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Phagocytic Activity of Human Macrophages and *Drosophila* Hemocytes after Exposure to the Neonicotinoid Imidacloprid

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ABSTRACT

Neonicotinoid insecticides are increasingly used in modern pest control and in conventional agriculture. Their residues are frequently found in our environment and in our food leading to chronic exposure of pollinating insects and humans. Indeed, evidence has become stronger that chronic exposure to neonicotinoids might have a direct impact on the immune response of invertebrates and vertebrates. Therefore, we compared the cellular immune response of human macrophages (THP-1) and *Drosophila melanogaster* hemocytes (Schneider 2 cells) after exposure to four different concentrations of the neonicotinoid imidacloprid. Cells were immune activated with LPS (lipopolysaccharide) of *Escherichia coli* to compare the phagocytic activity of immune activated and non-activated cells during pesticide exposure. *Drosophila* cells were more strongly affected by the insecticide than human macrophages. Even though imidacloprid showed an adverse effect on phagocytosis on both cells while immune activated, it decreased phagocytosis in *Drosophila* cells at shorter exposure time and without immune activation.

Keywords

Imidacloprid, macrophage, phagocytosis, THP-1, *Drosophila*

1. INTRODUCTION

29 Having been launched on the market by Bayer Crop science in 1991, Imidacloprid (IMI) was the very
30 first insecticide of a new group of pesticides: the neonicotinoids (Mason et al., 2013). Neonicotinoid
31 insecticides are nicotinic acetylcholine receptor agonists in insects and are lethal through disruption
32 of the nervous system. All neonicotinoids are systematic insecticides with a high solubility in water
33 leading to the uptake of the pesticide by the plant's tissues. The pesticide's active molecule, which is
34 spread in the entire plant, thus acts directly on any sucking insect that would feed on the plant. Once
35 distributed in the whole plant it is also available for non-target insects such as pollinating insects as
36 they feed on contaminated nectar and pollen (Simon-Delso et al., 2015).

37 In the late 1980's the global insecticide market was dominated by carbamates, organophosphates
38 and pyrethroids, to which many pest insects have developed increasing resistance (Simon-Delso et
39 al., 2015). The neonicotinoids rapidly took over the global insecticide sales and by 2010 were
40 responsible for more than 25 percent of the market share with imidacloprid becoming the second
41 largest selling pesticide worldwide after Glyphosate (Jeschke et al., 2010). Despite the ban of
42 imidacloprid, clothianidin and thiamethoxam for outdoor application (they are still authorized in
43 greenhouses) in the European Union in May 2018 (Official Journal of the European Union, L 132/31,
44 2018), imidacloprid is still one of the most used insecticides in the world. In fact 75 percent of
45 neonicotinoids were used in Latin America, North America and Asia. Europe only accounted for 11
46 percent of the worldwide use (Bass et al., 2015).

47 The most common application and spread of neonicotinoids in agriculture is the use of seed coatings
48 or dressings which generates toxic dust during the process of sowing and can lead to contamination
49 of surrounding vegetation (Bonmatin et al., 2015). Further they are also used against sucking insects
50 in fruit and vegetable cultivations as well as in viticulture and hop growing. Residues of imidacloprid
51 and other neonicotinoids have been found in honeybees, honey and their pollen (Chauzat et al.,
52 2009; Codling et al., 2016; David et al., 2016; Hladik et al., 2016; Kasiotis et al., 2014) as well as fruits
53 and vegetables we consume (Fernández-Alba et al., 2000; Ko et al., 2014; Obana et al., 2002).

54 In the last decade neonicotinoids have been controversially discussed in literature as they have been
55 associated with a worldwide pollinator decline. While acute toxicity occurs rather seldom the cause
56 of this decline was associated with chronic pesticide exposure. There is a growing awareness that
57 neonicotinoids could weaken the immune response of pollinators. Indeed several studies have
58 shown a weakening effect of neonicotinoids and pathogens to pollinators (Abbo et al., 2017; Alaux et
59 al., 2010; Fauser-Misslin et al., 2014; Pettis et al., 2012; Vidau et al., 2011). The suppression of the
60 immune response to infectious diseases by neonicotinoids was not only shown for pollinators
61 (Brandt et al., 2016; Di Prisco et al., 2013; López et al., 2017) but also hypothesized for insectivorous
62 vertebrates like amphibians, birds, fish and mammals, when comparing disease outbreaks with the
63 use of neonicotinoids in the concerned regions and countries (Mason et al., 2013). A study of

Sánchez-Bayo and Goka (2005) supports this idea, claiming that medaka fish (*Oryzias latipes*) exposed to imidacloprid were massively infested by a *Trichodina* ectoparasite when compared to control groups without pesticides and groups exposed to zinc pyrrithione.

Also other reviews support this hypothesis and Gibbons et al. (2015) stated that imidacloprid and clothianidin exert sublethal effects on vertebrates ranging from genotoxic and cytotoxic effects, and impaired immune function, to reduced growth and reproductive success. Mostafalou and Abdollahi (2013) mentioned human chronic diseases like cancer, Alzheimer, Parkinson or autoimmune diseases that could be linked to sublethal pesticides exposure, even though they did not mention imidacloprid in their work. Though Cimino et al. (2016) cited several studies of chronic exposure to imidacloprid which showed adverse health effects on humans, still not much is known about the effect of neonicotinoids on human health.

Innate immune responses in mammals and insects do show several functional similarities. Cells, especially phagocytes, of these innate immune systems are comparable both in terms of morphology and reactivity towards pathogen recognition (Browne et al., 2013). An important role in the primary immune response is given to phagocytes. These cells are responsible among others for the recognition and removal of foreign pathogens and the recruitment of other immune cells through the release of cytokines (Chanput et al., 2014). Insect hemocyte's main function is phagocytosis of pathogens and apoptotic material (Feldhaar and Gross, 2008) therefore being of major importance to the organism. The most dominant hemocytes in *Drosophila* are plasmatocystes which are highly motile and resemble monocytic macrophages in humans (Feldhaar and Gross, 2008). Insect hemocytes are able to change their state from non-adherent to adherent upon pathogen recognition and respond by "spreading" which enhances phagocytosis of small antigens (Negri et al., 2013). In contrast to human monocytes, insect hemocytes are able to perform nodulation and encapsulation of larger foreign objects like parasites as they adhere and spread over the surface of the foreign object until they form overlapping layers (Negri et al., 2013). The encapsulation is often followed by melanisation of the antigen (Schmid et al., 2008) and hemocytes are also able to secrete cytotoxic compounds into the capsule (Negri et al., 2013). Imidacloprid could have a suppressive effect on this cellular immune response of invertebrates as well as of vertebrates.

Hence in this study we investigate the impact of imidacloprid (IMI) on the phagocytic activity of immune system cells of one invertebrate and one vertebrate species. We chose the cell lines Schneider-2 from *Drosophila melanogaster* and THP-1, a human monocytic cell line that can be differentiated into macrophages. To simulate an immune reaction we activated the cells with lipopolysaccharide (LPS) of *Escherichia coli*.

2. MATERIALS AND METHODS

2.1. Cell culture and exposure

Both human THP-1 (Ref. ACC 16) and *D. melanogaster* Schneider-2 (Ref. ACC 130) cells were purchased from Leibnitz – Institute DSMZ- Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH. THP-1 cells were maintained in RPMI 1640 with 10 percent heat inactivated FBS (fetal bovine serum), 2mM L-Glutamine, 50 U/mL penicillin and 50 µg/mL streptomycin at 37 °C and 5 percent CO₂. Schneider cells were kept in 44.75 percent Schneider's insect medium, 44.75 percent TC-100 insect medium, 10 percent heat inactivated FBS, 50 U/mL penicillin and 50 µg/mL streptomycin at 20 °C.

Imidacloprid (37894 Sigma-Aldrich, powdery analytical standard) was dissolved in PBS (pH 7.4) at 300 µg/mL prior to experiments. Imidacloprid was further diluted in RPMI or Schneider medium in order to obtain the following concentrations: 1; 25; 50 and 100 µg/mL IMI. During each experiment all cell solutions have been tested with HPLC to determine the actual concentration of IMI that has been in contact with cells to avoid measuring errors. An analytical standard of Imidacloprid solution (46341 Sigma-Aldrich) of 100 µg/mL in acetonitrile was used to obtain a standard curve for HPLC analyses. Cells were incubated with imidacloprid at the cell particular conditions for 5 or 24 hours. Cell viability was examined after 5 and 24 hours with a trypan blue solution (2 g/L in 0.9 percent NaCl solution). Trypan blue is a diazo dye used for vital staining of cells (Tolnai, 1975). After the exposure to imidacloprid cells were washed with PBS and incubated for 16 hours with LPS (lipopolysaccharide) from *Escherichia coli* at concentrations of 0.1 µg/mL or 1 µg/mL for THP-1 and 1 µg/mL for Schneider cells. LPS is an endotoxin and a component of the outer membrane of Gram-negative bacteria resulting in a strong inflammatory response of the immune system.

2.2. Phagocytosis

Phagocytosis of THP-1 and Schneider cells was examined by using amine-modified polystyrene, fluorescent yellow-green latex beads (1 µm mean particle size) after slight modifications of the methodology of Bennisroune et al. (2012).

THP-1 cells were differentiated to their macrophage phenotype with 5 ng/mL of PMA (phorbol 12, myristate 13-acetate) for 48 hours and washed once with PBS prior to pesticide exposure. Schneider cells and differentiated THP-1 cells were grown on glass coverslips at a concentration of 7×10^5 cells/mL in six-well or twelve-well plates. Cells were exposed to different concentrations of imidacloprid from 1 µg/mL to 100 µg/mL, including a negative control without IMI, for either 5 or 24 hours at the particular cell conditions. 4 hours prior to the phagocytosis measure, 100 latex beads per cell diluted in the cell particular medium were added to each well. After 4 hours of incubation cells were washed with 3 percent BSA (Bovine serum albumin) dissolved in PBS in order to eliminate

latex beads which have not been absorbed by the cells (Komiyama et al., 2003). The glass coverslips of differentiated THP-1 cells and Schneider cells were dipped three times in the BSA solution before being dipped into PBS. Glass coverslips were then mounted on object plates and phagocytosis was measured under a fluorescent microscope. A minimum of 250 cells were counted for each sample. Phagocytosis was calculated as follows:

$$\text{cells (phagocytosis)} = \frac{\text{number of cells ingesting at least 1 bead}}{\text{number of total cells}} * 100$$

2.3. Statistical analyses

All data was analysed using Addinsoft (2019) XLSTAT statistical and data analysis solution, Long Island, NY, USA. For all tests a probability level of 0.05 was considered to be statistically significant. After checking for normality and variance homogeneity using the Shapiro-Wilk's test and the Bartlett's test, a one-way ANOVA was performed to identify differences between different IMI groups of each LPS treatment, followed by a Dunnett's test for comparison of different IMI groups to the control group or a Tukey's test of multiple comparison between different IMI groups of the same LPS treatment. Data for viability of human THP-1 cells after 5 hours exposure time were log transformed in order to fit parametric conditions. As parametric conditions were not given for the viability of human THP-1 cells after 24 hours exposure time, even after log or arcsine transformation, we used a non-parametric Kruskal-Wallis test.

Results were expressed as means of all replicates \pm SE (standard error).

3. RESULTS

3.1. *D. melanogaster* Schneider cells

Cell viability in Schneider cells of *D. melanogaster* was higher after 5 hours than after 24 hours exposure to IMI. Cell viability varied between 88.4 ± 0.47 percent and 90.9 ± 0.96 percent after 5 hours and between 84.6 ± 1.73 percent and 80.4 ± 1.84 percent after 24 hours. No statistically significant differences were found between the control without IMI and different IMI concentrations neither after 5 hours (ANOVA $p=.233$ with $n=9$) nor after 24 hours (ANOVA $p=.480$ with $n=9$) (Figure 1).

Phagocytosis of *D. melanogaster* Schneider cells was tested after 5 and 24 hours of exposure to IMI with or without immune activation with $1 \mu\text{g/mL}$ LPS of *Escherichia coli*.

Phagocytosis after 5 hours and no LPS activation showed a decline of phagocytosis with increasing concentrations of IMI. 24.84 ± 1.71 percent of cells from the control group were able to phagocytize a minimum of one latex bead. Percentages decreased continuously with increasing pesticide concentrations until phagocytosis reached only 5.02 ± 0.86 percent at the highest IMI concentration, which was statistically significant from the control (Dunnett $p=.035$, with $n=3$). When immune activated with $1 \mu\text{g/mL}$ LPS, phagocytosis increased slightly up to 30.74 ± 3.26 percent in the control group. IMI treated groups revealed decreasing percentages of phagocytosis compared to the control. Statistically significant differences were determined between 25, 50 and $100 \mu\text{g/mL}$ IMI and the control group (Tukey $p=.009$; $p=.025$; $p=.004$ respectively). Phagocytosis in these three treatment groups varied from 21.37 ± 1.15 percent to 18.11 ± 0.95 percent.

After 24 hours of exposure to IMI and no immune activation phagocytosis ranged from 42.90 ± 4.99 percent in the control group to 27.85 ± 1.38 percent after IMI exposure, showing no statistical significant difference between control and IMI (ANOVA $p=.222$ with $n=3$).

After immune activation with $1 \mu\text{g/mL}$ LPS for 16 hours following a 24 hours exposure to IMI we observed a statistically significant decrease of phagocytosis with increasing pesticide concentrations upon the lowest used IMI concentration (Tukey $p=.033$, $p=.0001$, $p=.0001$, $p<.0001$ for 1; 25; 50 and $100 \mu\text{g/mL}$ IMI respectively). Values varied between 31.55 ± 1.92 percent in the control group and 12.92 ± 1.19 percent at the highest IMI concentration. At the lowest used IMI concentration of $1 \mu\text{g/mL}$ only 24.05 ± 1.01 percent of cells were capable of phagocytosis, which is about a quarter less compared to the control group. At $25 \mu\text{g/mL}$ the ability of cells for phagocytosis was reduced by one third and at $50 \mu\text{g/mL}$ it was already reduced by more than half compared to the control (Figure 2, b).

3.2. Human THP-1 cells

Cell viability in THP-1 was measured after 5 and 24 hours exposure to IMI. Viability in human THP-1 cells was between 92.9 ± 1.06 and 90.3 ± 2.05 percent after 24 hours exposure while it ranged between 84.9 ± 2.61 percent and 80.6 ± 2.27 percent after 5 hours. No statistical significant differences in THP-1 viability were found between the control group and any tested IMI concentration at 24 hours (Kruskal-Wallis $p=.857$ with $n=12$) or 5 hours (ANOVA $p=.88$ with $n=9$) exposure to IMI (Figure 1).

Phagocytosis of differentiated THP-1 cells was stimulated by immune activation of either $0.1 \mu\text{g/mL}$ or $1 \mu\text{g/mL}$ LPS. Percentages of phagocytizing cells of the same population increased in average about 16.4 percent while stimulated with LPS. Phagocytosis measured after an exposure of 5 hours to IMI and no immune activation with LPS was ranging between 37.3 ± 5.02 percent and 55.3 ± 4.78 percent. Phagocytosis increased with immune activation and varied between 62.5 ± 4.31 percent to

72.2 \pm 4.76 percent at 0.1 μ g/mL LPS and between 52.1 \pm 1.62 percent and 68.3 \pm 3.21 percent at 1 μ g/mL LPS activation. No statistically significant difference between the control group and the treatment groups was found for all tests after 5 hours exposure without, with 0.1 μ g/mL or 1 μ g/mL LPS (ANOVA, p=.161, p=.624 and p=.070 respectively, with n=3).

No statistically significant differences in phagocytosis between different IMI concentrations and the control were found after 24 hours of exposure and no immune activation with LPS (ANOVA p=.102 with n=3). Values varied between 84.07 \pm 2.15 percent and 70.23 \pm 2.65 percent. However, differences between different IMI concentrations were found after immune activation with either 0.1 μ g/mL or 1 μ g/mL LPS. After activation with 0.1 μ g/mL LPS phagocytosis decreased after 24 hours IMI exposure in treated groups compared to the control group for up to 17.1 percent. The biggest decreases in phagocytosis were found at 25 μ g/mL and 100 μ g/mL IMI which was also confirmed by statistical analyses (Tukey p=.038 and p=.044 respectively). 81.33 \pm 1.43 percent of cells of the control group were capable of phagocytizing at least one latex bead while this percentage decreased with increasing IMI concentrations except for cells at 50 μ g/ml IMI which were able to phagocytize more latex beads than cells at 25 μ g/mL IMI. Phagocytosis after 24 hours and later activation with 1 μ g/mL LPS (Figure 2) ranged from 81.21 \pm 4.69 percent at the control group to 67.2 \pm 4.99 percent at 50 μ g/mL IMI, which was statistically different from the control group (Tukey p=.034).

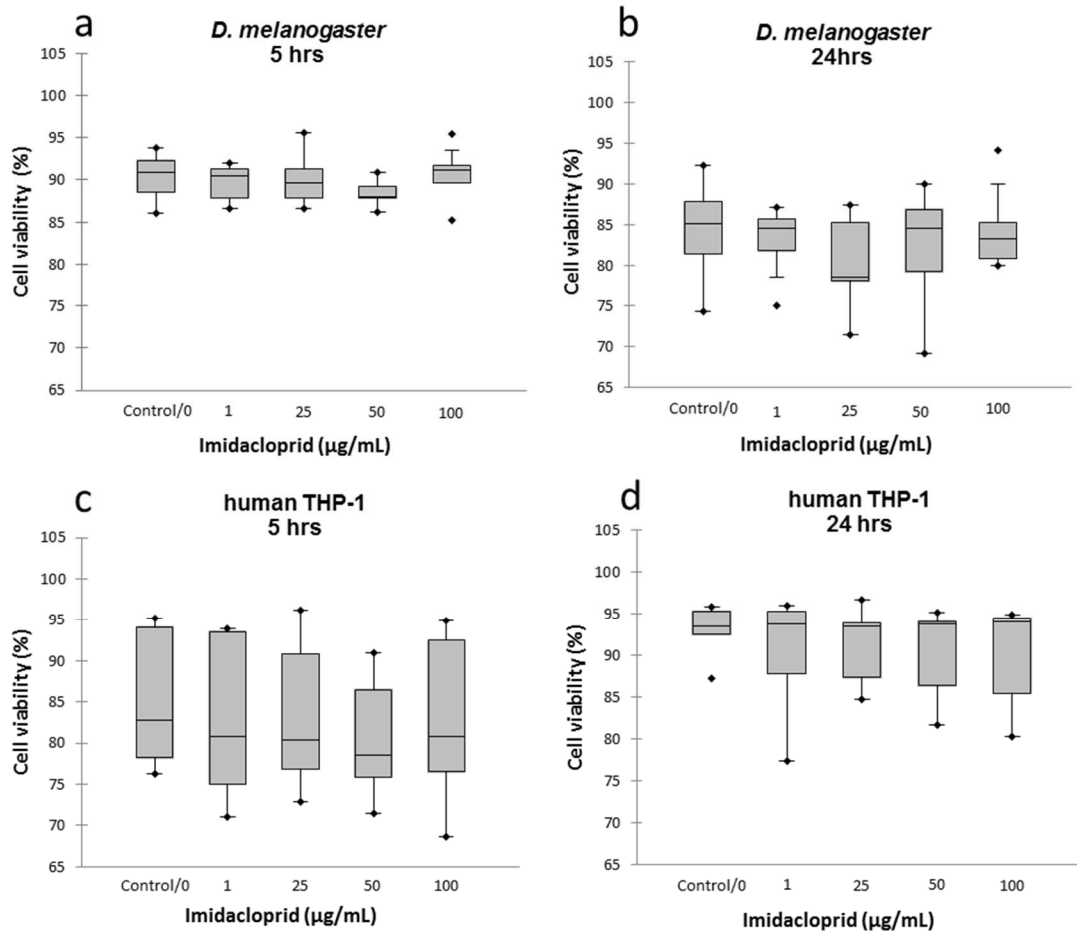


Figure 1: Viability of *D. melanogaster* Schneider cells (a,b) and human THP-1 cells (c,d) after 5 and 24 hours exposure to IMI. No statistical differences were found between the control group without IMI and the IMI concentrations neither for Schneider after 5 and 24 hours nor THP-1 after 5 and 24 hours (ANOVA: $p=.233$; $p=.480$; $p=.880$; Kruskal-Wallis $p=.857$ and $n=9$; $n=9$; $n=9$; $n=12$ respectively).

4. DISCUSSION

In the last decade neonicotinoid insecticides have been associated with a worldwide pollinator decline as they might act as an immune suppressor in insects. But evidence has become stronger that they might not only weaken the immune response in insects but also in insectivorous vertebrates (Mason et al., 2013). Further residues of neonicotinoids have also been detected in human urine as we are exposed to contaminated food (Nomura et al., 2013; Taira et al., 2013). A few studies exist on human chronic exposure to neonicotinoids but still not much is known on their impact on human immune cells (Han et al., 2018). To investigate this effect, macrophage-like immune system cells of two different species, *Drosophila melanogaster* and human, were tested *in vitro* on their cellular reactivity when confronted with four different concentrations of IMI (1, 25, 50 and 100 µg/mL). The cells of the two organisms had similar characteristics and were able to

phagocytize particles. Thus we measured the phagocytic capacity of the two cell lines after pesticide exposure. Further cells were exposed to LPS of *Escherichia coli*, which acts as a pathogen associated molecular pattern (PAMP). Hence cells confronted with LPS were immune activated leading to an immune reaction or an inflammatory response. We were then able to compare the effect of IMI on immune activated and non-activated cells.

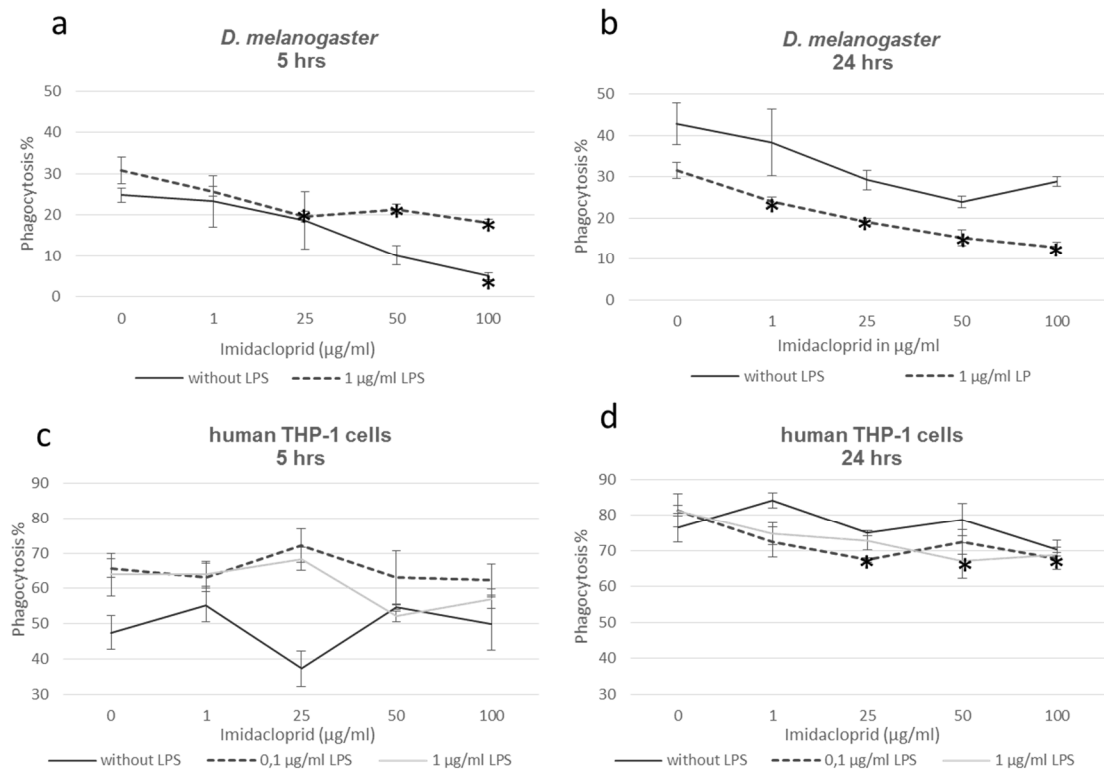


Figure 2: Phagocytosis of *D. melanogaster* Schneider cells (a,b) and human THP-1 cells (c,d) after 5 and 24 hours exposure to IMI and immune activation with LPS. Stars indicate statistically significant differences from the control group without IMI (ANOVA $p < .05$ with $n = 3$).

Without immune activation IMI significantly decreased phagocytosis of Schneider cells after 5 hours of exposure at the highest tested concentration. Immune activation with LPS increased this inhibiting effect leading to a significant decrease of phagocytosis upon 25 μg/mL IMI. These results match with results of Di Prisco et al. (2013) who found clothianidin, another neonicotinoid insecticide, to negatively affect the activation of the Toll-pathway of *Drosophila melanogaster*. The Toll-pathway in turn plays an important role on immune responses including the activation of phagocytosis (Valanne et al., 2011). Similar results on phagocytosis were also obtained after an exposure of 24 hours. IMI significantly decreased phagocytosis in Schneider cells after immune activation upon our lowest tested concentration of 1 μg/mL. It thus seems that the immune activation with LPS enforces the toxic effect of IMI in *Drosophila* cells as immune stress increases.

As for human macrophages IMI significantly decreased phagocytosis only after 24 hours exposure at higher IMI concentrations when cells were immune activated with either 0.1 $\mu\text{g/mL}$ or 1 $\mu\text{g/mL}$ LPS. No significant alteration of phagocytosis was found in macrophages after the shorter exposure time of 5 hours with or without immune activation. Several studies have shown a dose-dependent suppression of phagocytosis of phagocytic vertebrate cells alongside other pollutants (Bennasroune et al., 2012; Couleau et al., 2012; Dunier et al., 1991; Roszell and Anderson, 1994) suggesting that concentrations used were not high enough to induce effects on phagocytosis of THP-1 cells after short time exposure. In THP-1 cells LPS results in an activation of the NF- κB transcription factor (Chanput et al., 2014). Di Prisco et al. (2017) tested clothianidin on the THP-1 cell line showing that NF- κB was down regulated by the neonicotinoid insecticide. However, it is to mention that we were only differentiating between cells that were able to phagocytise at least one fluorescent bead and those who did not phagocytize any beads at all. We did not consider the number of beads that were ingested by the cells. As shown in Figure 3 cells were able to engulf many beads, making it impossible to count their number. We did though see cells that clearly ingested less beads than others when observed under the microscope. Therefore, it is possible that with our method we might not have been able to detect an effect of IMI on the efficiency of phagocytosis. IMI could be influencing different aspects of the phagocytic process. With our method we cannot clearly say if IMI can influence the rate of the uptake of bacteria or the degradation of bacteria once engulfed.

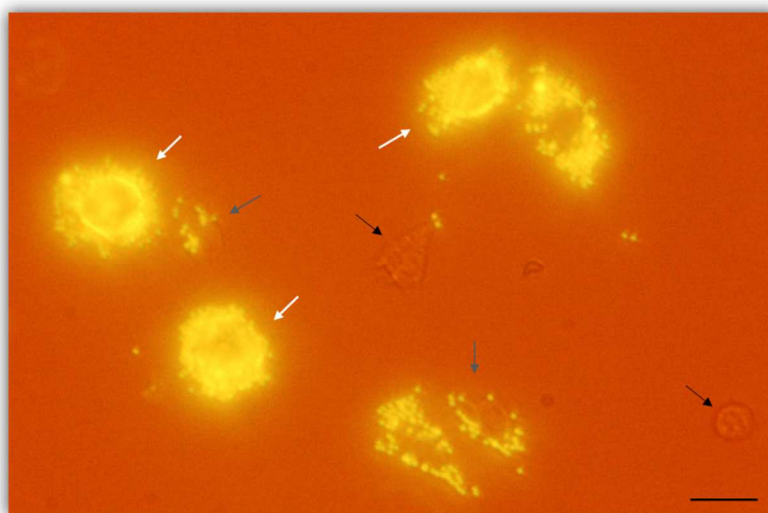


Figure 3 : Phagocytized fluorescent latex beads by Schneider 2 cells of *D. melanogaster*. White arrows show cells that engulfed a high number of beads, grey arrows show cells that engulfed a smaller number of beads and black arrows show cells that did not phagocytise any fluorescent latex bead. The scale bar is 10 μm .

IMI already showed an effect on *Drosophila* Schneider cells even when cells were not stimulated with LPS whereas effects in human THP-1 cells only occurred if cells were confronted with LPS and thus initiating an immune reaction. Having in mind that IMI is an insecticide, with insects being the

desired target, it is not surprising that Schneider cells are reacting more sensitive to it than human THP-1 cells. Cresswell *et al.* (2012) interpreted in their results that temperature might play a role during IMI intoxication in honey and bumblebees. Schneider cells as well as bee hemocytes were incubated at 22 °C while THP-1 cells were incubated at 37 °C. These temperature differences are due to different cell characteristics but it is not to neglect that this may play a role in the intoxication processes in the two cell lines. Camp and Buchwalter (2016) and Song *et al.* (1997) found higher chronic toxicity of IMI on aquatic invertebrates at lower temperatures while Van den Brink *et al.* (2016) found that summer populations of aquatic insects were stronger effected by IMI than winter populations. Apart from intoxication processes temperature also plays a role during the immune response. Indeed Linder *et al.* (2008) suggested that colder temperatures had rather beneficial effects on the immune response of *Drosophila* as heat shock proteins may improve immune function. According to our results IMI did not reduce viability of human THP-1 nor *Drosophila*'s Schneider cells at any tested concentration from 1 µg/mL to 100 µg/mL IMI. We were thus working with sublethal doses in our experimental conditions. Hence EC₅₀ values are dependent on cell and tissue type as it was already assumed by Benzidane *et al.* (2011). Concentrations used during experiments were ranging from 1 µg/mL to 100 µg/mL, which were quite high when compared to environmental concentrations. Chauzat *et al.* (2009) and Kasiotis *et al.* (2014) detected 5.7 to 73.9 ng/g imidacloprid in pollen while LD₅₀ values for honey bees varied from 4.5 ng – 81 ng/bee for oral ingestion (Nauen *et al.*, 2001; Cresswell, 2011) and from 18 ng – 102 ng/bee during contact (Iwasa *et al.*, 2004; Nauen *et al.*, 2001). In *Drosophila* the LD₅₀ at oral ingestion was estimated at 0.41 - 1.39 µg/g (Arain *et al.*, 2014). It is, though, to mention that IMI has a very short half-life in organisms. IMI showed a similar metabolism in honey bees as in *Drosophila melanogaster*. In bees the half-life of IMI accounts of 4.5 hours (Chauzat *et al.*, 2009) and is reduced to several metabolites like 6-chloronicotinic acid and urea derivative (Suchail *et al.*, 2004). Consequently concentrations of IMI in honeybees could be way higher than the actual ones found, depending on when the bee has ingested contaminated nectar for the last time. Further these metabolites might have other toxicological effects on the host organisms. Indeed Suchail *et al.* (2004) claims that the mortality of bees is more due to the action of the metabolites than the actual parent molecule. The EC₅₀ value of honey bee Kenyon cells was estimated at around 6.3 µg/mL IMI (Déglise, *et al.*, 2002). As IMI is an agonist on the nicotinic acetylcholine receptors effects on neuronal cells are expected to be higher than on immune system cells. It is therefore not implausible that effects on the two cell types used appeared at higher concentrations. These indications should be kept in mind when analysing *in vitro* toxicity test as we are missing metabolic processes.

To conclude we can summarize that the concentrations from 1 µg/mL to 100 µg/mL of IMI did reduce phagocytic activity in both human macrophages and *Drosophila* hemocytes after immune activation

with LPS of *Escherichia coli*. LPS clearly intensified the inhibiting effects of IMI on cell phagocytic activity. Phagocytosis in *Drosophila* cells was more strongly inhibited than in human macrophages. While we observed no effect of IMI on phagocytosis of human macrophages without immune activation, IMI did reduce phagocytosis in *Drosophila* cells after even short exposure and no immune activation.

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Conflict of interest

The authors report no conflicts of interest to be declared.

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