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ASPARTAME INCREASES FOOD DEMAND AND IMPACTS BEHAVIOR: A STUDY USING ANTS AS MODELS

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ABSTRACT

Using ants as biological models, we showed that aspartame, probably the most consumed sweetener in the world, appeared to have unwanted ethological and physiological effects. Compared to a diet made of meat and sugar, it increased the ants' meat consumption, speed of locomotion, and 'audacity'. It drastically impacted visual and olfactory memory, reduced the precision of reaction, response to pheromones, brood caring behavior and cognition. When having to choose between a solution of aspartame and one of sugar, the ants largely chose the latter one. The physiological and ethological perturbations seen in ants may be attributed to the fact that though being not a glycoside, aspartame gives to the brain the 'presence of sugar' information. Using ants as models, we conclude that aspartame, consumed in moderate amount, does not impact health but induces severe disturbances in behavior and cognitive abilities.

INTRODUCTION

Many persons consume sweeteners as a substitute of sugar or as a constituent of 'light' food and drinks (pastries, creams, yogurts, sodas …). Today, the most and even nearly exclusively used sweetener instead of sugar is aspartame, a molecule made of two amino acids and a methyl function (Figure 1). Aspartame is present in at least 6,000 products [1-2] and its possible adverse effects still lead to unsolved questions. Some of these adverse effects are due to the fact that, in water, at moderate temperature and at some pH, aspartame hydrolyzes into aspartic acid, phenylalanine and methanol [1-2], the latter component having been claimed to produce formaldehyde adducts in animal live tissues [3]. Aspartame also produces formaldehyde and formic acid as well as diketopiperazine under temperature well above 37°. Diketopiperazine appears of course when the sweetener is warmed while cooking and is thus unlike to appear in humans' body (at 37°C) [2-3]. However, the intact molecule itself might have some (even still unknown) unwanted effects. It is not ruled out that studies already made on the impact of aspartame on health [4-5-6-7-8 for instance] could have been performed under conflict of interest. They might have been done on behalf of aspartame furnishers who would make known that aspartame has no adverse effect, or by practitioners who would prefer that humans do not use potentially dangerous artificial food substances.

We aimed thus to examine – on ants as a natural biological model – what are the effects of the aspartame solutions commonly used by humans and maintained at room temperature – on the complete food consumption (and consequently, the potential food demand), the learning capability and memory, the locomotion, precision of reaction, response to a pheromone, audacity, brood caring, and cognition, as well as the natural acceptance of aspartame instead of sugar, – and without any conflict of interest.

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Why using ants as biological models?

Most biological processes are quite similar for all animals, including humans (i.e. genetics, metabolism, nervous cells functioning). Consequently, a lot of invertebrates and vertebrates are used as models for studying biological subjects [9-10-11]. Invertebrates are more and more used for this goal because they offer scientists many advantages, among others a short life cycle, a simple anatomy, and being available in large numbers [12-13]. Some species are largely used as biological models, for instance, the flatworm *Dendrocelium lacteum*, the nematode worm *Caenorhabdotes elegans*, the mollusk *Aplysia californica*, the beetle *Tribolium castaneum*, the fruit fly *Drosophila melanogaster*, and the domestic bee *Apis mellifera*. Among the invertebrates, insects, especially social hymenoptera and among them, bees, are advantageously used as biological models [14-15], but ants too can be used. Indeed, colonies containing thousands of ants can easily be maintained in laboratories, at low cost and very conveniently, throughout the entire year. Ants are among the most complex and social invertebrate animals as for their morphology, physiology, social organization and behavior. They are among the most morphologically evolved hymenoptera, having indeed a unique resting position of their labium, mandibles and maxilla [16], as well as a lot of glands emitting numerous efficient pheromones [17]. Their societies are highly organized with a strong division of labor, an age-based polyethism and a social regulation [18]. Their behavior is well developed: they care for their brood, build sophisticated nests, chemically mark the inside of their nest, and, differently, their nest entrances, nest surroundings and foraging area [19]. They generally use an alarm signal, a trail pheromone, and a recruitment signal [19]; they are able to navigate using memorized visual and olfactory cues [20 and references therein]; they efficiently recruit nestmates where, when and as long as it is necessary [21], and, finally, they clean their nest and provide their area with cemeteries [22]. According to the complexity of their society and their behavior, it looks reasonable to use ants as biological models for studying physiological and ethological effects of substances, treatments or situations.

During many years, we worked on ant's species belonging to the genus *Myrmica*, and among others, on *Myrmica rubra* Linnaeus 1758. We know about its annual cycle [23], its ecological traits, eye morphology [24], subtended angle of vision [25], visual perception [26], navigation system [20], visual and olfactory conditioning capabilities [27], and recruitment strategy [28]. The ontogenesis of cognitive abilities of *Myrmica* species has also been approached [29-30-31-32-33-34]. Studies on the impact of age, activity and diet on the conditioning capability of the related species *M. ruginodis* [35] led to presume that ants could be good biological models.

This was confirmed by the study of the effects of caffeine, theophylline, cocaine, and atropine [36], of nicotine [37], of morphine and quinine [38], of fluoxetine (an 'ISRS' antidepressant) [39], of anafranil (an 'ACT' antidepressant) and of efexor (an 'IRSNa' antidepressant) [40], of carbamazepine [41], and finally of buprenorphine and methadone [42]. Each time, we observed effects related to those observed on humans, and brought information and precision on them. Here, we used ants, and more precisely those of the species *M. rubra*, once more as biological models for examining effects of aspartame.

Why aspartame stays at moderate temperature during the present study, and what is the consequence?

The temperature of the laboratory was maintained at about 20°C (extremes: 18 – 22 °C) and the aspartame solution had a pH of 7.5. The kinetics of the aspartame hydrolyzation depends on the temperature and the pH of the solution, aspartame being most stable at low temperature and a pH of 4 – 5 [1-2-43]. According to Homler [43], after 15 hours at 40°C, only ca 10% of intact aspartame still remains in an aspartame solution. However, hydrolyzation is dramatically reduced with decreasing temperature. At 25°, under a pH of 7.5, the aspartame solution stability lasts one day [43]. At the laboratory temperature of 20° C, it should last longer, perhaps two days, and the solution was indeed renewed each two days (see section 'Aqueous solution of aspartame'). Ants were thus under some aspartame diet during the time periods they received aspartame instead of sugar or pure water.

Secondly, ants are invertebrates and have thus cold blood (haemolymph), i.e. blood at the ambient temperature, that is about 20°C, a temperature at which aspartame should not much hydrolyze into dangerous compounds [1-2-43].

Why are the planned assessments possible?

The ants' food being given on their foraging area, at a clearly visible place, it is easy to assess food consumption. The ants' acquisition of a visual and an olfactory conditioning, as well as their visual and olfactory memory can be quantified using an already set up experimental protocol. The ants' locomotion (linear and angular speeds), precision of reaction (orientation towards an alarm signal), response to a pheromone (trail following behavior), audacity, brood caring, and preference between two kinds of liquid food are also easy to assess using well-tried analytical techniques [42 + references therein].

Why have we no conflict of interest?

We have no conflict of interest since we do fundamental research on the ethology of ants without external funding.

We can thus scientifically use ants for studying some ethological and physiological effects of aspartame.

EXPERIMENTAL PLANNING AND PRESENTATION OF THE RESULTS

The entire experimental protocol is detailed here below and schematized in Figure 2.

The ants were first fed with sugar water (an aqueous solution of saccharose) and *Tenebrio molitor* larva, and their meat (complete) food consumption was assessed. Then, they were fed with an aqueous solution of aspartame and *T. molitor* larvae, and their complete meat consumption was again assessed, exactly as previously (see below in the 'Material and Methods' section). While the ants were still under aspartame diet, and before making exactly the same observations while feeding the ants with pure water and *T. molitor* larvae, we undertook visual then olfactory operant conditioning and assessed, in the course of time, the ants' ability in acquiring such conditioning as well as, after removal of the visual or olfactory cues, and if the case arises, their visual and olfactory memory. The ants appeared to forage more than usual, to move erratically, and to re-enter their nest less often than usually. For keeping the experimental nests in good health, we thus did not set them continuously under aspartame diet and performed the planned experiments in the following order. The ants were then fed with pure water and *T. molitor* larvae, and their complete meat food consumption was assessed exactly as previously, i.e. as under sugar water or aspartame diet. The ants' locomotion (speed and sinuosity), precision of reaction, response to trail pheromone, audacity, brood caring and cognition were then assessed, making so seven control assessments. Thereafter, a choice experiment was performed for quantifying the ants' acceptance of aspartame, when a solution of this substance was presented together with a solution of sugar. The ants were then provided with sugar water and again, their locomotion (speed and sinuosity), precision of reaction, trail following behavior, audacity, brood caring and cognition were assessed, thus making seven other (different) control experiments. After that, the ants were again set under aspartame diet, and the seven assessments made under sugar water and under pure water diets were identically performed (locomotion, precision of reaction, trail following behavior, audacity, brood caring, cognition), this consisting of course in making seven test assessments. Finally, the experiment ended and the ants were definitively fed with sugar water and *T. molitor* larvae.

The results were presented in another order, that is:

- the ants' complete meat consumption under sugar water, pure water and aspartame diet,
- the ants' ability in acquiring visual and olfactory conditioning, as well as their visual and their olfactory memory under aspartame diet. These results were compared to those previously obtained under sugar water diet [27] and under pure water diet [35].
- the ants' locomotion, precision of reaction, trail following behavior, and audacity under sugar water, pure water, and aspartame diet, the three series of results being compared to one another.
- the ants' brood caring and cognition under sugar water, pure water, and aspartame diet, the three results being compared to one another.
- the ants preference between sugar water and an aqueous solution of aspartame.

The results were then summarized, discussed and compared to what has already been observed and/or studied about effects of aspartame.

MATERIAL AND METHODS

Collection and maintenance of ants

The effects of aspartame were studied using six colonies of *M. rubra* labeled A to F (Figure 3 A). Colony A was collected in an abandoned slate quarry located in the Aise valley (Ardenne, Belgium). Colony B was collected at Dour (Hainaut, Belgium) on the abandoned coal mining slag heap St Charles. The colonies C - F were collected at Haine St Paul (Hainaut, Belgium), on the slag heap named Chef Lieu. All the colonies were maintained in the laboratory in artificial nests made of one to three glass tubes half-filled with water, a cotton-plug separating the ants from the water. The glass tubes were deposited in trays (34 cm x 23 cm x 4 cm), which internal sides were slightly covered with talc to prevent the ants from escaping (Figure 3 A). These trays served as foraging areas, food being delivered in them. The ants were fed with sugar-water provided ad libitum in a small glass tube plugged with cotton, and with two half-cut *Tenebrio molitor* larvae (Linnaeus 1758) provided twice a week on a glass slide. During experiments, the sugar water was replaced by either pure water, or an aqueous solution of aspartame (see below) each two being delivered to the ants exactly as their usual sugar water. Temperature was maintained between 18°C and 22°C with a relative humidity of circa 80% all over the course of the study. Lighting had a constant intensity of 330 lux while caring for the ants, training and testing them. During other time periods, lighting was dimmed to 110 lux. The ambient electromagnetic field had an intensity of 2-3 µW/m2. All the members of a colony are here named nestmates, as commonly done by researchers on social hymenoptera.

Aqueous solution of aspartame

Aspartame was furnished by the pharmacist J. Cardon (1050, Bruxelles) in the form of tablets (0.085 gr = 85 mg) containing 8.5 mg of aspartame, made by the manufacturer 'Canderel', one of the most available source of aspartame and the most consumed one. The aqueous solution of that sweetener given to the ants was prepared according to, on one hand, the concentration in sugar (so the sugared taste) of the sugar water usually consumed by ants in nature as well as in laboratory, and, on the other hand, the quantity of aspartame commonly consumed by humans. On one hand, the sugar water usually consumed

by ants is a nearly saturated solution of glycosides (glucose, saccharose …). For instance, for usual maintenance of ants in laboratory, we poor ten small spoons (= 5 gr x 10 = 50 gr) of brown sugar into 150 – 200 ml of tap water for obtaining a sugared solution the ants obviously appreciate. During the experimental time period, the water had a pH of 7.5. For obtaining the same sugared taste using aspartame, ten tablets of 'Canderel' (850 mg = 85 mg of aspartame mixed to lactose; www.canderel.ch/FR/informations/faq.html) should be dissolved into 150 ml of water. On the other hand, the quantity of aspartame consumed by humans is one tablet of 'Canderel' with each small cup (or glass) of a drink, so one tablet into 150 – 180 ml of liquid. Insects consume about ten less water than mammals. It could be estimated that the most appropriate solution of aspartame to be given to ants for being in agreement with the ants' usual sugar food and with the amount of aspartame commonly consumed by humans would be ten tablets of 'Canderel' into 150 – 180 ml of water. Therefore, it can be deduced that the best aqueous solution of aspartame which should be given to the ants should be made by scratching then dissolving ten tablets of 'Canderel' into 150 ml of water. We dissolved three tablets of 'Canderel' into 45 ml of water and poured 5 ml of that solution into six small tubes usually used for providing sugar water to the ants. The solution of aspartame was 10 times more concentrated than those commonly used by humans, an important experimental detail since aspartame progressively hydrolyzes into not sweetened compounds (see the 'Introduction' and [1-2-3]). The tubes containing the aspartame solution were plugged with cotton which was refreshed each day. The entire solution was renewed every two days, so largely before it lost sweeten savor i.e. before aspartame splits into amino acids, what was checked by tasting it. It was also checked each day if ants actually consumed the aqueous solution of aspartame. The ants effectively and spectacularly consumed that solution, even more than their usual sugar water, and this until the day the solution was renewed (Figure 3 B).

Ants' complete food consumption

For assessing the ants' food consumption under sugar water, pure water and aspartame diet, we counted for each diet (one diet at a time, a time lapse of three days between each diet) during five consecutive days, and twice each day, exactly at the same time o'clock and under identical conditions (giving food or not, temperature, humidity, light), the ants of the six used experimental colonies present on the provided half-cut *T. molitor* larvae given on days 1, 3 and 5 (Table 1, daily counts; Figure 3 C). We then established the mean values per day (= mean of 12 i.e. 6 x 2 counts; in total 5 mean values; Table 1, daily means), as well as the mean of all the counts performed under each diet (Table 1, total mean). As foraging is a collective task performed by nearly always the same individuals, the five mean values per day obtained for each diet could be compared to one another using the non parametric test of Wilcoxon [44].

Ants' conditioning ability and memory

These traits were examined on the experimental colonies A, B, D and E.

Briefly, at a given time, either a green hollow cube or pieces of fennel were set above or aside respectively the pieces of *T. molitor* larvae given as food to four of the six used colonies. The ants of these colonies so underwent each time either visual or olfactory operant conditioning. Tests were then performed in the course of time, while the ants were expected acquiring conditioning then, after having removed the green hollow cube or the pieces of fennel, while the ants were expected to partly lose their conditioning.

In detail, ants were collectively visually trained to a green hollow cube constructed of strong paper (Canson ®) according to the instructions given in [45, Figure 3, upper part] and set over the meat food which served as a reward. The color has been analyzed to determine its wavelengths reflection [46]. The ants could see the cube and easily enter it. Choosing the way with the green arch (see below) was considered as giving the 'correct' choice when ants were tested as explained below. The ants were olfactory conditioned by setting pieces of fennel aside the pieces of *T. molitor* larvae. Choosing the way with the pieces of fennel was then considered as giving the 'correct' choice when ants were tested as explained below.

Ants were individually tested in a Y-shaped apparatus (Fig. 3 D) constructed of strong white paper according to the instructions given in [45, Figure 3, middle part], and set in a small tray (30 cm x 15 cm x 4 cm), apart from the experimental colony's tray. Each colony had its own Y apparatus. The apparatus had its own bottom and its sides were slightly covered with talc to prevent the ants from escaping. In the Y-apparatus, the ants deposited no trail since they were not rewarded. However, they could utilize other chemical secretions as traces. As a precaution, the floor of each Y-apparatus was changed between tests. The Y-apparatus was provided with either a green arch [45, Figure 3, upper part), or pieces of fennel, in one or the other branch. Half of the tests were conducted with the arch or the odorous plant in the left branch and the other half with the arch or the odorous plant in the right branch of the Y maze, and this was randomly chosen. Control experiments had previously been made on never conditioned ants and on trained ants of colonies being under sugar water diet [27, Tables 1, 2]. During another work, conditioning has been tempted on ants under pure water diet [35]. This had to be done because, once an animal is conditioned to a given stimulus, it becomes no longer naïve for such an experiment. It was thus impossible to perform, on the same ants, conditioning first under sugar diet, then under aspartame diet, and later on, under pure

water diet. The only solution was to use previous results obtained in the course of identical experiments made on similar colonies being under sugar diet [27], and to use previous results obtained on colonies maintained on pure water diet [35].

To conduct a test on a colony, 10 workers - randomly chosen from the foragers of that colony - were transferred one by one onto the area at the entrance of the Y-apparatus. Each transferred ant was observed until it turned either to the left or to the right in the Y-tube, and its choice was recorded. Only the first choice of the ant was recorded and this only when the ant was entirely under the cube, i.e. beyond a pencil drawn thin line indicating the entrance of a branch (Figure 3 D). Afterwards, the ant was removed and transferred into a polyacetate cup, in which the border was covered with talc, until 10 ants were so tested, this avoiding testing twice the same ant. All the tested ants were then placed back on their foraging area. For each test, the numbers of ants belonging to the four used colonies (n = 10 x 4 = 40 ants) which turned towards the "correct" green arch or the pieces of fennel, or went to the "wrong" empty branch of the Y were recorded. The percentage of correct responses for the tested ant population was so established (Table 2). The results here obtained for ants under aspartame diet were compared to those previously obtained for ants under sugar water diet [27], using the non parametric Wilcoxon test [44]. They were also compared to results previously obtained on ants receiving only pure water [35]. The values of N, T, and P, according to the nomenclature given in the here above reference, are given in the results section.

Ants' locomotion (linear and angular speed) and ants' precision of reaction (orientation towards an alarm signal)

The assessments were made on ants freely moving on their foraging area. For each assessment, the movement of five ants of each nest (n = 6 x 5 = 30 ants) was analyzed. Ants' linear and angular speed was assessed without presenting any stimulus to the ants. Ants' orientation towards an alarm signal was assessed by presenting to the ants, on their foraging area, an isolated worker's head. Such a head, with widely opened mandibles, is a source of alarm pheromone identical to that of an alarmed worker, in terms of the dimensions of the emitting source (the mandibular glands' opening) and the quantity of pheromone emitted [47].

Trajectories were manually recorded using a water-proof marker pen, on a glass slide horizontally placed 3 cm above the experimental tray area, where the tested individuals were moving. A metronome set at 1 second was used as a timer for assessing the total time of each trajectory. Each trajectory was recorded until the ant reached the stimulus or walked for about 6 cm. All the trajectories were then copied with a water-proof marker pen onto transparent polyvinyl sheets. These sheets could then be affixed to a PC monitor screen and remained in place due to their own static electricity charge. The trajectories were then analyzed using specifically designed software [48], each trajectory being entered in the software by clicking as many points as wanted with the mouse (for instance, 20 points in a trajectory length of 5 cm) and by entering then the location of the presented worker's head. After that, the total time of the trajectory was entered, and the software was asked to calculate three variables defined as follows:

The linear speed (V) of an animal is the length of its trajectory divided by the time spent moving along this trajectory. It was here measured in mm/s.

The angular speed (S) (i.e. the sinuosity) of an animal's trajectory is the sum of the angles, measured at each successive point of the trajectory, made by each segment 'point i to point i – 1' and the following segment 'point i to point i + 1', divided by the length of the trajectory. This variable was here measured in angular degrees/cm.

The orientation (O) of an animal towards a given point (here a small blank piece of paper used as a control or an ant's head) is the sum of the angles, measured at each successive point of the recorded trajectory, made by each segment 'point i of the trajectory - given point' and each segment 'point i - point i + 1', divided by the number of measured angles. This variable was here measured in angular degrees. When such a variable (O) equals 0°, the observed animal perfectly orients itself towards the given (source) point; when it equals 180°, the animal fully avoids the source point; when O is lower than 90°, the animal has a tendency to orient itself towards the source point and when it is larger than 90°, the animal has a tendency to avoid the source.

Each distribution of 30 variables was characterized by its median and quartiles (since being not Gaussian) and the distributions obtained for ants under aspartame diet, pure water diet and sugar water diet were compared to one another using the non-parametric χ^2 test [44]. Two distributions were considered as statistically different when $P < 0.05$.

Ants trail following behavior

This behavior was assessed on colonies A, B, D, and E for examining the ants' response to pheromones. The trail pheromone of *Myrmica* ants is produced by the workers' poison gland. Ten of these glands were isolated in 0.5 ml (500µl) hexane and stored for 15 min at -25 °C. To perform one experiment, 0.05 ml (50µl) of the solution was deposited, using a metallic normograph pen, on a circle (R = 5 cm) pencil drawn on a piece of white paper and divided into 10 angular degrees arcs (= ang. deg.). One minute after being prepared, the piece of paper with the artificial trail was placed in the ants' foraging area. When an ant came into contact with the trail, its movement was observed (Figure 3 E). Its response was assessed by the

number of arcs of 10 angular degrees it walked without departing from the trail, even if it turned back while walking on the trail. If an ant turned back when coming in front of the trail, its response was assessed as "zero arc walked"; when an ant crossed the trail without following it, its response equaled "one walked arc". Before testing the ants on a trail, they were observed on a 'blank' circumference imbibed with 50µl of hexane, and the control numbers of walked arcs were so obtained (Table 3, C = control, T = test). On experimental trails, *Myrmica* workers do not deposit trail pheromone because they do so only after having found food or a new nest site. Each time, these handlings were made on ants under aspartame diet, under pure water diet, and under sugar water diet. For each control and test experiment, 10 individuals of each used nest were observed (n = 4 x 10 = 40). Each distribution of values was characterized by its median and quartiles (since being not Gaussian). The distribution of values obtained for ants under aspartame diet, pure water diet and sugar water diet were compared to one another using the non parametric χ^2 test [44].

Ants' audacity

This trait was assessed on colonies A, B, D, and E. For ants under pure water, sugar water, or aspartame diet, a cylindrical tower built in strong white paper (Steinbach ®, height = 4 cm; diameter = 1.5 cm) was set on the foraging area, and the ants present on it, at any place, were counted 10 times, in the course of 10 min. The mean and the extreme values of the obtained values were established each time (Table 3, audacity) and the three series of values were compared to one another using the non parametric Mann-Whitney U test [44].

Ants' brood caring

This trait was assessed on colonies C and F which contained a large amount of small larvae. For ants under pure water, or sugar water, or aspartame diet, a few larvae were removed from the inside of the nest and deposited in front of the nest tube entrance. Five of them were carefully observed, as well as the ants' behavior in front of a larva. The numbers of the five observed larvae still remaining out of the nest were counted after 5 seconds, 2, 4, 6, 8, and 10 minutes, and the numbers recorded for each tested colonies were summed (Table 4, brood caring). The results obtained for ants maintained under each three kinds of diet were compared to one another using the non parametric Wilcoxon test [44], the values of N, T and P being given in the results section.

Ants' cognition

The assessment was made on ants of colonies B and E, maintained under pure water, or sugar water, or aspartame diet, using an adequate experimental apparatus schematically presented in the figure 3 of [37]. This apparatus consisted in a small tray (15 cm x 7 cm x 4.5 cm) inside of which pieces of white extra strong paper (Steinbach ®, 12 cm x 4.5 cm) were inserted in order to create a way with twists and turns between a loggia too narrow for 15 ants at a time (the initial loggia) and a larger one (the free loggia). Two such experimental apparatus were built and used, each one, for one of the two colonies. Each time, for each nest and each feeding situation, 15 ants were collected from their colony and set all together, at the same time, in the initial loggia of the apparatus, and those located in this loggia as well as in the free loggia were counted after 5 seconds, 2, 4, 6, 8 and 10 minutes. The numbers obtained for the two used colonies were added (Table 4, cognition). The total numbers obtained for ants under the three kinds of diet were statistically compared to one another using the non parametric Wilcoxon test [44], the values of N, T, and P being given in the results section.

Ants' acceptance of aspartame

During the time period of the present experiment, fifteen ants of colony B and of colony E were transferred into a small tray (15 cm × 7 cm × 5 cm), the borders of which had been covered with talc to prevent escape, and in which two tubes (h = 2.5 cm, diam. = 0.5 cm) were laid, one containing sugar water, the other an aqueous solution of aspartame (the same solution as that used in the course of the whole experimental work), each tube being plugged with cotton. In one of the trays, the tube containing aspartame was located on the right; in the other tray, it was located on the left (Figure 3 G). The ants drinking each liquid food were counted 12 times in 15 min, the mean values being then established for each kind of food (Table 4, acceptance). They were statistically compared to those expected if ants randomly went drinking each kind of food, using the non parametric goodness of fit χ^2 test [44].

RESULTS

Food consumption

Under sugar water diet, the ants were not very numerous on their meat food. Their numbers were similar to those commonly observed during our 45 years of work on ants. Under pure water diet, the ants appeared to be a little more numerous in coming on the meat food, but this unexpected observations leaded to non significant results (Table 1, columns 'sugar water' and 'pure water', N = 5, T = 11, P ~ 0.22, NS). Under aspartame diet, abnormally large numbers of ants were often seen on the meat food. Meanly, they were nearly three times more numerous under aspartame than under sugar water diet (Table 1, columns 'sugar water' and 'aspartame'). This time, the numerical results were statistically significant: N = 5, T = 15, P = 0.031. Moreover, the numerical results reflected the observed fact that more ants came onto the meat food while being under aspartame diet than while being under pure water (N = 5, T = 15, P = 0.031).

Conditioning ability and memory

Under sugar water diet, the ants easily acquired visual conditioning, reaching a score of 33/40 = 82.5% in about 36 hours (Table 2 and [27]). Under aspartame diet, the ants never acquired such a conditioning. After 7 hours for instance, they presented a score of 47.5% instead of 67.5% under sugar water diet, and after 36 hours, their score was still unchanged (45%) (Table 2: visual conditioning, aspartame diet). During the tests in the Y apparatus, the ants under aspartame diet often presented displacement behaviors (cleaning legs, antennae, mouth parts) when reaching the choice place of the apparatus (Figure 3 D). The difference between ants' conditioning ability under the two diets (sugar water and aspartame) was statistically significant (N = 6, T = 21, P = 0.016). Let us add that under sugar water diet, the ants presented a long lasting visual memory, still responding to the visual cue after 50 hours, while under aspartame diet, they developed no visual memory at all. Under pure water diet also, the ants never acquired visual conditioning, even after more than 36 hours, and presented so no visual memory at all [35].

Before trying to condition the ants to fennel, we have used onion as olfactory cue. The ants immediately eat (drunk the juice of) the pieces of onion set aside their meat food. Half an hour later, the ants already began to correctly respond to that olfactory cue. Onion contains some amount of sugar. We immediately removed the pieces of onion from the ants' meat food and used pieces of fennel for trying to olfactory condition them.

Under sugar water diet, they could easily acquire olfactory conditioning, affecting a score of 67.5% after 7 hours, and a score of 77.5% after 36 hours (Table 2 and [27]). Under aspartame diet, they could not do so. After 7 hours, they affected a score of 45%, and after 36 hours, a score of 47.5 % (Table 2). This difference of ants' capability under the two kinds of diet was statistically significant (N = 6, T = 21, P < 0.016). Also, under aspartame diet, the ants had no olfactory memory at all while under sugar diet, they still retained 22.5% of their conditioning after 36 hours. Let us note that, under pure water diet, the ants also could never acquire olfactory conditioning [35].

Locomotion

Under sugar water diet, the ants presented usual locomotion, with a commonly observed linear and angular speed (Table 3, column 'sugar water', lines 1, 2). Under pure water diet (Table 3, column 'pure water, lines 1, 2), the ants walked with a nearly similar speed (meanly 14.2 mm/s *vs* 13.7 mm/s, the difference being not significant: χ^2 = 2.53, df = 2, NS) and a similar sinuosity (meanly 133 ang. deg./cm *vs* 135 ang. deg./cm). They simply foraged a little more intensively than usually. Under aspartame diet, the increase in locomotion and in foraging was obvious, and clearly reflected by the numerical results (Table 3, column 'aspartame', lines 1, 2). The ants' linear speed meanly reached the value of 19.6 mm/sec, statistically different from that under sugar water diet (χ^2 = 28.55, df = 2, P < 0.001), while their sinuosity did not change (139 ang. deg/cm *vs* 135 ang.deg./cm; χ^2 = 1.32, df = 2, NS). The difference between the ants' speed of locomotion under pure water and aspartame diet was also obvious, this trait being significantly higher under aspartame diet (χ^2 = 22.13, df = 2, P < 0.001). In fact, the ants very actively foraged under that sweetened diet.

Precision of reaction

Under sugar water diet, the ants perfectly oriented themselves towards an isolated worker's head (Table 3, column 'sugar water', line 3). Under pure water diet, and even more under aspartame diet, the ants did not so well oriented themselves, though obviously perceiving the alarm pheromone and responding to it. This impact of the insects' diet on their precision of reaction was significant: pure water *vs* sugar water: χ^2 = 7.5, df = 1, P < 0.01; aspartame *vs* sugar water: χ^2 = 11.93, df = 1, P < 0.001. The difference of ants' orientation between pure water and aspartame diet was not significant: χ^2 = 0.71, df = 2, NS.

Response to pheromones

Under sugar water diet, the ants followed the presented trail under rather long distances (meanly 10 arcs of 10°, Table 3, column 'sugar water, line 4). Under pure water diet, the ants did not so well follow the trail, moving along meanly only 6 arcs. This decrease in trail following behavior consequently to pure water diet was statistically significant (χ^2 = 20.81, df = 2, P < 0.001). Under aspartame diet, the ants still more failed in following the trail along long distances; they meanly followed it along 2 arcs of 10°. This decrease in trail following behavior due to aspartame diet was highly significant: χ^2 = 46.17, df = 2, P < 0.001. A supplementary experiment was performed for checking this unexpected result. The numerical result was 3.0 (2.0 – 4.0) arcs walked along the trail, what confirmed the first experiment (Figure 3 E). The difference of ants' trail following behavior between pure water and aspartame diet was also significant: χ^2 = 14.51, df = 2, P < 0.001. Thus, without sugar in their diet, the ants did not correctly follow a trail, this being probably due to their large tendency to forage everywhere. Note that under aspartame diet, this impact was larger than under a simple 'no sugar' diet.

Audacity

Numerical results are given in Table 3, line 5. Under sugar water diet, only few ants came onto the risky apparatus: meanly 0.85 ants were there counted. Under pure water diet, about twice more ants came on the apparatus and a few ones were seen climbing onto the tower. This increase in 'audacity' was statistically significant (U = 379, Z = -4.3686, P = 0.000013) and may be due to the ants' slight increase of foraging behavior.

Under aspartame diet, the ants were even more numerous in coming onto the risky apparatus (meanly 1.88 ants), and many ones were seen climbing on the tower (four ants for instance, were seen at the same time, on a tower). This large increase in 'audacity' was statistically significant (U = 341, Z = -4.6671, P = 000005), and clearly corresponded to the ants' large tendency in foraging. The difference of ants' audacity between pure water and aspartame diet was not significant (U = 695.5, Z = -1.08814, P = 0.2765).

Brood caring

Numerical results can be found in Table 4, 'brood caring'. Under sugar water diet, ants very quickly re-entered the larvae experimentally removed from the nest (Figure 3 F). After the ten experimental minutes, no more larva among the ten observed ones were still lying on the foraging area. Under pure water diet, the ants also took care of their larvae, and re-entered them, but not as quickly as under sugar water diet since two larvae were still not re-entered after the ten experimental minutes. The difference was however not significant (N = 5, T = 6, NS). Under aspartame diet, the ants also took care of their larvae but re-entered them with a rather longer delay, probably due to their increased tendency in foraging. After six experimental minutes, six larvae among the ten observed ones were still lying on the foraging area, and after the ten experimental minutes, two ones were still not re-entered. This time, the result was statistically different from that obtained with ants under sugar water diet (N = 5, T = 15, P = 0.031), as well as under pure water diet (N = 5, T = 15, P = 0.031).

Cognition

Table 4 gives the numerical results at the line 'cognition'. Under sugar water diet, the ants could quickly find their way through the twists and turns of the experimental apparatus. After the ten experimental minutes, five among the 30 tested ants were moving in the free loggia, beyond the twists and turns while only nine ants were still in the initial loggia. Under pure water diet, their ability in doing so was somewhat lower, only two ants among the thirty tested ones having reached the free loggia while 13 ones being still in the initial loggia after the ten experimental minutes. This slight decrease in cognitive ability between sugar water and pure water diets was significant: initial loggia: N = 6, T = 21, P = 0.016; free loggia: N = 5, T = 15, P = 0.031. Under aspartame diet, the ants were still less able to reach the free loggia, beyond the twists and turns. After the ten experimental minutes, only two ants were moving in the free loggia while 18 ones were still in front of the twists and turns. The numbers of ants counted in the initial loggia in the course of time statistically differed from those counted for ants under sugar water (N = 6, T = 21, P = 0.016) as well as from those counted for ants under pure water diet (N = 6, T = 19.5, P ~ 0.039). The numbers counted in the free loggia did not statistically differ (*vs* sugar water diet: N = 4, T = 10, P = 0.06; *vs* pure water diet: N = 2, NS), this being due to the small numbers of individuals observed. In fact, under aspartame diet, the ants largely moved inside the twists and turns once towards the free loggia, then back on their way, then again towards the free loggia, coming so often back into the initial loggia.

Aspartame acceptance

The numerical results are presented in Table 5. For colony B, only one ant was seen drinking the solution of aspartame while 62 ones were counted on the sugar water. This corresponded to 1.6% of ants accepting to drink the aspartame solution *vs* 98.4% of ants preferring sugar water. For colony E, 26 ants were counted on the sugar water while two ones were seen on the solution of aspartame, i.e. 92.9% of ants choosing the sugar water *vs* 7.1% accepting the solution of aspartame. Meanly, 96.4% of ants were counted while consuming sugar water and 3.6% of ants were counted while drinking the solution of aspartame. This difference was highly significant (χ^2 = 71.44, df = 1, P < 0.001). Ants thus clearly preferred true sugar to aspartame (Figure 3 G).

Table 1. Ants' meat consumption under three kinds of diet. Six colonies were successively provided with sugar water, pure water, and an aqueous solution of aspartame, as well as, each time with pieces of *T. molitor* larvae given at days 1, 3, 5. During five days, the ants present on the larvae were twice counted (daily counts). The mean of these twelve counts was established (daily means), and finally the total mean was calculated. Experimental details and statistical analyses are given in the text. Briefly, ants consumed more food under pure water diet and still more food under aspartame diet.

Diets :	**sugar water**						**pure water**						**solution of aspartame**					
Colonies :	**A**	**B**	**C**	**D**	**E**	**F**	**A**	**B**	**C**	**D**	**E**	**F**	**A**	**B**	**C**	**D**	**E**	**F**
daily counts																		
Day 1	1	1	2	0	2	1	1	6	0	4	2	1	2	3	0	2	12	2
	0	3	2	0	1	1	7	10	1	1	0	1	6	4	0	5	12	4
Day 2	0	0	0	1	2	0	1	1	0	1	1	1	0	4	0	1	3	1
	0	1	0	0	1	0	2	2	0	2	0	0	4	1	0	7	3	1
Day 3	2	1	0	0	4	0	2	4	0	2	1	1	3	2	0	5	4	2
	1	2	0	2	1	1	3	4	0	1	1	2	5	1	0	9	10	2

Day 4	0 0 0 0 5 0 1 0 0 0 3 0	1 5 0 1 0 0 1 5 0 1 1 0	3 1 0 2 4 1 4 1 0 2 4 1
Day 5	3 3 0 0 9 1 5 2 0 3 9 1	4 2 0 2 1 1 5 3 0 2 2 2	4 6 0 4 5 2 4 4 0 4 8 2
daily means			
Day 1	1.17	2.83	3.67
Day 2	0.42	0.92	2.08
Day 3	1.17	1.75	3.58
Day 4	0.75	1.25	1.92
Day 5	3.00	2.00	3.58
total mean			
Days 1-5	1.30	1.75	2.97

Table 2. Ants' visual and olfactory conditioning, as well as visual and olfactory memory under sugar water, pure water and aspartame diet. Ants of four colonies under aspartame diet were collectively trained to a hollow green cube, or to pieces of fennel, and 10 ants of each colony were individually tested in a Y apparatus, one branch of which was provided with the visual or the olfactory cue. The table gives the numbers of ants of each colony correctly responding and the scores (%) reached by the experimented population. Identical experiments have previously been made on four colonies under sugar water diet [27]. Similar experiments have previously been made on two colonies under pure water diet [35]. Details and statistical analysis are given in the text. Briefly, under aspartame diet, the ants could never acquire conditioning, as it also occurred under pure water diet.

Time (hours)	N° of correct responses among 10 for each colony, and proportion of correct responses for all the colonies, this under three different diets:		
	sugar water [25]	pure water [33]	aspartame
Visual conditioning			
Control	5 5 4 6 50%		
1	6 6 5 5 55%		5 4 6 5 50%
2	8 3 7 5 57.5%		5 4 5 6 50%
7	8 6 5 8 67.5%	45%	4 4 5 6 47.5%
13	7 8 7 9 77.5%	50%	3 6 5 6 50%
25	7 7 9 8 77.5%	50%	4 4 5 4 42.5%
36	8 9 7 9 82.5%	50%	5 4 4 5 45%
Loss:			
2	8 8 7 8 77.5%		
13	7 9 8 7 77.5%	no memory	no memory
50	6 7 7 6 65%		
Olfactory conditioning			
1	6 6 5 6 57.5%		3 5 4 5 42.5%
2	6 6 6 6 60%		4 4 6 5 47.5%
7	6 6 9 8 67.5%	55%	5 3 5 5 45%
13	7 8 7 8 75%	50%	3 5 5 5 45%
25	7 8 7 8 75%	45%	5 5 4 4 45%
36	7 8 8 8 77.5%	55%	5 6 4 4 47.5%
Loss:			
2	7 6 9 8 75%		
13	8 6 6 5 62.5%	no memory	no memory
36	7 6 8 8 72.5%		

Table 3. Median (and quartile) values of five ethological and physiological ants' traits, under sugar water, pure water, and aspartame diet. Experimental details and statistical significance are given in the text. Briefly, water diet somewhat affected, and aspartame diet largely affected the ants' locomotion speed, orientation ability, trail following behavior, and audacity. C = control, i.e. a blank circumference; T = test, i.e. a circumference traced with trail pheromone.

Diet → Traits ↓	sugar water	pure water	aspartame
Linear speed (mm/sec)	13.7 (12.3 – 15.4)	14.2 (13.1 – 15.8)	19.6 (17.7 – 21.1)
angular speed (ang.deg/cm) orientation (ang. deg)	135 (115 – 157) 30.8 (27.7 – 38.3)	133 (116 – 157) 41.3 (30.0 – 49.9)	139 (123 – 150) 44.8 (28.7 – 57.6)
trail following (n° of walked arcs) 'audacity'(mean n° of ants)	C: 1.0 (1.0-1.0) T: 10.0 (8.0 – 16.0) 0.85 (0 – 3)	C: 1.0 (1.0 – 1.0) T: 6.0 (4.0 – 9.3) 1.60 (1 – 3)	C: 1.0 (1.0 – 1.0) T: 2.0 (1.0 – 5.2) 1.88 (0 – 4)

Table 4. Two ethological ants' traits assessed under sugar water, pure water, and aspartame diet. The table gives the sums of the scores of two colonies. Explanation and statistical values are given in the text. Briefly, pure water diet somewhat, and aspartame diet largely affected the two traits, i.e. ants brood caring and cognitive ability.

Diet → Traits ↓		Sugar water		Pure water		Aspartame	
Brood caring:							
n° of not re-entered larvae after:	5 s	9		9		10	
	2 min	8		7		8	
	4 min	6		5		7	
	6 min	4		3		6	
	8 min	2		3		4	
	10 min	0		2		2	
Cognition:							
n° of ants in front and beyond twists and turns after:		in front	beyond	in front	beyond	in front	beyond
	5 s	25	0	30	0	28	0
(in front = a small initial loggia; beyond = a large free loggia)	2 min	18	1	21	0	26	0
	4 min	15	1	20	0	22	0
	6 min	14	2	18	1	22	2
	8 min	13	4	16	1	21	2
	10 min	9	5	13	2	18	2

Table 5. Ants' preference between an aqueous solution of sugar and one of aspartame. Fifteen ants of two colonies were confronted, apart from their colony, with the two aqueous solutions. The ants drinking each solution were counted 12 times, during 10 minutes. The table gives the sums of the obtained numbers and the corresponding proportions revealing the choice of the ants between the two sweetened solutions. Obviously, ants preferred sugar and not aspartame.

Colonies	Aqueous solutions	N^{os} of ants	% of choices
B	aspartame	1	1.6%
	sugar	62	98.4%
E	sugar	26	92.9%
	aspartame	2	7.1%
B + E	aspartame	3	3.6%
	sugar	88	96.4%

Figure 1. Chemical structure of aspartame.

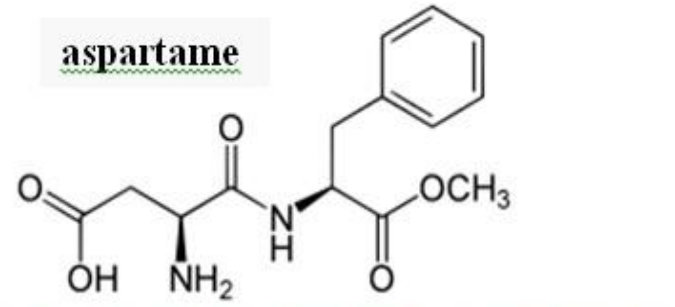


Figure 2. Protocol used and presentation of the results. Experiments were so performed to avoid giving continuously aspartame to the ants, perturbed by such a diet. Results were more user-friendly presented.

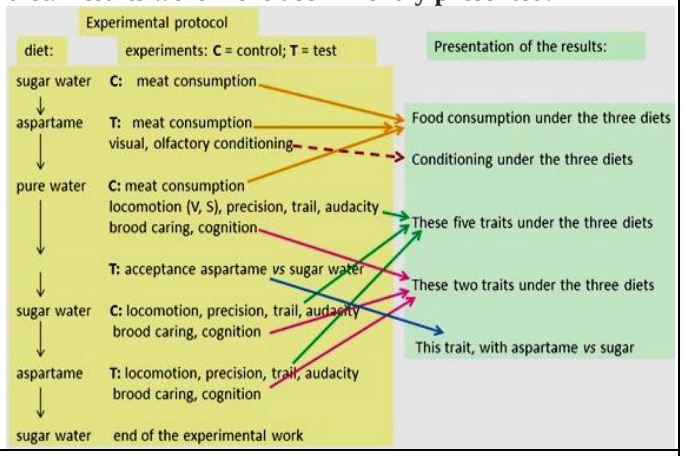
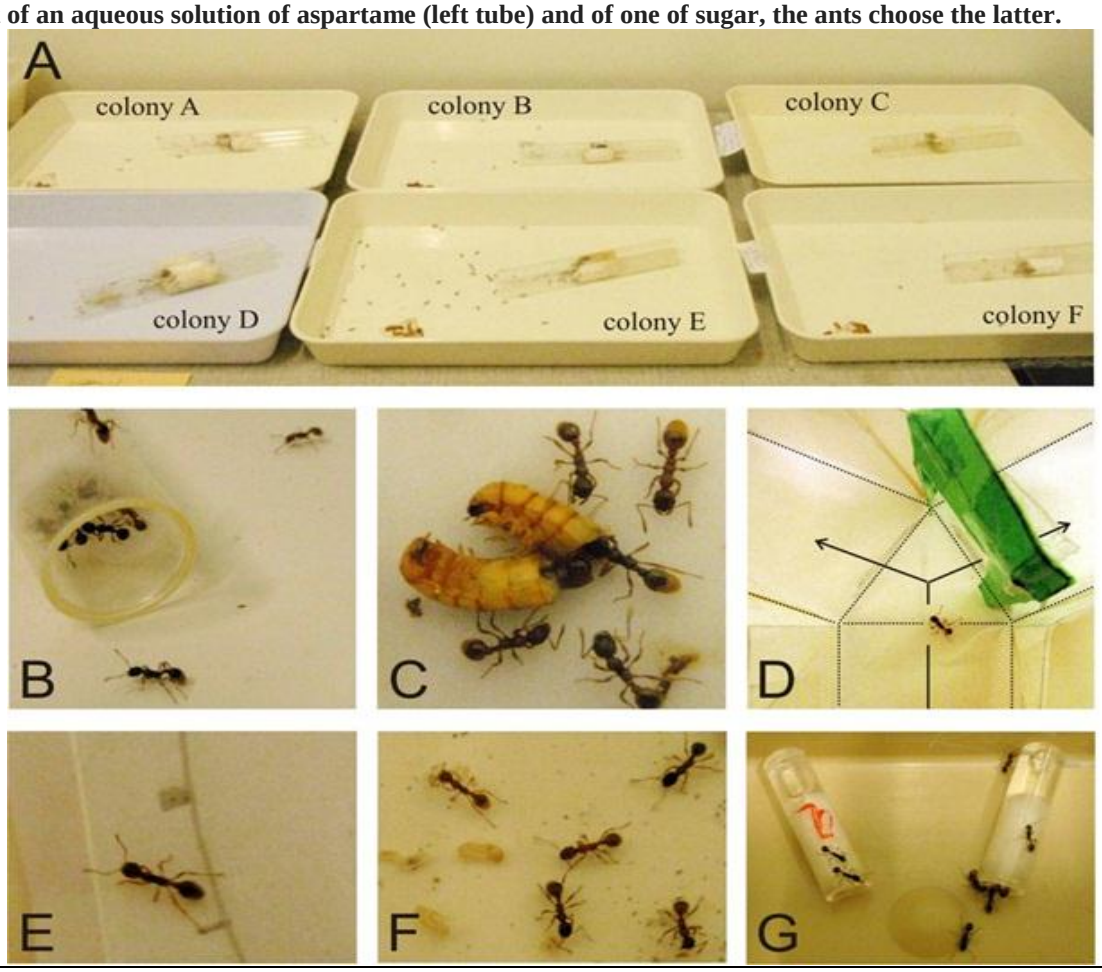


Figure 3. Some views of the experiments. A: the six used colonies. B: ants drinking the aqueous solution of aspartame. C: under aspartame diet, ants eagerly consumed meat; note their enlarged gaster. D: an ant, under aspartame diet and visual conditioning is tested in a Y maze: reaching the choice place, the ant exhibited a displacement behavior, i.e. cleaning its left antennae. The limits of the way in the Y maze have been highlighted by dotted lines on the photo and the possible ways indicated with black arrows. E: an ant under aspartame diet departing from a pheromonal trail. F: ants under sugar water diet taking care of their brood, i.e. re-entering larvae experimentally removed from the nest. G: in front of an aqueous solution of aspartame (left tube) and of one of sugar, the ants choose the latter.



DISCUSSION AND CONCLUSION

Aspartame is today the most consumed and easily available sweetener, essentially in Europe and North America, either as a substitute of sugar or as a constituent of numerous drinks, creams and cakes. It is presently known that this substance hydrolyses into toxic compounds (see the introduction section). Its effects on the individuals' physiology and behavior, while being still or almost intact, have little been examined and/or divulgated. Humans commonly look for information on internet sites, e.g. [2-49-50-51-52] among many other ones. Internet information can be misleading, being not always truly scientific, perfectly exact, objective or updated [1]. In the present work, using ants as biological models, we precise some behavioral and physiological effects of 'canderel' tablets, a common source of aspartame, we used exactly as it is used by humans. Here below, we recall an important point of our experimentation, then we summarize and somewhat discuss our findings. After that, we give a short review of the actual scientific literature on the subject, and finally we conclude.

Let us recall that the here used aspartame solution was 10 times more concentrated than the solutions commonly consumed by humans. The temperature of the laboratory was about 20°C, what was also the inner temperature of the 'models' ants. The aspartame solutions given to the ants were renewed each two days. Even if some hydrolyzation had begun in these solutions, intact aspartame was still present. These solutions had still a sweetened pronounced taste, and ants went on drinking them. The only consequence on our results of a possible partial hydrolyzation of aspartame was that these results should have been more pronounced, more obvious if hydrolyzation did not have occurred.

Here, we showed that in ants and under our experimental conditions, aspartame increases food consumption, reduces visual and olfactory memory, increases speed of locomotion, reduces precision of reaction, decreases response to pheromones, increases audacity, i.e. the tendency in moving under risky situation, reduces brood caring behavior and decreases cognitive ability. In fact, under aspartame diet, the ants continuously foraged everywhere in their tray, often drunk and eat, obviously searching for food, and so, were less able to correctly perform the different behaviors they commonly present. Such behavioral changes also occurred under pure water diet, but at a lower extent, and never under sugar water diet.

Let us remark that an ants' life span is about 30 times shorter than that of humans. Giving aspartame during one or two weeks to ants, as we did in the present work, corresponds to give continuously aspartame to humans during seven or fourteen months. Moreover, during our experiments, ants under aspartame diet received no carbohydrate, thus no source of glucose, what was not the case during experiments on humans (see below). Consuming aspartame instead of sugar all the time deprives the organism from glucose, though giving to the brain the 'presence of sugar' information, what leads to several ethological and physiological perturbations. On the other hand, the brain requires glucose for correctly and efficiently functioning. Moreover, we showed that ants, a perfectly objective model, largely preferred consuming true sugar instead of aspartame. Having often used ants in such choice tests (references in the introduction), we know that their response is valid, is in agreement with their physiological requirement. Thus, instinctively, naïve individuals choose true sugar instead of aspartame.

Among the scientific studies already made, Saravis et al. [53] have experimented on children 9 – 10 years old. After having got breakfast, the children received at 12:00 either (experiment 1) a meal with aspartame or cyclamate, or (experiment 2) a meal with sucrose or aspartame. Children's learning, calculation, activity, social interactions, and mood were then measured and appeared to be equivalent in experiment 1, but in experiment 2, the only significant difference was that motor behaviors were more frequent under aspartame than under sucrose. The authors concluded that the effect observed under aspartame is only due to its absence of metabolic consequences, what is in agreement with our results. Examining the vast literature about effects of aspartame on general health (cancer, depression, dementia …) and on avoiding obesity, Lean et al. [54] concluded that, under moderate consumption, aspartame does not affect humans' general health, but that its action on avoiding obesity is inconclusive. Once more, this report is in agreement with our observations on ants: they stayed in good health under aspartame diet but did not eat less, on the contrary.

Wolraich et al. [55] tested the effect of aspartame, sucrose and saccharin on motor behavior (writing and drawing, these traits examined for a possible hyperactivity) and cognitive performance of 3 to 10 years old children, each sweetener in a complete but controlled diet during three weeks at a time. They did not detect any significant difference between the three sweeteners. However, the children went on receiving all the time usual amounts of carbohydrates as a source of glucose. This situation differs from that of our present work which shows that the effects of aspartame on behavior and cognition are due to a lack of sugar (glucose).

Mattes [56] made an excellent experimental work on adults for assessing effects of aspartame on hunger and energy intake, and failed in finding an appetite stimulating effect of the sweetener. But the sweetener was added to cereal, an important source of glucose, a situation which also differed from that of our present work.

In their scientific evaluation of the safety of aspartame during its post marketing period, Butchko and Stargel [57] concluded that aspartame is safe for its use as

a sweetener. Very probably, in the numerous studies, reports and issues they used for finalizing this evaluation, aspartame was consumed as an additive to usual food, maybe without sugar but surely accompanying common carbohydrates as sources of glucose. In a more recent review, Abegaz et al. [1] also state that aspartame does not affect general health, at least when consumed at moderate doses.

However, it is known that aspartame may trigger some kinds of headache [58], and may increase the amount of phenylalanine in plasma, this substance being potentially neurotoxic [59 and references therein]. The conclusion of the here above mentioned studies is that, due to its inevitable hydrolyzation, aspartame should not be consumed in large amount, and not at all by persons suffering from phenylketonuria.

Moreover, it has been shown on rats receiving 10 mg/kg aspartame 14C-labelled in the methanol carbon [3] that the later component is, at 37° C, oxidized in several tissues to formaldehyde which forms adducts of proteins and nucleic acids. For the authors [3], this could explain neurological effects such as headache, notwithstanding the potential danger of carcinogenic effects. However, the quantity of methanol released by aspartame in this study did not exceed the control values of usual blood methanol concentrations [60].

Finally, thoroughly reviewing medical and cellular biochemical literature, Humphries et al. [61] examined at a cellular level the known effects, at ambient temperature, of aspartame on the brain and concluded that it disturbs amino acid metabolism, protein structure, integrity of nucleic acids, neuronal function, endocrine balances and that it changes the brain concentrations of catecholamines. Aspartame leading to overall oxidative stress and neurodegeneration, the authors proposed that excessive ingestion of this sweetener might be involved in the pathogenesis of certain mental disorders, and might also compromise learning and emotional functioning.

On the other hand, it must be said that aspartame has nevertheless a beneficial effect when used for preventing the genotoxicity of ochratoxin A, a mycotoxin produced by some *Penicillium* and *Aspergillus* moulds and which affects brain and kidneys [62].

In conclusion, on the basis of our study on ants and on many others performed on humans and animals, aspartame is not a sweetener above all criticisms. Of course, at ambient temperature, it stays more or less intact, has a very nice sweetened taste and apparently does not severely impact health. But we showed that, in ants used as a biological model, it affects several ethological and physiological traits (food consumption, activity, cognition, memory), likely because it is not a true glycoside though giving to the central nervous system the information that it is sugar. Thus, if aspartame is consumed together with a normal intake of carbohydrates, such adverse effects should not occur. An unexpected convincing argument for this hypothetical explanation was our observation that ants under aspartame diet, once consuming onion juice (which contains a very small amount of sugar), immediately acquired conditioning when we started olfactory conditioning using onion as cue. Then, for correctly experimenting, we had to promptly change the onion cue by fennel. Furnishing no glucose to the organism, aspartame should be used by diabetic persons, and could be added to drugs. For non diabetic persons we propose that it should not be used all the time instead of true sugar, in any kind of food and drinks, without a sufficient (normal) yield of glucose. For healthy persons, a small amount of true sugar or carbohydrates, together with a small quantity of aspartame, is thus far better than the equivalent amount of aspartame without true glycosides. As aspartame soon hydrolyzes into, among others, phenylalanine, persons suffering from phenylketonuria must avoid consuming aspartame, but also anyone consuming large amounts of phenylalanine (contained in meat, cheese, eggs, for instance) should avoid consuming large amounts of aspartame since phenylalanine becomes a neurotoxin when present in large amount. The contribution of aspartame to the formation of formaldehyde must also be taken into account. Anyway, precautions must be taken as for aspartame consumption in large quantities or over long lasting periods. Our ants were still in good health after the entire experimental work because we have alternated sugar diet and aspartame diet. Moreover, when they had the choice between aspartame and sugar, they chose sugar.

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