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Research Article

Side effects of Apranax (Naproxen) Studied on Ants as Models

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Abstract

On ants used as models, the anti-inflammatory drug Apranax (the active substance of which is Naproxen) appeared to have more, and more severe, adverse effects than those people can easily find, e.g. in the notice for use and on several internet sites. Indeed, this drug impacted the ants' food intake, locomotion, sensory perception, social relationships, cognition, and short-term memory. No adaptation occurred to Apranax side effects. Habituation to its wanted analgesic effect occurred. Ants acquired need of water, and developed some dependence on this drug consumption. After its consumption was stopped, the effect of Apranax vanished in a total of 12-13 hours, rapidly decreasing soon after weaning. Such adverse effects may exist for humans. Therefore, all the above information should be known by practitioners who should monitor their patients treated with Apranax (Naproxen) as for their food intake, movements, sensory perception, social interactions, cognitive abilities, memorization, dehydration, dependence on the drug consumption, among others. The practitioners should use the smallest dose and the shortest treatment time possible for sufficiently care of their patients. Provided that these advices are taken into account, Apranax (Naproxen) could be used since, despite its numerous, severe and not adequately divulgated adverse effects, it is one of the most efficient anti-inflammatory drug.

Keywords: Addiction; Cognition; *Myrmica sabuleti*; Sensory Perception; Social Interaction

Abbreviations

ang.deg.: Angular Degrees; ang.deg./cm: Angular Degrees per cm; mm/s: Millimeter Per Second; χ^2= chi-Square; *vs:* Versus; n°: Number; cm: Centimeter; mm: Millimeter; ml: Milliliter; mg: Milligram; s: Second; min: Minute; h: Hour; t: Time; %: Percentage

Introduction

The active substance of Apranax is Naproxen-Natrium. This drug has been authorized in 1983. It is a largely used anti-inflammatory drug allowing caring of persons suffering, among others, from arthritis, rheumatism, periarthritis, tendonitis, low back pain, stomatological inflammation, as well as from a few other dolors. In the notice joined to Apranax packages as well as on the numerous internet sites devoted to this drug or, more generally, to Naproxen, the efficiency and usefulness of these products are affirmed and advocated, while several side effects are also reported, but without insistence. These side effects are, among others, digestive problems, headache, drowsiness, hearing and eye problems, dyspnea, and edema. No information are given as for a potential impact of Apranax on the individuals' food intake, activity, sensory perception, stress, cognition, learning, memorization, adaptation to side effects, habituation to wanted effects, dependence on the drug

consumption, and decrease of its effect after its consumption was stopped. However, according to the mode of action of Naproxen [1], impact on such physiological and ethological traits may occur, and should be cautiously considered for safely and correctly caring of patients treated with this medicinal substance. We thus intended to fill this gap, working on ants as models, since we are accustomed to make such studies, having until now examined the side effects of fifty products used by humans [2-6].

Before relating our methods and results, we here below recall why using ants as models, which species we used and what we know on it, as well as which physiological and ethological traits Apranax could affect we aimed to examine.

Why using ants as models

The most important biological processes, such as genetics, muscles contraction, nervous impulses, memorization, and sensory perception, are similar for any animal species, including the humans. Several invertebrate and vertebrate animals are consequently used as biological models [7,8]. Invertebrates are preferably used because they are small, easily maintained in a laboratory, and have a short generation time [9]. Insects, for examples the locusts, mealworms, fruit flies, bees, are often used [10]. Ants can also be used. They are advantageously so because their maintenance is easy and cheap, and because they detain several evolved ethological capabilities on which the effects of substances or environmental factors can be examined. As for the ants' capabilities, let us cite their use of specific pheromones for communicating with congeners, their ability to memorize visual and olfactory cues and to use them for finding their way, their establishing of efficient recruitment systems, their brood caring behavior, their specific territorial markings, nest building, and cemeteries managing [11,12].

Which species we used, what we know on it

Here we used the species *Myrmica sabuleti* Meinert, 1861 we particularly well known. We know its recruitment system, navigating strategy, visual perception, visual and olfactory conditioning [13]. We studied the ontogenesis of several of their knowhow [14], and discovered their self recognizing in a mirror [15]. Also, we found that they detain many cognitive abilities, such as having a number line, acquiring the notion of zero, counting elements, adding numbers, acquiring numerical symbolisms, expecting future events on the basis of previously experienced ones, associating perceived visual and olfactory cues with their time period of occurrence [16,17: and references therein], [18]. In addition, the distance effect, size effect and Weber's law can be applied to their perception [19,20]. However, their cognitive abilities always remained at a concrete level, i.e. they never reach abstraction.

Which physiological and ethological traits we intended to examine

We here intended to examine the potential effects of Apranax on the ants' food consumption, general activity, locomotion, orientation ability, audacity, tactile (pain) perception, social relationships, stress, cognition, learning and memory. We also intended to study the ants' potential adaptation to adverse impacts of Apranax, their potential habituation to the wanted effect of this drug, and their possible dependence on its consumption. In the course of our experimental work, we estimated that we should check the ants' potential need of water. Finally, we studied the loss of the effect of Apranax after its consumption was stopped. The experimental methods were identical to those previously used for studying the effects of fifty substances [2-6: references therein]. However, for the readers' convenience, they are here again briefly related, and some self plagiarism could thus not be avoided.

Materials and Methods

Collection and maintenance of ants

The controls, then the test experiments were performed on two colonies of *M. sabuleti* collected in May 2021 in an abandoned quarry located in the Aise valley (Belgium, Ardenne). The colonies contained about 500 workers, a queen and brood. They were maintained in one to three glass tubes which were half filled with water, a cotton plug separating this compartment and that devoted to the ants. The nest tubes of each colony were deposited in a tray (34 cm x 23 cm x 4 cm) which served as a foraging area. In this area, *Tenebrio molitor* larvae (Linnaeus, 1758) were deposited three times per week, and a small tube filled of sugar water was permanently set. The lighting of the room equaled *ca* 330 lux while working on ants and 110 lux during the other time periods. The temperature equaled 20°C, the humidity 80%, and the electromagnetic field 2 μWm2. All these conditions are suitable to *M. sabuleti*. The ants are here often named 'workers' or 'nestmates' as commonly do researchers on social insects.

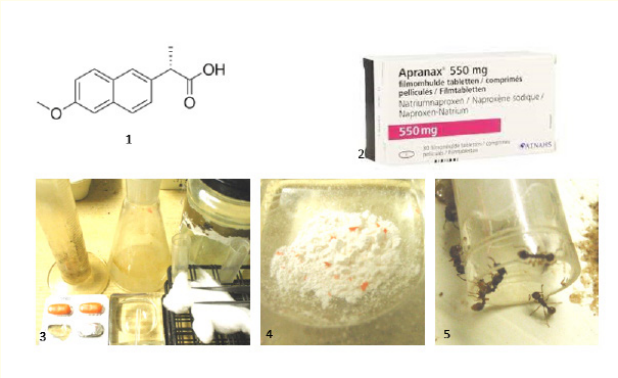


Figure 1: Successively, chemical structure of Naproxen, the active substance of Apranax, a package of Apranax 550 mg, realization of the solution of Apranax given to the ants, half a tablet of Apranax duly crushed, and ants drinking the sugared solution of Apranax.

Solution of Apranax (Naproxen-Natrium) given to the ants

A package of Apranax (Naproxen-Natrium) 550 mg (manufacturer: Roche Farma S. A., 28914 Leganes, Madrid) was furnished by the pharmacist Wera (1170 Bruxelles, Belgium). For humans, the most usually advised daily dose of this drug is 550 mg per day. Mammals consume a daily large amount of water; humans, among others, consume about one liter of water per day. Therefore, patients who are cared thanks to Apranax consume 550 mg of this drug together with one liter of water. Due to their anatomy (cuticle) and their physiology (excretory system), the insects consume about ten less water than mammals. Therefore, to maintain the ants under a diet with Apranax similar to the commonly one of humans, they must receive a solution of one tablet of 550 mg of Apranax in 100 ml of water, or of half such a tablet in 50 ml of water. Half a tablet '550 mg' Apranax was thus duly crushed, then dissolved into 50 ml of the ants' sugar water, and this solution was provided to the ants in their usual cotton-plugged tubes (Figure 1). The plug of these tubes was refreshed every 2-3 days, and the entire solution was renewed every 7 days. Each day, we looked if ants drunk the Apranax solution, and they did. The control experiments were firstly made on the two colonies maintained under normal diet. After that, the tubes containing sugar water were replaced by tubes contained the sugared solution of Apranax, and the test experiments started after the ants had the drug solution at their disposal since 24 hours.

Meat and sugar water consumption, general activity

While ants were under normal maintenance, then while they had Apranax at their disposal, we counted, for each two colonies, eight times per day during six days, the ants which were on the meat food, which were in front of the sugar water tube, and which were active at any place in their environment (foraging area, nest entrance, inside the nest). Four each kind of count, the total of counts equaled thus: 4 counts x 2 colonies = 8 counts per day, this being done during 6 successive days. For each kind of count and of diet, the daily mean of the eight counts was established (Table 1, lines 1 - 6). These six daily means obtained for ants under normal diet on one hand and for ants consuming Apranax on the other hand were compared to one another using the non-parametric test of Wilcoxon [21]. Also, for each diet and each kind of count, the mean of the six daily means was established (Table 1, last line).

Linear and angular speeds; orientation to a tied nestmate

These traits were quantified on ants freely walking in their foraging area. The linear and the angular speeds were quantified without stimulating the ants; the orientation was quantified while stimulating them with a nestmate tied to a piece of paper (Figure 2 A). Such a tied nestmate emits its mandible glands attractive alarm pheromone which induces ants approaching. For the ants' speeds on one hand and for their orientation on the other hand, 40 trajectories were manually recorded, and were then analyzed using appropriate software. The latter was set up on the basis of the following definitions. The linear speed (in millimeter per second = mm/s) is the length of a trajectory divided by the time spent to travel it; the angular speed (in angular degrees per centimeter = ang.deg./cm) is the sum of the angles made by successive adjacent segments, divided by the length of the trajectory; the orientation (in angular degrees = ang. deg.) to a location is the sum of successive angles made by the direction of the trajectory and the direction towards the location, divided by the number of measured angles. An orientation value lower than-90° means that the observed animal tends to approach the location. An orientation value higher than 90° means that the observed animal tends to avoid the location. For the three considered variables, the median and quartiles of the 40 recorded values were established (Table 2, lines 1, 2, 3). For each variable, the distribution of values obtained for ants consuming Apranax was compared to that obtained for ants living under normal condition using the non-parametric χ^2 test [21].

Audacity

This trait was assessed through the ants' coming onto a risky unknown apparatus. A cylinder (height = 4 cm; diameter = 1.5 cm) vertically tied to a squared platform (9 cm^2), each made of Steinbach® white paper, was deposited in the ants' foraging area (Figure 2 B), and the ants present on this apparatus were counted 10 times over 10 minutes (number of counts: 10 x 2 = 20). The mean and extremes of these counts were established (Table 2, line 4). Also, the numbers obtained for the two colonies were correspondingly added, and the ten sums were then chronologically added by two, what provided five successive numbers. These latter five numbers obtained for ants consuming Apranax were compared to the five numbers obtained for ants normally maintained using the non-parametric Wilcoxon test [21].

Tactile (pain) perception

When correctly perceiving the rough character of a substrate, the ants walk on it slowly, sinuously, with difficulty, and often touch the substrate with their antennae (Figure 2 C1). When poorly perceiving such an uncomfortable character of a substrate, the ants walk on it frankly, not very sinuously and rather rapidly, seldom touching the substrate with their antennae. Consequently, for evaluating the ants' tactile (pain perception), their linear and angular speeds were assessed while they walked on a rough substrate. To do so, a piece (3 cm x 2 + 7 + 2 = 11 cm) of n° 280 emery paper duly folded was inserted inside a tray (15 cm x 7 cm x 4.5 cm), what divided the tray in a first 3 cm long zone, a second 3 cm long one containing the emery paper, and a last 9 cm long zone. Such a design was elaborated for each colony. To perform an experiment, 12 ants of each colony were transferred into the first zone of their own apparatus, and their trajectories were recorded while they walked on the emery paper. Their linear and angular speeds were then assessed as usually (see the subsection which concerns these traits). For ants not consuming Apranax and for those consuming this drug, the 24 values of linear and of angular speeds obtained for ants walking on the rough substrate were compared to those previously obtained for ants walking in their foraging area using the non-parametric χ^2 test [21]. These two comparisons allowed evaluating the impact of Apranax on the ants' tactile perception. In addition, for each kind of ants' maintenance, the 24 recorded values of linear and of angular speeds of ants moving on the rough substrate were characterized by their median and quartiles (Table 2, lines 5, 6).

Brood caring behavior

For each colony, a few larvae and nymphs were removed from the inside of the nest and were deposited in front of the entrance. Each time, five of these larvae or nymphs were observed during five minutes, and during this time, the number of not re-entered larvae or nymphs among the five ones were counted after 30 seconds, 1, 2, 3, 4, and 5 minutes (Table 3, line 1). In addition, the ants' behavior towards these larvae and nymphs was carefully observed (Figure 2 D). Only five larvae or nymphs of each colony were used because all of them must be looked simultaneously. The experiment was made only once because removing brood from the nest triggers a great disturbance. The six numbers of not re-entered larvae or nymphs obtained for the two colonies were correspondingly added, and the six sums obtained for ants consuming Apranax were compared to the six ones obtained for ants normally maintained using the non-parametric test of Wilcoxon [21].

Social relationships (interactions between nestmates)

Under normal conditions, the ants belonging to the same colony do not aggress themselves. Several ants' physiological changes or environmental perturbing factors may imperil this common peaceful behavior. To know if this occurs when ants consume Apranax, for each colony, five dyadic encounters were performed (of course while the colonies were under normal condition, then while they had the drug at their disposal), the total number of encounters for each kind of maintenance equaling thus ten. These encounters were performed in a cup (diameter = 2cm, height = 1.6cm), the borders of which having been covered with talc to prevent ants climbing on them. For each encounter, one ant of the pair was carefully observed during 5 minutes, and its behavior was characterized by the numbers of times it did nothing (level 0 of aggressiveness), touched the other ant with its antennae (level 1), opened its mandibles (level 2), gripped and/or pulled the other ant (level 3), and tried to sting or stung the other ant (level 4) (Table 3, line 2; Figure 2 E). The numbers obtained for the five observed ants of the two colonies were correspondingly added, and the distribution of the recorded values obtained for ants consuming Apranax was compared to that obtained for normally maintained ants using the non-parametric χ^2 test [21]. Also, for each kind of maintenance, a variable 'a' evaluating the ants' aggressiveness towards nestmates was calculated, this variable 'a' equaling the number of aggressiveness levels 2 + 3 + 4 divided by the number of aggressive levels 0 + 1 (Table 3, line 2).

State of stress and cognition through escaping from an enclosure

To be able to escape from an enclosure, an individual must stay calm, not stress, and cautiously look for an exit. It must also have some intact cognitive ability. To quantify the state of stress and the cognition of ants normally maintained and maintained with Apranax at their disposal, six ants of each colony were enclosed under a reversed cup (made of polyacetate; height = 8cm, bottom diameter = 7 cm, ceiling diameter = 5 cm) set in their foraging area. The inside surface of these cups were slightly covered with talc to prevent ants climbing on it. Also, a notch (3 mm height, 2 mm width) has been made in the rim of the bottom of the cup, giving so to the ants the possibility of escaping (Figure 2 F). For each two colonies, the ants among the six enclosed escaped after 2, 4, 6, 8, 10 and 12 minutes were counted, and the numbers obtained for the two colonies were correspondingly added (Table 3, line 3). The six sums obtained for ants consuming Apranax were compared to the six ones obtained for ants normally maintained using the non-parametric Wilcoxon test [21].

Cognition

This physiological trait was quantified through the ants' ability in crossing a twists and turns path. Two pieces (4.5 cm x 12 cm) of strong white paper (Steinbach®) were duly folded and inserted in a tray (15 cm x 7 cm x 4.5 cm) in order to create a twists and turns path between a 2cm long zone in front of this 'difficult' path and a 8 cm long zone beyond it (Figure 3 A). Such an apparatus was built for each colony. To perform an experiment on a colony, 15 ants were transferred into the zone located in front of the twists and turns path. Then, the ants still there, and those having reached the zone lying beyond the twists and turns path, were counted after 2, 4, 6, 8, 10 and 12 minutes. The numbers obtained for the two colonies were correspondingly added (Table 3, line 4). For each two considered zones, the added numbers obtained for ants consuming Apranax were compared to those previously obtained for ants normally maintained using the non-parametric Wilcoxon test [21].

Visual operant conditioning and memory

At a recorded time, a blue hollow cube, made of strong paper (Canson®) was placed above the entrance of the tube containing sugar water and the meat was set close to this tube (Figure 3 Ba). Having done so, the ants underwent operant visual conditioning. The control experiment, i.e. on ants living under normal diet, has previously be done on another similar colony of *M. sabuleti*, collected on the same site and maintained under normal diet. This is required because, as soon as a *M. sabuleti* worker has acquired conditioning to a stimulus, it keeps its conditioning during two to three days, and even after having lost it, the worker more rapidly than usually acquires it again. Consequently, it becomes no longer possible to again assess its conditioning acquisition. All over the ants' conditioning acquisition, then, after the blue cube removal, while the ants lost their conditioning, the ants of the two used colonies were tested in an own Y-maze. This Y-maze was made of strong white paper, was set in a tray (not in the ants' foraging area), and had its sides slightly covered with talc. Also, a blue hollow cube was located in randomly its left or right branch. To conduct a test on a colony, 10 ants were one by one deposited in the maze, three to four cm before its division into two branches, and the ants' choice of one or the other branch of the Y-apparatus was recorded (Figure 3 Bb). Choosing the branch containing the blue cube was giving the correct response. After having tested, to avoid testing twice the same ant, the ant was kept in a glass until 10 ants of its colony were tested, and when all the 10 ants were tested, they were transferred again into their foraging area. Six such successive tests were made while ants acquired condition, and six ones were again performed while ants lost their conditioning (Table 4). For each of these twelve conducted tests, the ants' responses obtained for the two colonies were added, and the proportions of correct responses each time established. The proportions obtained for ants consuming Apranax were compared to those previously obtained for ants normally maintained using the non-parametric Wilcoxon test [21].

Adaptation (tolerance) to Apranax adverse effects

Adaptation to a drug occurs when an individual consuming this drug less and less suffers over time from the side effects of the drug. For studying adaptation to a drug, a side effect of the drug must be quantified soon after the individual has consumed the drug and again later, after it has consumed the drug for a time, and the two assessments must be compared. In the present work, the ants' linear speed was largely affected by Apranax consumption. Therefore, to study potential adaption to this drug, the ants' linear speed was again assessed after the ants had Apranax at their disposal during eight days, exactly as this trait had been assessed after one day of this drug consumption. The median and quartiles of the recorded values were established (Table 5, first part), and the distribution of these values was compared to that obtained af-

ter the ants had consumed the drug during one day using the non-parametric χ^2 test [21].

Ants' potential habituation to Apranax

Habituation to a drug occurs when the consumer of this drug becomes less and less sensitive to the wanted effect of the drug over its consumption. To study habituation to a drug, a trait favorably affected by the drug must be assessed soon after the start of the drug consumption, and again later after some time of its consumption. The results of the two assessments must then be compared. In the present work, Apranax is expected to decrease the pain perception. Therefore, this trait, already assessed after the ants had Apranax at their disposal for three days, was again assed, exactly in the same way, after the ants consumed the drug during nine days. The median and quartiles of the recorded 24 values of linear and angular speeds were established (Table 5, second part). Also, the distribution of these two kinds of recorded values was compared to the corresponding ones previously obtained for ants consuming the drug for three days using the non-parametric χ^2 test [21].

Possible need of water

Ants consuming Apranax appeared to stay on their sugar water during more and more times over their consumption. This may be due to a need of water or of the drug. The need of the drug, i.e. a dependence on its consumption, was examined thanks to the next experiment (see below). Here, we describe the experiment made to know if ants under a diet with Apranax needed water intake. Instead of their tube filled with a sugared solution of Apranax, the ants of the two colonies were provided with two smaller tubes (length = 2.5 cm, diam. = 0.5 cm), one again filled with the sugared solution of Apranax, the other filled with a pure water solution of the drug (Figure 3 F). The ants sighted on these two Apranax solutions were separately counted every two minutes during forty minutes (number of counts = 20). The numbers obtained for the two colonies were correspondingly added, and for each kind of count, the sum of these added numbers was established (Table 5, third part). The results of these additions allowed calculating the proportion of ants having gone on one hand on the drug sugared water solution, and on the second hand, on the pure water solution of the drug. Moreover, the results of the additions, i.e. a result for each kind of drug solution, so for each kind of tube, were compared to those expected if the ants randomly went onto the two provided tubes using the non-parametric χ^2 goodness-of-fit test [21].

Dependence on Apranax consumption

Dependence on a drug occurs when the consumers want to have this drug at their disposal at any time, when they continuously consume the drug whatever its adverse effects, and finally when they can no longer live without consuming it. In this work on Apranax, the ants' dependence on this drug consumption was examined after the ants had consumed it during 10 days. To reveal any potential dependence on Apranax, 15 ants of each colony were transferred in an own tray (15cm ×7 cm × 5cm) inside of which two cotton-plugged small tubes (length = 2.5 cm, diam. = 0.5 cm) had been set. One of the tubes contained sugar water, and the other contained the sugared solution of Apranax used all over the present experimental work. The tube containing the drug was set on the right of the tray for colony A, and on the left of the tray for colony B (Figure 3 G). Half a minute after the ants were in their tray, for each colony, those sighted at the entrance of each tube were counted 15 times over 15 minutes. For each colony and each kind of count, the recorded numbers were added. Then, the two sums obtained for each two colonies were correspondingly added, and the two resulting sums allowed calculating the proportion of ants having gone onto the tube containing the drug and the tube free of drug (Table 5, fourth part). In addition, the two resulting sums were compared to the two numbers expected if ants randomly visited each two provided tubes using the non-parametric χ^2 goodness-of-fit test [21].

Decrease of the effects of Apranax after its consumption was stopped

The decrease of the effect of Apranax was examined after the ants had consumed this drug during twelve days. The physiological trait impacted by the drug chosen for conducting this study was the ants' linear speed. Twelve hours before the start of the study, the ants received a fresh sugared solution of Apranax. After these 12 hours, the ants' linear speed was assessed as this trait was assessed after the ants had the drug at their disposal during one as well as seven days, except that 20 instead of 40 ants' trajectories were recorded and analyzed. This reduction of the number of analyzed trajectories was made for being able to assess the successive linear speed values the ants presented over the decrease of the effect of Apranax, and thus to permanently evaluate the current situation. After this initial assessment, i.e. that made at t = 0, the ants' tubes containing the sugared drug solution were replaced by similar tubes but free of drug, and this constituted the weaning. Thereafter, the ants' linear speed was assessed every two hours until the

obtained speed value was similar to the control one, i.e. to that of ants normally maintained. For each assessment, the median and quartiles of the 20 recorded values were established (Table 6). The distributions of the successively recorded linear speed values were compared to the distribution obtained at t = 0 and to that obtained for ants under normal diet using the non-parametric χ^2 test for independent samples (Table 6) [24]. Also, we researched the mathematical function which could best match the observed increase of the ants' linear speed, i.e. the decrease of the effect of Apranax on ants as models. This possible function is given in the text, and is illustrated in figure 4.

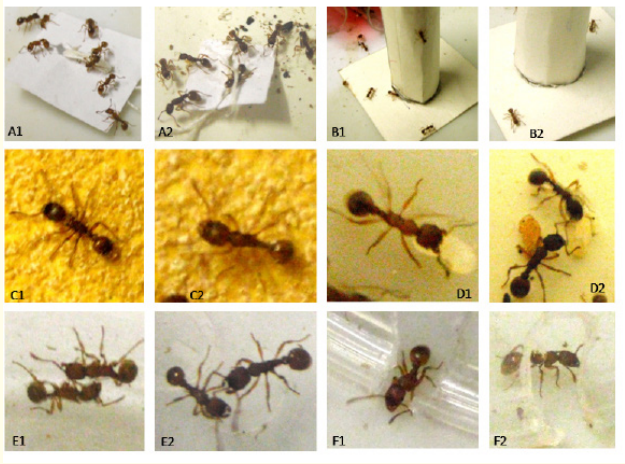


Figure 2: Photos taken while performing experiments for knowing the effect of Apranax (Naproxen) on six ants' physiological and ethological traits. The corresponding numerical results are given in Tables 1 and 2. Photos 1: ants under normal diet; photos 2: ants consuming Apranax. A: ants coming to a tied nestmate, not well orienting them while consuming the drug. B: ants coming onto a risky apparatus, less inclined to do so while consuming the drug. C: ants walking on a rough substrate, with less difficulty while consuming the drug. D: ants taking care of their brood, but poorly doing so while consuming the drug. E: nestmates presenting some aggressive reaction while consuming the drug. F: enclosed ants not escaping while consuming the drug.

Results and Discussion

Meat and sugar water consumption, general activity

These three physiological traits were affected by Apranax consumption (Table 1). While consuming this drug, the ants eat far less meat than while living under normal condition, this being statistically significant (N = 6, T = 21, P = 0.016). They also drunk les sugar water than when not consuming Apranax, and this was also statistically significant (N = 6, T = 21, P = 0.016). Over time, the ants having Apranax at their disposal appeared to increase their sugar water intake (Table 1), and to stay on this water during rather long times (personal observation). It was thus examined, in two following experiments, if the ants needed water, or wanted consuming the drug, having develop some dependence on its consumption (see the paragraphs relative to 'potential need of water' and to 'dependence'). While consuming Apranax, the ants were largely less active than while maintained under normal condition, and this was significant (N = 6, T = 21, P = 0.016). In the course of their maintenance under the drug diet, the ants became less and less active (Table 1, personal observation). These three important adverse effects of Apranax are not in favor of its use, and should be considered while treating patients with this drug.

Days	Normal diet			Diet with Apranax		
	On meat	On sugar water	Active	On meat	On sugar water	Active
I	3.25	5.62	20.50	0.38	0.38	7.00
II	3.00	5.75	18.00	0.38	0.25	7.25
III	2.13	7.00	12.62	0.25	3.75	7.00
IV	3.75	7.37	17.25	0.25	4.25	6.25
V	3.00	13.38	24.37	0.25	4.00	5.00
VI	2.88	12.00	26.25	0.25	4.25	3.75
I-VI	3.00	8.52	19.83	0.29	2.81	6.04

Table 1: Effect of Apranax (Naproxen) on ants' food intake and general activity. The table gives the mean numbers of ants counted during six days on their meat, on the sugar water, and being active at any place (lines I to VI), as well as the mean of these six mean numbers for each kind of counts (last lines). Apranax (Naproxen) appeared to impact these three physiological traits, the effect on sugar water intake and the activity having a tendency to increase over time.

Linear and angular speeds

These traits were largely impacted by Apranax consumption (Table 2, lines 1, 2). This was obvious to observers: the ants had dif-

ficulty in walking, sometimes falling down, trebling, and stopping, keeping their body very near the ground, touching the substrate with their antennae, and seldom folding their body. The difference between ants consuming Apranax and ants under normal maintenance as for their linear and angular speeds was significant: linear speed: $\chi^2 = 80.00$, dif = 1, $P < 0.001$; angular speed: $\chi^2 = 24.54$, dif = 2, $P < 0.001$. On the basis of observations, numerical results and statistical analyses, it could be affirmed that it was essentially the linear speed (the speed of locomotion) which was impacted by Apranax consumption, and that the effect on the angular speed may simply be a consequence of this impact on the linear speed. Such an adverse effect of Apranax allows presuming impact on other biological traits (a presumption checked in the following here below related experiments), and is not in favor of Apranax use.

Orientation to a tied nestmate

This ethological trait was affected by Apranax consumption (Table 2, line 3; Figure 2 A). Indeed, while consuming this drug, the ants poorly oriented themselves towards a tied nestmate, and their orientation values statistically differed from those presented by ants living under normal maintenance ($\chi^2 = 46.03$, df = 1, $P < 0.001$). This may be due to a decrease of the olfactory perception and/or of the usual social relationships of the ants consuming Apranax. These two potential causes were examined in the course of three following experiments (see the paragraphs concerning the ants' tactile perception, brood caring behavior and social relationships).

Audacity

This ethological was impacted by Apranax consumption (Table 2, line 4; Figure 2 B). While consuming this drug, the ants hesitated to come onto the provided risky apparatus and, if coming on it, did not stay there a long time. The difference between the ants under a diet with Apranax and those under a normal diet as for their presence on the risky apparatus was statistically significant (N = 5, T = 15, P = 0.031). This result was in agreement with that on the ants' activity which was decreased by Apranax consumption (see the above paragraph relative to the ants' activity).

Tactile (pain) perception

This important physiological trait was highly affected by Apranax consumption (Table 2, lines 5, 6; Figure 2 C). Ants under normal diet, perceiving the uncomfortable character of the rough substrate, walked on it statistically far more slowly and more sinuously than on their foraging area. This was significant: linear speed: $\chi^2 = 52.05$, df = 1, $P < 0.001$; angular speed: $\chi^2 = 64.00$, df = 1, $P < 0.001$. On the contrary, while consuming Apranax, the ants walked on the emery paper at a linear and an angular speed statistically similar to that presented on a normal substrate: linear speed: $\chi^2 = 1.58$, df = 1, $0.20 < P < 0.30$; angular speed: $\chi^2 = 0.61$, df = 2, $0.30 < P < 0.50$. They thus not at all perceive the uncomfortable character of the substrate. This is due to the mode of action of Apranax, and not perceiving pain is effectively the wanted effect of this drug. However, on ants, it appeared that the sensitive perception was really largely decreased, what may potentially have dangerous consequences. Such an affect of Apranax must be known and considered when treating patients with this drug.

Traits	Normal diet	Diet with Apranax
Linear speed (mm/s)	10.5 (9.7 - 12.1)	5.2 (4.6 - 6.1)
Angular speed (ang.deg./cm)	116 (99 - 128)	164 (142 - 195)
Orientation (ang.deg.)	30.4 (24.2 - 38.3)	75.4 (57.8 - 89.4)
Audacity (n°)	3.65 [3 - 5]	1.35 [0 - 2]
Tactile perception:		
Linear speed	5.1 (4.8 - 5.6)	5.8 (5.5 - 6.3)
Angular speed on a rough substrate	267 (246 - 294)	167 (154 - 196)

Table 2: Effect of Apranax (Naproxen) on five ants' physiological and ethological traits. The table gives the median (and quartiles) or the mean and [extremes] of the recorded data. Apranax (Naproxen) statistically impacted all these traits. See explanation in the text, and photos in figure 2.

Brood caring behavior

This behavior was affected by Apranax consumption (Table 3, line 1; Figure D). Under normal diet, the ants rapidly found the larvae removed from the nest, held them with their mandibles, and transported them towards the nest entrance. While consuming Apranax, the ants delayed in finding the larvae, in taking them with

their mandibles, and if succeeding in doing the latter act, they could not always find the nest entrance. Such a behavior was in agreement with that presented in the vicinity of a tied nesmate (see the subsection relative to the ants' orientation). The numbers of not re-entered larvae among the 10 experimentally removed from the nest statistically differed between the ants maintained under normal and maintained under a diet with Apranax (N = 6, T = 21, P = 0.016). Such an impact of the drug may lead to social relations problem - a hypothesis examined in the next experiment -, and is not in favor of this drug use.

Traits	Normal diet	Diet with Apranax
Brood caring: n° of not re-entered larvae over 5 minutes	30'' 1' 2' 3' 4' 5' 10 9 8 4 0 0	30'' 1' 2' 3' 4' 5' 12 11 9 7 5 5
Social relationships: n° of 0 to 4 aggressive levels; variable 'a'	0 1 2 3 4 a 70 52 14 0 0 0.11	0 1 2 3 4 a 24 32 63 0 0 1.13
Stress and cognition: n° of ants escaped over 12 minutes	2 4 6 8 10 12 min 2 5 8 9 11 12	2 4 6 8 10 12 min 0 0 0 0 2 4
Cognition: n° of ants in front and beyond a twists and turns path over 12 minutes	2 4 6 8 10 12 In front 25 19 16 14 11 12 Beyond 0 4 6 8 10 12	2 4 6 8 10 12 In front 27 25 23 21 19 17 Beyond 0 0 0 0 1 2

Table 3: Effect of Apranax (Naproxen) on four ants' physiological and ethological traits. The table gives, for each trait, the numbers (of ants, larvae, and aggressive levels according to the examined trait) recorded over a given time. Apranax appeared to impact all these traits, i.e. the social relationships, the state of stress, and the cognition. Photos can be seen in Figure 2 and 3. Details are given in the text.

Social relationships towards nestmates

As presumed in the previous experiment, the ants' social relation was affected by Apranax consumption (Table 3, line 2; Figure 2 E). The experimented under normal diet ants often stayed side by side, contacting each other with their antennae, and seldom slightly opening their mandibles. The ants consuming Apranax seldom stayed side by side, and when briefly doing so, they often largely opened their mandibles. The distribution of the recorded values of aggressive levels statistically differed between the ants maintained normally or maintained with Apranax at their disposal ($\chi^2 = 57.73$, df = 2, $P < 0.001$). Such an impact of the drug on the individuals' relationships is not in favor of its use, and should be known by patients and by practitioners treating patients thanks to Apranax.

State of stress and cognition through escaping from an enclosure

Apranax affected this physiological and ethological trait (Table 3, line 3; Figure 2 F). While ants lived under normal diet, the 12 enclosed ones could escape over the twelve experimental minutes.

Figure 3: Photos of experiments allowing to know the effect of Apranax (Naproxen) on the ants' cognition (A), learning and memorizing (B), adaptation to the side effect of the drug (C), habituation to its wanted effect (D), death of ants (E), need of water (F), and dependence on its consumption (G). 1: ants under normal diet, 1: ants consuming Apranax. The drug affected the ants' cognition and learning. No adaptation occurred. Habituation occurred. The ants needed water (blue dot). The ants developed some dependence on Apranax consumption (the drug solution is indicated thanks to a red dot).

While the ants consumed the drug, only 4 among the twelve enclosed ones could escape during the same time period. The difference between the ants maintained under one and the other kind of diet as for the numbers of escaped ants over time was significant: N = 6, T = 21, P = 0.016. This important adverse effect of Apranax on the cognition was again examined thanks to the following experiment (see below).

Cognition

Apranax affected the ants' cognition (Table 3, line 4; Figure 3 A). Over the twelve experimental minutes, 13 ants under normal diet could cross the twists and turns path. Over the same time period, only 2 ants consuming the drug could cross the same path. The numbers of ants present over time in front as well as beyond the twists and turns path statistically differed between the ants maintained under one and the other kinds of diet: in front: N = 6, T = 21, P = 0.016; beyond: N = 5, T = 15, P = 0.031. This result was in agreement with that of the previous experiment, and the impact of Apranax on the ants' cognitive abilities was again examined in the following experiment (see below).

Visual operant conditioning and memory

The ants' conditioning acquisition was affected by Apranax consumption (Table 4; Figure 3 B). Ants under normal diet acquired a conditioning score of 70% after 31 training hours, and of 85% after such 72 hours. Ants consuming the drug kept a conditioning score of 45 - 55% all along the 72 training hours. The difference between the acquisition of conditioning by the ants under one and the other kinds of diet was significant: N = 6, T = 21, P = 0.016. Apranax impacted thus the ants' short-term memory. Since the ants consuming this drug here learned nothing, their middle-term memory could not be assessed. Nevertheless, these ants went on going to their food sites and returning to their nest; their long-term memory was thus probably not, or only little, affected by Apranax consumption. This adverse effect of Apranax on the short-term memory should be taken into account while treating patients with this drug.

Adaptation (tolerance) to Apranax adverse effects

The ants did not adapt themselves to the adverse effect of Apranax on their speed of locomotion. Indeed, their linear speed after seven days of the drug consumption was statistically similar to that presented after one day of consumption: $\chi^2 = 0.069$, df = 1, P ~ 0.80 (Table 5, first part). This was very obvious to the observers: the ants walked slowly all over the entire experimental work and often presented difficulties in moving (Figure 3 C). This not adaptation should be considered when treating humans with Apranax: the time period of the treatment should be as short as possible.

Habituation to Apranax wanted effect

Habituation to the reducing effect of Apranax on pain perception occurred (Table 5, second part; Figure 3 D). After eight days of this drug consumption, the ants walked on a rough substrate with far more difficulty than after three days of the drug consumption. Their linear speed was statistically lower ($\chi^2 = 17.03$, df = 2, P < 0.001), and their angular speed statistically larger ($\chi^2 = 37.09$, df = 2, P < 0.001). This was obvious to the observer, is not in favor of the

Time (hours)	Normal diet	Diet with Apranax: correct *vs* wrong responses colony A, colony B
7 hs	6 *vs* 4 60%	5 *vs* 5, 4 *vs* 6 45%
24 hs	6 *vs* 4 60%	5 *vs* 5, 4 *vs* 6 45%
31 hs	7 *vs* 3 70%	4 *vs* 6, 4 *vs* 6 40%
48 hs	7 *vs* 3 70%	5 *vs* 5, 5 *vs* 5 50%
55 hs	8 *vs* 2 80%	4 *vs* 6, 4 *vs* 6 40%
72 hs	9 *vs* 1 85%	6 *vs* 4, 5 *vs* 5 55%
Cue removal		
7 hs	9 *vs* 1 85%	
24 hs	8 *vs* 2 80%	
31 hs	8 *vs* 2 80%	
48 hs	8 *vs* 2 80%	The ants having not learned, their memory could not be assessed
55 hs	8 *vs* 2 80%	
72 hs	8 *vs* 2 80%	

Table 4: Effect of Apranax on the ants' conditioning acquisition and memorization. The table gives the numbers of trained ants of the two used colonies which gave the right and the wrong responses when tested in a Y maze, and on this basis, the obtained collective levels of conditioning. Apranax decreased the ants' learning, and thus their short-term memory. See details in the text and two photos in Figure 3.

Traits	Recorded values
Adaptation to side effect	Ants' linear speed after having consumed the drug during: one day: 5.2 (4.6 - 6.1); seven days: 5.6 (4.7 - 6.1)
Habituation to wanted effect	Ants' linear and angular speeds on a rough substrate after they consumed the drug for: three days: linear speed: 5.8 (5.5 - 6.3); eight days: linear speed: 4.3 (3.8 - 4.7) angular speed: 167 (154 - 196); angular speed: 320 (282 - 330)
Potential need of water	n° ants on the sugared solution; n° of ants on the aqueous solution of the drug colony A: 8 colony B: 14 → 25.88%; colony A: 27 colony B: 36 → 74.12%
Potential dependence	n° ants on the drug solution; n° of ants on the drug-free solution colony A: 35 colony B: 39 → 68.5%; colony A: 18 colony B: 16 → 31.5%

Table 5: Ants' adaptation to the side effect of Apranax (Naproxen), habituation to its wanted effect, potentially induced need of water, and potential dependence on its consumption. Briefly, no adaptation occurred, habituation occurred, ants needed water, and ants developed some dependence on Apranax consumption. More information can be found in the text, and six photos in figure 3.

drug use, and should be considered when treating patients with Apranax (i. e. the patients, less feeling the drug wanted effect, may be tempted to increase their drug daily dose, what would accentuate the drug adverse effects).

Unexpected not quantified observation

Nine days after the ants had Apranax at their disposal, we discovered 10 to 20 dead ants at the nest entrance (Figure 3 E). There was no dead ant in the other twelve colonies maintained exactly in the same way, in the same room, with the same food. The only possible cause of such deaths in colonies provided with Apranax was the presence of this drug in the ants' sugar water. Dehydration might be at least one of the causes of such deaths. This presumption was examined thanks to the next experiment (see below).

Potential need of water under Apranax maintenance

Effectively, ants consuming Apranax needed drinking water (Table 5, third part; Figure 3 F). During the experiment, 8 ants of colony A were sighted on the sugared solution of the drug and 27 ones on its aqueous solution. At the same time, 14 ants of colony B were sighted on the sugared solution of Apranax and 36 ones on its aqueous solution. In total, 25.88% of the ants chose the sugared solution of Apranax and 74.12% chose its aqueous solution. The obtained numbers of counted ants (8 + 14 = 22, 27 + 36 = 63) statistically differed from those (42.5, 42.5) expected if the ants randomly went onto the two kinds of solution ($\chi^2 = 9.50$, df = 1, $0.001 < P < 0.01$). The ants wanted thus drinking water. However, this was not highly significant, and ants may thus also want to consume Apranax. This latter presumption was examined in the following experiment (see below).

Dependence on Apranax consumption

Briefly, the ants developed some dependence on Apranax consumption (Table 5, fourth part; Figure 3 G), and this was statistically significant though not very highly significant ($\chi^2 = 6.92$, df = 1, $0.001 < P < 0.01$). In details, 35 ants of colony A were counted on the drug solution while 18 ones were counted on the drug-free solution. Similarly, 39 ants of colony B were sighted on the drug solution while 16 ones were sighted on the drug-free solution. These numbers leaded to 68.5% of ants' visits to the drug solution and 31.5% of ants' visits to the drug-free solution. The added numbers recorded for the two solution (74 and 34) statistically differed from those expected if the ants randomly went onto each presented solution (54, 54) at a level of probability lower than 0.01. The ants' habituation to the wanted effect of Apranax (see the previous paragraph) and their dependence on this drug consumption may occur in humans. If these two events occur in humans, the latter will be tempted to increase their daily dose of Apranax, and to continue consuming this drug over time, what will accentuate the adverse effects of the drug. Potential habituation and dependence should thus be checked in patients treated with Apranax. In other words, no larger dose and no longer time than those previously decided should be used.

Decrease of the effects of Apranax after its consumption was stopped

Briefly, after weaning, the effect of Apranax rapidly decreased in a total of 12-13 hours. In details, as soon as 4 hours after weaning, the effect of this drug was statistically ($P < 0.01$) lower than its initial one, and 6 hours after weaning, it was highly significantly lower ($P < 0.001$). In fact, in 6 hours, the drug lost 65.12% of its efficiency. This could be perceived by consumers who could then be tempted to intake the drug before the initially advised delay. After the six first hours following the weaning of Apranax, the effect of this drug went on decreasing. It lost 15.49% of its value from 6 to 10 hours (so, in 3 hours), then 29.27% of its value from 10 to 16 hours (so, in 6 hours). Consequently, the decrease of the effect of Apranax after weaning was initially very rapid, then slightly less rapid over time. Twelve hours after weaning, the effect of Apranax was statistically still slightly different from or nearly similar to the control value (in fact, $P = 0.05$). We could thus conclude that this drug lost its effect in about 12-13 hours. Indeed, 14 then 16 hours after weaning, the effect of Apranax was statistically similar to that of the control (respectively $P \sim 0.20$, $0.50 < P < 0.70$). Such a decrease accounted for the development of dependence on Apranax consumption (see the Discussion and Conclusion subsection).

Mathematically, the decrease of the effect of Apranax after its consumption was stopped could be well described by a linear function ($R^2 = 0.9862$), but, although statistically not significantly, could be somewhat better described by a quadratic function ($R^2 = 0.9914$). Thus, to describe the loss of the effect of Apranax after weaning, the following function fits well:

$$V_t = V_i + t^{0.3858}$$

With V = linear speed (mm/s), t = time (hours), V_i = initial speed, V_t = speed at time t

These experimental and mathematical results are graphically presented in figure 4.

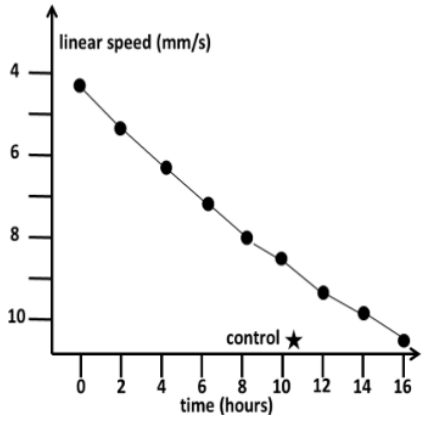
Time (hours)	Median (quartiles) of linear speed (mm/s)	Statistical results *vs* t = 0			Statistical results *vs* control			
		χ^2	df	P	χ^2	df	p	
T = 0 h	4.3 (4.1 - 4.7)	1.99	--	< 0.50	60.00	1	<0.001	
2 hrs	5.4 (5.1 - 5.9)	8.77	2	< 0.01	60.00	1	<0.001	
4 hrs	6.2 (5.8 - 6.9)	22.55	1	< 0.001	51.42	1	<0.001	
6 hrs	7.1 (6.4 - 7.7)	32.73	1	< 0.001	47.08	1	<0.001	
8 hrs	8.0 (7.6 - 8.7)	32.73	1	< 0.001	23.34	1	<0.001	
10 hrs	8.2 (7.3 - 9.0)	32.73	1	< 0.001	23.34	1	<0.001	
12 hrs	9.6 (8.9 - 10.8)	40.00	1	< 0.001	3.84	1	= 0.05	
14 hrs	9.8 (8.6 - 10.5)	25.57	1	< 0.001	1.61	1	~ 0.20	
16 hrs	10.6 (9.3 - 11.4)	60.00	1	< 0.001	0.027	1	< 0.70	
Control	10.5 (9.7 - 12.1)							

Table 6: Decrease of the effect of Apranax after its consumption was stopped. The table gives the median (and quartiles) of the values of ants' linear speed recorded over this decrease. These numerical results are also illustrated in Figure 4. Briefly, the effect of Apranax decreased in a total of 12-13 hours, rapidly during the first hours, somewhat less rapidly thereafter. Details can be found in the text.

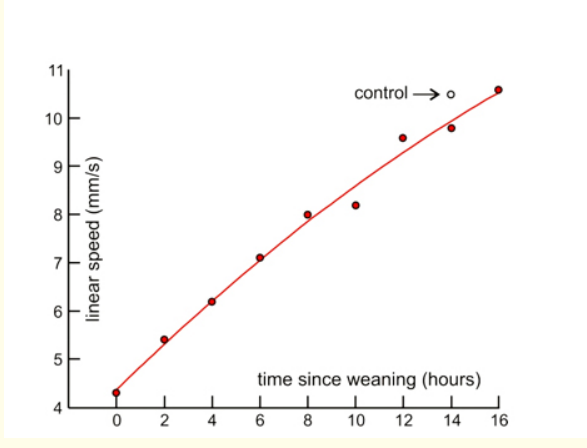


Figure 4: Decrease of the effect of Apranax (Naproxen) after its consumption was stopped. The numerical results are given in table 6, and information is furnished in the text. The effect of the drug vanished in 12-13 hours, according to a quadratic function (nevertheless approaching a linear function), and already became very different from its initial effect 6 hours after weaning, what accounted for the development of dependence on this drug consumption.

Discussion

On the basis of personal experience, i.e. feeling side effects while consuming Apranax, and finding no easily available and precise information about such potential side effects, we aimed to examine, on ants as models, the impact of Apranax on nine ethological and physiological traits, as well as the decrease of the effect of this drug after its consumption was stopped. We found that Apranax impacted the ants' locomotion, orientation ability, audacity, tactile (pain) perception, social relationships, cognition, learning and memory; that no adaptation occurred to the side effects, but that habituation occurred to the wanted analgesic effect of the drug. We also pointed out the need of water induced by Apranax, and the development of dependence on its consumption. Many ants died in the course of our experimental work. Finally, we showed that the effect of Apranax rapidly decreased as soon as just after weaning (see a few lines below), and totally vanished in 12-13 hours according to a quadratic function. In several internet sites devoted to this drug, it is generally admitted that the half-live of Naproxen equals 12-15 hours. This perfectly agrees with our finding, i.e. the effect of the drug became just not different from that of the control 12 hours after weaning, and not longer efficient thereafter. In ad-

dition, we showed that, during the six first hours after weaning, Apranax rapidly lost its effect which became, at the end of these six hours, highly significantly distinct from its initial effect. Such a decrease could have adverse consequences on the consumers' intake of the drug and on their potential development of dependence on its use. Indeed, dependence on a product occurs when the effect of this product rapidly decreases after its use was stopped [22].

Despite all the adverse effects we found, Naproxen (Apranax) has been numerously proved to have an efficient anti-inflammatory effect and to present few adverse effects [23-25]. Even when compared to other anti-inflammatory drugs, Naproxen is estimated as being the best or at least among the best anti-inflammatory drug(s) [23,26].

Only few experimental works concerns the adverse effects of Naproxen. One of them examined the side and severe effect of Naproxen and Rofecoxib on the upper part of the gastrointestinal track, and showed that these side effects were lower for Rofecoxib than for Naproxen [27]. The side effects of Naproxen are also reported in a clinical study made on patients with osteoarthritis [28]. Another experimental work concerns the recognized adverse effect of Naproxen and Celecoxib on the kidney; it showed that Naproxen is far more toxic than Celecoxib [29]. In addition, the latter work reports several other side effects of Naproxen, and underlines the frequent use of anti-inflammatory drugs by elderly persons together with the great susceptibility of these persons for such drugs. Attention should thus be paid while treating patients, essentially the older ones, with Apranax. However, the few works, as those reported here above, scientifically relating some precise adverse effects of Naproxen (without naming Apranax), are not easily available and understandable by most people, and therefore, the severe adverse physiological and ethological effects of Naproxen (so of Apranax) are not commonly known. Moreover, this state of fact contrasts with the marketing and praise for this drug [30].

Finally, let us add that Naproxen (and thus Apranax), which are largely used, present some not negligible ecotoxicity, and systems for reducing this harmful consequence of the drug use should be set up [31,32]. This kind of pollution is valid for many products used by humans [33,34].

Conclusion

Apranax (the active substance of which is Naproxen) is a largely used anti-inflammatory drug. It is really very efficient and so, very useful for many patients. However, it has several severe adverse effects, impacting the food intake, the locomotion, the sensory perception, the social relationships, the cognition, the learning and memory. No adaption to its side effects occurs, but habituation to its analgesic effect occurs. It leads to water requirement and to dependence on its consumption. People are not sufficiently and correctly informed about these adverse effects of Apranax. Practitioners should become acquainted with our findings, and accordingly take care of the health of their patients, essentially of their elderly patients, treated with Apranax. They must try to use, case by case, the smallest possible dose, during the shortest possible time period, and even, if necessary, they must try to employ another less toxic anti-inflammatory drug. Apranax (Naproxen) contributes with plenty of other drugs, to pollute the natural environmental water, imperiling so the life of vertebrate and invertebrate animals.

Conflict of Interest

We affirm having no conflict of interest concerning the use of Naproxen (Apranax). We work on ants, on their biology, and we use them as biological models for examining several subjects. We receive no money for making our research.

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