PERMITTED DAILY EXPOSURE FOR SILDENAFIL CITRATE

1. OBJECTIVE & SEARCH STRATEGY:

Determination of Health based exposure limits for a residual active substance through the derivation of a safe threshold value like Permitted daily exposure (PDE) or threshold of toxicological concern are used to determine the risk of the active pharmaceutical substance. For determination of PDE, all the available pharmacological and toxicological data including both non-clinical and clinical data should be evaluated. This involves hazard identification by reviewing all relevant data, identification of critical effects, determination of NOAEL of the findings that are considered to be critical effects.

In this document, brief summary of pharmacological, pharmacokinetics and toxicity data of Sildenafil Citrate have been presented based on the published data. The data were extracted from PubMed, PubChem, TOXLINE, Drugdex, RTECS (Registry of Toxic effects of Chemical Substances), National Toxicology Program (NTP) and FDA.

2. INTRODUCTION:

Sildenafil is a medication used to treat erectile dysfunction and pulmonary arterial hypertension. It is unclear if it is effective for treating sexual dysfunction in women. It is taken by mouth or injection into a vein. Onset is typically within 20 minutes and lasts for about 2 hours.

Common side effects include headaches, heartburn, and flushed skin. Caution is advised in those with cardiovascular disease. Rare but serious side effects include a prolonged erection that can lead to damage to the penis, vision problems, and hearing loss. Sildenafil should not be taken by people on nitrates such as nitroglycerin (glycerin trinitrate), as this may result in a serious drop in blood pressure. Sildenafil acts by blocking phosphodiesterase 5 (PDE5), an enzyme that promotes breakdown of cGMP, which regulates blood flow in the penis. It requires sexual arousal, however, to work. It also results in dilation of the blood vessels in the lungs.

3. IDENTITY OF THE ACTIVE SUBSTANCE:

Sildenafil citrate is a white to off-white crystalline powder.

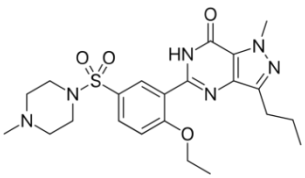
IUPAC name: 5-{2-Ethoxy-5-[(4-methylpiperazin-1-yl)sulfonyl]phenyl}-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one

Chemical Abstract Services (CAS) Registry Number: 139755-83-2

Molecular Weight: 474.5764 g/mol g·mol−1

Chemical Formula: $C_{22}H_{30}N_6O_4S$

Molecular Structure:



4. HAZARDS IDENTIFIED:

CATEGORIZATION:			
TOXICITY	**YES**	**NO**	**UNKNOWN**
Genotoxicant	-	√	-
Carcinogen	-	√	-
Reproductive/Developmental Toxicant	-	√	-
Highly Sensitizing potential	-	√	-

PERMITTED DAILY EXPOSURE FOR SILDENAFIL CITRATE

SUMMARY OF HAZARD IDENTIFICATION:	
Pharmacodynamics data	Effects of Sildenafil Citrate) on Blood Pressure-Oral Administration Single oral doses of sildenafil (100 mg) administered to healthy volunteers produced decreases in supine blood pressure (mean maximum decrease in systolic/diastolic blood pressure of 8.3/5.3 mmHg). The decrease in blood pressure was most notable approximately 1-2 hours after dosing, and was not different than placebo at 8 hours. Similar effects on blood pressure were noted with 25 mg, 50 mg and 100 mg doses of sildenafil, therefore the effects are not related to dose or plasma levels within this dosage range. Larger effects were recorded among patients receiving concomitant nitrates. Single oral doses of sildenafil up to 100 mg in healthy volunteers produced no clinically relevant effects on ECG. After chronic dosing of 80 mg t.i.d. to patients with pulmonary arterial hypertension, no clinically relevant effects on ECG were reported. After chronic dosing of 80 mg t.i.d. sildenafil to healthy patients, the largest mean change from baseline in supine systolic and supine diastolic blood pressure was a decrease of 9.0 mmHg and 8.4 mmHg respectively. After chronic oral dosing of 80 mg t.i.d. sildenafil to patients with systemic hypertension, the mean change from baseline in systolic and diastolic blood pressure was a decrease of 9.4 mmHg and 9.1 mmHg respectively. After chronic oral dosing of 80 mg t.i.d. sildenafil to patients with pulmonary arterial hypertension, lesser effects in blood pressure reduction were observed (a reduction in both systolic and diastolic pressure of 2mm Hg). This may be due to improvements in cardiac output secondary to the beneficial effects of sildenafil on pulmonary vascular resistance. In a study of the hemodynamic effects of a single oral 100 mg dose of sildenafil in 14 patients with severe coronary artery disease (CAD) (>70% stenosis of at least one coronary artery), the mean resting systolic and diastolic blood pressures decreased by 7% and 6% respectively compared to baseline. Mean pulmonary systolic blood pressure decreased by 9%. Sildenafil showed no effect on cardiac output, and did not impair blood flow through the stenosed coronary arteries.
Pharmacokinetics data	Sildenafil Citrate is rapidly absorbed after oral administration, with mean absolute bioavailability of about 41%. After oral three-times-daily (t.i.d.) dosing of Sildenafil Citrate, AUC and Cmax increase in proportion with dose over the dose range of 20-40 mg t.i.d. After 80 mg t.i.d., a slightly more than dose-proportional increase of sildenafil plasma levels has been observed. It is eliminated predominantly by hepatic metabolism (mainly cytochrome P450 3A4) and is converted to an active metabolite with properties similar to the parent, sildenafil. The concomitant use of potent cytochrome P450 3A4 (CYP3A4) inhibitors (e.g. ritonavir ketoconazole, itraconazole) as well as the nonspecific CYP inhibitor, cimetidine, is associated with increased plasma levels of sildenafil. Both sildenafil and the metabolite have terminal half-lives of about 4 hours. The pharmacokinetic profile of Sildenafil Citrate solution for injection has been characterized following intravenous administration. A 10 mg dose of

PERMITTED DAILY EXPOSURE FOR SILDENAFIL CITRATE

SUMMARY OF HAZARD IDENTIFICATION:	
	Sildenafil Citrate solution for injection is predicted to provide exposure of sildenafil and its N-desmethyl metabolite and pharmacological effects comparable to those of a 20 mg oral dose. **Absorption:** Sildenafil Citrate is rapidly absorbed. Maximum observed plasma concentrations are reached within 30 to 120 minutes (median 60 minutes) of oral dosing in the fasted state. When Sildenafil Citrate was administered with a high-fat meal, the rate of absorption was significantly decreased, with a 29% mean reduction in Cmax and a 60-minute mean delay in Tmax, however, the extent of absorption was not significantly affected (AUC decreased by 11%). This is not clinically relevant for chronic dosing in this patient population. An intravenous dose of 10 mg is required in order to achieve plasma concentrations similar to those observed following oral administration of 20 mg. **Distribution:** The mean steady state volume of distribution (Vss) for sildenafil is 105 L, indicating distribution into the tissues. Sildenafil and its major circulating N-desmethyl metabolite are both approximately 96% bound to plasma proteins. Protein binding is independent of total drug concentrations. Based upon measurements of sildenafil in semen of healthy volunteers 90 minutes after dosing, less than 0.0002% (average 188 ng) of the administered dose may appear in the semen of patients. **Metabolism:** Sildenafil is cleared predominantly by the CYP3A4 (major route) and CYP2C9 (minor route) hepatic microsomal isoenzymes. The major circulating metabolite (UK-103,320) results from N-desmethylation of sildenafil at the N-methyl piperazine moiety. This metabolite has a phosphodiesterase selectivity profile similar to sildenafil and an in vitro potency for PDE5 approximately 50% of the parent drug. In healthy volunteers, plasma concentrations of this metabolite are approximately 40% of those seen for sildenafil, so that the metabolite accounts for about 20% of sildenafil's pharmacologic effects. In patients with pulmonary arterial hypertension, however, the ratio of UK-103,320 to sildenafil is higher. Plasma concentrations of UK-103,320 are approximately 72% those of sildenafil after 20 mg t.i.d. oral dosing (translating into a 36% contribution to sildenafil's pharmacological effects). The subsequent effect on efficacy is unknown. In healthy volunteers, the plasma levels of the N-desmethyl metabolite following intravenous dosing are significantly lower than those observed following oral dosing. **Excretion:** The total body clearance of sildenafil is 41 L/h with a resultant terminal phase half life of 3-5 hours. After either oral or intravenous administration, sildenafil is excreted as metabolites predominantly in the feces (approximately 80% of administered dose) and to a lesser extent in the urine (approximately 13% of the administered dose) **Case Study 1:** **Species:** Sprague Dawley

PERMITTED DAILY EXPOSURE FOR SILDENAFIL CITRATE

SUMMARY OF HAZARD IDENTIFICATION:

Route: Oral
Dose: mg/kg/day (60)
Animals/dose level: 5 Sex
Duration: 14 days
This study was conducted to provide an estimate of the pharmacokinetic exposure of rats over 24 hours. Plasma concentrations of sildenafil were higher in females than in males, while concentrations of the metabolite, UK-103,320, were higher in males than in females.

Acute Toxicity

Case Study 1:

Species	Route	End Point	Dose (mg/kg)
Rat	Oral	LDmin.	300-500
Mouse	Oral	LDmin.	500-1000
Rat	Dermal	LD_{50}	>2000

Case Study 2:
Species: Sprague Dawley rat CD1 mice
Route: Oral (gavage)
Dose: mg/kg/day (Rat: 300 500 1000 Mice: 500 1000)
Animals/dose level: 5/sex
Duration: 1 day
At 1000 mg/kg one male mouse died within 24 hours after drug administration. In rats, mortality occurred in three females at 1000 mg/kg and in one female at 500 mg/kg. The dose of 1000 mg/kg induced clinical signs in both species, generally within 24 hours following the administration, which persisted less than 24-48 hours. Some of these signs were similar in mice and rats and consisted of partially-closed eyes, hunched posture, tremours, depression, coldness to the touch (with pallor of ears and paws in rats) and prostration. Female rats were more affected than male rats. Dyspnea was limited to one mouse, and chromodacryorrhea to four female rats. Clinical signs at 500 mg/kg included partially-closed eyes in one mouse and subdued behaviour in the female rat which died. No clinical signs were observed in rats at 300 mg/kg. In both species, the doses administered induced no changes in body weight gain and there were no treatment related macroscopical changes at gross necropsy. These results indicate that the no observed adverse effect level (NOAEL) was 500 mg/kg in mice and 300 mg/kg in rats.

Case Study 3:
Species: Sprague Dawley rat CD1 mice
Route: Intravenous
Dose: mg/kg/day (Rat: 10; Mice: 20)
Animals/dose level: 5/sex
Duration: 1 day
AII animals survived the treatment and gained weight over the 14-day study period. There were no clinical signs during the study and no abnormalities at necropsy. Under the conditions of this study, the no observed effect level (NOEL) after intravenous administration was 20

PERMITTED DAILY EXPOSURE FOR SILDENAFIL CITRATE

SUMMARY OF HAZARD IDENTIFICATION:

mg/kg in mice and 10 mg/kg in rats.

Repeated Dose Toxicity (Chronic Toxicity)

Case Study 1:

Duration	Species	Route	Dose (mg/kg/day)	End Point	Target Organ
6 months	Rat	Oral	3	NOAEL	Adrenal gland, Liver, Thyroid
6 months	Dog	Oral	15	NOAEL	Cardiovascular system

Case Study 2:
Species: CD1
Route: Oral (Gavage)
Dose: mg/kg/day (10,50,100, 200)
Animals/dose level: 10/sex
Duration: 3 months
The exposure to Sildenafil and its metabolite UK-103,320 was similar in males and females and approximately dose-related. Treatment-related mortality occurred in 3/20 animals in each group given 50, 100 or 200 mg/kg. A marked gastrointestinal dilation was the cause of the death and was associated with a number of clinical signs, in particular dyspnea and/or swollen abdomen. This dilation resulted in gastrointestinal inflammation, fatty changes and focal/multifocal necrosis in the liver, atrophy of adipose tissues and hemoconcentration. There was also a mild gastrointestinal dilation in a few survivors of these groups. In males treated with 50, 100 or 200 mg/kg, there was an apparent decrease in body weight gain. However, in the absence of dose relationship and consistent statistical significance, the association with treatment is questionable. Plasma cholesterol was slightly increased in females treated with 50, 100 or 200 mg/kg and plasma triglycerides were slightly decreased in males treated with 100 or 200 mg/kg. However we consider these changes to be of minor toxicological importance. The NOAEL in this study was 10 mg/kg, given the mortality and gastrointestinal dilation at higher doses.

Case Study 3:
Species: CD1
Route: Oral (Gavage)
Dose: mg/kg/day (20,40,100)
Animals/dose level: 10/sex
Duration: 3 months
The exposure to sildenafil and its metabolite UK-103,320 was similar in males and females and increased super proportionally with dose level. Treatment-related mortality occurred in 1/20 animals in each group given 4

PERMITTED DAILY EXPOSURE FOR SILDENAFIL CITRATE

SUMMARY OF HAZARD IDENTIFICATION:	
	or 100 mg/kg. A marked gastrointestinal dilation was the cause of the death and was associated with a number of clinical signs, in particular dyspnea and/or swollen abdomen. There was also a transient abdominal swelling in a few survivors of these groups. The NOAEL in this study was 20 mg/kg, given the mortality and gastrointestinal dilation at higher doses. **Case Study 4:** **Species:** Sprague Dawley **Route:** Oral (Gavage) **Dose:** mg/kg/day (50,150,500) **Animals/dose level:** 5/sex **Duration:** 10 days Measurement of plasma concentrations of sildenafil and UK-103,320 showed that females were exposed predominantly to the drug while males were exposed mainly to the metabolite, UK-103,320, and a lower level of unchanged compound. Concentrations of UK-95,340 were generally below the limit of determination (30 ng/mL). Exposure increased with dose but not in linear manner. At 500 mg/kg, 1/5 females died after the second dose with no apparent cause of death. Of the animals used for plasma drug determination, 1/10 rats at 150 mg/kg and 2/10 rats at 500 mg/kg died after the first or second dose. As these animals died after taking blood samples, they were not considered in the analysis of mortality. Food consumption was decreased between day 1 and 4 in mid- and high-dose males and in all treated female groups. A dose-related decrease of plasma triglycerides occurred in males, and an increase of plasma cholesterol was seen in high-dose females. Blood urea increased in mid- and high-dose males and in the 3 treated female groups. Relative heart weight was slightly increased in high-dose males. Kidney and liver weights were increased in mid- and high-dose females, and in high-dose males. The increase of liver weight was associated with centrilobular hypertrophy. Changes in red blood cell parameters were seen in females. They indicate a decrease of circulating red blood cells at the 3 dose levels, with some evidence of regenerative response at the high dose. An increase of white blood cell counts was recorded at the mid dose in females and at the high dose in both sexes. Changes at the dose of 50 mg/kg were considered minor. The NOAEL in this study was 150 mg/kg, based on the mortality at 500 mg/kg. **Case Study 5:** **Species:** Sprague Dawley **Route:** Oral (Gavage) **Dose:** mg/kg/day (10,45,200) **Animals/dose level:** 10/sex **Duration:** 1month Plasma concentrations of sildenafil were higher in females than in males,

SUMMARY OF HAZARD IDENTIFICATION:	
	while concentrations of the metabolite, UK-103,320, were higher in males than in females. As a result, females were exposed predominantly to the unchanged drug and males to an almost equal balance of drug and metabolite. These data indicate that N-demethylation of sildenafil to UK-103,320 is an important route of sildenafil biotransformation in male rats. Concentrations of UK-95,340 were generally below the limit of determination (30 ng/ml). One of the high-dose females used for plasma drug level determination died after the first dose, before blood samples had been taken. Clinical signs were limited to a few high-dose animals and consisted of chromodacryorrhea and palpebral closure. Slight increases in water and food intake were seen generally in mid- and high-dose animals. A mild dose related decrease in circulating red blood cells with evidence of a regenerative response was found in mid- and high-dose females and, to a smaller extent, in high-dose males. A moderate neutrophilia was seen in high-dose males, while a moderate lymphocytosis occurred in mid- and high-dose females. Plasma chemistry changes at the high dose consisted of increases in urea, decreases in triglycerides (males) and increases in cholesterol (females), but remained within our normal range of values. Doses of 45 and/or 200 mg/kg were associated with an increase in liver weight and centrilobular hypertrophy in both sexes. Hypertrophy of the zona glomerulosa of the adrenal glands was seen in the high-dose males and in the mid- and high-dose females. Thyroid follicular hypertrophy occurred at the high dose in both sexes. In addition, mesenteric arteritis was found in two mid-dose and one high-dose males, but was not considered to be related to the treatment. The NOAEL was 45 mg/kg in this study. **Case Study 6:** **Species:** Sprague Dawley **Route:** Oral (Gavage) **Dose:** mg/kg/day (0,60,120) **Animals/dose level:** 10/male group **Duration:** 28 days A 2-year rat carcinogenicity study with sildenafil citrate at a contract laboratory (Study No. 911/002), at doses of 1.5, 5 and 60 mg/kg, was terminated after unexpectedly high mortality and severe toxic effects in high-dose males during weeks 3 and 4. An exploratory study was performed to confirm that the batch of sildenafil used at the contract laboratory did not induce severe toxicity. The only treatment-related effects were a mild dose-related increase in liver and kidney weights and possibly a slight decrease in body weight gain. Importantly, the absence of death in this study confirms the results of previous studies up to 200 mg/kg, and contrasts with the results of the study at the contract laboratory. Subsequently, it was shown that the mortality in the carcinogenicity study (Study No. 911/002) was due to dosing with a cytotoxic compound from another company and not sildenafil.

PERMITTED DAILY EXPOSURE FOR SILDENAFIL CITRATE

SUMMARY OF HAZARD IDENTIFICATION:	
	Consequently, the contracted carcinogenicity study was invalid. **Case Study 7:** **Species:** Sprague Dawley **Route:** Oral (Gavage) **Dose:** mg/kg/day (200) **Animals/dose level:** 10/Female group **Duration:** 1 month Following the appearance of thyroid follicular hypertrophy in rats, an investigative study was conducted to examine the relationship between liver enzyme induction and thyroid hypertrophy in rats. Two groups of 10 female rats were treated orally with sildenafil citrate at 200 mg/kg for 29 days, and two control groups received the vehicle alone. One treated group and one control group were used for assessment of exogenous thyroxine clearance. The other treated group and the other control group were used for measurement of plasma TSH and thyroid hormones, for histopathological examination of the liver and thyroid, and for determination of UDP-glucuronyl transferase (UDPGT) activity in the liver. The treatment caused the deaths of 2/20 rats on days 2 or 3. In the treated group, there was an increase in the weight of liver and thyroid, associated with minimal centrilobular hypertrophy of the liver and thyroid follicular cell hypertrophy. There was also an increase in hepatic UDPGT activity, an increase in TSH, and a decrease in T3 and T4 hormones. In addition, the clearance of exogenous thyroxine was increased in treated animals. These results are consistent with the view that the thyroid hypertrophy associated with treatment of rats with sildenafil was due to induction of hepatic UDPGT which increased the clearance of thyroid hormone and consequently caused a compensatory increase in plasma TSH which stimulated the thyroid gland. **Case Study 8:** **Species:** Sprague Dawley **Route:** Oral (Gavage) **Dose:** mg/kg/day (3,12,60) **Animals/dose level:** 20/Sex **Duration:** 6 month Drug and metabolite plasma level determinations showed that females were exposed predominantly to sildenafil while males were exposed almost exclusively to the metabolite. No treatment-related deaths were recorded. Chromodacryorrhea was seen in the 3 treated groups. Body weight gain and food consumption were increased at the low dose and, to a lesser extent, at the mid dose. A trend towards a reduced body weight gain was seen at the high dose; however, the relationship to compound administration cannot be ascertained. Decreases of plasma bilirubin and triglycerides, and increases in plasma urea, total proteins and cholesterol were seen at the high dose. These changes suggest compound-induced metabolic changes in the liver. Increase in liver weight associated with

SUMMARY OF HAZARD IDENTIFICATION:	
	mild centrilobular hypertrophy indicate an adaptive response. Thyroid hypertrophy occurred at the high dose in both sexes and at a lower incidence in mid-dose males. This change was considered to be a secondary phenomenon related to increased hepatic clearance of thyroid hormone. Although thyroid hormones and hepatic clearance were not measured in this study, changes in these parameters were demonstrated in an exploratory study (Study No. 96010). Hypertrophy of the zona glomerulosa of the adrenal gland occurred with a dose-related incidence at the mid and high doses and was associated with an increase in the weight of the organ at 60 mg/kg. The NOAEL in this study was 60 mg/kg. **Case Study 9:** **Species:** Sprague Dawley **Route:** Intravenous **Dose:** mg/kg/day (2.5,5,10) **Animals/dose level:** 5/Sex **Duration:** 13 days No deaths occurred during the treatment period. The only clinical sign noted was a transient redness of the ears in a few treated animals, notably in the high-dose male group. The NOAEL in this study was 10 mg/kg. **Case Study 10:** **Species:** Sprague Dawley **Route:** Intravenous **Dose:** mg/kg/day (0.5,2,4) **Animals/dose level:** 10/Sex **Duration:** 1 month No compound-related changes were seen at the doses of 0.5 and 2 mg/kg. At the dose of 4 mg/kg, the incidence and severity of mild myocardial inflammation was slightly increased compared to the control group; the relationship to treatment cannot be ascertained. The NOAEL in this study was 2 mg/kg. **Case Study 11:** **Species:** Beagle **Route:** Oral (Gavage) **Dose:** mg/kg/day (10,30,100) **Animals/dose level:** 1 M & 2 F **Duration:** 10 days Plasma concentrations of sildenafil and UK-103,320 were similar in males and females and increased with dose, although subproportionally at the high dose. The proportion of UK-103,320 relative to sildenafil varied minimally (18-24%) over the dose range examined and indicates no detectable saturation of this metabolic pathway. Concentrations of UK-95,340 were generally below the limit of determination (30 ng/mL). Emesis and salivation occurred at the dose of 100 mg/kg, and lacrimation, conjunctival redness and a transient decrease in amplitude of the pupillary reflex were seen at all dose levels. There was no evidence of a convincing

SUMMARY OF HAZARD IDENTIFICATION:

change in blood pressure, given the spontaneous variation in this parameter. Heart rate was increased at 30 and 100 mg/kg, and probably represents a reflex response to the vasodilating properties of the compound. Decreases in PQ and QT intervals of the ECG at these doses were secondary to the heart rate changes. Two high-dose animals showed a moderate increase of plasma cholesterol which was not considered to be toxicologically important. An arteritis of an extramural branch of a coronary artery was found in one high-dose female. This is considered to be a spontaneous finding considering the morphological features and the background incidence in Beagle dogs in our laboratories. The NOAEL in this study was therefore 100 mg/kg.

Case Study 12:
Species: Beagle
Route: Oral (Gavage)
Dose: mg/kg/day (5,20,80)
Animals/dose level: 3/Sex
Duration: 1month
The dogs were exposed to concentrations of sildenafil and UK-103,320, which increased with dose, although sub proportionally at the high dose. The proportion of UK-103,320 relative to sildenafil varied minimally (15-19%) over the dose range examined and indicates no detectable saturation of this metabolic pathway. Concentrations of UK95, 340 were generally below the limit of determination (30 ng/mL). At the mid and high doses, the compound induced a low incidence of emesis and transient salivation. A moderate incidence of soft and liquid feces was noted at all doses. There was no evidence of consistent changes in blood pressure, although there were increases in heart rate at 20 and 80 mg/kg. Changes in the ECG (increased P-wave amplitude and decreases in PQ and QT intervals) were expected from the increases in heart rate. There was a moderate increase in plasma cholesterol at the high dose. A mild coronary arteritis was seen in one high-dose animal, but considering the morphological features of this finding, and the high background incidence in Beagle dogs in our laboratories, this was not thought to be treatment-related. The NOAEL was 80 mg/kg in this study.

Case Study 13:
Species: Beagle
Route: Oral (Gavage)
Dose: mg/kg/day (3,15,50)
Animals/dose level: 4/Sex
Duration: 6 month
Analyses of plasma sildenafil and UK-103,320 showed dose-related concentrations in the dog. The proportion of UK-103,320 relative to sildenafil varied minimally (15-23%) as the dose increased, indicating no saturation of this process. Salivation, emesis and resistance to compound administration were seen when the animals were treated with an initial

SUMMARY OF HAZARD IDENTIFICATION:	
	high dose of 80 mg/kg, and reflected gastric intolerance to the compound at this dose level. These signs were rare after reducing the high dose to 50 mg/kg. A moderate increase in heart rate, associated with decreases in PQ and QT intervals, occurred at the high dose and is considered to be a reflex response to the vasodilatory properties of the drug. Increases in plasma cholesterol and in liver weight were seen in animals treated with 15 and 50 mg/kg. A high-dose male showed a number of clinical signs and changes in hematological parameters and plasma chemistry associated with a disseminated arteritis. These changes correspond to Idiopathic Juvenile Arteritis Syndrome (Beagle Pain Syndrome) which occurs sporadically in Beagle dogs. Another high-dose male showed arteritis in the thymus which indicated a less severe expression of the same disease. It is probable that the high dose precipitated the expression of this latent spontaneous disorder. The NOAEL in this study was 15 mg/kg, given the appearance of Idiopathic Juvenile Arteritis Syndrome at higher doses. **Case Study 14:** **Species:** Beagle **Route:** Oral (Gavage) **Dose:** mg/kg/day (3,10,50) **Animals/dose level:** 4/Sex **Duration:** 12 month The dogs were exposed to approximately dose-related concentrations of sildenafil and its N-demethylated metabolite, UK-103,320. The proportion of UK-103,320 relative to sildenafil varied minimally as the dose increased. Features typical of a syndrome of Idiopathic Juvenile Arteritis occurred in all high-dose males. In 3/4 high-dose males, there was arteritis which affected several organs. In one of these dogs, arteritis was associated with a number of clinical signs, body weight loss and hematological changes. In the other two animals, there were no clinical or hematological correlates to arteritis. In addition, the fourth high-dose male presented clinical signs and clinical pathology changes typical of the syndrome though no vascular lesion was found at histopathology. Focal coronary arteritis occurred in one low-dose and one high-dose female; neither finding was considered treatment-related. The treatment produced an increase in the amount of lipogenic pigments in renal tubular epithelium in 1/8 animals at the mid dose and 7/8 animals at the high dose, a dose-related decrease in plasma creatine kinase, mainly in males, and a decrease in plasma myosin in high-dose animals. However, these changes were considered of no toxicological importance. A dose-related increase in heart rate occurred at the high and mid doses, and was considered to be due to compensatory mechanisms occurring in response to the vasodilatory properties of the compound. The NOAEL in this study was 10 mg/kg, given the appearance of Idiopathic Juvenile Arteritis Syndrome at higher doses. **Case Study 15:**

PERMITTED DAILY EXPOSURE FOR SILDENAFIL CITRATE

SUMMARY OF HAZARD IDENTIFICATION:	
	Species: Beagle **Route:** Intravenous **Dose:** mg/kg/day (2.5,10,10) **Animals/dose level:** 1 M & 2 M **Duration:** 14 Days The doses of 5 and 10 mg/kg were associated with liquid feces and an inhibition of the pupillary reflex. An increase in heart rate was observed at the high dose and, to a lesser extent, at the mid dose. This change was probably related to the vasodilator effect of the compound. Evidence of vasodilatation was provided by the peripheral redness seen in two high-dose animals. An increase in plasma cholesterol occurred in 2/3 high-dose animals but was not considered to be toxicologically important. At the dose of 2.5 mg/kg, there were no treatment-related changes. The NOAEL was 10 mg/kg in this study. **Case Study 16:** **Species:** Beagle **Route:** Intravenous **Dose:** mg/kg/day (0,0.5,2,4) **Animals/dose level:** 3 Sex **Duration:** 1 month The treatment induced no adverse effects. The NOAEL is therefore 4 mg/kg in this study **Case Study 17:** **Species:** Beagle **Route:** Oral **Dose:** mg/kg/day (300) **Animals/dose level:** 1 M & 1 F **Duration:** 1 month The aim of the current study was to assess, in the dog, the oral bioequivalence of a suspension of the base, and of capsules of the citrate. The base was suspended in a 5% aqueous solution of methylcellulose 4000 cps containing 0.1% Tween 80 and acidified with hydrochloric acid 0.1M (final concentration). The citrate salt was administered in gelatin capsules. On day 1, a first group of one male and one female beagle dogs was treated with the base and the second group of one male and one female was treated with the citrate. On day 8, the first group received the citrate, and the second group the base. The animals were regularly examined for clinical signs and weighed before each administration. Blood was sampled 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 11 and 24 hours after each administration. Plasma levels of UK-92,480 and two metabolites, UK-95-340 and UK-103,320, were measured. One male dog vomited after each administration and its drug and metabolite plasma concentrations were therefore considered not to be relevant. In other dogs, maximal plasma concentrations and AUCs of UK-92,480 and of UK-103,320, observed after administration of the citrate in capsules were similar to or

PERMITTED DAILY EXPOSURE FOR SILDENAFIL CITRATE

SUMMARY OF HAZARD IDENTIFICATION:

higher than those seen after administration of the base in a suspension. All the plasma concentration of UK-95,340 were below the limit of detection of the assay. These data indicate that bioavailability of the citrate in the dog is identical to or better than that of the base.

Carcinogenicity

Duration	Species	Route	Dose (mg/kg/day)	End Point	Target Organ
24 months	Mouse	Oral	5	NOAEL	Not Carcinogenic
24 months	Rat	Oral	60	NOAEL	Not Carcinogenic

Sildenafil was not carcinogenic when administered to rats for 24 months at a dose resulting in systemic drug exposure (AUC) of 110- and 146-times, respectively, for male (unbound sildenafil and its major metabolite) and female (unbound sildenafil) rats. The exposures observed in humans given the Recommended Human Dose (RHD) of 20 mg t.i.d. sildenafil was not carcinogenic when administered to mice for 18-21 months at dosages up to the Maximum Tolerated Dose (MTD) of 10 mg/kg/day, approximately 1.1 times the RHD on a mg/m^2 basis.

Case Study 1:
Species: CDI
Route: Oral
Dose: mg/kg/day (3,10,30,1,3,5)
Animals/dose level: 55 Sex
Duration: 3 & 10 mg: males 649 days females 558 days 30 mg: males 453 days females 404 days 1, 3, 5 mg males and females 719-730 days.

The exposure to the parent compound and the demethylated metabolite, UK-103,320 was dose-related. The compound produced an increase in mortality rate with consequent decreases in survival times and percent of survival. The effect was marked at the mid dose in females and at the high dose in both sexes. In addition, the percent of survival was also slightly decreased in mid-dose males, at the end of the study. Because of the lower survival in mid- and high-dose animals interim sacrifices were decided. When the survival in the high-dose group reached about 20%, the survivors were sacrificed, on day 405 (females) or 454 (males). Control, low- and mid-dose groups were sacrificed on day 559 (females) or 650 (males), when the survival at the mid dose was about 20%. In a number of animals, especially high-dose males (40%), unscheduled death was preceded by abdominal swelling and/or dyspnea. Gastrointestinal dilation and gavage accident were identified as causes of unscheduled death related to treatment. Additionally, the number of deaths without explanatory macroscopic or histopathological changes was higher in mid- and high-dose groups than in the control groups. In high-dose males and females, there was also a trend to body weight decrease compared to controls (10 and 18%, respectively). In addition, there was an abrupt body

PERMITTED DAILY EXPOSURE FOR SILDENAFIL CITRATE

SUMMARY OF HAZARD IDENTIFICATION:

weight loss in most animals dying prematurely which was more marked in mid- and high-dose females. The treatment produced no increase in the incidence of neoplastic lesions. Furthermore, in the animals sacrificed at the various interim and final sacrifices, there were no differences in the incidence of non-neoplastic lesions between control and treated groups. In conclusion, the doses of 10 and 30 mg/kg produced signs of toxicity consisting mainly of a dose-related increase in mortality. At the dose of 3 mg/kg, although there was no compound effect on group mortality, 2 animals died from gastrointestinal dilation. There were no carcinogenic effects at any dose.

Case Study 2:
Species: Sprague Dawley
Route: Oral
Dose: mg/kg/day (1.5,5,60)
Animals/dose level: 60 Sex
Duration: 24 months
The rats were exposed to plasma concentrations of sildenafil and UK-103,320 that increased with dose levels. Male rats were exposed predominantly to UK-103,320, whereas unchanged drug was the major circulating form in females. Overall, the total exposure to drug and metabolite was higher in females than in males. The treatment produced no mortality. Survival at the end of the study ranged between 18 and 42% in males and between 15 and 25% in females. The body weight was decreased in high-dose animals, compared to controls. A transient decrease in body weight occurred also in mid-dose females. There was a dose-related decrease in plasma bilirubin which, in our view, is related to the enzyme-inducing properties of the compound. In high-dose males there was an increased incidence of proliferative changes in the thyroid which was mainly related to an increase in follicular cell hyperplasia. We consider that these changes are the consequence of an increased turnover of thyroid hormones due to hepatic enzyme induction and bear no relevance to man. To conclude, the dose of 60 mg/kg was associated with a toxicologically significant decrease in body weight and with an increase in follicular proliferative changes in the thyroid in males. At 5 mg/kg there was only an inconsistent decrease in the body weight of females. There were no compound effects at 1.5 mg/kg. There were no indications of a carcinogenic potential of sildenafil.

In vivo/In vitro Genotoxicity Studies

Study Type	Cell Type/Organism	Dose	Result
In Vitro Bacterial Mutagenicity (Ames)	S. typhimurium TA 1535, 1537, 98, 100	0.002 - 1 mg/plate	Negative
in vitro mammalian cell mutagenicity	Chinese Hamster Ovary /HGPRT	65-240 µg/mL	Negative
in vitro clastogenicity	Human lymphocytes	10, 20, 25 µg/mL -S9 100, 125, 250 µg/mL + S9	Negative

PERMITTED DAILY EXPOSURE FOR SILDENAFIL CITRATE

SUMMARY OF HAZARD IDENTIFICATION:

in vivo clastogenicity	Mouse bone marrow	0, 500, 1000, 2000 mg/kg	Negative

Sildenafil has been studied in a comprehensive battery of tests designed to detect genotoxic activity. Sildenafil did not display mutagenic activity in bacterial or mammalian cells in vitro, or clastogenic activity in vitro or in vivo.

Reproductive/Developmental Toxicity

Case Study 1:

Study Type	Species	Route	Dose (mg/kg/day)	End Point	Effect
Reproductive & Fertility	Rat	Oral	60	NOEL	No effects at maximum dose
Embryo/ Fetal Development	Rat	Oral	50	NOEL	Maternal Toxicity, Not Teratogenic
Embryo/ Fetal Development	Rabbit	Oral	50	NOEL	Maternal Toxicity, Not Teratogenic

No evidence of teratogenicity, embryotoxicity or fetotoxicity was observed in rats and rabbits which received up to 200 mg/kg/day during organogenesis. These doses represent, respectively, about 32 and 68 times the RHD on a mg/m2 basis in a 50 kg subject. In the rat pre- and postnatal development study, the no observed adverse effect dose was 30 mg/kg/day given for 36 days. In the non-pregnant rat the AUC at this dose was about 24 times unbound human AUC. In pups of rats which were pre- and postnatally treated with 60 mg/kg sildenafil, a decreased litter size, a lower pup weight on day 1 and a decreased 4-day survival were seen at exposures which were approximately fifty times the expected human exposure at 20 mg three times a day. Effects in non-clinical studies were observed at exposures considered sufficiently in excess of the maximum human exposure indicating little relevance to clinical use.

Case Study 2 (Maternal toxicity study in rats by the oral route):
Species: Sprague Dawley
Route: Oral (gavage)
Dose: mg/kg/day (10,50,200)
Animals/dose level: 7 females
Duration: Gestation days 6-17
Hematological, biochemical (plasma) and pathological changes were recorded only at 200 mg/kg. Hematological changes consisted of a moderate decrease in hemoglobin, red blood cell count and packed cell volume accompanied by an increase in the mean red blood cell distribution width. The only variation observed in plasma chemistry was a decrease in mean plasma triglycerides. Finally, a mild hepatic weight increase with hepatic centrilobular hypertrophy was noted after pathological examination. With regard to the fetuses, there was a decrease in the mean male body weight at 200 mg/kg. In male fetuses at 10 and 50 mg/kg and in female fetuses at all dose levels, the mean body weights were similar to

SUMMARY OF HAZARD IDENTIFICATION:	
	those of the control group. The NOAEL was 50 mg/kg in dams and fetuses given the changes in plasma chemistry and fetal weight of males at 200 mg/kg. **Case Study 3** (Study of fertility and early embryonic development to implantation in rats by the oral route): **Species:** Sprague Dawley **Route:** Oral (gavage) **Dose:** mg/kg/day (3,12,60) **Animals/dose level:** 20 Sex **Duration:** Males: from 9 weeks before mating to gestation day 20 Females from 2 weeks before mating to gestation day 6 The treatment produced no adverse effects on the fertility of either sex. In addition, there was no evidence of maternal, embryo- or fetotoxicity. The only finding was a moderate reduction in plasma triglycerides in females treated with 60 mg/kg. Therefore the NOAEL in this study was 60 mg/kg. **Case Study 4** (Study for effects on pre- and post-natal development, including maternal function, in rats by the oral route): **Species:** Sprague Dawley **Route:** Oral (gavage) **Dose:** mg/kg/day (10,30,60) **Animals/dose level:** 20 Females **Duration:** from gestation day 6 until 20 days after birth The only noteworthy finding was a toxicologically significant decrease in the ratio of viable pups at birth, with consequently a decreased litter size of viable pups, at 60 mg/kg. At this high-dose level, there was a toxicologically significant decrease in the 4-day survival index, in the F1 pups body weight on day 1 p.p. and some delay in a developmental landmark, the appearance of upper incisors. There were no findings in the reproductive function of the F1 generation, and in the F2 generation. The NOAEL was 30 mg/kg for F0 females and F1 pups, given the minimal maternal toxicity and the effect on pup development during the first 2 weeks of life. The NOAEL for the F2 generation is 60 mg/kg. **Case Study 5** (Study for effects on embryo-foetal development in rats by the oral route): **Species:** Sprague Dawley **Route:** Oral (gavage) **Dose:** mg/kg/day (10,50,200) **Animals/dose level:** 20 Females **Duration:** Gestation days 6-17 There were detectable levels of sildenafil and UK-103,320 in maternal plasma, amniotic fluid and fetal homogenates at all dose levels. Treatment at 200 mg/kg produced salivation and a reduction in mean body weight gain between days 6 and 9 p.c., accompanied by a decrease in food intake on day

PERMITTED DAILY EXPOSURE FOR SILDENAFIL CITRATE

SUMMARY OF HAZARD IDENTIFICATION:	
	9 p.c. On day 18 p.c., the mean food consumption increased. Hematological changes consisted of a slight decrease in hemoglobin, red blood cell count and hematocrit accompanied by an increase in the mean red blood cell distribution width at 200 mg/kg. A dose-related increase in the reticulocyte count was present, reaching statistical significance at the high-dose only. The only variation in plasma chemistry was a dose-related decrease in mean plasma triglycerides, at most moderate and statistically significant at the high-dose only. The body weight of male fetuses was reduced at 200 mg/kg. There were no treatment-related external, skeletal or visceral anomalies. Treatment with 200 mg/kg produced a slight maternal toxicity without embryotoxicity but a slight toxicity in male fetuses only. There was no maternal, fetal or embryotoxicity after treatment with 10 or 50 mg/kg. There were no teratological effects at any dose. The NOAEL in this study was 50 mg/kg in dams and fetuses, given the slight toxicity at 200 mg/kg. **Case Study 6** (Maternal toxicity study in rabbits by the oral route): **Species:** New Zealand White (Rabbit) **Route:** Oral (gavage) **Dose:** mg/kg/day (50,100,200) **Animals/dose level: 7** Females **Duration:** Gestation days 6-18 Pregnant females and fetuses were exposed to the drug. The only noteworthy findings in dams were an increase in plasma glucose and a decrease in plasma cholesterol at the high dose. This is indicative of a minimal toxicity in dams. There were no adverse effects on embryo or fetal development. The NOAEL was 100 mg/kg in dams given the changes in plasma chemistry values at 200 mg/kg. The NOEL was 200 mg/kg in the developing embryos and fetuses. **Case Study 7** (Study for effects on embryo-foetal development in rabbits by the oral route): **Species:** New Zealand White (Rabbit) **Route:** Oral (gavage) **Dose:** mg/kg/day (10,50,200) **Animals/dose level:** 20 Females **Duration:** Gestation days 6-18 Sildenafil and UK-103,320 were found in the plasma of pregnant females. The presence of sildenafil was also detected in amniotic fluid. At the high-dose, there were reductions in body weight and body weight gain late in gestation, compared to the control group, which are indicative of minimal maternal toxicity. A reduction in food intake in high-dose females during the same period may have contributed to the body weight changes. The plasma chemistry changes, encountered in the preliminary study, were not found in this study. The treatment had no adverse effects on the developing conceptus. The NOAEL in this study was 50 mg/kg for dams, given the effect on body weight at 100 mg/kg. The NOEL was 100 mg/kg in the

PERMITTED DAILY EXPOSURE FOR SILDENAFIL CITRATE

SUMMARY OF HAZARD IDENTIFICATION:	
	developing embryos and fetuses.
Highly Sensitizing Potential	Rare cases of Stevens-Johnson's Syndrome (SJS), Toxic Epidermal Necrolysis (TEN) and Erythema Multiforme (EM) have been reported during the post-marketing period

IDENTIFICATION OF CRITICAL EFFECTS:	
Sensitive Indicator of an adverse effect seen in non-clinical toxicity data	No any adverse effect seen in non-clinical toxicity data.
Clinical therapeutic and adverse effects	**Adverse effects:** Adverse effects most commonly reported in clinical use include difficult digestion (dyspepsia), nose bleed, headache, flushing, insomnia, abnormal redness of skin (erythema), difficulty breathing, muscle pain, fever, gastrointestinal irritation, tingling/itching (paresthesia), transient changes in light perception and color vision, effects on hearing, and effects on vision.

NOAEL/LOAEL	NOAEL value of 3 mg/kg/day of Rat for 6 months selected.

APPLICATION OF ADJUSTMENT FACTORS:		
F1: Extrapolation between species	5	For extrapolation from rats to humans.
F2: Inter Individual Variability	10	Used for differences between individuals in the human population.
F3: Duration of Toxicity (Repeat Dose Toxicity)	2	6 months duration study in rodent.
F4: Severe Toxicity (1-10)	1	No any toxicity (Genotoxicity/Reproductive toxicity/ Carcinogenicity) observed
F5: NOAEL or LOAEL (10 if LOAEL)	5	NOAEL value of 3 mg/kg/day of Rat for 6 months selected.
PK Correction	For PDE calculation no pharmacokinetic correction was carried out	

CALCULATION	
PDE Calculation	$\frac{\text{NOEL or NOAEL or LOAEL (mg/kg/day) x Body Weight (kg)}}{\text{F1 x F2 x F3 x F4 x F5}}$ $= \frac{\text{3 (NOAEL) x 50}}{\text{5 x 10 x 2 x 1 x 5}}$ = 0.3 mg/day

5. REFERENCES:

- https://en.wikipedia.org/wiki/Sildenafil
- https://www.pfizer.com/files/products/material_safety_data/PZ01668.pdf
- https://www.pfizer.ca/sites/default/files/201909/Revatio_PM_E_22Aug2019_L3.pdf