

## Effect of the Environmental Temperature on the Degradation Period of Carbaryl

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**Abstract:** In this study, the effect of the temperature on the degradation period of Carbaryl (Sevin), a widely used outdoor and indoor insecticide, was investigated in a laboratory in soil and on green plants by using a new quantitative TLC (Thin Layer Chromatography) method. The experimental temperature conditions were 40°C, 16°C and 5°C for soil and 15°C for green plants. The results which were evaluated by photocopying of the TLC spots on a graph paper and square counting showed that carbaryl degradation is a first order reaction and the temperature dependent half-life of the degradation takes greater than four weeks under the investigated conditions.

### Karbarilin Parçalanma Süresine Çevresel Sıcaklığının Etkisi

**Özet:** Bu çalışmada, oldukça geniş bir spektrumda kullanılan Karbaril (Sevin) adlı zirai mücadele ilacının toprakta ve yeşil yapraklı bitkiler üzerindeki parçalanma süresine sıcaklığın etkisi laboratuvarında kurulan modeller üzerinde incelenmiştir. Deneysel sıcaklık şartları toprak için 40°C, 16°C ve 5°C; yeşil yapraklı bitkiler için 15°C olarak seçilmiştir. İnce Tabaka Kromatografisinde karbarilin oluşturduğu lekelerin grafik kağıdına fotokopisi çekilmiş ve kare sayma metoduyla kantitatif değerlendirme yapılmıştır. Parçalanma reaksiyonunun birinci dereceden olduğu ve incelenen şartlar altında sıcaklığa bağlı parçalanma yarı ömrünün 4 haftadan daha uzun bir süre aldığı tesbit edilmiştir.

### Introduction

Carbaryl (1-naphthyl methyl carbamate), was first synthesised by Dr.A.J.Lambrech in 1953 and introduced as an agricultural insecticide in 1958. Since then it is one of the most widely used insecticides in the world against fruit, vegetable, cereals, timber forest, cotton; domestic animals and livestock insects (1-3). In Turkey, in particular at The Black Sea Region, it is widely used for various crops under the commercial names of Agrovin 5, Hektavin, Karbavin, Kovin. The use of carbaryl for other agricultural harmfuls is also encouraged by regional studies. Such commonly used material must be safe in terms of human, animal and environmental health.

Actually, it is reputed to have a relatively short degradation period and a wide safety margin. In the past, it was reported that carbaryl degrades in short time in water, soil and on various plants (1,2,4) by a hydrolysis reaction (see Figure 1). However, physical and chemical properties of the experimental surroundings of the degradation, in those experiments have not been clearly described. The further studies showed that the half life of

the degradation in water could be extended up to four years depending on the pH ( $\leq 5$ ) and 1/24 of carbaryl would remain in soil even after twenty years (5).

In recent years, the chronic toxicity of carbaryl has come into discussion and adverse effects in human brain, muscles and nerves were investigated (3,6). The chronic toxicity potential of any insecticide is related to the residue remained on the environment or in other words to the half-life of its degradation. In the past no evidence was found on the direct carcinogenic potential of carbaryl (3,7), but in recent years, there is some belief that carbaryl reacts with nitrites in slightly acidic media and carcinogenic nitroso derivatives, which are highly mutagenic in vitro system are formed. In addition, it was reported that carbaryl may contain 2-naphthyl carbamates formed during manufacturing process and produce carcinogenic naphthol as a degradation product (8). In this study, variation of the residue of carbaryl in soil and on green leafed plants with temperature have been investigated on models in a laboratory.

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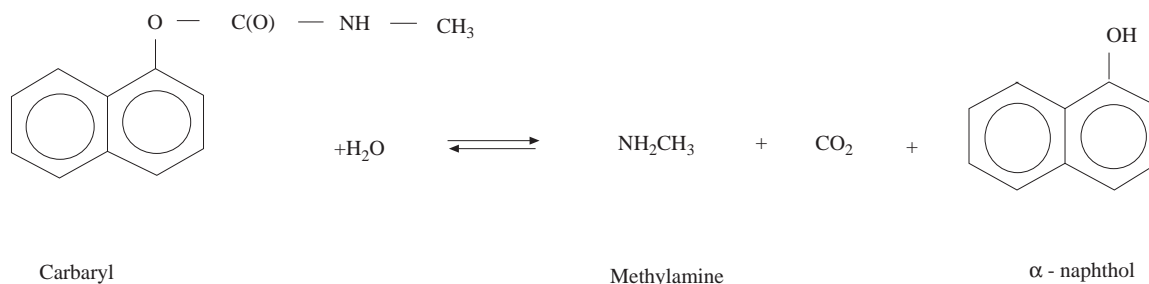


Figure 1. Hydrolysis reaction of carbaryl (1-naphthyl methyl carbamate) in water

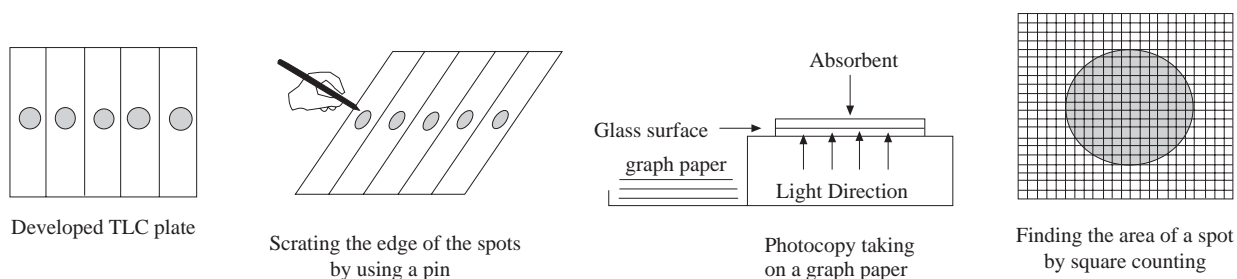


Figure 2. Simple display of the evaluation sequence of TLC spots by photocopy method

## Materials and Methods

### Materials

About 5 kg soil mixture (collected from different fields), 10 large green leafed *Canna indica* L, which were planted into pots, and technical carbaryl (98%) obtained from Hektaş-Turkey were used, and solvents (ethanol, acetone *etc.*) involved throughout the experiments were purchased from Mecrk and Sigma. The technical carbaryl dissolved in ethanol (9) was further purified by successive flash crystallisation and vacuum drying until satisfactory analytical purity (usually attained after four times) was observed by the UV spectra (Varian D.M.S. 100 UV spectrometer). Experimental temperature conditions were continuously by using a hygrotermograph (New Sigma Sato Keiryoki NS 307) placed at the experimental sites.

*Experiments on soil:* The collected soil (pH=7.4) was cleaned, dried and sterilised for two days at 120°C to prevent the microbial activity interfering and spreaded in 1.5-kg portions at wooden boxes (30x40x10 cm). The boxes then placed into the experimental sites (Elektromag temperature conditioner, Arçelik refrigerator) and waited for two weeks for the experimental atmospheres

to come to an equilibrium. Following the equilibrium, carbaryl was applied by spraying 75 ml of 0.5% (w/v) technical carbaryl-acetone solution onto the soil samples (0.25 mg carbaryl/g soil) and mixed thoroughly and before placing it into the experimental site the first sample was taken for the analysis. In the sampling, the soil boxes were finger divided into four equal parts and 15 g soil from the middle of each part was scooped, homogenised and two times  $20.00 \pm 0.01$  g soil weighed for the extraction step. The mean experimental temperature conditions measured during the experiments were 40°C, 16°C, 5°C respectively.

*Experiments on plants:* 10 large leafed green plants (*Canna indica* L) were brought to the laboratory and planted into pots and kept there until the end of the experiments. 25 ml of 0.5% (w/v) technical carbaryl was sprayed on the plants at a high spraying rate (to protect the green leaves from acetone) and the first sample were taken immediately after the application. The green leaf samples were randomly cut at least ten different part of the plants and homogenised to represent the whole population and two times  $5.00 \pm 0.01$  g green leaves weighed for the extraction step. The mean experimental tempera-

ture conditions measured along the experiment were 15°C.

**Extraction:** the extraction of the samples were carried out according to a standard method (Williams, 1984) [9] and the obtained solution was taken into a rotary vacuum evaporator (Büchi Rotavapor R110, Germany) at 40°C to obtain the dry residue. The flask was then rinsed three times with 1 ml acetone and content transferred into a conical graduated tube and concentrated to 1 ml for soil; 1ml for green samples by N<sub>2</sub> blowing. The green mossy precipitates, observed in the concentration process of plant extracts was removed by centrifuging, no precipitating was observed with the soil samples.

**TLC method:** standard carbaryl solutions at 1, 2, 3, 4, 5, 6, µg/µl concentrations were prepared in acetone and refreshed every week. TLC (Thin Layer Chromatography) plates (20x20cm) were prepared (0.25 mm thickness) by slurring 30 g silicagel 60<sub>HF254</sub> in 65 ml water and activated, divided into 2 cm columns before using. A 5µl aliquots of the standards (once) and extraction solution (three times) were applied on TLC plates by using a micro syringe (Cosge, Australia) and plates were developed (R<sub>Fcarbaryl</sub>=5.5; R<sub>Fα-naphthol</sub>=8.5) in a saturated tank atmosphere of 40 % hexane-60% diethyl ether (v/v) liquid mixture. Developed plates (10 cm) were subjected to Br<sub>2</sub> vapour exposure for at least 2 minutes and then plates were sprayed with 1% fluorescein-ethanol solution. This resulted green carbaryl spots appearing on pink coloured absorbent [if plate is hold in Br<sub>2</sub> vapour again (just for a moment) the colours become even brighter].

**Evaluation of the TLC spots:** the edge of the coloured spots were scratched carefully by a thin needle and made ready for photocopying in 5 minutes. Glass side of the plates (not the adsorbent coated side) placed on the glass surface of the photocopy machine (see Fig.2) and copied at A4-auto normal copy mode (smaller spots may be magnified) on normal graph papers without closing the lid (closing the lid improves the copy quality but damages the plates). The area of the TLC plates spots was expressed as the number of the squares form the spots on graph paper and the concentration vs spot area graph (calibration graph) were constructed for each plate. Carbaryl content of the extracts (µg/µl) were obtained from the callibration graph of each plate. The amount of carbaryl (mg) in 100 g original sample was calculated by the following equation:

$$\% \text{ Carbaryl} = \frac{C.V}{W} \cdot 100$$

C=concentration found from the calibration graph (µg/µl)

V=last volume of the concentrated extract (ml)

W=amount of the sample taken (g)

Each week two samples, taken from soil and green plants were applied on TLC three times, thus, the obtained results indicate the mean of the six concentration values. The amount of carbaryl found immediately after the spraying was accepted 100 and degradation results were stated relative to this amount as remained carbaryl percent.

**Reproducibility, repeatability and accuracy of photocopy evaluation:** TLC plates were prepared in six different occassion and divided into ten columns. Standards (once) and known concentration of 3mg/ml carbaryl solution (four times) were applied on each plate. Calibration curves were constructed for each plate and concentration of the developed spots was calculated from the calibration graphs. The %Relative Standard Deviation (%RSD) of the calculated concentration on each plate represents repeatability (*within-run precision*), the %RSD of the calculated concentration between six different application represents reproducibility (*between-run precision*), the calculated %Relative Error (%RE) of known concentration represents the accuracy within 95% confidence limits (10).

## Result and Discussion

Quantification of the TLC spots may be performed by using several methods based on the concentration-spot area relation. Photocopying of TLC spots on graph paper and calculating the spot area by square counting is simple, cheaper and allows more accurate quantitative analysis. Calculated reproducibility (2.13%, n=24), repeatability (2.62%, n=4) and accuracy (3.08%, n=24) show that the proposed method may be used for analytical purposes with advantages. A remarkable error, however, arise from the miscounting of the number of the squares forming spots. Omitting the residual squares or assuming residual squares or assuming residual squares as a whole square brings an extra 2% (> 3 mg/ml) and 4% (>2mg/ml) error on the calculated values. If whole squares are formed by addition of the residual parts by the rule of thumb, the analysis may be performed with minimum calculation error. Magnifying the spots during photocopy process could be very helpful to see residual parts clearly. The concentraton-spot area relation produces a concave shaped calibration curve in the experimental concentration region (see Figure 3). This limits

the applied concentration over 7mg/ml. If more concentrated samples need to be analysed the thickness of the plates should be increased or the column width (2 cm) should be enlarged. Since calibration curve is plotted for

each plate, the variation in the properties of plates from one to another is not very important. The most important thing is that the homogeneity of each plate which is usually attained.

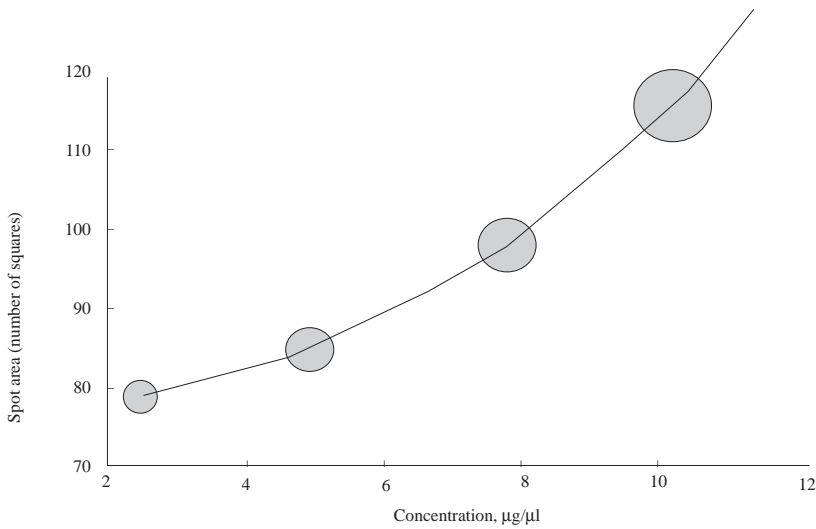


Figure 3. A typical concentration-spot area relation graph for TLC analysis.

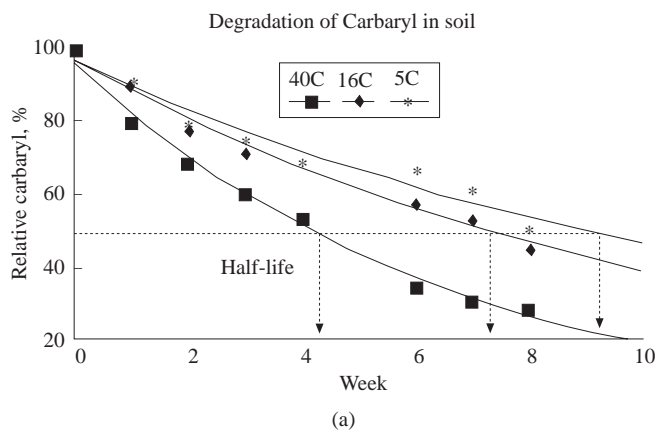
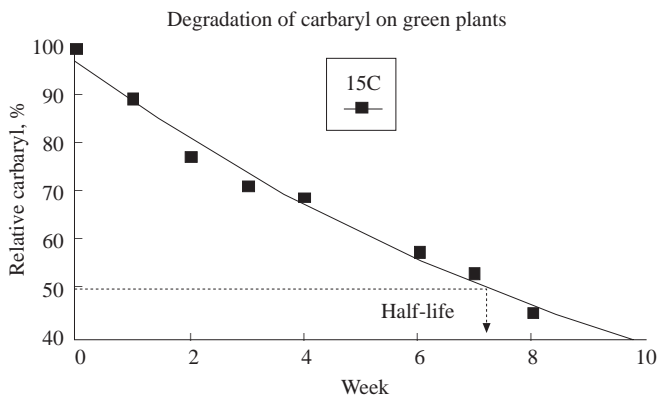


Figure 4. Degradation half-life of carbaryl in soil (a) and on green plants (b)



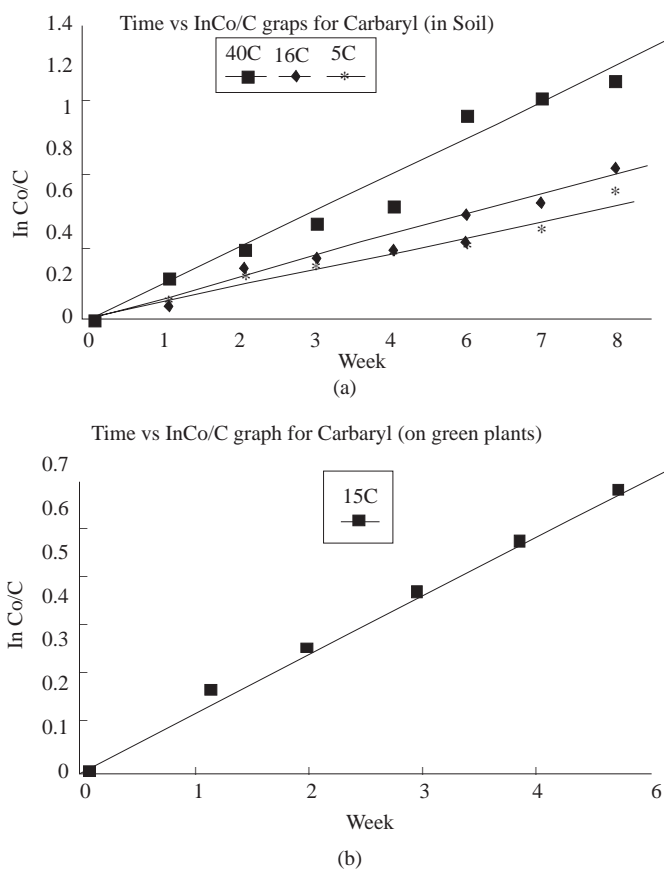


Figure 5. Time vs lnCo/C graphs for soil (a) and for green plant degradations (b)

Obtained degradation graphs given in Figure 4 (a), (b) show that half-lives of the degradation reactions of carbaryl at the investigated temperatures were never so short as it was reported by some investigators in the past and the environmental temperature plays an important role in the degradation process. The half-life of the reaction was remarkably lowered by increasing the experimental temperature. This was the expected result from the thermodynamics point of view. The time vs lnCo/C graphs (see Figure 5 (a), (b)) indicate that the degradation reaction follows a first order reaction kinetics. Thus, half-life of the degradation reaction ( $t_{1/2}$ ) reversely depends on the rate constant of the reaction ( $t_{1/2}=0.693/k$ ) and so as temperature according to the Arrhenius equation ( $k=k_0 e^{-E/RT}$ ). It must be noted that this results show the decomposition of carbaryl by only temperature effect. Since carbaryl is a stable substance in the absence of water, temperature can only speed up the rate of the hydrolysis reaction. The humidity may play an important role upto the effective number of the water molecules enough to start the hydrolysis reaction. After

this point the increased number of water molecules do not help in the rate of the hydrolysis. But increasing the temperature in the presence of hydrolysis increases the rate constant,  $k$ , of the degradation reaction. In addition, it has been observed that carbaryl degrades on green plants in shorter time than does in soil at the same temperature.

Despite its longer half-life, the application period of carbaryl for insects is generally in summer time in hazelnut, various fruits and vegetable corps (May, June). Since higher temperatures are likely to occur in the following months of the application and carbaryl degrades in shorter time on green plants in comparison with soil, it could be said that this common insecticide of the world and The Black Sea Region could reasonably be used, but one cannot easily say that this insecticide is 'safe' by only referring to the short half-life values with undefined physical and chemical conditions. Since the degradation in soil takes more time, using the powder formulation of carbaryl by mechanical spreading should be avoided and wettable powder formulation by spraying should be pre-

ferred for applications. This will reduce the amount of carbaryl added to soil. This is particularly important from the environmental point of view because all the added carbaryl turns into  $\alpha$ -naphthol sooner or later after the degradation. Since this substance is resistant to decom-

position and insoluble in water that cannot be removed by washing a remarkable  $\alpha$ -naphthol accumulation may occur in soil over the years. No one can clearly say that  $\alpha$ -naphthol will not be an environmental problem in the coming years.

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