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The influence of size on the toxicity of an encapsulated pesticide: a comparison of micron- and nano-sized capsules

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ABSTRACT

Encapsulation technology involves entrapping a chemical active ingredient (a.i.) inside a hollow polymeric shell and has been applied to commercial pesticide manufacturing for years to produce capsule suspension (CS) formulations with average particle sizes in the micron-scale. The few literature sources that investigate the environmental fate and toxicity to non-target organisms of encapsulated commercially available pesticide products with regard to capsule size report on average sizes between 20 and 50 μm. Here, we have identified a CS formulation with an average capsule size of approximately 2 μm with some capsules extending into the nanometer scale (~200 nm). Determining how carrier size influences toxicity is important to understanding if current pesticide risk assessments are sufficient to protect against products that incorporate encapsulation technology. Here, a commercial pyrethroid CS pesticide with lambda-cyhalothrin (λ-Cy) as the a.i. was separated into two suspensions, a fraction consisting of nano-sized capsules (~250 nm) and a fraction of micron-sized capsules (~2200 nm) in order to investigate the influence of capsule size on toxicity to embryonic zebrafish, *Danio rerio*. Toxicity was evaluated 24 h after exposure to equivalent amounts of a.i. by the presence and severity of pyrethroid-specific tremors, 14 sublethal developmental impacts and mortality. Fish exposed to greater than 20 μg a.i. L^{-1} technical λ-Cy or formulated product experienced curvature of the body axis, pericardial edema, craniofacial malformations, and mortality. Exposure to the unfractionated formulation, micro fraction, nano fraction and technical a.i. resulted in no significant differences in the occurrence of sublethal impacts or mortality; however, the technical a.i. exposure resulted in significantly less fish experiencing tremors and shorter tremors compared to any of the formulated product exposures. This suggests that the capsule size does not influence the toxic response of the entrapped λ-Cy, but the presence or absence of the capsules does. Testing across other encapsulated products is needed to determine if size does not have influence on toxicity regardless of encapsulation technology.

1. Introduction

Nanotechnology's involvement in agriculture is not limited to nanoparticulate active ingredients (a.i.), but includes a wide array of formulation chemistries and nanocarriers intended to better protect and disperse already on the market chemical a.i. Nanotechnology-based pesticides include formulations that incorporate nanoscale shells, capsules, coatings, particulate materials such as nano clays, inorganic additives and others (Kah et al., 2013). Pesticides engineered to utilize such complex formulation chemistries have the potential for unforeseen consequences to the environment and public health (Stone et al., 2010; Grillo et al., 2015; Mehrazar et al., 2015).

In pesticide risk assessment, toxicity and exposure are often well understood for the chemical a.i. alone, with little environmental data required to assess the risk of the complete formulation (Surgan and Cox, 2006; Kookana et al., 2014; Mullin et al., 2015). Meanwhile, many pesticide formulations are being developed with novel chemistry and nanotechnology to change the way the a.i. interacts with the environment and biota, limiting the applicability of a.i. specific partitioning coefficients (like K_{ow}) and degradation rates for estimating environmental persistence, mobility, bioconcentration potential and other risks after formulated.

Encapsulation technology involves entrapping a chemical a.i. inside a hollow polymeric shell and has been applied to commercial pesticide manufacturing for years to produce capsule suspension (CS) formulations with average particle sizes in the micron-scale. The few literature sources that investigate the environmental fate and toxicity to non-target organisms of encapsulated commercially available pesticide products with regard to capsule size report on average sizes between 20 and 50 μm (Jarvinen and Tanner, 1982; Sibley and Kaushik, 1991; Stejskal et al., 2009). Here, we have identified a CS formulation with an average capsule size of approximately 2 μm with some capsules

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extending into the nanometer scale (~200 nm). Pesticide toxicity, partially due to capsule rigidity and release, can be dependent on particle size (Tsuji, 2001; Roy et al., 2014; Mehrazar et al., 2015), yet little data exists on the toxicity or fate of nano-scale capsules from commercial pesticides. Entrapping the chemical a.i. in a nano-sized polymer capsule has the potential to change the biological distribution and persistence of the chemical, even relative to micron-sized particles of the same composition.

Size is known to influence biological mobility in terms of adsorption, distribution, metabolism and excretion (ADME) (Zolnik and Sadrieh, 2009). For example, the subcellular fate of particles is size dependent with particles greater than 500 nm being engulfed by phagocytes, while smaller particles are taken up by pinocytosis (Zhao, Zhao et al., 2011; Oh and Park, 2014). Polymeric microspheres with diameters between 2 and 3 μm have been shown to exhibit maximal phagocytosis compared to larger and smaller particles of the same composition (Champion et al., 2008). It is likely that entrapping a chemical in a nano-sized organic carrier can result in altered uptake, biodistribution and toxicity compared to submicron-sized organic carriers and the non-encapsulated chemicals.

The growing body of literature on nanoencapsulations for the targeted delivery of therapeutics supports the hypothesis that the toxic response of a chemical can be influenced by the size of its polymeric carrier (Kowalczuk et al., 2014). The Food and Drug Administration (FDA) requires extensive research and development to bring a drug reformulated with a nanocarrier to clinical trials including reevaluation of ADME and toxicity (Zolnik and Sadrieh, 2009). An equivalent safety assessment is not required for nano-sized carriers in pesticide formulations (USEPA, 2015). This is problematic considering the widespread use of these formulations and their inevitable increased presence in surface water and sediment (Stone et al., 2014; Tu et al., 2014; Stehle and Schulz, 2015) and as residues on crops intended for consumption (Ripley et al., 2001).

Size dependent toxicity for inorganic nanoparticles is well documented in the literature (Jiang et al., 2008; Jin et al., 2009; Oh and Park, 2014), but there has yet to be efforts to understand the relative toxicological differences of micron- and nano-sized polymeric capsules of commercial pesticide formulations. Extraction and concentration of nanocapsules from existing pesticide products allows for experimentation into both the risks and benefits of nanoencapsulation technology in relation to currently employed microencapsulation technology. Here, a pyrethroid CS insecticide was separated into two fractions, differing only in size, to investigate the influence of carrier diameter on the toxicity of λ-cyhalothrin (λ-Cy) to embryonic zebrafish. The aim of this paper is to provide some of the first data on the relative toxicity of micro- and nano-sized polymeric capsules that are commercially used as carriers for agricultural pesticides.

Chemical λ-Cy was first marketed in 1985 and in addition to its current use as an agricultural pesticide, it also has registered uses for controlling public health pests (Farmer et al., 1995; WHO, 2013). According to the Environmental Protection Agency (EPA), there are an additional 3500 pyrethrin and pyrethroid products registered in the United States, many of which are also encapsulated formulations and are used globally. Therefore, contamination by encapsulated pyrethroids, including nano-size capsules, in surface water is plausible. Currently, pyrethroids can be detected in natural waters throughout the world after agricultural, urban and residential applications (Weston et al., 2009; Domagalski et al., 2010; Weston and Lydy, 2012; Jabeen et al., 2015; Stehle and Schulz, 2015).

Class II pyrethroids, including λ-Cy are known to have detrimental neurotoxic effects on aquatic organisms (Toumi et al., 2013; Tu et al., 2014), including fish (Bradbury and Coats, 1989; Haya, 1989). As such, we are performing our toxicity assessments with embryonic zebrafish (*Danio rerio*). Zebrafish are commonly utilized for nanotoxicology studies (Harper et al., 2011; Lin et al., 2013; Rizzo et al., 2013) and as a developmental model for nervous system physiology and neurotoxicity studies (Ton et al., 2006; Chopra et al., 2010).

2. Materials and methods

2.1. Materials

An EPA registered capsule suspension insecticide with 22.8% λ-cyhalothrin was used (EPA Reg. Number 100-1295, Greensboro, NC, USA). Analytical standard grade λ-cyhalothrin [3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyano(3-phenoxyphenyl)methyl cyclopropanecarboxylate], 97.8% purity (CAS number 91,465-08-6) was purchased from Sigma-Aldrich (St. Louis, MO, USA). For enzymatic removal of the chorionic membrane of the zebrafish, protease enzyme from *Streptomyces griseus* (cat #81,750) was purchased from Sigma-Aldrich. 3-aminobenzoate ethyl ester methanesulfonate salt (tricaine, cat # A-5040) and dimethyl sulfoxide (DMSO) (CAS number 67-68-5) were also purchased from Sigma-Aldrich.

2.2. Isolating and concentrating capsules by size

In order to isolate and concentrate the nano-sized capsules in the commercial CS formulation, the formulation (2.08 lbs. a.i./gallon per product label) was diluted to 1000 mg a.i. L^{-1} with Milli-Q water (Milli-Q Gradient A10 water purification system equipped with a Q-Gard® 2 and a Quantum™ IX Ultrapure Organex cartridge, Millipore Corp., Billerica, MA, USA). Three 10 mL aliquots of the diluted stock were placed in 15 ml tubes and centrifuged for 7 min at 1454 g with a benchtop Eppendorf 5430 centrifuge. For two of the aliquots, the supernatants were collected to represent the nano fraction (NF). The remaining pellets in the two aliquots were resuspended in Milli-Q water, combined and labeled the micro fraction (MF). The pellet and the supernatant of the remaining aliquot were mixed back together to provide an unfractioned formulation (UF) control that contained both the nano and micron-sized capsules which had been subjected to the same centrifugation process as the other fractions. The UF, MF and NF were diluted with Milli-Q water to similar opaqueness and stored in the dark at 4 °C in glass vials.

2.3. Fraction characterization

The hydrodynamic diameter, polydispersity index (PDI) and zeta potential of the three suspensions (UF, NF and MF) were measured in triplicate using a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) at 25 °C after dilution to 50 mg a.i. L^{-1}. Statistical differences between fractions were determined with a one-way ANOVA. To quantify the amount of a.i. in the three suspensions, λ-Cy was extracted from the capsules by mixing with toluene and continually agitating for 1 h. Gas chromatography (GC) analysis was performed on a 1 μL sample with a Varian 3800 GC equipped with an electron capture detector and a 15 m × 0.53 mm ID RTX-200 column. Standard grade λ-Cy was run at 0.02, 0.1, 0.5, 1.0, and 2.0 mg L^{-1} and a calibration curve was generated before analysis of the samples. Samples were diluted to fit within the curve. The primary size and capsule morphology was examined using a FEI Quanta 600 FEG (FEI Co., Hillsboro, OR, USA) scanning electron microscope (SEM) operating at 15 kV using samples prepared by dropping 20 μL of each suspension onto a Si substrate and drying before imaging.

2.4. Embryonic zebrafish assay

Adult zebrafish (*D. rerio*) were maintained at the Sinnhuber Aquatic Research Laboratory at Oregon State University. Zebrafish embryos were collected from group spawns of wild-type 5D fish. To eliminate possible exposure differences from the pores of the chorionic barrier, at 6 h post fertilization (hpf) the embryos were dechorinated with

Table 1
Capsule characterization.

	HDD (nm)	ZP (mV)	PDI
UF	1925 ± 153	−54 ± 9	0.71
MF	2188 ± 145	−48 ± 9	0.48[b]
NF	251 ± 13[a]	−52 ± 8	0.34[a]

Average hydrodynamic diameter (HDD), zeta potential (ZP) and polydispersity index (PDI) for the UF, MF and NF. [a] indicates significant difference compared to the MF and UF. [b] indicates significant difference compared to UF and NF.

protease in fish water (FW) (Truong et al., 2011). FW exposure media was prepared by dissolving 0.26 g L^{-1} Instant Ocean salts (Aquatic Ecosystems, Apopka, FL) in reverse osmosis water and adjusting pH to 7.2 ± 0.2 using ~0.1 g sodium bicarbonate (conductivity 480–600 μS cm^{-1}). The dechorinated embryos were group housed at 26.5 °C under a 14:10 h light:dark photoperiod. At 24 hpf, embryos were staged according t Kimmel et al. (1995) to ensure all embryos were at the same developmental time point. Embryos were exposed individually in clear 96-well plates ($n = 12$ per concentration per suspension) to150 μL/well of 0, 20, 40, 60, 80, 400 and 2000 μg a.i. L^{-1} of the UF, NF and MF. This non-linear range in concentrations was selected to get good responses for both morbidity and mortality endpoints. In the same manner and at matched a.i. concentrations, embryos were exposed to technical λ-Cy in 0.1% dimethyl sulfoxide (DMSO) carrier solvent, a concentration known to not induce toxicity in the zebrafish embryos. FW and 0.1% DMSO controls were also prepared. All exposures were kept at 26.5 °C under a 14:10 h light:dark photoperiod. Parafilm was placed under the lid of each plate to prevent volatilization and evaporation. In entirety, 2 experimental replicates on two separate days were performed for a total $n = 24$. To ensure that λ-Cy was contained in the capsules and not free in solution, and to account for the potential toxicity of the other ingredients in the product, the UF stock was centrifuged at 10,000 g for 15 min to remove all capsules, both nano- and micron-sized. Embryos were exposed to the supernatant to ensure no toxicity was present from the solution alone. In addition, a stock solution of λ-Cy at an equivalent a.i. concentration underwent the same centrifugation treatment. This control was to ensure that a force of 10,000 g for 15 min did not force free chemical out of solution or promote the chemical adhering to the plastic centrifuge tubes. Embryos exposed to the resulting supernatant had a similar toxicological response as those exposed to the same solution not centrifuged. Free a.i. above the water solubility of λ-Cy (0.005 mg L^{-1}) is not expected for the capsule exposures used in this study. This amount contributed minimally and equally to the toxicity assessments.

2.5. Toxicological evaluation of embryonic zebrafish

At 56 hpf (day 1 of exposure), a 30 s video was taken of each embryo to quantify the occurrence and duration of embryonic tremoring. At 56 hpf, control embryos remained still with no spontaneous movement and there was 100% survivability in all exposures providing a good time point for behavioral analysis. Total time spent tremoring during each 30 s video was calculated and the mean time for each concentration within each treatment group was calculated. Fish were categorized as non-tremoring if no motion was detected for the complete duration of the 30 s video. Fish not tremoring but unresponsive to touch were considered paralyzed. The results were analyzed by a 3-way ANOVA to look for differences between experiments, exposure concentration and material. Results from experiment 1 and 2 were pooled because there were no statistical differences between experimental replicates ($p = 0.266$). At 120 hpf (day 4 of exposure), embryos were assessed for 14 developmental sublethal impacts including malformations to the body axis, brain, eyes, caudal and pectoral fins, jaw, ears, snout, trunk and somite. Abnormal circulation, pericardial edema, yolk sack edema and pigmentation were also evaluated. Mortality was assessed on day 5 of exposure (144 hpf) and embryos were determined to be dead by the visual absence of cardiac activity. LC_{50} and EC_{50} values were derived by linear extrapolation of the dose–response curves. The significant occurrence of tremors, tremor duration, pericardial edema, jaw and axis malformations and mortality between control and pesticide exposures was determined by Fisher's exact statistical test. The significance level in all calculations was set at $p < 0.05$. Representative images were taken at 144 hpf of each exposure condition before

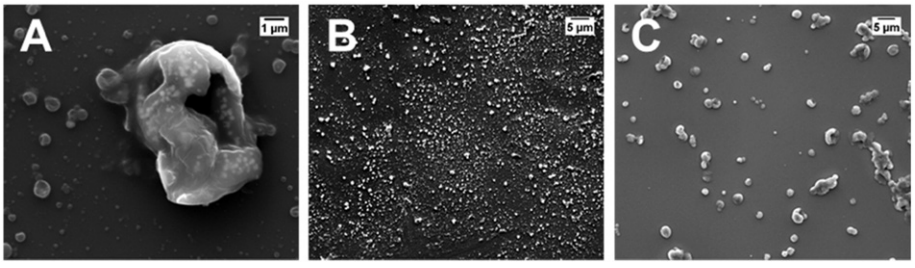

Fig. 1. Representative SEM images showing both nano- and micron-sized capsules. A) Unfractioned formulation with broken capsule. Photo credit: Dr. Louisa Hooven B) NF and C) MF at 5 mg a.i. L^{-1}. Scale bars are 1, 5 and 5 μm respectively.

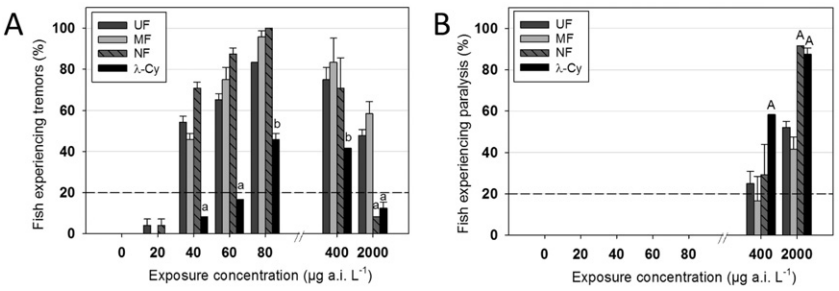

Fig. 2. Pyrethroid-specific tremor response on day 1 of exposure (48 hpf). All responses above 20% are significantly different from control. A) Percent of fish experiencing tremoring. a and b indicate significant differences compared to the formulated exposures (UF, MF and NF). B) Percent of fish experiencing paralysis. A indicates significant differences compared to the other exposures at the same concentration ($n = 24$).

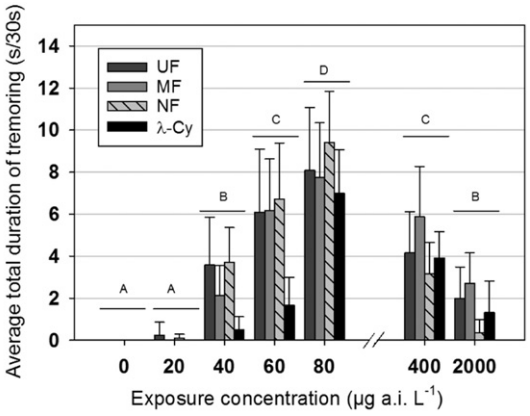

Fig. 3. Average total duration of tremoring after analysis of 30 s videos (n = 24). A, B, C and D depict a concentration dependent increase in average time spend tremoring for all exposures from 20 to 80 µg a.i. L^{-1}. Average total time spent tremoring decreased at the two highest concentrations as fish began to reach paralysis.

experiment termination, when zebrafish were euthanized with 4 mg/mL tricaine.

3. Results and discussion

3.1. Pesticide fractionation and characterization

The UF had an average hydrodynamic diameter (HDD) of 1925 ± 153 nm and a polydispersity index (PDI) of 0.71 indicating a broad particle size distribution within the suspension (Table 1). After removal of the nano-sized capsules, the average HDD increased to 2188 ± 145 nm and the PDI lowered to 0.48, suggesting a more homogeneous size distribution. The NF had an average HDD of 251 ± 13 nm, had the lowest PDI of 0.34 and was significantly smaller than the MF ($p = 0.0001$). The average zeta potential of the UF, MF and NF were −54 ± 9, −48 ± 9 and −52 ± 8 mV respectively (Table 1), indicative of good particle stability and low potential to form agglomerates (Clogston and Patri, 2011). This was expected considering the necessary shelf life of a concentrated product and is desirable to create a flowable formulation.

SEM images were taken in order to confirm the capsules were spherical and to justify the use of HDD measurements from dynamic light scattering where calculations are based on this assumption. The capsules of this formulation are specifically engineered to break open upon drying, so the spherical nature is assumed based on the images acquired (Fig. 1) although some capsule shells are collapsed from sample preparation for SEM microscopy.

3.2. Embryonic tremoring

In order to compare the toxicity of non-encapsulated and encapsulated a.i. with nano- or micro-sized capsules using the zebrafish, we focused on the pyrethroid-specific response of physical tremoring after exposure to λ-Cy. This response is due to disruption of the voltage-gated sodium channels of the peripheral nervous system by λ-Cy and has been observed by others investigating the toxicity of class II pyrethroids with zebrafish (DeMicco, Cooper et al. 2010; Yang, Ma et al. 2014). Alterations to the nerve from the prolonged opening of the voltage-gated sodium channels and repetitive discharge of nerve signals results in convulsions or tremors and eventually paralysis (Soderlund and Bloomquist 1989). Since we do not have access to a capsule only control in our experimental design, looking at an endpoint specific to the a.i. allows us to link the toxic response to the active chemical and not the other ingredients of the commercial formulation.

The proportion of fish experiencing tremors increased (Fig. 2A) as concentration of a.i. increased for all exposures with exception of the two highest concentrations, in which the fish were beginning to reach paralysis (Fig. 2B). For the exposures to formulated products, the NF at 2000 µg a.i. L^{-1} was the only condition to result in a statistically lower percent of fish tremoring compared to the UF and the MF due to the high percent having reached paralysis. The λ-Cy exposures resulted in a significantly lower percent of fish tremoring at 40, 60 and 80 µg a.i. L^{-1} compared to the capsule suspensions. At 2000 µg a.i. L^{-1}, the λ-Cy exposure resulted in a significant decrease in tremoring fish, but only compared to the UF and MF. The NF could be behaving similar to the non-encapsulated chemical, only slightly more toxic, because the smaller capsules may release their contents more rapidly after being taken up by the fish compared to larger capsules (Perrin, 2000; Mehrazar et al., 2015).

Average total time spent tremoring was also quantified (Fig. 3). A significant increase in total time spent tremoring (s/30s) was observed as concentration increased; however, the embryos were reaching paralysis at the two highest concentrations (400 and 2000 µg a.i. L^{-1}) (Fig. 2B) resulting in a decrease in time spent tremoring (Fig. 3). For average total time spent tremoring, no significant differences were noted between UF, MF or NF; however, embryos exposed to λ-Cy spent significantly less time tremoring than the UF ($p = <0.001$), MF ($p = <0.001$) and NF ($p = 0.002$). Tremors were not present in any of the control exposures (FW, DMSO and UF filtrate). The no observable adverse effect level (NOAEL) for tremoring was 20 µg a.i. L^{-1} for all exposures and the lowest observable adverse effect level (LOAEL) was 40 µg a.i. L^{-1}, the onset of significant tremoring for all technical and formulated λ-Cy exposures (Supplemental Table 1).

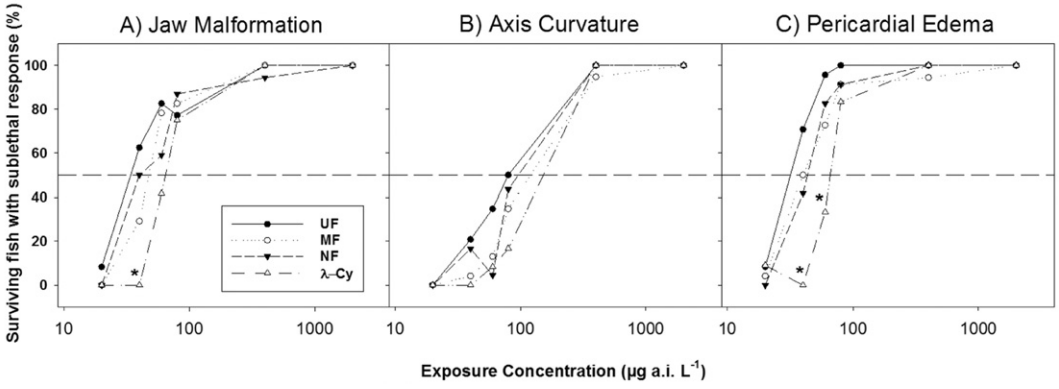

Fig. 4. Occurrence of sublethal developmental endpoints as a function of exposure concentration on day 4 of exposure (120 hpf). A) Jaw Malformation B) Axis Curvature and C) Pericardial Edema. * indicates significantly less fish with toxic response compared to other exposures at the same concentration.

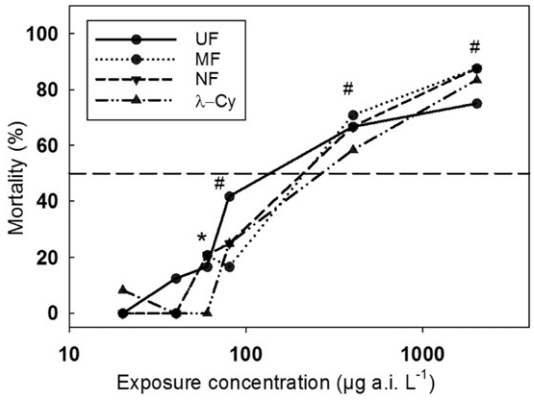

Fig. 5. Cumulative percent mortality on day 5 of exposure (144 hpf) as a function of exposure concentration. * indicates significantly differences in the λ-Cy exposure compared to the formulated exposures (UF, MF, NF) at 60 μg a.i. L^{-1}. # indicates significant differences between all exposures and control, (but not each other) at 80, 400, and 2000 μg a.i. L^{-1}.

3.3. Sublethal impacts and mortality

On day 4 of exposure, embryos were assessed for 14 developmental sublethal endpoints. Surviving embryos experienced a significant increase in curved body axis, pericardial edema, and jaw malformations at 40 μg a.i. L^{-1} for the technical and formulated a.i. exposures compared to controls (Fig. 4). Embryonic tremoring decreased at the two highest exposure concentrations as the fish reached paralysis on day 1 of exposure, yet on day 4 nearly 100% of the embryos at these concentrations had malformed jaws, pericardial edema and severely curved body axis for technical and formulated λ-Cy exposures (Fig. 4). After disruption of the voltage-gated sodium channels, ionic balance is disrupted throughout the organism, likely resulting in the swelling of the pericardium and abnormal axis curvature. More variability was observed at the lower concentrations between the percent of fish experiencing the sublethal responses, but at 80 μg a.i. L^{-1} and higher, there were no significant differences between UF, MF, NF and λ-Cy exposures. For the 4 exposures, the fish had visually similar patterns of malformations and similar degree of axis curvature as displayed in Fig. 6. There was no difference in the acute toxicity of nano- and micron-sized capsules of λ-Cy, even considering that more nanocapsules are needed to reach an equivalent amount of a.i. compared to micron-sized capsules with greater relative volume.

Table 2
Summary EC_{50} values for tremor response and pericardial edema (PE) and LC_{50} values for the UF, MF, NF and λ-Cy exposures.

	24 h Tremor EC_{50} (μg L^{-1})	96 h PE EC_{50} (μg L^{-1})	144 h LC_{50} (μg L^{-1})
UF	37	31	150
MF	42	39	216
NF	32	43	212
λ-Cy	66[a]	>80[a]	273

[a] Indicates a significant difference compared to other exposures.

On day 5, significant mortality was observed at 60 μg a.i. L^{-1} for the formulated exposures, but not until 80 μg a.i. L^{-1} for the technical a.i. exposure (Fig. 5). At 80, 400 and 2000 μg a.i. L^{-1} there was no significant differences in mortality for the 4 exposures. There was no mortality and no sublethal impacts observed in control fish (FW, 0.1% DMSO and UF supernatant).

Table 2 provides a summary of the approximate EC_{50} and LC_{50} values for the technical and formulated λ-Cy exposures. The encapsulated formulation exposures (UF, NF, MF) caused pyrethroid-specific effects in the fish at slightly lower concentrations than the technical a.i. exposure. This could be due to efficacy differences from the presence of the capsule improving the biological persistence of the a.i. or from preventing the degradation of the chemical in the exposure media by light, microorganisms and other factors (Tsuji, 2001). Therefore, the capsule may not have a direct influence on the toxicity of the chemical inside, but rather change the dynamics of the organism's exposure over the duration of the experiment.

We noticed significant differences in tremor response between the technical and formulated a.i. exposures, yet compared to the toxicity differences between other non-capsule containing formulations and the corresponding a.i., here, the differences in mortality and sublethal responses are minor (Beggel et al., 2010; Mesnage et al., 2014). This is likely due to CS formulations containing low solvent concentrations (Perrin, 2000; Shirley et al., 2001) and having relatively non-toxic other ingredients at the concentrations tested, which can cause drastic

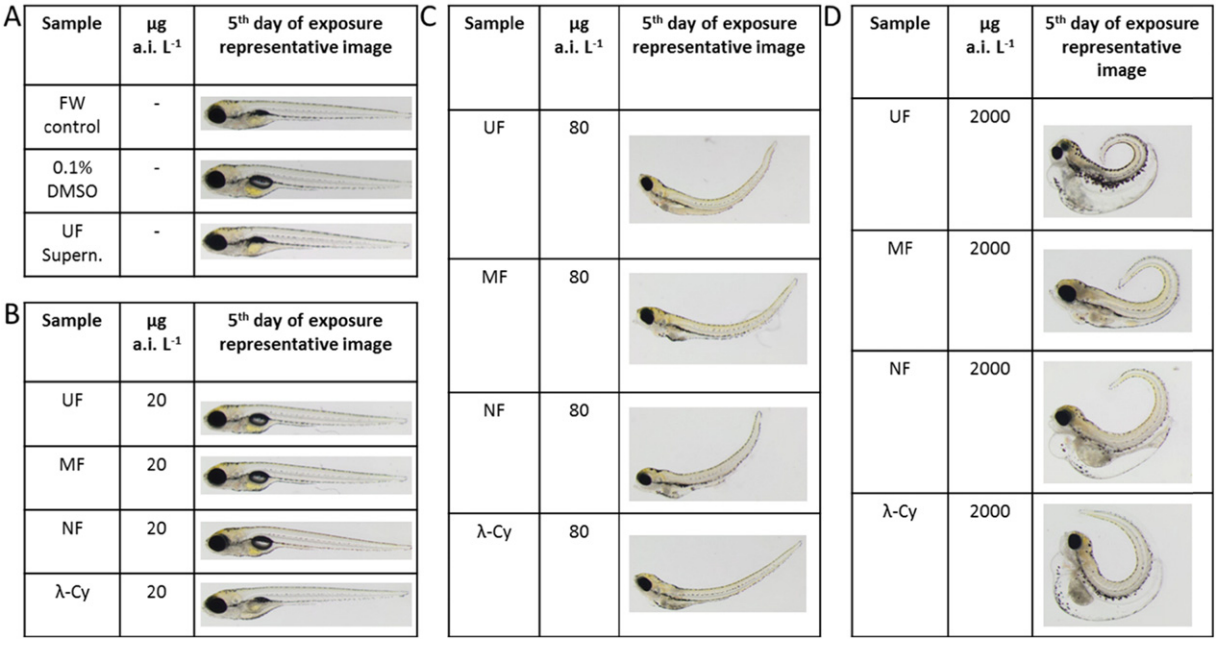

Fig. 6. Representative images of embryos on day 5 of exposure (144 hpf).

differences in the toxicity of formulations versus their corresponding a.i. For this pesticide we can conclude that capsules with diameters between 200 and 2500 nm do not result in significant acute toxicological differences to zebrafish when exposures are normalized for a.i. concentration. However, it is uncertain if capsule size will affect chronic toxicity and bioconcentration of the pyrethroid in aquatic organisms. To better understand the influence of size on the toxicity of pesticides to aquatic non-target species, it is necessary to test across other pesticides that implement different encapsulation technology to see if our observed trend holds true regardless of crosslinker chemistry, shape, surface charge, stabilizers and capsule wall thickness. Working with other encapsulated a.i. chemicals, including other pyrethroids with unique modes of action, can also determine if capsule size becomes an influence on toxicity when dealing with a site of action less ubiquitous than the voltage-gated sodium channels, such locations across the blood–brain barrier.

Although differences in toxicity presented here are small between various sized capsules and the corresponding technical a.i., exposure differences from differential environmental fate through air, soil and water could be significant. To achieve the same application of a.i., more nano-capsules are needed compared to micron-sized capsules because of loading capacity differences based on volume. The result of formulations with average particle sizes in the nano range is a greater propensity to be more easily transported through the small pore spaces of soil and through the air as drift. The colloidal properties are expected to differ for larger particles versus their smaller counterparts also and could result in greater bioavailability to aquatic organisms as the water stable capsules remain suspended in the water column.

4. Conclusions

Pesticides are among the most well-studied chemicals in the world, yet little information is available to assess possible elevated risks after formulated. For the pesticide presented here, the nano-sized capsules only account for a small percent of the total capsules in the formulation, with the rest of the capsules in the submicron size range. Investigations into the toxicological risks and benefits of pesticides that incorporate nanotechnology will allow for the precautious engineering and application of any products coming to market in the near future and to assess whether a unique evaluation is needed under Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Food and Environment Protection Act (FEPA) or other similar agencies worldwide for pesticides that contain nanoscale capsules. After isolating and concentrating the nano-sized capsules of a commercial pesticide, we found no difference in the acute toxicity compared to larger particles of the same composition. However, studies investigating the fate and persistence of nano-capsules of pesticides in the environment relative to the a.i. alone and to their larger sized counterparts are necessary to understand the environmental risks associated with implementing nanotechnology in agriculture.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2015.10.012.

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