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Ethological and Physiological Harmful Effects of Metformin, An Antidiabetic Used to Treat Type 2-Diabetes: A Study on Ants as Biological Models

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Abstract

Metformin is a drug mainly used for caring of persons suffering from type 2-diabetes. Over time, it was found to be efficient for treating others illness. Its use increasing, it is nowadays the second active pharmaceutical ingredient more present pollutant in natural water. It could therefore affect the biology of the aquatic fauna through harmful physiological effects. Using ants as biological models, we studied the potential physiological and ethological adverse effects of Metformin. We found that it largely reduced or impacted these insects' food intake, activity, audacity, social relationships, state of stress, cognition, and learning abilities. No adaptation and no habituation to the effects of Metformin were observed, and ants developed some dependence on its consumption. After weaning, the effect of Metformin became significantly lower than its initial one as soon as after four hours and fully vanished in a total of 13 hours. Metformin could thus harmfully impact the freshwater fauna rich in insect species, especially if chronic exposures occur. As regards patients treated with Metformin, our study suggests that they may suffer from side effects not mentioned in the drug notice. For instance, they may develop dependence and increase their daily dose, accentuating so the drug side effects, e.g., they may suffer from anorexia. Practitioners should know the side effects of Metformin and monitor patients as for their occurrence.

Keywords: Pharmaceutical pollution, freshwater health, biomonitoring, cognition, locomotion, memory, *Myrmica sabuleti*, social interactions

1. Introduction

1.1 Pollution of Freshwater Ecosystems by Pharmaceutical Products

Pollutions of freshwater ecosystems by pharmaceutical products and active pharmaceutical ingredients (APIs) occur in all countries in the world regardless of the level of GDP or the specificities of the economy (Wilkinson et al., 2022). Numerous products used or consumed by humans are present in the environment and all ecosystems (Arnold et al., 2014), mainly in aquatic ecosystems (freshwater but also in coastal ecosystems (Prichard and Granek, 2016)). These products affect all species and ecological processes (Richmond et al., 2017), including in the most remote areas (Krief et al., 2022). As it has been shown that these APIs can have (potentially in some case study) harmful impacts on ecosystems health (Richmond et al., 2017; Grenni et al., 2017; Prichard and Granek, 2016, Arnold et al., 2013), the effects of these substances on aquatic organisms and ecological processes are a worldwide subject of concern (Wilkinson et al., 2022; EEB, 2018 and references therein; Jones et al., 2004).

Investigations on potential harmful effects of APIs on freshwater organisms are not easy to conduct, due to experimental constraints and logistical challenges of this type of study, although if small-scale (microcosm) experiments using, for example, daphnia (Damasceno de Oliveira et al, 2015; Kim et al., 2007), or other freshwater invertebrates, such as mussels (Aguirre-Mart ńez et al., 2013) are common. A growing number of mid-scale experiments (using mesocosm experimental designs) are being carried out (Bartmentlo et al., 2021), but they remain very expensive, time-consuming and difficult to replicate.

If physiological impacts of APIs can be quite well evaluated through the use of microcosm experimental designs, it is often more complicated to investigate ethological effects of drugs on freshwater vertebrates and invertebrates (Brodin et al., 2014). The majority of the latter ones are insects, and some of them display complex, even almost social, behaviors.

This is the case for some caddis flies or stoneflies for instance (Johnstone, 2009; Hanada et al., 1994; Stewart & Zeigler, 1984). It is therefore relevant to rely on a biological model organism easy to experiment in order to bio monitor harmful effects of drugs and APIs on ethological and physiological traits of freshwater invertebrates. To carry out this type of study, we used a species of ants, *Myrmica sabuleti* Meinert, 1861, we have previously used in the course of many experimental works during which we examined the impact of APIs and drugs on several ethological and physiological traits. For instance, we so studied the effects of some drugs that are largely distributed in freshwater ecosystems, namely fluoxetine (Cammaerts & Cammaerts D, 2015a), carbamazepine (Cammaerts & Cammaerts D., 2015b), paroxetine (Cammaerts & Cammaerts, 2016), ibuprofen (Cammaerts &Cammaerts, 2018), and fluvoxamine (Cammaerts & Cammaerts, 2021). We present here the result of a study dedicated to the second most common drug found worldwide in freshwater ecosystems: Metformin, a drug largely used for caring of patients with type 2-diabetes, and the use of which nowadays increases.

1.2 Metformin, the Second More Frequent Polluting API in Freshwater Worldwide

Metformin, the complete name being Metforminhydrochlorid, the chemical structure of which is given in Figure 1, is a hypo- or normo- glycerinate, i.e., a drug which reduces the amount of sugar in blood (Silvio et al, 1998; Ferrannini, 2014). Its mode of action is not yet fully elucidated. It does not increase the production of insulin, but increases the insulin sensitivity (in other words, it reduces the insulin resistance) of muscles and fat tissues (Silvio et al, 1998). Metformin also reduces the neoglucogenesis, inhibiting the glycerophosphate dehydrogenase, and decreases the absorption of glucose (Mdiraju et al, 2014). In addition, it inhibits the propagation of glucagon into the blood (Silvio et al., 1998). Over time, practitioners and researchers have found other properties of Metformin. It is sometimes used for treating polycystic ovary syndrome (Lord et al., 2003) but the effect seems to be very limited (Legro et al., 2007). It may be efficient for preventing and/or reducing some cancers (Ben Sahra et al., 2010). Metformin has also been shown to be efficient for losing weight (Frieling et al., 2021). However, though being a useful drug, Metformin appears to present some side effects, the most divulgated ones (in the notice joined to the drug package, and on several internet links, e.g. https://www.revmed.ch › revue-medicale-suisse-394 › t...) being digestive problems, decrease of the amount of vitamin B12, metallic taste in the mouth, anorexia, and kidney dysfunction (Wei-Hao et al. 2018).

Since over time Metformin was found to be efficient for treating other illness and symptoms than type 2-diabetes, it progressively became more and more used. This drug is nowadays the secondly most present API pollutant in the natural water, the first most present one being Carbamazepin (Wilkinson et al., 2022). Metformin is for instance widely distributed in the German water cycle (Trautwein et al., 2014) and in south-eastern United States wadable streams (Bradley et al., 2016). In drinking water system treatment plants, Metformin is transformed thanks to chlorination process. The chlorination by-products of Metformin have led to serious problems as for human's and animal's health due to direct toxicity and cell tissues damages (Zhang et al., 2021). These products also cause endocrine disruption in reproductive tissues of fishes, leading to the development of intersex gonads in males, and reduction of fecundity in treated pairs of fishes (Niemuth and Klaper, 2015). Some negative effects of Metformin on photosynthetic activity of the freshwater chlorophyte, *Chlorella vulgaris* have also been reported (Cummings et al., 2018). Nevertheless, another study revealed no physiological consequence on the health of a freshwater gastropod - the big ramshorn snail (*Planorbarius corneus*) - due to an exposition to Metformin and to its transformed product guanylurea at environmentally relevant concentrations (Jacob et al., 2019).

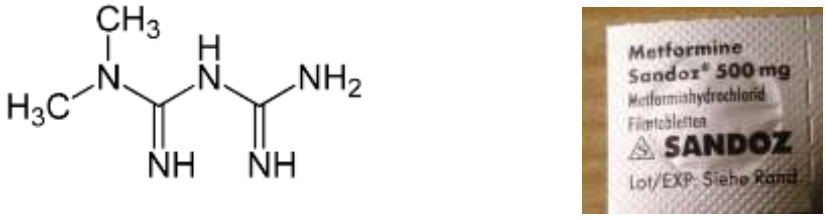


Figure 1. Chemical structure and a commercial tablet of Metformin

1.3 Aim of the Study

We aimed to know which kind of harmful effects Metformin can cause to animal species and to ecological process when released in the environment. Alongside to potential physiological disorders, Metformin appears to also cause some ethological impairments to freshwater invertebrates (Godoy et al 2018). As the latter include numerous insect species, we aimed to study potential ethological effects of Metformin on terrestrial insects, what is easier than on aquatic ones. More

precisely, we intended to make our research on our usual biological model, the ant *M. sabuleti*, which is very suitable to do so for several reasons reported in the subsections 1.4 and 2.1. To summarize, in the present paper, we report our study of some potential harmful ethological and physiological effects of Metformin on an ant species used as a biological model organism. In the subsection *2.2.*, we precise the ethological and physiological traits on which we focused our investigation. In total, 13 biological traits were considered, from food intake to dependence on the drug consumption and to the decrease of its effect after its consumption was stopped.

1.4 Ants as Biological Organism Models

Fundamental biological processes, such as embryology, proteins synthesis, nervous functioning, muscular functioning, conditioning acquisition, are similar in most animal species including humans. Therefore, several animal species are used as biological models for physiological and ethological studies (Wehner & Gehring, 1999; Russel & Burch, 2014). Invertebrates are generally preferred because they have a small size, can be easily maintained out of their natural environment, and have a short generation time (Wolf & Heberlein, 2003). Insects are often used, e.g., locusts, mealworms, fruit flies, bees (Andre et al., 1989). Ants can thus be used, the more so since they can easily be maintained in any room, at low cost, and since they detain a lot of evolved skills on which the effect of events or substances can be studied (Passera & Aron, 2005). They can memorize visual and olfactory cues and use them for navigating; they use pheromones to communicate with congeners; they are able to rapidly recruit congeners for killing enemies, collecting food, hunting for prey; they differently mark zones of their territory. They take care of their brood and queens; they build complex nests; they clean their nests and manage cemeteries at the limits of their territory.

2. Materials and Methods

2.1 Which Species We Used and What We Know on It

We worked on the species *Myrmica sabuleti* Meinert, 1861. We know rather well its biology as regards to physiological, ethological and cognitive traits. Indeed, we have studied its visual perception, conditioning acquisition, recruitment system (Cammaerts & Cammaerts D., 2014), ontogenesis of some of their skills (Cammaerts & Cammaerts, 2015a), their recognition in a mirror (Cammaerts & Cammaerts, 2015b), as well as several of their numerosity abilities (Cammaerts & Cammaerts, 2020a, 2020b, 2022). For example, we showed that they have a number line, can acquire the notion of zero, can count and add numbers of elements, and can expect the next element of an increasing or decreasing arithmetic or geometric sequence. The distance effect, the size effect and Weber's law can be applied to their perception (Cammaerts & Cammaerts, 2020c, 2020d). The physiological and ethological effects of drugs and APIs can thus valuably be examined on such a species detaining so many sophisticated skills, even if the latter always stay at a concrete level. Effectively, until now, we have successively examined on the workers of the ant *M. sabuleti* ants the side effects of 54 products or situations (e.g., Cammaerts, 2016, 2017, 2018a, 2019, 2021a, 2022).

2.2 Which Traits We Intended to Examine

Here we aimed to study the adverse effects of Metformin on *M. sabuleti* workers' meat and sugar water consumption, general activity, linear and angular speeds, orientation ability, audacity, tactile perception, brood caring behavior, congeners' relationships, stress, cognition, learning and memory, adaptation to these side effects, and dependence on its consumption. We also aimed to precise the manner according to which Metformin loses its effect after its consumption was stopped. The materials and methods were similar to those used for conducting our previous works (e.g., Cammaerts, 2021b, c; Cammaerts & Cammaerts D, 2015b, c; Cammaerts & Cammaerts R, 2016, 2018, 2020e, 2021). For the readers' convenience, we however briefly related them, but without avoiding some inevitable self-plagiarism.

2.3 Collection and Maintenance of Ants

The present experimental work was conducted on two colonies of *M. sabuleti* collected since a year in Ardenne (Belgium) from an old quarry situated near the Aise. These colonies contained 500-600 workers, 1-2 queens, and brood. Each one was maintained in one to three glass tubes half-filled with water, a cotton plug separating the ants from the water. The nest tubes of each colony were set in a tray (34 cm x 23 cm x 4 cm), the borders of which having been covered with talc to prevent ants from escaping. These trays served as foraging areas. In them, pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) were provided three times per week, and a cotton-plugged tube filled with sugar (~ 15% of sugar) water was permanently set. The lighting of the laboratory varied between 330 and 110 lux, the ambient temperature constantly equaled 20-21 ℃, the humidity 80%, and the electromagnetic field 2 μWm2. These conditions are favorable to *M. sabuleti*. The word 'ant' is here often replaced by 'worker' or 'nestmate' as do researchers on social insects.

2.4 Solution of Metformin Given to the Ants

One tablet of Metformin 500 mg (Metformin chlohydrate, Sandoz®; autorisation: viatris sante, 1 rue de Turin, 69007 Lyon; manufacturer: MC Dermott Laboratories T/A Gerard Laboratories, Dublin, Ireland) was furnished by the pharmacist Wera (1170 Bruxelles). People treated with this drug are advised to consume minimally one tablet of 500 mg

per day. As most mammals, they consume a rather large amount of water. Generally, one human consumes about *at minimally* one litter of water (not including the water contained in his food) per day. Insects, and thus ants, due to their anatomy and physiology (cuticle, excretory apparatus) generally drink *ca* 10 times less water. Therefore, for living under a diet with Metformin similar to that of people treated with this dug, the ants must be provided with a solution of 1 tablet 500 mg Metformin in 100 ml water. We thus duly crushed such a tablet of Metformin and dissolved the obtained powder into 100 ml of the sugar water provided to the ants (Figure 2). The obtained solution was delivered to the ants in their usual cotton-plugged tubes. The plugs of these tubes were refreshed every 2-3 days, and the entire content of the tubes was renewed every seven days. Several times per day, we checked if ants drunk the delivered solution of Metformin, and they did. All the control experiments were first conducted on the two colonies normally maintained. Then, the tubes filled with sugar water were replaced by those filled with the sugared solution of Metformin, and the test experiments started after the ants had the latter tubes at their disposal for one day.

Figure 2. Realization of the sugared solution of Metformin given to the ants. Successively, the material used to make the solution, a tablet in the process of being crushed, one ant drinking the provided drug solution

2.5 Assessment of the 13 Considered Biological Traits

2.5.1 Meat and Sugar Water Consumption, Activity

On ants living under normal diet, then while they consumed Metformin, we separately counted those sighted on the meat food, sighted at the entrance of the sugar water tube, and being active in the foraging area, near the food, at the nest entrance and inside the nest, all this twice during the day and twice during the night (n ° of counts = 4 x 2 colonies = 8 counts per day for each kind of count). Such counting was made during 6 successive days. For each kind of count and each kind of diet, the daily mean was established (Table 1, lines 1 - 6). For each kind of considered trait, these six mean values obtained for ants intaking Metformin were compared to the six mean values recorded for ants normally maintained by using the non-parametric test of Wilcoxon (Siegel & Castellan, 1988). In addition, for each kind of diet and of count, the mean of the six daily means was calculated (Table 1, last line I-VI). Note that, each time the Wilcoxon test was used in this work, we gave, in the results section, the values of N, T, P as defined by Siegel and Castellan (1988).

2.5.2 Linear and Angular Speeds, Orientation

These three traits were quantified as usually (Cammmaerts, 2021b, c: Cammaerts & Cammaerts, 2015a, b; Cammaerts & Cammaerts, 2016, 2018, 2020e, 2021) on ants walking in their foraging area, the speeds without stimulating the ants, the orientation when stimulating them with a nestmate tied to a piece of paper (Figure 3 a). Such a tied nestmate emits its attractive alarm mandible glands pheromone, attracting so the ants located at 1 to 8 cm from it. For quantifying the ants' speeds, then their orientation, 40 ant trajectories were collected and treated with adequate software (Cammaerts et al., 2012). The latter assessed the three required variables on the basis of the three following criteria. An individual's linear speed (assessed for instance in millimeter per second = mm/s) equals the length of its trajectory divided by the time it spend to travel it; its sinuosity (quantified for instance in angular degrees per centimeter = ang.deg./cm) is the sum of the angles made by the successive parts of its trajectory divided by the length of this trajectory; its orientation (quantified for instance in angular degrees = ang. deg.) towards a given point is the sum of the successive angles it makes with its own direction and that to the given point, divided by the number of measured angles. An orientation value lower than 90 ° means that the animal tends to orient itself toward the location; an orientation value higher than 90 °means that the animal tends to avoid the location. For the three kinds of quantified variable, the median and the quartiles of the 40 recorded values were established (Table 2, lines 1, 2, 3), and the distribution of values obtained for ants consuming Metformin was compared to that obtained for ants living under normal diet using the non-parametric χ^2 test (Siegel & Castellan, 1988).

2.5.3 Audacity

This trait was studied, as usually (Cammmaerts, 2021b, c: Cammaerts & Cammaerts, 2015a, b; Cammaerts & Cammaerts, 2016, 2018, 2020e, 2021) by quantifying the ants' tendency to come onto an unknown apparatus. To do so, a cylinder (height = 4 cm; diameter = 1.5 cm) orthogonally attached to a square (9 cm^2), both of Steinbach® white paper, was deposited in the foraging area of each colony, and the ants coming onto this apparatus were counted 10 times over 10

minutes (Figure 3 b). The mean and the extremes of these counts were established (Table 2, line 4). The numbers obtained for the two colonies were correspondingly added. Then, the obtained sums relative to every two successive minutes were added, what provided five successive sums. The five sums obtained for ants consuming Metformin were compared to those obtained for ants under normal diet using the non-parametric Wilcoxon test (Siegel & Castellan, 1988).

2.5.4 Tactile Perception

An ant perceiving the uncomfortable character of a substrate moves on it slowly, sinuously, and often touches the substrate with its antennae (Figure 3 c1). An ant unable to correctly perceive the rough character of a substrate walks on it rather easily, rapidly, not vey sinuously, and seldom touches the substrate with its antennae. Therefore, for quantifying the ants' tactile perception, we assessed their linear and angular speeds while they walked on an uncomfortable substrate exactly as we assessed these two traits on ants moving in their foraging area (see the subsection relative to the ants' speeds). For making such an assessment, as in previous works (Cammmaerts, 2021b, c: Cammaerts & Cammaerts, 2015a, b; Cammaerts & Cammaerts, 2016, 2018, 2020e, 2021), a piece (3 cm x 2 + 7 + 2 = 11 cm) of n °280 emery paper was inserted in a tray (15 cm x 7 cm x 4.5 cm) in order to divide this tray in a first small 3 cm long zone, a second 3 cm long one covered with the emery paper, and a third 9 cm long zone. For each colony, 12 ants were transferred in the first small zone of the tray, and their trajectories were recorded while they walked on the emery paper. The ants' linear and angular speeds were then quantified. The 24 recorded values of linear and of angular speeds were characterized by their median and quartiles (Table 2, lines 5, 6). The distributions of the values of these two variables obtained for ants consuming Metformin were compared to those obtained for ants normally maintained using the non-parametric χ^2 test (Siegel & Castellan, 1988).

2.5.5 Brood Caring Behavior

This social task, which occurs in the nest, was evaluated through the ants' behavior in front of larvae experimentally removed from the nest. Normally, the ants soon re-entered such larvae. To make the required evaluation, as in e.g., Cammmaerts, 2021b, c: Cammaerts and Cammaerts, 2015a, b; Cammaerts and Cammaerts, 2016, 2018, 2020e, 2021, for each colony, a few larvae were taken out of the nest and set in front of its entrance. For each colony, the workers' behavior towards five of these larvae was observed during five minutes, and the not re-entered ones were counted after 30 seconds, 1, 2, 3, 4, 5 minutes (n °of counts = 5 x 2 =10) (Table 3, line 1; Figure 3 d). We looked only at five larvae for each colony because we had to look at all of them at the same time. The experiment was made only once because removing brood from the nest perturbs the ants and imperils the larvae's survival. The six numbers obtained for the two colonies were correspondingly added, and the six sums obtained for ants consuming Metformin were compared to those obtained for ants normally maintained using the non-parametric test of Wilcoxon (Siegel & Castellan, 1988).

2.5.6 Social Relationships

Ants belonging to the same colony are not aggressive towards each other. Several factors may alter this peaceful social behavior. For examining if Metformin has such an impact, as in e.g., Cammmaerts, 2021b, c: Cammaerts and Cammaerts, 2015a, b; Cammaerts and Cammaerts, 2016, 2018, 2020e, 2021, five dyadic encounters were performed for each colony in a small cup (diameter = 2 cm, height = 1.6 cm) the borders of which having been slightly covered with talc to prevent ants from escaping. During each encounter, one ant of the pair was observed during 5 minutes, and we recorded its amounts of 'level 0 = doing nothing', 'level 1 = making antennal contacts with its congeners', 'level 2 = opening its mandibles', 'level 3 = gripping its congener', and 'level 4 = trying to sting or stinging its congeners' (Table 3, line 2, Figure 3 e). The numbers recorded for each ant and each colony were correspondingly added, and the distribution of values obtained for ants consuming Metformin was compared to that obtained for ants maintained under normal conditions using the non-parametric χ^2 test (Siegel & Castellan, 1988). For each kind of diet, the ants' social interactions were also assessed by a variable 'a' equaling the number of aggressiveness levels 2 + 3 + 4 divided by the number of aggressive levels 0 + 1 (Table 3, line 2).

2.5.7 Stress and Cognition

Escaping from an enclosure requires staying calm, not stressing, meticulously looking for an exit, and detaining some cognitive ability. Therefore, to assess the state of stress and the cognitive ability of the ants, for each colony, six ones were enclosed under a reversed polyacetate cup (height = 8cm, bottom diameter = 7 cm, ceiling diameter = 5 cm, the inside surface of the cup having been slightly covered with talc) set in their foraging area. This protocol has been often used (e.g., Cammmaerts, 2021b, c: Cammaerts & Cammaerts, 2015a, b; Cammaerts & Cammaerts, 2016, 2018, 2020e, 2021). A notch (3 mm height, 2 mm broad) was created in the bottom rim of the enclosure for allowing the ants to escape (Figure 3 f). For each colony, the numbers of ants which could escape after 2, 4, 6, 8, 10 and 12 minutes were recorded, then the numbers obtained for the two colonies were correspondingly added (Table 3, line 3). The six sums obtained for ants consuming Metformin were compared to those obtained for ants normally maintained using the non-parametric Wilcoxon test (Siegel & Castellan, 1988).

2.5.8 Cognition

This trait was evaluated, as usually (Cammmaerts, 2021b, c: Cammaerts & Cammaerts, 2015a, b; Cammaerts & Cammaerts, 2016, 2018, 2020e, 2021), through the ants' skill in crossing a twists and turns path. For each colony, two pieces of Steinbach® paper (12 cm x 4.5 cm) duly folded were inserted into a tray (15 cm x 7 cm x 4.5 cm) in order to create a twists and turns path between a 2 cm long zone in front of this path and an 8 cm long zone beyond it. For making an experiment on a colony, 15 ants were deposited into the zone lying in front of the twists and turns path, and the ants still there as well as those having reached the zone lying beyond the 'difficult' path were counted after 2, 4, 6, 8, 10 and 12 minutes (Figure 4 a, Table 3, line 4). The numbers obtained for the two colonies were correspondingly added, and for each zone of the design, the numbers obtained for ants consuming Metformin were compared to those obtained for ants normally maintained using the non-parametric Wilcoxon test (Siegel & Castellan, 1988).

2.5.9 Conditioning Acquisition, Memory

We proceeded as previously (e.g., Cammmaerts, 2021b, c: Cammaerts & Cammaerts, 2015a, b; Cammaerts & Cammaerts, 2016, 2018, 2020e, 2021). For each colony, at a recorded time, a green hollow cube (constructed in strong paper (Canson®)) was deposited above the entrance of the tube filled of sugared solution of Metformin and the meat food was relocated near that cube (Figure 4 b a). Since this deposit, the ants underwent operant visual conditioning. The control experiment on ants normally maintained, was previously performed on another similar colony *of M. sabuleti* collected in the same site. Such a doing was required because since a worker is conditioned to a stimulus, it keeps its conditioning during several days, and even after having lost it, it acquires it again more rapidly than initially, and its conditioning acquisition can no longer be validly assessed. Over the ants' conditioning acquisition, then after the green cube removal, over the ants' loss of conditioning, 10 workers of each colony were tested in a Y-apparatus. A Y-maze was built for each colony in strong white paper, with its sides slightly covered with talc, and was set in a separated tray (15 cm ×7 cm ×5cm). A green hollow cube was deposited randomly in the left or the right branch of these Y-apparatus. For making a test on a colony, 10 workers were transferred one by one in the area lying in front of the Y-maze division into its two branches. The first choice made by each experimented ant between one or the other branch of the Y-apparatus was recorded (Figure 4 b b). Of course, choosing the branch containing the green cube was considered as giving the correct response. After having been tested, each ant was kept in a cup, until 10 ants of its colony were tested to avoid testing twice the same ant. After having tested 10 ants of a colony, all of them were transferred back into their foraging area. For each considered time period (Table 4), the responses obtained for the two experimented colonies were correspondingly added. The 10 control responses and the 10 summed responses obtained for ants consuming Metformin were compared to each other using the non-parametric test of Wilcoxon (Siegel & Castellan, 1988). Also, for each considered time period and each diet, the ants' conditioning score (i.e., the proportion of correct responses) was established (Table 4).

2.5.10 Adaptation to a Side Effect of Metformin

The reasoning is identical to that used in previous works (e.g., Cammaerts, 2021b, c: Cammaerts & Cammaerts, 2015a, b; Cammaerts & Cammaerts, 2016, 2018, 2020e, 2021). An individual adapts itself to the side effects of a drug when, over this drug consumption, it less and less suffers from these side effects. To study such an adaptation, a trait affected by the drug has to be assessed soon after the start of the consumption, then again after some time of such a consumption, and the results of the two assessments must be compared. In the present work, Metformin appeared to affect the ants' locomotion. Therefore, the ants' linear and angular speeds were assessed after the ants had the drug at their disposal during seven days, exactly as they had been after one day, and the distribution of the values each time recorded were compared to one another using the non-parametric χ^2 test (Siegel & Castellan, 1988). Also, for each kind of speed, the median and quartiles of the values recorded after seven days of ants' maintenance under a diet with Metformin were established as they had been after one day of such a maintenance (Table 5, upper part).

2.5.11 Habituation to a Wanted Effect of Metformin

The reasoning is identical to that used in previous works (e.g., Cammmaerts, 2021b, c: Cammaerts & Cammaerts, 2015a, b; Cammaerts & Cammaerts, 2016, 2018, 2020e, 2021). An individual becomes habituated to an expected effect of a drug when it less and less perceives this effect over its drug consumption. To evaluate such a habituation, an expected effect of the drug has to be assessed soon after the individual begins consuming the drug, and then after it had consumed the drug during some time, and the two assessments must be compared. In the present work, the ants' less intake of sugar water may be an expected effect of Metformin. Consequently, to evaluate the ants' habituation to Metformin, their sugar water intake was assessed after they had that drug at their disposal during eight days, exactly as it had been assessed during the first six days of their maintenance under a diet with Metformin, except that the six assessments were made over 24 hours (6 x 8 counts) instead of over six days. The values of the six assessments made after the ants had the drug at their disposal for eight days were compared to the six values of the assessments performed over the six first days of the ants' drug

consumption, using the non-parametric test of Wilcoxon (Siegel & Castellan, 1988). Also, the mean of the last six assessments was established as had been the mean of the first six assessments (Table 5, middle part).

2.5.12 Dependence on Metformin Consumption

An individual develops dependence on a drug when it "enjoys" consuming this drug, tries to have it at its disposal at any time, consumes the drug even if it suffers from some adverse effects, and becomes unable to live without consuming the drug. In the present work, the ants' dependence on Metformin was studied after they had this drug at their disposal during 9 days, this using a protocol already often used (e.g., Cammmaerts, 2021b, c: Cammaerts & Cammaerts, 2015a, b; Cammaerts & Cammaerts, 2016, 2018, 2020e, 2021). The cotton plug of the tubes containing the sugared solution of Metformin was not refreshed on the tenth day, and at the end of that day, 15 ants of each two colonies were deposited in an own tray (15 cm ×7 cm ×5 cm) containing two cotton-plugged small tubes (h = 2.5 cm, diam. = 0.5 cm), one full of sugar water, the other full of the sugared solution of Metformin used all over the work. The tube containing the drug was located on the right in one tray and on the left in the other tray (Figure 4 e). This having been done, for each sample of 15 ants of each colony, those present near each two presented tubes were counted 15 times over 15 minutes, and the 15 counts obtained for each colony were correspondingly added. Also, the 15 added counts were summed for each kind of tube (i.e., of each solution) what allowed establishing the proportions of ants having visited one and the other tubes (Table 5, lower part). In addition, the sums of the 15 added counts corresponding to each presented tubes were compared to the numbers expected if ants randomly approached each tube, using an adequate non-parametric test, i.e., the χ^2 goodness-of-fit one (Siegel & Castellan, 1988).

2.5.13 Decrease of the effect of Metformin After Its Consumption Was Stopped

This decrease was studied after the ants had consumed Metformin during 10 days, and the trait used for conducting this study was the ants' angular speed which was largely impacted by the drug consumption. The protocol was similar to that already often used (e.g., Cammmaerts, 2021b, c: Cammaerts & Cammaerts, 2015a, b; Cammaerts & Cammaerts, 2016, 2018, 2020e, 2021). One day before starting the study, a fresh sugared solution of Metformin was delivered to the ants, and the day after, the ants' angular speed was assessed as it had been assessed after 1 and 7 days of this drug consumption, except that 20 instead of 40 ants' trajectories were recorded and analyzed. This sample reduction was done for having time enough to analyze the recorded data all along the decrease of the effect of the drug, and so to evaluate the level of the decay of the impact of Metformin on the ants' angular speed. Just after this first assessment made at t = 0, the weaning started: the ants' tubes full of the sugared solution of Metformin were replaced by tubes full of pure sugared water. From then on, the ants' sinuosity was quantified each two hours until this trait value was identical to that obtained for ants normally maintained (= to the control values). For each quantification, the median and quartiles of the 20 recorded values of sinuosity were established (Table 6). Also, each successively obtained distribution of values was compared to that obtained at t = 0 and to the control one using the non-parametric χ^2 test (Siegel & Castellan, 1988). In addition, the mathematical function best describing the decrease of the effect of Metformin on the ants' angular speed was empirically sought and statistically analyzed, and is given in the text. The result of the present study is illustrated in Figure 5.

3. Results and Discussion

3.1 Food Consumption, General Activity

These physiological traits were impacted by Metformin consumption (Table 1). Under that drug diet, the ants eat less meat ($N = 6$, $T = 21$, $P = 0.016$), drunk less sugar water ($N = 6$, $T = 21$, $P = 0.016$), and were less active ($N = 6$, $T = 19$, $P = 0.047$) than when living under normal diet. The most impacted trait was the sugar water intake; the less impacted trait was the individuals' activity. These effects of Metformin should be taken into account when caring of patients with this drug, although eating less, and essentially intaking less sugar, may be one of the expected and wanted effects of the drug.

3.2 Linear and Angular Speeds

The ants' locomotion was affected by Metformin consumption (Table 2, lines 1, 2). While consuming this drug, the ants walked more slowly ($\chi^2 = 28.99$, $df = 2$, $P < 0.001$) and essentially more sinuously ($\chi^2 = 59.02$, $df = 2$, $P < 0.001$) than when living under normal diet. This was very obvious to observers: the walking ants continuously changed of direction, and made some abnormal movements with their legs and their body. Such a potential effect of Metformin on the individuals' displacements should be considered when caring of humans with this drug.

Table 1. Effect of Metformin on ants' food intake and activity. The table gives, for each kind of diet, the mean numbers of ants counted over six days on their meat, on their sugar water and being active at any place (lines I to VI), as well as the mean of these six recorded means (line I-VI). The drug decreased the three considered traits, essentially the ants' sugar water intake

Days	Normal diet			Diet with Metformin		
	on meat	on sugar water	in activity	on meat	on sugar water	in activity
I	1.00	1.50	12.00	0.75	0.50	11.38
II	0.75	1.38	9.50	0.50	0.38	10.38
III	1.75	1.75	17.00	0.38	0.00	10.25
IV	1.50	1.75	14.63	0.25	0.25	9.75
V	1.63	2.25	15.50	1.00	0.50	9.50
VI	1.75	2.50	14.25	0.75	0.75	8.50
I-VI	1.39	1.85	15.31	0.60	0.39	9.96

3.3 Orientation

While consuming Metformin, the ants less well oriented themselves towards a tied nestmate than when living under normal diet (Table 2, line 3; Figure 3 a). This was obvious to observers and was statistically significant ($\chi^2 = 24.04$, df = 2, $P < 0.001$). The experiment was repeated, and a median of 53.2 angular degrees was obtained, confirming thus the results of the initial experiment. This decrease of orientation ability may be due to the ants' large sinuosity of movement (see the above subsection), but may also result from a weaker ants' olfactory or general sensory perception, a hypothesis examined thanks to a following experiment (see below the subsection devoted to the ants' tactile perception).

3.4 Audacity

Metformin impacted the ants' tendency to come onto the presented unknown apparatus (Table 2, line 4; Figure 3 b). While ants under normal diet rather frankly came onto such an apparatus, those consuming the drug were reluctant to do so. They hesitated to come on the apparatus; if they did so, they soon went away from it; if they tempted to climb on the tower, they soon went back or even felt down. Such an observation was in agreement with that made for the ants' locomotion (see the above subsection relative to the ants' linear and angular speeds). The numbers of ants sighted on the apparatus over the experimental time statistically differed between the ants consuming Metformin and those normally maintained ($N = 5$, $T = 15$, $P = 0.031$). Such an impact of the drug should be considered when caring of patients thanks to Metformin.

Table 2. Effect of Metformin on five ants' ethological and physiological traits. For each kind of diet and each examined trait, the table gives the median (and quartiles) or the mean [and the extremes] of the recorded data. The drug affected the ants' locomotion, orientation and audacity, but did not impact their tactile (maybe their general sensory) perception. Photos can be seen in Figure 3

Traits	Normal diet	Diet with Metformin
Linear speed (mm/s)	9.7 (8.8 – 10.9)	7.5 (6.6 – 8.1)
Angular speed (ang.deg./cm)	106 (92 – 129)	212 (191 – 238)
Orientation (ang.deg.)	30.4 (24.2 – 37.9)	56.1 (42.7 – 70.7)
Audacity (n°)	2.50 [1 – 4]	1.20 [0 – 2]
Tactile perception:		
linear speed	3.5 (3.3 – 4.2)	3.6 (3.2 – 3.9)
angular speed	334 (286 – 358)	318 (286 – 359)
on a rough substrate		

3.5 Tactile Perception

This important physiological trait was not affected by Metformin consumption (Table 2, lines 5, 6; Figure 3 c). When ants under normal diet walked on a rough substrate, their linear speed was smaller and their angular speed was higher than when they walked on their foraging area, and these differences were statistically significant (linear speed as well as

angular speeds: $\chi^2 = 64.00$, df = 1, $P < 0.001$). Such differences also occurred for ants consuming Metformin, the differences being simply somewhat smaller because these ants' linear speed was already smaller and their angular speed already larger when they walked in their foraging area. The differences between their walking on one and the other kinds of substrate were however highly significant: linear speed: $\chi^2 = 23.39$, df = 1, $P < 0.001$; angular speed: $\chi^2 = 29.57$, df = 1, $P < 0.001$). This result is in favor of the drug use.

3.6 Brood Caring

This ethological trait was affected by Metformin consumption (Table 3, line 1; Figure 3 d). When living under normal diet, the ants soon found the larvae removed from the nest, held them with their mandibles, and re-entered them in the nest. While consuming Metformin, the ants had some difficulty in holding the larvae; they tried several times to do so and did not succeed each time. If they finally succeeded in holding a larva, they presented obvious difficulties for transporting them. All this leaded to only few larvae re-entered in the nest over the five experimental minutes. The difference as for the numbers of not re-entered larvae over time between the ants maintained under one and the other kinds of diet was significant: N = 6, T = 21, P = 0.016. This result was in agreement with that on the ants' locomotion and that on their capability in climbing on a tower (see the two above subsections relative to the ants' speeds and to the ants' audacity).

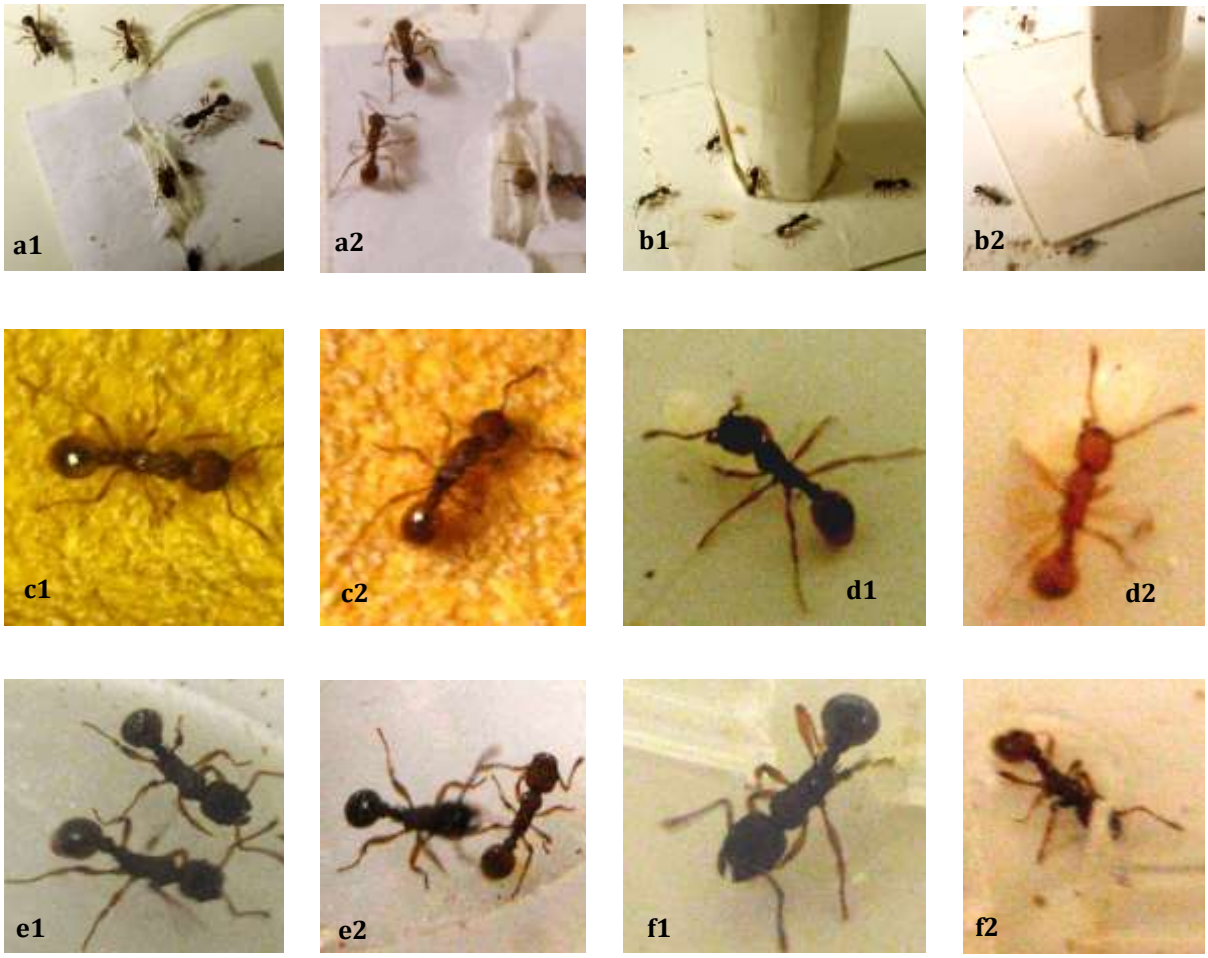


Figure 3. A few photos of the experiments made to know the adverse effects of Metformin on six ants' physiological and ethological traits. 1: ants under normal diet; 2: ants under a diet with Metformin. a: ants stimulated by a tied nestmate, coming towards it under normal diet, not doing so while consuming the drug. b: while consuming the drug, the ants were less inclined to come onto an unknown apparatus. c: ants under each kind of diet perceiving the uncomfortable character of a substrate and walking on it with difficulty. d: ants easily transporting a larva when being under normal diet, and with difficulty while consuming the drug. e: two nestmates staying peacefully side by side when living under normal diet, and being slightly aggressive when consuming the drug. f: ants enclosed, escaping when under normal diet, not doing so when consuming the drug

3.7 Social Relationships

This ethological trait appeared to be somewhat affected by Metformin consumption (Table 3, line 2; Figure 3 e). Ants normally maintained generally stayed near its congeners, touching them with their antennae. Ants consuming the drug did not stay side by side during long time periods, though not really avoiding their nestmates. When they were very near from each other, they rather often opened their mandibles, but never gripped or tried to sting their opponent. Nevertheless, the difference as for the numbers of presented levels of aggressiveness between the ants under one and the other kinds of diet was significant: $\chi^2 = 10.60$, df = 2, $0.001 < P < 0.01$. The variable assessing the ants' aggressiveness equaled 0.05 and 0.21 for ants maintained under respectively a normal diet and a diet with Metformin. This slight but significant impact of the drug on the individuals' social interactions should be taken into account when caring of patients with Metformin.

3.8 Escaping Ability

This ethological trait was impacted by Metformin consumption (Table 3, line3; Figure 3 f). When maintained under normal diet, the ants walked along the rim of the enclosure, found the exit, and escaped. When consuming Metformin, the ants also walked along the rim of the enclosure and also found the exit, but then, they hesitated to escape, they seemed undecided and they often walked back then away from the exit. Consequently, only 4 among 12 ants were escaped after the 12 experimental minutes while, when living under normal diet, the 12 experimented ants could escape. The difference between the ants living under one and the other kinds of diet as for the numbers of escaped ants over time was statistically significant: N = 6, T = 21, P = 0.016. This result was in agreement with that relative to the ants' audacity (see the above subsection concerning this trait), and should be considered when treating patients with Metformin.

3.9 Cognition

This trait was impacted by Metformin consumption (Table 3, line 4; Figure 4 a). Ants living under normal diet soon entered the twists and turns path, and 10 among 30 ants could cross this difficult path over the twelve experimental minutes. When consuming Metformin, the ants hesitated to enter the twists and turns path, and delayed in progressing inside this difficult-path. They often came back on their way. Consequently, after the twelve experimental minutes, only 3 ants could cross the twists and turns path, and were beyond it. The difference between the ants maintained under one and the other kinds of diet as for their presence in front and beyond the difficult path was statistically significant: in front: N = 6, T = 21, P = 0.016; beyond: N = 4, T = 10, P = 0.063). This result was in agreement with those on the ants' audacity and escaping ability (see the subsections relative to these two traits): the ants hesitated, were undecided, and seemed reluctant to make novel tasks. Such an impact of Metformin should be considered when caring of patients with this drug.

Table 3. Effect of Metformin on four ethological and physiological traits. The significance of what is written in column 1 is given in the 'Materials and Methods' section. The drug impacted the ants' social interactions (brood caring, social relationships), state of stress and cognition. Photos are shown in Figures 3 and 4

Traits	Normal diet						Diet with Metformin					
Brood caring: n° of not re-entered larvae among 12 ones over time	30"	1'	2'	3'	4'	5'	30"	1'	2'	3'	4'	5'
	8	7	5	2	0	0	10	10	10	7	5	3
Social relationships: n° of 0 to 4 aggressive levels; variable 'a'	0	1	2	3	4	a	0	1	2	3	4	a
	70	64	7	0	0	0.05	60	51	23	0	0	0.21
Stress, cognition: n° of escaped ants among 12 ones over time	2	4	6	8	10	12 min	2	4	6	8	10	12 min
	2	4	7	9	11	12	0	1	2	2	3	4
Cognition: n° of ants in front and beyond a twists and turns path over 12 minutes		2	4	6	8	10	12		2	4	6	8	10	12
	in front	23	20	16	14	12	9	in front	24	21	19	18	17	16
	beyond	0	0	4	6	7	10	beyond	0	0	0	0	0	3

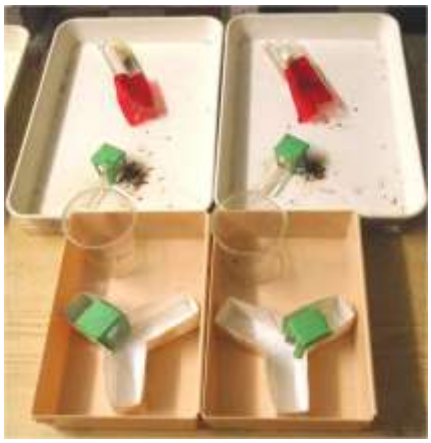
3.10 Conditioning Acquisition, Memory

These abilities were affected by Metformin consumption (Table 4; Figure 4 b2). Ants maintained under normal diet soon acquired conditioning: they reached a score of 70% and 85% after respectively 31 and 72 training hours; they still detained a score of 80% 72 hours after the cue removal. When consuming Metformin, the ants never acquired conditioning; they still presented a score of 45% after 72 training hours. The difference of reached conditioning scores

over time between the ants living under one and the other kinds of diet was significant: N = 6, T = 21, P = 0.016. The ants consuming the drug having learned nothing, their middle-term memory could not be assessed. When such ants were in the Y-maze, they were reluctant in entering a branch of the maze, above all that containing the green cube. Their hesitation recalled that presented near the risky apparatus (see the subsection relative to the ants' audacity), near the exit of the enclosure (see the subsection relative to the ants' escaping ability), as well as in front and inside the twists and turns path (see the subsection relative to the ants' cognition). Obviously, Metformin induced hesitation, reluctance in performing novel tasks, and this should be considered when treating patients with this drug.

Table 4. Impact of Metformin on the ants' conditioning acquisition and memory. The table gives the numbers of correct *versus* wrong responses (for colonies A; B under a diet with Metformin), and the resulting proportion of correct responses (= the conditioning scores). The ants' responses under normal diet were previously obtained on another similar colony. Under Metformin diet, the ants did not acquire conditioning, and this prevented assessing their middle-term memory. Two more photos are shown in Figure 4

Time (hours)	Normal diet		Diet with Metformin
7 hrs	6 *vs* 4	60%	5 *vs* 5 ; 5 *vs* 5 → 50%
24 hrs	6 *vs* 4	60%	5 *vs* 5 ; 5 *vs* 5 → 50%
31 hrs	7 *vs* 3	70%	5 *vs* 5 ; 5 *vs* 5 → 50%
48 hrs	7 *vs* 3	70%	6 *vs* 4 ; 6 *vs* 4 → 60%
55 hrs	8 *vs* 2	80%	4 *vs* 6 ; 5 *vs* 5 → 45%
72 hrs	9 *vs* 1	85%	3 *vs* 7 ; 6 *vs* 4 → 45%
Cue removal			
7 hrs	9 *vs* 1	85%	
24 hrs	8 *vs* 2	80%	
31 hrs	8 *vs* 2	80%	could not be examined
48 hrs	8 *vs* 2	80%	
55 hrs	8 *vs* 2	80%	
72 hrs	8 *vs* 2	80%	



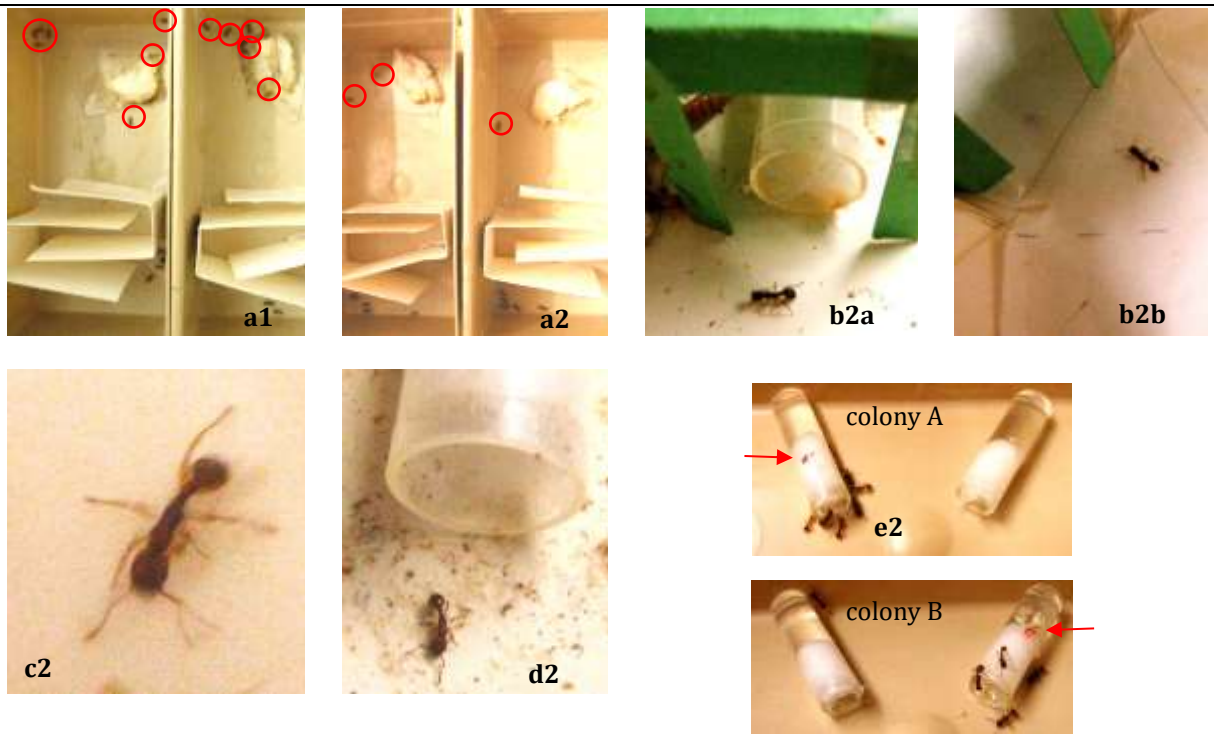


Figure 4. Some views of experiments made for knowing the impact of Metformin on the ants' cognition (a), and learning (b), their adaptation to the impact of the drug on their locomotion (c), their habituation to the effect of the drug on their sugar water consumption (d), and their dependence on Metformin consumption (e, drug solution is indicated by red dots and arrows). Successively: a: the drug impacted the ants' cognition; b: under the drug diet, the ants

hesitated to approach the green cube; c: ants did not adapt themselves to the impact of the drug on their locomotion; d: ants consuming the drug still drunk little sugar water; e: ants developed dependence on the drug consumption

3.11 Adaptation to the Impact of Metformin on the Ants' Locomotion

After seven days of Metformin consumption, the ants had not adapted themselves to the impact of Metformin on their linear and angular speeds (Table 5, upper part; Figure 4 c). Indeed, at that time, the ants' linear speed was even smaller, though statistically not smaller than the speed they presented after one day of this drug consumption ($\chi^2 = 0.27$, df = 1, $0.50 < P < 0.70$). Also, after seven days of the drug consumption, the ants' angular speed was even larger than that presented after one day of consumption and this increase was statistically significant ($\chi^2 = 12.34$, df = 2, $0.001 < P < 0.01$). Such a non-adaptation to the side effects of the drug is not in favor of its use. This effect is not mentioned in the notice for use joined to the drug package (on the contrary, it is reported that side effects may occur only at the start of treatment). This should be known by practitioners and taken into account while treating patients with Metformin.

3.12 Habituation to the Impact of Metformin on the Ants' Sugar Water Consumption

After having consumed Metformin during eight days, the ants went on drinking very little sugar water (Table 5, middle part; Figure 4 d). The six successive mean numbers of workers sighted on the sugar water were 0.13, 0.25, 0.25, 0.38, 0.13, 0.25, and these six means did not statistically differ from the six ones obtained during the six first days of the drug consumption: N = 6, T = +4.5, -16.5, P = 0.133. The mean of the six recorded means equaled 0.39 for the six first days of Metformin, and 0.23 for the eighth day. It could thus be concluded (and this was obvious to the observers: Figure 4 d) that the ants did not habituate themselves to the effect of the drug on their sugar water intake. If this decrease of sugar intake is an expected effect of Metformin, then, it can be stated that the drug keeps it effect over time on ants used as biological model organisms, and at the concentration used in this experiment. However, patients treated with this drug should be monitored as for their potential acquisition of anorexia and as for a too large loss of weight.

Table 5. Ants' adaptation to the side effect of Metformin on their locomotion, habituation to the drug effect on their sugar water consumption, and dependence on this drug consumption. Ants did not adapt themselves to the impact of the drug on their locomotion, did not habituate themselves to the impact of the drug on their sugar water intake, and developed dependence on the drug consumption. Photos are shown in Figure 4. mm/s: millimeter per second; ang.deg./cm: angular degree per centimeter

Adaptation	Normal diet	+ Metformin for 1 day	+ Metformin for 7 days
linear speed (mm/s)	9.7 (8.8 – 10.9)	7.5 (6.6 – 8.1)	6.6 (5.8 – 7.6)
angular speed ang.deg./cm)	106 (92 – 129)	212 (191 – 238)	241 (220 – 271)
Habituation	Normal diet	+ Metformin for 1-6 days	+ Metformin for 8 days
n° of ants on the sugar water	1.85	0.39	0.23
Dependence: n° of ants on drug *vs* drug-free solutions	colony A: 69 *vs* 28	colony B: 33 *vs* 12	total: n^{os}: 102 *vs* 40 → 71.83% *vs* 28.77%

3.13 Dependence on Metformin Consumption

Numerical results are reported in Table 5, lower part, and two photos are shown in Figure 4 e. Ants developed dependence on Metformin consumption. In the course of the experiment devoted to the exam of such a potential dependence, 69 ants of colony A were counted on the drug solution and 28 ones on the drug-free solution, while 12 ants of colony B were counted on the drug-free solution and 33 ones on the drug solution. In total, 102 ants were sighted on the drug solution and 40 ones on the drug-free solution, what leaded to 71.83% of ants having chosen the drug solution and 28.17% having chosen the drug-free solution. The recorded numbers (102 *vs* 40) were statistically different from the numbers (71 *vs* 71) which should be obtained if ants had randomly gone onto the two provided solutions ($\chi^2 = 13.31$, df = 1, $P < 0.001$). This revealed dependence, and consequently the 'non- stop' consumption of the drug, may, at least partly, explain the occurrence of anorexia and other symptoms in patients treated with this drug. Such patients should thus be imperatively monitored as for their potential development of dependence on the drug.

3.14 Decrease of the Effect of Metformin After Its Consumption Was Stopped

Table 6 gives the numerical and the statistical results; Figure 5 graphically illustrates the results. In short, the effect of Metformin totally vanished in a total of about 13 hours after its consumption was stopped. In details, 2 hours after weaning, the effect of the drug was still statistically similar to its initial one, but 4 hours after weaning, it was already lower than its initial effect, and this could be perceived by consumers. The effect of Metformin went on decreasing over time after weaning. It still differed from the control situation until 12 hours after weaning (after 10 hours: $P < 0.001$; after 12 hours: $P < 0.01$), and became statistically similar to the control situation 14 hours after weaning ($P < 0.50$). It could thus

be stated that the effect of Metformin vanished in a total of about 13 hours after its consumption was stopped. The assessment made 20 hours after weaning confirmed this vanishing. From 0 to 6 hours after weaning, Metformin lost '51 ang. deg./cm' of its effect, so 8.5 ang.deg./cm per hour. From 6 to 12 hours after weaning, Metformin lost '70 ang. deg./cm' of its effect, so 11.67 ang.deg./cm per hour. From 12 to 14 hours after weaning, Metformin lost '25 ang. deg./cm' of its effect, so 12.5 ang.deg./cm per hour. Metformin effects presented thus a decrease which slightly increased over time, but this slight increase was not statistically significant, and *in fine*, a mathematical and statistical analysis of the recorded data revealed that the decrease of the angular speed values recorded over time nearly obeyed to a linear function, and could best obey to the following function:

$$E_t = E_I - a\,t \qquad \text{or} \qquad E_t = 254 - 10.21\,t$$

with E_t = effect at time 't'; E_I = initial effect; t = time (in hours)

Note that, using the experimental values of 'a' here above reported (8.5, 11.67, 12.5), we obtained, for a linear function, a value of 'a' equaling 32.67/3 = 10.89, what approached the value provided by the mathematical analysis.

Such a rapid decrease of the effect of Metformin after weaning could account for the development of dependence on that drug consumption (Cammaerts, 2018b).

Table 6. Decrease of the effect of Metformin after its consumption was stopped. The table gives the median (and quartiles) of ants' angular speed values (in angular degrees per centimeter) obtained over the decrease (column 2), as well as the results of the statistical analysis (column 3). Briefly, the effect of the drug soon became different from its initial one, and total vanished in about 13 hours. These results are illustrated in Figure 5

Time	Ants' angular speed	*vs* t = 0 statistics			*vs* control		
(hours)		χ^2	df	P	χ^2	df	P
0 hrs	257 5230-288)		--		60.00	1	<0.001
2 hrs	230 (204 – 253)	2.92	1	<0.30	52.11	1	<0.001
4 hrs	206 (175 – 229)	13.26	1	~0.001	41.98	1	<0.001
6 hrs	201 (188 – 246)	13.34	2	~0.001	26.45	1	<0.001
8 hrs	173 (146 – 196)	26.27	1	<0.001	23.08	1	<0.001
10 hrs	161 (125 – 204)	21.54	1	<0.001	19.01	2	<0.001
12 hrs	131 (116 – 148)	40.00	1	<0.001	6.72	1	<0.01
14 hrs	106 (95 – 116)	40.00	1	<0.001	0.64	1	<0.50
20 hrs	104 (88 – 127)	40.00	1	<0.001	0.40	1	<0.90
control	106 (92 – 129)	60.00	1	<0.001		--	

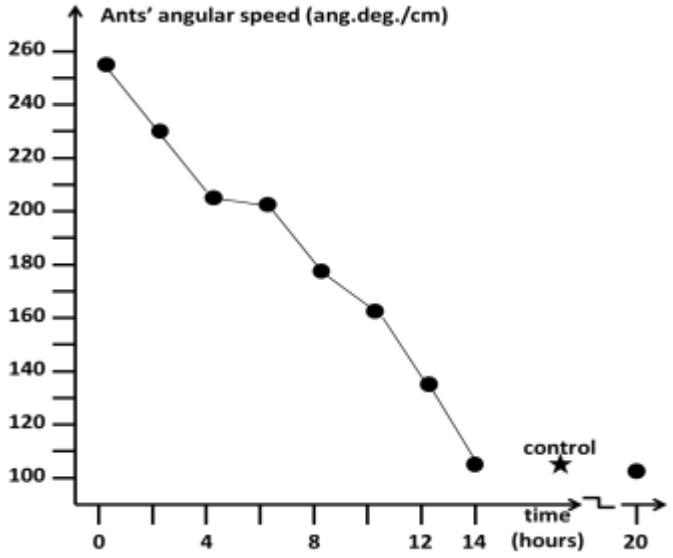


Figure 5. Decrease of the effect of Metformin after its consumption was stopped. Numerical and statistical results are given in Table 6. Briefly, the effect of the drug rapidly decreased, becoming significantly lower than its initial one as soon as 4 hours after weaning, and totally vanishing in about 13 hours. This decrease could be best described thanks to a linear function of the passing time

4. Discussion, Conclusion

Nowadays, Metformin is used for caring of persons suffering from type-2-diabetes (as initially) and several other illnesses, and is the second more frequent API pollutant of the natural watercourses. Few adverse effects are reported for Metformin, and most of them are not easily available to general public. On ants, this drug largely impacted the individuals' food consumption (mainly reducing the sugar water consumption), activity, orientation, audacity, social interactions, state of stress, cognition, learning and memorization. Ants did not adapt and did not habituate to the effect of Metformin, and they developed dependence on its consumption. After weaning, the effect of Metformin soon differed from its initial one and fully vanished in 13 hours. The results of our different experiments agreed with one another, and were also in agreement with those of other researchers (see the introduction section), considering the fact that we used a different model organism that other researchers. Before concluding, we below give some more bibliographical information on Metformin.

Metformin is effectively very efficient for caring of persons suffering from type 2-diabetes, and is so thanks to several modes of action more and more elucidated over practitioners' researches (Wiemsperger & Bailey, 2012). It is efficient for adults (same reference as above), and also for children (Kenneth et al., 2002). Metformin has also been proved to be efficient for treating obesity: the obtained results are really scientifically valid, and its modes of action are now known (Paolisso et al., 1998). Metformin also appeared to be useful for helping caring of persons suffering from some kinds of cancer (Jun et al., 1998; Legros et al., 2007). Research on this promising subject is going on, and, generally, best efficiency is obtained by combining Metformin with other drugs (Jun et al., 1998; Kailin et al., 2020).

Concerning the adverse effects of Metformin, the best-known ones are those on the intestine and on the liver; they are considered as being very important and are still now largely studied (Tongzhi et al., 2017). As for the effect of Metformin on the kidneys, let us recall (see the introduction section) that Hsu and co-authors have shown that this drug has an adverse effect on the renal function in patients suffering from type 2-diabetes and moderate chronic kidney disease (Wei-Hao, 2018). However, other researchers have shown that, in some cases of kidney illness, thanks to its numerous clinical effects, Metformin can act as a therapeutic drug (Heaf, 2014).

As for its environmental impact, being a largely used drug, Metformin is present in wastewater, and, together with its degraded compound guanylurea, contributes to the natural water pollution by API (Kosma et al., 2015: a study made in Greece and lasted four years). In their review, Elizalde-Velazquez and co-authors explain how Metformin is present in natural water and is toxic, report perspectives for reducing the adverse impacts of this drug on the environment, but do not relate any ethological or physiological effect of the drug on the aquatic fauna (Elizalde-Velazquez & Gomez-Olivan, 2020). In the same year, several co-authors found that Metformin present in water can be degraded by photocatalysis, and that the degradation products are not toxic (Carbuloni et al., 2020). Although we looked for published works on the biological impacts of Metformin on the aquatic fauna or on any other animals used as models, using several actual systems of research, we could not find any. The present work brings thus novel unpublished and useful information.

In the present paper, we reveal that, in experimental conditions and under a rather high concentration, Metformin caused adverse physiological and ethological harmful effects to ants. Therefore, the drug could very likely affect any insects. Since most species constituting the macrobenthos and inhabiting watercourses are insects, there is a risk that a prolonged exposition to Metformin induces changes in the behavior and/or in some physiological traits of these insect species, with consequences on the whole community living in the river bed and on the ecological processes which there occur. For instance, a decrease of general activity, orientation, audacity, and food consumption (as we observed in ants), occurring in preys as well as in predators, could affect the whole trophy chain in watercourses, reducing the velocity of organic matter processing and thus of self-depuration efficiency. Other products used by humans also affect the environment. We have studied some of them (see the introduction section) and, to cite one work among the numerous ones made on the subject, a study has been recently made on the ant *Lasius niger*, the authors concluding that the use of neonicotinoids induce severe environmental and ecological risks (Schläppi at al., 2020).

To conclude, Metformin, with its several medicinal properties, must be used by practitioners. However, the patients must imperatively be monitored as for their general activity, audacity, social interactions, cognition, memorization, and above all their potential dependence on this drug consumption and the occurrence of anorexia. Also, wastewater should be submitted to photocatalysis in order to degrade the drug for not affecting the health of organisms, those living in the water and those consuming natural water, this including thus humans.

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Conflict of interest

We affirm having no conflict of interest as for the use of Metformin or any similar drugs. Marie-Claire Cammaerts make

research on ants, on their behavior and cognition, and uses them as models for examining several topics. David Cammaerts works on natural water quality and related subjects, and also teaches and makes researches on these subjects. None of us receive money for making our researches.

We have no other data than those written in our paper, in the text, the tables and the figures. So, all our data are available

The two authors equally worked on the reported work and agree with the content of the presented paper. David Cammaerts essentially provided the references, wrote the introduction section, and largely corrected a first version of the discussion section. Marie-Claire Cammaerts made the experiments and write a first version of the paper.

References

Aguirre-Martinez, G. V., Buratti, S., Falbri, E., Del Valls, A. T., & Martin-Diaz, M.L. (2013). Using lysosomal membrane stability of haemocytes in *Ruditapes philippinarum* as a biomarker of cellular stress to assess contamination by caffeine, ibuprofen, carbamazepine and novobiocin. *Journal of Environmental Sciences, 25*(7), 1408-1418. https://doi.org/10.1016/S1001-0742(12)60207-1

Andre, R. G., Wirtz, R. A., & Das, Y. T. (1989). Insect Models for Biomedical Research In: *Nonmammalian Animal Models for Biomedical Research*. Woodhead AD, editor, Boca Raton, FL: CRC Press, 1989.

Arnold, K. E., Brown, A. R., Ankley, G. T., & Sumpter, J. P. (2014). Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. *Philosophical Transactions of the Royal Society B, 369*, 20130569. https://doi.org/10.1098/rstb.2013.0569

Barmentlo, H., Schrama, M., de Snoo, G., Van Bogedom, P., Van Nieuwenhuizen, A., & Vijver, M. (2021). Experimental evidence for neonicotinoid driven decline in aquatic emerging insects. *Proceedings of the National Academy of Science, 118*(44), e2105692118. https://doi.org/10.1073/pnas.2105692118

Ben Sahra, I., Le Marchand-Brustel, Y., Tanti, J. F., & Bost, F. (2010). *Metformin in cancer therapy: a new perspective for an old antidiabetic drug? International Journal of Molecular Sciences, 9*(5), 1092-1099. https://doi.org/10.1158/1535-7163.MCT-09-1186

Bradley, P. M., Journey, C. A., Button, D. T., Carlisle, D. M., Cark, J. M., Mahler, B. J., ... VanMetre, P. C. (2016). Metformin and Other Pharmaceuticals Widespread in Wadeable Streams of the Southeastern United States. *Environmental Science & Technology Letters, 3*, 243-249. https://doi.org/10.1021/acs.estlett.6b00170

Brodin, T., Piovano, S., Fick; J., Klaminder, J., Heynen, M., & Jonsson, M. (2014). Ecological effects of pharmaceuticals in aquatic systems—impacts through behavioral alterations. *Philosophical Transactions of the Royal Society B, 369*, 1-10. http://dx.doi.org/10.1098/rstb.2013.0580

Cammaerts, M.-C. (2016). Ants as biological models for studying effects of substances used by humans. *JSM Anatomy and Physiology, 1*(1003), 8 pages. Retrieved from https://www.jscimedcentral.com›anatomy-1-1003

Cammaerts, M.-C. (2017). Some findings on ants as models, which should be considered for caring of humans. *MOJ Biology and Medicine, 1*(5), 00027. https://doi.org/10.15406/mojbm.2017.01.00027

Cammaerts, M.-C. (2018a). Ants as models for examining potential adverse effects of products used by humans. *JSM Anatomy and Physiology*, *3*(1), 1016. Retrieved from https://www.jscimedcentral.com › anatomy-3-1016

Cammaerts, M.-C. (2018b). Physical dependence on a substance occurs when the effect of this substance rapidly decreases after withdrawal. *JSM Anatomy and Physiology*, *3*(1), 1017. Retrieved from https://www.jscimedcentral.com>anatomy-3-1017

Cammaerts, M.-C. (2019). Brief report of the effects of seven human drugs studied on ants as models. *MOJ Biology and Medicine*, *4*(2), 42-47. https://doi.org/10.15406/mojbm.2019.04.00112

Cammaerts, M.-C. (2021a). Harmful effects of humans' environmental factors and drugs, and advices for a safer live; a study on ants as models. *World Journal of Pharmaceutical Sciences*, *9*(1), 34-45. Retrieved from http://www.wjpsonline.org

Cammaerts, M.-C. (2021b). Side effects of ivermectin, a drug recently used to treat humans suffering from the Covid-19 illness, a study on ants as models. *AS Pharmacology*, *2*(10), 40-53. Retrieved from https://www.actascientific.com>ASPC-02-0154

Cammaerts, M.-C. (2021c). Physiological and ethological impacts of the antidepressant escitalopram studied on ants as models. *Acta Scientific Pharmaceutical Sciences, 5*(6), 02-16. https://doi.org/10.31080/ASPS.2021.05.0723

Cammaerts, M.-C. (2022). Side effects of drugs studied on ant models: a mini review. *MOJ Biology and Medicine*, *7*(1), 1-7. https://doi.org/10.15406/mojbm.2021.06.00156

Cammaerts, M.-C., & Cammaerts, D. (2014). Comparative outlook over three *Myrmica* species' biotopes and foragers' know-how. *Biologia*, *69*, 1051-1058. https://doi.org/10.2478/s11756-014-0399-z

Cammaerts, M.-C., & Cammaerts, D. (2015a). Physiological and ethological effects of fluoxetine, on ants used as biological models. *International Journal of Biology, 7*(2), 1-18. https://doi.org/10.5539/ijb.v7n2p1

Cammaerts, M.-C., & Cammaerts, D. (2015b). Potential harmful effects of Carbamazepine on aquatic organisms, a study using ants as invertebrate models. *International Journal of Biology*, *7*(3), 75-93. https://doi.org/10.5539/ijb.v7n3p75

Cammaerts, M.-C., & Cammaerts, R. (2015a). The acquisition of cognitive abilities by ants: a study on three *Myrmica* species (Hymenoptera, Formicidae). *Advanced Studies in Biology*, *7*, 335-348 + synopsis: 349-350. https://doi.org/10.12988/asb.2015.5424

Cammaerts, M.-C., & Cammaerts, R. (2015b). Are ants (Hymenoptera, Formicidae) capable of self-recognition? *Journal of Sciences, 5*(7), 521-532. https://doi.org/10.12988/asb.2015.5424

Cammaerts, M.-C., & Cammaerts, R. (2016). Ethological and physiological effects of paroxetine, the nowadays most consumed antidepressant. A study on ants as models. *Research Trends*, *12*, 107-126.

Cammaerts, M.-C., & Cammaerts, R. (2018). Ethological and physiological effects of the recently most used analgesic, ibuprofen; a study on ants as models. *EC Pharmacology and Toxicology, 6*(4), 251-267. Corpus ID: 212573593

Cammaerts, M.-C., & Cammaerts, R. (2020a). Ants' numerosity ability defined in nine studies. *Journal of Biology and Life Science, 11*(1), 121-142. https://doi.org/10.5296/jbls.v11i1.16278

Cammaerts, M.-C., & Cammaerts, R. (2020b). Summary of seven more studies on numerosity abilities in an ant, four of them relating to human competence. *Journal of Biology and Life Science, 11*(2), 296-326. https://doi.org/10.5296/jbls.v11i2.17892

Cammaerts, M.-C., & Cammaerts, R. (2020c). Non-numerical distance and size effects in an ant. *Journal of Biology and Life Science*. *11*(2), 13-35. https://doi.org/10.5296/jbls.v11i2.16895

Cammaerts, M.-C., & Cammaerts, R. (2020d). Weber's law applied to the ants' visual perception. *Journal of Biology and Life Science, 11*(2), 36-61. https://doi.org/10.5296/jbls.v11i2.16896

Cammaerts, M.-C., & Cammaerts, R. (2020e). Side effects of mirabegron studied on ants as models. *MOJ Biology and Medicine*, *5*(1), 18-29. https://medcraveonline.com>MOJBM-05-00117

Cammaerts, M.-C., & Cammaerts, R. (2021). Side effects, studied on ants as models, of fluvoxamine nowadays used for treating persons suffering from the Covid-19. *EC Pharmacology and Toxicology, 9*(11), 03-25. Retrieved from https://actascientific.com>ASPC

Cammaerts, M.-C., Cammaerts, R. (2022). A synthesis of six recent studies on numerosity abilities in an ant. *Journal of Biology and Life Sciences*, *13*(1), 1-23. https://doi.org/10.5296/jbls.v13i1.19346

Cammaerts, M.-C., Morel, F., Martino, F., & Warzee, N. (2012). An easy and cheap software-based method to assess two-dimensional trajectories parameters. *Belgian Journal of Zoology*, *142*, 145-151. Retrieved from www.naturalsciences.be› BJZ

Carbuloni, C. F., Savoia, J. E., Santos, J. S. P., Pereira, C. A. A., Marques, R. G., Ribeiro, V. A. S., & Ferrari, A. M. (2020). Degradation of metformin in water by $TiO_2 – ZrO_2$ photocatalysis. *Journal of environmental Management, 262*(15), 110347. https://doi.org/10.1016/j.jenvman.2020.110347

Cummings, B. M., Needoba, J. A., & Peterson, T. D. (2018). Effect of metformin exposure on growth and photosynthetic performance in the unicellular freshwater chlorophyte, *Chlorella vulgaris. PloS ONE, 13*(11), e0207041. https://doi.org/10.1371/journal.pone.0207041

Damasceno de Oliveira, L. L., Antunes, S. C., Goncalves, F., Rocha, O., & Nunes, B. (2015). Acute and chronic ecotoxicological effects of four pharmaceuticals drugs on Cladocera *Daphnia magna*. *Drug Chemistry and Toxicology, 39*(1), 1-9. https://doi.org/10.3109/01480545.2015.1029048

Elizalde-Velazquez, G. A., & Gomez-Olivan, L. M. (2020). Occurrence, toxic effects and removal of metformin in the aquatic environments in the world: Recent trends and perspectives. *Science of the Total Environment, 702*, 134924.

European Environmental Bureau (2018). The environmental and health impacts caused by emissions of APIs to the Environment. https://doi.org/10.1016/j.scitotenv.2019.134924

Ferrannini, E. (2014). The target of metformin in type 2 diabetes, *New England Journal of Medicine, 371*, 1547-1548. https://doi.org/10.1056/NEJMcibr1409796

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Frieling, K., Monte, S.V., Jacobs, D., & Paolini Albanese, N. (2021). Weight loss differences seen between glucagon-like peptide–1 receptor agonists and sodium–glucose cotransporter–2 inhibitors for treatment of type-2 diabetes. *Science and Practice Research, 61*(6), 772-777. https://doi.org/10.1016/j.japh.2021.06.015

Godoy, A. A., Domingues, I., Noguienen A. J. A., & Kummrow, F. (2018). Ecotoxicological effects, water quality standards and risk assessment for the anti-diabetic Metformin. *Environnemental Pollution, 243*, 534-542. https://doi.org/10.1016/j.envpol.2018.09.031

Grenni, P., Ancoma, V., & Caracciolo, A. B. (2017). Ecological effects of antibiotics on natural ecosystems: A review. *Microchemical Journal, 2689*, 1-47.

Hanada, S., Isobe, Y., Wada, K., & Nagoshi, M. (1994). Drumming Behavior of Two Stonefly Species, *Microperla brevicauda* Kawai (Peltoperlidae) and *Kamimuria tibialis* (Pictet) (Perlidae), in Relation to Other Behaviors. *Aquatic Insects, 16*(2), 75-89. https://doi.org/10.1080/01650429409361539

Heaf, J. (2014). Metformin in Chronic Kidney Disease: Time for a Rethink. *Peritoneal Dialysis International, 34*(4), 353-357. https://doi.org/10.3747/pdi.2013.00344

Inzucchri, S. E., Maggs, D. G., Spollett, G. R., Page, S. L., Rife, F. S., & Walton, V. (1998). Efficacy and Metabolic Effects of Metformin and Troglitazone in Type II Diabetes Mellitus. *The New England Journal of Medicine, 338*(13), 867-872. https://doi.org/10.1056/NEJM199803263381303

Jacob, S., Köhler, H. R., Tisler, S., Zwiener, C., & Triebskorn, R. (2019). Impact of the Antidiabetic Drug Metformin and Its Transformation Product Guanylurea on the Health of the Big Ramshorn Snail (*Planorbarius corneus*). *Frontiers in Environmental Science, section Environmental Toxicology, 7*, 45. https://doi.org/10.3389/fenvs.2019.00045

Johnstone, G.W. (1964). Stridulation by larval *Hydropsychidae* (*Trichoptera*). *Proceedings of the Royal Entomological Society of London (A), 39*(10-12), 146-150. https://doi.org/10.1111/j.1365-3032.1964.tb00997.x

Jones, 0. A. H., Voulvoulis, N., & Lester, J. N. (2004). Potential Ecological and Human Health Risks Associated with the Presence of Pharmaceutically Active Compounds in the Aquatic Environment. *Critical Reviews in Toxicology, 34*(4), 335-350. https://doi.org/10.1080/10408440490464697

Jun, D., Mei, P., Zhiren, W., Sichun, Z., Di, X., Jiating, D., Xue, Y., Jingyuan, P., & Xiaoping, Y. (2019). Novel application of Metformin combined with targeted drugs on anticancer treatment. *Cancer Science (JCA), 110*(1), 23-30. https://doi.org/10.1111/cas.13849

Kailin, C., Yajun, L., Zhen, G., Yong, Z., Wei, Z., & Hui, W. (2020). Metformin: current clinical applications in nondiabetic patients with cancer. *Aging (Albany NY). 12*(4), 3993–4009. https://doi.org/10.18632/aging.102787

Kenneth, L. J., Arslanian, S., Peterokova, V. A., SoonPark, J., & Tomlinson, M. J. (2002). Effect of Metformin in Pediatric Patients with Type 2-Diabetes: A randomized controlled trial. *Diabetes Care, 25*(1), 89-94. https://doi.org/10.2337/diacare.25.1.89

Kim, Y., Choi, K., Jung, J., Park, S., Kim, P. G., & Park, J. (2007). Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. *Environnemental International, 33*, 370-375. https://doi.org/10.1016/j.envint.2006.11.017

Kosma, C. I., Lambropoulou, D. A., & Albanis, T. A. (2015). Comprehensive study of the antidiabetic drug Metformin and its transformation product guanylurea in Greek wastewaters. *Water Research, 70*(1), 436-448. https://doi.org/10.1016/j.watres.2014.12.010

Krief, S., Iglesias-Gonzalez, A., Appenzeller, B. M. R., Rachid, L., Beltrame, M., Azalu, E., ... Spirhanzlova, P. (2022). Chimpanzee exposure to pollution revealed by human biomonitoring approaches. *Ecotoxicology and Environmental Safety, 233*, 113341. https://doi.org/10.1016/j.ecoenv.2022.113341

Legro, R. S., Barnhart, H. X., Schlaff, W. D., Carr, B. R., Diamond, M. P., Carson, S. A., ... Myer, E. R. (2007). Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. *New England Journal of Medicine. 356*, 551-566. https://doi.org/10.1056/NEJMoa063971

Lord, J. M., Flight, I. H., & Norman, R. J. (2003). Metformin in polycystic ovary syndrome: systematic review and meta-analysis. *British Medical Journal, 327*, 951-953. https://doi.org/10.1136/bmj.327.7421.951

Madiraju, A. K., Erion, D. M., Rahimi, Y., Zhang, X-M., Braddock, D. T., Albright, R. A., …. Shulman, G. I. (2014). Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature, 510*, 542-546. https://doi.org/10.1038/nature13270

Niemuth, N. J., & Klaper, R. D. (2015). Emerging wastewater contaminant Metformin causes intersex and reduced fecundity in fish. *Chemosphere, 135*, 38-45. https://doi.org/10.1016/j.chemosphere.2015.03.060

Passera, L., & Aron, S. (2005). *Les fourmis : comportement, organisation sociale et évolution*. Les Presses Scientifiques du CNRC, Ottawa, Canada, 2005.

Paolisso, G., Gambardella, A., Tagliamonte, M. R., Varricchio, G., Carella, C., Giugliano, D., & D'Onofrio, F. (1998). Effect of Metformin on food intake in obese subjects. *European Journal of Clinical Investigation, 28*(6), 441-446. https://doi.org/10.1046/j.1365-2362.1998.00304.x

Prichard, E., & Granck, E. F. (2016). Effects of pharmaceuticals and personal care products on marine organisms: from single-species studies to an ecosystem-based approach. *Environmental Science Pollution Research, 23*, 22365-22384. https://doi.org/10.1007/s11356-016-7282-0

Richmond, E. K., Grace, M. R., Kelly, J. J., Reisinger, A. J., Rosi, E. J., & Walters, D. M. (2017). Pharmaceuticals and personal care products (PPCPs) are ecological disrupting compounds (Eco DC). *Elementa Science Anthropocene, 5*, 66. https://doi.org/10.1525/elementa.252

Russell, W. M. S., & Burch, R. L. (2014). *The Principles of Humane Experimental Technique*. Johns Hopkins University, ISBN-13: 978-0900767784

Schläppi, D., Kettler, N., Straub, L., Glauser, G., & Neumann, P. (2020). Long-term effects of neonicotinoid insecticides on ants. *Communications Biology, 3*, 335. https://doi.org/10.1038/s42003-020-1066-2

Siegel, S., & Castellan, N. J. (1988). *Nonparametric statistics for the behavioural sciences* Singapore: McGraw-Hill. 1988.

Stewart, K. W., & Zeigler, D. D. (1984). The use of larval morphology and drumming in *Plecoptera* systematics, and further studies of drumming behavior. *Annal of Limnology, 20*(1-2), 105-114. http://dx.doi.org/10.1051/limn/1984001

Tongzhi, W., Horowitz, M., & Rayner, C. K. (2017). New insights into the anti-diabetic actions of Metformin: from the liver to the gut. *Expert Review of Gastroenterology & Hepatology, 11*(2), 157-166. https://doi.org/10.1080/17474124.2017.1273769

Trautwein, C., Berset, J-D., Wolschke, H., & Kümmerer, K. (2014). Occurrence of the antidiabetic drug Metformin and its ultimate transformation product Guanylurea in several compartments of the aquatic cycle. *Environnemental International*, 203-212. http://dx.doi.org/10.1016/j.envint.2014.05.008

Wehner, R., & Gehring, W. (1999). *Biologie et physiologie animales* Eds. De Boeck Université, Thieme Verlag, Paris, Bruxelles. Retrieved from www.unitheque.com › ... › Animaux › Zoologie

Wei-Hao, H., Pi-Jung, H., Pi-Chen, L., Szu-Chia, C., Mei-Yuch, L., & Shyi-Jang, S. (2018). Effect of metformin on kidney function in patients with type-2 diabetes mellitus and moderate chronic kidney disease. *Oncotarget, 9*(4), 5416-5423. https://doi.org/10.18632/oncotarget.23387

Wiernsperger, N. F., & Bailey, C. J. (1999). The Antihyperglycemic Effect of Metformin. *Drugs, 58*, 31–39. https://doi.org/10.2165/00003495-199958001-00009

Wilkinson, J. L., Boxall, A. B. A., Kolpin, D. W., Leung, K. M. Y., Lai, R. W. S., Galban-Malagon, C., Adell, A. D., & Mondon, J. (2022). Pharmaceutical pollution of the world's rivers. *Environmental Sciences, 119* (8), e2113947119. https://doi.org/10.1073/pnas.2113947119

Wolf, F. W., & Heberlein, U. (2003). Invertebrate models of drug abuse. *Journal of Neurobiology, 54*, 161-178. https://doi.org/10.1002/neu.10166

Zhang, R., He, Y., Yao, L., Chen, J., Zhu, S., Rao, X., ... Wu, L. (2020). Metformin chlorination byproducts in drinking water exhibit marked toxicities of a potential health concern. *Environmental International*, 1-9. https://doi.org/10.1016/j.envint.2020.106244