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Controlled-Release Systems for the Delivery of Cyromazine into Water Surface

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Controlled-release systems (CRSs) for the insect growth regulator cyromazine (Neporex), as a larvicide against *Culex pipiens* (Culicidae) mosquitoes, are under development. Despite promising results obtained previously for both in vitro (dissolution tests) and in vivo systems (mosquito larvae floating on water surfaces), it was evident that the CRSs did not remain afloat for sufficiently long times. The present study was thus conducted to determine the relationship between the process parameters and the potential contact time of the active ingredient with the mosquito larvae. A series of CRSs were prepared by an extrusion process, alone or in combination with a supplementary coating. The active ingredient was incorporated into the matrix or the coating. The matrix comprised low-density polyethylene 600 and perlite, and the coating, a polyurea with or without the addition of paraffin wax. The study showed that the cumulative release of the active ingredient into water could be controlled by manipulating the preparation techniques, the types and concentrations of inert materials, and the concentration of the active ingredient.

KEYWORDS: Controlled-release systems; insect growth regulators; cyromazine; Neporex; encapsulation; matrix; coating; pesticide formulations

INTRODUCTION

For pesticide applications, controlled-release formulations are defined as depot systems that continuously release their toxic constituents into the environment over a specified period of time, usually months to years (*1*). One of the main advantages of controlled-release systems (CRSs) for agricultural chemicals, including pesticides or fertilizers, is that the active ingredient is maintained in the environment at the appropriate dosage for the desired period of time. An additional—if not equally important—advantage is the reduction of damage to the environment (*2*, *3*), particularly because the increasing use of pesticides is causing ongoing harm to the environment.

Over the years, the synthetic insecticides used to control mosquitoes, which are vectors in the transmission of human tropical diseases, such as malaria and yellow fever, have varied greatly in terms of structure, toxicity, persistence, and environmental impact (*4*). These chemicals include organochlorines, organophosphates, pyrethroids, carbamates, natural insecticides, and insect growth regulators (IGRs). The latter constitute a group of pesticides that are primarily active against the immature lifestages of insects (larvae or pupae), including those of species that have developed resistance to conventional insecticides (*5*, *6*). These substances act by disrupting the normal development of insects by mimicking juvenile hormone (JH) or molting hormone or by interfering with chitin synthesis (*7*, *8*), typically resulting in larval or pupal mortality. The hormonal disruption and/or growth development of IGRs against immature insects may differ among affected species. The main advantage of IGRs over conventional pesticides is that they have minimal toxicity to mammals, birds, fish, and bees. The IGR that constitutes the subject of this study, cyromazine (Larvadex Trigard, Novartis Crop Protection, Inc.) (*9*) is used as a larvicide against the mosquito *Culex pipiens* (Culicidae). Cyromazine (*9*) can be metabolized by dealkylation reactions in both plants and animals, and it can undergo environmental degradation by various mechanisms to form melamine (*10*, *11*). Once the chemical is introduced into the environment, it may undergo physical, chemical, or biological processes that generate environmentally hazardous compounds (*11*). For protection of the environment it can be advisable to formulate cyromazine into a CRS in order to prevent this IGR from relatively rapid degradation and to facilitate a reduction of the dose by increasing the efficacy. In commercial terms, the increased costs of the CRS formulation

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are set off by the greater efficacy, making the proprietary preparation cost-effective.

Various technologies for the controlled release of a variety of bioactive agents for pest control in aquatic and terrestrial environments can be found in the literature (*12*). The choice of a particular process depends on the intended size of the encapsulation system and the physical and chemical properties of both the substance to be encapsulated and the other components of the system, such as polymers, additives, and other inert materials (*13*).

In our previous study (*14*), we reported a simple extrusion technique for preparing CRSs for cyromazine, which is a hydrophilic water-soluble triazine (*6*). Because the CRS is intended to act against mosquitoes at the larval stage, it is necessary to develop a formulation that will float on water, as do the larvae. The formulations developed in the previous study exhibited good release characteristics, but partial sinking of grains was observed after 3 weeks. In the current study, perlite, an inert material with a much lower density (0.032 g/cm^3) than that of water, was used in some of the formulations to improve the ability of the CRS to remain afloat. Low-density polyethylene (LDPE) was used in some of the formulations because it was judged to have suitable characteristics, that is, hydrophobicity, low density, lack of reactivity with the other components within the system, suitability for extrusion, low cost, and ease of handling. The choice of polyurea and paraffin wax for the coating was based on their hydrophobic properties (*14*).

The objective of this study was to develop CRSs incorporating cyromazine as active ingredient by a technique consisting of an extrusion process and/or coating with a polymeric envelope. To evaluate the in vitro efficacy of the series of CRSs developed in this study, their cumulative release profiles were examined, as was the influence of a combination of various coating layers and additives on the release of the active ingredient.

EXPERIMENTAL PROCEDURES

Materials. Cyromazine (Neporex; *N*-cyclopropyl-1,3,5-triazine-2,4,6-triamine; solubility in water of 13 g/L at 20 °C and pH 7.1; melting point of 219−222°C, formulated into product of 50% cyromazine and 50% sodium polyphosphate) was produced by Novartis Crop Protection AG (formerly Ciba-Geigy AG). Two different types of polyurea were prepared by interfacial polymerization, in the presence of water, between a polyisocyanate designated PAPI 1 [polyisocyanate diphenyl methane-4,4′-diisocyanate (known as Voranate 580)] or PAPI 7 (Izonatem M342), both produced by Dow Benelux N.V. and a polyfunctional amine obtained by reaction between ethylenediamine (Fluka Chemical Corp.) and tetraethylenepentamine (Fluka Chemical Corp.) (*15*−*19*). The inert materials used in the preparation of the CRSs were pure paraffin wax (Farmitalia Carlo Elba) and perlite (the particle diameter being 2.5 mm) (Hagarin).

CRS Systems. To incorporate cyromazine into CRSs that are suitable from the sustained release and floatability points of view, two different technological methods were used, that is, extrusion and/or coating. With these techniques three types of CRSs were prepared, as follows:

System I. A technologically simple extrusion process was used to prepare polymeric material in which the active ingredient was dispersed homogeneously. A mixture of cyromazine, low-density polyethylene having a molecular weight of 600 Da (LDPE 600), and perlite was melted and homogenized in an extruder (Modern Plastic Machinery Corp., Clifton, NJ; type 100-20) (*20*). The solid mixture was pumped through a die and then extruded at 130−150 °C in the desired form. The extrusion temperature was carefully controlled because it affects the properties of the final product, such as degradability, distortions of the polymeric mixture, and matrix structure (*21*, *22*). The extrudate, in the form of filament, was then taken up on the spool of a pelletizer (Accrapak Systems Ltd., model BM-15-HD), where it was cut into slabs of the following dimensions (±3%): length = 3.64 mm, width = 3.14 mm, and height = 2.2 mm. Three different matrix formulations, CE7−CE9, were prepared by varying the proportions of perlite and LDPE 600 mixed with cyromazine (**Table 1**). The choice of the ratios was based on our previous study on CRSs comprising cyromazine encapsulated in a polyethylene matrix (*14*).

Table 1. Composition (Percent) of Systems I and III Formulations

formulation	cyromazine	LDPE	perlite	polyurea I	wax	polyurea II
			System I			
CE7	10	40	50			
CE8	10	35	55			
CE9	10	30	60			
			System III			
CC10	10		65	25		
CC11	10		45	25	20	
CC12	10		25	25	40	
CC13	10		20	25[a]	20	25[a]

[a] The two polyurea coatings are identical from the compositional point of view.

Table 2. Composition (Percent) of Polyurea Coatings in the Formulations of Systems II and III

formulation	PAPI 1	PAPI 7	ethylene-diamine	tetraethylene-pentaamine
		System II		
CS7(1)	70		15	15
CS7(2)		70	15	15
CS8(1)	70		15	15
CS8(2)		70	15	15
CS9(1)	70		15	15
CS9(2)		70	15	15
		System III		
CC10	70		15	15
CC11	70		15	15
CC12	70		15	15
CC13	70		15	15

System II. Slabs of each of the formulations CE7−CE9 obtained by extrusion were rotated in a Multipex coating pan (Apex Engineering Industries Ltd.) together with a mixture of the a polyisocyanate (PAPI 1 or PAPI 7) in acetone sprayed over the granules. A mixture of equal parts of ethylenediamine and tetraethylenepentamine dissolved in the acetone was then added (**Table 2**), and the coating pan was rotated at 40 rpm. The interfacial polymerization condensation process was carried out for 1 h in the rotating coating pan at room temperature. Talcum powder was added to prevent the granules from sticking together. On the basis of our previous experience, the coating constituted 20% of the total weight of each slab (matrices).

System III. Perlite was coated first with polyurea (prepared from PAPI 7) into which cyromazine had been incorporated (formulations CC10−CC12, **Table 2**) in the same manner as in system II and then with paraffin wax (**Table 1**). For formulation CC13, perlite was coated sequentially, first with polyurea containing the active ingredient, then with paraffin wax, and finally with polyurea (**Table 1**).

Experimental Setup. In vitro dissolution tests were performed to determine the chemical release of the active ingredient from the obtained formulations. The tests were carried out in a dissolution test system (model 2100B, Distek, North Brunswick, NJ), comprising six glasses (11.5 cm high × 10.2 cm i.d.), each filled with 800 mL of distilled water and containing a basket that held 3 g of one of the CRSs containing cyromazine. The granules of the three types of formulation had approximately the same size and shape. The dissolution system was held at 25 °C and operated at 50 rpm. At fixed intervals (in 1, 4, and 8 h and then daily), liquid samples were withdrawn from each of the glasses. The samples were taken from the liquid surface, but because the aqueous solution was stirred, the concentration of cyromazine was the same in any part of the solution. The amount of active material released into the water was determined on an HPLC system (Shimadzu,

Table 3. Cumulative Release of Cyromazine from the Analyzed Formulations versus Saturation Time

formulation	release of cyromazine[a] (%)	time (days)
	System I	
CE7	80 ± 4.5	∼4
CE8	91 ± 6.2	∼2
CE9	90 ± 8.8	∼1
	System II	
CS7(1)	90 ± 7.7	∼12
CS8(1)	93 ± 6.5	∼11
CS9(1)	91 ± 8.1	∼6
CS7(2)	85 ± 5.9	∼14
CS8(2)	89 ± 5.3	∼12
	System III	
CS9(2)	85 ± 7.4	∼6
CC10	100	∼2
CC11	96 ± 7.5	∼21
CC12	91 ± 5.2	∼21
CC13	89 ± 4.5	∼21

[a] Values are means ± confidence intervals (95% confidence coefficient for *t* distribution).

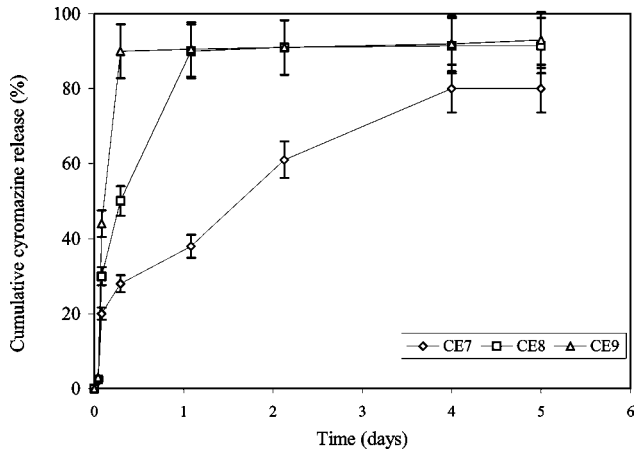

Figure 1. Release of cyromazine into water from extruded formulations CE7–CE9 as a function of time. Bars represent confidence intervals (for 95% confidence coefficient for *t* distribution).

Tokyo, Japan), equipped with a Hypersil ODS 5-μm column, 250 × 4.6 mm (Runcorn, U.K.). The eluent used was acetonitrile/0.05 M sulfuric acid, 50:50 v/v, at a flow rate of 1 mL/min.

For each formulation the weight percent of the active ingredient was plotted versus the saturation time.

For the dissolution tests performed in six glasses, three samples of each formulation were taken from two different batches prepared under the same conditions to exclude random factors, such as technological conditions (e.g., temperature), nonhomogeneous distribution of the IGR in the matrix, and variations in coating thickness. Average values are given in the tables and figures. The variations did not exceed 11%.

RESULTS AND DISCUSSION

Analysis of the release results of cyromazine from the CRSs into water showed that effective and even optimal control of the CRS formulation may be obtained by manipulating both the manufacturing technology and the constituents, that is, the concentrations of the active ingredient and the additives and the type and concentration of polymer used (*14*, *23*).

The formulations for the CRS of cyromazine produced by the simple extrusion technique, designated system I (formulations CE7−CE9, **Table 1**), gave very fast release of cyromazine (**Table 3**; **Figure 1**) and exhibited a tendency to sink (∼10% of the granules were sinking after the third day; up to 80% after

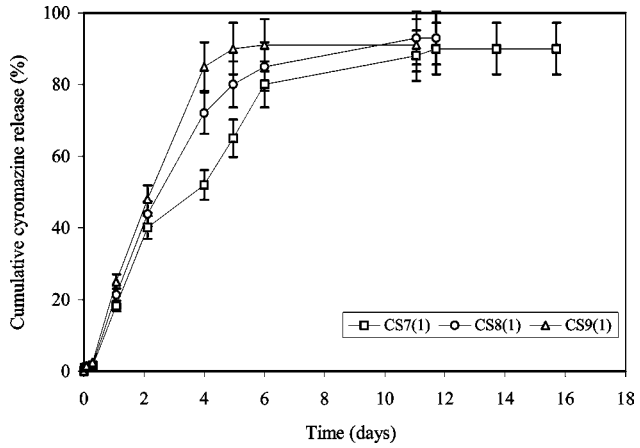

Figure 2. Release of cyromazine into water from formulations CS7(1)–CS9(1) coated with polyurea as a function of time. Bars represent confidence intervals (for 95% confidence coefficient for *t* distribution).

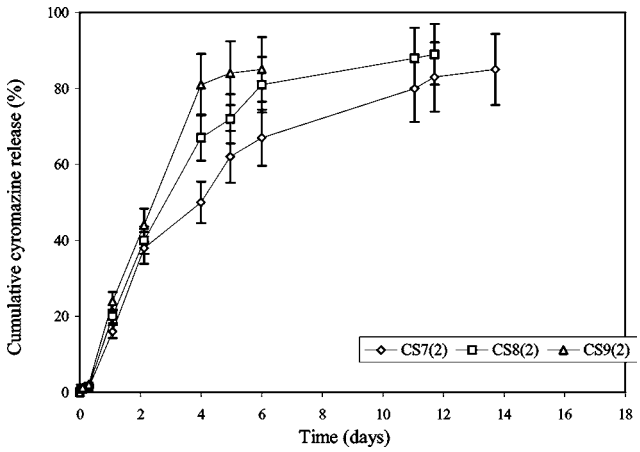

Figure 3. Release of cyromazine into water from formulations CS7(2)–CS9(2) coated with polyurea as a function of time. Bars represent confidence intervals (for 95% confidence coefficient for *t* distribution).

3 weeks). These findings can be attributed to a combination of the low matrix hydrophobicity and high water solubility of cyromazine. To prolong the release of the active agent, a supplementary hydrophobic material was used to coat the matrix [CS 7−9(1) and (2), **Table 2**]. As can be seen from **Table 3**, the percentages of the cyromazine released from these system II formulations remained approximately within the same range (85−93%) as those of the system I extruded formulations, but the period activity was increased to 12 days.

As was expected from our previous experience with different hydrophobic coatings, the formulations prepared with the polyisocyanate PAPI 1 [CS7(1) and CS9(1)] gave faster release of cyromazine into water than those prepared with PAPI 7 [CS7-(2)−CS9(2) (**Figures 2** and **3**; **Table 3**). However, the differences between the six formulations were not statistically significant (**Figures 4**−**6**).

Although the supplementary coating did improve the sustained-release rate of the active ingredient, it did not prevent sinking of the slabs. To overcome this disadvantage, system III formulations were developed: they were based on coating light granules of perlite with a hydrophobic coating, containing a high weight percent of polyurea (CC10) into which cyromazine had been incorporated or with a combination of polyurea, cyromazine, and wax (CC11, CC12, and CC13). As can be seen from **Table 3** and **Figure 7**, the simple polyurea coating (formulation CC10) was not efficient, because the cyromazine

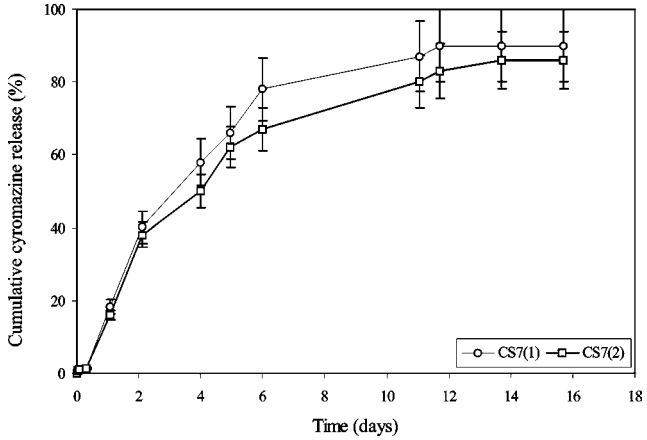

Figure 4. Comparison of cyromazine release into water from formulations CS7(1) and CS7(2) as a function of time. Bars represent confidence intervals (for 95% confidence coefficient for *t* distribution).

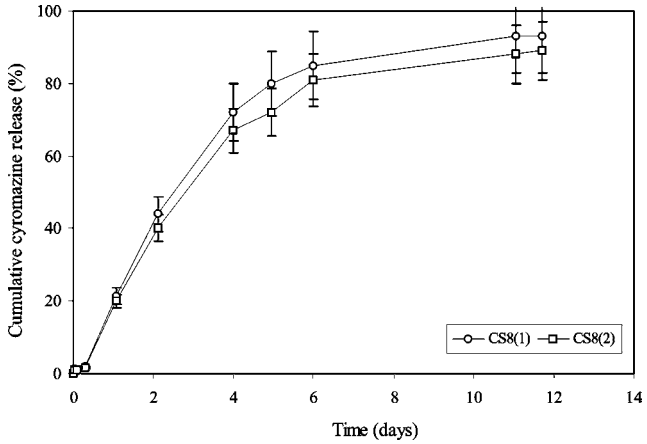

Figure 5. Comparison of cyromazine release into water from formulations CS8(1) and CS8(2) as a function of time. Bars represent confidence intervals (for 95% confidence coefficient for *t* distribution).

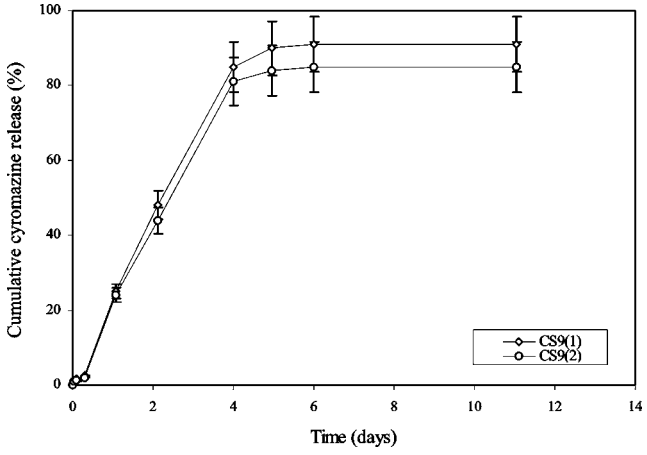

Figure 6. Comparison of cyromazine release into water from formulations CS9(1) and CS9(2) as a function of time. Bars represent confidence intervals (for 95% confidence coefficient for *t* distribution).

was completely released in ~2 days. Addition of a hydrophobic polymer, such as paraffin wax (CC11, CC12), to the coating lengthened the period of release (**Table 3**; **Figure 8**) but with a slight reduction in the total mass of cyromazine released, that is, 96% from CC11 and 91% from CC12, in ~21 days. In an attempt to improve these results, formulation CC13 was produced, in which the perlite was coated sequentially first with polyurea containing cyromazine, then with paraffin wax, and finally with polyurea. This formula gave an ~89% release of cyromazine in ~21 days (**Table 3**; **Figure 8**). Although the floatability of this formula was superior to that of coated formulations CC11 and CC12, there were no significant differences among the three formulations in terms of cumulative cyromazine release (**Figure 8**).

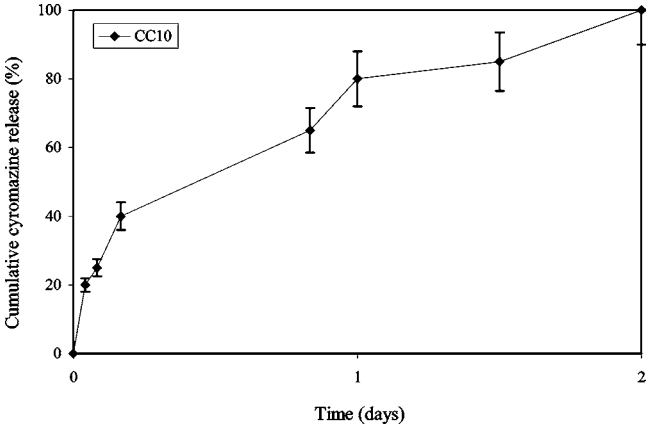

Figure 7. Release of cyromazine into water from formulation CC10 coated with polyurea as a function of time. Bars represent confidence intervals (for 95% confidence coefficient for *t* distribution).

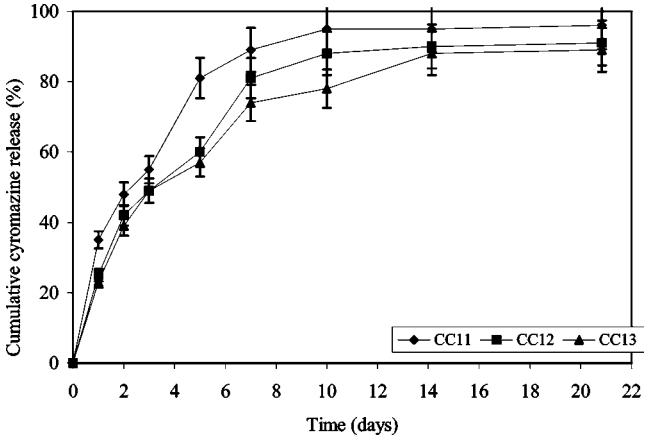

Figure 8. Release of cyromazine into water from formulations CC11–CC13, coated with polyurea and wax, as a function of time. In the case of CC13, a second layer of polyurea was applied on the wax coating. Bars represent confidence intervals (for 95% confidence coefficient for *t* distribution).

The results thus show that the extrusion/coating combination is superior to the coating process alone from the point of view of cyromazine release rates. The problem of the ability of the CRSs to float was solved by applying a coating of a light inert material containing the active compound. However, the drawback of the latter formulations was the faster release of cyromazine. This problem was partially solved by using a combination of two hydrophobic polymers, polyurea and wax, in the coating process. It is possible that suitable formulations could be obtained by preparing a light very porous hydrophobic matrix that would improve the floatability of the final product.

The products will be tested under laboratory and mostly under field conditions on mosquito larvae under the open system. It was shown that by producing CRSs through the mentioned methods, satisfactory results from an in vitro point of view can be obtained.

It is extremely important to consider biological parameters and to take into consideration the environmental conditions (temperature or humidity) when developing CRSs formulations.

The temperature is an important factor in larvicide and mosquito growth (in this study the laboratory temperature—about 22 °C—was used). Therefore, by constructing different types of CRSs with different rates of release, it will be possible, depending on the climate conditions or the developmental stage of larvae, to combine different systems to obtain the optimal result. In other words, a "programming" of these CRSs can be possible by taking into consideration different parameters. Depending on the field conditions the CRSs will be produced with modified properties with enhanced or hindered release of cyromazine. Therefore, future work will be devoted to further improvement of CRSs based on an extrusion/supplementary coating combination.

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