control and treated animals. Some haematological findings were observed: significantly increased haemoglobin at 50 and 250 µg/kg bw per day and a significantly increased number of red blood cells and haematocrit in these dose groups. Although these changes did not show strong dose dependency, there was a degree of biological consistency. There were also significantly decreased numbers of platelets, absolute neutrophil counts and monocyte numbers at 50 and 250 µg/kg bw per day.

A NOAEL of 20 µg/kg bw per day was identified, based on changes in haematological parameters at 50 µg/kg bw per day (Auletta, 2003).

(b) Rats

In a GLP-compliant study, five groups of 20 male and 20 female Sprague-Dawley rats weighing about 178 g for males and 157 g for females and 6 weeks of age were given zilpaterol HCl (purity 99.7%) incorporated into the feed at a dose of 0, 25, 50, 125 or 250 µg/kg bw per day for 52 consecutive weeks. Every week, the concentration of zilpaterol HCl was adjusted to achieve the nominal doses. Achieved doses were very close to the theoretical values. An additional 10 males

and 10 females in the control and 250 µg/kg bw per day groups were kept for a further 4-week treatment-free (recovery) period. Two satellite groups of 10 males and 10 females received doses of 25 or 250 µg/kg bw per day for determination of toxicokinetics, and plasma samples collected on day 3 and then in weeks 27 and 52 were assayed for zilpaterol using a validated radioimmunoassay method.

Animals were observed daily for clinical signs of toxicity and mortality. Feed consumption and body weight were recorded on a weekly basis. Thereafter, achieved doses were calculated. Ophthalmic examination, ECG and blood pressure were recorded throughout the study. Haematology and blood biochemistry were also monitored on 10 animals of each sex per group throughout the study. At termination, following necropsy, a complete macroscopic examination was performed, and selected organs were collected, weighed and subjected to histopathological examination. Electron microscopic examinations of the heart were performed on three animals of each sex per group.

No mortality or clinical signs attributed to the test item were observed. Plasma levels of zilpaterol ranged from 0 to 5 ng/mL, with concentrations increasing in a dose-related fashion. Systemic exposure was slightly higher in females. Treatment had no significant impact on body weight, and the feed consumption was similar between treated and control animals, including the treatment-free period. Ophthalmological examinations revealed no impact of the treatment. ECG revealed slight decreases in heart rate, without impact on waveform traces, in males and females at 125 and 250 µg/kg bw per day; these were fully reversible at the end of the recovery period. A slightly increased systolic blood pressure, which was fully reversible, was also observed in the high-dose group. Haematology and blood biochemistry revealed no findings attributable to the test item, and all individual data remained within the range of the background values. At necropsy, macroscopic examination revealed no non-neoplastic or neoplastic findings attributable to the test item, and microscopic examinations revealed no impact of the treatment. Treated animals displayed no difference in either absolute or relative organ weights when compared with control rats.

The NOAEL was 50 µg/kg bw per day, based on decreased heart rate (Richard, 1999a).

In a GLP-compliant carcinogenicity study, five groups of 66 male and 66 female Sprague-Dawley rats weighing about 178 g for males and 157 g for females and 6 weeks of age were given zilpaterol HCl (purity 99.7%) incorporated into the feed at a dose of 0, 25, 50, 125 or 250 µg/kg bw per day for 104 consecutive weeks. Every week, the concentration of zilpaterol HCl was adjusted to achieve the nominal doses. Achieved doses did not deviate from the theoretical ones by more than 0.5%. Ten males and 10 females per dose group were sampled during the last treatment week to assess zilpaterol plasma concentration using a validated radioimmunoassay method. Animals were observed daily for clinical signs of toxicity and mortality; after 6 months of treatment, all animals were palpated every 2 weeks to record the date of appearance of any palpable mass. Feed consumption and body weight were recorded on a weekly basis until week 69, then once every 2 weeks. Thereafter, achieved doses were calculated. Haematology was also

monitored throughout the study. At termination, following necropsy (including all animals found dead or moribund during the course of the experimental phase), a complete macroscopic examination was performed, and selected organs were collected, weighed and subjected to histopathological examination. All masses and macroscopic lesions were also subjected to microscopic examinations.

The incidence, nature and onset of the clinical signs, as well as palpable masses, did not show any indication of a relationship with treatment or dose. Distribution of mortality and factors contributing to mortality were similar between controls and animals in all but the highest-dose group. In the highest-dose group, survival decreased in the second half of the study, being about 10–20% lower than in the control group, reaching statistical significance in the males. In plasma, zilpaterol concentrations ranged from 0 to 9 ng/mL and increased with dose. In females, concentrations were slightly higher than in males. Feed consumption was slightly increased in the females at 250 µg/kg bw per day. Body weights were slightly lower in males and females from the highest-dose group, especially in the second half of the study. The test item had no qualitative or quantitative impact on haematological parameters. Macroscopic changes in decedent and surviving animals were similar in incidence and nature between treated and control rats. Organ weights were similar between treated and control animals, other than a marked increase in the weight of the ovaries in the 125 and 250 µg/kg bw per day dose groups. Histopathologically, this may have a possible link to an increased incidence of follicular cysts and the occurrence of leiomyomas. There were no microscopic non-neoplastic or neoplastic lesions attributable to the test item in males. In 3% (2/63 females examined) of the 125 µg/kg bw per day dose group and 8% (5/64) of the 250 µg/kg bw per day dose group, leiomyomas of the suspensory ligament of the ovary were observed. This is a benign tumour known to be associated with the pharmacological activity of β_2 -agonist compounds in rats. The severity of this lesion was not increased with the dose.

The NOAEL in this study was 50 µg/kg bw per day, based on increased weight of ovaries with increased incidence of ovarian cysts and increased incidence of ovarian leiomyomas (Richard, 1999b).

Ovarian leiomyomas are tumours that are well known to be related to the use of β_2 -adrenoceptor agonists in rodents. The proliferation of the mesovarian smooth muscle is considered to be an adaptive physiological response to prolonged stimulation of the β -receptors, with muscle relaxation as a consequence, because the induction of this type of neoplastic lesion in rodents was blocked by the concomitant use of propranolol, a β_2 -adrenoceptor antagonist (Nelson, Kelly & Weikel, 1972; Jack, Poynter & Spurling, 1983; Gibson et al., 1987).

Little or no relaxant response to β_2 -agonists can be demonstrated in the uteri of non-pregnant women (Cantox, 2000). There is no evidence in humans of any increased incidence of smooth muscle tumours such as leiomyomas among users of β_2 -agonists (Jack, Poynter & Spurling, 1983; Gibson et al., 1987; Gopinath & Gibson, 1987). Thus, the occurrence of ovarian leiomyomas in rats treated with zilpaterol HCl may represent a species-specific effect, and it is considered unlikely that oral exposure in humans would result in ovarian leiomyomas.

2.2.4 Genotoxicity

A comprehensive range of genotoxicity studies using either zilpaterol HCl or its main metabolite, deisopropyl zilpaterol (free base), has been performed in microorganisms, mammalian cultured cells or in vivo in the mouse. Most of these studies complied with GLP. For zilpaterol HCl, the purity of the test items ranged from 99.4% to 99.7%; for deisopropyl zilpaterol free base, the purity either was 88.7%, with impurities containing 10.3% water, or ranged from 96.5% to 97.5%. When required (lack of intrinsic metabolic activity of the test system), a metabolic activating system (liver S9 fractions, from Aroclor-treated rats) was also used for testing the effect of any metabolic activation on potential genotoxicity. The results for zilpaterol HCl and deisopropyl zilpaterol free base are provided in [Tables 7](#) and [8](#), respectively.

Based on comprehensive tests of genotoxic activity, mainly performed under GLP-compliant conditions, in microorganisms, in cultured mammalian cells or in vivo in mice, it can be concluded that zilpaterol HCl shows no evidence of any genotoxic potential. This is also true of its main metabolite, deisopropyl zilpaterol.

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration reproductive toxicity

(i) Rats

In a preliminary GLP-compliant study, four groups of 10 male and 10 female Sprague-Dawley rats approximately 9 weeks of age with average body weights of 344 g for males and 225 g for females were fed with feed supplemented with zilpaterol HCl at concentrations of 0, 0.9, 3.6 and 14.4 mg/kg. The tested doses corresponded to 0, 0.06, 0.23 and 0.94 mg/kg bw per day for males and 0, 0.10, 0.40 and 1.61 mg/kg bw per day for females, respectively. Administration started 15 days before mating and during mating for males and during mating, pregnancy and lactation for females, lasting until termination. For mating purposes, each male was paired with one female from the same dose group until mating or for a duration not exceeding 7 days. Day 0 of gestation was set when vaginal lavage revealed the presence of spermatozoa. Males and females in the F₀ generation were observed for clinical signs and mortality or morbidity on a daily basis. Feed consumption and body weight were recorded on a weekly basis until termination for males and, for females, on a weekly basis until day 21 postpartum and then on days 64 and 71 of the post-weaning period. Females were allowed to deliver normally; after weaning of the last F₁ litter, F₀ parents were terminated and subjected to complete macroscopic examination. The number of implantation sites was recorded in all F₀ females. Microscopic examination was performed on selected organs and on all macroscopic lesions. Each litter was observed daily for clinical signs and for number and sex of live, dead and cannibalized pups, from day 1 postpartum until days 22–23, and body weights were recorded on days 1, 4, 7, 14 and 21 postpartum. Weights of live pups were recorded on days 1, 4, 7, 14 and 21 postpartum. On day 4, random selection was used to adjust each litter size to four males and four females, and pups not selected were preserved in Bouin's fluid. All surviving pups, killed between day 22 and day 25 postpartum, together with pups that had died,

Table 7. Results of genotoxicity studies performed with zilpaterol HCl

Test system	Test object	Concentrations/ doses	Results	Reference
Microorganisms				
In vitro reverse mutation in bacteria	<i>Salmonella typhimurium</i> TA98, TA100	100–5 000 µg/plate (±S9)	Negative (±S9)	Vannier & Chantot (1984)
	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	10–4 000 µg/plate (±S9)	Negative (±S9)	Loquet (1987a)
	<i>S. typhimurium</i> TA98, TA100, TA102, TA1535, TA1537 <i>Escherichia coli</i> WP2uvrA	312.5–5 000 µg/plate (±S9)	Negative (±S9)	Haddouk (1997)
Cultured mammalian cells				
Chromosomal aberration	CHO/HGPRT locus assay	500–5 000 µg/mL (±S9)	Negative (±S9)	Adams (1992)
Unscheduled DNA synthesis	Primary Fischer 344 rat hepatocytes	2.50–5 000 µg/mL	Negative	McKeon (1991)
Forward mutation	L5178Y/ <i>tk</i> ^{+/-} mouse lymphoma cell	250–5 000 µg/mL (±S9)	Negative (±S9)	Mitchell (1998d)
		500–3 000 µg/mL (±S9)		
In vivo tests				
Micronucleus test	Swiss Cr1:CD-1 (1CR) BR mouse bone marrow cells	400 mg/kg bw	Negative	Loquet (1987b)
Micronucleus test	ICR mouse bone marrow cells	100–400 mg/kg bw	Negative whatever the sacrifice time (24, 48 or 72 h after administration of the test item)	Murli (1991a, 1991b)
Micronucleus test	Swiss Webster mouse bone marrow cells	31.25–500 mg/kg bw	Negative	Mitchell (1998c)

bw: body weight; CHO: Chinese hamster ovary; DNA: deoxyribonucleic acid; HGPRT: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate

Table 8. Results of genotoxicity studies performed with deisopropyl zilpaterol free base

Test system	Test object	Concentrations/doses	Results	Reference
Microorganisms				
In vitro reverse mutation in bacteria	<i>Salmonella typhimurium</i> TA98, TA100, TA102, TA1535, TA1537 <i>Escherichia coli</i> WP2uvrA	312.5–5 000 µg/plate (–S9) 37.5–5 000 µg/plate (+S9)	Negative (±S9)	Molinier (1993)
Cultured mammalian cells				
Unscheduled DNA synthesis	Wistar rat hepatocytes	0.064–5 000 µg/mL	Negative	Ward (1994)
Forward mutation	L5178Y/ <i>tk</i> ^{+/–} mouse lymphoma cells	78–5 000 µg/mL (–S9) 400–1 100 µg/mL (+S9)	Weak potential to induce mutation in the mouse lymphoma assay (+S9) ^a	Riach (1994)
Forward mutation	L5178Y/ <i>tk</i> ^{+/–} mouse lymphoma cells	100–2 500 µg/mL (–S9) 250–1 700 µg/mL (+S9)	Negative (±S9)	Mitchell (1998a)
In vivo tests				
Micronucleus test	Swiss OF1 mouse bone marrow cells	1 500 mg/kg bw	Negative	Molinier (1994)
Micronucleus test	Swiss Webster mouse bone marrow cells	125–2 000 mg/kg bw	Negative	Mitchell (1998b)

bw: body weight; DNA: deoxyribonucleic acid; S9: 9000 × *g* supernatant fraction from rat liver homogenate

^a Non-biologically relevant because observed at only highly toxic concentrations (at 900 µg/mL), and other two experiments show negative results.

were subjected to complete macroscopic examination. Male and female mating index and fertility index were calculated. Gestation, live birth, viability (day 4 postpartum) and lactation (day 21 postpartum) indices were also calculated.

F₀ parents did not display any clinical signs, and no deaths were observed. Feed consumption was similar between treated and untreated animals, but body weight was significantly increased in all treated males, mainly during the 1st week of treatment. During the premating period, body weight only of females from the high-dose group was increased. This difference lasted during the pregnancy and lactation periods and was attributed to the pharmacological activity of the test item. There was no effect of zilpaterol HCl on mating and fertility (males and females) or on gestation

index and duration of gestation. The mean number of liveborn pups as well as the viability index (day 4 postpartum) were similar in all groups. On day 21 postpartum, the lactation index was similar in all groups, and no clinical signs were registered in pups. Macroscopic examination of pups dying between day 5 and day 21 postpartum revealed no abnormalities. Macroscopic and microscopic examinations performed on both F_0 and F_1 animals revealed no treatment-related changes.

In this reproductive toxicity study with zilpaterol HCl, the only effect observed was an increase in body weight. The LOAEL for parental toxicity was 0.9 mg/kg feed (equal to 0.06 mg/kg bw per day), based on increased body weight in F_0 males at 3.6 mg/kg feed (equal to 0.23 mg/kg bw per day). The NOAEL for reproductive and offspring toxicity was 14.4 mg/kg feed (equal to 0.94 mg/kg bw per day), the highest dose tested (Richard, 1995).

In a multigeneration study aiming to evaluate potential effects of the test item on male and female reproductive performance and on growth and development of the offspring, four groups of 30 male Sprague-Dawley rats approximately 7 weeks of age with an average body weight of 249 g and 30 female Sprague-Dawley rats about 10 weeks of age with an average body weight of 248 g were fed with feed supplemented with zilpaterol HCl (purity 99.7%) at concentrations of 0, 0.9, 3.6 or 14.4 mg/kg in a GLP-compliant study. F_0 treatment started 71 days before mating for males and until termination (after the weaning of F_1 litters) and 15 days before mating for females and until termination after the weaning of F_1 litters (treatment period included mating for males and mating, pregnancy and lactation for females). For mating purposes, each male was paired with one female from the same dose group until mating or for a duration not exceeding 7 days. After mating, females were allowed to deliver normally and to rear their progeny until weaning. After weaning, 25 males and 25 females were selected to constitute the F_1 generation and were administered the same treatment until the end of the study. Achieved doses were 0, 0.06, 0.23 and 0.94 mg/kg bw per day for F_0 males and 0, 0.10, 0.40 and 1.61 mg/kg bw per day for F_0 females. For the F_1 generation, 0, 0.08, 0.32 and 1.26 mg/kg bw per day were administered to F_1 males and 0, 0.12, 0.45 and 1.77 mg/kg bw per day to F_1 females.

Males and females in the F_0 generation were observed for clinical signs and mortality and morbidity on a daily basis. Feed consumption and body weight were recorded on a weekly basis until termination for males and, for females, on a weekly basis during the premating period, during the gestation period at intervals of days 0–6, days 6–13 and days 13–21, and during lactation at intervals of days 1–7, days 7–14 and days 14–21. Females were allowed to deliver normally. F_1 litters were examined daily (number and sex of live, dead and cannibalized, clinical signs and gross abnormalities). Body weight of live pups was recorded on days 1, 4 (before selection), 7, 14 and 21 postpartum. On day 4 postpartum, the size of the litter was randomly adjusted to four males and four females, the remaining pups being killed. Pups were then observed for developmental steps (pinna unfolding and hair growth on day 5 postpartum, incisor eruption on day 13, eye opening and auricular duct opening on day 17) and for reflexes (surface righting reflex on day 5, cliff avoidance on day 11, air righting reflex on day 17). At weaning, one male and

one female from each group and from 25 surviving litters were randomly selected (day 22 postpartum corresponding to day 1 of the F_1 generation). They were further fed with feed supplemented with the same concentrations of zilpaterol HCl as for the F_0 parents until termination (after weaning of the F_2 generation). F_1 males and females were allowed to mate when they were about 12 weeks old and subjected to the same examination protocol (clinical signs, mortality, body weight and feed consumption) as for the parent F_0 generation. F_1 females were allowed to deliver normally. Litter size and the numbers of live, dead and cannibalized F_2 pups were recorded, as well as clinical signs on a daily basis. F_2 pups were weighed on days 1, 4 (before selection), 7, 14 and 21 postpartum and were also observed for developmental steps and for reflexes, as for the F_1 pups. On day 4 postpartum, the size of the litter was randomly adjusted to four males and four females, the remaining animals being terminated. F_0 and F_1 parents were terminated after weaning of most F_1 and F_2 litters, respectively. After weaning, F_2 pups were also killed. At necropsy, F_0 , F_1 and F_2 animals were observed macroscopically, and selected organs were collected, weighed and subjected to microscopic examination, together with all macroscopic lesions (for F_0 and F_1 animals only). The male and female mating and fertility indices, female gestation index as well as live birth and viability indices were calculated, together with preimplantation and postimplantation losses.

There were no clinical signs in treated animals, and the test item had no effect on mating, fertility or gestation parameters (F_0 and F_1 generations). During the lactation period, there was no effect on litter size, pup survival, clinical signs, body weight gain, sex ratio or physical or reflex development (F_1 and F_2 pups). At all tested concentrations, the test item induced higher feed consumption and body weight gain in both F_0 and F_1 parents, associated with the pharmacological activity of zilpaterol HCl. Macroscopic and microscopic examinations revealed no treatment-related effect of toxicological relevance (F_0 , F_1 and F_2 animals).

In this multigeneration study, the only effect produced by zilpaterol HCl was induction of a slight increase in feed consumption and body weight gain in F_0 and F_1 parent animals at all dose levels. The LOAEL for parental toxicity was 0.9 mg/kg feed (equal to 0.06 mg/kg bw per day), the lowest dose tested, for effects on feed consumption and body weight gain. The NOAEL for offspring and reproductive toxicity was 14.4 mg/kg feed (equal to 0.94 mg/kg bw per day), the highest dose tested (Richard, 1998).

(b) *Developmental toxicity*

(i) *Mice*

A GLP-compliant tolerance study in pregnant mice was split into two trials. In the first one, two groups of 10 mated female Swiss CD-1 mice about 13–14 weeks of age were orally (by gavage) administered zilpaterol HCl (purity 99%) in water at either 0 or 300 mg/kg bw per day from days 6 to 18 of pregnancy, day 0 being the day of the presence of the vaginal plug. In the second trial, and based on the results from the first one, two groups of 12 mated females were

administered orally (by gavage) zilpaterol HCl at 0 or 450 mg/kg bw per day from days 6 to 18 of pregnancy. General condition and behaviour of dams were recorded on a daily basis. Body weights were recorded on days 0, 6, 10, 14 and 18, prior to termination. Litter parameters were recorded, and live fetuses were subjected to external examinations.

There was no effect of zilpaterol HCl on general condition or behaviour of the dams or on body weight. Four females died due to dosing incidents, three in the 450 mg/kg bw per day dose group and one in the control group. Pregnancy rates were 100%, and no litter parameters were affected by the test item. No external malformation attributable to the test item was recorded.

The NOAEL for maternal and embryo/fetal toxicity was 450 mg/kg bw per day, the highest dose tested (Fournex & Vannier, 1990).

(ii) *Rats*

Four groups of 10 female Sprague-Dawley rats weighing 200–225 g were allowed to mate with male Sprague-Dawley rats. The day when vaginal smears revealed the presence of spermatozoa was set as day 0 of pregnancy. Pregnant females were then orally administered zilpaterol HCl (purity 99%) to achieve a dose of 0, 200, 400 or 600 mg/kg bw per day, from day 6 to day 15 of gestation. They were observed for clinical signs, mortality and abortion signs, and body weights of the dams were recorded on days 0, 6, 10, 15, 18 and 20 of gestation. On gestation day 20, animals were killed, and gravid uterus weight, number of corpora lutea, number of early and late resorptions, number of live and dead fetuses as well as number of implantations were recorded. Viable fetuses were observed for external malformations and anomalies. Preimplantation and postimplantation losses were further calculated.

Ten minutes after treatment, some dams from the treated groups showed signs of hypersalivation, with the number of animals affected increasing with the dose. There were no deaths or abortions due to treatment, and the fertility index was similar between treated and untreated females. Females from the 200 mg/kg bw per day dose group showed lower body weight gain than controls. This was considered incidental, because it was not observed in the two highest dose groups. At necropsy, no adverse effects associated with the test item were observed. Slightly increased postimplantation losses were noted in females from the high-dose group, reflecting slight embryotoxicity. One fetus from the 200 mg/kg bw per day dose group and two fetuses from the 600 mg/kg bw per day dose group showed malformations, including amelia of forelimb and right hindlimb; omphalocele, microphthalmia and absence of mandible; and ectrodactyly and cleft lip, respectively.

The LOAEL for maternal toxicity was 200 mg/kg bw per day, based on increased occurrence of salivation at all doses. The NOAEL for embryo/fetal toxicity was 400 mg/kg bw per day, based on the increase in postimplantation loss at 600 mg/kg bw per day, but further studies (see below) were required to clarify whether the compound causes teratogenicity (Bussi, Milone & Fumero, 1987a).

In a GLP-compliant study performed to evaluate the potential embryo and fetal toxicity and teratogenic effects of zilpaterol HCl, 100 mated female Sprague-Dawley rats 12 weeks of age and weighing about 250 g were randomly allocated to four groups of 25 animals. Zilpaterol HCl (purity 99%) was orally administered (by gavage) at a dose of 0, 50, 150 or 450 mg/kg bw per day from day 6 to day 15 of pregnancy (inclusive) in distilled water, day 0 being the day of detection of spermatozoa in the vaginal smear. Dams were observed daily for clinical signs, including abortion, morbidity and mortality. Body weights were recorded periodically throughout the pregnancy. On day 20 of pregnancy, dams were terminated. Fetuses were delivered by caesarean section, and dams were subjected to macroscopic examinations. Litter parameters were recorded. Half of the live fetuses were submitted to soft tissue examination, and the remainder to skeletal examination.

Signs of hypersalivation were observed in all treated dams during the last 2 days of treatment, with dose-related incidence, and one dam died because of a dosing incident. No abortions were observed, and the fertility index was unaffected by zilpaterol HCl. Body weight gain as well as final absolute body weight (excluding gravid uterus) were slightly higher in all treated groups, when compared with the control, being significantly higher in the 50 and 150 mg/kg bw per day dose groups. This was associated with the pharmacological activity of the test item. No embryotoxic effect of zilpaterol HCl was observed, and litter data were similar between groups, sometimes slightly decreased without statistical significance in the treated groups. Fetuses with external malformations were found in all treatment groups without treatment-related incidence. Increased numbers of fetuses with skeletal anomalies were observed in all treated groups, being statistically significant in the high-dose group. Significantly increased numbers of skeletal variants were also found in the treated groups. Visceral variants were also present in all treated groups, with higher frequency in the high-dose group, and were related only to the urinary tract.

The LOAEL for maternal toxicity was 50 mg/kg bw per day, based on the increased occurrence of salivation at all doses. The NOAEL for embryo and fetal toxicity was 150 mg/kg bw per day, based on increased external anomalies at 450 mg/kg bw per day (Bussi, Conz & Fumero, 1988b).

In a GLP-compliant preliminary study aiming to assess the effect of zilpaterol HCl on pregnancy and in utero development in rats, 40 (10 animals per group) female Sprague-Dawley rats about 8–10 weeks old with weights ranging from 170 to 225 g were treated with zilpaterol HCl in distilled water at 0, 400, 600 or 800 mg/kg bw per day from day 6 to day 15 of pregnancy. Dams were then terminated on day 20 and subjected to macroscopic examination. Litter values were determined, and fetuses were examined for gross abnormalities. Treated dams presented signs of hypersalivation, together with a dose-related increase in water consumption. Transient changes in feed intake were observed in treated animals, and body weight gain was transiently lowered in the high-dose group. Litter and fetal weights were reduced in the high-dose group only. The dose of 800 mg/kg bw per day was considered to be the maximum tolerated dose.

In the definitive test, 100 pregnant female Sprague-Dawley rats 8–10 weeks of age and weighing about 161–235 g were allocated to four groups of 25 animals. Zilpaterol HCl was orally administered at a dose of 30, 150 or 750 mg/kg bw per day from day 6 to day 15 of pregnancy, one group receiving the vehicle alone (control group). Dams were observed daily for clinical signs and weighed about every 2 days. Feed and water intakes were determined concomitantly. On day 20 of pregnancy, dams were terminated and subjected to macroscopic examination, and litter values were determined. Fetuses were examined for visceral and skeletal abnormalities. In the 150 and 750 mg/kg bw per day dose groups, some females showed signs of hypersalivation, with the saliva sometimes being stained. The incidence was dose related. From day 8, water intake was increased in treated females, and feed consumption was decreased from day 3 to day 6 at 30 and 150 mg/kg bw per day and from day 6 to day 10 at the high dose. This was associated with reduced body weight gain during the same periods. Thereafter, feed consumption was slightly increased in all treated animals. At necropsy, staining of fur and dirty tails were observed in dams from the high-dose group only. There was no other finding. Litters from the high-dose group only displayed slightly reduced weights, together with a significantly reduced average mean fetal weight. Postimplantation losses were higher in the high-dose group, but not statistically significantly. Sex ratio as well as litter size remained unaffected by the test item. Fetal examination showed significant dose-related increases in skeletal changes in fetuses from all treated dams, including wavy ribs, sternebral anomalies, reduced longbones/digits as well as misshapen scapulas. In fetuses from dams treated with 750 mg/kg bw per day, there was a possible suggestion of visceral changes (two fetuses displaying intraventricular septal defects).

The LOAEL for both maternal toxicity and embryo/fetal toxicity was 30 mg/kg bw per day, the lowest dose tested, based on the results observed (Hughes & Myers, 1990).

In a pivotal, GLP-compliant study aimed at assessing the potential effect of zilpaterol HCl on embryonic and fetal development after oral gavage, mated female Sprague-Dawley rats 12 weeks of age and weighing about 210–230 g were randomly allocated to five groups with 28 animals per group. Zilpaterol HCl (purity 99.2%) was orally administered via gavage in water at a dose of 0, 0.2, 2, 10 or 50 mg/kg bw per day from day 6 to day 15 of pregnancy. Dams were observed daily for clinical signs, morbidity and mortality. Feed and water consumption as well as body weights were recorded periodically throughout the pregnancy. On day 20 of pregnancy, dams were terminated. Fetuses were delivered by caesarean section, and dams were subjected to macroscopic examinations. Litter parameters were recorded. Half of the live fetuses were submitted to soft tissue examination, the remainder to skeletal examination.

Hypersalivation related to the test item was observed in dams from the 2, 10 and 50 mg/kg bw per day dose groups, with a higher incidence in the highest-dose group. No treatment-related deaths or abortions were observed, and there were no findings at necropsy. Feed consumption was higher at 2 mg/kg bw per day during the last treatment days. In the 10 and 50 mg/kg bw per day dose groups,

feed consumption was lower on days 6–9 and then increased thereafter, which was attributed to zilpaterol HCl. Treatment-related increased water consumption was observed in dams from the highest-dose group. Increased body weight was observed in the 2, 10 and 50 mg/kg bw per day dose groups. Feed consumption was increased at 2 mg/kg bw per day; at 10 and 50 mg/kg bw per day, feed consumption decreased transiently and then increased. Zilpaterol HCl displayed no effect on litter data, including mean numbers of corpora lutea, implantation sites and live fetuses, mean fetal weight and postimplantation loss. The test item had no impact on the incidence of external malformations or soft tissue anomalies/malformations when compared with the control group. In the 50 mg/kg bw per day dose group, however, an increased incidence of delayed ossification was observed. This concerned mainly the 5th and 6th sternbrae and the skull. Wavy ribs were also evident. These findings were associated with maternal toxicity of the test item. These results demonstrated slight (2 and 10 mg/kg bw per day) to moderate (50 mg/kg bw per day) maternal toxicity, mainly characterized by increased growth, related to the pharmacological activity of zilpaterol HCl. As a consequence, fetal development was impacted in the highest-dose group only, without embryotoxicity.

The NOAEL for maternal toxicity was 0.2 mg/kg bw per day, based on the change in body weights and the increased occurrence of hypersalivation at 2 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 10 mg/kg bw per day, based on delayed ossification at 50 mg/kg bw per day (Savary, 1992).

(iii) *Rabbits*

In a preliminary dose range-finding developmental toxicity study, 32 mated female New Zealand White rabbits weighing about 3.10–3.20 kg were randomly allocated to four groups of eight animals each. Zilpaterol HCl in distilled water was orally administered via gavage at 0, 30, 100 or 300 mg/kg bw per day from day 6 to day 18 of pregnancy, day 0 being the day with a positive vaginal smear. Does were observed daily for clinical signs, morbidity, mortality and possible abortion. Body weights were recorded periodically throughout the pregnancy. On day 29 of pregnancy, animals were terminated and subjected to macroscopic examination. Fetuses were delivered by caesarean section and observed for external malformations. Litter parameters were determined.

No dams displayed clinical signs, and there were no abortions or drug-related mortality. The fertility index was similar between treated and untreated females. Treated does showed a lower body weight increase compared with untreated dams, being more marked in the high-dose group. Necropsy of the animals revealed no findings. Early resorptions were observed in all treated groups, being significantly higher from 100 mg/kg bw per day. In the high-dose group, there were two dams with 100% resorption. There was no dam with dead fetuses. The number of does with only viable fetuses was significantly decreased in the 100 and 300 mg/kg bw per day dose groups. Zilpaterol HCl had no effect on the number of implantations. Early resorption frequency and number of viable fetuses versus number of implantations were significantly higher and lower, respectively, in dams from the high-dose group. External examination of the fetuses revealed no substance-related effect.

The LOAEL for maternal toxicity and embryo/fetal toxicity was 30 mg/kg bw per day, based on effects observed at all doses (Bussi, Milone & Fumero, 1987b).

In a GLP-compliant study aiming to assess the potential effect of zilpaterol HCl on embryonic and fetal development after oral gavage, 80 mated female New Zealand White rabbits weighing about 3.40–3.70 kg were randomly allocated to four groups of 20 animals, to ensure at least 12 gravid does per group. Zilpaterol HCl (purity 99%) in distilled water was orally administered via gavage at a dose of 0, 20, 60 or 180 mg/kg bw per day from day 6 to day 18 of pregnancy, day 0 being the day with a positive vaginal smear. Does were observed daily for clinical signs, morbidity, mortality and possible abortion. Body weights were recorded periodically throughout the pregnancy. On day 29 of pregnancy, animals were terminated and subjected to macroscopic examination. Fetuses were delivered by caesarean section and observed for external malformation. Litter parameters were determined. Fetuses were eviscerated for macroscopic examination of internal organs. One third of the fetuses were decapitated for further cranial examination. The rest of the carcass as well as the carcass from all other fetuses were used for skeletal examination. Observations were classified as malformations, anomalies or variants.

No dams displayed clinical signs. Some cases of death, not substance related, were recorded in all groups. One doe from the control group and another from the 20 mg/kg bw per day dose group were found dead on the day of abortion on days 22 and 25, respectively. One doe from the high-dose group aborted on day 24 and survived. No pathological change was recorded in any of these three does. There was no difference between groups regarding the fertility index. Body weights as well as final body weights (excluding gravid uterus) were slightly higher in treated does, the increase being statistically significant in the 60 mg/kg bw per day dose group. Body weight gain was higher, although not statistically significantly, in all treated groups. This was associated with the pharmacological activity of the test item. At necropsy, there were no macroscopic findings. The frequency of does with either early or late resorptions was similar between groups. In each treated group, there was one doe with 100% postimplantation loss. There was a significantly lower number of corpora lutea compared with number of implantations in treated animals. This was, however, not associated with the test item, because treatment started after the implantation phase. The frequency of early resorption was higher in all treated groups, being statistically significant in the 20 and 60 mg/kg bw per day dose groups. The observed increase in postimplantation losses in the high-dose group was associated with the doe that aborted and survived, although the does that aborted in the control and 20 mg/kg bw per day dose groups died. There was no external malformation observed in fetuses, but one fetus from the 20 mg/kg bw per day dose group presented skeletal malformation (sutural bones near to the fontanella bregmatica, lack of central parietal ossification, complete ossification of frontal sutura, partial ossification of sutura between frontals and parietals, marked ossification of lumbar processes). This was considered incidental. There was no visceral malformation. There was no visceral anomaly, but the number and ratio of skeletal anomalies were higher in all treated groups, reaching statistical significance in the low- and high-dose groups. Skeletal variants were similar in all groups.

The LOAEL for maternal and embryo/fetal toxicity was 20 mg/kg bw per day, based on increased body weight in does and increased ratio of skeletal anomalies at all doses (Bussi, Conz & Fumero, 1988a).

2.2.6 *Special studies*

(a) *Relay pharmacology*

As demonstrated by short- and long-term toxicological studies, zilpaterol HCl is a compound of low intrinsic toxicity. The observed effects, mainly on the cardiovascular system, are associated with its β_2 -adrenoceptor agonist activity. Thus, a relay pharmacology study was performed in dogs.

A relay pharmacology study was performed to investigate the possible pharmacological effects on blood pressure and heart rate in dogs ingesting meat (muscle, liver) from steers treated orally with zilpaterol HCl. Beagle dogs (13–26 months old and weighing 10–16 kg, groups of one male and one female implanted with transmitters) were fed daily for 5 consecutive days with 200 g of muscle or liver from untreated steers or from steers treated for 50 consecutive days with zilpaterol HCl at 0.2 mg/kg bw per day and slaughtered immediately or 7 days after the end of treatment. A daily complementary ration of 100 g cereal flakes was also provided to all animals. A positive control group of dogs was treated orally with a capsule containing zilpaterol HCl (purity 99.2%) at a dose of 3 μ g/kg bw per day, together with muscle or liver from untreated steers and cereal flakes ration.

The ingested dose of zilpaterol HCl when dogs were fed with meat from treated steers was calculated from zilpaterol concentrations determined using a radioimmunoassay method. The impact of the feed on both heart rate and blood pressure was calculated from respective AUC values (global and daily). When dogs were fed with 200 g liver from steers slaughtered immediately after 50 days of daily oral zilpaterol HCl doses of 0.2 mg/kg bw, the ingested doses ranged from 1742 to 1985 ng/kg bw per day. The doses ranged from 252.5 to 275.6 ng/kg bw per day when dogs were fed with 200 g muscle. The corresponding doses when dogs were fed with liver and muscle from steers slaughtered 7 days after treatment was withdrawn were 11.3 ng/kg bw per day with liver and 1.5–1.7 ng/kg bw per day with muscle.

Whatever the feeding regimen (liver or muscle from treated steers slaughtered at the end or 7 days after the end of the treatment), there was no effect on either systolic/diastolic blood pressure or heart rate of dogs. In the positive control group treated with zilpaterol HCl at 3 μ g/kg bw per day, a slight increase in the global AUC and daily AUC was observed for heart rate, but not for blood pressure. Thus, ingestion of muscle and liver from zilpaterol-treated steers (50 days at 0.2 mg/kg bw per day) does not induce changes in blood pressure and heart rate in dogs (Vacheron et al., 1995).

2.3 *Observations in humans*

Four studies were conducted in which human volunteers, either healthy or asthmatic, were administered zilpaterol HCl. The study protocols were approved by ethics committees and carried out according to the guidelines in the Declaration of Helsinki (revised Tokyo version).

Table 10. NOAELs and LOAELs derived from the ascending dose tolerance study in healthy volunteers

End-point	NOAEL (mg/head)	LOAEL (mg/head)
Cardiovascular	–	0.25
- Systolic blood pressure	0.50	1.00 (16.3 mmHg ^a)
- Diastolic blood pressure	0.25	0.50 (5 mmHg)
- Stroke volume	0.25	0.50 (25.5 mL)
- Heart rate	–	0.25 (5.75 bpm)
Bronchodilatation	0.25	0.50
Tremor	0.25	0.50
Metabolism (blood glucose)	–	0.25 (2 mmol/L)

bpm: beats per minute; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level

^a 1 mmHg = 133.3 Pa.

Source: Sutton & Budhram (1987)

In the first single-blind and placebo-controlled study, the tolerance of zilpaterol HCl was assessed in eight healthy male volunteers aged 28–55 years given ascending single oral doses of 0, 0.25, 0.50, 1.00 and 2.00 mg zilpaterol HCl dissolved in aqueous vehicle in a four-way cross-over design. The volunteers fasted from midnight on the evening before the study, although water was allowed. Taking individual body weights into consideration, the corresponding doses were 0, 3.6, 7.1, 14.3 and 28.6 µg/kg bw. ECG, arterial blood pressure, cardiac output, stroke volume and ejection velocity were recorded. Blood biochemistry, airway calibre and haematology factors were also recorded.

Heart rate was slightly increased (6 beats per minute [bpm]) at a dose of 0.25 mg and significantly increased at higher doses (44 bpm at 2.00 mg). Systolic blood pressure was significantly increased from 1.00 mg zilpaterol HCl, and airway calibre was significantly increased from 0.50 mg. Blood glucose level was increased in a dose-dependent manner at all doses, from 2 mmol/L at 0.25 mg to 4.81 mmol/L at 2.00 mg, although haematology remained unaffected by the treatments. The occurrence of tremors was evaluated (subjective and inspective), and increased frequency was observed only at a dose of 0.50 mg and above.

The LOAEL was 0.25 mg/person (equal to 3.6 µg/kg bw), based on increased heart rate and blood glucose level observed at all doses (Sutton & Budhram, 1987) (Table 10).

In a second double-blind, comparative placebo-controlled study, 13 healthy male volunteers were administered placebo ($n = 4$) or zilpaterol HCl ($n = 9$) at a dose of 0.25 mg/person, corresponding to 3.68 µg/kg bw, 3 times/day for 7 consecutive days (i.e. a daily dose of 0.75 mg/person or about 11.04 µg/kg bw). One of the nine volunteers was removed from the trial prior to completing the dosing regimen, due to adverse pharmacological effects. This

subject was replaced in the study. Zilpaterol HCl was swallowed, and then blood pressure, heart rate, clinical chemistry parameters and bronchodilatation were measured. Heart rate increased significantly on average by 13 bpm after the first and second doses of the day, then by 6 bpm after the third dose of the day. There was also a significant bronchodilating effect of the test item, and a trend for tremors was also observed.

The LOAEL was 0.25 mg/person (equal to 3.68 µg/kg bw), based on cardiovascular effects, bronchodilatation and tremors (Strauch et al., 1989).

A double-blind, randomized, placebo-controlled cross-over trial was performed in 12 adult asthmatic volunteers given a single oral dose of 0 or 0.25 mg zilpaterol HCl (equal to 0 or 3.85 µg/kg bw). The volunteers fasted before dosing. The aim was to assess both the bronchodilating activity (measured by the forced expiratory volume in 1 second [FEV₁]) and tolerance (heart rate, blood pressure, tremors – clinical examination). Zilpaterol HCl significantly increased bronchodilatation (measured by FEV₁) up to 4 hours after dosing. Heart rate was slightly but significantly increased up to 1.5 hours post-dosing, with no major palpitation or ECG changes. Diastolic blood pressure was slightly but significantly decreased at 0.5 and 1 hour after dosing. Short-lasting finger tremors were seen in 2/12 patients after treatment. Another patient experienced tremulousness without, however, objective tremor of the fingers. A fourth subject who had previously displayed mild tremor following zilpaterol HCl administration showed no tremor increase after drug intake.

A LOAEL of 0.25 mg/person (equal to 3.85 µg/kg bw) for cardiovascular effects, bronchodilatation and tremor was derived from the results of this study (Vivet, 1988).

A double-blind, randomized, placebo-controlled four-way cross-over trial was performed in 11 asthmatic volunteers receiving three single oral doses of 0, 0.05, 0.10 or 0.25 mg zilpaterol HCl (equal to 0, 0.76, 1.52 and 3.79 µg/kg bw, respectively). The volunteers fasted before dosing. Several parameters were assessed during this study, such as bronchodilatation, blood pressure, heart rate, tremor occurrence, blood glucose and potassium. Statistically significant increased bronchodilatation, as measured by FEV₁, was observed after doses of 0.10 or 0.25 mg, from 30 minutes to 4 hours post-dosing. However, at 0.10 mg/person, bronchodilatation was much less pronounced and of shorter duration. The dose of 0.25 mg was associated with a small and asymptomatic increase in heart rate, from 1 to 6 hours after dosing. Blood pressure remained unaffected at all doses. Mild tremor was seen when evaluated clinically by a physician (two patients at 0.05 and 0.10 mg/person and eight patients at 0.25 mg/person). When the tremor was assessed by drawing a line between two parallel sinusoids, no significant differences were observed at any time between the three doses of zilpaterol HCl and the placebo.

The LOAEL was 0.05 mg/person (equal to 0.76 µg/kg bw), based on the increased incidence of tremor (Vivet, 1989) ([Table 11](#)).

Table 11. NOAELs and LOAELs derived from the study of bronchodilating activity of zilpaterol HCl in adult asthmatic volunteers

End-point	NOAEL (mg/person)	LOAEL (mg/person)
Cardiovascular	0.10	0.25
- Heart rate	0.10	0.25
- Blood pressure	0.25	–
Bronchodilatation	0.05	0.10
Tremor	–	0.05
Metabolism (blood glucose and potassium)	0.25	–

LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level
Source: Vivet (1989)

Table 12. NOAELs and LOAELs derived from studies performed in adult healthy and asthmatic volunteers

End-point	NOAEL (mg/person)	LOAEL (mg/person)
Cardiovascular	0.10	0.25
- Systolic blood pressure	0.25	0.50
- Diastolic blood pressure	0.25	0.50
- Stroke volume	0.25	0.50
- Heart rate	0.10	0.25
Bronchodilatation	0.05	0.10
Tremor	–	0.05
Metabolism (blood glucose)	0.25	–

LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level
Source: Sutton & Budhram (1987); Vivet (1988, 1989); Strauch et al. (1989)

In all studies performed in human volunteers (16 healthy and 23 asthmatic), the observed effects after an oral dose were slight and transitory and typically related to the β_2 -adrenergic agonist activity of zilpaterol HCl. Considering all the human data, the LOAEL for zilpaterol was 0.05 mg/person (equal to 0.76 $\mu\text{g/kg bw}$), the lowest dose tested. An overall NOAEL could not be determined ([Table 12](#)).

3. COMMENTS

The Committee considered the results of studies on blood protein binding in vitro, pharmacokinetics, pharmacological effects in vitro and in vivo, acute, short-term and long-term toxicity, genotoxicity, reproductive and developmental toxicity and relay pharmacology, as well as observations in humans. The majority of the studies were performed in accordance with GLP.

3.1 Biochemical data

In rats, dogs and humans, the binding of zilpaterol HCl to serum albumin was low (14–15%). In human red blood cells, the bound fraction was 55%, irrespective of the concentration and the presence of plasma. Zilpaterol HCl was rapidly absorbed via the oral route, with almost 100% bioavailability in rats and dogs. In rats, peak blood concentrations were reached within 1 hour of a single oral gavage dose and by 3–23 hours when given in the diet. Total AUC correlated approximately with dose. The half-life in plasma ranged from 2.4 to 5.5 hours. After repeated dosing, the pharmacokinetic profile in rats was similar to that following a single dose. Systemic exposure was slightly higher in females than in males. In a study in human male volunteers in which zilpaterol HCl was given as a single dose in drinking-water, the time to reach the peak concentration was 1 hour, and the half-life in plasma was 4–5 hours, independent of the dose.

In studies in rats using radiolabelled zilpaterol HCl, approximately 50–55% of the dose was eliminated in the urine and 40–42% in the faeces over 8 days. In urine, unchanged zilpaterol predominated, accounting for at least half of the radioactivity, with the metabolites deisopropyl zilpaterol, acetylated deisopropyl zilpaterol, hydroxy-zilpaterol, a glucuronide conjugate of hydroxy-zilpaterol and two unidentified metabolites each accounting for between 2% and 20% of the radioactivity. Deisopropyl zilpaterol was the main metabolite present. In faeces, parent compound accounted for 10–45% of the radioactivity present and hydroxy-zilpaterol for 60–80%, with a small fraction present as deisopropyl zilpaterol. After 8 days, residues in liver and carcass were less than 0.1% and less than 1%, respectively, of the administered dose; radioactivity could not be detected in fat or muscle.

In studies using rat hepatic microsomal fractions, unchanged zilpaterol was present, together with deisopropyl zilpaterol and hydroxy-zilpaterol. The metabolites were shown to be largely the products of cytochromes P450; deisopropyl zilpaterol was formed preferentially by members of the CYP1A subfamily, whereas hydroxy-zilpaterol was formed less specifically, possibly involving members of the CYP2B subfamily. Zilpaterol HCl did not induce drug-metabolizing enzymes.

Many *in vitro*, *ex vivo* and *in vivo* studies have clearly demonstrated the β_2 -agonist effect of zilpaterol HCl and its main metabolite, deisopropyl zilpaterol (as free base or hydrochloride form). These effects are manifested as contraction of cardiac muscle and relaxation of the smooth muscles of the vasculature and the bronchi. There is little or no affinity of zilpaterol or its main metabolite for α_1 - or α_2 -adrenoceptors or for dopaminergic receptors D_1 and D_2 . Studies on guinea-pig lung membranes have demonstrated that zilpaterol HCl and deisopropyl zilpaterol are only partial agonists at the β_2 -adrenoceptor in terms of adenylyl cyclase activation.

In rat studies, zilpaterol HCl given intravenously to anaesthetized, pithed animals induced a dose-dependent decrease in diastolic blood pressure from 0.01 mg/kg bw, with a maximum effect observed at 0.1 mg/kg bw, and an increase in heart rate from 0.3 mg/kg bw. Deisopropyl zilpaterol (in either its free base or hydrochloride form) caused a dose-dependent decrease in diastolic blood pressure from 0.1 mg/kg bw, with a steady-state maximum effect observed at 0.3 mg/kg bw, but had no effect on the heart rate. These data indicate that deisopropyl zilpaterol

has 10-fold lower β_2 -agonist activity on the cardiovascular system, compared with the parent compound. Use of specific antagonists for β_1 - and β_2 -adrenoceptors demonstrated that the effect of the compounds on blood pressure was mediated by β_2 -adrenoceptors, whereas the activity of zilpaterol HCl on the heart was associated with the β_1 -adrenoceptor.

3.2 Toxicological data

A comprehensive set of toxicological studies was performed, mainly in compliance with GLP, in both rodent (mice, rat) and non-rodent species (dog, Cynomolgus monkey and microswine). The purity of the test item was higher than 90% in all studies. The pivotal toxicological studies are summarized in [Table 13](#).

Table 13. Studies relevant to risk assessment

Species/study type (route)	Doses (mg/kg bw per day ^a)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day ^a)
Mouse				
Eighteen-month study of toxicity and carcinogenicity (gavage)	0, 0.01, 0.02, 0.05, 0.25	Increased haemoglobin, red blood cell counts, haematocrit; decreased numbers of platelets, absolute neutrophil counts and monocyte numbers	0.02	0.05
Rat				
Thirteen-week study of toxicity (gavage)	0, 0.05, 0.5, 1	Decreased mean heart rate	–	0.05 ^b
Two-year study of toxicity and carcinogenicity (dietary)	0, 0.025, 0.05, 0.125, 0.25	Increased weight of ovaries with increased incidence of ovarian cysts; increased incidence of ovarian leiomyomas	0.05	0.125
Two-generation reproductive toxicity study (dietary)	0, 0.06, 0.23, 0.94 (F ₀ males); 0, 0.10, 0.40, 1.61 (F ₀ females); 0, 0.08, 0.32, 1.26 (F ₁ males); 0, 0.12, 0.45, 1.77 (F ₁ females)	Parental toxicity: Increased body weight and feed consumption	–	0.06 ^b
		Reproductive and offspring toxicity: No effects	0.94 ^c	–
Developmental toxicity study (gavage)	0, 0.2, 2, 10, 50	Maternal toxicity: Increased occurrence of hypersalivation, increased body weight gain	0.2	2
		Embryo and fetal toxicity: Delayed ossification	10	50

Table 13 (continued)

Species/study type (route)	Doses (mg/kg bw per day ^a)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day ^a)
Rabbit				
Developmental toxicity study (gavage)	0, 20, 60, 180	Maternal toxicity: Increased body weight Embryo and fetal toxicity: Increased incidence of skeletal anomalies	–	20 ^b
Dog				
Thirty-day study of toxicity (capsule)	0, 0.5, 5, 50	Increased peripheral vasodilatation, increased heart rate, decreased blood pressure	–	0.5 ^b
Microswine				
Thirteen-week study of toxicity (gavage)	0, 0.001, 0.05, 1, 10	No effects	10 ^c	–
Cynomolgus monkey				
Four-week study of toxicity (gavage)	0, 0.005, 0.01, 0.05, 5	Increased heart rate with a decreased QT interval	0.01	0.05
Human				
Four-way cross-over design, single-blind and placebo-controlled study (healthy adult)	0, 3.6, 7.1, 14.3, 28.6 µg/kg bw	Increased heart rate and blood glucose level	–	3.6 µg/kg bw ^b
Double-blind, randomized, placebo-controlled four-way cross-over study (asthmatic patient)	0, 0.76, 1.52, 3.79 µg/kg bw	Increased incidence of tremor	–	0.76 µg/kg bw^{b,d}

^a Except where otherwise noted.

^b Lowest dose tested.

^c Highest dose tested.

^d Pivotal study value (Vivet, 1989).

Zilpaterol HCl and deisopropyl zilpaterol were of relatively low acute toxicity by the oral route. The oral LD₅₀ values of zilpaterol HCl were about 1100 mg/kg bw in rats and about 500 mg/kg bw in mice. The main metabolite, deisopropyl zilpaterol free base, had an oral LD₅₀ value in mice of about 1000 mg/kg bw, indicating that its acute toxicity is about half that of the parent compound. Zilpaterol HCl

is not irritating to skin and is slightly irritating to eyes. It is not a skin sensitizer in guinea-pigs.

In short-term toxicity studies performed in mice, rats, dogs, microswine and Cynomolgus monkeys, the main effects observed were those classically associated with β -adrenoceptor agonists, such as cardiovascular effects (increased heart rate and decreased diastolic blood pressure). In parallel, increased body weight associated with increased feed consumption was also observed.

In mice, zilpaterol HCl administered orally for 4 weeks at a dose of 0, 0.2, 2, 4 or 40 mg/kg feed (equal to 0, 0.046, 0.46, 0.895 and 8.867 mg/kg bw per day for males and 0, 0.047, 0.483, 0.986 and 9.601 mg/kg bw per day for females, respectively) induced no treatment-related effects except body weight change. There was a small but statistically significant increase in body weight in female mice at the highest dose, but body weight had returned to control values by the end of treatment. The NOAEL was 4 mg/kg feed (equal to 0.986 mg/kg bw per day), based on increased body weight in female mice.

In a 30-day study, rats were given zilpaterol HCl at a dose of 0, 1, 10 or 100 mg/kg bw per day by oral gavage. Body weight was increased at 10 and 100 mg/kg bw per day in males and at 100 mg/kg bw per day in females, with no change in feed consumption. A dose-dependent decrease in heart rate was observed at all doses in female rats. In male rats, total leukocyte count was increased at the highest dose, and activated partial thromboplastin time was slightly decreased at all doses. Decreased prothrombin time was observed at 10 and 100 mg/kg bw per day in females. Microscopic examination revealed no treatment-induced lesions. The LOAEL was 1 mg/kg bw per day, the lowest dose tested, based on decreased heart rate in females and decreased activated partial thromboplastin time in males.

In a 90-day study, rats were given zilpaterol HCl at a dose of 0, 1, 10 or 100 mg/kg bw per day by oral gavage. At 10 and 100 mg/kg bw per day, dose-dependent hypersalivation in both sexes was observed. Statistically significant increases in body weight gain were seen in females at 10 and 100 mg/kg bw per day and in males at 100 mg/kg bw per day, and an increase in feed consumption in males was observed at 10 and 100 mg/kg bw per day. There was a slight increase in blood urea at all doses in both sexes, but it was not accompanied by histopathological changes. Increased plasma creatinine was found at 10 and 100 mg/kg bw per day in males and females on week 5, but levels returned to normal at 10 mg/kg bw per day in males and at 100 mg/kg bw per day in females on week 13. The LOAEL was 1 mg/kg bw per day, the lowest dose tested, based on increased blood urea. In a follow-up study, when zilpaterol HCl was administered to rats at 0, 0.05, 0.5 or 1 mg/kg bw per day for 4 weeks, body weight was significantly increased at the highest dose without significant change in feed consumption in female rats. The NOAEL was 0.5 mg/kg bw per day, based on body weight change in females.

In a further short-term study, when rats were given zilpaterol HCl for 13 weeks at 0, 0.05, 0.5 or 1 mg/kg bw per day by oral gavage, increased body weight was observed in males and females at 0.5 and 1 mg/kg bw per day. Significantly lower mean heart rates were observed at all doses in females and at the highest dose in males on week 3, 4, 8 or 13. This was associated with longer

PQ intervals at all doses in females and at 0.5 or 1 mg/kg bw per day in males. Lower systolic blood pressure was observed at 0.5 or 1 mg/kg bw per day on week 3 or 4 in males. A LOAEL of 0.05 mg/kg bw per day, the lowest dose tested, was derived from the results of this study.

In a 7-day study, dogs were given zilpaterol HCl at 100 mg/kg bw per day in a capsule by oral gavage. Clinical signs included frequent vomiting, reduced motor activity, apathy, vasodilatation and diarrhoea, accompanied by reduced body weight, changes in haematology and blood biochemistry, and morphological changes in the liver.

In a 30-day study, dogs were given zilpaterol HCl at 0, 0.5, 5 or 50 mg/kg bw per day in a capsule by oral gavage. A dose-dependent peripheral vasodilatation was observed at all doses. Reduced blood pressure and increased heart rate were observed at 1 hour, but blood pressure and heart rate had returned to normal values by 24 hours after dosing at all doses. Irregular vomiting and reduced motor activity were found at 5 and 50 mg/kg bw per day. The LOAEL was 0.5 mg/kg bw per day, the lowest dose tested, based on peripheral vasodilatation, increased heart rate and decreased blood pressure.

Microswine (one of each sex per group) were given increasing doses of zilpaterol HCl of 0.05, 0.5, 1, 5 and 10 mg/kg bw per day by oral gavage, each dose being given for 4 days with a 3-day withdrawal period between doses. A slight increase in heart rate associated with a decreased QT interval, without any change in blood pressure, was found from 0.5 mg/kg bw per day in a male and at 10 mg/kg bw per day in a female. In a 4-week toxicity study in which microswine were given zilpaterol HCl at 0, 0.005, 0.01, 0.05 or 5 mg/kg bw per day by oral gavage, the NOAEL was 5 mg/kg bw per day, the highest dose tested, as no treatment-related adverse effects were found. In a 13-week toxicity study in which microswine were given zilpaterol HCl at 0, 0.001, 0.05, 1 or 10 mg/kg bw per day by oral gavage, non-statistically significant increases in body weight were observed only in males at 1 and 10 mg/kg bw per day. The NOAEL was 10 mg/kg bw per day, the highest dose tested.

Cynomolgus monkeys (one of each sex per group) were given increasing doses of zilpaterol HCl of 0.05, 0.5, 1, 5 and 10 mg/kg bw per day by oral gavage, each dose being given for 4 days with a 3-day withdrawal period between doses. A slight increase in heart rate associated with a decreased QT interval, without any change in blood pressure or dose-response relationship, was found at all doses.

In a 4-week toxicity study in which Cynomolgus monkeys were given zilpaterol HCl at 0, 0.005, 0.01, 0.05 or 5 mg/kg bw per day by oral gavage, a reduction in blood pressure, accompanied by an increased heart rate with an associated decrease in QT interval, was observed at 0.05 and 5 mg/kg bw per day. The NOAEL was 0.01 mg/kg bw per day, based on cardiovascular effects.

In a chronic toxicity and carcinogenicity study in female mice, zilpaterol HCl was given orally by gavage at doses of 0, 10, 20, 50 and 250 µg/kg bw per day for 18 months. Significantly increased haemoglobin, associated with significantly increased red blood cell counts and haematocrit, was found at 50 and 250 µg/kg bw per day. There were also significantly decreased numbers of platelets, absolute

neutrophil counts and monocyte numbers at 50 and 250 µg/kg bw per day. There were no neoplastic or preneoplastic findings. The NOAEL was 20 µg/kg bw per day, based on haematological changes.

In a chronic toxicity study in rats, zilpaterol HCl was given in the feed at concentrations adjusted to achieve doses of 0, 25, 50, 125 and 250 µg/kg bw per day for 52 weeks. There were slight decreases in heart rate, without impact on waveform traces, at 125 and 250 µg/kg bw per day and slight increases in systolic blood pressure at the highest dose in both sexes. These effects were fully reversible after a 4-week recovery period. The NOAEL was 50 µg/kg bw per day, based on the decrease in heart rate.

In a chronic toxicity and carcinogenicity study in rats, zilpaterol HCl was given in the feed at concentrations adjusted to achieve doses of 0, 25, 50, 125 and 250 µg/kg bw per day for 104 weeks. Slightly reduced body weight gains were found in males and females at the highest dose. The number of surviving male animals was significantly reduced at the highest dose. A marked increase in the weight of ovaries with increased incidence of ovarian cysts was observed at 125 and 250 µg/kg bw per day. An increased incidence of ovarian leiomyomas of the suspensory ligament was also found in the two highest dose groups. The NOAEL was 50 µg/kg bw per day, based on the increased ovarian weight with increased incidence of cysts and the increased incidence of leiomyomas.

Ovarian leiomyomas are benign tumours known to be related to the use of β_2 -adrenoceptor agonists in rodents. The proliferation of the mesovarian smooth muscle is considered to be an adaptive physiological response to prolonged stimulation of the β -receptors, with muscle relaxation as a consequence. The occurrence of this neoplastic lesion in rodents was reported to be blocked by concomitant treatment with propranolol, a β_2 -adrenoceptor antagonist. Little or no relaxant response to β_2 -agonists can be demonstrated in the uteri of non-pregnant women. There is no evidence in humans of any increased incidence of smooth muscle tumours such as leiomyomas among users of β -agonists. Thus, the occurrence of ovarian leiomyomas in rats treated with zilpaterol HCl may represent a species-specific effect, and it is considered unlikely that oral exposure in humans would result in ovarian leiomyomas.

In an adequate range of tests of genotoxic activity, mainly performed under GLP-compliant conditions, zilpaterol HCl showed no evidence of genotoxic potential in microorganisms, in cultured mammalian cells or in vivo in mice. Like the parent compound, its main metabolite deisopropyl zilpaterol showed no evidence of genotoxicity in an adequate range of tests.

Considering the absence of any evidence of genotoxicity in vitro and in vivo, together with the likelihood that induction of mesovarian leiomyomas observed with zilpaterol HCl is associated with species-specific pharmacological β_2 -adrenergic activity, the Committee concluded that zilpaterol HCl is unlikely to pose a carcinogenic risk to humans.

In a one-generation reproductive toxicity study in rats, zilpaterol HCl was given in the feed at a concentration of 0, 0.9, 3.6 or 14.4 mg/kg (equal to 0, 0.06, 0.23 and 0.94 mg/kg bw per day for males and 0, 0.10, 0.40 and 1.61 mg/kg bw per

day for females, respectively) from 15 days prior to and during mating, pregnancy and lactation. There were no effects attributable to the treatment on F_0 reproduction or F_1 litters, apart from significant increases in body weight in all treated males, mainly during the 1st week of treatment, and in females during the entire treatment period at the highest dose. Based on body weight increases, the LOAEL for parental toxicity was 0.9 mg/kg feed (equal to 0.06 mg/kg bw per day in F_0 males), the lowest dose tested. The NOAEL for offspring and reproductive toxicity was 14.4 mg/kg feed (equal to 0.94 mg/kg bw per day), the highest dose tested.

In a two-generation reproductive toxicity study in rats, zilpaterol HCl was given in the feed at a concentration of 0, 0.9, 3.6 or 14.4 mg/kg from 71 days prior to mating and until weaning in F_0 or F_1 males and from 15 days before mating until the end of lactation for F_0 or F_1 females (equal to 0, 0.06, 0.23 and 0.94 mg/kg bw per day for F_0 males; 0, 0.10, 0.40 and 1.61 mg/kg bw per day for F_0 females; 0, 0.08, 0.32 and 1.26 mg/kg bw per day for F_1 males; and 0, 0.12, 0.45 and 1.77 mg/kg bw per day for F_1 females). There were no effects attributable to the treatment on F_0 or F_1 reproduction or on F_1 and F_2 litters, apart from slightly higher feed consumption and body weight gain in both F_0 and F_1 parents at all doses. The LOAEL for parental toxicity was 0.9 mg/kg feed (equal to 0.06 mg/kg bw per day), the lowest dose tested, for effects on feed consumption and body weight gain. The NOAEL for offspring and reproductive toxicity was 14.4 mg/kg feed (equal to 0.94 mg/kg bw per day), the highest dose tested.

In a limited but GLP-compliant developmental toxicity study in mice, zilpaterol HCl was given at a dose of 0, 300 or 450 mg/kg bw per day by oral gavage from days 6 to 18 of pregnancy. There were no effects on general condition or behaviour of the dams or on body weight. No litter parameters were affected, and there were no malformations attributable to zilpaterol HCl. The NOAEL for maternal and embryo/fetal toxicity was 450 mg/kg bw per day, the highest dose tested.

In a series of three developmental toxicity studies in rats, zilpaterol HCl was given at doses ranging from 30 to 750 mg/kg bw per day by oral gavage from days 6 to 15 of gestation. A dose-dependent increase in maternal salivation was seen in all three studies (from 50 mg/kg bw per day). At the two highest doses used (600 and 750 mg/kg bw per day), there was an increase in postimplantation loss and a reduction in fetal weight, respectively. In two of the studies, there were significant increases in the numbers of fetuses with skeletal or visceral anomalies and/or variants, reaching statistical significance at the highest doses (450 and 750 mg/kg bw per day).

In a developmental toxicity study in rats using lower doses, zilpaterol HCl was given at a dose of 0, 0.2, 2, 10 or 50 mg/kg bw per day by oral gavage from days 6 to 15 of pregnancy. Hypersalivation was observed at 2, 10 and 50 mg/kg bw per day, with a more marked incidence in the high-dose group. Maternal body weight gains were increased in a dose-dependent manner from 2 mg/kg bw per day. Feed consumption was increased at 2 mg/kg bw per day; at 10 and 50 mg/kg bw per day, feed consumption decreased transiently and then increased. Water consumption was increased at 50 mg/kg bw per day. The incidences of delayed ossification of some bones (5th and 6th sternbrae, skull) and of wavy ribs were higher at

50 mg/kg bw per day. The NOAEL for maternal toxicity was 0.2 mg/kg bw per day, based on hypersalivation and increased body weight gain. The NOAEL for embryo and fetal toxicity was 10 mg/kg bw per day, based on delayed ossification.

In a developmental toxicity study in rabbits, zilpaterol HCl was given at a dose of 0, 20, 60 or 180 mg/kg bw per day by oral gavage from days 6 to 18 of pregnancy. Maternal body weights as well as final body weights (excluding gravid uterus) were slightly higher at all doses, with statistical significance at 60 mg/kg bw per day. The incidence of skeletal anomalies was increased in all treated groups, reaching statistical significance in the low- and high-dose groups. The LOAEL for maternal and embryo/fetal toxicity was 20 mg/kg bw per day, the lowest dose tested.

In a relay pharmacology study in dogs, one male and one female were fed daily for 5 days with either 200 g liver or 200 g muscle from steers treated orally with a zilpaterol HCl dose of 0.2 mg/kg bw per day for 50 days and then slaughtered immediately after the end of the treatment or 7 days later. A positive control group was fed with 200 g muscle from untreated cattle together with zilpaterol HCl (by oral capsule) at 3 µg/kg bw per day. The achieved doses of zilpaterol HCl were 1742–1985 ng/kg bw per day for dogs fed with 200 g liver from steers killed immediately after the 50 days of treatment and 252.5–275.6 ng/kg bw per day for dogs fed with 200 g muscle from steers killed immediately after the 50 days of treatment. The achieved doses of zilpaterol HCl were 11.3 ng/kg bw per day for dogs fed with 200 g liver and 1.5–1.7 ng/kg bw per day for dogs fed with 200 g muscle from steers killed 7 days after the 50 days of treatment. There was no effect of this treatment regimen on either heart rate or blood pressure. In the positive controls treated with 3 µg/kg bw per day, a marginal effect on heart rate, but not on blood pressure, was observed.

3.3 Observations in humans

Studies were conducted in which human volunteers, either healthy or asthmatic, were administered zilpaterol HCl. The study protocols were approved by ethics committees, and the studies were carried out according to the guidelines in the Declaration of Helsinki (revised Tokyo version). The pivotal studies in humans are summarized in [Table 13](#) above.

In a four-way cross-over design, single-blind and placebo-controlled study involving eight healthy male volunteers, ascending single doses of 0, 0.25, 0.50, 1.00 and 2.00 mg/person (equal to 0, 3.6, 7.1, 14.3 and 28.6 µg/kg bw, respectively) were given orally in aqueous vehicle. The volunteers fasted before dosing. The dose of 0.25 mg/person slightly increased heart rate, and higher doses provoked significant increases in heart rate. Airway calibre was significantly increased at 0.50, 1.00 and 2.00 mg/person, and systolic blood pressure was significantly increased at 1.00 and 2.00 mg/person. Haematology remained unaffected, but blood glucose was increased in a dose-dependent manner at all dose levels. Tremor frequency was significantly increased from 0.50 mg/person (equal to 7.1 µg/kg bw). Based on this study, the LOAEL was 0.25 mg/person (equal to 3.6 µg/kg bw), the lowest dose tested, for cardiovascular effects and higher blood glucose levels.

In a double-blind, comparative placebo-controlled study, 13 healthy male volunteers were given placebo or zilpaterol HCl at a dose of 0.25 mg/person (equal to 3.68 µg/kg bw), 3 times a day for 7 consecutive days (i.e. a daily dose of 0.75 mg/person or about 11.04 µg/kg bw). This dosing regimen had significant effects on the cardiovascular system, tremor and bronchodilatation.

A double-blind, randomized, placebo-controlled cross-over trial was performed in 12 adult asthmatic volunteers given a single oral dose of 0 or 0.25 mg zilpaterol HCl per person (equal to 0 or 3.85 µg/kg bw). The volunteers fasted before dosing. Zilpaterol HCl significantly increased bronchodilatation (measured by FEV₁) up to 4 hours after dosing. Heart rate was slightly but significantly increased up to 1.5 hours post-dosing, with no major palpitation or ECG changes. Diastolic blood pressure was slightly but significantly decreased at 0.5 and 1 hour after dosing. Short-lasting finger tremors were seen in 2/12 patients after treatment. The LOAEL was 0.25 mg/person (equal to 3.85 µg/kg bw), the only dose tested, for bronchodilatation, tremors and cardiovascular effects.

In a double-blind, randomized, placebo-controlled, four-way cross-over trial performed in 11 asthmatic volunteers, three single oral zilpaterol HCl doses of 0, 0.05, 0.10 or 0.25 mg/person (equal to 0, 0.76, 1.52 and 3.79 µg/kg bw, respectively) were tested. The volunteers fasted before dosing. Bronchodilatation was observed at 0.10 and 0.25 mg/person, remaining slight and transient, although only the highest dose induced a slight but significant change in heart rate. Blood pressure remained unaffected at all doses. Mild tremor was seen when evaluated clinically by a physician (two patients at 0.05 and 0.10 mg/person and eight patients at 0.25 mg/person). When the tremor was assessed by drawing a line between two parallel sinusoids, no significant differences were observed at any time between the three doses of zilpaterol HCl and the placebo. The LOAEL was 0.05 mg/person (equal to 0.76 µg/kg bw), based on the clinical assessment of tremor.

In all studies performed in human volunteers (16 healthy and 23 asthmatic), the observed effects after an oral dose were slight and transitory and typically related to the β₂-adrenergic agonist activity of zilpaterol HCl. Considering all the human data, the LOAEL for zilpaterol HCl was 0.05 mg/person (equal to 0.76 µg/kg bw). An overall NOAEL could not be identified.

4. EVALUATION

The Committee considered tremors observed in humans, which were consistent with the compound's β₂-adrenergic agonist activity, as the most relevant adverse effect for establishing an ADI for zilpaterol HCl. The LOAEL for tremor was 0.05 mg/person (equal to 0.76 µg/kg bw); the effect was slight at this dose. The Committee established an ADI of 0–0.04 µg/kg bw per day by applying an uncertainty factor of 20, comprising a default uncertainty factor of 10 for human individual variability and an additional uncertainty factor of 2 to account for use of a LOAEL for a slight effect instead of a NOAEL. The Committee noted that the ADI is based on an acute effect. The Committee also noted that the upper bound of the ADI provides a margin of safety of at least 1250 with respect to the NOAEL of 50 µg/kg bw per day for the formation of leiomyomas in rats.

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