Nano-pesticides: the lunch-box principle—deadly goodies (semio-chemical functionalised nanoparticles that deliver pesticide only to target species)

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Abstract

Nature contains many examples of “fake promises” to attract “prey”, e.g., predatory spiders that emit the same sex-attractant-signals as moths to catch them at close range and male spiders that make empty silk-wrapped gifts in order to mate with a female. Nano-pesticides should ideally mimic nature by luring a target and killing it without harming other organisms/species. Here, we present such an approach, called the lunch-box or deadly-goodies approach. The lunch-box consists of three main elements (1) the lure (semio-chemicals anchored on the box), (2) the box (palatable nano-carrier), and (3) the kill (advanced targeted pesticide). To implement this approach, one needs to draw on the vast amount of chemical ecological knowledge available, combine this with recent nanomaterial techniques, and use novel advanced pesticides. Precision nano-pesticides can increase crop protection and food production whilst lowering environmental impacts.
Background
Some of the biggest challenges for modern society, e.g., sustainable increase crop protection, elimination of vector borne diseases, all whilst keeping or promoting biodiversity. For example, FAO estimate that 20–40% of all crop production is lost to pests [1] and WHO estimate that globally vector borne diseases are responsible for 17% of all infections [2]. To reduce the damage caused by these pests conventional pesticides are widely used. These pesticides are spread directly in nature as chemicals in various formulations, in the order of three billion tonnes [3, 4]. This application approach entails a uniform cover of chemicals on the target environment at a defined time. However, a large proportion of the pesticide never reaches the target organisms but instead reach non-target organisms, ground water, etc. To deal with some of these issues, progress has been made in the area of nano-pesticides, aimed at reducing general spreading of the pesticide and providing timed release, hence reducing overall emissions [5]. In recent years, nano-delivery-systems, e.g., chitosan, pectin or zein-nano-carriers containing pesticides, have been developed as a way to use smaller amounts of pesticides. The aim is to distribute the pesticide in a more time resolved and targeted way [6–12]. In this approach, the encapsulated pesticide is released from its nanocarrier upon an environmental trigger, e.g., moisture. The pest organisms (or any other animal) may randomly contact the released pesticide or consume the encapsulated material. However, no study has yet examined whether it is possible to entice a specific pest-species to contact the nanocarrier.

We describe a concept, the nano lunch-box approach that eliminates the described randomness of encountering the pesticide by combining a nano-delivery system with a semio-chemical, i.e., pheromone, allomone, kairomone or synomone. The aim is to make the pest organism wish to approach the encapsulated pesticide, i.e., using the attract-to-kill approach [13, 14] at the nanoscale. The attract-to-kill approach has been commonly used bait traps [13], but never on nanocarriers. The pest organism sets out on a deliberate quest to find the lunch-box, without knowing the contents are deadly. Hence, the target is to make an attractive lunch-box that contains a targeted killer. The attractiveness is obtained by anchoring species-specific semiochemicals on the surface of the nanocarrier. The box is a nanocarrier of a highly palatable material that can be digested in the midgut of the target pest. The killer is a pesticide that is species and life-stage specific.

From a purely natural perspective, the lunch-box approach is not very novel since nature has many examples carriers with chemotaxis—the novel part here is that human may be able to utilise this approach. In line with this, Nature contains many examples where “fake promises” are used to attract “prey”, e.g., predatory spiders that emit the same sex-attractant-signals as moths for catching them at close range [15], male spiders that make empty silk-wrapped gifts in order to mate with a female [16], and plants that emit an odour that attracts certain species of insects [17]. Hence, the lunch-box approach mimics nature and we can draw on a vast amount of chemical ecological knowledge in the development of this approach.

The lunch-box approach consists of three main steps—(1) the lure, (2) the box and (3) the kill (Fig. 1).
due to pH changes induced by sweat. The anchors were When the clothes were worn, the odour was released odours to functionalised cotton surfaces in clothes. [36] showed that it was possible (via anchors) to bind low cost [33, 34]. Obtaining an optimal anchoring (from cultures) can be used to produce sufficient amounts at novel synthetic biology methods (e.g., engineered yeast Once attractants (semio-chemicals) have been identified, effective detection techniques [32] or reverse chemical ecology can be used to identify, effective compounds. Where these compounds are not yet known, novel sensitive detection techniques [32] or reverse chemical ecology [27] can be used to identify, effective compounds. Once attractants (semio-chemicals) have been identified, novel synthetic biology methods (e.g., engineered yeast cultures) can be used to produce sufficient amounts at low cost [33, 34]. Obtaining an optimal anchoring (from a loading- and release-rate perspective) is important. However, binding semio-chemical cues to the surface of a nanocarrier may be challenging, e.g. when trying to maintain the correct chirality and general stereochemistry of the attached chemicals [15, 35]. Goncalves et al. [36] showed that it was possible (via anchors) to bind odours to functionalised cotton surfaces in clothes. When the clothes were worn, the odour was released due to pH changes induced by sweat. The anchors were in this case carbohydrate-binding modules with an attach spacer (repetition of glycine-glutamine residues) to confer conformational mobility [36]. Such a pH dependent approach can also be used for pheromones, which show pH dependent reversed binding to receptors via the C-terminal [37]. It may, depending on the specific cases, be considered whether release is necessary (and how much) or whether it is enough for the semio-chemical to be attached to the “box”. For example, if the media (air, soil or water) transport the nano-pesticide to the pest species, the pest species will detect the semio-chemical loaded carrier and a release of semio-chemicals may not be advantageous. Previous studies in related areas are: (1) studies on the surface modelling and functionalisation of nanomaterials providing information on how strong and weak binding sites can be formed on the nano surfaces [38–41], e.g., via cross-linker [42]. Further, models show that nanocarriers, depending on the size and material, may contains tens of thousands surface atoms, i.e. potential functionalisation sites [43]. (2) Studies on nanomaterial (bio-)corona interactions providing information on how organic molecules bind to nano-surfaces [44, 45]. (3) Studies on the reversible binding of pheromones to insect surfaces revealing how semio-chemicals can be reversibly bound to nanocarriers [21]. By integrating these three areas, we showed that it was possible to load semio-chemicals on nanocarriers and at the same time ensure their controlled release (Fig. 2). The reader may be reminded that the above “binding semio-chemical cues to the surface of a nanocarrier may be challenging” refers to challenging for humans, but for nature this is ubiquitous occurring. We may obviously also learn from nature here, e.g., volatile compounds or semio-chemicals from surfaces of bacteria or pollen [46, 47].

**The lure**

The lunch-box approach requires that the pest organism senses an advantage in finding or being close to the “lunch” and is therefore lured into this “belief”. There are no previous reports on this approach, although studies have shown that pheromones can be embedded in polymer fibres [18] or nanogels [19]. The approach involves anchoring highly attractive chemicals on the surface of a nanocarrier. Potential attractants include semio-chemicals, e.g., volatile compounds that signal attraction and mating, that signal food, or more general host detecting chemicals. These chemicals can be highly species specific, can be detected by organisms even at very low concentrations and can induce a response that overrides many of the natural “fears” within an organism [14, 20]. Semio-chemical compounds are known for some of the main pest species [21] (e.g., see lists of the European Food Safety Authority (EFSA) published October 2019 of top pests for plant species [22], or for well-known global human pests [23–31]). For species where these compounds are not yet known, novel sensitive detection techniques [32] or reverse chemical ecology [27] can be used to identify, effective compounds. Once attractants (semio-chemicals) have been identified, novel synthetic biology methods (e.g., engineered yeast cultures) can be used to produce sufficient amounts at low cost [33, 34]. Obtaining an optimal anchoring (from a loading- and release-rate perspective) is important. However, binding semio-chemical cues to the surface of a nanocarrier may be challenging, e.g. when trying to maintain the correct chirality and general stereochemistry of the attached chemicals [15, 35]. Goncalves et al. [36] showed that it was possible (via anchors) to bind odours to functionalised cotton surfaces in clothes. When the clothes were worn, the odour was released due to pH changes induced by sweat. The anchors were

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**Fig. 1** Principle of components of the Lunch-Box approach. Three components are required (1) the lure (chemical ecology) based on semio-chemicals, (2) the box (nanotechnology), which include novel nanocarriers made of palatable natural materials, and (3) the kill (pesticides), which can include smart pesticide that are more targeted and ensure a protected environment until it reaches the target species. *For visual reasons we use a bucky-ball to illustrate the nano-container, we will not use bucky-balls but polymers but in a drawing, these would simply be opaque.

**The box**

In this approach, if a pest organism is attracted to the lunch-box, it must then “open” it, which requires that the carrier is made of a palatable/digestible material, e.g., cellulose or pectin. The “opening” could be triggered by gut digestive enzymes or physical–chemical parameters [48–50]. Hence, the material properties are important for the carrier’s stability, the potential to be opened, and, in particular, the timing of the box’s opening is crucial. The encapsulation should consider materials already present in nature, i.e., nanomaterials based on compounds, such as sugars or polysaccharides (cellulose derivatives, chitosan, pectin, lignin, etc.), proteins (zein, casein, etc.) or inorganic materials (silica, etc.). These may be produced/extracted either directly or by recombinant methods [51, 52]. Many natural materials have properties suitable for nanocarrier systems and are degradable by enzymes present in organisms. Hence, they are good candidates...
for promoting the release of active ingredients in site-specific pest-control. In a recent review, Fraceto and co-workers [53] presented an overview of the development of stimuli-responsive nanomaterials that can be used for nanocarriers. Such systems can enable the site-specific release of active ingredients (insecticides, repellents, acaricides, etc.) under biotic (fungi, insects, weeds, nematodes, etc.) and abiotic stress conditions (pH, temperature, drought, salinity, etc.). Most of these site-specific release systems were inspired from drug delivery and food science research, whereas systems that promote agricultural release applications are still at an early stage. A few papers have reported that enzymes present in the salivary glands and midgut of larvae and insects are good candidates for triggering the release owing to the presence of carbohydrates, glycans and proteases [54–58]. For example, Oliveira et al. [58] showed that carrier systems based on zein nanoparticles (loaded with botanical
insecticides) had a potential dual advantage: (1) when consumed by larvae, they released the active compound (trypsin based hydrolyse in the midgut), and (2) when not consumed, there was only very slow release of the active compound. In another example, Kaziem et al. [59] developed a system based on cyclodextrin anchored in hollow mesoporous silica loaded with avermectin where the release was controlled by the α-amylase activity of Plutella xylostella. In summary, strategies to deliver pesticides using site-specific nanoparticles are extremely interesting because they enable targeted effects on an organism whilst avoiding non-effective release of the active compound.

**The kill**

Once the lunch-box is open, the pesticide can perform its action at the target site without harming other organisms. The approach goes beyond conventional chemicals and allows the use of more benign and sophisticated approaches. For example, Bt (Bacillus thuringiensis) can be used against various pest species, Bt by inducing lethal midgut lesions, which kills the organism. Hence, Bt can be encapsulated in a pheromone-loaded carrier and used as an insecticide [60], a nanocarrier if the crystal is used and a microcarrier if the spores are used. Within the nanocarriers, novel natural or biosynthetic “compounds” can also be employed, e.g., natural chemicals [7], small-molecule agonists [61], or novel synthetic RNAi virus like strings [62–64]. Alternatively, they can be used as a platform for delivering CRISPR ribonucleoprotein for gene editing in the target [65] as for example used for vector-borne diseases from Mosquitoes [29, 66]. With this system, the pesticides can be more accurately targeted and are generally less damaging than conventional pesticide chemicals because the carrier system can protect and ensure proper functioning. Since nanocarriers may cross the midgut membrane, the lunch-box may even be designed to target specific tissues before release, although development of the latter may take longer. Obviously, by controlling the size of the carrier, e.g., between nano and micro size, it is possible adjust what can be inside the carrier but also to enhance or inhibit cellular internalisation [67]. Finally, the expiry date of the kill substance should be considered, i.e., the degradation rate of the kill material should be faster than that of the nanocarrier (when not triggered) as this will also help to prevent undesirable release of unused pesticide [68].

**Lunch-box example—based on combining previous research**

Rice is one of the world’s most important foods, with 750 million tonnes being produced globally, but rice is infested by numerous pests [69, 70]. We here show how the lunch-box principle can be applied to rice by using essential oil semio-chemicals, polymer nanocarriers and various pesticides.

**The lure**

Kuhnt et al. [71] showed a sustained rose fragrance (semio-chemical) release from functionalised cellulose nanocrystals (CNC) (10–30 nm × 100–300 nm) decorated with β-damascone. They linked the fragrance via a short thioether that served to bind the fragrance molecules to the hydroxyl bonds on the CNCs. The release was pH dependent, controlling release under neutral or basic conditions. The chemical group which damascene belong to, i.e., damascenone, contains closely related chemical compounds, i.e., damascone and ionone, hence this indicates that β-ionone may also be bound to the cellulose by the same thioether technique. The β-ionone is an attractant [72] for the white-backed planthopper, Sogatella furcifera (Horváth) (Hemiptera: Delphacidae), one of the main agricultural insect pests in China.

**The box**

The CNC is made of cellulose, which is a natural polysaccharide with many hydroxy groups on the surface. To this group belong other polysaccharides such as chitosan, alginate and pectin, which also contain many hydroxyl groups on the surface. These polysaccharides are well known as nanocarriers for pesticides [73].

**The kill**

The polysaccharide nanocarriers have been loaded with a wide variety targeted insecticide, e.g., the neonicotinoids thiamethoxam (nanocellulose carrier) [74], the bactericide Iproloxacin-HCl (nanochitosan carrier) [75], the botanical compound Geranolium (nanochitosan carrier) [76], and in human health studies the gene silencing siRNA [64]. These polysaccharide nanocarriers also show controllable release properties [73].

Hence, we here outline a lunch-box pesticide, where an attractant in the form of essential oils are attached to polysaccharide nanocarriers, a nanocarrier that is able to deliver a wide range of traditional and more benign pesticides. This lunch-box pesticide can target a pest species (white-backed planthopper, Sogatella furcifera) affecting one of the main global agricultural crops (rice, Oryza sativa), which has shown resistance to traditional pesticides. The lunch-box system will enable delivery of more targeted and at the same time more generally benign pesticides directly to the pest species.
Nano-pesticide

The global pesticide usage has been estimated to two billion tonnes, but predictions are that this usage has currently increased to 3.5 billion tonnes [3, 4]. The current non-specific pesticide approach is to spread chemicals over a vast area, reaching both target and non-target organisms [81–85].

Thus, a large proportion of the pesticide does not reach the pests efficiently and may promote resistant populations. Novel methods of using naked or functionalised nanocarriers containing pesticides reduce this problem and enable novel pesticides to be used. However, these novel methods are not species specific and rely on random encounters. The lunch-box or deadly goodies concept eliminates this randomness by using species-specific attractants on the nanocarriers, which may also be life-stage specific (Fig. 2A) and can target different species (Fig. 2B). Hence, the lunch-box approach can (1) be highly species-specific, (2) lower pesticide use, (3) utilise “benign” pesticides, (4) ensure the diversity of other species, and (5) decrease pesticide residues in food and the environment (Fig. 3). Nevertheless, it is important also for such an approach to fully understand the life-cycle fate of the nanocarriers-/nanopesticides. How do they impact (benefits/risk) the environment in which they are introduced, are they indeed able to provide better sustainability and less collateral ecotoxicity.

Wider perspective

The lunch-box approach can also help to sustain beneficial species (e.g., important pollinators). For example, bees are reported to be increasingly affected by parasites (e.g., host specific Crithidia biomb, Paenibacillus larvae, Nosema ceranae, etc. [86]). Hence, a bee specific lunch-box that contains anti-parasitical compounds could help the bee population and in turn pollination while minimising effects on other species. The above approach is, to some extent, in line with the principle of nano-medicine, utilising functionalisation to reach the target, e.g., functionalised zein or virus-like nanoparticles [8, 87–89]. However, for nano-pesticides, we aim to make the pest organism do the work of coming to the “medicine/cure”.

In summary, compared to present approaches the lunch-box concept seems to be highly promising for developing precision nano-pesticides that enable targeted release, increased efficacy and avoid widespread undesirable effects of pesticides. The approach benefits from the interplay between chemical, nano-technological, and ecological sciences.
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