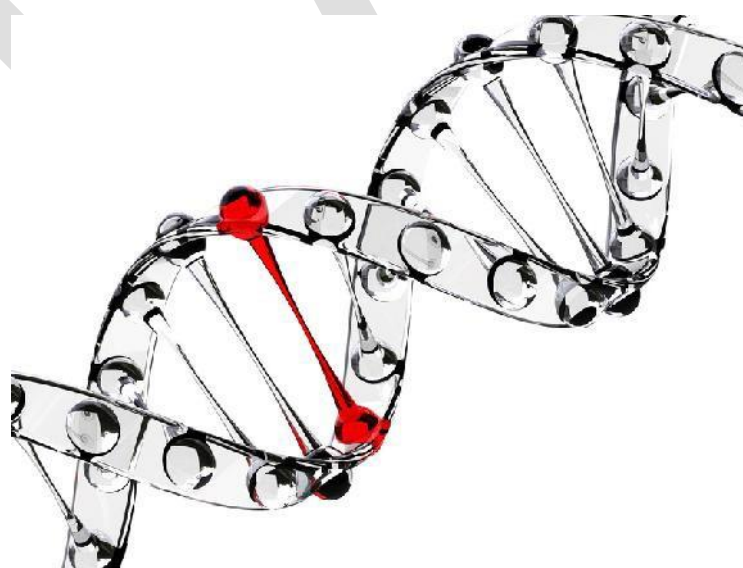




# PIPEMIDIC ACID: PDE DETERMINATION STRATEGY

LAB



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## 1. BASIC INFORMATION

<b>Company name:</b>	LAB.
<b>Company address:</b>	
<b>Expert name:</b>	
<b>Signature:</b>	
<b>Date:</b>	
<b>Assessment review data:</b>	
<b>Chemical name:</b>	8-Ethyl-5,8-dihydro-5-oxo-2-(1-piperazinyl)pyrido(2,3-d)pyrimidine-6-carboxylic acid
<b>Drug product:</b>	Pipemidic acid (oral)

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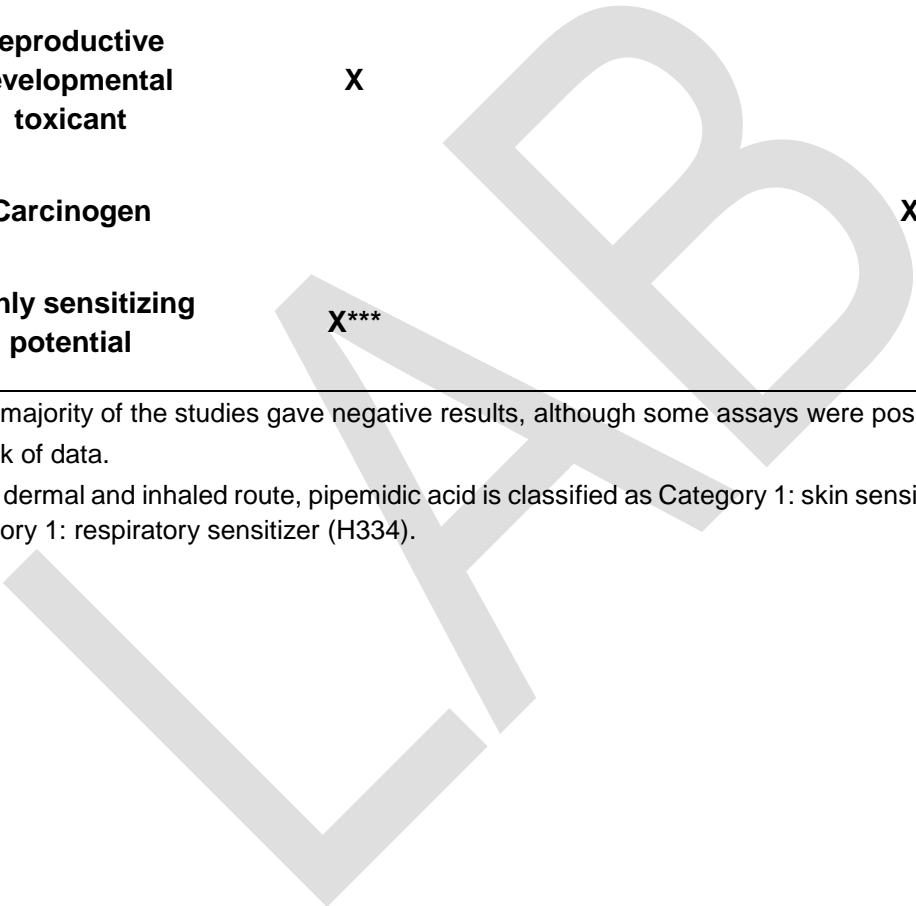
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**2. HAZARDS IDENTIFIED**

	Yes	No	Unknown
<b>Genotoxicant</b>			<b>X*</b>
<b>Reproductive developmental toxicant</b>	<b>X</b>		
<b>Carcinogen</b>			<b>X**</b>
<b>Highly sensitizing potential</b>	<b>X***</b>		

\* The majority of the studies gave negative results, although some assays were positive.  
 \*\* Lack of data.  
 \*\*\* By dermal and inhaled route, pipemidic acid is classified as Category 1: skin sensitizer (H317); Category 1: respiratory sensitizer (H334).



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### 3. SUMMARY OF ASSESSMENT PROCESS (CALCULATION OF PDE VALUE)

<b>PDE value (oral)</b>	0.42 mg/day
-------------------------	-------------

<b>HAZARD IDENTIFICATION</b>	
<b>Pharmacodynamics data</b>	Pipemidic acid is a pyridopyrimidine antibiotic derivative of piromidic acid with activity against gram-negative bacteria, as well as some gram-positive bacteria. Pipemidic acid interferes with DNA gyrase, and thus inhibit DNA synthesis during bacterial replication.
<b>Acute toxicity</b>	Pipemidic acid showed a low level of toxicity. The LD <sub>50</sub> values ranges between 300-16000 mg. See acute toxicity values in Table 1.
<b>Repeat-dose toxicity</b>	Pipemidic acid has been reported to be a compound of low toxicity.  Pipemidic acid caused abnormalities in gait and joints in young dogs, but it did not in mature dogs. No abnormality in joints was seen in a study performed in rats and rabbits. In young monkeys, small changes like blister or erosion in the articular cartilage surface of digits were seen. In 7-day-old mice subcutaneously treated with pipemidic

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	acid, articular lesions were observed; weakness and decreases in body weight were also reported.
<b>Carcinogenicity</b>	No carcinogenic information of pipemidic acid is available.
<b>“In vitro”/“in vivo” genotoxicity studies</b>	<p>Quinolones appear to be nonmutagenic. Some quinolones including pipemidic acid were reported to produce positive results in some genotoxicity studies with eukaryotes.</p> <p>Pipemidic acid gave negative results on the <i>E. coli</i> PolA-/PolA+ test, comet assay and micronucleous test. Pipemidic acid did not produce frameshift mutations in TA98, or base-pair substitutions in <i>S. typhimurium</i> hisG46 strains TA100, or UTH8414. It induced mutations in <i>S. typhimurium</i> hisG48 strains only when they had an efficient DNA excision repair system. After oral administration at high doses it produced DNA alkali-labile sites in granuloma tissue cells. After direct injection, DNA damage was absent (this suggest that pipemidic acids is activation-dependent compound).</p>
<b>Reproductive/developmental toxicity</b>	<p>Due to the arthropathy observed in adolescent animal species, the administration of quinolones to children and pregnant women is contraindicated.</p> <p>Impaired spermatogenesis and/or testicular damage have been observed</p>

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	<p>in animal studies with quinolones, including pipemidic acid. Delay in ossification and dilatation of the ureters and renal pelves in fetuses were observed in rats.</p>
<b>IDENTIFICATION OF CRITICAL EFFECTS</b>	
<p><b>Most sensitive indicator of an adverse effect seen in non-clinical toxicity data</b></p>	<p>Possible targets of quinolone toxicity include the juvenile joint, the kidney, the central nervous system, the eye, and the cardiovascular system.</p> <p>The main effects seen with pipemidic acid were arthropathy and gait abnormalities.</p>
<p><b>Clinical therapeutic and adverse effects</b></p>	<p>Pipemidic acid is a broad-spectrum antibacterial agent, effective principally against Gram-negative organisms, <i>Proteus</i> included. It is protein bound and concentrated in bile and urine and used for gastrointestinal, biliary, and urinary infections.</p> <p>It is practically free of any side-effect in man. No serious side effects were observed in two clinical studies. Severe quinolone-induced arthropathy has been reported rarely in man, transient arthralgia, characterized by joint swelling or tendonitis, has also been observed.</p>

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<b>NOAEL</b>	10 mg/Kg/day
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<b>APPLICATION OF ADJUSTMENT FACTORS</b>		
<b>F1: Extrapolation between species (2-12)</b>	<b>2</b>	For extrapolation from dogs to humans.
<b>F2: Inter-individual variability (10)</b>	<b>10</b>	Conventionally used to allow for differences between individuals in the human population.
<b>F3: Toxicological study chronic or acute (1-10). Not included genotoxicity, carcinogenicity, neurotoxicity and teratogenicity</b>	<b>10</b>	Because the study was of short duration in dogs.
<b>F4: for severe toxicity (1-10)</b>	<b>6</b>	As a safety margin, due to the possible toxic effects on the fetuses (maybe no related to maternal toxicity), the lack of information regarding the carcinogenic potential, and the positive results obtained in genotoxicity studies.
<b>F5: NOAEL vs LOAEL (10 if LOAEL)</b>	<b>1</b>	As the NOAEL was extracted.

<b>PK CORRECTION</b>	Not applied
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#### 4. IDENTITY OF THE ACTIVE SUBSTANCE

Synonyms: 5,8-Dihydro-8-ethyl-5-oxo-2-(1-piperazinyl)pyrido(2,3-d)pyrimidine-6-carboxylic acid; Deblaston; Palin; Pipemid; Uromidin

Chemical Abstracts Service (CAS) Registry Number: 51940-44-4

Chemical Description and Physical Properties: Yellowish-white, odorless, bitter-tasting crystals. Very slightly soluble in water and alcohols, soluble in acids and basis (PDS TOKU-E, 2016)

Molecular formula: C<sub>14</sub>H<sub>17</sub>N<sub>5</sub>O<sub>3</sub>

Molecular weight: 303.32 g/mol

Melting point: 253-255 °C

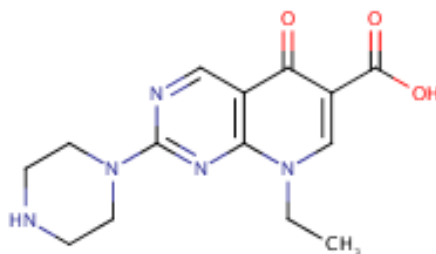


Figure 1. Structure of pipemidic acid (ChemIDplus, 2016)

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## 5. OBJECTIVE AND SEARCH STRATEGY

In accordance with the “Guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities” (EMA/CHMP/CVMP/SWP/169430/2012) the determination of health based exposure limits for a residual active substance is based on the calculation of the Permitted Daily Exposure (PDE). Determination of a PDE involves (i) hazard identification by reviewing all relevant data, (ii) identification of “critical effects”, (iii) determination of the no-observed-adverse-effect level (NOAEL) of the findings that are considered to be critical effects, and (iv) use of several adjustment factors to account for various uncertainties.

The NOAEL value has been used to calculate a PDE in this study.

It is the purpose of this document to provide a brief summary of the scientific information relative to pipemidic acid compound. All the information presented in this document is fully based on published data.

With this aim, several pharmaceutical and medical databases were scanned to reduce the risk of some reports missing. They include databases such as Pubmed, PubChem, Toxline, Drugdex, RTECS (Registry of Toxic Effects of Chemical Substances), NTP (National Toxicology Programm), CPDB (Carcinogenic Potency Database), Classification by the monograph of IARC (monograph on the evaluation of carcinogenic risk to human, International Agency for Research on Cancer monograph), DART (Development and Reproductive Database), HSDB (Hazardous Substance Data Bank) and data from medical agencies such as AEMPS (Agencia Española de Medicamentos y Productos Sanitarios), CIMA (Centro de Información on-line de medicamentos), EMA (European Medicinal Agency), FDA (Food and Drug Administration) and ECHA (European Chemical Agency). In addition, the reference book Goodman and Gilman (2006) was also consulted. The searched term was “pipemidic acid”.

## 6. INTRODUCTION

Pipemidic acid is an antibacterial agent related structurally to piromidic and nalidixic acids (Shimizu et al., 1975). It is a broad-spectrum antibacterial agent, effective principally against Gram-negative organisms, *Proteus* included (Adamowicz et al., 1976). It is effective against Gram negative and some Gram positive bacteria. It is protein bound and concentrated in bile and urine and used for gastrointestinal, biliary, and

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urinary infections (ChemIDplus, 2016). Its toxicity is low and it is practically free of any side-effect in man (Adamowicz et al., 1976).

Its ATC code is J01MB04. Pharmacotherapeutic group: other quinolones (Quinolone antibacterials. Antibacterials for systemic use) (WHO ATC/DDD Index, 2016).

## 7. HAZARD IDENTIFICATION

In this section, an evaluation of all pertinent information relative to the substance's potential to cause harm in humans is performed. This section includes an expert discussion with respect to the critical end-points, a rationale for the discussion of the choice of end points and dose. Pivotal animal and human studies were sources to the original references when possible. The study design, description of findings and accuracy of the report were revised.

### a. Pharmacodynamics data

Pipemidic acid is a pyridopyrimidine antibiotic derivative of piromidic acid with activity against gram-negative bacteria, as well as some gram-positive bacteria. Pipemidic acid exhibits greater activity than piromidic acid or nalidixic acid and shows moderate activity against bacteria that are resistant to piromidic acid and nalidixic acid (PubChem, 2016). Quinolones inhibit DNA synthesis during bacterial replication: this effect results from interference with DNA-gyrase activity (Maura and Pino, 1988).

Pipemidic acid targets bacterial DNA gyrase, an enzyme which reduces DNA strain during replication. Because DNA gyrase is required during DNA replication, subsequent DNA synthesis, and ultimately cell division is inhibited (PDS TOKU-E, 2016).

Pipemidic acid is effective after oral administration in systemic infection with *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Morganella morganii* and *Pseudomonas aeruginosa* in the mouse. Furthermore, the compound was effective in a urinary bladder-kidney infection with Gram-negative bacteria in the mouse and in pulmonary and dermal infections of the mouse with *P. aeruginosa*. Orally or subcutaneously administered pipemidic acid was more active in all model infection with *P. aeruginosa* than carbenicillin subcutaneous, but less active than gentamicin subcutaneous (Jucker, 1977).

Clinically pipemidic acid was effective in urinary tract infections with *E.coli*, *Klebsiella*,

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*Proteus* and *Pseudomonas*. In uncomplicated cases cure rates of up to 90% were obtained. In the case of chronic infections the cure rate was 30-40%. In addition, in pediatrics therapeutic success was obtained in dysentery with ampicillin-resistant strains at a dose level of 30-50 mg/Kg/day, the duration of therapy being 5 days. The side effects observed were gastrointestinal disturbances and, in some cases, skin rash (Jucker, 1977).

#### **b. Acute toxicity**

The toxicity of pipemidic acid was examined in mice and rats. The mean lethal dose values after a single dose were 707 mg/kg intravenously, 2244 mg/kg subcutaneously, and more than 16000 mg/kg orally. The toxicity test revealed that the oral toxicity of pipemidic acid was low (Shimizu et al., 1975).

The toxicity of pipemidic acid is slight. The LD<sub>50</sub> values in mice were 707 mg/Kg (intravenous), 2.24 g/Kg (subcutaneous), 1 g/Kg (intraperitoneal), 5-6 g/Kg and >16 g/Kg (oral). In rats no mortality is established at doses up to 10 g/Kg, oral administration; or 2 g/Kg intraperitoneal route. Dogs tolerate oral doses of up to 200 mg/Kg. Mice which were given oral daily doses of up to 4 g/Kg for 4 weeks and rats at daily doses of 1.6 g/Kg for 2 weeks showed no abnormalities in weight development or hematological findings (Jucker, 1977).

According to ChemIDplus (2016), Table 1 summarizes acute toxicity values of pipemidic acid in different organism and routes of administration.

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**Table 1. Acute toxicity values of pipemidic acid in different organisms and routes of administration.**

Organism	Administration route	Test type	Value (mg/Kg)	Effects
Dog	Oral	LD <sub>50</sub>	> 2000	
Monkey	Oral	LD <sub>50</sub>	> 2000	
Mouse	Intraperitoneal	LD	> 1000	
	Intravenous	LD <sub>50</sub>	300	
	Oral	LD <sub>50</sub>	5000	
	Subcutaneous	LD <sub>50</sub>	1213	
Rat	Intravenous	LD <sub>50</sub>	529	
	Oral	LD <sub>50</sub>	16000	
	Subcutaneous	LD <sub>50</sub>	1438	
Woman	Oral	TDL <sub>0</sub>	8	Skin and appendages: "dermatitis, other: after systemic exposure"

### c. Repeated dose toxicity

No abnormalities were observed in mice orally receiving pipemidic acid once a day for 4 weeks at doses of 1000, 2000, and 4000 mg/kg/day, and in rats orally receiving the drug once a day for 2 weeks at doses of 400 and 1600 mg/kg/day. The toxicity test revealed that the oral toxicity of pipemidic acid was low (Shimizu et al., 1975).

Pipemidic acid, was used to treat 14 dogs and 2 cats with recurrent urinary tract infection caused by multiresistant strains of *Escherichia coli* and *Proteus spp.* Animals were orally administered pipemidic acid at a dose rate of 10 mg/kg in two divided doses per day for two weeks. During the pipemidic acid treatment of all 16 patients, no side effects were mentioned by the owners, and in all patients hematuria and dysuria were abolished and did not recur at long-term follow-up (Van Oosterom et al., 1986).

Quinolone-induced arthropathy has been investigated most extensively in immature rats and adolescent dogs. The toxicity is characterized by lameness associated with histopathological evidence of chondrocyte loss, matrix degeneration, blistering, ulcerative erosion, or cavitation of the articular cartilage of the weight-bearing joints

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(Linseman et al., 1995).

Gait abnormalities were observed after oral administration of nalidixic acid, oxolinic acid and pipemidic acid for 1-15 days at doses of 200-1000 mg/Kg to immature dogs. At autopsy, lesions were confined to the major articulations, such as hip or shoulder joints. Initially, blisters in the articular cartilage were evident, which then progressed to ulcerative erosions. In most animals clinical recovery occurred within 2 or 3 weeks, however, the cartilage lesions were present up to 3 months after withdrawal of the drug. Microscopic examination showed hypertropic as well as shrunken or vacuolated chondrocytes (Andriole, 1988).

Similar results were reported in a comparative study of oxolinic acid (100 and 500 mg/Kg/day) and pipemidic acid (500 mg/Kg/day) effects on the cartilage in 3-month-old beagle dogs. A greater arthropathogenic potential of pipemidic acid was found in this comparative study (Andriole, 1988).

Pipemidic acid has been reported to be a compound of low toxicity as determined by subacute toxicity tests in rats, dogs and monkeys, and chronic toxicity tests in rats and dogs. However, it was found that the compound, when administered to beagle dogs of about 3 months old, induced abnormal gait with alterations in diarthrodial joints (Tatsumi et al., 1978).

A study to evaluate the effect on diarthrodial joints in experimental animals (dogs, rats, rabbits and monkeys) was performed by Tatsumi et al. (1978):

- Dogs

When young beagle dogs (2.5-3.5 months) were orally given 300 mg/Kg/day or more of pipemidic acid in two divided doses, the animals showed abnormal bending in articulations of hand and lame gait about 2 days after medication, and laid down on the side, making staggering gait when forced and broken down readily on loading within 3 days. Such abnormal gait was not noted in a dose of less than 30 mg/Kg/day. The abnormal gait manifested in massive doses was gradually recovered to nearly normal state in about 4 weeks in all animals. Autopsy disclosed abnormalities in dogs with gait disturbances: increased in synovial fluid in synovial cavity of the four limbs, blisters of various sizes under the outer layer of articular cartilage. On microscopic examination, fibrous portions with regressive cells developed near the outer layer of articular cartilage with a general orientation parallel to the joint surface, where fluid substance was

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retained. Microscopic alteration was hardly observed in the synovial membrane. Histopathologically, the articular cartilage was thin at the injured portion and there were chondrocytes divided like giant cells in the peripheral cartilage tissue and uneven stroma in some surrounding area of the lesion. At autopsy on the 91<sup>st</sup> day of medication, neither the X-ray radiographic findings of appendicular skeleton nor the length of the femur and humerus gave any difference between medicated and control groups.

Abnormal gait was marked in a dose of 300 mg/Kg/day or more, slight at 100 mg/Kg/day, and not found at 30 mg/Kg/day or less. Autopsy disclosed arthropathy in the limbs in a dose of 100 mg/Kg/day or more, less frequent manifestation of blisters and erosions in a dose of 30 mg/Kg/day, and no abnormal finding in the joints in a dose of 10 mg/Kg/day, even if a pipemidic acid was given for a long period of 90 days.

Although pipemidic acid caused the abnormality in gait and joints in young beagle dogs, it failed to cause abnormalities in mature beagle dogs (12 months or older). When an oral dose of 1000 mg/Kg/day was given to beagle dogs of 9 months old for 7 days, there were no abnormal gait, nor, slight if any, abnormality in the articular cartilage. Beagle dogs of 6 months old orally receiving 1000 mg/Kg/day showed abnormality in gait and in large joints similar to that observed in beagle dogs of 2.5-3.5 months old, though arthropathy in distal joints was low in incidence and severity. It was not possible to observe the gait of young beagle dogs of 4 weeks old, but autopsy demonstrated abnormality in the articular cartilage. However, the abnormality was not observed in younger dogs of 2 weeks or 1 week old.

When a spitz and a pomeranian of 2 months old were orally given pipemidic acid in a dose of 1000 mg/Kg/day for 4 days and in dose of 500 mg/Kg/day for 3 days, respectively, abnormal gait, increased synovial fluid, and blister formation in the outer layer of articular cartilage were observed as in beagle dogs. Similar abnormalities were noted in a young mongrel dog of the presumptive age of 3 months which was given pipemidic acid in a dose of 500 mg/Kg/day for 3 days.

- Rats, rabbits and monkeys

When Sprague-Dawley rats of 3 weeks old were orally given pipemidic acid in a dose of 1000 mg/Kg/day for 20 days, any abnormality in joints was not detected.

When pipemidic acid was orally given to groups of albino rabbits of 4 weeks old, in doses of 30, 100 and 300 mg/Kg/day for 30 days, there was no abnormality in joints.

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Administration of more than 300 mg/Kg/day was not possible because of its toxicity.

When adult monkeys (5-10 years of presumptive age) were given 1000 mg/Kg/day pipemidic acid for 30 days intragastrically via nose, there was abnormality probably due to premedication in articular cartilage surface of articulations humeri and coxae, but no abnormality in joints ascribable to pipemidic acid.

When young monkeys (5-10 months and 9-12 months of presumptive age) were given pipemidic acid in a dose of 1000 mg/Kg/day intragastrically via nose, there was no abnormal finding in behavior locomotion. No abnormalities in joints were seen. However, disc eting-microscopic examination incidentally demonstrated a small change like blister or erosion in the articular cartilage surface of digits of some animals.

Seven-day-old CF-1 mice were treated subcutaneously with either pipemidic acid (50, 400, or 3150 mg/kg/day) for 7 or 14 days or ciprofloxacin (50 or 200 mg/kg/day) for 5, 7, or 14 days. Lameness was observed only after high-dose pipemidic acid treatment for 2-7 days. Histopathological assessment of the principal weight-bearing joints revealed a lesion characterized by chondrocyte loss, matrix degeneration, and erosion of the articular cartilage in mice treated with pipemidic acid at 400 or 3150 mg/kg/day for 7 days or ciprofloxacin at 200 mg/kg/day for 5 days. Mice treated for 14 days with 400 mg/kg/day pipemidic acid demonstrated a lower incidence of lesions than mice treated for 7 days, suggesting the potential for reversibility during ongoing treatment. The results suggest that neonatal mice are sensitive to quinolone-induced arthropathy, but less so than previously reported for adolescent dogs. Subcutaneous administration of 50 and 400 mg/kg/day pipemidic acid had no adverse effects on the general appearance or body weight. However, treatment with 3150 mg/kg/day pipemidic acid resulted in overt toxicity characterized by general weakness and statistically significant decreases in body weight gain during the first 5 days (Linseman et al., 1995).

#### **d. Carcinogenicity**

No information regarding the carcinogenic potential of pipemidic acid is available.

#### **e. *In vitro* / *in vivo* genotoxicity studies**

To exclude the possibility of damage to mammalian DNA, mutagenicity studies have been performed. Since all but two tests (which may give false-positive results) have been negative, quinolones appear to be nonmutagenic. Photosensitivity has occurred in

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humans given quinolones (Christ et al., 1988).

Nalidixic acid, pipemidic acid, oxolinic acid, ofloxacin, norfloxacin, ciprofloxacin, levofloxacin and lomefloxacin were reported to produce positive results in some genotoxicity studies with eukaryotes (Itoh et al., 2006).

Pipemidic acid and norfloxacin were tested for their capacity to induce point mutations using the Ames test and DNA damage on *Escherichia coli* PolA-/PolA+. At non-toxic doses, all of the drugs studied were negative on the *E. coli* PolA-/PolA+ test with or without *in vitro* metabolic activation with induced arochlor 1254 rat liver (S9). They did not produce frameshift mutations in TA98, or base-pair substitutions in *S. typhimurium* hisG46 strains TA100, or UTH8414. Pipemidic acid induced mutations in *S. typhimurium* hisG48 strains only when they had an efficient DNA excision repair system (Arriaga-Alba et al., 1997).

The mutagenic and DNA-damaging activities of oxolinic acid and pipemidic acid were evaluated in the rat granuloma pouch assay. Oxolinic acid and pipemidic acid were administered to adult rats at various doses either orally or directly into the pouch, and isolated granuloma cells were scored for their mutation frequency at the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus by measuring their resistance to 6-thioguanine (6-TG). Moreover, oxolinic and pipemidic acids, administered by gavage in intact animals, were tested for a possible DNA-damaging activity in liver and kidneys, chosen as metabolizing and excretory organs. After oral administration at high doses both the chemicals produce DNA alkali-labile sites in granuloma tissue cells, and with oxolinic acid an increase of liver and kidney DNA elution rate was also observed. In contrast, after direct injection into the granuloma tissue, DNA damage was absent. The simultaneous analysis of 6-thioguanine-resistant cells in the granuloma tissue revealed no statistically significant mutation induction either after local treatment or oral administration. No clear dose-response curve was obtained. However, after oral application in some of the animals an enhanced mutation frequency was detected, and the cloning efficiency of cells exposed to the drugs was reduced even after culturing them for 6 days. The most likely explanation is that functional multilocus mutations are induced which cannot be recovered efficiently at the HGPRT locus. Taken as a whole, the results suggested that oxolinic and pipemidic acids are activation-dependent compounds, not directly transformed into reactive species by granuloma cells, but susceptible to activation to stable alkylating intermediates in other tissues, presumably mainly in the liver (Maura and Pino, 1988).

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Four old and four new quinolones were selected to investigate their effects on DNA (Itoh et al., 2006).

- Nalidixic acid, pipemidic acid, oxolinic acid, piromidic acid, enoxacin, ofloxacin, norfloxacin and ciprofloxacin were evaluated by the *in vitro* alkaline single-cell gel electrophoresis (comet) assay. WTK-1 cells (mutant p53) were treated with each of the eight quinolones at 62.5-1000 µg/mL for 2, 4 and 20 hours. Norfloxacin and ciprofloxacin significantly induced DNA damage concentration-dependently after 4 and 20 hours treatment, but this damage was recoverable. No increase in migration was observed for nalidixic acid, pipemidic acid, oxolinic acid, piromidic acid, enoxacin and ofloxacin.
- In the *in vitro* micronucleus test, WTK-1 cells were treated with nalidixic acid, pipemidic acid, norfloxacin and ciprofloxacin. Norfloxacin significantly increased micronucleus in the cells, but no changes were noted in the cells treated with three other quinolones.

#### **f. Reproductive and developmental toxicity**

Administration of quinolones to children and pregnant women is contraindicated by the observation of acute arthropathy in developing adolescents of several animal species (Linseman et al., 1995).

Impaired spermatogenesis and/or testicular damage (e.g., reduced spermatogenesis, azoospermia; reduced weight of the prostate, of the epididymis, and of seminal vesicle; testicular atrophy) have been described in long-term toxicity studies with some of the quinolones (e.g., pipemidic acid, rosoxacin, norfloxacin, perfloxacin, enoxacin, fleroxacin). In these investigations, the drugs were administered orally for a period of 4-26 (-52) weeks to rats, dogs, and monkeys at doses ranging from 80 to 3000 mg/Kg/day (Siporin et al., 1990).

Pipemidic acid was given to pregnant rats by gavage in doses of up to 3200 mg/Kg on days 8 through 14 of gestation. At the highest dose, a slight decrease in fetal weight and delay in ossification occurred. Dilatation of the ureters and renal pelves were significantly more common in the fetuses exposed to 800 and 3200 mg/Kg. Postnatally 8 of 132 pups in the 3200 mg/Kg groups showed dilatation of the renal pelves (Shepard and Lemire, 2004).

Results in experiments suggest that pipemidic acid may cause disorders in the

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development of the embryo or fetus, even when no signs of poisoning show in the mother (MSDS Santa Cruz Biotechnology, 2010).

## **8. IDENTIFICATION OF CRITICAL EFFECTS**

The steps deliberated to identify the nature of the adverse effect include its severity and persistence. If the substance causes multiple types of adverse effects, the critical effect is one that meets the severity and persistence criteria at the lowest intake.

### **a. Most sensitive indicator of an adverse effect seen in non-clinical toxicity data**

Possible targets of quinolone toxicity include the juvenile joint, the kidney, the central nervous system, the eye, and the cardiovascular system (Christ et al., 1988).

The main effects seen with piperimidic acid were arthropathy and gait abnormalities.

### **b. Clinical therapeutic and adverse effects**

Piperimidic acid is a broad-spectrum antibacterial agent, effective principally against Gram-negative organisms, *Proteus* included (Adamowicz et al., 1976). Piperimidic acid is an antimicrobial against Gram negative and some Gram positive bacteria. It is protein bound and concentrated in bile and urine and used for gastrointestinal, biliary, and urinary infections (ChemIDplus, 2016).

Its toxicity is low and it is practically free of any side-effect in man. The average recommended dosage is 400 mg twice a day (Adamowicz et al., 1976).

Although severe quinolone-induced arthropathy has been reported only rarely in man, transient arthralgia, characterized by joint swelling or tendonitis, has also been observed (Linseman et al., 1995).

Twenty-seven cases of urinary tract infection were treated with daily dose of 1.5 g of piperimidic acid trihydrate. Duration of drug administration varied between 7 and 28 days. The patients were consisted of acute cystitis, underlying disease and complicated infection after long period catheterization. Following results were obtained: 1. Organisms cultured before treatment were sensitive to piperimidic acid trihydrate. 2. First and second classes of patients were cured by the treatment, however, patients having indwelling catheter were not changed on the view of culture. 3. No serious side effect was observed

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including hepatic and renal function, and blood (Iwama et al., 1977).

In a clinical study, 23 females and 2 males, ages varied from 17-47 years were treated with pipemidic acid. Seventeen had acute and 8 chronic urinary tract infection. In patients with acute urinary tract infection, all except one patient were symptom free at the end of 7-10 days therapy and the drug was effective in 94% cases. In patients with chronic urinary tract infection, no side effects were observed except nausea in patient and the drug was effective in 87.5% cases (Kamran et al., 1984).

### Sensitization

According to the European Chemical Agency (ECHA, 2016), pipemidic acid is included in the Hazard Category 1 as respiratory and skin sensitizer:

- Skin sensitizer 1: H317 (may cause an allergic skin reaction).
- Respiratory sensitizer 1: H334 (may cause allergy or asthma symptoms or breathing difficulties if inhaled).

Contact allergies quickly manifest themselves as contact eczema, more rarely as urticaria or Quincke's edema. The pathogenesis of contact eczema involves a cell-mediated (T lymphocytes) immune reaction of the delayed type (MSDS Santa Cruz Biotechnology, 2010).

Allergic reactions involving the respiratory tract are usually due to interactions between IgE antibodies and allergens and occur rapidly. Allergic potential of the allergen and period of exposure often determine the severity of symptoms. Exogenous allergic alveolitis is induced essentially by allergen specific immune-complexes of the IgG type; cell-mediated reactions (T lymphocytes) may be involved. Such allergy is of the delayed type with onset up to four hours following exposure (MSDS Santa Cruz Biotechnology, 2010).

According to Septidron capsules SPC, hypersensitivity reactions, such as eruption, urticaria, pruritus, fever and anaphylactic shock, can occur, in which case treatment should be discontinued.

## **9. RATIONALE FOR NOAEL VALUES SELECTION**

Pipemidic acid has been reported to be a compound of low toxicity. The main adverse

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effects seen were on the joints. Dogs seem to be more sensitive than other animal species. No information about its carcinogenic potential is available. The quinolones appear to be non-mutagenic. The majority of genotoxicity studies gave negative results. Some quinolones including pipemidic acid gave positive results in some genotoxicity studies with eukaryotes. Pipemidic acid induced mutations in *S. typhimurium* hisG48 strains only when they had an efficient DNA excision repair system; and it produced DNA alkali-labile sites in granuloma tissue cells after oral administration at high doses. Due to the arthropathy observed in adolescent animal species, the administration of quinolones to children and pregnant women is contraindicated. Impaired spermatogenesis and/or testicular damage have been observed in animal studies with quinolones, including pipemidic acid. Delay in ossification and dilatation of the ureters and renal pelvis in fetuses were observed in rats.

A NOAEL of 10 mg/Kg/day was used for PDE calculation. This value is derived from a study performed in young beagle dogs, orally administered pipemidic acid, and based on the adverse effects in the joints. At 10 mg/Kg/day, neither abnormal gait nor abnormal findings in the joints were seen.

Young beagle dogs (2.5-3.5 months) were orally given pipemidic acid. Abnormal gait was marked in a dose of 300 mg/Kg/day or more, slight at 100 mg/Kg/day, and not found at 30 mg/Kg/day or less. Autopsy disclosed arthropathy in the limbs in a dose of 100 mg/Kg/day or more, less frequent manifestation of blisters and erosions in a dose of 30 mg/Kg/day, and no abnormal finding in the joints in a dose of 10 mg/Kg/day, even if a pipemidic acid was given for a long period of 90 days (Tatsumi et al., 1978).

## **10. APPLICATION OF ADJUSTMENT FACTORS (rationale for the adjustment factors)**

A series of modifying, or safety factors, are used when the NOAEL is based on studies of differing types and durations in differing species to provide a risk assessment for human exposure. These factors were generally established according to Appendices 3 of the ICH Q3C (R5) and VICH GL 18.

### **a. F1: Interspecies differences**

This factor takes into account the comparative surface area: body weight ratios for the species concerned and for man. Surface area (S) was calculated as:  $S = k M^{0.67}$  where

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M is the body mass and the constant, k, has been taken to be 10 according to the appendices 3 of the ICH guideline. For a 50 kg person the equation gives a surface area of 137.5 dm<sup>2</sup>; the surface area: body weight ratio is thus 2.76.

Applying the same calculation to other species and expressing the results as multiples of the human surface area: body weight ratio gives factors for the mouse = 12; for the rat = 5; for the monkey = 3; for the rabbit = 2.5; for the dog = 2. For other species, where the data are not so well established the factor F1 is taken as 10.

A value of F1= 2 is used for extrapolation from dogs to humans.

**b. F2: Inter-individual differences**

A value of F2= 10 is conventionally used to allow for differences between individuals in the human population.

**c. F3: Duration of exposure**

A variable factor up to 10 takes into account the differing durations of exposure in the reported studies. For reproductive studies, a factor of 1 is used if the whole period of organogenesis is covered. A factor of 2 has been used for a 6-month study in rodents, or a 3.5-year study in non-rodents. A factor of 5 has been used for a 3-month study in rodents or a 2-year study in non-rodents and a factor of 10 for studies of a shorter duration. In all cases, the higher factor has been used for study durations between the time points e.g. a factor of 2 for a 9-month rodent study.

A value of F3= 10 is established as the value is derived from a short duration study in dogs (90 days).

**d. F4: Nature of toxicity**

A variable factor is applied when the toxicity produced is irreversible in nature i.e. carcinogenicity, neurotoxicity or teratogenicity. A factor of 10 is used when oncogenic or neurotoxic responses are present. A variable factor is used for reproductive toxicity effects as follows: 1 for embryo or fetal toxicity or mortality associated with maternal toxicity; 5 for embryo or fetal toxicity or mortality without maternal toxicity; 5 for a teratogenic effect with maternal toxicity and 10 for a teratogenic effect in the absence of accompanying maternal toxicity.

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A value of F4= 6 is established, as a safety margin, due to the possible toxic effects on the fetuses that might not to be related to maternal toxicity; the lack of carcinogenicity information; and the positive results seen in some genotoxicity studies.

**e. F5: Quality of data**

A variable factor, up to 10, applied to results in which a NOAEL has not been established, the PDE being derived from a LOAEL.

As the NOAEL was extracted, a value of F5= 1 is used in this report.

The PDE calculation is generally presented in the format:

$$\text{PDE (mg/day)} = \frac{\text{NOAEL or LOAEL (mg/kg/day)} \times \text{Body weight (kg)}}{\text{F1} \times \text{F2} \times \text{F3} \times \text{F4} \times \text{F5}}$$

$$\text{PDE (oral)} = \frac{10 \text{ (mg/kg/day)} \times 50 \text{ (kg)}}{2 \times 10 \times 10 \times 6 \times 1} = 0.42 \text{ mg/day}$$

**11. PK CORRECTION**

No PK correction was established since the same administration route was used for PDE calculation.

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## **ANNEX 1: PHARMACOKINETICS AND METABOLISM**

According to Shimizu et al. (1975) the pharmacokinetics properties are as follows:

### **Absorption**

Pipemidic acid was absorbed well by the oral route. Its peak levels in plasma ranged from 4 to 12 µg/ml at an oral dose of about 50 mg/kg in mice, rats, dogs, monkeys, and men.

### **Distribution**

The protein binding of pipemidic acid was about 20% in dog plasma and about 30% in human serum. Pipemidic acid was distributed to most of the organs and tissues tested at the concentrations comparable to or higher than the plasma level. Its concentrations in bile and urine were much higher than the plasma level.

### **Metabolism and excretion**

About 25 to 88% of orally administered pipemidic acid was excreted into urine in a bacteriologically active form, the percentage depending on the animals and doses employed. The remainder was excreted into feces in men. The main active principle *in vivo* was unchanged pipemidic acid itself.

As pipemidic acid orally administered to men was recovered from urine and feces in nearly 100% in a bacteriologically active form, it was expected that the drug was neither accumulated in a body nor inactivated metabolically.

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## ANNEX 2: GLOSSARY

ADI: Acceptable daily intake

AUC: Area under the curve

GRAS: Generally regarded as safe

GLP: Good laboratory practice

GMP: Good manufacturing practice

LD: Lethal dose

LED: Lowest-effective dose

TDLo (Toxic Dose Low,): Lowest published toxic dose

LOAEL: Lowest-observed-adverse-effect level

LOEL: Lowest-observed-effect level

MSDS: Material safety data sheet

MTD: Maximum tolerable dose

MPDD: Maximum permissible daily dose

MTEL: Maximum tolerable exposure level

NEL: No-effect level

NOAEL: No-observed-adverse-effect level

NOEL: No-observed-effect level

OEL: Occupational exposure limit

QSAR: Quantitative structure–activity relationship

SDS: Safety data sheet

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**ADI:** Acceptable daily intake. Estimate by JECFA; the amount of a food additive, expressed on a body weight basis that can be ingested daily over a lifetime without appreciable health risk.

**Area under the curve (AUC):** Area between a curve and the abscissa (horizontal axis), i.e., the area underneath the graph of a function; often, the area under the tissue (plasma) concentration curve of a substance expressed as a function of time.

**Bioaccumulation:** progressive increase in the amount of a substance in an organism or part of an organism that occurs because the rate of intake exceeds the organism's ability to remove the substance from the body.

**Bioavailability:** biological and physiological availability. Extent of absorption of a substance by a living organism compared to a standard system.

**Biological half-life:** for a substance, the time required for the amount of that substance in a biological system to be reduced to one-half of its value by biological processes, when the rate of removal is approximately exponential.

**Carcinogen:** agent (chemical, physical, or biological) that is capable of increasing the incidence of malignant neoplasms, thus causing cancer.

**Clastogen:** agent causing chromosome breakage and (or) consequent gain, loss, or rearrangement of pieces of chromosomes.

**Clearance:** volume of blood or plasma or mass of an organ effectively cleared of a substance by elimination (metabolism and excretion) divided by time of elimination.

**Cmax:** used in pharmacokinetics referring to the maximum (or peak) serum concentration that a drug achieves in a specified compartment or test area after the drug has been administered and before the administration of a second dose.

**Critical dose:** dose of a substance at and above which adverse functional changes, reversible or irreversible, occur in a cell or an organ.

**Critical effect:** for deterministic effects, the first adverse effect that appears when the threshold (critical) concentration or dose is reached in the critical organ: Adverse effects with no defined threshold concentration are regarded as critical.

**Dose (of a substance):** total amount of a substance administered to, taken up, or

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absorbed by an organism, organ, or tissue.

**Draize test:** evaluation of materials for their potential to cause dermal or ocular irritation and corrosion following local exposure; generally using the rabbit model (almost exclusively the New Zealand White) although other animal species have been used.

**Elimination (in toxicology):** disappearance of a substance from an organism or a part thereof, by processes of metabolism, secretion, or excretion.

**Embryotoxicity:** production by a substance of toxic effects in progeny in the first period of pregnancy between conception and the fetal stage.

**Fetotoxicity:** production by a substance of toxic effects in progeny in the second period of pregnancy between fetal stage and delivery.

**First-pass effect:** biotransformation and, in some cases, elimination of a substance in the liver after absorption from the intestine and before it reaches the systemic circulation.

**Gavage:** administration of materials directly into the stomach by esophageal intubation.

**Generally regarded as safe (GRAS):** phrase used to describe the USFDA philosophy that justifies approval of food additives that may not meet the usual test criteria for safety but have been used extensively and have not demonstrated that they cause any harm to consumers.

**Genotoxic:** capable of causing a change to the structure of the genome.

**Good laboratory practice (GLP) principles:** fundamental rules incorporated in OECD guidelines and national regulations concerned with the process of effective organization and the conditions under which laboratory studies are properly planned, performed, monitored, recorded, and reported.

**Good manufacturing practice (GMP) principles:** fundamental rules incorporated in national regulations concerned with the process of effective organization of production and ensuring standards of defined quality at all stages of production, distribution, and marketing.

**Hazard identification:** determination of substances of concern, their adverse effects, target populations, and conditions of exposure, taking into account toxicity data and knowledge of effects on human health, other organisms, and their environment.

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**Hypersensitivity:** state in which an individual reacts with allergic effects following exposure to a certain substance (allergen) after having been exposed previously to the same substance.

**In silico:** phrase applied to data generated and analyzed using computer modeling and information technology.

**In vitro:** in glass, referring to a study in the laboratory usually involving isolated organ, tissue, cell, or biochemical systems.

**In vivo:** In the living body, referring to a study performed on a living organism.

**Lethal dose (LD):** amount of a substance or physical agent (e.g., radiation) that causes death when taken into the body.

**Lowest-effective dose (LED):** lowest dose of a chemical inducing a specified effect in a specified fraction of exposed individuals.

**Lowest published toxic dose (Toxic Dose Low, TDLo):** the lowest dosage per unit of bodyweight (typically stated in milligrams per kilogram) of a substance known to have produced signs of toxicity in a particular animal species.

**Lowest-observed-adverse-effect level (LOAEL):** lowest concentration or amount of a substance (dose), found by experiment or observation, that causes an adverse effect on morphology, functional capacity, growth, development, or life span of a target organism distinguishable from normal (control) organisms of the same species and strain under defined conditions of exposure.

**Lowest-observed-effect level (LOEL):** lowest concentration or amount of a substance (dose), found by experiment or observation, that causes any alteration in morphology, functional capacity, growth, development, or life span of target organisms distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure.

**Material safety data sheet (MSDS):** compilation of information required under the U.S. OSHA Hazard Communication Standard on the identity of hazardous substances, health and physical hazards, exposure limits, and precautions.

**Maximum permissible daily dose (MPDD):** maximum daily dose of substance whose

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penetration into a human body during a lifetime will not cause diseases or health hazards that can be detected by current investigation methods and will not adversely affect future generations.

**Maximum tolerable dose (MTD):** highest amount of a substance that, when introduced into the body, does not kill test animals (denoted by LD0).

**Maximum tolerable exposure level (MTEL):** maximum amount (dose) or concentration of a substance to which an organism can be exposed without leading to an adverse effect after prolonged exposure time.

**Maximum tolerated dose (MTD):** high dose used in chronic toxicity testing that is expected on the basis of an adequate subchronic study to produce limited toxicity when administered for the duration of the test period.

**Median lethal dose (LD50):** statistically derived median dose of a chemical or physical agent (radiation) expected to kill 50 % of organisms in a given population under a defined set of conditions.

**Mutagenicity:** ability of a physical, chemical, or biological agent to induce (or generate) heritable changes (mutations) in the genotype in a cell as a consequence of alterations or loss of genes or chromosomes (or parts thereof).

**No-effect level (NEL):** maximum dose (of a substance) that produces no detectable changes under defined conditions of exposure.

**No-observed-adverse-effect level (NOAEL):** greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development, or life span of the target organism under defined conditions of exposure.

**No-observed-effect level (NOEL):** greatest concentration or amount of a substance, found by experiment or observation, that causes no alterations of morphology, functional capacity, growth, development, or life span of target organisms distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.

**Quantitative structure–activity relationship (QSAR):** quantitative structure–biological activity model derived using regression analysis and containing as parameters

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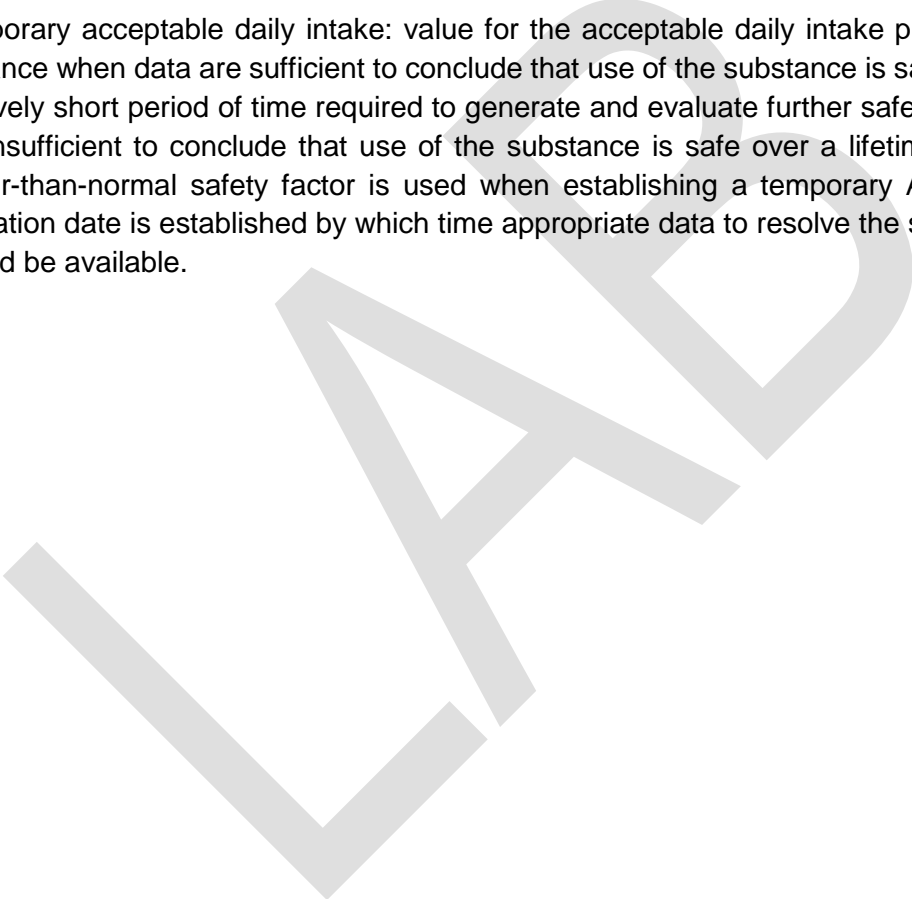


physicochemical constants, indicator variables, or theoretically calculated values.

**Safety data sheet (SDS):** single page giving toxicological and other safety advice, usually associated with a particular preparation, substance, or process.

**Target (in biology):** any organism, organ, tissue, cell or cell constituent that is subject to the action of an agent.

**Temporary acceptable daily intake:** value for the acceptable daily intake proposed for guidance when data are sufficient to conclude that use of the substance is safe over the relatively short period of time required to generate and evaluate further safety data, but are insufficient to conclude that use of the substance is safe over a lifetime. Note: A higher-than-normal safety factor is used when establishing a temporary ADI and an expiration date is established by which time appropriate data to resolve the safety issue should be available.



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**ANNEX 3. SUMMARY OF THE EXPERT CV**



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