Anxitane

Product Profile

Zen in tablets

The natural response to stress and anxiety

Virbac

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Foreword

Phobia, anxiety, deprivation syndrome... these terms are now current veterinary vocabulary. Dog behavioural disorders are indeed frequent. When they are not the very reason for consulting, they are at least mentioned to the practitioner. They are estimated to be responsible for half of euthanased or abandoned adult dogs.

For a long time, owners left vets "on the sideline" preferring to see educators and other trainers. Vets have now invested this new field.
This is indeed logical, as these dogs undergo a true psychological suffering, and emotional disturbances can only be a doctor's field. They must provide global caring and bring a solution, especially given that owners confronted to this pathology are often pressing, because unhinged and suffering as well.

Therefore, if owners consult for the resulting nuisances (vocalising, dirtiness, destroying, aggressiveness etc.), the veterinary must attend globally to the responsible phobic or anxious states. This is an absolute requisite for solving symptoms and restoring the wellbeing of the animal (and the family).

In other circumstances, the animal is brought in consultation to treat organic disorders and the veterinary, after careful semiology, will detect – and treat – the causes, which will prove to be behavioural: licking granuloma or irritable bowel syndrome are some classics. Once again, global caring for the animal not only improves symptoms, but also wellbeing.

Many etiologies may cause emotional disturbances. Among others are early developmental anomalies, "family-pack" communication disorders, disturbances related to the dog's unstable hierarchical positioning, foster home changes, and some organic causes (endocrinal, metabolic, algetic, etc.).

Somatic disorders (called psychosomatic in human medicine), or psychic disorders require, as any pathology, a semeiological approach. This is to set a diagnosis, basic requisite for appropriate treating. Usually, the practitioner combines two complementary therapies: behavioural and medical.
The former requires patience and contribution from the owner and is usually indispensable. Depending on the case, it will involve counter-conditioning, communication clarification, "directed social regression" (DSR) techniques, etc.
Medical therapy helps this process by reducing fear – a permanent feature in these emotional disturbances – and/or diminishing the excessive vigilance of the anxious or phobic animal.

Recently, veterinary medical therapy for emotional disturbances gained an original molecule – L-theanine – a synthetic nutraceutical resulting from the Virbac laboratories' clinical research.
This molecule, with a proven gabaergic effect on man and laboratory animals, revealed, through clinical trials made on dogs, a true anxiolytic effect. It was especially efficient on dogs suffering sensory deprivation syndromes or social phobias and, in general, those with an inhibition.

It is extremely palatable, has no side effect (unlike psychotropic drugs), and is totally harmless, making it a first intention treatment for dog anxious emotional disturbances.

The veterinary's armamentarium now includes an original, palatable, and efficient molecule devoid of side effects, which will be very useful for treating dog anxiety...

Doctor Laurent Kern
Veterinary Behaviourist
Clinical Referral Practice, Paris

ANXITANE™ KEY POINTS

- Theanine: a neurologically active amino acid, naturally found in green tea
- Anxitane™: a unique, patented, chirally pure supplement based on the active l-isomer form of theanine
- Supported by extensive research and documentation
- Tested in numerous human and animal studies
- Helps to alleviate anxiety-related behaviors in pets
- Supports calm and relaxation in fearful, tense dogs and cats
- Helps pets to cope with changes in their environment
- Does not induce drowsiness
- Extremely safe, non-addictive
- Uniquely palatable

In calmness there should be activity, in activity there should be calmness.
Daisetsu Suzuki

From green tea to theanine

The secret hidden in green tea

Taiyo

Life Extension Magazine

Taiyo

- Green tea is certainly one of the oldest and most appreciated drinks of mankind.
- Its tradition dates back to around 2700 B.C when the Chinese discovered the infusion of leaves from the tea plant and used this as a medicine[1].
- In the early 8th century buddist monks imported the plant to Japan. Very rapidly the "chado" (the way of the tea) became an art associated with a very simple, but extremely codified ceremony, in which boiling water is poured onto the "matcha" (green tea powder). Chado is perceived as a way of life that enables an appeased and peaceful mind to discover the true beauty of things[2].
- Dutch missionaries discovered the beverage in the 16th century and introduced it to Europe from Japan.

- The tea plant (*Camelia sinensis*) is a large shrub with evergreen leaves that grows in tropical and temperate regions in Asia. The 4 varieties of tea (green, oolong, white and black) all originate from the same plant and are distinguished according the degree of fermentation of the leaves during processing. Green tea is the most virgin type : during its preparation the leaves are quickly dried by steaming after being rolled, hindering fermentation and thus preserving enzymes and the original olive-green color.
- The contribution of unfermented green tea to good health maintenance and its valued taste in Asia are related to caracteristic chemical constituents of the tea leaf[3].

Chemical components of green tea
Polyphenols : especially catechins that have antioxidant properties and are responsible for the slight astringent and bitter taste of green tea infusion
Xanthins : among which caffeine a cerebral and cardiac stimulant, diuretic
Nitrogenous compounds : especially free amino acids (theanine, aspartic acid, arginine..) and to a lesser extent amides, tannin-binded proteins and nucleic acids
Vitamins : A, B1, C, E, K, P and **inorganic elements** : Al, F, Mn, Fe, K
Carbohydrates : cellulosis and starch **Lipids** : fatty acids

- Even though tea contains significant amounts of caffeine, Asians have long enjoyed their daily tea drinking ritual for its many benefits, including its calming influence on the mind and body. How this is possible ?

- The answer is found in a special ingredient from the leaves of the tea plant : theanine, a unique amino-acid with amazing relaxing effects. Theanine accounts for about 50% of all free amino-acids in green tea.

Suntheanine™ : a patented pure active form of theanine

- The tea leaf is the single natural resource of theanine reported to present. Large scale extraction of the compound historically has been an expensive process because the neurologically active isomeric form, L-theanine, constitutes only 1 to 2 % of the dry weight of tea.

- In 1990, a Japanese company Taiyo Kagaku developed a patented enzymatic method to obtain isomerically pure L-theanine (free of D-theanine), which it trademarked as Suntheanine™. Standardised mass production became possible and the stress-relieving compound was then introduced into several products for human consumption in Japan. Research developed demonstrating the relaxing effect of Suntheanine™ in humans and animal models, for which the ingredient was awarded two prestigious prizes at the Food Ingredient Europe (1998) and Nutracon (2000) conventions. These awards recognize new, applied ideas that are truly breakthrough in nature and lead the industry toward safe, effective, useful products.

Taiyo green tea powder main workshop

Taiyo researcher

- Suntheanine™ is protected by over 40 international patents for its various physiological activities and L-isomer-specific production processes.

- Recent chemical analyses performed by an independant laboratory at Iowa State University on 6 commercially available products labelled as "L-theanine" found that 5 of them contained significant amount of D-theanine (as much as 50%). The only material that proved to be pure L-theanine (>98%) was the Suntheanine™ brand[4].

- This is all the more important since the stereochemistry of amino-acids have a dramatic influence on their metabolism and biological activity. Hence, another recent study in the journal Chirality demonstrates the higher absorption of orally administered L-theanine in the blood of rats, as compared to a racemate LD mixture supplemented at equal dose. Results indicated that D- and L-theanine exhibit a competitive effect with respect to intestinal absorption[5]. Because virtually all of the animal and human clinical research with theanine was performed with Suntheanine™, it is therefore uncertain whether generic ingredients may yield the same efficacy and safety.

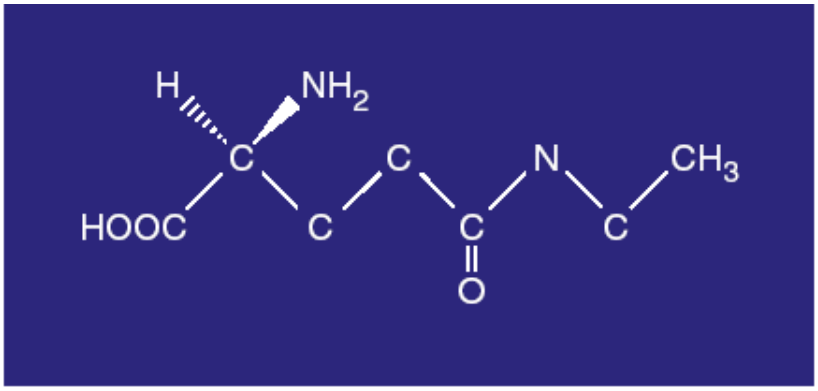
- In 2001, Taiyo obtained a patent on the use of theanine to reduce some behaviour problems in pets[6]. Symptoms like astasia, shivering, dysbasia or kinesia associated with car travel were improved in dogs following theanine administration.

- It was Virbac initiative to build on Taiyo's experience with theanine to bring this innovative ingredient in veterinary medicine. A new "nutraceutical" (health promoting dietary supplement), Anxitane™, was developed to help relieve signs associated with anxiety in pets, small companions that share so closely their owner daily life – and stress.

Theanine : the stress-relieving amino acid

A mimetic molecule with connections in the brain

- L-theanine, also known as γ-ethylamino-L-glutamic acid, is a natural structural analogue of **glutamate**, a brain excitatory neurotransmitter. This similarity enables theanine to physically block glutamate binding to neuronal receptors, or influence glutamate transport. In vitro receptor-binding studies on rat cortical neurons indeed demonstrate that theanine acts as a competitive antagonist on three subtypes of glutamate receptors (AMPA, kainate and NMDA)[7,8,9].



Chemical structure of theanine[10]

- In addition, administration of theanine significantly increases the concentration of **GABA** (gamma amino butyric acid), an important inhibitory neurotransmitter, in the brain of rats[11]. GABA serves a calming function by bringing balance to neuronal excitability in the central nervous system and is associated with relaxation and a feeling of well-being.

- It is essential to note that glutamate and GABA are neurotransmitters used by a majority of neurons in the mammalian brain and that they represent key elements of the pathways modulating excitation and inhibition in the central nervous system.

- More recently, microdialysis techniques revealed that administration of theanine also induce increased dopamine release in certain areas of the rat brain (eg striatum)[12]. **Dopamine** is another brain chemical with mood-enhancing properties. This effect is deemed to be indirect, as a consequence of the changes in the NMDA glutamate receptors associated with theanine, since glutamatergic and dopaminergic neurons co-exist and share physiological connections in the striatum.

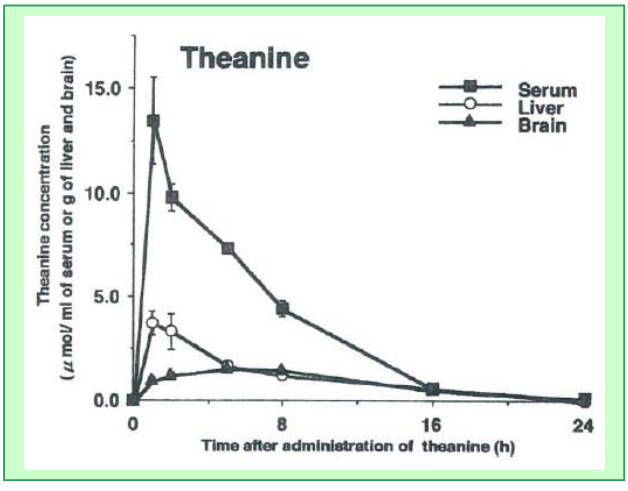
- In summary, L-theanine was shown to influence the release and concentration of several neurotransmitters in the brain, under experimental conditions. Much remain to be elucidated on L-theanine's precise mechanism of action, but it is likely that each of the central neurotransmitters cited above play a role in mediating the physiological effects of the ingredient.

The metabolic fate of theanine in the body

- In vivo animal studies have revealed the fate of theanine after it is orally ingested.

- **Absorption**: theanine is absorbed into the blood through the brush border membrane of the small intestinal tract via a Na^+-coupled cotransporter[13,14].

- **Distribution**: theanine concentration in plasma increase rapidly to peak 30-60 minutes after oral administration, then gradually decrease over 8-12h[10,14,27]. Theanine is incorporated in the brain and liver tissues without metabolic changes[11]. The compound is transported in a dose-dependant manner into the brain via the leucine-preferring transport system of the blood-brain barrier[10]. The maximum concentration in the brain is achieved 5h after administration in rats[27].



Time-dependant changes of theanine concentrations in the serum (■), liver (❍), and brain (▲) of rats[27]

- **Elimination**: L-theanine does not appear to accumulate, being metabolized by an enzyme in the kidney (hydrolyse to ethylamine) and completely eliminated in the urine within 24h[14,15].

The physiological activity of theanine

- A number of human and animal studies document the ability of L-theanine to induce relaxation and relieve emotional or pharmacological stress.

- **Relaxation**: theanine intake causes a feeling of relaxation in human volunteers. This correlates with changes in electric wave patterns of the brain of patients as recorded by the electroencephalogram (EEG)[16].
  - ✓ The brain emits weak electrical impulses (brain waves) that can be measured on the surface of the head. The predominant frequency of electrical impulses correlates with different types of mental states and correspond to 4 categories of brain waves.
  - ✓ Delta waves are seen only in the deepest stages of sleep. Theta waves are seen in light sleep and drowsiness. Alpha waves are indicative of relaxed effortless alertness, while beta waves are seen in highly stressful situations, where mental concentration and focus is difficult[10].

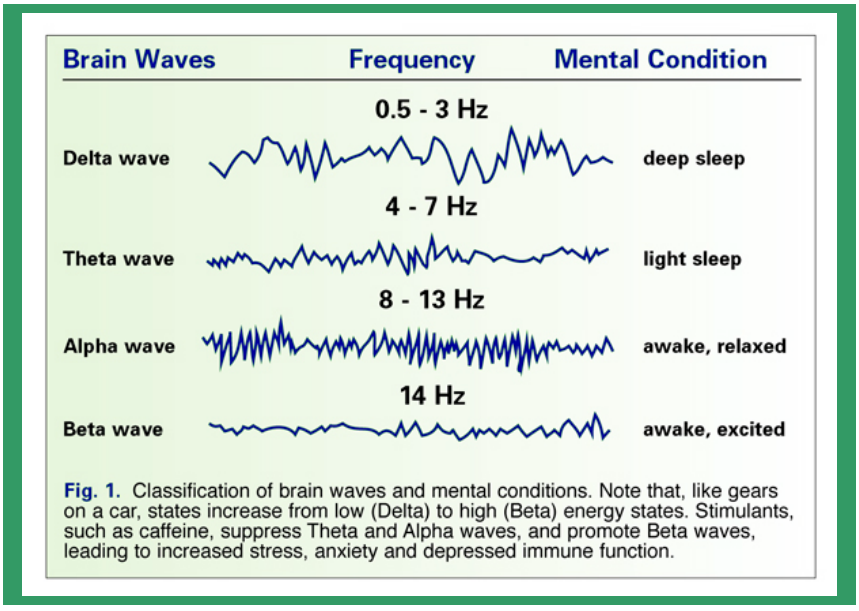
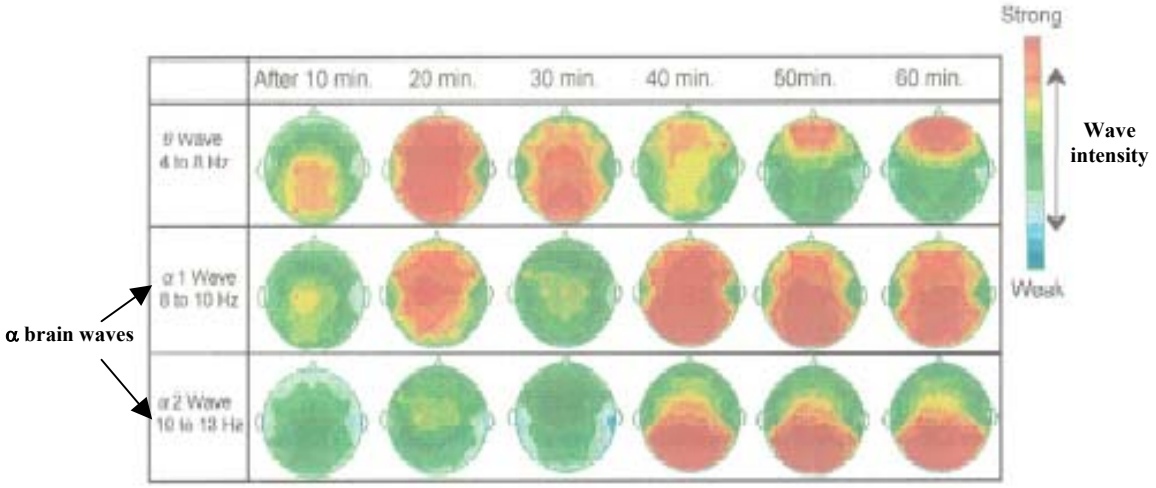
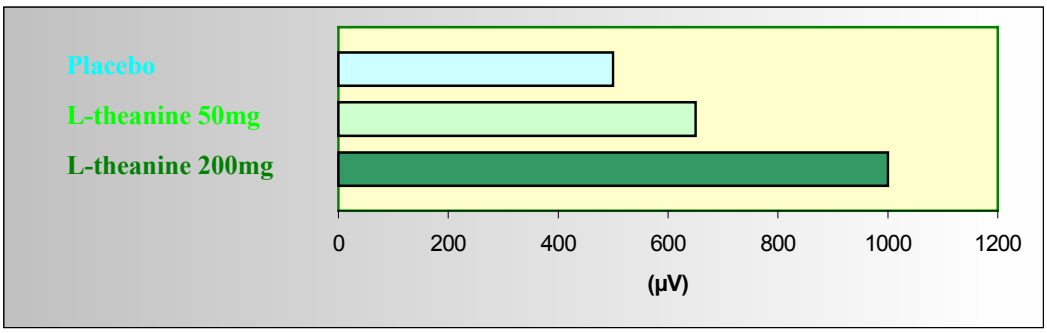


Fig. 1. Classification of brain waves and mental conditions. Note that, like gears on a car, states increase from low (Delta) to high (Beta) energy states. Stimulants, such as caffeine, suppress Theta and Alpha waves, and promote Beta waves, leading to increased stress, anxiety and depressed immune function.

  - ✓ In two blinded studies of the mental response to theanine, marked generation of alpha brain waves was observed from the occipital to the parietal region of the brain within 40 minutes after the subjects had taken 200 mg of theanine, while fewer alpha brain waves was recorded in the placebo groups. The response was especially marked in the high-anxiety subgroups of patients. Accumulated intensity of alpha brain waves showed clear tendency of theanine dose-dependant generation of alpha waves over the one hour measurement period[10, 16, 17]. This was considered as indicative of an induced awake, alert and relaxed mental state. By contrast, the level of theta waves was not increased in the studies, therefore the compound did not promote drowsiness.



Topography of electric wave generation on the brain surface as measured over 60 min after theanine intake[16]



Cumulative alpha brain wave generation on the brain surface as measured over 60 min after theanine intake

- ✓ Based on these findings, 200 mg of theanine intake is proposed in man to promote relaxation and mood enhancement. The calming effects are felt half an hour after the supplement intake and last for several hours without affecting the person's motor skills[10].

- **Improvement of learning ability and memory**: by shutting down the "worry" mode, theanine was found to increase concentration and focus thought in animals.
  - ✓ The Operant test to evaluate memory and learning ability in rats is performed under the condition that feed comes out when a lever is pushed and a light turns on.
  - ✓ Theanine (180 mg/day) was added in feed of weanling male Wistar rats for a period of 4 months, while no supplementation was provided in the control group. The frequency of correct responses to the test was greater in the theanine group compared with the control group[10].
  - ✓ The Passive Avoidance test to evaluate memory is based on the inherent behaviour of rats to move from a light area to a dark place. An electric shock is applied soon after the rat moves from the light room to the dark one.
  - ✓ Animals that were administered theanine hesitated more readily before moving to the dark (significantly increased latency time) and showed a tendency to remain in the light room for longer than the non-supplemented control group[10,18].
  - ✓ By contrast locomotion, standing, the animal tendency to explore a novel environment and grooming analysed by an open-field hole-poke apparatus, were unaltered by theanine administration[18]. This confirmed the lack of sedative effect of the compound.

- **Antagonistic effect on caffeine stimulation**: as a component of green tea, l-theanine offsets the stimulatory action of caffeine with its calming effect.
  - ✓ In one study in rats, a small dose of caffeine (5 µmol/l) induced central nervous system excitation, as evidenced by EEG with electrode implantation. Theanine completely inhibited caffeine stimulatory effect at equivalent molar concentration[19].
  - ✓ Other studies document the antagonistic effect of theanine on spontaneous activity caused by caffeine administration in rats[20,21].

- **Blood pressure lowering effect** : high blood pressure is a feature of stress. Regulation of blood pressure is dependant on neurotransmitters both in the brain and peripheral nervous systems. Theanine intake may be beneficial by exerting an antihypertensive action.
  - ✓ Various doses of theanine were administered to hypertensive rats and blood pressure was monitored before and 60 min after intake. A dose-related reduction of blood pressure was recorded at end-point. The decrease was significant for high theanine doses (1500 mg/kg), with values maintained in the normal range. The heart rate was not affected by theanine administration. L-glutamine was used as one of the controls in the study. Although glutamine share structural similarities with theanine, it did not exhibit any antihypertensive action in the same model[22,23].

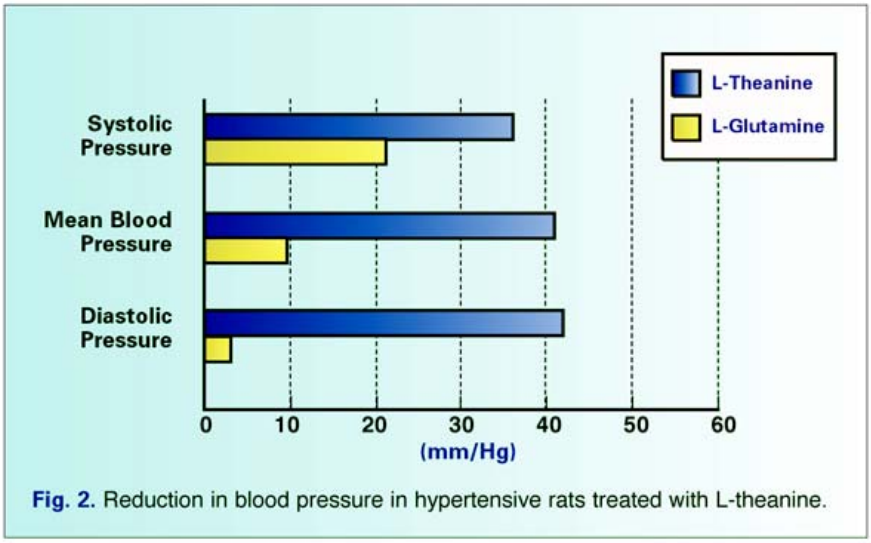


Fig. 2. Reduction in blood pressure in hypertensive rats treated with L-theanine.

  - ✓ The same experiment performed on normotensive rats did not reveal any impact if theanine administration on blood pressure[23].

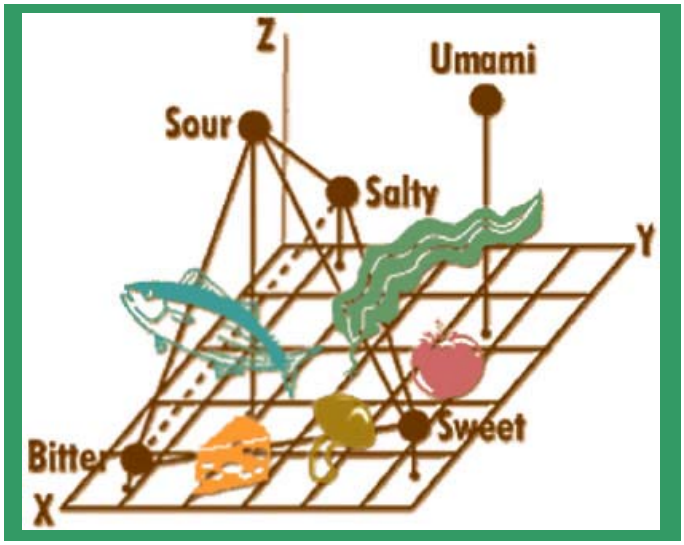
- The extensive research on theanine, mainly conducted in Japan, and the approval by the Japanese Ministry of Health and Welfare for **"unlimited use" of the compound in all sort of human foods** (except baby food) made the supplement popular in this country, where the ingredient has been incorporated in more than 50 food products including candy, beverages and supplements for its relaxing effects[10]. Experience suggested that l-theanine may be helpful in the following **applications**: reduce stress and anxiety, calm nervous agitation, promote relaxation and heighten mental acuity, antagonise the negative effects of caffeine naturally present in various drinks.

The taste of theanine

- The structural similarity of theanine and glutamate is also responsible for another important physiological property of theanine unrelated to stress and anxiety reduction.

- **The Umami taste**

  - ✓ It is theanine that gives its specific exotic taste (called "umami" in Japanese) to green tea infusion[24]. The concept of the umami taste was first promulgated more than 90 years ago in Japan and is of such interest that there is a Society for Research on Umami Taste in this country (founded in 1982). In relation to standard taste groups (bitter, sweet, salty, sour) umami is described as close to sweet and salty and opposite to bitter on a three-dimensional scale.



  - ✓ In green tea, the bitter taste of polyphenols and caffeine is mellowed and broadened by the umami flavor of theanine. If tea is brewed for too long, the concentration of polyphenol increase and the umami taste is finally overcome.

  - ✓ Meats and other foods high in glutamine and related amino acids, are classified in the traditional system as having a sweet taste. This designation indicates that the taste is pleasant and desirable. Humans and animals are naturally attracted to foods that have pleasant tastes, such as sweet and umami, as these food substances play critical nutritional roles (by contrast, bitter tastes are often avoided as they signal toxicity potential).

  - ✓ The umami taste receptors of the tongue have been isolated in rats. These are amino acid taste receptors which respond to each of the 20 amino acids, but are most sensitive to glutamate[25]. Other studies in humans indicate that the sweet and umami taste receptors have similar subunits[26].

  - ✓ These findings are the rationale for **the excellent palatability of supplements containing theanine**. Theanine has long been appreciated as a flavouring agent in candies, ice creams, cakes, jellies, chewing gum in Japan[10].

Toxicological data on theanine

- Tea is the most consumed beverage worldwide after water. People have taken theanine as a component of green tea for thousand years. It is estimated that a heavy tea drinker (6 to 8 cups daily) consumes between 200 and 400 mg of L-theanine daily. Substantial theanine intake over long periods of time without adverse effects is an historical fact in humans[24].

- Theanine is considered as a safe functional additive to human foods. No dietary exposure limits were imposed on L-theanine by the Japan Food Additive Association[10]. Suntheanine™ is an ingredient for use in human dietary supplements in Europe and the US (GRAS Self Affirmation).

Acute toxicity

- LD50 is a term used to describe acute ingestion toxicity. The term defines a dose of a substance (mg/kg of body weight) that has a 50% probability of causing death. The lower the LD50 the more acutely toxic the chemical.
- **The LD50 of Suntheanine™ was determined as > 5000 mg/kg** in rats (upper dose tested). Actually, only one female rat died during the tests, and this was determined to be accidental (neck injury)[10,28].
- Examples of LD50s of typical food ingredients include caffeine (192 mg/kg) and sodium chloride (3000 mg/kg).
- The European Union classification of chemical toxicity based on acute LD50 is as follows:
  - ✓ Very toxic: < 25 mg/kg
  - ✓ Toxic: 25-200 mg/kg
  - ✓ Harmful: 200-2000 mg/kg
- Therefore L-theanine is classified as a safe substance.

Subacute toxicity

- 2000 mg/kg of L-theanine (Suntheanine™) was administered orally to rats for **28 consecutive days**.
- The effects of theanine were evaluated by repeated clinical examinations, body weight and feed consumption measurement, urinalysis, blood biochemistry, cell blood count, ophtalmic testing and histopathology. Comparison with a non-supplemented control group was performed.
- **No detectable toxicological effects were recorded[28].**

Chronic toxicity

- Three groups of 50 mice were allowed to consume freely a basal diet containing 0, 2.5% or 5% of Suntheanine™ over a period of **78 weeks**.
- Measurement of body weight and feed consumption were recorded every 4 weeks.

- Mice that developed malignant tumours during the test and surviving mice at end-point were submitted to histopathological examination of each internal organ. The rate of tumour occurrence was compared between the test and control groups.
- Despite long-term administration of L-theanine, **no findings suggestive of carcinogenicity was observed**[28].

Mutagenicity

- The use of the Ames test is based on the assumption that any susbtance that is mutagenic (for the bacteria used in the test) may also turn out to be a carcinogen.
- It was concluded that Suntheanine™ was not mutagenic as determined by the Ames tests performed at the Tokyo Metropolitan Research Laboratory of Public Health[28].

Adverse reactions, precautions for use

- There are no known adverse reactions to theanine.
- Pregnant or nursing females should avoid L-theanine supplements as a precaution (because of the lack of available safety data).

Review on safety data for Suntheanine™

- Based on specific toxicological studies, purity data (chemical compliance with L-theanine standard) and observations made during animal and human research using this ingredient formulation, a panel of experts concluded that Suntheanine™ consumption causes "no significant or unreasonable risk of illness or injury"[28].
- Nonetheless, specific safety studies were additionally performed by Virbac on Anxitane™ the veterinary product containing Suntheanine™ to further check tolerance in the target species (see "tolerance studies in dogs" section hereunder).

Anxitane™ and anxiety in dogs: efficacy studies

Preliminary exploration

- The US patent no. 6,297,280 "Composition and method for suppressing behaviour problems in pets" (Oct. 2, 2001) describes clinical tests conducted with various concentrations of L-theanine, on dogs affected by a number of behavioral problems associated with anxiety.

- Doses of L-theanine ranging from 0.04 to 110 mg/kg of dog body weight administered in feed over a period of 7 days were tested.

- L-theanine doses above 0.8 mg/kg improved the following behaviour traits, with no clear dose-effect relationship:
  - ✓ Altered motor behaviour, shivering, vomiting, vocalising associated with car travel or visit to the veterinary clinic
  - ✓ Excessive barking, inappropriate elimination behaviour, destruction of chair and sofa after the owner has left home.
- Under the same conditions, no improvement was recorded in dogs non-supplemented with theanine.
- These preliminary tests, however, were conducted on small number of patients (9 per behaviour condition tested), with single individuals evaluated at each treatment dose and using a simple 4-point arbitrary scale for outcome (no change/slight suppression/marked suppression/cure).
- It was concluded that the dietary ingredient deserved further full scale testing in dogs with well defined anxiety-related disorders.

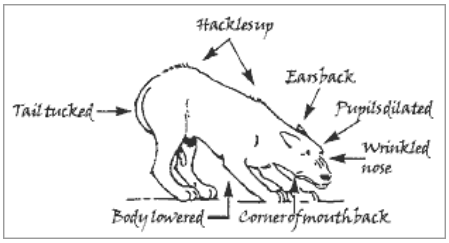
Open clinical field trial on anxious and phobic emotional states in dogs

This study was presented at the joint meeting of Zoopsy (association of French veterinary behaviourists)- ESVCE (European Society of Veterinary Clinical Ethology)- ECVBM (European College of Veterinary Behavioural Medicine), Marseille, October 5-9, 2005[29].

- **Study objectives**: investigate the efficacy of a "nutraceutical" based on L-theanine in the management of anxious states in dogs.

- **Methods**:
  - ✓ The study was designed and monitored by Laurent Kern (Paris, France) and Valérie Dramard (Lyon, France), 2 veterinarians certified in animal behaviour and running full time behavioural referral practices.

  Animal recruitment
  - ✓ **Dogs** aged between 6 months and 15 years, of all breeds, gender, origin or living conditions were eligible to the study.
  - ✓ **Inclusion criterion :** the occurrence for at least one month of one or more of the following manifestations: fear in the street (sensory deprivation syndrome), fear of humans, fear of dogs (social phobia), or signs of intermittent anxiety (hypervigilance, tenseness, jumping at the slightest sound), digestive problems (e.g. hypersalivation) and



finally, sustained displacement activities such as licking of the body or bulimia.

- ✓ **Exclusion criteria**: chronic incapacitating disease, infectious disease, and the use of psychotropic drugs, hormones, pheromones or anti-inflammatory drugs in the preceding month.

Treatment

- ✓ The dogs were followed for 2 months during which the owners gave them oral L-theanine tablets (Anxitane™) twice a day at a total daily dosage of 5 mg/kg (doses of 50-200 mg depending on dog body weight). The test theanine daily dosing regimen had been established based on kinetics data in rats, preliminary clinical experience in dogs and doses usually recommended in man to relieve anxiety.
- ✓ Any other modality to address behavioural problems, be it pharmacological, dietary or pheromonal was forbidden. The owners were not given any instructions on behavioural therapy.

Theanine 50 and 100 mg tablets

Evaluation

- ✓ Clinical and behavioural examinations were performed on D0, D15, D30 and D60.
- ✓ A codified grid (anxiety grading scale) with 17 items was filled in by the veterinarians on the basis of the dog's behaviour during the consultation and information given by the owners.
- ✓ The evaluation addressed:
  - Autonomic manifestations: panting, shivering, mydriasis, yawning, vomiting, hypersalivation, fear-induced urination or defecation.
  - Behavioural manifestations: whining, whimpering, excessive demands for attention, seeking of the owner, threats/growling or even aggressive behaviour because of irritation or fear, aggression redirected against people, objects, furniture, avoidance behaviour.
  - The animal's emotional state and associated postures: inhibition with flattened ears/tail between the legs/tendency to cringe, agitation, hypervigilance and hyperaesthesia, tendency to panic/run away or hide, inhibition with licking/stereotypical behaviour.

BEHAVIOURAL EXAM GRID	D0	D15	D30	D60
Autonomic manifestations				
Panting	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Shivering	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Mydriasis	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Yawning	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Vomiting	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Hypersalivation	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Fear-induced urination / defecation	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Behavioural manifestations				
Whining, whimpering	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Excessive demands for attention, seeking of the owner	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Threats/growling or aggressive behaviour because of irritation or fear	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Aggression redirected against people, objects, furniture	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Avoidance behaviour	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Emotional state and associated postures				
Inhibition with flattened ears/tail between the legs/tendency to cringe	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Agitation	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Hypervigilance and hyperaesthesia (jumps at slightest sound, excess. alertness)	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Tendency to panic/run away or hide	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Inhibition with licking / stereotypical behaviour	0-1-2-3	0-1-2-3	0-1-2-3	0-1-2-3
Total				

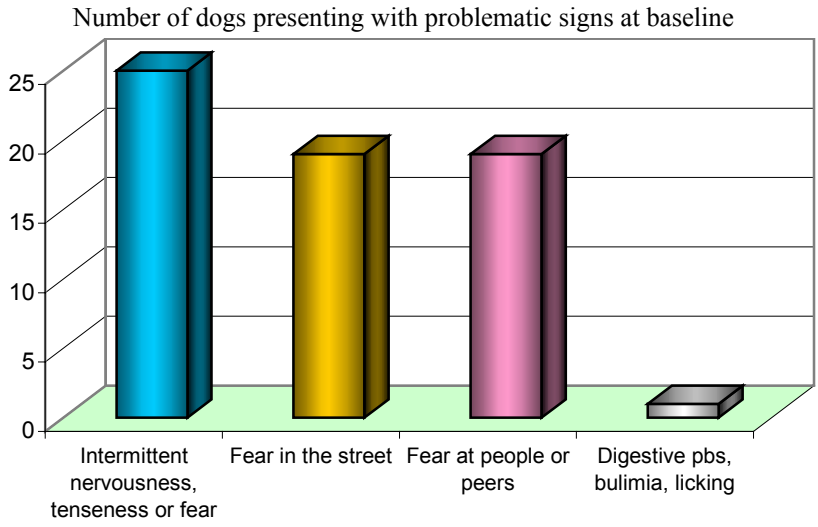
- ✓ Each symptom was scored on a scale of 0 to 3 according to its intensity and all the scores were added together to give an aggregate clinical score reflecting the severity of the problems observed.
- ✓ The investigators were also asked to make a subjective estimate of the degree of improvement at each visit.
- ✓ The opinion of owners on treatment efficacy was recorded at end-point.

- ✓ Any adverse event occurring during the study period was to be reported with clear description of the clinical signs and outcome.

▪ Results

Animal characteristics at baseline

- ✓ **Thirty-two dogs** aged between 7 months and 13 years of age were studied. A large variety of breeds was represented: crossbreds, Labradors, poodles, Yorkshires, setters, King Charles, Lhassa Apso… The sex ratio was balanced (18F /13M /1 NA).
- ✓ **The Origins and socio-familial environments** of the dogs in the study were comparable with those of the general population in France. Mostly acquired by relations (37.5%), from breeders (25%) or a shelter (22%), the dogs had been adopted at an average age of 7 months, often into a home with two or three people.
- ✓ **Main motives for inclusion**: intermittent signs of nervousness, tenseness or fear were recorded in 78.1% of the subjects. Manifestations of fear in the street or at people or peers were also reported in many cases (59.4%). Only one dog had digestive problems, namely bulimia and licking.



- ✓ **The average age at which the problems had appeared** was 18 months (from adoption to 10 years), less than or equal to 1 year for 70% of the dogs. In most cases, the first signs were noticed by the owners at the time of adoption.
- ✓ **The circumstances which were associated with onset of problems that appeared later on** in adult dogs were a change in location (moving house) or the animal's immediate environment (owner's parental leave, death of another pet, etc.) and traumatic events (e.g. an accident).
- ✓ In some cases, there were **other, intercurrent behavioural problems**: related to separation (37.5%), hyperactivity (9.3%), a tendency to run away (9.3%) and inappropriate barking (6.3%).
- ✓ **Anamnesis**: most commonly, little change in the emotional picture was observed. No physical disease was associated, except overweight in 2 cases.

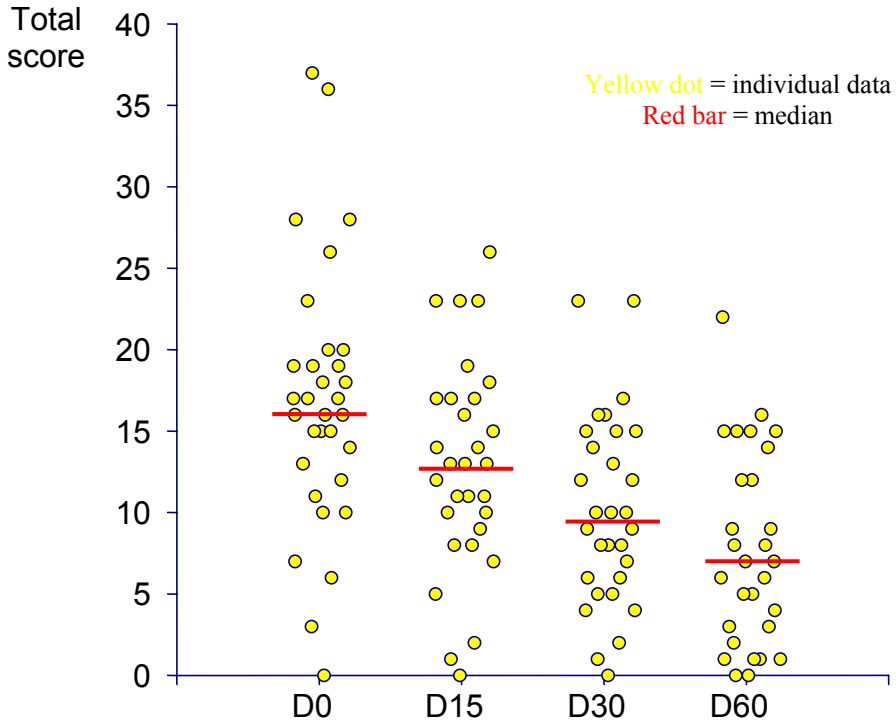
✓ Basal **T4 levels** were measured in 29 dogs, 23 of which had normal levels and six had readings slightly below normal.

Clinical outcome

✓ A **significant reduction of the aggregate clinical score** was observed over the course of the study.

Aggregate clinical score: median (95% confidence interval)					
D0	D15	D30	D60	P*	% reduction D60
16.5 (14-19)	13 (10-16)	9.5 (7-13)	7 (3-9)	<0.0001	59.2 (30-66.7)

* Friedman ANOVA



✓ A **substantial objective response** was observed to L-theanine supplementation (over 50% reduction in the global clinical score) in 64% of the dogs within two months.

✓ **Sign prevalance** was reduced at end-point.

Moderate to marked manifestation	% dogs D0	% dogs D60
Shivering	43.8%	15.6%
Mydriasis	43.8%	15.6%
Excessive demands for attention	59.4%	37.5%
Avoidance behaviour	68.8%	25.0%
Inhibition	59.4%	18.8%
Hypervigilance, hyperaesthesia	65.6%	25.0%
Tendency to panic, run away, hide	62.5%	15.6%

- ✓ The **veterinarians' subjective estimates** also clearly showed an impression of improvement vis-à-vis problems of fear in the street, fear of people and peers and in general signs of tenseness and nervousness in respectively 50%, 60.8% and 42.2% of cases.
- ✓ A total of 54.8% of the **owners** thought that the treatment had been effective.
- ✓ **Tablet palatability** was judged very good in 93.8% of cases with spontaneous ingestion or the animal asking for the tablet.
- ✓ **Tolerance of the supplement** was recorded as good or very good in all cases with no side effects reported, except one incident of a dog showing aggressiveness at people outside the family as a consequence of fear reduction.

- **Discussion**
  - ✓ This clinical trial points up **the value of Anxitane™ (L-theanine) supplementation to mitigate symptoms of anxiety in dogs**.
  - ✓ **A majority of dogs respond within 3 to 6 weeks of Anxitane™ supplementation**, however some cases remain refractory and requires stronger treatment measures.
  - ✓ Despite the absence of an untreated control group, the magnitude of the effects as measured by the scores (reduction of the severity of symptoms by more than 50% in the majority of cases) together with the investigators' general impressions place the efficacy of the supplement beyond the type of effects typically seen with placebo.
  - ✓ **Anxitane™ would seem to be particularly effective in fearful or tense dogs or dogs that have been perturbed by a one-off or permanent change in their environment.**
  - ✓ By contrast, little improvement was seen incidentally in other types of behavioural problem presented concomitantly by certain of the dogs in this study (problems due to separation, hyperactivity).

Open clinical field trial on anxiety related to car travel in dogs

This study is still undergoing at the time this product profile is elaborated.

Tolerance studies with Anxitane™ in dogs

Specific safety trial in dogs: clinical impact

Virbac Animal Unit internal study no. 691.05/70001

- **Study objectives**: evaluate the tolerance of Anxitane™ tablets administered orally for 2 months at a dosage of 5 mg/kg (label dose) or 25 mg/kg (5-fold the label dose) in dogs.

- **Methods**:
  Animals
  - ✓ **10 beagle dogs:** 4 females / 6 males, aged 1.5 to 5 years, size: 7.9 to 11.4 kg, housed under the same standard conditions in individual boxes of the animal unit kennel.
  - ✓ Fed with standard dry food (Virbac Vet Complex®), allowed free access to water.

Treatments

- ✓ **Randomisation** in 2 groups of 5 dogs, according to sex and weight.
- ✓ **Group 1**: L-theanine tablets 5 mg/kg daily dose administered orally 1h before feeding, for 63 days.
- ✓ **Group 2**: L-theanine tablets 25 mg/kg daily dose administered orally 1h before feeding, for 63 days.

Records

- ✓ Animals under constant surveillance 6 days a week during the study period.
- ✓ **Clinical examination, weight measurement** once a week.
- ✓ **Blood biochemistry profile: blood sampling at D-7, D0, D7, D28 and D63**. Analysis performed by an independent veterinary laboratory (Vebiotel, Arcueil, France) from plasma (lithium heparinate tube).

- **Results**:
  - ✓ No significant differences were detected between the groups at baseline for any of the parameters, except creatinine values (but the difference in the later case were clinically irrelevant: group 1: average 8.8 mg/l, group 2: average 7.5 mg/l).
  - ✓ **No particular change of the animal habits was observed during supplementation in the 2 groups. Isolated events that lasted few days included:**
    - ➢ In group 1: slight reddening of conjunctive membranes, watery faeces
    - ➢ In group 2: excitation, watery faeces, dysorexia
    - ➢ Such events are commonly observed from time to time in the kennel on animals left untreated
  - ✓ A slight general tendency to weight gain was recorded in both groups over time, although limited numerically (average +400 g). Physiological variations are normally observed on dogs in the kennel, depending on the period of the year.
  - ✓ **BUN, creatinine, ALAT, ASAT, ALP, total proteins, glucose remained within or near normal range** in all dogs during the study period.

- **Conclusions**:
  - ✓ Two months of Anxitane™ supplementation is very well tolerated by dogs at 5 to 25 mg/kg/day. No side effects could be detected in the canine species in the dose range tested.

Specific safety trial in dogs: haematology

Virbac Animal Unit internal study no. 691.05/70002

- **Study objectives**: evaluate the impact of Anxitane™ tablets administered orally for 2 months at a dosage of 5 mg/kg (label dose) on blood cell counts in dogs.

- **Methods**:

  Animals
  - ✓ **5 beagle dogs:** 4 females / 6 males, aged 1.5 to 5 years, size: 7.9 to 11.4 kg, housed under the same standard conditions in individual boxes of the animal unit kennel.

- ✓ Fed with standard dry food (Virbac Vet Complex®), allowed free access to water.

Treatment

- ✓ L-theanine tablets 5 mg/kg daily dose administered orally 1h before feeding, for 63 days.

Records

- ✓ Animals under constant surveillance 6 days a week during the study period.
- ✓ **Blood cell count: blood sampling at D-7, D0, D7, D28 and D63**. Analysis performed by an independent veterinary laboratory (Vebiotel, Arcueil, France) from whole blood (EDTA and citrate tubes).

- **Results**:
  - ✓ **No particular change of the animal habits was observed during supplementation.**
  - ✓ **Hemoglobin, hematocrit, red blood cell, white blood cell and platelet counts remained within or near normal range** in all dogs during the study period.

- **Conclusions**:
  - ✓ Complete blood count is unaffected by two months of Anxitane™ supplementation at label dose.

Safety in clinical trials

Anxitane™ tablets (L-theanine 5 mg/kg divided BID) administered by dog owners to their pets were very well tolerated under field conditions (see above).

Palatability study of Anxitane™ tablets in dogs

Panelis study no. FDOG02

- **Study objectives**: evaluate Anxitane™ tablets intake and consumption by dogs.

- **Methods**:

Animals

- ✓ **33 dogs** (small, medium and large breeds).
- ✓ Housed in individual boxes.

Treatments

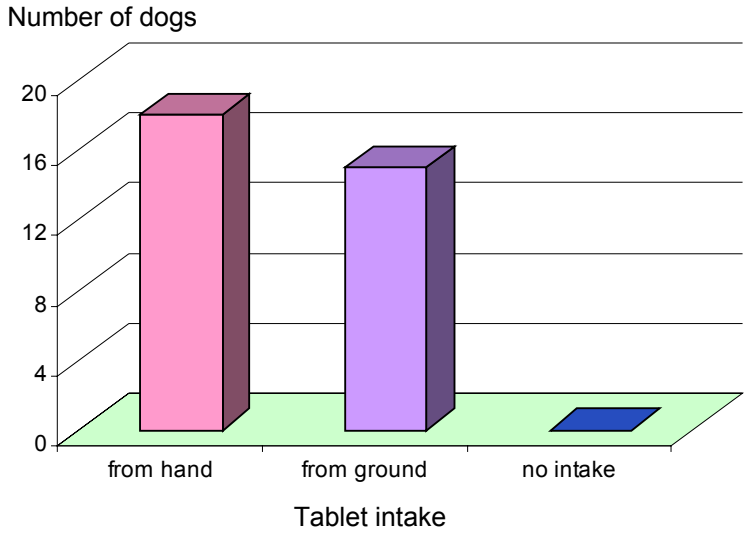
- ✓ 1 or 2 Anxitane™ tablets depending on dog body weight.
- ✓ **First given by hand, then placed on the ground.**

Records

- ✓ Observation of dog reaction over ½ h.

- ✓ % tablet prehension in hand or on the ground.
- ✓ % consumption.

- **Results**:
  - ✓ **Excellent tablet acceptability**: 31/33 dogs (94%) spontaneously ingested the tablets.
  - ✓ **A majority of dogs took the tablets on hand (55%)**, the others took the tablets on the ground (45%).



  - ✓ No major difference were detected between small and large dogs for tablet consumption. Small breeds were more represented in the group of dogs that took tablets from ground.

- **Conclusions**:
  - ✓ Anxitane™ tablets are very well accepted by dogs, spontaneous consumption is the rule.
  - ✓ This is related to strong inherent tasteful effect of the active ingredient theanine (see p.10), as well as an additional flavouring agent in the formula.
  - ✓ The oblong shape of Anxitane™ tablets moreover facilitates handling by the owner and acceptance by the animal.

Anxitane™: practical recommendations

Field of application

- Anxitane™ is a dietary aid for the management of excessive emotional reactions in pets.
- Chronic stress/anxiety, fear in the street, fear of people or of other dogs, fear associated with travel by car are conditions for which Anxitane™ has proven to be beneficial in clinical settings on dogs.
- Noise related phobias (thunderstorms, fireworks), fearful puppy adaptation may be other situations where Anxitane™ supplementation reveals helpful in the canine species.

- Potential for product use in cats include anxiety-related conditions such as: idiopathic cystitis, alopecia, asthma, fear of man (social phobia), cohabitation problems, adaptation to changing environment problems,…Further testing is required in this area.

Profile of responders

- Fearful dog (pattern of avoidance reaction, inhibition).
- Tense dog (hypervigilance).
- Dog perturbed by a permanent change in its environment (moving house, adoption, …).
- Dog perturbed by a one-off change in its environment (grooming, visit to the veterinarian, holiday sheltering, canine beauty show, gatherings…).
- Dog perturbed by changes in the family (death of another pet, owner's parental leave, …).
- Dog sensitised to car sickness.

Doses

- Dogs

Body weight	Daily dose
≤ 10 kg	½ Anxitane™ S tablet twice a day
11-24 kg	½ Anxitane™ L tablet twice a day
≥ 25 kg	1 Anxitane™ L tablet twice a day

Anxitane™ S breakable tablets contain 50mg of L-theanine.
Anxitane™ L breakable tablets contain 100mg of L-theanine.

- Cats: ½ Anxitane™ S tablet once a day.

Treatment duration

- Chronic stress, intermittent anxiety
  - ✓ Daily administration for a period of 2 months, which may be repeated according to the veterinarian.
  - ✓ Progressive improvement of behaviour is expected over 3-6 weeks.
- Temporary fears
  - ✓ Administration 12h and 2h before the challenging situation.
  - ✓ If the situation is prolonged then repeat administration every 6h.
  - ✓ The decrease in the intensity of signs is progressive over repetitions of challenges (desensitisation).
- Adapted behaviour instructions given concurrently will improve efficacy.

Limits

- Preliminary observations suggest that Anxitane™ provides only limited improvement in dogs presented primarily for separation-related problems or hyperactivity.

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