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Inclusion Complexation of Carbaryl and β-Cyclodextrin in Solution and in the Solid State

FRANCESCO BARBATO, MARIA IMMACOLATA LA ROTONDA*, AGNESE MIRO, PATRIZIA MORRICA and FABIANA QUAGLIA
Dipartimento di Chimica Farmaceutica e Tossicologica, Università degli Studi di Napoli "Federico II", Via D. Montesano 49, 80131 Napoli, Italia

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Abstract. This study was carried out with the aim of investigating the interactions between β-cyclodextrin and carbaryl, a carbamate pesticide, and their effect on some physico-chemical properties of carbaryl, such as aqueous solubility and lipophilicity. The interactions between carbaryl and β-cyclodextrin were thoroughly investigated both in solution and in the solid state. The effect of β-cyclodextrin on the aqueous solubility of carbaryl was evaluated by the phase solubility method. The amount of carbaryl dissolved increased linearly with the addition of β-cyclodextrin according to an A_L type plot and without precipitation of the complex. The apparent stability constant of the complex was 289 ± 21 M^{-1}, assuming a 1 : 1 stoichiometry; this value was confirmed by a method based on circular dichroism measurements. Equimolar carbaryl/β-cyclodextrin solid systems were prepared by physical-mixing and freeze-drying, and fully characterised by Differential Scanning Calorimetry, X-ray powder diffractometry and Fourier Transform Infra-Red analysis. The results of the solid state study demonstrated that the freeze-drying method yields a system with a high degree of amorphisation and yields an inclusion complex. The dissolution profile of the pesticide was affected by the physico-chemical properties of each solid system, the freeze-dried form dissolving more rapidly. However, the physical association of β-cyclodextrin and carbaryl enhanced the aqueous solubility of the insecticide as well.

Key words: carbaryl; β-cyclodextrin complex; aqueous solubility; environmental impact.

1. Introduction

When a crop protection chemical is applied, its performance, convenience of use and safety to applicators, to the environment and to other people who may be nearby, are affected by the way the product is formulated [1]. Aqueous solubility is certainly a critical property of chemicals, inasmuch as it affects their environmental fate, particularly when considering sorption and transport processes. In fact, it is very difficult to define the optimal aqueous solubility value for a given pesticide, as it changes continuously during the pesticide cycle in the environment. Nevertheless, the possibility to reversibly modulate this property by an adequate formulation type appears a very interesting task in the agrochemical field.

* Author for correspondence.

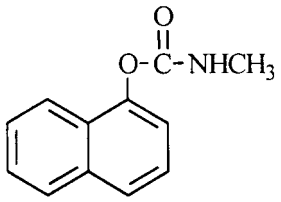

Chart 1.

A suitable strategy to approach this complex problem is based on the modulation of the aqueous solubility of a chemical by using biocompatible macromolecules such as cyclodextrins. Cyclodextrins (CDs) have been successfully employed in the pharmaceutical field [2–6] and have recently been the focus of agrochemical research. CDs are cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity. Due to their cone-shaped structure, many guest molecules can interact with CDs to form an inclusion complex, leading to an improvement of the apparent aqueous solubility of the guest. CDs have been successfully used to accelerate detoxification processes, as they catalyse decomposition of many toxic substances [6, 7]. Moreover, CDs can accelerate the biological detoxification of industrial and domestic wastewater. In fact, when the concentration of a polluting agent exceeds a given level, microflora is paralysed and the biological detoxification process decreases irreversibly. CDs can in this case form a complex with the polluting agent, temporarily masking its presence toward microflora: the large, hydrophilic complex is not absorbed into the micro-organism cells, thus leaving the course of normal biodegradation phenomena unaffected [8]. CDs are able to immobilise environmental polluting substances, such as heavy metals [9] and polynuclear aromatic hydrocarbons from solutions, an effect applicable also to gaseous phases [10]. Since the effectiveness of CDs in pesticide formulations depends on their capacity to form an inclusion complex, a comprehensive study of the physico-chemical characteristics of the pesticide-CD system is a fundamental step before any on-field application is attempted.

In this study, we have considered carbaryl (α-naphthyl-*N*-methylcarbamate) (Chart 1), a broad-spectrum insecticide with contact and stomach action and β-cyclodextrin (βCD) because it is natural and inexpensive, with the aim to thoroughly investigate the influence of βCD on the physico-chemical properties of carbaryl. The interactions between CAR and βCD were studied in solution by both the phase solubility and circular dichroism methods. CAR/βCD solid systems in equimolar ratio were prepared by physical mixing and freeze-drying, and fully characterised by thermal analysis (DSC), Fourier transform infrared analysis (FTIR) and X-ray powder diffractometry. The influence of the physico-chemical properties of the binary systems on the dissolution profiles of carbaryl are highlighted and discussed.

2. Experimental

2.1. CHEMICALS

Carbaryl (CAR) was extracted from a commercial formulation. β-Cyclodextrin (βCD) was kindly supplied by Roquette Frères (Lestrem, France). All chemicals were of analytical reagent grade. Distilled water was used throughout the study.

2.2. STUDIES IN SOLUTION

2.2.1. *Solubility studies*

Solubility studies were performed according to Higuchi and Connors [11]. An excess of CAR (50 mg) was added to 25 mL of water containing amounts of βCD ranging from 1.0×10^{-3} to 12×10^{-3} M, and shaken in screw-capped glass vials at 25 °C until equilibrium. An aliquot was withdrawn, filtered (filter HA-0.45 μm, Millipore) and analysed for CAR content by spectrophotometry at the wavelength of 298 nm (PU 8700 Philips spectrophotometer).

The apparent stability constant ($K_{1:1}$) was calculated from the linear graph obtained by plotting the CAR molar concentration in the solution *versus* each βCD molar concentration according to the equation:

$$K_{1:1} = \text{slope/intercept (1-slope)}. \tag{1}$$

Each experiment was performed in triplicate; the coefficient of variation associated with each measurement never exceeded 3%.

2.2.2. *Circular dichroism*

The spectra were collected on a Jasco J710 spectropolarimeter in the range 240–350 nm on aqueous solutions containing βCD ranging from 1.5×10^{-3} to 1.0×10^{-2} M and CAR 5.0×10^{-5} M. The instrument was calibrated using (+)-10-camphorsulfonic acid. A quantitative analysis of the ellipticity variation as a function of βCD concentration was performed according to the modified Scott equation [12]:

$$\frac{[\mathrm{G}] \cdot [\mathrm{CD}] \cdot d}{\Delta\psi} = \frac{1}{\Delta\theta} \cdot [\mathrm{CD}] + \frac{1}{K_{1:1}\Delta\theta}, \tag{2}$$

where [G] is the total molar concentration of the guest, [CD] is the molar concentration of uncomplexed βCD (which can be considered equivalent to total CD concentration), $\Delta\psi$ is the difference between experimental ellipticities of the guest in the absence and presence of cyclodextrins at a definite wavelength, $\Delta\theta$ is the difference in the molar ellipticity coefficient between included and free guest, $K_{1:1}$ is the apparent stability constant and d is the path-length of the cell. For the CAR/βCD system, ψ and θ of CAR are zero.

Each experiment was performed in triplicate; the coefficient of variation associated with each measurement never exceeded 3%.

2.3. PREPARATION OF CAR/βCD SOLID SYSTEMS

CAR and βCD were sieved and the corresponding 75–150 μm granulometric fraction collected. The CAR/βCD stoichiometric ratio employed to prepare solid systems was 1 : 1 (mol:mol).

For the preparation of the physical mixture, CAR and βCD powders were blended in an agate mortar to obtain a homogeneous mixture. The freeze-dried product was prepared by dissolving 1 g of physical mixture in 1 L of water, freezing the solution at -70 °C and freeze-drying in a Modulyo Edwards apparatus. The absence of CAR degradation products in the βCD containing systems was assessed by HPLC analysis.

Reversed phase chromatography was performed on a Waters 6000E liquid chromatograph equipped with a 7125 Rheodyne injection valve and a 440 spectrofluorimetric detector (Waters). The chromatograms were recorded by a 746 Data Module (Millipore). The column was a Spherisorb ODS2 (25 $\times$ 4.6 mm) and the mobile phase a mixture of acetonitrile-water (55 : 45 v/v) at a flow rate of 1 mL/min. Fluorimetric detection of CAR was carried out at 230 nm excitation and 330 nm emission wavelengths. Under these experimental conditions CAR and its major degradation product, α-naphthol, eluted at different retention times (t_r = 6.021 and t_r = 5.503 minutes, respectively).

2.4. STUDIES IN THE SOLID STATE

2.4.1. *Differential scanning calorimetry*

DSC measurementments were carried out with a Mettler DSC 30 apparatus equipped with a TC II probe. Samples ranging from 7 to 10 mg were placed in pierced aluminium pans and scanned at a rate of 10 °C/min. Dry nitrogen was used as the purge gas.

2.4.2. *Infrared spectroscopy*

FTIR spectra (KBr disk) were obtained on a Bruker Model IFS-48 apparatus applying Fourier transformation of 8 scans.

2.4.3. *X-ray analysis*

X-ray diffraction powder patterns were collected on a Philips PW 3710 diffractometer in the 2–40° 2θ range. Kα radiation of Cu was generated at 40 kV and 30 mA.

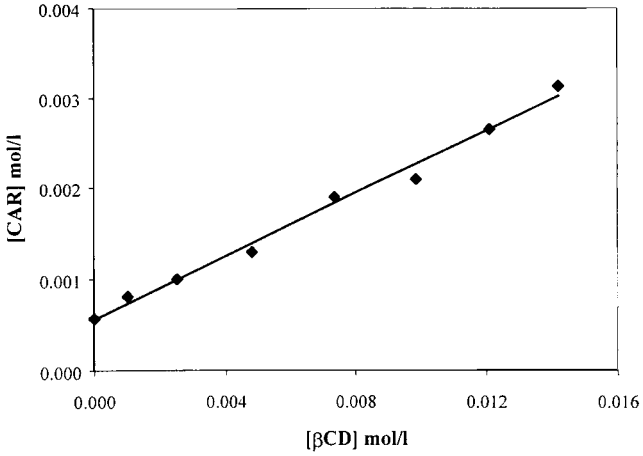

Figure 1. Phase solubility diagram of the CAR/βCD system.

2.5. DISSOLUTION STUDIES

The dissolution profiles of CAR and CAR/βCD systems were evaluated according to the USP 23 method [13]. Ten mg of CAR or equivalent amounts of CAR/βCD formulations were added to 1 L of water at 37.0 ± 0.1 °C in a Sotax ATII apparatus. Suitable aliquots were removed at specified times, filtered and spectrophotometrically analysed at 298 nm for CAR content. Each experiment was performed in triplicate; the coefficient of variation associated with each measurement never exceeded 3%.

3. Results and Discussion

The equilibrium phase solubility plot for the CAR/βCD system is reported in Figure 1. The increase in βCD molar concentration enhanced CAR solubility. The diagram was of A_L type indicating the formation of a CAR/βCD complex with a 1 : 1 stoichiometry [11]. The apparent stability constant calculated from the regression Equation (1) reported in the experimental section, and assuming the formation of a complex with a 1 : 1 stoichiometry, was $310 \pm 5\ M^{-1}$.

Figure 2 shows the effect of different concentrations of βCD on the circular dichroism spectrum of CAR. It is well known that complexes formed between chiral non-absorbing cyclodextrins and achiral light-absorbing guests, such as CAR, can induce Cotton effects on the dichroism spectra. These effects can be referred mainly to the optical activity of the guest molecule induced by inclusion of its chromophore portion into the chiral cavity and partly to conformational changes of cyclodextrin [14–17]. The CAR curve in an aqueous solution containing βCD showed both a maximum and minimum in the same range of UV absorption maxima. Increasing the amount of βCD dramatically changed the ellipticity of both extrema: the value of the negative peak increased and the value of the positive peak

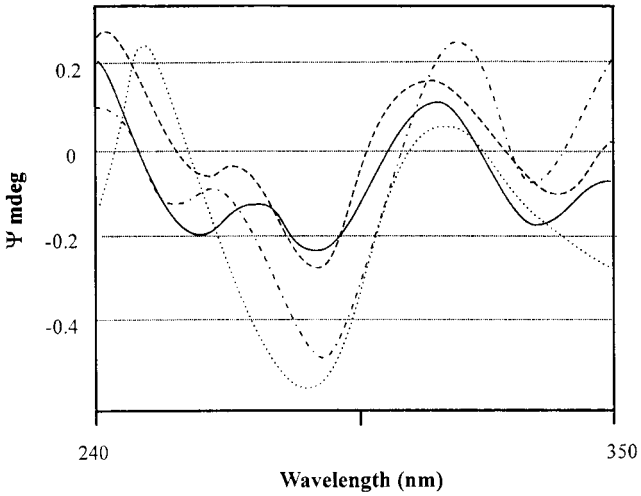

Figure 2. Circular dichroism spectra of CAR in the presence of increasing amounts of βCD. CAR concentration 5.0×10^{-5} M; βCD 1.5×10^{-3} M (A) (—); 2.0×10^{-3} M (B) (– –); 5.0×10^{-3} M (C) (- - -); 1.0×10^{-2} M (D) ($\cdots$).

decreased. The negative band at 287 nm could be attributed to the Cotton effect induced on CAR, and generated by the formation of an inclusion complex with βCD. Nevertheless, according to the symmetry rule [18], the sign of the Cotton effect induced is governed by the configuration of the asymmetric centre and spatial relationship with the chromophore. The negative effect observed for the CAR/βCD system allowed us to make a hypothesis that the transition dipolar moment of the CAR molecule was perpendicular to the z-axis of βCD, thus determining a perpendicular orientation of the guest within the host cavity [19]. Figure 3 illustrates the plot of [CAR][CD]/$\Delta\psi$ *versus* [βCD]. The linearity of the plot was once again in agreement with the hypothesis of a complexation with a 1 : 1 stoichiometry. The apparent stability constant was calculated from the ratio slope/intercept assuming a complex with a 1 : 1 stoichiometry and according to Equation (2) reported in the experimental section. The constant value was 268 ± 8 M^{-1} which is in good agreement with the value determined by the phase solubility method.

The studies in solution indicated that CAR forms a complex with βCD having an apparent stability constant falling within the range 200–5000 M^{-1}, considered by various authors as adequate to achieve an inclusion complex in the solid state [20].

Thus, CAR/βCD solid systems were prepared by physical mixing and freeze-drying, this last procedure being effective for achieving complexes of βCD and many drugs in the solid state.

The results of Differential Scanning Calorimetry analysis on CAR, βCD and their solid systems are reported in Figure 4. As can be seen, CAR showed a fusion endothermic peak at 144 °C, corresponding to the melting point of its crystalline

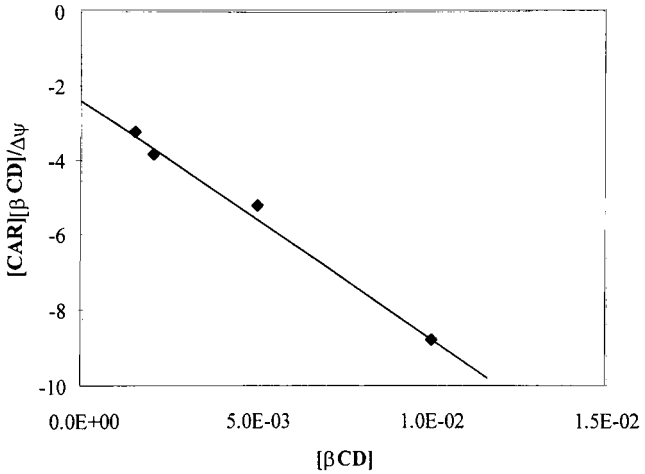

Figure 3. Plot of circular dichroism data for CAR/βCD system according to Scott's equation. Ellipticity values were taken at 287 m.

form, followed by a second broad endothermic peak at about 200 °C. The second peak is due to the decomposition of the solid after melting during the calorimetric scan as demonstrated by TGA (data not shown). The thermogram of βCD exhibited a very broad endothermic peak with a maximum at ca. 100 °C, corresponding to the dehydration peak of the compound. The DSC pattern of the physical mixture was the superimposition of those of CAR and βCD, whereas the CAR fusion peak completely disappeared in the freeze-dried product. This indicates an almost complete loss of crystallinity of this binary system – a typical consequence of the modification of pre-existing hydrogen bonds of the crystalline drug and the occurrence of interactions with βCD, probably due to the complexation of CAR with βCD.

The X-ray powder diffraction patterns of CAR, the physical mixture and the freeze-dried product are shown in Figure 5. The pattern of the physical mixture suggested that CAR maintains its crystallinity also in the presence of βCD. On the other hand, a total amorphisation was observed for the freeze-dried product, due to the disruption of the cyclodextrin crystal lattice by CAR molecules.

The behaviour of the physical mixture of CAR and βCD separately freeze-dried was also investigated by DSC and X-ray (data not shown). The results showed a substantial equivalence of this product to the physical mixture and indicated that the freeze-drying process did not affect the properties of the untreated physical mixture.

Infrared spectra in the C=O stretching region of CAR (1600–1800 cm^{-1}) and βCD-containing solid systems are illustrated in Figure 6. The carbonyl stretching band observed at 1713 cm^{-1} for CAR shifted to 1717 cm^{-1} in the freeze-dried product, suggesting a modification of the electronic environment of the C=O group. Our finding indicated that a monomolecular dispersion of the active compound

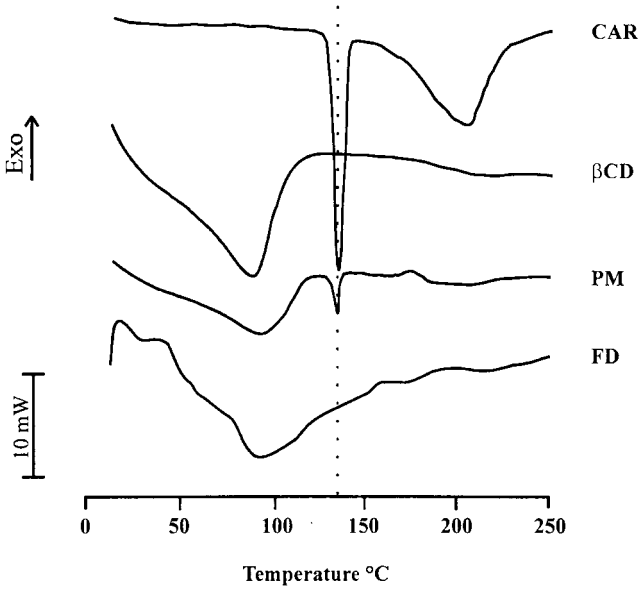

Figure 4. DSC thermograms of CAR; βCD; physical mixture (PM); freeze-dried product (FD).

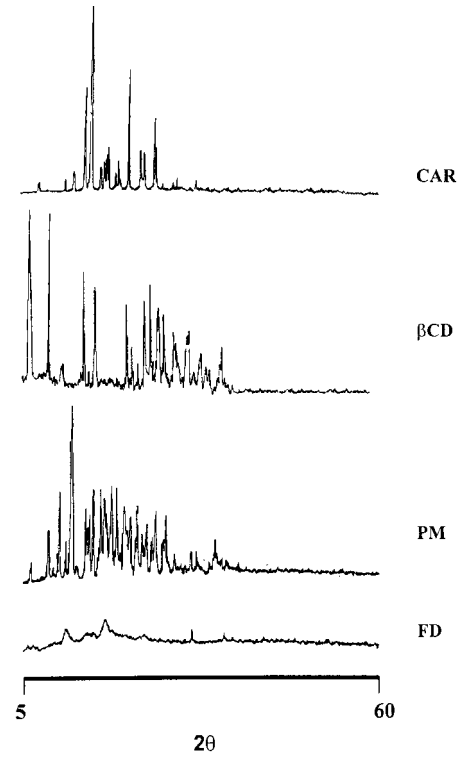

Figure 5. X-ray powder diffraction patterns of CAR; βCD; physical mixture (PM); freeze-dried product (FD).

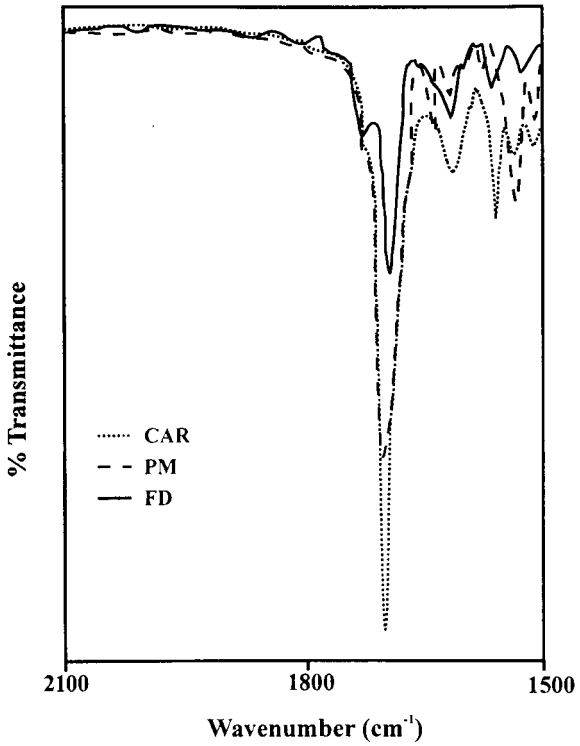

Figure 6. FTIR spectra of CAR; physical mixture (PM); freeze-dried product (FD).

within βCD determined an ordering of the molecules in the binary system, which is in agreement with the increase generally observed in the frequency of a specific peak on complexation [21]. Therefore, in the CAR/βCD freeze-dried product the shift could be ascribed to the interaction between the carbonyl group of the CAR molecule and the hydroxyl group of the βCD. On the basis of these results and the fact that the freeze-dried product is prepared by a technique widely recognised to preserve in the solid state the inclusion complex existing in solution, we made a hypothesis that CAR was also present in the solid state as an inclusion complex. The lack of C=O shift of CAR in the spectrum of the physical mixture confirmed that in this case no interaction between host and guest was achieved.

The dissolution profiles of CAR and different CAR/βCD solid systems are reported in Figure 7. At each time, the amount of CAR dissolved from βCD-containing binary systems was higher than CAR alone, with the freeze-dried product showing the highest amount of pesticide dissolved. The amount of CAR dissolved from the freeze-dried product after 120 minutes was slightly greater than from the physical mixture, whereas a very low amount of CAR was dissolved from its sieved powder. The increase in CAR solubility in the presence of βCD could be ascribed to the capability of CD to form a rapidly soluble complex in solution whereas the differences displayed by the various solid systems could be mainly

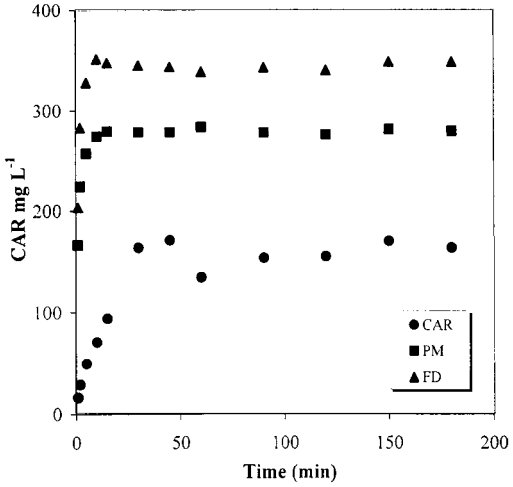

Figure 7. Dissolution profiles of carbaryl CAR; physical mixture (PM); freeze-dried product (FD).

related to their different degree of amorphisation. It is worth noting that one can achieve a rapidly dissolving CAR/βCD system simply by physical mixing.

As the dissolution rate is certainly crucial to achieve a rapid absorption of CAR in the stomach of an insect and consequently a faster onset of pesticide action, the pre-existence of an inclusion compound is expected to affect insecticide activity. Moreover, the apparent improvement of CAR hydrophilicity could favourably affect the environmental distribution of the pesticide.

4. Conclusions

We have demonstrated that β-cyclodextrin can be successfully used to increase the water solubility of carbaryl. This effect can be ascribed to the formation of a complex between carbaryl and βCD, with a stability constant of 289 ± 21 M^{-1}. The characterisation of different CAR/βCD solid systems suggested that in the freeze-dried product a molecular inclusion probably takes place. The dissolution of CAR from all the βCD-containing systems is enhanced due to both the amorphisation degree of the powders and formation of a rapidly soluble complex in solution. It is worthy of note that the improvement of CAR aqueous solubility by simply mixing the pesticide with βCD can be considered as a very interesting tool to modulate the activity and environmental fate of the pesticide, mostly in view of industrial applications.

Acknowledgement

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