Exposure to sublethal doses of insecticide and their effects on insects at cellular and physiological levels

Alexandre Bantz, Jérémy Camon, Josy-Anne Froger, Delphine Goven, Valérie Raymond

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Insect Science

Exposure to sublethal doses of insecticide and their effects on insects at cellular and physiological levels

Alexandre Bantz, Jérémy Camon, Josy-Anne Froger, Delphine Goven and Valérie Raymond

Insecticides were used as pest management tools for a long time. The appearance of resistant insects has led the scientific community to rethink their use and to study the mechanisms underlying the resistance in order to circumvent it. However, we know now that sublethal doses of insecticide induce many effects which should be taken into account for pest control. In this review, we summarized current knowledge on mechanisms used by insects to deal with exposure to sublethal dose of insecticides. Physiological and cellular changes could contribute to the adaptation of the insect to its environment making the challenge of managing pests difficult.

Address
Laboratoire « Signalisation Fonctionnelle des Canaux Ioniques et Récepteurs » (SiFCIR), UPRES-EA2647 USC INRA 1330, SFR 4207 QUASAV, UFR Sciences, Université d'Angers, 2 Bld Lavoisier, 49045 Angers Cedex 01, France

Corresponding author:
Raymond, Valérie (valerie.raymond@univ-angers.fr)

Current Opinion in Insect Science 2018, **30**:73–78

This review comes from a themed issue on **Neuroscience**

Edited by **David Sattelle** and **Kazuhiko Matsuda**

For a complete overview see the Issue and the Editorial

Available online 27th September 2018

https://doi.org/10.1016/j.cois.2018.09.008

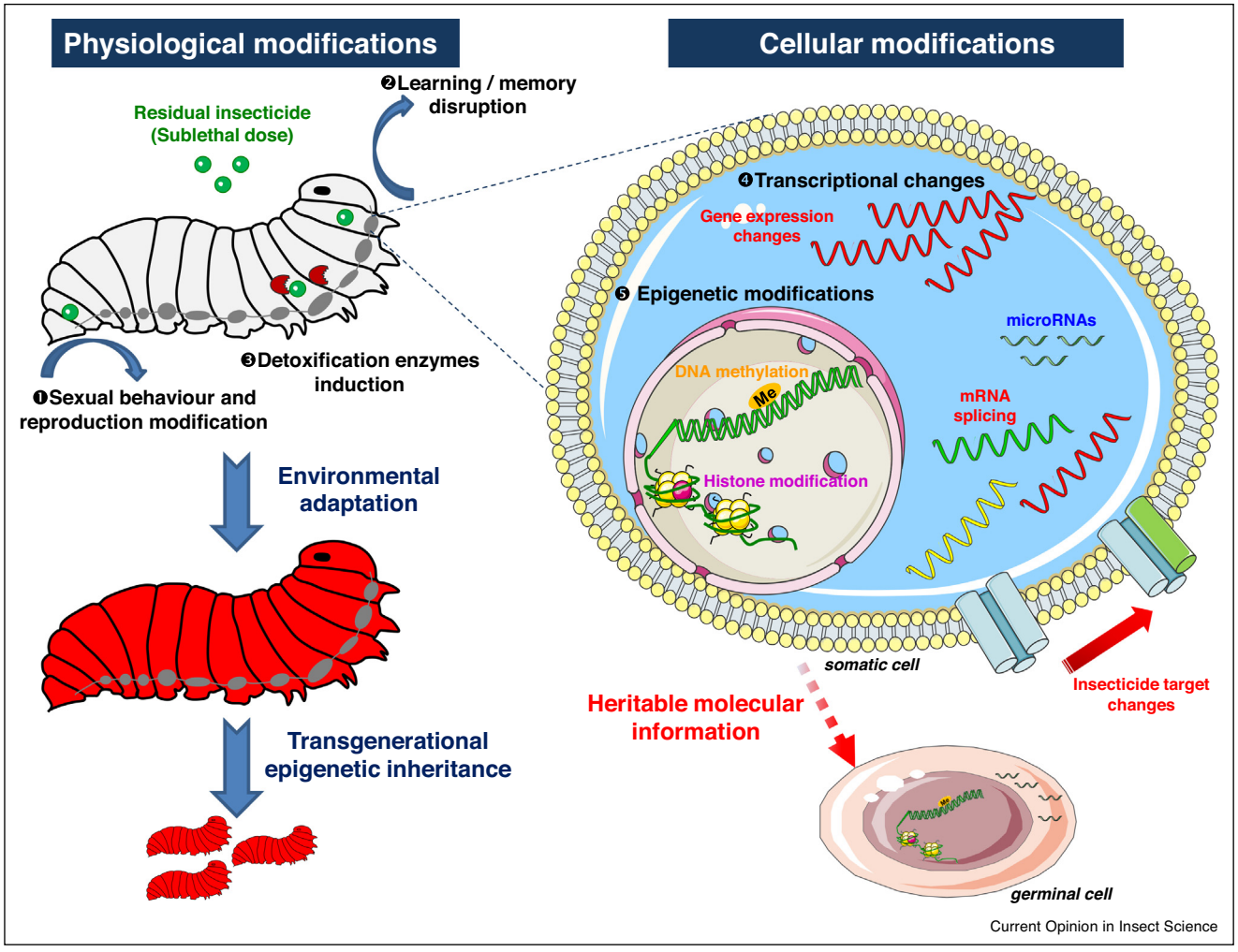
Introduction

Insecticides are pesticides which mainly act on neuronal ion channels and receptors [1,2]. Their use was considered as the main pest management strategy over a long period. Indeed, the insecticides block physiological functions through their actions directly on molecular targets or indirectly on detoxification enzymes. However, one major problem linked to their widespread use is the emergence of resistance to insecticides. The mechanisms of resistance such as behavioural avoidance, thickening of the cuticle, increased activity of detoxification enzymes or mutation of the insecticide targets have been well studied [1,3,4]. Pests and disease vectors which undergo strong insecticide selection pressure, develop resistance to the used compounds and often proceed cross-resistance to compounds with similar chemical structure or with the same mode of action. The levels of resistance or cross-resistance depend on the mechanisms involved. To circumvent this problem, agrochemical industries try to develop new chemicals to replace the ineffective insecticides. However, we know now that not only the use of lethal insecticide concentration but also sublethal exposure can induce physiological changes favouring the development of resistant or adapted insects. Following a field application of insecticides, insects may be exposed to different doses. Also, insecticides degrade over time or can volatilize with wind. Consequently, the concentration of products initially used to kill pests and disease vectors decreases until it becomes sublethal [5,6]. The degradation of this initial product can also generate metabolites exhibiting biological activity. The effect of low residual doses of these compounds that can affect both targeted species but also beneficial insects became a major concern [7]. Indeed, exposure to low dose of insecticide can induce different effects on insects. This brief review provides an insight into the knowledge essential to understand how exposure to sublethal dose of insecticide modifies the insects at physiological and cellular levels. The behavioural studies as well as the transcriptomic and genomic studies have helped to increase our knowledge on the changes induced by sublethal exposure to insecticides (Figure 1).

Exposure to sublethal dose of insecticides: a good opportunity for the pests and disease vectors to stay alive

Insecticides are initially used to control pest insects. However, studies demonstrated that exposure to low dose of insecticides can benefit several insects. Thus, the effect of insecticides is biphasic. This phenomenon is known as hormesis. According to Cutler and Guedes [8], hormesis is defined as 'a biphasic dose-response, characterized by high-dose inhibition and low-dose stimulation during or following exposure to a toxicant'. Study of insecticide-induced hormesis demonstrated that low doses of insecticide stimulate biological processes in order to increase insect survival and reproduction [9–12]. Indeed, sublethal dose of sulfoxaflor, a sulfoximine insecticide which is an agonist of nicotinic acetylcholine receptors, shortened the developmental time of the small brown planthopper, *Laodelphax striatellus* and increased their fecundity [10]. In the cotton leafworm *Spodoptera littoralis*, males exposed to sublethal dose of deltamethrin,

Figure 1



General scheme summarizing adaptation mechanisms of insect through different physiological and cellular modifications in response to sublethal dose of insecticides.
Exposure to low dose of insecticides affects life history traits such as physiological functions (❶, ❷, ❸) that are due to cellular alterations by gene expression modifications (❹, ❺). These mechanisms can be passed to offspring.

an insecticide acting on voltage-gated sodium channels, shown a higher rate of reproductive success compared to untreated males. This positive effect on male sexual behaviour is linked specifically to a change of pheromonal detection [11]. All these data shown that insects adapt their physiology to be able to cope with sublethal exposure to insecticides in order to keep their population growth.

However, these sublethal exposures can also cause adverse effects. Sublethal concentrations of cyantraniliprole, a novel insecticide targeting ryanodine receptors, decreased growth speed and population reproduction of *Agrotis ipsilon* [13]. It was also shown that a sublethal dose of imidacloprid, which belong to the neonicotinoid class of insecticides, is cytotoxic for the honeybee brain and causes damage to the honeybee midgut [14]. This sublethal exposure also has an impact on foraging ability of bumblebees [15]. To understand the observed effect at cellular levels, further studies have been done on the mushroom body cells called Kenyon cells which are involved in learning and memory in insects. Thus, exposure to sublethal doses of insecticides reduced microglomerular density of mushroom bodies [16] and induced Kenyon cell alterations [14,17]. In these conditions, imidacloprid caused abnormal neural connectivity and damaged the development of mushroom bodies [16]. These effects could explain learning and memory disruption elicited by neonicotinoids [18–20].

In addition to stimulating different life history traits [9–12,21], exposure to a sublethal dose of insecticides could

induce production of enzymes that detoxify insecticides and this effect could participate in the development of insecticide tolerance or resistance in pest insects. There are several detoxification mechanisms in insects such as Cytochrome P450 monooxygenases, glutathione S-transferases (GST) and esterases. Biochemical and proteomic analysis demonstrated that the exposure to sublethal dose of insecticides modifies protein level and activity [22] and by consequent physiology and behaviour of insects. It was shown that the positive effect on male *S. littoralis* sexual behaviour is linked to three detoxification proteins such as GST or antennal esterase which were overexpressed in these conditions [11]. The fecundity increase of the gnat, *Bradysia odoriphaga*, induced by a sublethal dose of chlorfenapyr was also correlated to the rise of GST activity [12]. However, sublethal doses of insecticides do not always have a positive effect on physiology and behaviour of insects. Thus, contrasting effects of sublethal doses have been reported in the literature. Nevertheless, the question is how this opposite effect can be explained. Can it be only related to detoxification enzymes? Genomic, transcriptomic and proteomic analysis could help to fill this gap in our knowledge.

Changes of gene expression after exposure to sublethal dose of insecticides: is it the key event which makes the physiological difference?

The use of chemical insecticides on an increasing scale has led to the widespread development of resistance. Organisms have to adapt in order to face the toxicity of xenobiotics and to minimize negative effects on their life traits and fitness. A large amount of studies demonstrated that one of the main physiological response of insects was to up regulated the expression of genes coding for detoxification enzymes after lethal as well as sublethal exposure [11,23,24••,25,26]. Among the other strategies used by the insects, many cellular and molecular factors such as insecticide-induced target point mutation, subunit combination or involvement of auxiliary subunits, can modify the insecticide efficacy [27]. Exposure to a sublethal dose of insecticide doesn't seem to induce target point mutation [28••] but leads to altered gene expression. For insecticides acting mainly on neuronal ion channels and receptors, changes in transcript levels of these targets could be expected in this condition. So far, the main studied target of insecticide is the nicotinic acetylcholine receptors (nAChRs). Transcriptomic studies shown that expression of genes coding for nAChR subunits was modified after a sublethal exposure to neonicotinoids [24••,25,29,30,31•]. Moreover, changes of nAChR subunit expressions were correlated to a decrease of imidacloprid sensitivity on the cockroach *Periplaneta americana* neurones [32•]. Cockroach dorsal unpaired median neurones expressed several nAChRs. Among them, two nAChR subtypes called nAChR1 and nAChR2 differ in their sensitivity to imidacloprid [32•,33]. Exposure to a sublethal dose of imidacloprid decreased the sensitivity of nAChR1 to imidacloprid without affecting subunit combination whereas this treatment modified the expression of the subunit involved in nAChR2 structure in order to optimize its function [32•]. Thus, insects such as cockroaches could be able to trigger adaptive mechanisms by reducing the participation of imidacloprid-sensitive nAChRs and by optimizing functional properties of nAChRs which are insensitive to this insecticide. Interestingly, it was reported in *Locusta migratoria* that transcripts of proteins interacting with nAChRs were differentially regulated when the locusts were exposed to sublethal dose of imidacloprid [24••]. Among these interactive proteins, transcript of RIC-3, ubiquilin-1 and PICK1, which potentially regulated the expression and the function of nAChRs, were overexpressed [24••]. Thus, expression modifications of insecticide targets as well as these of interactive proteins should be considered in the strategy of insect adaptation. However, it was also reported that sublethal exposure to neonicotinoids or spinosyns, both targeting nAChRs [1], could alter the expression of genes coding for other receptors. For instance, exposure to a sublethal dose of thiamethoxam or spinosad modify respectively the expression of NMDA glutamate receptor subunit in *Apis mellifera* [30] and the expression of GABA receptors subunits in *Plutella xylostella* [34]. These data suggest that intracellular signalling pathways could be involved when insects are exposed to sublethal dose of insecticides. Transcriptomic analysis demonstrated that, according to the insecticide used and the studied insect, more than 300 genes were differentially expressed [24••,30,31•]. Interestingly, expression of intracellular factor transcripts, well-known to modulate insecticide sensitivity such as kinases and phosphatases [27], were changed [24••,31•]. Thus, exposure to sublethal dose of insecticide can modify the insect physiological responses using changes of various intracellular factors. However, it is important to note that these factors involved in sublethal response can be different from those elicited by a lethal dose [24••]. To improve pest management programs, it is necessary to know if these modifications induced by sublethal doses of insecticides can be maintained over generations.

Can modifications induced by low doses of insecticide be transgenerationally inherited?

It is acknowledged now that an adaptation to a new environment can occur through both genetic and epigenetic means across all organisms [35,36]. The rapidity of this adaptation suggests that mechanisms different from mutation can be involved [37••]. Even if researches have been shown that cells are able to raise their mutagenesis level onto DNA [36], the probability to obtain the expected mutation remains too long. Therefore, the involvement of post-transcriptional and epigenetic regulations, which modify gene expression without changing the underlying DNA sequence, can be suggested.

However, our concern is to determine if these phenomena can occur after an exposure to sublethal dose of insecticides. Transcriptomic studies, described above, showed a multitude of modulated genes in that condition.

Several epigenetic mechanisms such as DNA methylations [38], histone modifications and heritable noncoding RNAs could allow activation or inhibition of genes [39,40]. It is known that the presence of DNA methylations near to the promoter region of genes is associated with gene silencing and loss of methylation induces rise of transcription whereas histone acetylations make accessible the genes for their expression [41]. The post-transcriptional regulation is a direct regulation on mRNAs by mRNA silencing. Recently, small noncoding RNAs, named microRNAs (miRNAs), have been shown to be involved in the regulation of mRNA translation by blocking or cleaving the mRNA [42].

All these types of regulation have also been found in insects [43]. Moreover, it has been demonstrated that such changes in gene expression can be transmitted to the offspring [36,44,45]. This transmission called the transgenerational epigenetic inheritance (TEI) describes the transmission of alternative functional states through multiple generations in the presence of the same genomic DNA sequence [46]. Studies demonstrated that stress and memory can be transmitted by TEI [47–49]. TEI has been also reported in insects [50] and it has been demonstrated that the epigenetic inheritance influences offspring caste fate [51]. In organisms, adult acquired transcriptomic alteration in somatic cells and this alteration is spermatogenically inherited across generations suggesting that all life experiences could be transmitted through DNA methylations [45].

In insects, few studies have been done on epigenetics and inheritance involved in adaptive mechanisms after sublethal exposure to pesticides [37••]. Most of the data came from studies using lethal dose of insecticide leading to the development of resistant insects. For instance, when the diamondback moth, *P. xylostella* larvae resistant to the pyrethroid, deltamethrin, were fed with miRNA called miR-2b-3p to rise its level into the cells, treated moth became more sensitive to deltamethrin. Indeed, the miRNA may suppress transcript levels of genes coding for detoxification enzymes [43]. Other two studies demonstrated that inhibiting histone deacetylation in *A. mellifera* or activating DNA methylation in *Aedes albopictus*, both increased resistance to imidacloprid used at LC_{50} [52,53]. Similar results were obtained using sublethal doses of insecticides. Treatment with the neonicotinoid clothianidin at sublethal dose accelerated the development of F1 generation of *Bemisia tabaci* but reduced its survival and fecundity [54]. Low dose of the neonicotinoid cycloxaprid decreased adult longevity and fecundity of *Aphis gossypii*. Indeed, transgenerational effects in offspring were to reduce adult fecundity and net reproductive rate [55]. Study on aphids, *Myzus persicae*, shown that exposure to sublethal dose of imidacloprid reduced their sensitivity to this insecticide across four generations without affecting sensitivity to spirotetramat, another insecticide acting as lipid biosynthesis inhibitor. This result suggests a specific adaptation to one molecule which is not in line with a non-specific chemical resistance. Moreover, no mutation was found in all five subunits which compose nAChR targeted by neonicotinoids [28••]. This work demonstrated that changes induced by exposure to sublethal dose of insecticides were not linked to mutation but to epigenetic mechanisms. Thus, the mechanism offering the fastest way of adaptation and the transgenerational inheritance is the direct modulation of gene expression via epigenetics. Moreover, the transgenerational studies suggest that species, which have shorter reproductive cycles and higher reproductive rate, may promote epigenetics to trigger insecticide adaptation. In fact, faster the offspring with inherited information from parents appear, the better the next offspring of this species will adapt and then survive.

Conclusions

Exposure to sublethal dose of insecticides induced physiological modifications that could be linked to changes in gene expression levels via epigenetic mechanisms. The advantage of these mechanisms is that they are faster than genetic mutation adaptations. Moreover, epigenetic modifications can be transmitted to the offspring, offering not only faster adaptation but also a persistent adaptation across the next generations. Today, researches are still incomplete and some points have to be resolved. After exposure to sublethal dose of pesticides, mutations occurred in fungi and weed species whereas this is not seen in insects [28••]. Further studies are needed to determine under which conditions mutations can occur. Moreover, several studies have demonstrated the role of detoxification and the immune system in insecticide resistance [25,29,56,57]. Further studies should be done to determine if the immune system can play an important role in transgenerational adaptations to insecticides. In summary, different aspects of the adaptive mechanisms must be explored in order to improve pest control.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was supported by INRA, la Région Pays de la Loire and the RFI objectif végétal.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Ffrench-Constant RH, Williamson MS, Davies TG, Bass C: **Ion channels as insecticide targets**. *J Neurogenet* 2016, **30**:163-177.

2. Casida JE: **Neonicotinoids and other insect nicotinic receptor competitive modulators: progress and prospects**. *Ann Rev Entomol* 2018, **63**:125-144.

3. Ffrench-Constant RH: **The molecular genetics of insecticide resistance**. *Genetics* 2013, **194**:807-815.

4. Zalucki MP, Furlong MJ: **Behavior as a mechanism of insecticide resistance: evaluation of the evidence**. *Curr Opin Insect Sci* 2017, **21**:19-25.

5. Vryzas Z: **Pesticide fate in soil-sediment-water environment in relation to contamination preventing actions**. *Curr Opin Environ Sci Health* 2018, **4**:5-9.

6. Zheng S, Chen B, Qiu X, Chen M, Ma Z, Yu X: **Distribution and risk assessment of 82 pesticides in jiulong river and estuary in south china**. *Chemosphere* 2016, **144**:1177-1192.

7. Fernandes MES, Alves FM, Pereira RC, Aquino LA, Fernandes FL, Zanuncio JC: **Lethal and sublethal effects of seven insecticides on three beneficial insects in laboratory assays and field trials**. *Chemosphere* 2016, **156**:45-55.

8. Cutler GC, Guedes RNC: **Occurrence and significance of insecticide-induced hormesis in insects**. In *Pesticide Dose: Effects on the Environment and Target and Non-Target Organisms.* Edited by Duke SO, Kudsk P, Solomon K. American Chemical Society; 2017:101-119.

9. Rix RR, Ayyanath MM, Cutler GC: **Sublethal concentrations of imidacloprid increase reproduction, alter expression of detoxification genes, and prime myzus persicae for subsequent stress**. *J Pest Sci* 2016, **89**:581-589.

10. Xu L, Zhao CQ, Zhang YN, Liu Y, Gu ZY: **Lethal and sublethal effects of sulfoxaflor on the small brown planthopper laodelphax striatellus**. *J Asia-Pac Entomol* 2016, **19**:683-689.

11. Lalouette L, Pottier MA, Wycke MA, Boitard C, Bozzolan F, Maria A, Demondion E, Chertemps T, Lucas P, Renault D, Maibeche M *et al.*: **Unexpected effects of sublethal doses of insecticide on the peripheral olfactory response and sexual behavior in a pest insect**. *Environ Sci Pollut Res Int* 2016, **23**:3073-3085.

12. Zhao Y, Wang Q, Ding J, Wang Y, Zhang Z, Liu F, Mu W: **Sublethal effects of chlorfenapyr on the life table parameters, nutritional physiology and enzymatic properties of bradysia odoriphaga (diptera: Sciaridae)**. *Pesticide Biochem Physiol* 2018, **148**:93-102.

13. Xu C, Zhang Z, Cui K, Zhao Y, Han J, Liu F, Mu W: **Effects of sublethal concentrations of cyantraniliprole on the development, fecundity and nutritional physiology of the black cutworm agrotis ipsilon (lepidoptera: Noctuidae)**. *PloS One* 2016, **11**:e0156555.

14. Catae AF, Roat TC, Pratavieira M, Silva Menegasso ARD, Palma MS, Malaspina O: **Exposure to a sublethal concentration of imidacloprid and the side effects on target and nontarget organs of apis mellifera (hymenoptera, apidae)**. *Ecotoxicology* 2018, **27**:109-121.

15. Feltham H, Park K, Goulson D: **Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency**. *Ecotoxicology* 2014, **23**:317-323.

16. Peng YC, Yang EC: **Sublethal dosage of imidacloprid reduces the microglomerular density of honey bee mushroom bodies**. *Sci Rep* 2016, **6**:19298.

17. Jacob CR, Soares HM, Nocelli RC, Malaspina O: **Impact of fipronil on the mushroom bodies of the stingless bee scaptotrigona postica**. *Pest Manag Sci* 2015, **71**:114-122.

18. Samuelson EE, Chen-Wishart ZP, Gill RJ, Leadbeater E: **Effect of acute pesticide exposure on bee spatial working memory using an analogue of the radial-arm maze**. *Sci Rep* 2016, **6**:38957.

19. Tison L, Holtz S, Adeoye A, Kalkan O, Irmisch NS, Lehmann N, Menzel R: **Effects of sublethal doses of thiacloprid and its formulation calypso((r)) on the learning and memory performance of honey bees**. *J Exp Biol* 2017, **220**:3695-3705.

20. Alkassab AT, Kirchner WH: **Impacts of chronic sublethal exposure to clothianidin on winter honeybees**. *Ecotoxicology* 2016, **25**:1000-1010.

21. Qu Y, Xiao D, Li J, Chen Z, Biondi A, Desneux N, Gao X, Song D: **Sublethal and hormesis effects of imidacloprid on the soybean aphid aphis glycines**. *Ecotoxicology* 2015, **24**:479-487.

22. Roat TC, Carvalho SM, Palma MS, Malaspina O: **Biochemical response of the africanized honeybee exposed to fipronil**. *Environ Toxicol Chem* 2017, **36**:1652-1660.

23. Xiao D, Tan X, Wang W, Zhang F, Desneux N, Wang S: **Modification of flight and locomotion performances, respiratory metabolism, and transcriptome expression in the lady beetle harmonia axyridis through sublethal pesticide exposure**. *Front Physiol* 2017, **8**:33.

24. •• Wang X, Sun H, Zhang Y, Liu C, Liu Z: **Transcriptional changes in nachrs, interactive proteins and p450s in locusta migratoria manilensis (orthoptera: Acrididae) cns in response to high and low oral doses of imidacloprid**. *J Insect Sci* 2015:15.
This article shown modifications of gene expressions in*Locusta migratoria* exposed to low and high doses of imidacloprid. Among the gene differentially expressed, the genes coding for proteins interacting with nicotinic acetylcholine receptors were upregulated after exposure at low dose.

25. Christen V, Fent K: **Exposure of honey bees (apis mellifera) to different classes of insecticides exhibit distinct molecular effect patterns at concentrations that mimic environmental contamination**. *Environ Pollut* 2017, **226**:48-59.

26. Alptekin S, Bass C, Nicholls C, Paine MJ, Clark SJ, Field L, Moores GD: **Induced thiacloprid insensitivity in honeybees (apis mellifera l.) is associated with up-regulation of detoxification genes**. *Insect Mol Biol* 2016, **25**:171-180.

27. Raymond V, Goven D, Benzidane Y, List O, Lapied B: **Influence of cellular and molecular factors on membrane target sensitivity to insecticides**. *Curr Med Chem* 2017, **24**:2974-2987.

28. •• Rix RR, Cutler GC: **Does multigenerational exposure to hormetic concentrations of imidacloprid precondition aphids for increased insecticide tolerance?** *Pest Manag Sci* 2018, **74**:314-322.
This study demonstrated that sublethal dose of imidacloprid induced an adaptation of aphids over four generations without mutation onto the genes which compose the nicotinic acetylcholine receptors.

29. Christen V, Mittner F, Fent K: **Molecular effects of neonicotinoids in honey bees (apis mellifera)**. *Environ Sci Technol* 2016, **50**:4071-4081.

30. Shi TF, Wang YF, Liu F, Qi L, Yu LS: **Sublethal effects of the neonicotinoid insecticide thiamethoxam on the transcriptome of the honey bees (hymenoptera: Apidae)**. *J Econ Entomol* 2017, **110**:2283-2289.

31. • Wu MC, Chang YW, Lu KH, Yang EC: **Gene expression changes in honey bees induced by sublethal imidacloprid exposure during the larval stage**. *Insect Biochem Mol Biol* 2017, **88**:12-20.
This article are based on whole transcriptomic analysis to identify down or up regulated genes induced by sublethal exposure of imidacloprid in bee larvae. An exhaustive and informative list of all regulated genes is built.

32. • Benzidane Y, Goven D, Abd-Ella AA, Deshayes C, Lapied B, Raymond V: **Subchronic exposure to sublethal dose of imidacloprid changes electrophysiological properties and expression pattern of nicotinic acetylcholine receptor subtypes in insect neurosecretory cells**. *Neurotoxicology* 2017, **62**:239-247.
In this study, exposure of cockroaches*Periplaneta americana* to a sublethal dose of imidacloprid, differently affect electropharmacological properties and subunit expression pattern of DUM neuron nicotinic acetylcholine receptors, which thereby impact their physiological functions.

33. Sun H, Liu Y, Li J, Cang X, Bao H, Liu Z: **The potential subunits involved in two subtypes of alpha-bgt-resistant nachrs in cockroach dorsal unpaired median (dum) neurons**. *Insect Biochem Mol Biol* 2017, **81**:32-40.

34. Yin XH, Wu QJ, Zhang YJ, Long YH, Wu XM, Li RY, Wang M, Tian XL, Jiao XG: **Analysis of persistent changes to gamma-aminobutyric acid receptor gene expression in plutella**

xylostella subjected to sublethal amounts of spinosad. *Genet Mol Res: GMR* 2016:15.

35. Elhamamsy AR: **DNA methylation dynamics in plants and mammals: overview of regulation and dysregulation**. *Cell Biochem Funct* 2016, **34**:289-298.

36. Xue Y, Acar M: **Mechanisms for the epigenetic inheritance of stress response in single cells**. *Curr Genet* 2018 http://dx.doi.org/10.1007/s00294-018-0849-1.

37. •• Brevik K, Lindstrom L, McKay SD, Chen YH: **Transgenerational effects of insecticides-implications for rapid pest evolution in agroecosystems**. *Curr Opin Insect Sci* 2018, **26**:34-40.
This review report that epigenetic modifications which are due to xenobiotic stress, are heritable to germ lines and insecticide-induced effects can be transgenerationally inherited

38. Zhang H, Lang Z, Zhu JK: **Dynamics and function of DNA methylation in plants**. *Nat Rev Mol cell Biol* 2018, **19**:489-506.

39. Lawrence M, Daujat S, Schneider R: **Lateral thinking: how histone modifications regulate gene expression**. *Trends Genet: TIG* 2016, **32**:42-56.

40. Burggren WW: **Epigenetics in insects: Mechanisms, phenotypes and ecological and evolutionary implications**. In *Insect Epigenetics.* Edited by Verlinden H. Academic Press; 2017:1-30.

41. Allis CD, Jenuwein T: **The molecular hallmarks of epigenetic control**. *Nat Rev Genet* 2016, **17**:487-500.

42. Denzler R, McGeary SE, Title AC, Agarwal V, Bartel DP, Stoffel M: **Impact of microrna levels, target-site complementarity, and cooperativity on competing endogenous rna-regulated gene expression**. *Mol Cell* 2016, **64**:565-579.

43. Etebari K, Afrad MH, Tang B, Silva R, Furlong MJ, Asgari S: **Involvement of microrna mir-2b-3p in regulation of metabolic resistance to insecticides in plutella xylostella**. *Insect Mol Biol* 2018, **27**:478-491.

44. Houri-Ze'evi L, Korem Y, Sheftel H, Faigenbloom L, Toker IA, Dagan Y, Awad L, Degani L, Alon U, Rechavi O: **A tunable mechanism determines the duration of the transgenerational small rna inheritance in C. elegans**. *Cell* 2016, **165**:88-99.

45. Chalker DL: **Transgenerational inheritance: Parental guidance suggested**. *Curr Biol: CB* 2018, **28**:702-704.

46. Heard E, Martienssen RA: **Transgenerational epigenetic inheritance: Myths and mechanisms**. *Cell* 2014, **157**:95-109.

47. Seong KH, Li D, Shimizu H, Nakamura R, Ishii S: **Inheritance of stress-induced, atf-2-dependent epigenetic change**. *Cell* 2011, **145**:1049-1061.

48. Dias BG, Ressler KJ: **Pacap and the pac1 receptor in post-traumatic stress disorder**. *Neuropsychopharmacology* 2013, **38**:245-246.

49. D'Urso A, Brickner JH: **Epigenetic transcriptional memory**. *Current Genet* 2017, **63**:435-439.

50. Xing Y, Shi S, Le L, Lee CA, Silver-Morse L, Li WX: **Evidence for transgenerational transmission of epigenetic tumor susceptibility in drosophila**. *PLoS Genet* 2007, **3**:1598-1606.

51. Matsuura K, Mizumoto N, Kobayashi K, Nozaki T, Fujita T, Yashiro T, Fuchikawa T, Mitaka Y, Vargo EL: **A genomic imprinting model of termite caste determination: Not genetic but epigenetic inheritance influences offspring caste fate**. *Am Nat* 2018, **191**:677-690.

52. Hu YT, Wu TC, Yang EC, Wu PC, Lin PT, Wu YL: **Regulation of genes related to immune signaling and detoxification in apis mellifera by an inhibitor of histone deacetylation**. *Sci Rep* 2017, **7**:41255.

53. Oppold A, Kress A, Vanden Bussche J, Diogo JB, Kuch U, Oehlmann J, Vandegehuchte MB, Muller R: **Epigenetic alterations and decreasing insecticide sensitivity of the asian tiger mosquito aedes albopictus**. *Ecotoxicol Environ Saf* 2015, **122**:45-53.

54. Fang Y, Wang J, Luo C, Wang R: **Lethal and sublethal effects of clothianidin on the development and reproduction of bemisia tabaci (hemiptera: Aleyrodidae) med and meam1**. *J Insect Sci* 2018, **18**.

55. Yuan HB, Li JH, Liu YQ, Cui L, Lu YH, Xu XY, Li Z, Wu KM, Desneux N: **Lethal, sublethal and transgenerational effects of the novel chiral neonicotinoid pesticide cycloxaprid on demographic and behavioral traits of aphis gossypii (hemiptera: Aphididae)**. *Insect Sci* 2017, **24**:743-752.

56. Pamminger T, Botías C, Goulson D, Hughes WOH: **A mechanistic framework to explain the immunosuppressive effects of neurotoxic pesticides on bees**. *Funct Ecol* 2018 http://dx.doi.org/10.1111/1365-2435.13119.

57. Brandt A, Gorenflo A, Siede R, Meixner M, Buchler R: **The neonicotinoids thiacloprid, imidacloprid, and clothianidin affect the immunocompetence of honey bees (apis mellifera l.)**. *J Insect Physiol* 2016, **86**:40-47.