

Recent insights into sublethal effects of pesticides on insect respiratory physiology

Reet Karise
Marika Mänd

Institute of Agricultural and
Environmental Sciences, Estonian
University of Life Sciences, Tartu,
Estonia

Abstract: Determination of the sublethal effects of pesticides on insects is a challenging topic because of the vast number of different possible end points. Sublethal effects can manifest themselves through changes in motor activity, behavior, or, in the case of social insects, learning ability. These are still the outcomes of physiological changes. As autonomic processes like respiration assure the normal functioning of any organism, the estimation of disturbances of these processes can give valuable data for toxicology researchers. This review reports a variety of effects of pesticides on insect respiratory patterns, metabolic rate, and water loss rate. Although the tested pesticides and target subjects belong to very different groups, the results of the reviewed studies indicate several common effects. We conclude that the study of the pesticide effects on insect respiratory physiology has potential for further development as a methodology for measurement of basic physiological changes as it allows measurement of the intact living insect, the result is obtained rapidly, and several parameters can be measured simultaneously. At the same time, the method has its shortcomings: the equipment is expensive and complicated, the results can be affected by the experimental conditions, and as yet there are no standardized end points for data comparison.

Keywords: sublethal effects, respiratory physiology, DGE, metabolic rate, water loss rate

Introduction

Agricultural practice with intensive land use and extensive pesticide input influences the agrienvironment by harming beneficial fauna.¹ Mortality tests and determination of the lethal dose 50% value for each pesticide alone do not give sufficient information to protect them. Therefore, new technologies and study methods are being developed to gain more detailed information about the sublethal effects of pesticides.

Determination of the sublethal effects of pesticides on insects is a challenging topic because of the vast number of different end points. Sublethal effects of pesticides involve both behavioral and physiological changes.² Among behavioral effects, insect mobility, navigation, and orientation ability as well as feeding and oviposition behavior are studied. In the case of bees, learning ability is also a commonly estimated parameter since it plays a major role in pollination. The physiological features that are commonly estimated are developmental success, morphological deformities, fecundity, and adult longevity.² More detailed studies address biochemistry and neurophysiology which affect cellular functions. Changes in the function of neural or muscular tissues lead to insufficiency in autonomic processes such as thermoregulation,³ respiration,⁴ and water balance.⁵

Correspondence: Reet Karise
Institute of Agricultural and
Environmental Sciences, Estonian
University of Life Sciences,
Kreutzwaldi 1, 51014 Tartu, Estonia
Tel +372 731 3457
Email reet.karise@emu.ee

Insect respiration is a highly sensitive system, which responds rapidly to any stressor.⁵ Kestler⁴ demonstrated that respiratory measurements can be used to detect reversible and irreversible toxicosis in insects. Understanding the real nonharming doses of pesticides on nontarget organisms should be an important component in calculating the suggested/allowed doses for controlling pests. Techniques based on changes in autonomic processes can add valuable data to fill gaps in our knowledge. The aim of this review is to collate data and clarify the general respiratory effects observed after treatment of insects with different pesticides.

Respiratory physiology in insects

Gas-exchange patterns and metabolic rate

The insect tracheal system consists of branching tubes through which air moves to the tissues, enabling gas exchange within the whole body. The external openings of the tracheal system are called spiracles, which act as muscular valves and control the amount of gases getting through. When metabolic needs are high, the spiracles are wide open and the insect breathes continuously. When metabolic demands are lower, less oxygen is needed,⁶ and continuous gas exchange is replaced by patterns during which spiracles are closed some of the time.^{7–10}

With the lowest energy demands, the insect keeps the spiracles closed most of the time and opens them for moments periodically. This pattern is called discontinuous gas exchange (DGE). DGE involves the constriction of the spiracles of the insect tracheae (constriction phase, C) during which no CO₂ release occurs; the C-phase is followed by fluttering (flutter phase, F) when the spiracles partly open and close in rapid succession keeping the tracheal oxygen at a certain level. Finally, the opening of spiracles (open phase, O) allows maximum gas exchange.^{11,12}

The pattern of CO₂ release reflects the activity level of the insect. DGE occurs only during the quiet state of the insect.^{13–16} During the deep resting state, the DGE cycles involve a very long closed phase and short rapid outbreaks of CO₂ release,¹⁷ a state that reflects the basic metabolic rate of the organism. If the quiescent state is disturbed and the activity level is raised, the pattern changes: the CFO cycles become shorter and the mean amount of CO₂ released usually increases.¹⁶ Eventually, the DGE pattern disappears and is replaced by cyclic gas exchange (CGE) or continuous (Cont) respiration.^{18–20}

DGE and water loss rate

Water loss is a mechanistic consequence of gas exchange.²¹ Increasing metabolic rate leads to an increase in water loss

rate.^{22,23} Insects often endure shorter or longer periods of immobility because of their developmental stage, unfavorable environmental conditions,^{23–27} paralysis due to contact with toxicants,^{28,29} or death feigning for protection^{15,30,31} during which it is important to decrease water loss rate to avoid desiccation.

The presence of DGE in insects and some other arthropod taxa is most often considered as an adaptation to reduce respiratory water loss rate.^{32–34} In DGE during C and F phases, the spiracles are closed or flutter and no outward movement of gas molecules (including H₂O) can occur.^{35–37} The water vapor leaves the insect body through the cuticle or spiracles. In most insect species studied, spiracular water loss makes up approximately 10% of all water loss.³⁸ This seems to be an inconsiderable amount, which still may prove to be critical in conditions where water is a limiting factor.^{39–42} There is proof that the varying patterns of DGE contribute differently to the rate of water loss.⁴³ However, in some experiments where the insects were forced to open or close the spiracles because of hyperoxic or hypoxic conditions, the presence or absence of DGE has been shown not to affect water loss rate.^{33,44,45}

In many studies, only the total water loss has been estimated. The gravimetric method of weighing the insect before and after the experiment does not allow separation of the gas-exchange patterns and phases within DGE.^{23,43} Modern gas analyzers (eg, LiCor 6262 or 7000, LiCor, Lincoln, NE, USA) allow simultaneous recording of different gases, which enable the measurement of water loss during the open and C+F phases of DGE.^{24,46–48} At that time, the only H₂O detected is evaporated through the cuticle. During the O phase of DGE, the additive peak indicates H₂O emergence through the spiracles.

Factors affecting insect respiratory physiology

The metabolic rate and gas-exchange patterns of any organism depend on several factors. For insects, the first effector is the life stage. The metabolic rate of the larva is generally higher than that of the pupa because the former is feeding intensively.^{49–52} Furthermore, larvae preparing for pupation have a higher resting metabolic rate than those not preparing for pupation.^{53–55} The metabolic rate may also change due to parasitism of the larva as was shown for *Manduca sexta* L.⁵⁶ The metabolic rate of bumble bee queens depends on whether they are pre- or postwintered⁵⁷ and also on their task in the nest.⁵⁸

The second aspect that must be taken into consideration in respiratory studies is the individual properties of the subject.

Metabolic rate has been shown to be affected by its sex,⁵⁹ behavior,^{16,30,31,47,60,61} and body mass,⁶² although some studies, for example, in the bumble bee *Bombus terrestris* L.^{16,48,47} failed to find the latter correlation.

Temperature affects the metabolic rate to a great degree.^{23,63} At higher temperatures, the metabolic rate is usually higher. This phenomenon has its limits. Each species has its own normal activity temperatures below and above which the vital functions are slowed down.^{24,27,64,65} In both situations, the insect usually hides quietly until the conditions become favorable again.⁶⁶ In addition, in some species, the activity level is not correlated directly with temperature. For part of the time, honey bees and bumble bees stay inside the nest, where temperature remains around 30°C, and is the temperature actively controlled by nest mates.⁶⁷ The foragers go out in the cooler temperatures. It has been observed that the metabolic rate of resting or walking bumble bees is higher at cooler than higher temperatures.⁵⁸

Water availability is a limiting factor for most terrestrial insects. Because of their relatively large body surface compared to their body mass, water evaporation is more hazardous to insects than to larger animals.^{13,68} Insects have adaptations to avoid water loss. The insects' cuticle with its waterproofing long-chain saturated hydrocarbons provides a barrier to water loss.⁶⁹ The excretion system involves a water reabsorption capability, and thus much of water can be saved.⁷⁰ Metabolic water production is essential not only for eggs and pupae but also, for instance, in insects living in stored grain consuming only dry food with no access to water.⁷¹ Restricting water loss through spiracles can contribute to restricting total water loss, although, in most insect species, respiratory water loss has been estimated to be much less than 20%.⁶⁸ Insects from arid regions certainly benefit from restricting respiratory water loss.^{39–42} Lowering metabolic rate in extremely dry conditions has been observed even with bumble bees,⁷² which inhabit the middle latitudes where water is mostly available.

Effects of lethal and sublethal doses of pesticides on insect respiration

The most common insecticides (organophosphorus compounds, pyrethroids, and neonicotinoids) target the nervous system, which controls automatic functions in an organism.⁵ Studies dealing with the effects on the autonomous functions in insects have involved several types of pesticides: synthetic insecticides,^{4,47,73–78} botanical extracts,^{36,74,79–81} bacterial toxins,^{82,83} biofungicides and -insecticides,⁴⁸ and

inert powders⁴⁸ (Table 1). These studies have been conducted with almost all developmental stages of insects from distinct systematic families.

Autonomic functions are highly sensitive, and alterations in metabolic rate and respiratory patterns occur long before death. Sláma and Miller⁵ demonstrated changes in the patterns of hemocoelic pulses after treatment with different doses of pyrethroids, carbamates, and organophosphorus insecticides. They demonstrated dose dependency and reversibility of the effects of very low doses of these pesticides in pupae of *Tenebrio molitor* L. Kestler⁴ indicated the use of insect respirometry to determine the sublethal effects of normal (eg, drought) and anthropogenic (eg, pollutants) stressors. He described the cyclic CO₂ release as an indicator for physiological stress in *Periplaneta americana* L. This insect typically displays classical DGE when at rest. The O phases may be accompanied by ventilation (V) movements. Normally within the regular pattern of DGE, some intracyclic activity occurs. After mechanical disturbance, extracyclic activity in-between the DGE was observed. Kestler⁴ suggested that this pattern is a sensitive index for stress on the central nervous system in the resting state of an insect. The chemical stress induced by the vapor of chlorpyrifos (insecticide) is expressed at first by an irregular pattern of extracyclic activity and continuous respiration due to nerve excitation, as Kestler deduced. This phase was followed by an irreversible phase when, as a result of nerve poisoning, the spiracles were constantly open because of paralysis. According to this study,⁴ the paralysis leads to exponential wash out of CO₂ from the tissues with some activity because of tremors. At the end, a smooth CO₂ release can be seen. The gradual loss of resting CFO(V) cycles in moribund insects, sometime after the onset of the reversible excitation phase, was described as the typical physiological effect. The very same pattern may occur due to desiccation or hard handling stress. If the effect of the stressor disappears before the end of cyclic respiration, the toxicosis may be reversible.

Woodman et al⁷⁶ found that the organophosphorus fumigant phosphine caused immediate loss of DGE in *P. americana* exposed to 800 ppm of phosphine in the air. The first reaction was the central nervous system response within 60 seconds, followed by high-frequency burstlike CO₂ release and then by stable and continuous CO₂ release. The nerve damaging effect of the phosphine was evident as, after returning to normoxia following the phosphine treatment, normal DGE pattern was not reinstated in any of the trials.

The treatments with pyrethroids also cause changes in normal patterns of DGE due to over excitation or paralysis

Table 1 The effects of different pesticides on metabolic rate, respiratory patterns, water loss rate, muscle work, and longevity in insects and a species of ticks (Acaridae)

Pesticide group	Target site	Active ingredient	Object	Metabolic rate	Changes in respiratory patterns	Water loss rate	Muscle work	Longevity	Reference
Organophosphate	Nerve poison	Chlorpyrifos	<i>Periplaneta americana</i> , adults	Decreases	DGE > CGE > Cont	Increases	Nerve excitation at first, lost afterward		4
Organophosphate	Nerve poison	Phosphine	<i>P. americana</i> , adults	Decreases	DGE disappears	Decreases	Nerve excitation at first, lost afterward		76
Pyrethroid	Nerve poison	Alpha-cypermethrin	<i>Bombus terrestris</i> , foragers	0.04%: decreases 0.02%: decreases	DGE disappears	Increases in most individuals Increases in some individuals	Decreases	Death within 1–3 days	47
Pyrethroid	Nerve poison	Deltamethrin	<i>Platynus assimilis</i> , adults <i>Tenebrio molitor</i> , adults	Increases	DGE > CGE		Increases at first, restored on day 3	Normal death rate	77
Pyrethroid	Nerve poison	Permethrin	<i>T. molitor</i> , pupae	Increases	DGE > CGE		Rare activity periods, vigorous pumping		75
Pyrethroid	Nerve poison	Permethrin	<i>T. molitor</i> , pupae	Increases	DGE disappears		Abnormal coordination, hyperactivity	Lethal neurotoxicosis	74
Pyrethroid	Nerve poison	Permethrin	<i>Amblyomma americanum</i> , adult	Increases	DGE very frequent	Major water loss			78
Synergist/ acaricide/ insecticide	Nerve excitation	amitraz		Increases	DGE > Cont	No change			
Mixture of previous two	Nerve excitation	Permethrin + amitraz		Increases, synergistic effect	DGE > chaotic CO ₂ release, synergistic effect	Major water loss at first and another after 12 h		Synergistic effect on MR, respiratory patterns	
Neonicotinoid	nAChRs	Imidacloprid	<i>T. molitor</i> , adults		DGE with lower peak amplitude, DGE > Cont		Ceases	Killed	75
Neonicotinoid	nAChRs	Imidacloprid	<i>Apis mellifera</i> , adults	Decreases			Muscular ventilation periods shorter	No change in behavior or longevity	73
Botanical	Nerve poison	<i>Azadirachta indica</i>	<i>Pieris brassica</i> , pupae		No change at first, DGE disappears after 2–3 days	Lethal desiccation	Muscle work disappears, no response to stimuli, heartbeats present	Death within 5–21 days	80
			<i>Hylobius abietis</i> , adults	No change	DGE > DGE with higher frequency > Cont; DGE not restored	Lethal desiccation	Irritation first, then irregular pumping movements	80% within 5 days, compared to 20% in untreated beetles	79
			<i>P. brassica</i> , pupae	Increases slightly	DGE > chaotic fluttering and Cont	Lethal desiccation	No movements, heartbeat pattern changes at fifth day		36
Botanical	Nerve poison	<i>Heracleum sosnowskyi</i>	<i>T. molitor</i> , pupae <i>Galleria mellonella</i> ,	Decreases	DGE disappears		Inhibition of muscular activity, no reaction to stimuli		74

Botanical	Juvenile hormone analog	<i>Nicotiana rustica</i> <i>Artemisia absinthium</i> <i>A. vulgaris</i> <i>Ledum palustre</i>	pupae	Decreases	DGE restores after some time, DGE > CGE DGE disappears	Muscular hyperactivity	74,81
			<i>T. molitor</i> , pupae	Increases		Muscular hyperactivity	74
			<i>G. mellonella</i> , pupae	Increases	DGE disappears	Muscular hyperactivity	82,83
	Bioinsecticide	<i>Bacillus thuringiensis</i>	<i>Spodoptera exigua</i> Resistant and susceptible larvae/pupae	In larvae increases; in pupae increases at first, normalizes after 4 days	DGE less frequent	More ventilation periods	
Clay	Physical barrier, affecting water loss	Kaolin	<i>B. terrestris</i> , foragers	No change	No change	No change	48
Biofungicide	Fungus + kaolin	<i>Gladiolus catenulatum</i>	<i>B. terrestris</i> , foragers	No change	No change	No change	48
Bioinsecticide	Fungus + inert material	<i>Beauveria bassiana</i>	<i>B. terrestris</i> , foragers	No change	No change	No change	48

Abbreviations: DGE, discontinuous gas exchange; CGE, cyclic gas exchange; Cont, continuous gas exchange; h, hours; nAChRs, nicotinic acetylcholine receptors; MR, metabolic rate.

of muscles.^{47,74,75,78} Muljar et al⁴⁷ treated bumble bees with the pyrethroid “Fastac 50 EC” in which alpha-cypermethrin is the active ingredient. They saw a decrease in metabolic rate in bumble bee *B. terrestris* foragers after topical treatment with a 0.004% and a 0.002% solution of the insecticide. In addition, with the higher concentration, the regular DGE cycles ceased after the first 30 minutes, whereas no change was observed with the lower concentration. The loss of DGE cycles occurred because of paralysis, since no muscle activity was detected. In contrast, Kivimägi et al⁷⁷ found that the same toxicant caused an increase in metabolic rate in *Platynus assimilis* Paykull, occurring due to the higher activity level. The application of deltamethrin in the experiment of Zafeiridou and Theophilidis⁷⁵ has caused a gradual increase in the frequency of the respiratory contraction of *T. molitor* adults. Kuusik et al⁷⁴ studied the effect of topical treatment with 0.1% permethrin solution on *T. molitor* pupae and described the increase in metabolic rate and loss of DGE cycles. This nerve poison caused lethal neurotoxicosis, which at first was seen by abnormal coordination and hyperactivity of treated pupae. Zheng et al⁷⁸ experimented with the effect of permethrin on the tick *Amblyomma americanum* L. In addition, they also studied the effect of amitraz, an acaricide and synergist, and found both permethrin and amitraz caused an increase in metabolic rate because of increasing frequency of DGE cycles or even replacement of DGE with continuous respiration. The permethrin treatment also caused major water loss. Since amitraz is often used mixed with insecticides for better pest control, Zheng et al⁷⁸ also tested the effect of the mixture of these two pesticides. This clearly showed the synergistic effect on the CO₂ release and metabolic rate. In the case of the mixture, the authors observed two major water loss periods: the first immediately and the second 12 hours after treatment.

The effects of the neonicotinoid imidacloprid on the insect respiratory system have been studied on *T. molitor*. Zafeiridou and Theophilidis⁷⁵ treated *T. molitor* adults with 50–500 ng of imidacloprid and found dose-dependent changes in the impact on gas-exchange cycles. Although both imidacloprid and deltamethrin affect the nervous system of the insect, the effects found were different. Unlike the deltamethrin, imidacloprid caused cessation of respiratory rhythms.⁷⁵

Insecticidal plant extracts may affect insects at the same toxic level as synthetic insecticides.^{36,74,79–81} Sibul et al⁷⁹ treated *Hylobius abietis* L. adults with 0.01% NeemAzal T/S solution and saw no change in metabolic rate, although an increase in the frequency of respiratory cycles occurred,

and the normal pattern was not restored within 5 days. Jõgar et al⁸⁰ found that in *Pieris brassicae* L. pupae 0.001% Neem EC solution led to disappearance of DGE within 2–3 days and all pupae died within 21 days. Both these experiments share the result (based on gravimetric recording) that the treated individuals lost more water, and in both cases the authors supposed that death came through desiccation. Harak et al³⁶ also worked with *P. brassicae* pupae and observed that regular DGE cycles were replaced by chaotic fluttering or continuous respiration after treatment with natural pyrethrum (derived from *Tanacetum roseum* Adam). Similar to azadirachtin, pyrethrum caused lethal desiccation in parallel with muscle paralysis. Kuusik et al⁷⁴ treated pupae of *T. molitor* and *Galleria mellonella* L. with extracts from *Heracleum sibiricum* (L.) Simonkai, *Nicotiana rustica* L., *Artemisia absinthium* L., and *A. vulgaris* L. All of these caused a decrease in metabolic rate and disappearance of DGE cycles, whereas with *A. absinthium* and *A. vulgaris*, the DGE pattern was restored after some time, and unlike the other plant extracts, they caused muscular hyperactivity instead of paralysis or inhibition. The lengthening of periods of muscular hyperactivity was also the cause of DGE disappearance after treatment with a natural juvenile hormone analog, extracts of *Ledum palustre* Marsh-Tea.^{74,81}

Insect respirometry measurements are suitable for detection of changes caused by many different kinds of pollutants or substances. Dingha et al^{82,83} published the results of treatment of *Spodoptera exigua* Hübner pupae and larvae with *Bacillus thuringiensis* Berliner Cry1C toxin. In Cry1C toxin-resistant larvae and pupae which were constantly exposed to the toxin, the metabolic rate was higher and, at the same time, body mass was lower, indicating the costs of detoxification of the toxin. They also studied the water loss rate of pupae and observed that Cry1C toxin-exposed pupae had higher water loss rate, which indicates their increased susceptibility to desiccation.

Some bioinsecticides have also been tested against the bumble bee *B. terrestris*. Karise et al⁴⁸ found no changes in metabolic rate or respiratory patterns, but saw an increase in water loss rate. They demonstrated that it was not the active substance/organism in biopreparations that affected bumble bee respiration, but the inert material kaolin which increased cuticular but not respiratory water loss, indicating some kind of cuticular disturbances due to its possible abrasiveness or lipid absorptive property. This study clearly demonstrates the need for testing not only active ingredients but also all the substances in pesticide formulations.

The respiratory system reacts readily even to very low doses of insecticides. Despite the many aspects affecting metabolic rate, the changes in respiratory patterns can help to ascertain the doses that are really harmful, causing reversible or irreversible injuries to insects. Using the insect respiratory system for toxicology testing has some drawbacks. First, the equipment is expensive and not readily available. However, when present, the experimental costs are minimal and a precise result can be obtained quickly. Second, there is no current experimental protocol and, probably, the experimental conditions, measurement times, and air flow rate in the system vary with species and its specific environmental needs. Some researchers use the mean metabolic rate over a certain time period, while others measure the basic metabolic rate. There are pros and cons for both. This review has revealed that only a few studies report all of these end points. There is a lack of standardized end points and study questions: mortality data are often not involved in the study – without this, no risk assessment can be given. Similarly, there is a lack of dose-specific data on the concentrations of active ingredients or pesticide formulations causing or not causing any change in metabolic rate, water loss rate, or muscular activity in the context of longevity. The newer equipment enables measurement of several characteristics in parallel without disturbing the subject. In older publications, water loss rate was determined gravimetrically, whereas in more recent studies water loss rate is measured simultaneously allowing separation of water loss through cuticle or spiracles. Third, the respiratory patterns largely depend on metabolic rate of the insect, which in turn may be affected by several factors leading to possible misinterpretations of the results.

Conclusion

This review reports a variety of effects of pesticides on insect respiratory patterns, metabolic rate, or water loss rate. Although both the tested pesticides and target subjects belong to very different groups, the results of the reviewed studies indicate several common effects. Nerve poisons induce rapid or delayed disappearance of DGE rhythms due to inhibition of muscle work at different level. Losing control over ventilatory muscle work leads to higher water loss and eventual death of the insect.

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Disclosure

The authors report no conflicts of interest in this work.

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