

Thermal and photolysis degradation of three profenofos formulations

Ismail, I. Ismail and Nasr Sobhy Khalil

Pesticides Analysis Research Department, Central Agricultural Pesticides Laboratory,
Agricultural Research Center, Dokki, Giza, Egypt.

Abstract

Three commercial profenofos emulsifiable concentrate (EC) formulations 720 g/l (72% w/v) currently applied in Egypt from three different companies were studied for the effect of sunlight exposure and storage on the stability of profenofos and its relevant impurity 4-bromo-2-chlorophenol content. Exposure to sunlight and storage for more than 14 days at 54 ± 2 °C indicated various degradation effects on profenofos content and increase the formation of its impurity 4-bromo-2-chlorophenol. Identification of profenofos content and degradation products were determined using GC-FID and GC-MS. From the obtained results the storage of profenofos at 54 ± 2 °C at intervals 28, 42, 56 and 70 days showed serious effects on the degradation rate of profenofos and increase the rate of formation of 4-bromo-2-chlorophenol compared to exposure to sunlight for all profenofos formulations under study. The results showed variable rate of degradation of profenofos and the amount of its relevant impurity 4-bromo-2-chlorophenol for all samples sources under investigation. Analysis after storage using GC-MS identified three degradation products: 4-bromo-2-chlorophenol, O-ethyl-O-(2-chloro-4-bromo-phenyl)-phosphate and O-(4-bromophenyl) O-ethyl S-propyl phosphorothioate.

Keywords: Profenofos, 4-bromo-2-chlorophenol, Degradation, Impurities, GC-MS.

INTRODUCTION

Profenofos (*O*-4-bromo-2-chlorophenyl *O*-ethyl *S*-propyl phosphorothioate) an organophosphorus insecticide, its label classification according to the Globally Harmonized System (GHS) is danger product, Harmful if swallowed and in contact with the skin, Class II moderately hazard (WHO, 2009). Profenofos showed highly toxicity to fish and macro-invertebrates (Akerblom, 2004), it inhibits and effect on acetylcholinesterase (Fukuto, 1990), also causing toxicity to humans (Costa *et al.*, 2008). The USA Environmental Protection Agency (USEPA) and research projects reported that profenofos is considered a strong and predictable contaminant in aquatic and terrestrial ecosystems (Jabbar *et al.*, 1993; Safiatou *et al.*, 2007; USEPA, 2006), and its residues have been found in foods and vegetables (Parekh *et al.*, 1994; Radwan *et al.*, 2005). In Egypt, profenofos has been classified as a restricted use pesticide (RUP) sprayed only on cotton crop for the management of cotton bollworm insects, and the use is through the Ministry of Agriculture and in specific required quantities (APC, 2020).

Profenofos degraded rapidly than chlorpyrifos through photodegradation by sunlight and UV-rays (Ali and Lamia, 2018). The degradation of profenofos in cotton crop leads to broken of O-P bond to form 4-bromo-2-chlorophenol, then conjugate with glucose (Capps *et al.*, 1996). The metabolic degradation of profenofos in plants, animals and soils are similar and occur via hydrolysis to give 4-bromo-2-chlorophenol which is then conjugated by many enzymatic reactions (JMPR, 2008). 4-bromo-2-chlorophenol is the main degradation product of profenofos and it can be formed via photolysis (Burkhard and Guth, 1979) and biodegradation (Talwar and Ninnekar, 2015). According to (FAO, 1998) the 4-bromo-2-chlorophenol is the main relevant impurity in profenofos due to its high toxicity compared to profenofos and its maximum limit should not be higher than 1% of profenofos content.

In the current study, three commercial profenofos (EC) formulations 720 g/l were investigated for the effect of sunlight exposure and storage at 54 ± 2 °C for different intervals 0, 14, 28, 42, 56 and 70 days on the stability of profenofos content and formation of its its degradation product 4-bromo-2-chlorophenol. The choice of these intervals depended on the degradation products produced after the accelerated storage at 54 ± 2 °C for 14 days according to FAO specifications. The identification and determination of profenofos content and its degradation products were confirmed using gas chromatography equipped with flame ionization detector (GC-FID) using external standard and gas chromatography mass spectrometer (GC-MS).

MATERIALS AND METHODS

Chemicals

Analytical standard of profenofos and 4-bromo-2-chlorophenol were obtained from Sigma Aldrich with 99% purity for both of them. The commercial formulations of profenofos 720 g/l EC were obtained from three different companies in Egypt (source I, II and III). Methanol HPLC Grade (Merck) was used.

Chemical structure of profenofos is shown in Fig. 1.

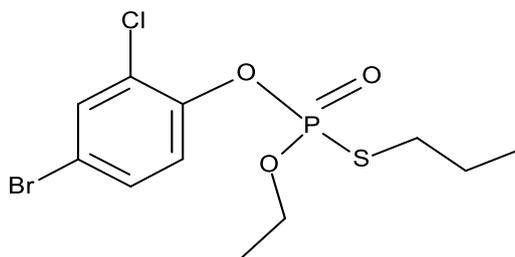


Fig. 1. Chemical structure of profenofos

Accelerated storage procedures (CIPAC MT 46, 1995)

Emulsifiable concentrate formulations of profenofos 720 g/l were placed in bottles (about 50 ml). These bottles were divided into two groups and subjected to different treatments

as follows: The first group was exposed to storage at 54 ± 2 °C for different intervals 0, 14, 28, 42, 56, and 70 days, while the second group was exposed to sunlight for the same intervals. Samples from the two tested groups were subjected to evaluate the stability of each formulation.

Preparation of samples:

1. Profenofos

Standard Preparation

Weight 10 mg of profenofos analytical standard of the known purity into a 25 ml grade (A) measuring flask, dissolve and complete with methanol.

Sample Preparation

A specific weight equivalent 10 mg of profenofos analytical standard was taken from each formulation and transferred into 25 ml grade (A) measuring flask and complete with methanol.

2. 4-bromo-2-chlorophenol

Standard Preparation

Ten mg of 4-bromo-2-chlorophenol analytical standard was weighed into a 25 ml grade (A) measuring flask, dissolve and complete to volume with methanol.

Sample Preparation

One gm from the formulation sample of profenofos was weighed into a 25 ml grade (A) measuring flask, dissolve and complete to volume with methanol.

Identification and determination of profenofos and its impurity 4-bromo-2-chlorophenol

1. Gas Chromatography Determination

The procedures were according to **CIPAC 461/TC/M/-(1996)**, using Agilent 7890B gas chromatograph with autosampler 7693. The conditions of GC were as follows:

Column	capillary column HP-50+ (30 m x 0.53 mm I.D., 1 µm film thickness)
Temperature program	held at 120 °C for 1 min, then ramp 20 °C /min to 260 °C and kept at that temperature for 2 min.
Detector	FID at 250 °C
Injector	250 °C with splitless mode
Carrier Gas	Nitrogen
Flow rate	8 ml/min.
The injection volume	1 µl
Retention times	4-bromo-2-chlorophenol: 3.725 min. Profenofos : 9.250 min.

2. GC-MS analysis and determination

The procedures were performed using GC-MS, model Aglient 7890B gas chromatograph equipped with 5977 A MSD, with a fused silica capillary column HP-5MS(30 m x 0.25 mm x 0.25 μ m film thickness). Carrier gas used was helium with 1.0 ml/min pulsed split mode. The injection volume was 1 μ l, temperature program was held at 50 $^{\circ}$ C for 0.5 min, then ramp 10 $^{\circ}$ C /min to 190 $^{\circ}$ C for 1 min. followed by ramp 10 $^{\circ}$ C /min to 300 and held for 2 min (total run time 28.5 min). The injector temperature was set at 280 $^{\circ}$ C. The mass spectra were identified using Wiley mass spectral data base Library.

3. Kinetic study

According to (FAO, 1998) the content of profenofos after storage should not be lower than 95% relative to the content of profenofos before storage. Shelf life of profenofos was calculated according to (Moye et al., 1987) and (Anderson and Scott 1991).

$$t_{95} \text{ for profenofos} = 0.0513/K$$

$$K = (1/t_x) \text{Ln} (a/b_x)$$

Where, t_{95} = the time required to reach 95% of the initial profenofos concentration.

K = rate of degradation

$$0.0513 = \text{Ln} (0.95)$$

a = initial concentration

t_x = time in days

b_x = concentration at x time

RESULTS AND DISCUSSION

1. Effect of storage on stability of profenofos 720 g/l EC content and the amount of impurity 4-bromo-2-chlorophenol.

Table 1 and Fig. 2 showed the effect of storage at 54 ± 2 $^{\circ}$ C for different intervals 0, 14, 28, 42, 56, and 70 days on stability of the three commercial profenofos formulations. The data of the three samples showed that no significant differences in profenofos and its impurity content before and after storage for 14 days at $54^{\circ}\text{C} \pm 2$ $^{\circ}$ C, suggesting that the formulation is stable at $54^{\circ}\text{C} \pm 2$ $^{\circ}$ C for 14 days where percentage of active ingredient were within recommended specifications. Increase period of storage, showed a significant increase in the rate of degradation for all sources and the loss percentages increased to reach 7.24, 9.45 and 10.02%, also shelf-lives were decreased to 46.64, 36.64 and 34.20 days for sources I, II, and III, respectively. Data presented in Table 1 showed the effect of storage on the amount of 4-bromo-2-chlorophenol at 54 ± 2 $^{\circ}$ C for the different interval of study. The amount of 4-bromo-2-chlorophenol was within the FAO limits for all sources before storage, where the maximum level of 4-bromo-2-chlorophenol in profenofos samples is 1% of profenofos content, and the results showed that the amount of 4-bromo-2-chlorophenol before storage was 0.784, 0.768

and 0.80% for source I, II, and III, respectively, and there was a significant increase in the amount of 4-bromo-2-chlorophenol after storage after 14 days and the amount of 4-bromo-2-chlorophenol increased to 2.97, 3.37 and 4.12 percentage for sources I, II, and III respectively as these values higher than the maximum allowed limits after storage for all sources. Profenofos was less stable after storage at 54 ± 2 °C than exposure to sunlight for the intervals of study. The data in Table 2 and Fig. 3 showed the effect of storage on the chemical stability of three commercial profenofos formulations and the amount of 4-bromo-2-chlorophenol after exposure to sunlight. The results indicated that the loss percentages at 70 days were found to be 5.48, 6.29 and 7.20%, and also the shelf-lives were 64.13, 57 and 46.64 days for source I, II, and III respectively. Also, there was an increase in the amount of 4-bromo-2-chlorophenol after exposure to sunlight for 70 days and the amount of 4-bromo-2-chlorophenol increased to reach 1.99, 2.79 and 3.07 for sources I, II, and III respectively. The obtained results showed that the amount of 4-bromo-2-chlorophenol was more than the maximum allowed limits after storage for all sources.

The previously mentioned results clearly showed that the rate of degradation of the three profenofos formulations under investigation and the amount of impurity were influenced by long period of storage at 54 ± 2 °C as well as exposure to sunlight. There is no significance difference in the rate of degradation for all profenofos formulations and the amount of 4-bromo-2-chlorophenol except profenofos from source III, as it was observed that the rate of degradation was faster, and its stability was less than other sources of profenofos and the amount of 4-bromo-2-chlorophenol was greater than other sources, however, these results of being affected by many factors such as manufacturing process, sources of starting materials, adjuvants, chemical structure, exposure to air, light, and elevated temperature, and also hydrolysis. So, all these factors can affect on the degradation of profenofos which leads to increase the amount of 4-bromo-2-chlorophenol. Much interest to note that the storage of profenofos at 54 ± 2 °C for 70 days showed serious effect on the degradation rate of profenofos and increase the rate of formation of 4-bromo-2-chlorophenol as compared to exposure to sunlight. Our findings are in harmony with (Ali and Lamia, 2018) they reported that profenofos degraded rapidly than chlorpyrifos after exposure to heat and sunlight, also the obtained results compatible with several investigators (Burkhard and Guth, 1979) and (Liu *et al.*, 2019) reported that 4-bromo-2-chlorophenol is the main degradation product of profenofos and it can be formed via photolysis and hydrolysis (Capps *et al.*, 1996).

2. Identification of the degradation products of profenofos by GC-MS

To identify the degradation products of profenofos formulations, samples were analysed before and after storage using GC-MS. Results from GC-MS analysis Table 3 detected the following degradation products i.e, profenofos 374 [M⁺], 4-bromo-2-chlorophenol 208 [M⁺], O-ethyl-O-(2-chloro-4-bromo-phenyl)-phosphate 314[M⁺-1] and O-ethyl S-propyl phosphorothioate 339 [M⁺].

According to the nature of profenofos chemical structure, it contain O-P bond, this bond easily cleavage leads to the formation of 4-bromo-2-chlorophenol. Profenofos can be easily degraded to 4-bromo-2-chlorophenol m/z 208 by hydrolysis and photolysis and that by cleavage the phosphorothioate ester bond (Burkhard and Guth, 1979; Capps *et al.*, 1996;

Zamy *et al.*, 2004 and Liu *et al.*, 2019), also Profenofos can be degraded after storage via hydrolysis and photolysis to form O-ethyl-O-(2-chloro-4-bromo-phenyl)-phosphate m/z, 314 [M⁺-1] (JMPR, 2008), moreover from analysis of our samples using GC-MS found that the characteristic ion at m/z, 339 was molecular ion of O-(4-bromophenyl) O-ethyl S-propyl phosphorothioate which it can be formed by loss of a chlorine atom from profenofos.

Table (1): Thermal degradation of three profenofos formulations stored at at 54 ±2 °C for 70 days.

Storage Periods (Days)	Source I			Source II			Source III		
	Profenofos content (w/v) %	Loss %	4-bromo-2-chlorophenol (%of profenofos)	Profenofos content (w/v) %	Loss %	4-bromo-2-chlorophenol (%of profenofos)	Profenofos content (w/v) %	Loss %	4-bromo-2-chlorophenol (%of profenofos)
0	71.91*	0	0.784**	71.84	0	0.768	71.98	0	0.80
14	71.20	0.987	0.838	71.40	0.612	0.859	71.28	0.972	0.894
28	70.40	1.42	1.11	70.24	2.23	1.17	70.20	2.47	1.33
42	68.53	4.70	1.85	68.14	5.15	2.12	68.09	5.40	2.86
56	67.53	6.09	2.44	67.23	6.42	2.85	66.79	7.21	3.40
70	66.70	7.24	2.97	65.05	9.45	3.37	64.77	10.02	4.12
t ₉₅ (days)	46.64***			36.64			34.20		

* Samples before and after storage stability test analyzed together to reduce the analytical error.

** Maximum level of 4-bromo-2-chlorophenol in tested samples = 1% of profenofos content.

***The time required to reach 95% of the initial profenofos content.

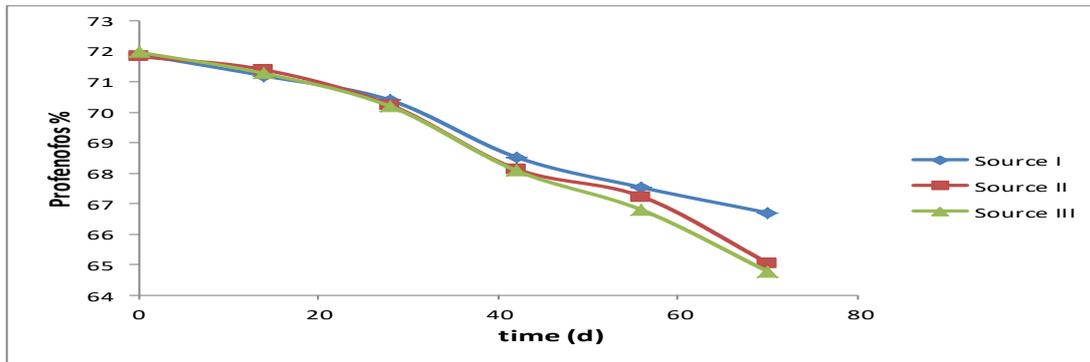


Fig. (2). Effect of storage stability on Profenofos 720 g/l EC (72 % (w/v)) from three different manufactured sources at 54 ±2 °C.

Table (2): Photolysis of three profenofos formulations (720 g/l EC) exposed to sunlight for 70 days.

Storage Periods (Days)	Source I			Source II			Source III		
	Profenofos content (w/v) %	Loss %	4-bromo-2-chlorophenol (%of profenofos)	Profenofos content (w/v) %	Loss %	4-bromo-2-chlorophenol (%of profenofos)	Profenofos content (w/v) %	Loss %	4-bromo-2-chlorophenol (%of profenofos)
0	71.91*	0	0.784**	71.84	0	0.768	71.98	0	0.80
14	71.53	0.528	0.801	71.15	0.960	0.822	71.18	1.11	0.833
28	70.82	1.52	1.07	70.78	1.48	1.25	70.59	1.93	1.49
42	69.61	3.20	1.35	69.42	3.37	1.61	69.01	4.13	1.99
56	68.89	4.20	1.81	68.61	4.50	1.91	68.34	5.06	2.30
70	67.97	5.48	1.99	67.32	6.29	2.79	66.80	7.20	3.07
t_{95} (days)	64.13***			57			46.64		

* Samples before and after storage stability test analyzed together to reduce the analytical error.

** Maximum level of 4-bromo-2-chlorophenol in tested samples = 1% of profenofos content.

***The time required to reach 95% of the initial profenofos content.

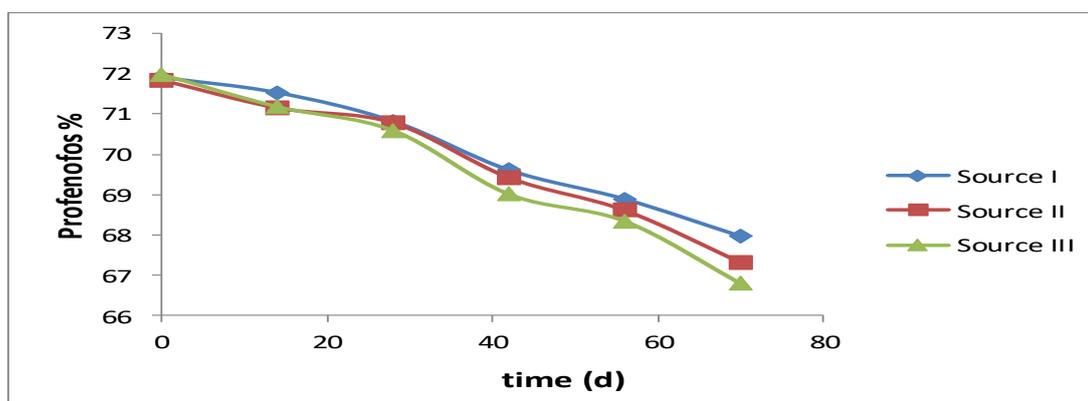
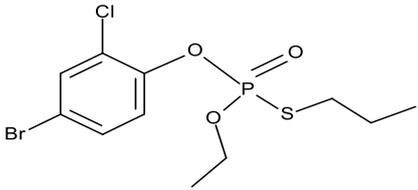
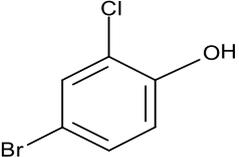
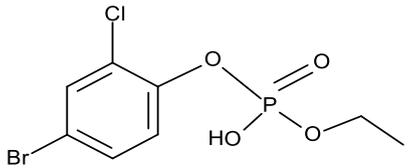
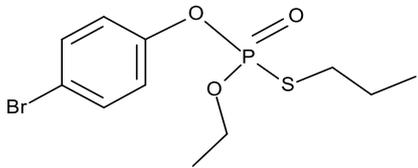


Fig. (3). Effect of storage stability on profenofos 720 g/l EC (72 % (w/v)) from three different manufactured sources in sunlight.

Table (3). Identification of the degradation products of profenofos by GC-MS

Structure	RT(min)	Characteristic ions (m/z)
	27.58	[M] ⁺ =374,339,208,139,97 profenofos
	15.43	[M] ⁺ =208,172,156,77 4-bromo-2-chlorophenol
	25.27	[M] ⁺ =314[M ⁺ -1], 286, 207,172,93 O-ethyl-O-(2-chloro-4-bromo-phenyl)-phosphate
	26.15	[M] ⁺ =339,295,266,172,93,77 O-(4-bromophenyl) O-ethyl S-propyl phosphorothioate

All the previous reactions can be explained in the Fig. 4 where shows the possible degradation pathways of profenofos.

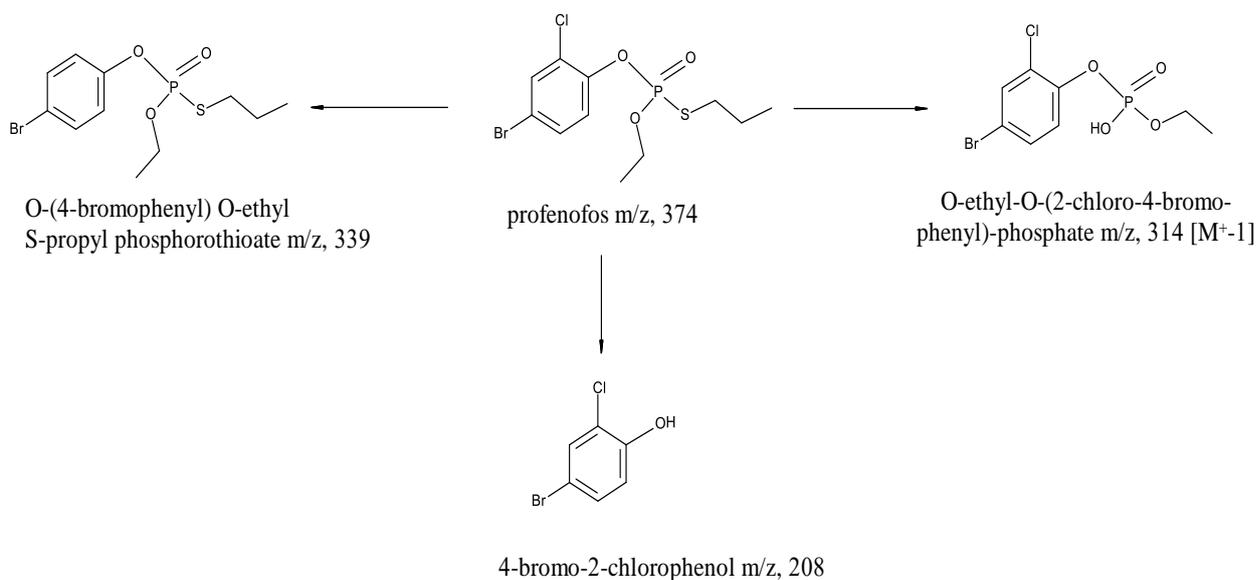


Fig (4). The possible degradation pathways of profenofos after storage.

CONCLUSIONS

Profenofos was affected by storage at 54 ± 2 °C for more than 14 days compared to exposure to sunlight which caused an increase in the rate of degradation and the amount of its impurity 4-bromo-2-chlorophenol. Profenofos can be degraded via hydrolysis and photolysis to some degradation products and the main degradation product of profenofos is 4-bromo-2-chlorophenol. There are many factors affected on the quality of profenofos formulation such as the manufacturing process, sources of technical materials, using different types of adjuvants and long period of storage in bad conditions.

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الملخص العربي

الانهيار الحراري والضوئي لثلاثة مستحضرات من مبيد البروفينفوس

اسماعيل ابراهيم اسماعيل، ونصر صبحي خليل

المعمل المركزى للمبيدات - مركز البحوث الزراعية - الدقى - جيزه - مصر

في هذا العمل تمت الدراسة على ثلاثة مستحضرات تجارية من المبيد الحشرى بروفينفوس 720 جم مادة فعالة/ لتر من المبيد، تم تجميعها من ثلاثة مصادر (شركات مختلفة) فى مصر وذلك لدراسة ثبات مبيد البروفينفوس بالاضافة الى تكوين الشائبة المصاحبة 4-برومو-2-كلوروفينول بعد التخزين عند 2 ± 54 م° والتعرض الي الشمس لفترات مختلفة. تم التعرف على محتوى مبيد البروفينوفوس و بعض نواتج التكسير بعد التخزين باستخدام جهازي GC – FID و GC/MS. من النتائج المتحصل عليها كان هناك تأثير خطير للغاية على ثبات البروفينفوس بعد التخزين عند 2 ± 54 م° لمدة أكثر من 14 يوم وعند فترات 28, 42, 56 و 70 يوم من التخزين وذلك بزيادة معدل انهياره وزيادة معدل تكون شائبته المصاحبه 4-برومو-2-كلوروفينول وذلك بالمقارنة بالتعرض الي الشمس لجميع المستحضرات قيد البحث . ووجد ايضا هناك تباين فى معدلات الانهيار وزيادة محتوى الشائبة للثلاثة مستحضرات قيد البحث. تم التعرف على ثلاثة نواتج تكسير باستخدام جهاز GC/MS بعد التخزين وهم:

أ. 4-bromo-2-chlorophenol

ب. O-ethyl-O-(2-chloro-4-bromo-phenyl)-phosphate

ج. O-(4-bromophenyl) O-ethyl S-propyl phosphorothioate