

## PRODUCT MONOGRAPH

**PR**TAMIFLU®

oseltamivir phosphate capsule

30 mg, 45 mg and 75 mg oseltamivir

oseltamivir phosphate powder for oral suspension

12 mg/mL oseltamivir when reconstituted

Antiviral Agent

Hoffmann-La Roche Limited  
2455 Meadowpine Boulevard  
Mississauga, Ontario  
L5N 6L7

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## PART II: SCIENTIFIC INFORMATION

### PHARMACEUTICAL INFORMATION

#### Drug Substance

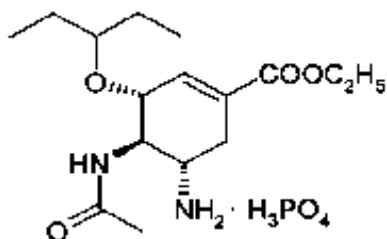
**Common Name** oseltamivir phosphate

**Chemical Name** Ethyl (3R, 4R, 5S)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate, phosphate (1:1)

**Molecular Formula** C<sub>16</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub> (free base)

**Molecular Weight** 312.4 for oseltamivir free base and 410.4 for oseltamivir phosphate salt

#### Structural Formula



**Physical Form** White crystalline solid

**Solubility** Freely soluble in water and methanol, slightly soluble in dimethylformamide and ethanol, and practically insoluble in acetone, 2-propanol and non-polar organic solvents.

**pKa and pH values** pKa: 7.75

**Partition Co-efficient** 1-octanol/aqueous phosphate buffer: logP=0.36

**Melting Point** 192-195°C with degradation

## CLINICAL TRIALS

### Treatment of Influenza

**Adult Patients:** Phase III clinical trials evaluated the safety and efficacy of TAMIFLU (oseltamivir phosphate) for the treatment of naturally occurring influenza during a period when influenza virus was known to be circulating in the community. A total of 1418 patients received any treatment (TAMIFLU or placebo) of whom 476 patients received 75 mg TAMIFLU twice daily for 5 days. Patients started treatment with TAMIFLU within 40 hours after reported onset of symptoms. The primary efficacy parameter was the time to alleviation of all symptoms. The population used in the primary analyses was the intent-to-treat-infected (ITTI) population. This population included only subjects who received at least one dose of study treatment and who had laboratory confirmed influenza. An intent-to-treat (ITT) population included all subjects who took at least one dose of study medication, regardless of whether they proved to have influenza. The results for two pivotal studies are shown in the table below.

**Table 6: Median Time (Hours) to Alleviation of All Symptoms in the ITTI and ITT Populations**

Study	Population	Placebo (95% CI)	TAMIFLU 75 mg twice daily (95% CI)	p-value*
WV15670	ITTI	n=161 116.5 (101.5 to 137.8)	n=157 87.4 (73.3 to 104.7)	0.017
	ITT	n=235 116.1 (99.8 to 129.5)	n=240 97.6 (79.1 to 115.3)	0.051
WV15671	ITTI	n=128 103.3 (92.6 to 118.7)	n=121 71.5 (60.0 to 83.2)	<0.0001
	ITT	n=200 97.0 (86.3 to 113.6)	n=204 76.3 (66.3 to 89.2)	0.004

ITT intent-to-treat

ITTI intent-to-treat infected

\* difference between medians

Treatment with TAMIFLU significantly reduced the duration by 1.3 days, of clinically relevant symptoms of influenza. The seven symptoms assessed were: feverish feeling, muscle aches or myalgia, headache, sore throat, cough, overall discomfort, and nasal stuffiness or runny nose.

***Pediatric Patients:*** One double-blind placebo controlled treatment trial was conducted in pediatric patients, aged 1 to 12 years (mean age 5.3), who had fever (>100°F) plus one respiratory symptom (cough or coryza) when influenza virus was known to be circulating in the community. In this study 67% of influenza-infected patients were infected with influenza A and 33% with influenza B. Treatment with TAMIFLU, started within 48 hours of onset of symptoms, significantly reduced the duration of illness by 1.5 days compared to placebo. Duration of illness was defined as time to alleviation of cough, alleviation of coryza, resolution of fever, and return to normal health and activity.

Pediatric patients receiving TAMIFLU returned to normal health and activity almost 2 days earlier than those receiving placebo.

### **Prevention of Influenza**

***Adult Patients:*** The efficacy of TAMIFLU in preventing naturally occurring influenza illness has been proven in four separate trials which are summarized below and in Table 4.

In a phase III study (WV15799) in adult and adolescent contacts of a household case of influenza, 75 mg of TAMIFLU once daily, started within 2 days of onset of symptoms in the household case and continued for 7 days, significantly reduced the incidence of influenza illness occurring in the contacts by 92% ( $p$  value = < 0.001).

In a double blind placebo controlled study (WV15673) conducted in unvaccinated otherwise healthy adults 18-65 years of age, 75 mg of TAMIFLU administered once daily significantly reduced the incidence of clinical influenza by 76% ( $p$  value = 0.0006) during a community outbreak of influenza. The subjects in this study received TAMIFLU for a period of 42 days. No additional benefit was demonstrated in this study using 75 mg of TAMIFLU twice daily.

In a double blind placebo controlled study (WV15825) in elderly residents of nursing homes, many of whom had chronic cardiac disease and/or respiratory disease, 80% received vaccine in the season of the study. The vaccine was a good match for circulating strains. The administration of 75 mg of TAMIFLU once daily significantly reduced the incidence of clinical influenza illness in these patients by 92% ( $p$  value = 0.0015). In the same study, TAMIFLU significantly reduced the incidence of influenza associated bronchitis, pneumonia and sinusitis by 86% ( $p$  value = 0.037). The subjects in this study received TAMIFLU for a period of 42 days.

In all three of these clinical trials, approximately 1% of subjects taking TAMIFLU for prevention developed influenza during the dosing period.

In a fourth phase III study (WV16193) it was demonstrated that TAMIFLU effectively prevents the secondary spread of influenza within households. In this study, the index case was treated with TAMIFLU and household contacts were randomized (by household) to receive either prophylaxis (P) with TAMIFLU or treatment (T) with TAMIFLU upon emergence of influenza-like illness. In households with infected index cases where subjects who were already shedding virus at baseline were excluded (ITTIINAB population) there was a 78.8% ( $p$  = 0.0008) reduction in the incidence of laboratory-confirmed influenza in P versus T. Amongst contacts, the outcome was analogous to that seen for households with a significantly lower number of infected

contacts in P versus T (84.5% reduction,  $p = 0.0002$ , ITTIINAB population). No virus shedding was detected in any subject in the prophylaxis group while 7% of contact in the treatment group (ITTIINAB) shed virus.

TAMIFLU also significantly reduced the incidence of virus shedding and successfully prevented virus transmission in families.

**Table 7: Clinical Summary of Prevention Studies**

Study	Number of Subjects	Dose	Reduction in Clinical Influenza (Protective Efficacy)
<b>Seasonal Studies</b>			
			ITT Population
WV15673/WV15697 Adults	1559	Placebo 75 mg o.d. 75 mg b.i.d. 42 days	76%, $p = 0.00055$ 72%, $p = 0.00125$
WV15825 Elderly	548	Placebo 75 mg o.d. 42 days	92%, $p = 0.00153$
<b>Post-exposure Studies</b>			
			ITTIINAB Population
WV15799 Adult/adolescent contacts, index case not treated	405	Placebo 75 mg o.d., 7 days	92%, $p = 0.000076$
WV16193 Index case treated, age $\geq 1$ year	89**	Prophylaxis: 75 mg o.d., 10 days* Treatment: 75 mg b.i.d., 5 days*	78.8%, $p = 0.0008$
WV16193 (children 1-12 years)	117	Prophylaxis: 30 mg (1-2 years) 45 mg (3-5 years) 60 mg (6-12 years) o. d., 10 days Treatment: 30 mg (1-2 years) 45 mg (3-5 years) 60 mg (6-12 years) b. i. d., 5 days	80.1 % (22.0-94.9), $p = 0.0206$

\*Pediatric dosing adjusted according to age

\*\* Number of households

ITT=Intent to treat

ITTIINAB=Intent to treat, index infected, not infected at baseline

**Pediatric Patients:** In the post-exposure prophylaxis study in the family (WV16193; see ‘Adult Prevention Studies’) there were 215 pediatric contact cases (>1 to 12 years of age). There was an even distribution of boys and girls with the majority being Caucasian. The mean age was 8 years (range 1 to 12). The data from this subset of pediatric patients was examined to determine if oseltamivir was effective in the prevention of influenza infection in this setting. When subjects who were already shedding virus at baseline were excluded (ITTIINAB population, 117), 17 pediatric contacts became infected, 2 in the prophylaxis group and 15 in the treatment group (see Table 4). The protective efficacy in the pediatric contacts was similar to that achieved in the overall population in this study.

The dosing schedule in this study was by age. The majority of children received the now recommended schedule of treatment by weight in children (see DOSAGE AND ADMINISTRATION). There were, however, some children who were under- or over-dosed (23% and 9%, respectively) in this study.

## **DETAILED PHARMACOLOGY**

### **Animal Pharmacology**

Oseltamivir phosphate produced effects in the non-clinical safety pharmacology studies only at oral doses well in excess of any clinically relevant therapeutic levels. These effects in the rat, were reduced gastrointestinal transit and gastric emptying at 1000 mg/kg. In the rodent toxicology studies these effects were not reported as any sign of gastrointestinal disturbance. Additionally, there were increases in excretion of electrolytes at 100 and 1000 mg/kg and increased urine production at 1000 mg/kg. Increases in electrolyte excretion were reported in a 27-week rat toxicology study at 1000 mg/kg/day and attributed to a high phosphate intake due to the salt of the test material. In the same study less pronounced effects were seen at 200 mg/kg/day, while in another rat toxicology study no significant effects were seen at 100 mg/kg/day. A statistically significant increase in response to a painful stimulus was seen but this was neither time nor dose related and therefore not thought to be of pharmacological significance.

The intravenous infusion of the active metabolite at 2, 15 and 100 mg/kg cumulatively produced statistically significant changes in heart rate, QT and QTc interval, QRS duration and pCO<sub>2</sub> when compared with time matched controls in the anaesthetized dog. The effects on heart rate and pCO<sub>2</sub> were at isolated time points and the decrease in QRS duration was not accompanied by any other relevant physiological changes and so not likely to be due to the drug treatment. The statistical differences in QT and QTc interval between the active metabolite and vehicle treated groups was seen in the predose and just after the start of the infusion suggesting no pharmacological significance. Because of this, significance was tested for by comparing percentage changes from predose values within the active metabolite treatment group. There were no significant differences detected. However, on comparison with the absolute values significant differences were seen during the infusion of the 100 mg/kg/dose. To clarify this situation a further test was performed in an isolated sheep Purkinje fibre study where no significant effects were observed on cardiac action potential parameters. Other than these findings, no additional effects were seen on the cardiovascular and respiratory dynamics of the anaesthetized dog.

In conclusion, oseltamivir phosphate produced significant pharmacological effects only at doses much greater than would be of clinical relevance. It is therefore concluded that oseltamivir phosphate and the active metabolite produced no clinically relevant pharmacological effects on the central nervous, cardiovascular, respiratory, gastrointestinal, smooth muscle, renal, hepatic and immune systems tested.

### **Human Pharmacology**

**QT/QTc:** A retrospective analysis of ECGs from 8 clinical pharmacology studies (n=182 subjects including 30 placebo) concluded that TAMIFLU (oseltamivir phosphate) does not cause prolongation of QT intervals in humans. Although some individuals were found to have some alterations in QTc measurements, none were of clinical significance and the frequency was similar among placebo and subjects treated with TAMIFLU.

In a study on ECG intervals in which healthy volunteers received daily doses of either 75, 225 or 450 mg TAMIFLU b.i.d. orally for 5 days, treatment with TAMIFLU had no impact on any ECG parameters

### **MICROBIOLOGY**

**Virology:** Oseltamivir was also tested for its effect on human T cell proliferation *in vitro*. Both antigen specific T cell lines and peripheral blood lymphocytes were isolated from whole blood. There was a slight but significant inhibition of influenza specific T cell line proliferation in the presence of 1 and 10  $\mu$ M active metabolite, while there was no effect on antigen stimulation of peripheral blood lymphocytes. This slight effect (<20 %) on T cell proliferation is unlikely to compromise the long-term immune status of the patient with respect to subsequent influenza infection.

The active metabolite inhibits neuraminidases of influenza viruses of both types A and B. Inhibitory concentrations *in vitro* are in the low nanomolar range. The 50% inhibitory concentration (IC<sub>50</sub>) was in the range of 0.1 to 2.6 nM. The relationship between the *in vitro* antiviral activity in cell culture and the inhibition of influenza virus replication in humans has not been established. The active metabolite also inhibits influenza virus infection and replication *in vitro* and inhibits influenza virus replication and pathogenicity in animal models.

#### ***Resistance:***

##### ***In vitro***

Extensive *in vitro* work has been completed with the active metabolite. Resistance to this compound does not arise readily *in vitro*. Several different resistance mutations in the viral neuraminidase have been selected *in vitro* in Roche studies or reported in the published literature. Resistance mutations tend to be viral sub-type specific. The degree of reduced sensitivity differs markedly for different mutations from 2 fold for I222V in N1 to 30,000 fold for R292K in N2. Influenza A virus H1N1 strains are associated with a histidine to tyrosine change at position 274 (H274Y) on the enzyme. In H3N2 subtypes the genetic alteration of interest is an arginine to lysine at position 292 (R292K) on the enzyme. *In vitro* these mutant viruses exhibit reduced growth potential compared to wild-type virus.

### **In vivo**

*In vivo* experiments of infectivity and pathogenicity have been conducted with mutated viruses in mice and ferrets. These experiments have demonstrated that the H274Y H1N1 mutant and the R292K H3N2 mutant have reduced ability to infect susceptible animals compared to wild-type virus and that infection is not associated with clinical evidence of pathogenicity in the ferret. Correlation of *in vitro* resistance patterns to resistance *in vivo* are not known. Viruses with resistant neuraminidase genotypes have varying degrees of loss of fitness compared to wild-type.

During the clinical program, resistance to therapy was assessed by neuraminidase enzyme inhibition assays and by genotyping of the neuraminidase enzyme itself. In addition, the potential for virus resistance mediated by changes in hemagglutinin was evaluated genotypically. The R292K mutation was the most commonly selected mutation in the *in vitro* studies and was also the predominant mutation occurring clinically (occurrence restricted to N2 subtype). The exception to the predictive pattern of *in vitro* experiments to the clinical emergence of resistance was the occurrence of E119V (glutamic acid to valine at position 119 on the enzyme) in influenza N2, that had not been seen previously *in vitro*. Characterisation of a clinical isolate carrying this mutation demonstrated that the mutation effected a reduction in infectivity/pathogenicity and is therefore unlikely to be of clinical concern. In the patients in whom resistant virus were selected the emergence of resistant virus did not appear to give a more severe or prolonged infection or illness as measured by a composite symptom score.

The potential for emergence of virus resistant to treatment continues to be evaluated. In clinical studies and viruses from TAMIFLU-treated patients, mutations in N1 neuraminidase giving resistance/reduced sensitivity to oseltamivir carboxylate are H274Y and in one instance N294S and E119V, R292K and in one instance each N294S and SASG245-248del in N2 neuraminidase. In influenza B neuraminidase one instance of G402S giving a 4 fold decrease in sensitivity has been reported and one instance of D198N (10 fold decrease) in an immunocompromised child has been reported.

The estimated incidence of oseltamivir-resistant virus in the adult/adolescent population is 0.32% (4/1245) by phenotyping alone and 0.4% (5/1245) by genotyping and phenotyping (full genotyping was not performed on all studies) and 4.1% (19/464) or 5.4% (25/464) respectively for pediatric patients aged 1 to 12 years. Insufficient information is available to fully characterize the risk of emergence of resistance to TAMIFLU in clinical use.

In the prophylactic trials with naturally acquired influenza infection, only 32 from 2155 drug treated individuals became productively infected with influenza virus (i.e. culture positive). Viral neuraminidase sensitivities were successfully determined from samples of 20 of these patients and none were found to be carrying resistant virus.

Naturally occurring mutations in influenza A/H1N1 virus with reduced *in vitro* susceptibility to oseltamivir have been detected in patients not exposed to oseltamivir. The clinical relevance of these mutations is unknown. The extent of reduction in sensitivity to oseltamivir and the incidence of such viruses can vary by season and region.

**Cross resistance:** Cross-resistance between zanamivir-resistant influenza mutants and oseltamivir-resistant influenza mutants has been observed *in vitro*.

Due to the limitations in the assays available to detect drug-induced shifts in virus susceptibility due to mutations in the viral hemagglutinin, an estimate of the incidence of oseltamivir resistance and possible cross-resistance to zanamivir in clinical isolates cannot be made. However, one of the three oseltamivir-induced mutations in the viral neuraminidase from clinical isolates is the same as one of the three mutations in the viral neuraminidase from clinical isolates observed in zanamivir-resistant virus.

Insufficient information is available to fully characterize the risk of emergence of resistance or cross-resistance to TAMIFLU (oseltamivir phosphate) in clinical use.

## **TOXICOLOGY**

### **Acute Dose Toxicity**

Acute oral administration was well tolerated by male and female adult rodents (mice and rats) and unweaned 14-day old male and female rats at 2000 mg/kg (~1000-fold the highest clinical dose). Single oral administration of 500 mg/kg (free base, corresponding to 657 mg/kg phosphate salt dose) or higher to juvenile 7-day old rats resulted in treatment-related mortality together with functional observation battery findings (FOB) and clinical signs indicative of general toxicity and imminent mortality (including low arousal, tremors, convulsions, alterations in general body posture, respiration, mucous membrane and skin coloration, and/or hypoactivity) and reduced body weight gain. The no effect level was 300 mg/kg (free base, corresponding to 394 mg/kg phosphate salt dose; ~150-fold the highest clinical dose) in juvenile rats in that study.

An intravenous range-finding study in mice (n=1/sex/dose) produced convulsions immediately after intravenous dosing with 250 mg/kg. The male died and the female recovered after 40 minutes. The maximum non-lethal dose of 100 mg/kg was confirmed in a further five males and five females observed for two weeks. Other than some evidence of a local reaction in the tail of two females, there were no significant adverse effects in this group.

### **Multiple Dose Toxicity**

In multiple dose rat studies, doses up to 500 mg/kg/day (2 weeks), 650 mg/kg/day (4 weeks), and 200 mg/kg/day (27 weeks) were generally well tolerated, with no significant toxicologic effects.

A dose of 1000 mg/kg/day in a two week range finding rat study in unweaned 7-9 day old rats resulted in a high rate of mortality (18/24). At 500 mg/kg, no adverse effects were seen in the 7-9 day old rats or repeated treatment (500 mg/kg/day administered from 7 to 21 days post partum).

In multiple dose rat studies, the highest doses examined ( $\geq 1000$  mg/kg/day) also induced two renal changes. One consisted of cortico-medullary mineralisation in the proximal tubules due to the imbalance of the calcium/phosphate ratio in the diet caused by dosing high levels of a phosphate salt. The second was a mild enhancement of chronic progressive nephropathy; rats are specifically sensitive to both these changes. A dose of 1000 mg/kg/day in the rat results in approximately 70 and 520 times the clinical exposure in humans, to the active metabolite and prodrug, respectively. In clinical studies, there was no biochemical evidence of renal effects in humans.

Marked gastrointestinal irritation was observed in marmosets at 2000 mg/kg/day, but not in four- and 39-week studies at 2 x 500 mg/kg/day. Emesis occurred at 500 mg/kg/day and above in marmosets, probably related to the concentration of the oral formulation. A reduction in incidence was associated with dividing the doses and halving the concentrations administered. This effect was seen at approximately 100 and 200 times the exposure values following clinical use in human, of the active metabolite and prodrug, respectively.

In the 39-week marmoset study, one 2 x 25 mg/kg/day group and two 2 x 100 mg/kg/day group animals were sacrificed prematurely. All three showed evidence of osteomalacia before dosing commenced, at autopsy and at the histopathological examination of the bones. No animal in the 2 x 500 mg/kg/day group was affected. Review of the clinical safety database, including the elderly, failed to reveal any biochemical evidence of skeletal effects in humans.

### **Reproduction and Teratology**

Fertility, teratology and pre- and post-natal studies were conducted to cover all phases of the reproductive process. There was no evidence of adverse effects on fertility or embryo-foetal development up to the highest dose of 1500 mg/kg/day in rats, or for teratogenicity testing in rabbits up to 500 mg/kg/day. These dose levels were associated with maternal toxicity. In rabbits mortalities occurred at 750 and 1500 mg/kg/day during a non-pregnant tolerance study. Some rabbits were sacrificed in the teratology range-finding and main studies at 500 mg/kg/day due to abortions associated with maternal toxicity. In the regulatory pre- and post-natal study in rats, maternal deaths occurred (9/25) at or immediately prior to delivery in the 1500 mg/kg/day group; prolonged parturition was also observed. Two further studies were therefore undertaken; although only 1/125 maternal deaths at parturition were seen in the combined 1500 mg/kg/day groups, extension of parturition was confirmed by these studies. It was concluded that the drug alone was not responsible for the maternal deaths in the first pre- and post-natal study.

At 1500 mg/kg/day in the rat teratology study, there was a slightly increased incidence of incomplete ossification of the 3<sup>rd</sup> sternebra in the exposed offspring, when compared to controls. Statistical significance was achieved, however, the majority of incidences occurred in one litter, where a general reduction in ossification was observed. In view of the isolated nature of this finding it was considered to be of doubtful toxicological significance.

### **Mutagenicity and Carcinogenicity**

There was no evidence of mutagenic potential in any study (doses up to 5000µg/plate), with or without metabolic activation. Separate bacterial cell gene mutation (Ames) tests were conducted for the pro-drug and active metabolite. A mouse lymphoma cell mutation test examined the active metabolite. The pro-drug was tested in a chromosome analysis assay with human lymphocytes, and in an *in vivo* micronucleus test in mice (oral dose of up to 2000 mg/kg). All the study systems were verified as sensitive by positive controls, and all the results were negative.

Two year rat and mouse studies and a six month transgenic Tg: AC mouse assay performed with the active metabolite were negative.

The tables presented on the following pages provide the findings of the main toxicology, reproductive, mutagenicity and various special studies performed with oseltamivir phosphate.

**Table 8: Acute Dose Toxicity**

<b>Species Strain Duration</b>	<b>Route/Doses (mg/kg) No./Group</b>	<b>Parameters Monitored</b>	<b>Treatment Related Effects</b>
Mouse CD-1 1 Dose	Oral gavage: 2000 5/sex	Mortality, clinical signs, bodyweight, food consumption, histopathology of gross lesions	2000: No deaths. ↓ Bodyweight gain (F)
Mouse CD-1 1 Dose	Intravenous: 5, 50, 100, 250 2/sex range-finder, 5/sex at MNLD	Mortality, clinical signs, bodyweight, autopsy, histopathology of gross lesions	250: 1/1 death (M). 1/1 M, 1/1F convulsed immediately, F recovered + 40 mins. 100: No systemic effects in 5M, 5F.
Rat Han-Wistar 1 Dose	Oral gavage: 2000 5/sex	Mortality, clinical signs, bodyweight, food consumption, histopathology of gross lesions	2000: No adverse effects
Rat (2w.old, unweaned) SD: CD 1 Dose	Oral gavage: 0, 250, 500, 1000, 1500, 2000 5/sex:	Mortality, clinical signs, bodyweight, autopsy, histopathology of gross lesions	2000: No adverse effects
Rat (7-day old, unweaned) SD:CD 1 Dose	Oral gavage: 0, 300, 500, 600, 700, 850, 1000 (free base, corresponding to 394, 657, 788, 920, 1117, and 1314 mg/kg phosphate salt dose) 10/sex + toxicokinetic satellites	Mortality, clinical signs, functional observational battery (FOB), bodyweight, necropsy, histopathology of gross lesions	300: No effects 500 and higher: lethality, FOB and/or behavioral findings indicative of general toxicity and imminent mortality (e.g., low arousal, tremors, convulsions, alterations in general body posture, respiration, mucous membrane and skin coloration, and/or hypoactivity), reduced body weight gain

**Table 9: Multiple Dose Toxicity**

<b>Species Strain Duration</b>	<b>Route/Doses (mg/kg) No./Group</b>	<b>Parameters Monitored</b>	<b>Treatment Related Effects</b>
Mouse CD-1 4 Weeks	Oral gavage: 0, 50, 250, 500, 1000, 1500 12/sex + toxicokinetic satellites	Mortality, clinical signs, bodyweight, food consumption, urinalysis, haematology, clinical chemistry, toxicokinetics, autopsy, organ weights, histopathology	50-1000: No adverse effects. 1500: ↑ Hb conc., RBC count, PCV (M). ↓ Plasma Na and Cl (M). ↑ Plasma cholesterol (M,F). ↑ Focal nephropathy (M,F).
Rat SD: CD 2 Weeks	Oral gavage: 0, 125, 500, 2000 10/sex + toxicokinetic satellites	Mortalities, clinical signs, bodyweights, food consumption, haematology, clinical chemistry, toxicokinetics, autopsy, organ weights, histopathology	125: No adverse effects 500: ↑ Salivation post dosing (M,F). ↓ Bodyweight gain (transient, M). ↑ Relative liver weight (F). 2000: ↑↑ Salivation post dosing (M,F). ↑+Discolouration of peri-urinogenital fur, second week (M,F). ↓Bodyweight gain (M), (transient, F). ↑ Hb conc., RBC count, PCV, RBC distribution width (M,F). ↑WBC count, segmented neutrophils (M). ↓ APTT (M). ↑ Plasma BUN, Ca, P, total protein, globulin (M,F). ↑ Plasma Cholesterol, glucose (M). ↑ plasma Creatinine, albumin (F). ↓ K, Cl (M,F). ↓ Na (F). ↑ Relative liver weight (F). ↑ Relative kidney weight (M,F). ↑ Renal tubule mineralisation (8/10 M). ↑ Alveolar macrophage accumulation (M,F).
Rat SD: CD 4 Weeks (+ 2w recovery)	Oral gavage: 0, 50, 250, 1500 10/sex + toxicokinetic satellites	Mortalities, clinical signs, bodyweight, food and water consumption, ophthalmoscopy, urinalysis, haematology, clinical chemistry, toxicokinetics, autopsy, organ weights, histopathology	50: No adverse effects 250: ↑ Salivation post dosing. 1500: ↑+ Salivation post dosing ↑ Water consumption. ↑ WBC count, lymphocytes, neutrophils, monocytes. ↓ APTT (F) ↑ Plasma urea. ↑ Urine NAG/creatinine ratio (M,F). ↓ Urine pH. ↑ Urine protein. ↑ Relative kidney weight (M,F). ↑+ Cortico-medullary mineralisation of kidneys (7/8 M). ↑↑ Cortico-medullary mineralisation (F). ↑+ Tubular basophilia, dilatation, granular casts (M,F).

**Table 9: Multiple Dose Toxicity**

Species Strain Duration	Route/Doses (mg/kg) No./Group	Parameters Monitored	Treatment Related Effects
Rat SD:CD 4 Weeks	Oral dietary: 0, 250, 650, 1500, 2500 6/sex	Mortalities, clinical signs, bodyweight, food and water consumption, urinalysis, haematology, clinical chemistry, toxicokinetics, autopsy, organ weights, histopathology	<p>250: ↑ Fur staining, rough hair-coat (M,F). ↑ Urinary phosphate (F). ↑ Cortico-medullary mineralisation of kidneys (F). ↑ Focal nephropathy (F).</p> <p>650: ↑ Fur staining, rough hair-coat (M,F). ↓ Bodyweight gain (M). ↑ Urine phosphate (F). ↑ Renal cortico-medullary mineralisation (F). ↑ Focal nephropathy (F)</p> <p>1500: ↑+ Fur staining, rough hair-coat (M,F). ↓ Bodyweight gain (M,F), ↑ Plasma Na (M). ↓ Plasma phosphate (M). ↑ Urine phosphate (F). ↑ Renal cortico-medullary mineralisation (M,F). ↑ Focal nephropathy (F)</p> <p>2500: ↑+ Fur staining, rough hair-coat (M,F). ↓+ Bodyweight gain (M,F). ↓+ Food consumption. ↑ Plasma Na, ALP (M). ↓ Plasma Phosphate (M). ↑ Urine phosphate (F). ↑ Adjusted liver, kidney weights (M). ↑+ Renal cortico-medullary mineralisation (M). ↑++ Renal cortico-medullary mineralisation (F). ↑ Focal nephropathy (F).</p>
Rat SD:CD 27 Weeks (+ 8w recovery + t/kinetic satellites maintained off treatment for 26 weeks)	Oral gavage: 0, 50, 200, 1000 20/sex + toxicokinetic satellites	Mortalities, clinical signs, bodyweight, ophthalmoscopy, neurology, food and water consumption, urinalysis, haematology, clinical chemistry, toxicokinetics, autopsy, organ weights, histopathology	<p>50: ↑ WBC count, lymphocytes, neutrophils (M). ↑ Serum globulin (F). ↓ A/G ratio (F).</p> <p>200: ↑ WBC count, lymphocytes, neutrophils (M). ↑ Serum globulin (F). ↓ A/G ratio (F). ↓ Urine pH (M). ↑ Urine Ca, Mg P (M). ↑ Relative kidney weights (M,F). ↑ Relative liver weight (M).</p> <p>1000: ↑ Unkempt, discoloured fur anogenital region (M,F). ↑+ Water consumption (M). ↑ Water consumption (F). ↑ WBC count, lymphocytes, neutrophils, RBC distribution width, platelets (M). ↑ Serum ALP, P (F). ↑ Serum bilirubin, BUN, albumin (M). ↑ Serum cholesterol, total protein, globulin, Mg (M,F). ↓ A/G ratio (F). ↓ Serum Na (M). ↓ Serum Cl (M,F). ↓ Urine pH (M). ↑++ Urine volume (M). ↑ Urine volume (F). ↓ Urine creatinine (M,F). ↓ Urine Ca (F). ↑ Urine P, Na, K, Cl (M,F). ↑ Urine Ca, Mg (M). ↑ NAG/creatinine ratio (M,F). ↑ Relative kidney, liver weights (M,F). ↑ Relative adrenal weights (F). ↑ Renal cortico-medullary mineralisation (M,F). ↑ Chronic progressive nephropathy (M).</p>

**Table 9: Multiple Dose Toxicity**

<b>Species Strain Duration</b>	<b>Route/Doses (mg/kg) No./Group</b>	<b>Parameters Monitored</b>	<b>Treatment Related Effects</b>
Marmoset 7 Days	Oral gavage: 0, 100, 500, 1000, 2000 2/sex	Mortalities, clinical signs, bodyweight, food consumption, urinalysis, haematology, clinical chemistry, toxicokinetics, autopsy, organ weights, histopathology	100: No adverse effects. 500/2x250: ↑ Emesis 1000/2x500: ↑+ Emesis. ↑+ Gastric mucosal atrophy (1/4). 2000: Death (1/4). Sacrificed in extremis (3/4). ↑++ Gastric mucosal degeneration (4/4). ↑++ Small intestinal mucosal degeneration (1/4).
Marmoset 4 Weeks (+2 w recovery)	Oral gavage: 2x0, 2x50, 2x150, 2x500 6/sex (control & high), 4/sex (low & mid)	Mortalities, clinical signs, bodyweight, food consumption, ECG, haematology, clinical chemistry, toxicokinetics, autopsy, organ weights, histopathology	2x50: ↑ Salivation 2x150: ↑ Salivation 2x500: ↑+ Salivation. ↑ Reddening at angle of mouth. ↑ Emesis.
Marmoset 39 Weeks (+ 9w recovery)	Oral gavage: 2x0, 2x25, 2x100, 2x500 5/sex	Mortalities, clinical signs, bodyweight, food and water consumption, ECGs, ophthalmoscopy, urinalysis, haematology, clinical chemistry, toxicokinetics, autopsy, organ weights, histopathology	2x25: ↓Heart rate (from Week 26). 2x100: ↓ Heart rate (from Week 26) 2x500: ↑+ Emesis. ↓ Bodyweight gain (F). ↓ Heart rate (from Week 26). ↑Urine volume. ↓ Urine Cl, K, Mg. ↓ Plasma total protein, albumin, K.
Rat (1w.old, unweaned) SD:CD 2 Weeks	Oral gavage: 0, 50, 150, 500, 1000 12/sex	Mortality, clinical signs, bodyweight, urinalysis, haematology, clinical chemistry, autopsy, organ weights, histopathology	5: No Adverse effects. 150: No Adverse effects. 500: No Adverse effects. 1000: Deaths, acute (8/12 M, 10/12 F). ↑ Cyanosis (3/8M, 3/10 F mortalities). ↑ Pulmonary oedema (4/8 M, 5/10 F mortalities) ↑ Hepatocyte vacuolation (18/24 mortalities).

**Table 9: Multiple Dose Toxicity**

Species Strain Duration	Route/Doses (mg/kg) No./Group	Parameters Monitored	Treatment Related Effects
Rat (7, 14, 24, 42 days old) Crl:CD (SD)	<u>Toxicity Phase</u> Oral gavage: 500, 700 or 1000mg/kg/day 3 groups: 7/sex single dose on day 7 3 groups: 7/sex single dose on day 14 <u>Toxicokinetic Phase</u> 28/sex single dose 1000/mg/kg on day 7 3 groups 14/sex single dose 1000mg/kg on day 14, 24 or 42 post-partum	Mortality, morbidity, bodyweight. In the Toxicity Phase, histological examination of selected tissues for inter-current mortalities, controls and high dose group animals	<u>Toxicity Phase</u> <b>Day 7 Post-Partum</b> 500: No clinical signs observed. 700: 1 F death, acute. 1 F prematurely sacrificed exhibiting signs of prostration, coldness and slow breathing. Clinical signs observed in 2 M and 2 F (hypoactivity, cold and slow and/or irregular breathing). 1000: 2 M deaths, acute. 1 M prematurely sacrificed exhibiting signs of prostration, coldness and slow breathing. 5 M developed hypoactivity and coldness. 5 M developed slow and/or irregular breathing. 7 F developed slow or irregular breathing, coldness and hypoactivity. Of these females, 1 F exhibited tremors while a second was prostrate. <b>Day 14 Post-Partum</b> 500: No clinical signs observed. 700: No clinical signs observed. 1000: 3 M cold, 2 of which were also hypoactive. 1 F cold.  <u>Toxicokinetic Phase - 1000 mg/kg/day</u> <b>Day 7 Post-Partum:</b> 7 deaths (5 M and 2 F). 6 pups (3 M and 3 F) observed to have clinical signs including coldness, pallor and hypoactivity. <b>Day 14 Post-Partum:</b> 1 death (F). No other clinical observations observed. <b>Day 24 Post-Partum:</b> 1 death (M) at dosing exhibiting no clinical signs. No other clinical observations observed. <b>Day 42 Post-Partum:</b> No clinical signs observed.
Rat (1w.old, unweaned) SD:CD 2 Weeks (+4w recovery)	Oral gavage: 0, 50, 150, 500 24/sex (control & high), 20/sex (low & mid)	Mortality, clinical signs, bodyweight, urinalysis haematology, clinical chemistry, toxicokinetics, autopsy, organ weights, histopathology	50: No adverse effects. 150: ↓Testes (M). ↓ Uterus (F). 500: ↓ Testes, thymus (M). ↓ Uterus (F). All findings of no toxicological significance, NOAEL = 500 mg/kg/day

**Table 9: Multiple Dose Toxicity**

<b>Species Strain Duration</b>	<b>Route/Doses (mg/kg) No./Group</b>	<b>Parameters Monitored</b>	<b>Treatment Related Effects</b>
Rat (3 w.old, weaned) SD:CD 4 Weeks (+4w recovery)	Oral gavage: 0, 50, 150, 500 24/sex (control & high), 20/sex (low & mid)	Mortality, clinical signs, bodyweight, food consumption, urinalysis, haematology, clinical chemistry, toxicokinetics, autopsy, organs weights, histopathology	50: ↓ Urine creatinine (M,F). 150: ↓ Urine creatinine (M,F). ↑ Urine volume, Na, K, Cl (M,F). ↑Serum P, Ca (M,F). ↑Serum Mg, K, globulin (F). ↓ A/G ratio (F). 500: ↓ Urine creatinine (M,F). ↑+ Urine volume, Na, K, Cl, Ca, Mg (M,F). ↑+ Urine P (M). ↑ Serum P, Na, Ca (M,F). ↑ Serum Mg, K, globulin (F). ↓ A/G ratio (F). All findings of no toxicological significance, NOAEL = 500 mg/kg/day.

**Table 10: Reproductive Toxicity Studies**

Study Type Species Strain	Route No./Group	Doses (mg/kg/day)	Treatment Related Effects
Fertility and Early Embryonic Development Rat SD:CD	Oral 20/sex	0, 50, 250, 1500	50: ↓Bodyweight gain (M). 250: ↓Bodyweight gain (M). ↑ Salivation post dosing (M,F). 1500: ↑+ Salivation post dosing, fur staining, rough coat. ↓ Bodyweight gain (M,F). ↓ Food consumption (F). No adverse effects on fertility, mating or early embryonic development.
Teratology Range finding Rat SD:CD	Oral 7 mated females + toxicokinetic satellites	0, 50, 250, 1500	50: No adverse effects. 250: ↓ Bodyweight gain, food consumption. 1500: ↓ Bodyweight gain, food consumption. No adverse effects on pregnancy and foetal parameters.
Teratology Rat SD:CD	Oral 22 mated females	0, 50, 250, 1500	50: No adverse effects. 250: No adverse effects. 1500: ↓ Bodyweight gain, food consumption. No adverse effects on pregnancy parameters. Incomplete ossification of 3 <sup>rd</sup> sternebra, mainly in one litter.
Tolerance Rabbit NZW	Oral 3 females (non-pregnant)	0, 50, 250, 500, 750, 1500	50: No adverse effects. 250: No adverse effects. 500: ↓+ Food consumption, faecal output (2/3). ↓ Bodyweight (1/3). ↓ Bodyweight gain (2/3). 750: Death (1/3). ↓++ Food consumption, faecal output (3/3). ↑++ Reddening, mucosal erosions, ulceration of stomach, intestinal fluid distension (1/3). ↑Fur in stomach, liquid caecal contents (2/3). ↑+ Reddened areas on gastric mucosa (1/3). 1500: Killed in extremis (3/3). ↑++ Hypoactivity, prostration, hunched posture, salivation (3/3). ↑++ Bodyweight, food intake, faecal output. ↑++ Reddening, mucosal erosion, ulceration of stomach, intestinal fluid distension (3/3). ↓++ Faecal pellets in colon.

**Table 10: Reproductive Toxicity Studies**

Study Type Species Strain	Route No./Group	Doses (mg/kg/day)	Treatment Related Effects
Teratology Range-finding Rabbit NZW	Oral 6 mated females + toxicokinetic satellites	0, 50, 250, 500	50: No adverse effects. 250: No adverse effects. 500: Abortion, premature sacrifice (1/8). ↓ Bodyweight gain, food consumption. ↓+ Faecal output No adverse effects on pregnancy and foetal parameters.
Teratology Rabbit NZW	Oral 23 mated females + toxicokinetic satellites	0, 50, 150, 500	50: No adverse effects 150: ↓+ Bodyweight gain, food consumption, faecal output. 500: Abortion and premature sacrifice (5/26). Premature sacrifice (2/26). ↓++ Bodyweight gain, food consumption, faecal output. No adverse effects on pregnancy parameters. No teratogenicity
Pre-and Post- Natal Rat SD:CD (regulatory)	Oral 25 mated females	0, 50, 250, 1500	50: No adverse effects. 250: No adverse effects. 1500: Deaths (3/25). Killed in extremis (6/25). All 9/25 showed some or all of: convulsions, hypoactivity, abnormal respiration, cold body surface, partly closed eyes. Deaths/KIE occurred day before, or day of expected parturition. ↑+ Salivation post dosing. ↑+ Duration of parturition (16/25). ↓ Pup viability. ↓ Pup bodyweight gain

**Table 10: Reproductive Toxicity Studies**

<p>Pre-and Post- Natal Rat Wistar RORO (investigatory)</p>	<p>Oral 21 mated females + satellites</p>	<p>0, 50, 500, 1500, 1500 to Day 19 of pregnancy</p>	<p>50: No adverse effects                      500: ↑ Salivation post dosing                      1500: ↑+ Salivation post dosing. ↓ Bodyweight gain, food consumption. ↑+ Urine volume. ↑+ Duration of parturition. ↓ Maternal care. ↓ Pup birthweights. ↓Pup viability. ↓+ Pup bodyweight gain. ↓ Developmental tests.                      1500 (to DG19): Deaths at parturition (1/18 with exceptional litter size of 19). ↑+ Salivation post dosing. ↓ Bodyweight gain, food consumption. ↑+ Urine volume. ↑ Delay in developmental tests.                      No mortalities at parturition occurred.</p>
<p>Pre-and Post- Natal Rat SD:CD (investigatory)</p>	<p>Oral 20 mated females (treated groups) 30 mated females (controls) + satellites</p>	<p>0, 500, 1500, 1500+, 1500+, 1500+ a</p>	<p>500: No adverse effects                      1500: ↑ Salivation post dosing. ↓ Bodyweight gain and food consumption. ↑+ Duration of parturition                      1500: ↑Salivation post dosing. ↓ Bodyweight gain and food consumption. ↑+ Duration of parturition                      1500: ↑ Salivation post dosing. ↓ Bodyweight gain and food consumption. One found dead Day 2 post-partum. ↑+ Duration of parturition                      1500: ↑ Salivation post dosing. ↓ Bodyweight gain and food consumption.                      There were no maternal deaths at parturition related to compound dosed at 1500 mg/kg/day</p>

+ = Mechanistic investigations (all dosed Day 6 of pregnancy to Day 21 of lactation) a = dosed from Day 6 to 17 of pregnancy

**Table 11: Mutagenicity Studies**

<b>Test</b>	<b>Compound</b>	<b>Concentration/Dose</b>	<b>Treatment Related Effects</b>
Bacterial Cell Gene Mutation (Ames Test)	Pro-drug (hydrochloride)	100-5000 mcg/plate ± S9 mix	No evidence of mutagenicity.
Bacterial Cell Gene Mutation (Ames Test)	Active compound	50-5000 mcg/plate ± S9 mix	No evidence of mutagenicity.
Mouse Lymphoma Cell Mutation	Active compound	23.4-3000 mcg/ml ± S9 mix	No evidence of mutagenicity.
Chromosome Aberrations in Human Lymphocytes	Pro-drug	62.5-2000 mcg/plate -S9 mix 500-4990 mcg/ml +S9 mix	No evidence of mutagenicity.
Micronucleus Test in Mice	Pro-drug	500, 100, 2000 mg/kg Evaluated 24, 48, 72 hours	No evidence of mutagenicity.

**Table 12: Special Studies**

<b>Study Type</b>	<b>Treatment Related Effects</b>
Rabbit Primary Eye Irritation	Reversible reddening, hyperaemia, swelling of conjunctivae (3/3). Reversible hyperaemia of sclera, watery/mucous discharge (3/3). Reversible iridic vascularisation and corneal opacity (1/3). Primary irritation score 4.11 (≡ “not irritating”, EU guidelines). Potential irritant to the eye in humans.
Guinea pig Skin Sensitisation	First challenge: Erythema in 56% (24h) and 78% (48h) animals Second challenge: Erythema in 44% (24h) and 56% (48h) animals. Potential for skin sensitisation in humans.
Dog 7 Day Gastrointestinal Tract Local Tolerance (75 mg capsule)	Erosion of the epithelium above a Peyer’s patch (1/2). Clinical relevance not known.
Antigenicity (pro-drug and active compound)	No immunogenicity potential (ASA, PCA tests). No specific antibodies (ELISA). No anaphylaxis elicited in ASA tests. Weak PCA elicited by pro-drug and active compound.

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