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**SISAPRONIL page 75-98**

# Toxicological evaluation of certain veterinary drug residues in food

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# Sisapronil

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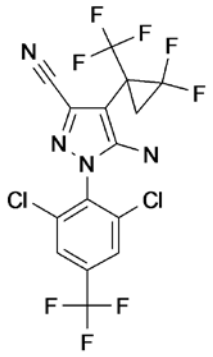
# 1. Explanation

Sisapronil, formerly known as phenylpyrazole, is the proposed International Non-proprietary Name (INN) for 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)-phenyl]-4-[2,2-difluoro-1-(trifluoromethyl) cyclopropyl]-1*H*-pyrazole-3-carbonitrile (International Union of Pure and Applied Chemistry), with Chemical Abstracts Service number 856225-89-3 (Fig. 1). It is a new member of the phenylpyrazole class of compounds. It is a long-acting subcutaneous injectable ectoparasiticide for control of cattle ticks. It also aids in the control of bot fly larvae, hornfly and screwworm. It is approved for use in cattle as a single subcutaneous injection of 2 mg/kg body weight (bw), with a withdrawal time of 120 days.

Fig. 1
**Chemical structure of sisapronil**




Sisapronil binds to ligand-gated chloride channels, in particular those gated by the neurotransmitter gamma-aminobutyric acid, thereby non-competitively blocking pre-synaptic and post-synaptic transfer of chloride ions across cell membranes in insects or acari. This mechanism of action results in uncontrolled activity of the central nervous system and death of the parasites.

Sisapronil has not previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Committee evaluated sisapronil at the present meeting at the request of the Twenty-second Session of the Codex Committee on Residues of Veterinary Drugs in Foods (FAO/WHO, 2015), with a view to establishing an acceptable daily intake (ADI) and recommending maximum residue limits (MRLs) in cattle tissues.

Information submitted to the Committee included studies on acute, repeated-dose, reproductive and developmental toxicity, genotoxicity and neurotoxicity. Pivotal studies were conducted according to good laboratory practice (GLP) standards and in accordance with Organisation for Economic

Co-operation and Development (OECD) and/or International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guidelines. A literature search provided no additional information.

# 2. Biological data

## 2.1 Biochemical aspects

### 2.1.1 Absorption, distribution and excretion

The pharmacokinetics of sisapronil was studied in rats, dogs and cynomolgus monkeys. Most of the investigations were done as part of the toxicological studies (see section 2.2).

#### (a) Rats

The concentration of sisapronil in plasma was investigated in all repeated-dose toxicity studies in rats, most extensively in the 28-day oral toxicity study (Hu, 2009) and the 90-day oral toxicity study (Rodríguez Gómez, 2012). Blood samples were taken at 4, 8 and 24 hours after gavage administration on day 1 in both studies. In addition, in the 28-day study, blood from animals from satellite groups was taken immediately after dosing on days 1, 4, 7, 14, 21, 28, 35, 38 and 42 and at 4, 8 and/or 24 hours after dosing on days 14 and 28. Remarkably, there was a 3- to 4-fold difference in the observed plasma concentrations at similar dose levels across the studies. The cause of these differences could not be determined. Nevertheless, the studies consistently showed that the plasma concentrations of sisapronil were dose dependent, but less than dose proportional. From the results, a plasma elimination half-life of approximately 14 days was estimated. The sponsor also cited a half-life of 20–39 days, derived from an exploratory oral study in rats with a sampling period of 14 days; however, that study was not provided. Owing to its slow elimination, sisapronil accumulated in plasma upon daily dosing. A time to steady state of approximately 100–150 days could be estimated from the time–concentration profile in plasma in rats from the 1-year repeated-dose toxicity study (Rodríguez Gómez, 2013). In that study, the mean sisapronil concentrations in plasma at the last time point were approximately 325, 567, 1172 and 3424 ng/mL for the 0.1, 0.3, 1.0 and 10 mg/kg bw per day dose groups, respectively.

Radioactive residues in liver and faeces were examined in male and female Sprague-Dawley rats orally dosed with $^{14}$C-labelled sisapronil for 1 day at a dose of 50 mg/kg bw or 4 days at 50 mg/kg bw per day. The radioactivity in faeces

was approximately 98% of the total recovery from excreta, and the remainder was found in urine, suggesting limited absorption (Lineham, 2012).

#### (b) Dogs

The concentrations of sisapronil in plasma from Beagle dogs were measured in the 28-day and 90-day oral toxicity studies (Heward, 2011, 2012). The plasma samples were taken at only one time point after administration of sisapronil in capsules (8 hours) on days 1, 8, 15 and 28 of the 28-day study and on days 1, 30, 60 and 90 of the 90-day study; therefore, the kinetic profile could not be determined. Sisapronil accumulated in plasma up to 90 days, the last time point of sampling.

In a non-GLP-compliant study, groups of three male and three female fasted Beagle dogs were given a single intravenous sisapronil dose of 0.5, 1.5 or 5 mg/kg bw. Blood samples were taken over a period of 268 days. The plasma concentrations of sisapronil were dose dependent, but less than dose proportional. The plasma elimination half-life for all three doses was approximately 100 days (Fisher, 2008).

#### (c) Cynomolgus monkeys

The pharmacokinetic profile of sisapronil was studied in male and female cynomolgus monkeys. Four animals were given a single dose of sisapronil by oral gavage (2 mg/kg bw) or intravenously (0.5 mg/kg bw). Blood was collected from a femoral vein 0.25, 0.5, 1, 2, 4, 8, 24, 48, 96, 168, 240, 336, 504, 672, 840, 1008, 1344 and 1680 hours post-dosing. After oral administration, the sisapronil concentration in plasma reached its maximum of approximately 17 ng/mL at 24 hours. Following intravenous administration, the area under the concentration–time curve from time 0 to infinity ($AUC_{0-\infty}$) was 1230 ng·d/mL, and the plasma elimination half-life was 12.4 days. The oral bioavailability in cynomolgus monkeys was 6.8% (Stuhler, Peterson & Starch, 2012).

### 2.1.2 Biotransformation

In the study in which Sprague-Dawley rats were orally dosed with $^{14}C$-labelled sisapronil for 1 day at a dose of 50 mg/kg bw or 4 days at 50 mg/kg bw per day (see section 2.1.1), the parent substance sisapronil accounted for more than 91% of the radioactivity in faeces at 24 hours after treatment, but the relative content of parent substance in faeces declined over the 24- to 144-hour study period. In liver, sisapronil was the primary residue. A metabolite was detected in the liver of rats, but could not be identified. It had the same retention time as the main metabolite found in cattle liver, which could not be identified because of the low quantities present (Lineham, 2012).

Table 1
**Acute toxicity studies on sisapronil**

| Species | No. of each sex per group | Route | Dose (mg/kg bw) | $LD_{50}$ (mg/kg bw) | Reference |
|---|---|---|---|---|---|
| Mouse | 3–4, sex not specified | Oral | 3, 10, 30 | >30 | Mill (2003) |
| Rat | 9 F | Oral | 175, 550, 2 000 | 552 | Beerens-Heijnen (2011a) |
| Rat | 2 M | Oral | 100, 250, 500, 1 000, 1 500 | Not estimated | Gagnon (2012a) |
| Rat | 3 M | Oral | 10 | Not applicable | Gagnon (2012b) |
| Rat | 20 M | Oral | 0, 100, 500, 1 000 | Not estimated | Ryan (2011) |
| Mouse | 4, sex not specified | Intravenous | 0.3, 1, 3 | >3 | Mill (2003) |

bw: body weight; F: females; $LD_{50}$: median lethal dose; M: males

## 2.2 Toxicological studies

### 2.2.1 Acute toxicity

#### (a) Lethal doses

The single-dose toxicity of sisapronil was investigated in mice and rats using the oral (mice and rats) and intravenous (mice) routes of administration (Table 1).

In a non-GLP-compliant study, groups of three or four mice (strain and sex not specified) were given sisapronil at a single oral dose of 3, 10 or 30 mg/kg bw. All animals appeared symptomatically normal (Mill, 2003).

In a non-GLP-compliant dose range–finding study, groups of two male Wistar rats were given sisapronil by gavage at a single oral dose of 100, 250, 500, 1000 or 1500 mg/kg bw. Body weights, feed consumption and clinical signs were recorded for 13 days after dosing. The animals that were found dead or euthanized moribund were examined histopathologically. Doses of 100 and 250 mg/kg bw were well tolerated, but rats dosed with 500 mg/kg bw and above showed observable clinical effects. Observations of hyperreactivity (to noise or cage opening) and sensitivity to touch were observed as early as 3 days post-treatment. Tremors upon stimulation were observed in 4/6 animals dosed at 500 mg/kg bw and above. At doses of 500 mg/kg bw and above, 4/6 animals were found dead, whereas the other two were euthanized moribund. The thymus of one animal at each dose of 500, 1000 and 1500 mg/kg bw had mild to moderate lymphoid depletion. In addition, the only animal examined histopathologically at the highest dose showed diffuse, mild vacuolation of the adrenocortical cells. A median lethal dose ($LD_{50}$) was not estimated (Gagnon, 2012a).

Another non-GLP-compliant study was conducted to compare the toxicity of sisapronil in Sprague-Dawley and Wistar rats given a single oral gavage dose of 10 mg/kg bw. The animals were examined twice daily for clinical signs, including mortality; however, the observation period was not specified, and the

study report did not contain raw data. It was stated that all animals appeared clinically normal (Gagnon, 2012b).

Sisapronil was tested for acute oral toxicity in female Wistar Crl:WI rats in a GLP-compliant study. Nine rats were given sisapronil by oral gavage at a dose of 175, 550 or 2000 mg/kg bw, following the up-and-down procedure with dosing intervals of 5 days and an observation period of 15 days for each rat. Hunched posture and piloerection were observed at all dose levels. Tremors were seen in mid-dose animals, whereas animals dosed at 2000 mg/kg bw additionally showed lethargy, chromodacryorrhoea, lean appearance and/or hypersensitivity to touch during the observation period until they died. The oral $LD_{50}$ was estimated to be 552 mg/kg bw (Beerens-Heijnen, 2011a).

In a non-GLP-compliant study, groups of four mice (strain and sex not specified) received sisapronil intravenously at a single dose of 0.3, 1 or 3 mg/kg bw. No animals died. One mouse of the high-dose group showed a slight and transient depression in body temperature (Mill, 2003).

#### (b) Dermal irritation

Three New Zealand White rabbits were exposed to 0.5 g of sisapronil moistened with 50% watery ethanol by application onto clipped skin for 4 hours using a semi-occlusive dressing. Skin reactions were assessed 1, 24, 48 and 72 hours after exposure. Very slight erythema was observed in the treated skin areas of the three rabbits, which resolved within 24–72 hours after exposure. Only one animal showed very slight oedema on day 1. No oedema was noted in the remaining two animals. No staining of the treated skin by the test substance was observed, and no test substance remnants were seen. On the basis of this study, sisapronil is considered not irritating to the skin of rabbits (Beerens-Heijnen, 2011b).

#### (c) Ocular irritation

Seventy milligrams of sisapronil was instilled into the conjunctival sac of one eye of each of three New Zealand White rabbits. Instillation of the test substance resulted in irritation of the conjunctivae, which consisted of redness and discharge. The irritation had completely resolved within 48 hours in two animals and within 72 hours in the other animal. Based on these results, sisapronil is considered not irritating to the eyes of rabbits (Beerens-Heijnen, 2011c).

#### (d) Dermal sensitization

In a local lymph node assay, groups of five female CBA/J mice were given sisapronil at a concentration of 0%, 0.5%, 1% or 2.5% weight per weight (w/w) on 3 consecutive days by open application on the ears. Three days after the last exposure, all animals were injected with [$^3$H-methyl]thymidine; after 5 hours,

the draining (auricular) lymph nodes were excised and pooled for each animal. No irritation of the ears was observed in any of the animals examined. All auricular lymph nodes of the animals of the experimental and control groups were considered normal in size, except for one animal at 2.5%, which showed larger lymph nodes. The stimulation index values calculated for the substance concentrations 0.5%, 1% and 2.5% were 0.9, 0.8 and 1.3, respectively. As there was no indication that the test substance elicits a stimulation index of 3 or higher when tested up to 2.5%, sisapronil was considered not to be a skin sensitizer (Beerens-Heijnen, 2011d).

### 2.2.2 Short-term studies of toxicity

#### (a) Rats

In a non-GLP-compliant study, sisapronil was given to groups of 10 male and 10 female Crl:CD(SD) rats by oral gavage at a dose of 0, 0.1, 1 or 10 mg/kg bw per day for 28 days. Observations included mortality, clinical signs, body weights, feed consumption, ophthalmic examinations, hormone analysis, clinical pathology and histology (only control and high-dose animals).

No animals died, and there were no effects on clinical signs. No treatment-related alterations in mean body weight, mean body weight gain or mean feed consumption were noted for animals given up to 10 mg/kg bw per day. No visible lesions were observed at ophthalmic examination. High-dose males had a decreased lymphocyte count and reticulocyte count. Higher plasma levels of thyroid stimulating hormone (TSH) and lower plasma levels of thyroxine ($T_4$) were observed in high-dose males and mid- and high-dose females. Higher levels of cholesterol and decreased levels of bilirubin were noted in all high-dose animals. Increased absolute and relative liver weights were observed in males and females of the high-dose group. In high-dose males, the absolute and relative weights of the thyroids were also increased, and the absolute and relative weights of the spleens were decreased. The effect on absolute lymphocyte count may have been associated with reduced spleen weight (no associated pathological findings). The effects on TSH, $T_4$, cholesterol and total bilirubin levels were consistent with metabolic enzyme induction and correlated with higher liver weights and hepatocellular hypertrophy, observed microscopically for animals given 10 mg/kg bw per day. The increased thyroid weights observed in high-dose males were accompanied by hypertrophy/hyperplasia of the thyroid follicular epithelium, characterized by a predominance of small follicles with decreased amounts of luminal colloid and increased height of thyroid epithelium at this dose level (Hu, 2009).

Short-term toxicity was further investigated in a non-GLP-compliant dose range–finding study. Groups of three female Wistar rats were given sisapronil by oral gavage at a dose of 0, 20, 40, 60, 80 or 100 mg/kg bw per day for 60 days. On day 27, animals previously assigned to the control group were reassigned to a 15 mg/kg bw per day dose group to further define dose tolerance. Body weight and feed consumption were measured weekly. Animals were observed multiple times daily for clinical signs of toxicity, including mortality.

At 40 mg/kg bw per day and higher, animals either were found dead or had to be euthanized. One animal of the 20 mg/kg bw per day group was euthanized on day 8; the other animals of that group and the animals of the 0/15 mg/kg bw per day group were terminal at day 60. Nineteen days after switching controls to 15 mg/kg bw per day, these animals started showing effects, including tense posture and hyperactivity, which continued throughout the study. Tense posture and hyperreactivity/aggressiveness were also observed at doses of 20–60 mg/kg bw per day. Additionally, tremors/seizures were noted at 40 mg/kg bw per day and higher, and decreased activity, red staining (face, paws) and occasional twitch were seen in animals of the 40, 60 and 100 mg/kg bw per day dose groups. Body weights were reduced at 40 mg/kg bw per day and above, and feed consumption was decreased at 60 mg/kg bw per day and above (Gagnon, 2012c).

In a GLP-compliant study, groups of 10 male and 10 female Wistar rats were given sisapronil by oral gavage at a daily dose of 0 (vehicle), 0.1, 0.3, 1.0 or 10 mg/kg bw per day for 13 weeks. Satellite groups were used to study the plasma kinetics of sisapronil (see section 2.1.1). The animals were checked for morbidity and mortality daily, whereas clinical signs were recorded weekly. A functional observational battery was performed on all animals prior to necropsy at the end of the study. Other observations included body weight, feed consumption, ophthalmology, haematology, clinical chemistry, urine analysis, gross pathology and histopathology (only the highest-dose animals and the controls).

No mortality occurred during the study. No treatment-related clinical signs were noted, including ophthalmology. Body weight and feed consumption were normal in all groups. The functional observational battery revealed no treatment-related effects. Blood parameters were also unaffected. Increased plasma levels of TSH and decreased plasma levels of $T_4$ were observed at 10 mg/kg bw per day (statistically significant and more pronounced in males). These changes were only slight and not statistically significant at 1.0 mg/kg bw per day. At lower doses, these parameters appeared unaffected. Males and females of the highest-dose group had slightly increased (<10%) levels of total protein and globulin. At 10 mg/kg bw per day, the absolute and relative (to body weight and brain weight) weights of the liver were increased in both sexes. This was also seen in females

of the 1.0 mg/kg bw per day group, but the differences relative to controls failed to attain statistical significance. The increase in liver weights was correlated with hepatocellular hypertrophy in 10/10 males and 5/10 females at the highest dose. Liver-specific clinical parameters, such as aspartate aminotransferase and alanine aminotransferase, were not affected. Thyroid follicular cell hypertrophy, observed in 9/10 males and 10/10 females of the highest-dose group, was characterized by a predominance of small follicles with decreased amount of luminal colloid and increased height of the thyroid epithelium. This hypertrophy was correlated with increased thyroid weights (absolute and relative to body weight and brain weight), decreased plasma levels of $T_4$ and increased plasma levels of TSH in animals in the highest-dose group. The study authors postulated that the thyroid hypertrophy may be secondary to the induction of thyroid metabolizing enzymes, such as uridine diphosphate-glucuronosyltransferase (UGT), occurring with hepatocellular hypertrophy in the liver.

The no-observed-adverse-effect level (NOAEL) in this study was 1.0 mg/kg bw per day, based on effects on the liver and thyroid at 10 mg/kg bw per day (Rodríguez Gómez, 2012).

In a 1-year oral toxicity study in rats, groups of 30 male and 30 female Wistar rats received sisapronil by daily gavage at a dose of 0 (vehicle), 0.1, 0.3, 1.0 or 10 mg/kg bw per day for 52 weeks. Satellite groups were added to investigate the plasma profile of sisapronil (see section 2.1.1). Animals were checked daily for mortality and morbidity, whereas clinical signs, body weight and feed consumption were recorded weekly. Haematological, clinical biochemistry and urine analysis examinations were carried out in 10 male and 10 female animals per group at 180 days and in the surviving animals at termination. Following gross pathology on all animals, tissues from animals in the control and highest-dose groups plus liver, thyroid and parathyroid glands, and macroscopic lesions from the remaining groups were evaluated by histopathological examination.

There was no mortality (except from deaths related to gavage trauma), and no clinical signs were noted. Body weights were unaffected, and feed consumption was increased in females in the highest-dose group only. Haematology and urine analysis revealed no treatment-related effects. Blood cholesterol levels appeared higher in 10 mg/kg bw per day animals, but this reached statistical significance in females only. Males and females of the 1.0 and 10 mg/kg bw per day dose groups had increased absolute and relative liver weights. These livers showed centrilobular hepatocellular hypertrophy and vacuolation. Increased relative thyroid weights were observed at 1.0 mg/kg bw per day in males only, and increased absolute and relative thyroid weights were seen at 10 mg/kg bw per day in both sexes. This was accompanied by a decrease in plasma levels of triiodothyronine ($T_3$) and $T_4$. Upon histopathology, thyroid follicular cell hypertrophy was noted in

all male dose groups, including controls, but the incidence was higher in males and females of the 1.0 and 10 mg/kg bw per day groups than in controls. Thyroid follicular cell hyperplasia was observed at 0.1, 0.3, 1.0 and 10 mg/kg bw per day in males, but only at 1.0 and 10 mg/kg bw per day in females. Thyroid follicular cell adenomas were observed at 0.1, 0.3, 1.0 and 10 mg/kg bw per day in both males and females, as well as in one male in the vehicle control group; the incidence was notably higher for the 10 mg/kg bw per day males. However, any observations at 0.1 and 0.3 mg/kg bw per day were small and not statistically significant. Although lung tissue was affected, this could be attributed to gastric reflux and aspiration following gavage.

The NOAEL in this study was 0.3 mg/kg bw per day, based on changes in the liver and thyroid at 1.0 mg/kg bw per day (Rodríguez Gómez, 2013).

The data from the 1-year rat study were used for benchmark dose (BMD) modelling. The analysis was performed using the Benchmark Dose Software (BMDS) from the United States Environmental Protection Agency (USEPA). A number of variables were used for the BMD analysis: TSH, total $T_4$, free $T_3$, relative (to body and brain weights) liver and thyroid weights as well as the occurrence of bilateral thyroid adenoma and hypertrophy. A benchmark response (BMR) of 10% extra risk above the background was selected as the basis for estimating BMDs. For the variables measured on a continuous scale (TSH, $T_3$, $T_4$, organ weights), all the available continuous variable models in BMDS were fit: exponential (models 2–5), Hill, linear, power and polynomial (limited to a quadratic) models. Similarly, for the variables measured on a dichotomous scale, all dichotomous models were fit: gamma, logistic, log-logistic, multistage, probit, log-probit, Weibull and quantal linear. Consistent with the guidance provided by the European Food Safety Authority (EFSA), the best-fitting models were chosen based on the nesting structure, smallest Akaike information criterion (AIC), small-scaled residuals, especially near the BMD and the control dose, parameter feasibility and visual check of the model fit. The outcome of this analysis is presented in Table 2.

Among the variables analysed, the BMDLs ranged from 0.26 to 0.79 mg/kg bw per day, which are supportive of the 0.3 mg/kg bw per day NOAEL identified in this study (Boucher, 2013).

### (b) Dogs

Groups of four male and four female Beagle dogs were given sisapronil in capsules at a daily dose of 0, 1, 5 or 25 mg/kg bw per day for 28 days. Observations included morbidity, mortality, injury, feed and water intake, clinical signs, body weight, ophthalmology, physical/neurological examinations and functional observational

Table 2
**Summary of BMD/BMDL results**

| Variable | Sex | BMR | BMD | BMDL |
|---|---|---|---|---|
| TSH | M/F | 10% | 0.921 | 0.631 |
| $T_3$ | M/F | 10% | 2.39 | 0.786 |
| $T_4$ | M/F | 10% | 1.65 | 0.547 |
| Liver/body weight | M/F | 5% | 0.626 | 0.447 |
| Thyroid/body weight | M/F | 10% | 0.919 | 0.454 |
| Liver/brain weight | M/F | 10% | 1.29 | 0.448 |
| Thyroid/brain weight | M/F | 10% | 0.486 | 0.298 |
| Thyroid hypertrophy | M/F | 10% | 0.440 | 0.257 |
| Thyroid adenoma | M | 10% | 2.10 | 0.305 |

BMD: benchmark dose; BMDL: lower 95% confidence limit on the benchmark dose; BMR: benchmark response; F: females; M: males; $T_3$: triiodothyronine; $T_4$: thyroxine; TSH: thyroid stimulating hormone
*Source*: Boucher (2013)

batteries (in weeks 2 and 4), and clinical pathology. At the end of the study, the animals were necropsied, organ weights were recorded and tissues were collected for microscopic examination (controls and high dose only). Additionally, the thyroid, thymus (female only) and liver of animals in all groups were examined histopathologically.

No mortality occurred, and no treatment-related clinical signs were noted. Ophthalmology, functional observational battery and neurological examinations revealed no effects. A trend to decreased body weight was apparent in high-dose males and in mid- and high-dose females, but those changes were slight and did not attain statistical significance. A slight and non-significant decrease in feed consumption was noted in the same groups. The tested parameters of haematology, clinical chemistry and urine analysis were unaffected. Relative liver weights were increased in high-dose males and females, accompanied by increased glycogen in hepatocytes. Absolute and relative thymus weights were decreased in females of the 5 and 25 mg/kg bw per day groups. These decreases were statistically significant in females of the 5 mg/kg bw per day group only. Microscopically, the decreased thymus weights correlated with an increased incidence of minimal to mild decreased cellularity of the lymphoid tissue.

The NOAEL in this study was 1 mg/kg bw per day, based on changes in the thymus at 5 mg/kg bw per day (Heward, 2011).

In a 90-day repeated-dose oral toxicity study, groups of four male and four female Beagle dogs were given sisapronil in capsules at a dose of 0 (vehicle), 0.3, 1 or 10 mg/kg bw per day. Observations included mortality, feed and water consumption, body weight, haematology, clinical chemistry, urine analysis,

ophthalmoscopy examinations, physical/neurological examinations, functional observational batteries, clinical signs, organ weights, gross pathology and histopathology (animals at 0 and 10 mg/kg bw per day and selected tissues for animals at 0.3 and 1 mg/kg bw per day).

In all treated male dogs, a dose-dependent increase in soft faeces was noted. There were no mortalities, and feed intake and body weight were not affected. Ophthalmology, physical/neurological tests and functional observational batteries revealed no effects. An increase in absolute and relative liver weights in high-dose males and females was noted. Two males and one female of this group showed vacuolar degeneration of individual hepatocytes in the centrilobular to midzonal regions. Males and females of the 1 and 10 mg/kg bw per day groups had an increased incidence of glycogen in hepatocytes. Minimal thyroid follicular cell hypertrophy was observed in 2/4 males of the mid-dose group and 4/4 males and 1/4 females of the high-dose group. One of four high-dose females had minimal thyroid follicular cell hypertrophy. Thyroid follicular cell hypertrophy was characterized by cuboidal to tall cuboidal follicular cells, frequently associated with increased vacuolation in the apical cytoplasm. This hypertrophy occurred without increased thyroid weight or changes in thyroid hormone levels in blood.

A NOAEL of 0.3 mg/kg bw per day was identified, based on minimal thyroid follicular cell hypertrophy and increased glycogen in hepatocytes at 1 mg/kg bw per day (Heward, 2012).

### 2.2.3 Long-term studies of toxicity and carcinogenicity

No 2-year toxicity studies or carcinogenicity studies were provided. The study with the longest duration was the 1-year repeated-dose oral toxicity study in rats (see section 2.2.2).

### 2.2.4 Genotoxicity

For the evaluation of the genotoxic properties of sisapronil, an in vitro bacterial reverse mutation assay, an in vitro test for chromosomal aberrations in mammalian cells and an in vivo micronucleus assay in mice (bone marrow) were available (Table 3). In addition to those experimental studies, an in silico analysis by Frank (2013) was submitted, using the DEREK software. No relevant structural alerts were identified.

### 2.2.5 Reproductive and developmental toxicity

#### (a) Multigeneration reproductive toxicity

A two-generation reproductive toxicity study was performed in rats in accordance with OECD Test Guideline 416. Three groups of Wistar Han rats (25 of each sex per dose) received sisapronil by daily oral gavage starting at least 70

Table 3
**Genotoxicity studies on sisapronil**

| Test system | Test object | Concentration/dose | Results | References |
|---|---|---|---|---|
| In vitro | | | | |
| Ames reverse mutation assay[a,b] | *Salmonella typhimurium* TA98, TA100, TA1535, TA1537; and *Escherichia coli* WP2 uvrA pKM101 | 15–5 000[c] µg/plate | Negative; not mutagenic | Cheung (2011) |
| Chromosomal aberration assay[a,d] | Human peripheral blood lymphocyte primary cell cultures | 9.73–1 000 µg/mL | Negative; not clastogenic | Gunther (2011) |
| In vivo | | | | |
| Micronucleus induction[e] | Mouse bone marrow | 62.5, 125 and 250 mg/kg bw[f] | Negative; not clastogenic | Engel (2011) |

bw: body weight; S9: 9000 × *g* supernatant fraction from rat liver homogenate
[a] With and without Aroclor 1254–induced rat liver S9 fraction.
[b] Positive controls were sodium nitrite, 9-aminoacridine, 2-nitrofluorene, nitrofurantoin and *N*-ethyl-*N*′-nitro-nitrosoguanidine in the absence of metabolic activation; and 2-anthramine in the presence of metabolic activation.
[c] Precipitation at 50 µg/plate and higher.
[d] Mitomycin C was used as a positive control without metabolic activation, and cyclophosphamide was used as a positive control in the presence of metabolic activation.
[e] Cyclophosphamide was used as a positive control.
[f] The maximum tolerated dose was 250 mg/kg bw. There was a dose-related decrease in the percentage of polychromatic erythrocytes.

days before mating (or starting at weaning for the $F_1$ animals). The doses tested were 0 (vehicle), 0.3, 2.0 and 15 mg/kg bw per day for the $F_0$ generation and 0.3 and 2.0 mg/kg bw per day for the $F_1$ generation. Because of the test substance–related effects on $F_1$ postnatal survival, an insufficient number of $F_1$ animals was available in the 15 mg/kg bw per day group to assign to the $F_1$ generation. Therefore, all pups in this group were euthanized on postnatal day 21, resulting in no $F_1$ generation at this dose level. The $F_0$ and $F_1$ females continued to receive the test substance throughout mating, gestation and lactation and through the day prior to euthanasia. $F_1$ and $F_2$ litters were culled to four pups of each sex, when possible, after weaning at postnatal day 21, and the non-selected pups were necropsied. Observations included clinical signs, body weight, feed consumption, reproductive parameters, functional observational battery, organ weights, gross pathology and histopathology.

There were no test substance–related deaths in the $F_0$ or $F_1$ adult generation. Sisapronil had no effect on body weight in the $F_0$ generation. Although the reproductive performance of the $F_0$ generation was not affected at any dose level, low fertility in males and females, decreased male copulation, decreased female conception indices and decreased ovarian follicle counts were noted in the $F_1$ generation at 2.0 mg/kg bw per day. Enlarged thyroid glands were observed in $F_0$ males at 2.0 mg/kg bw per day and in $F_0$ males and females at 15 mg/kg bw per day. This finding corresponded to higher mean thyroid gland weights and follicular cell hypertrophy and hyperplasia in the males and females of these groups. Thyroid follicular cell hypertrophy and hyperplasia were also noted at

0.3 mg/kg bw per day, although the finding was not statistically significant. In addition, nodular hyperplasia and follicular cell adenoma were observed in males in the 15 mg/kg bw per day group. Higher absolute and relative liver weights were observed in the 2.0 and 15 mg/kg bw per day groups. At 15 mg/kg bw per day, this was associated with hepatocellular hypertrophy, vacuolation, single-cell necrosis and, in males only, multinucleated hepatocytes. Whereas the $F_1$ postnatal survival was severely affected in the high-dose group, the survival, pup body weight and body weight gain were unaffected at 0.3 and 2.0 mg/kg bw per day.

The NOAEL for reproductive toxicity was 0.3 mg/kg bw per day, the NOAEL for offspring toxicity was 2.0 mg/kg bw per day and the NOAEL for parental toxicity was 0.3 mg/kg bw per day (Edwards, 2013a).

### (b) Developmental toxicity

#### (i) Rats

Sisapronil was given to groups of 25 pregnant Crl:WI (Han) rats by daily gavage on gestation days 6 through 20. The doses were 0 (vehicle), 0.3, 2.0 and 20 mg/kg bw per day. On gestation day 21, a laparohysterectomy was performed on each female. The uteri, placentae and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed and examined for external, visceral and skeletal malformations and developmental variations.

The only treatment-related effect noted was a lower mean body weight gain with corresponding reduced feed consumption at 20 mg/kg bw per day during the first week of treatment. The pups of these dams also had a lower body weight (up to 5.7%).

Based on these effects, the NOAEL for both maternal and embryo/fetal toxicity was 2.0 mg/kg bw per day (Edwards, 2012).

#### (ii) Rabbits

Groups of 25 pregnant New Zealand White (Hra:(NZW)SPF) rabbits were given sisapronil by daily gavage on gestation days 7 through 28 at a dose of 0 (vehicle), 0.3, 2.0 or 12.5 mg/kg bw per day. On gestation day 29, a laparohysterectomy was performed on each surviving female. The uteri, placentae and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed and examined for external, visceral and skeletal malformations and developmental variations.

The dams of the high-dose group showed severe body weight loss and reduced feed intake, resulting in moribundity, abortion and premature delivery in several dams. The fetuses at this dose had lower mean body weights (up to 12.2%). No substance-related effects were noted at the low and middle dose levels.

The NOAEL for both maternal and embryo/fetal toxicity was 2.0 mg/kg bw per day (Edwards, 2013b).

### 2.2.6 Special studies

#### (a) Acute neurotoxicity

The neurofunctional effects of sisapronil were evaluated following the administration of a single oral dose of 0, 100, 500 or 1000 mg/kg bw to groups of 20 male rats. Ten animals from each group underwent the functional observational battery, and the remaining 10 animals were tested for effects on motor activity.

Six high-dose animals were found dead on study day 3. Animals of the 500 and 1000 mg/kg bw groups ate less, lost weight and showed hindlimb retraction. Additionally, at 1000 mg/kg bw, the animals had tremors and showed signs of reduced grooming. Functional observational battery parameters were also affected at 500 and 1000 mg/kg bw, in particular reduced gait, tremors, convulsions (high dose), hyperactivity, vocalization, laboured breathing, reduced foot splay, reduced grip strength and altered reflex responses. The motor activity parameters were significantly affected in both 500 and 1000 mg/kg bw animals, in a dose-dependent manner. The animals of the 100 mg/kg bw group showed no effects on the functional observational battery or motor activity tests.

A NOAEL of 100 mg/kg bw was identified for single-dose neurotoxicity (Ryan, 2011).

#### (b) Mode of action for effects on the thyroid in rats

In a study specifically designed to reveal the mode of action for liver and thyroid toxicity, groups of 30 male Han Wistar/Crl:W1(Han) rats were given sisapronil daily by oral gavage at a dose of 0, 1, 7.5 or 15 mg/kg bw per day for 30 days. From each group, 10 animals were euthanized on day 8, 10 animals on day 15, and the remaining 10 on day 31. The observations included clinical signs, body weights, body weight changes, feed consumption, thyroid hormones (free and total $T_3$, free and total $T_4$, and TSH), UGT1A-mediated $T_4$ glucuronide formation, gross necropsy findings, organ weights and histopathological examinations.

Sisapronil produced no clinical signs of systemic toxicity and had no influence on body weight or feed consumption. Statistically significantly lower levels of free and total $T_4$ in plasma were recorded on day 15 at 15 mg/kg bw per day and on day 31 at 7.5 and 15 mg/kg bw per day. In addition, statistically significantly higher levels of TSH in plasma were recorded at 7.5 mg/kg bw per

day (day 31) and at 15 mg/kg bw per day (days 15 and 31). Liver weights were increased in high-dose animals at all euthanasia time points and in mid-dose animals only on day 31. The thyroid weights were increased in mid- and high-dose animals, but only at the end of the study. Induction of UGT1A-mediated $T_4$ glucuronide formation was observed in mid-dose animals on days 15 and 31 and in high-dose animals at all time points. Time- and dose-related follicular cell hypertrophy was noted in treated animals. In addition, centrilobular and diffuse hepatocyte hypertrophy was observed on day 31 in treated animals. Mitotic activity of the thyroid increased with both time and dose level (Robertson, 2012).

#### (c) Microbiological effects

Considering the chemical structure and the mode of action of sisapronil, the Committee did not anticipate any adverse effects of sisapronil residues on the human gastrointestinal microbiota.

### 2.3 Observations in humans

No data in humans are available. Sisapronil is not used in human medicine.

## 3. Comments

### 3.1 Biochemical data

The pharmacokinetics of sisapronil was studied in rats, dogs and cynomolgus monkeys. Most of the investigations were done as part of the toxicological studies.

Twenty-four hours after administration of $^{14}$C-labelled sisapronil by oral gavage to rats at a dose of 50 mg/kg bw, the radioactivity in faeces was approximately 98% of the total recovery from excreta, and the remainder was found in urine, suggesting limited absorption (Lineham, 2012).

An oral bioavailability of 6.8% was determined in cynomolgus monkeys by comparing the pharmacokinetics data in plasma following a single oral administration with those following a single intravenous administration (Stuhler, Peterson & Starch, 2012).

There was little information on the distribution and metabolism of sisapronil in laboratory animals. In a radiolabel study in rats, the parent sisapronil accounted for more than 91% of the radioactivity in faeces at 24 hours after oral administration. In addition, sisapronil was identified as the primary residue in the liver. A metabolite was detected in the liver of rats, but could not be identified. It had the same retention time as the main metabolite found in cattle liver, which could not be identified because of the low quantities present (Lineham, 2012).

These data suggest that sisapronil undergoes minimal metabolism and that the metabolite found in cattle is also present in the rat and therefore is inherently tested in the toxicity studies on rats.

In rats, the elimination half-life of sisapronil in plasma was approximately 14 days. This half-life was determined in a 28-day repeated-dose toxicity study in which blood samples were taken at 4, 8 and 24 hours post-dosing on several study days, including day 1 (Hu, 2009). The Committee considered that this estimated half-life in rats should be viewed with great caution, because it was based on only three time points and involved a large time extrapolation. The sponsor cited a half-life of 20–39 days, derived from an exploratory oral study in rats with a sampling period of 14 days; however, that study was not provided. The Committee noted that a time to steady state of approximately 100–150 days could be estimated from the time–concentration profile in plasma in rats from the 1-year repeated-dose toxicity study (Rodríguez Gómez, 2013). In the 1-year rat study, the mean concentrations of sisapronil in plasma at the last time point were approximately 325, 567, 1172 and 3424 ng/mL for the 0.1, 0.3, 1.0 and 10 mg/kg bw per day dose groups, respectively. The increase in sisapronil concentration in plasma was dose dependent, but less than dose proportional. Across the repeated-dose toxicity studies in rats, substantial differences in sisapronil concentrations in plasma were noted, but the cause of this could not be determined.

In a non-GLP-compliant study, dogs were given a single intravenous sisapronil dose of 0.5, 1.5 or 5 mg/kg bw. Blood samples were taken over a period of 268 days. The plasma elimination half-life for all three doses was approximately 100 days (Fisher, 2008).

The concentration of sisapronil in plasma was studied in cynomolgus monkeys over a period of 70 days after a single intravenous administration of 0.5 mg/kg bw. From this study, a plasma elimination half-life of 12.4 days was calculated (Stuhler, Peterson & Starch, 2012).

## 3.2 Toxicological data

Critical toxicity studies are summarized in Table 4.

Single-dose oral toxicity studies with sisapronil were conducted in mice and rats. The oral $LD_{50}$ in rats was 552 mg/kg bw (Beerens-Heijnen, 2011a). In mice, no mortality was observed at 30 mg/kg bw, the highest dose tested (Mill, 2003).

Studies in rabbits showed that sisapronil was not irritating to the skin (Beerens-Heijnen, 2011b) or the eyes (Beerens-Heijnen, 2011c). Sisapronil was not a skin sensitizer in the murine local lymph node assay (Beerens-Heijnen, 2011d).

Repeated-dose oral toxicity studies were conducted with sisapronil in rats (gavage) and dogs (capsules). The main studies in rats included a 90-day

Table 4
**Studies relevant to risk assessment**

| Species / study type (route of administration) | Doses (mg/kg bw per day) | Critical end-point | NOAEL (mg/kg bw per day) | LOAEL (mg/kg bw per day) |
|---|---|---|---|---|
| Rat | | | | |
| Three-month study of toxicity (gavage) | 0, 0.1, 0.3, 1.0, 10 | Hepatocellular hypertrophy; thyroid follicular cell hypertrophy | 1.0 | 10 |
| One-year study of toxicity (gavage) | 0, 0.1, 0.3, 1.0, 10 | Centrilobular hepatocellular hypertrophy; thyroid follicular cell hypertrophy, hyperplasia and adenoma | 0.3 | 1.0 |
| Two-generation reproductive toxicity study (gavage) | 0, 0.3, 2.0, 15 | Parental toxicity: Follicular cell hypertrophy and hyperplasia; increased liver weights | 0.3 | 2.0 |
| | | Reproductive toxicity: Low fertility in males and females, decreased male copulation, decreased female conception indices and decreased ovarian follicle counts in $F_1$ generation | 0.3 | 2.0 |
| | | Offspring toxicity: Decreased survival | 2.0 | 15 |
| Developmental toxicity study (gavage) | 0, 0.3, 2.0, 20 | Embryo/fetal toxicity: Lower pup weight due to lower body weight gain and lower feed consumption in dams | 2.0 | 20 |
| Rabbit | | | | |
| Developmental toxicity study (gavage) | 0, 0.3, 2.0, 12.5 | Maternal toxicity: Severe body weight loss, reduced feed intake, moribundity, abortion and premature delivery | 2.0 | 12.5 |
| | | Embryo/fetal toxicity: Reduced pup weight | 2.0 | 12.5 |
| Dog | | | | |
| Three-month study of toxicity[a] (capsule) | 0, 0.3, 1, 10 | Increased glycogen in hepatocytes; thyroid follicular cell hypertrophy | 0.3 | 1 |

[a] A plasma half-life of approximately 100 days was found in a non-GLP-compliant study in dogs.

study and a 1-year study. Studies in dogs included a range-finding 28-day study and a 90-day study.

The main target organs for toxicity in rats and dogs were the liver and thyroid.

In rats given an oral gavage sisapronil dose of 0, 0.1, 0.3, 1.0 or 10 mg/kg bw per day for 90 days, increased levels of TSH and decreased levels of $T_4$ in plasma were observed at the highest dose. Animals of this group also showed increased absolute and relative liver and thyroid weights, hepatocellular hypertrophy and thyroid follicular cell hypertrophy. The NOAEL in this study was 1.0 mg/kg bw per day, based on effects on the liver and thyroid at 10 mg/kg bw per day (Rodríguez Gómez, 2012).

In rats given an oral gavage sisapronil dose of 0, 0.1, 0.3, 1.0 or 10 mg/kg bw per day for 1 year, decreases in levels of $T_3$ and $T_4$ in plasma were observed at

the highest dose. Increased absolute and relative liver weights were observed in both sexes at 1.0 and 10 mg/kg bw per day. Increased relative thyroid weights were observed at 1.0 mg/kg bw per day in males only, and absolute and relative thyroid weights were seen at 10 mg/kg bw per day in both sexes. The livers of the animals at 1.0 and 10 mg/kg bw per day showed centrilobular hepatocellular hypertrophy and vacuolation. Thyroid follicular cell hypertrophy, hyperplasia and adenomas were statistically significantly increased at doses of 1.0 and 10 mg/kg bw per day. The NOAEL in this study was 0.3 mg/kg bw per day, based on changes in the liver and thyroid at 1.0 mg/kg bw per day (Rodríguez Gómez, 2013).

In a range-finding 28-day oral toxicity study, dogs were given sisapronil in capsules at a dose of 0, 1, 5 or 25 mg/kg bw per day. Relative liver weights were increased in high-dose males and females, accompanied by increased glycogen in hepatocytes. Absolute and relative thymus weights were decreased in females of the 5 and 25 mg/kg bw per day groups. These decreases were statistically significant in females of the 5 mg/kg bw per day group only. Microscopically, the decreased thymus weights correlated with an increased incidence of minimal to mild decreased cellularity of the lymphoid tissue. The NOAEL in this study was 1 mg/kg bw per day, based on changes in the thymus at 5 mg/kg bw per day (Heward, 2011).

In a 90-day repeated-dose oral toxicity study, dogs were given sisapronil in capsules at a dose of 0, 0.3, 1 or 10 mg/kg bw per day. Dogs had increased absolute and relative liver weights and vacuolar degeneration of individual hepatocytes at the highest dose. At 1 and 10 mg/kg bw per day, an increase in glycogen in hepatocytes was observed. Minimal thyroid follicular cell hypertrophy was observed at 1 and 10 mg/kg bw per day. Thyroid follicular cell hypertrophy was characterized by cuboidal to tall cuboidal follicular cells, frequently associated with increased vacuolation in the apical cytoplasm. This hypertrophy occurred without increased thyroid weight or changes in thyroid hormone levels in blood. A NOAEL of 0.3 mg/kg bw per day was identified, based on minimal thyroid follicular cell hypertrophy and increased glycogen in hepatocytes at 1 mg/kg bw per day (Heward, 2012).

The genotoxicity of sisapronil was investigated in an adequate array of in vitro and in vivo tests (Cheung, 2011; Engel, 2011; Gunther, 2011). No evidence of genotoxicity was found. In silico analysis revealed no structural alerts for genotoxicity (Frank, 2013). The Committee concluded that sisapronil is not genotoxic.

No specific 2-year toxicity studies or carcinogenicity studies were provided. The study with the longest duration was the 1-year repeated-dose oral toxicity study in rats.

The only test article–related proliferative lesions in the 1-year oral toxicity study in rats described above were thyroid hyperplasia and thyroid tumours

(adenomas). The NOAEL for these thyroid effects was 0.3 mg/kg bw per day. A study was conducted to determine a possible mode of action for thyroid hormone changes and to evaluate the microscopic liver and thyroid changes observed in rats (Robertson, 2012). This study supported a mode of action for sisapronil-induced thyroid tumour formation involving the disruption of homeostasis of the hypothalamic–pituitary–thyroid (HPT) axis by an extrathyroidal mechanism. Specifically, sisapronil induces UGT, which increases the conjugation of thyroid hormones ($T_3$ and $T_4$) in the liver. In response to the hypothyroid state, TSH synthesis and release are stimulated, and this is the key event that leads to thyroid follicular cell growth and hyperplasia. In response to the hypothyroid state and increased TSH levels, the thyroid gland is stimulated to produce more $T_4$, and, over time, there is compensatory thyroid gland enlargement. For sisapronil, the available data provide compelling evidence that increased hepatic clearance of $T_3$ and $T_4$ causes chronic stimulation of the HPT axis, seen acutely as an increase in TSH secretion, with long-term stimulation of the thyroid gland as a chronic consequence leading to follicular cell adenoma development.

The data from the 1-year rat study were used for BMD modelling to assist in the analysis of the proposed mode of action. A number of variables were used for the BMD analysis: TSH, total $T_4$, free $T_3$, relative (to body and brain weights) liver and thyroid weights as well as the occurrence of bilateral thyroid adenoma and hypertrophy. A BMR of 10% extra risk above the background was selected as the basis for estimating BMDs. Among the variables analysed, the BMDLs ranged from 0.26 to 0.79 mg/kg bw per day, which are supportive of the 0.3 mg/kg bw per day NOAEL identified in this study.

Although the Committee recognized that a specific carcinogenicity study had not been conducted, it concluded that sisapronil is not genotoxic and that the thyroid tumours in rats are a result of an indirect perturbation of the HPT axis by a mode of action not considered relevant to humans. Therefore, the Committee concluded that sisapronil does not pose a risk of carcinogenicity to humans at doses relevant to residues of veterinary drugs.

A two-generation reproductive toxicity study was conducted in rats given sisapronil by oral gavage at a dose of 0, 0.3, 2.0 or 15 mg/kg bw per day. The 15 mg/kg bw per day dose group was discontinued for the $F_1$ generation because of the high pup mortality in that group. Systemic toxicity was evidenced by significant effects on the liver (increased absolute and relative liver weights) and thyroid (follicular cell hypertrophy and hyperplasia) at 2.0 mg/kg bw per day and higher. Although the reproductive performance of the $F_0$ generation was not affected at any dose level, low fertility in males and females, decreased male copulation, decreased female conception indices and decreased ovarian follicle counts were noted in the $F_1$ generation at 2.0 mg/kg bw per day. The NOAEL for reproductive toxicity was 0.3 mg/kg bw per day, the NOAEL for offspring toxicity

was 2.0 mg/kg bw per day and the NOAEL for parental toxicity was 0.3 mg/kg bw per day (Edwards, 2013a).

Developmental toxicity studies were conducted in rats and rabbits. The rat study used oral gavage doses of 0, 0.3, 2.0 and 20 mg/kg bw per day. The only effects seen in this study were reduced body weight gain and feed consumption in dams at the high dose, which resulted in lower pup weights in that group. The NOAEL for both maternal toxicity and embryo/fetal toxicity was 2.0 mg/kg bw per day (Edwards, 2012).

The developmental toxicity study in rabbits used oral gavage doses of 0, 0.3, 2.0 and 12.5 mg/kg bw per day. The dams of the high-dose group showed reduced feed intake and severe body weight loss, resulting in moribundity, abortion and premature delivery in several dams. The fetuses of this dose group had lower mean body weights. The NOAEL for both maternal and embryo/fetal toxicity was 2.0 mg/kg bw per day (Edwards, 2013b).

The Committee concluded that sisapronil is not teratogenic.

The acute neurotoxicity of sisapronil was investigated in rats given an oral gavage dose of 0, 100, 500 or 1000 mg/kg bw. There was mortality at the highest dose, and animals of the 500 and 1000 mg/kg bw per day groups showed reduced gait, tremors, convulsions (high dose), hyperactivity, vocalization, laboured breathing, reduced foot splay, reduced grip strength and altered reflex responses. Motor activity parameters were also affected in these groups. The NOAEL in this study was 100 mg/kg bw per day (Ryan, 2011).

### 3.3 Microbiological data

Considering the chemical structure and the mode of action of sisapronil, the Committee did not anticipate any adverse effects of sisapronil residues on the human gastrointestinal microbiota.

## 4. Evaluation

The main findings in the livers of rats and dogs treated with sisapronil included increased liver weights, centrilobular hepatocellular hypertrophy, vacuolation and increased glycogen in hepatocytes. Hepatocellular hypertrophy and increased liver weights in rats are considered to be related to hepatic drug metabolizing enzyme induction, an adaptive response. It is well known that rats are particularly sensitive to developing thyroid tumours according to a mode of action, described above, that is considered not to be relevant to humans.

The Committee noted that the lowest relevant NOAEL was 0.3 mg/kg bw per day in both the 90-day oral toxicity study in dogs and the two-generation reproductive toxicity study in rats. However, the Committee considered this 90-

day study in dogs to be unsuitable to address long-term toxicity, in view of the very long elimination half-life of sisapronil in dogs (i.e. 100 days). Longer-term oral toxicity studies with sisapronil in dogs were not available. It remains uncertain how the toxic effects in dogs might progress and whether or not other effects might be triggered upon longer-term exposure. The Committee also concluded that the NOAEL from the reproductive toxicity study in rats may not be sufficient to protect against long-term effects of sisapronil.

The Committee concluded that a toxicological ADI could not be established because the Committee had no basis upon which to determine a suitable uncertainty factor to accommodate the lack of a long-term toxicity study.

### 4.1 Additional information that would assist in the further evaluation of the compound

- Data to address long-term toxicity relevant to humans (e.g. 1-year dog study)
- Comparative pharmacokinetics studies and an explanation of interspecies differences in the pharmacokinetic profiles.

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