EFFECT OF STORAGE CONDITION ON THE STABILITY OF PHYSICOCHEMICAL AND PROPERTIES OF DIAZINON INSECTICIDE FORMULATION AND ITS RESIDUE IN *OREOCHROMIC NILOTICUS*

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Abstract

The present study was undertaken to investigate the stability of a diazinon insecticide formulation (diazinon 60 % active ingredient EC) under different storage conditions at the temperatures 25°C, 54°C and sunny place for 14 days, and 72°C for 3 days, the persistence of active ingredient % of used insecticides was affected by storage condition and periods. The storage at room temperature for 14 days is not affected while storage at 72 ºC was the most effective in the chemical decomposition., with the residue analysis of diazinon insecticide in liver , gills and muscle flesh of fish at acute toxicity with ½LC50 (2.99 ppm) after 3,5, and 7days of treatment, and with chronic toxicity treatment at 1/10 LC50 (0.6 ppm) with air & without air in the aquaria after 7,14,21,28,35, and 42 days of treatments.

INTRODUCTION

Pesticides may fail to comply with the FAO/WHO meting specifications (2002) required if is improperly stored. Chemical and physical instability usually lead to the deterioration of the active ingredient content and emulsion stability under variable climatic conditions as well as several cases (El-Shemy *et al.,* 1992, El- Deeb *et al.,* 1991, Emara and Abdel Aziz 2007, and Radwan *et al.,* 2007). In addition, diazinon insecticide formulation may become concentrated in the organs of aquatic organisms, especially these at the top food chain. Diazinon commonly used for the control of agricultural pests in Egypt. Several publications revealed the existence of pesticide residues in various aquatic ecosystems were studies by several investigators (Radwan and Atalla 2005, Radwan and El- Said 2006, Radwan and Atalla 2008). The different components within diazinon formulation were identified, active ingredient percentage and finger print determination by using GC/MS to indicate any degradation in the active ingredient, FT-IR to indicate for any disappearance of function groups.

MATERIALS and METHODS

Chemicals

Diazinon formulation (diazinon 60 % EC) lot no.1 production date (Sept.2007) in Fig (1), which obtained from Syngenta- Switzerland Company. Chemical name: O, O-diethyl o-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate.

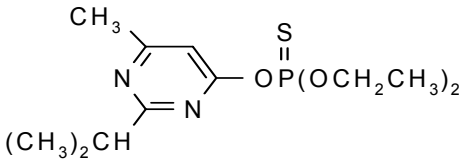

Fig. 1.

Sample of diazinon formulation was stored in glass package at room temperature and in sunny place for 14 days, in the oven at 54ºC ± 2ºC for 14 days according to FAO specifications (1988), and at 72 ± 2ºC for 3 days.

Experimental animals

Healthy of fresh water Oreochromis niloticus (weight 82.05±6.34g, length 13.6±0.43cm.)Purchased from the farm of the Central Research of Fish Laboratory-Abbasa, Sharkia governorate and boroughs to laboratory where acclimatized for 42 day under laboratory conditions. Physicochemical characteristics of the used water were analyzed PH (7.44±0.048), temperature (21.4±0.79 ° C), Electrical conductivity (342.601±2.292µm ho/cm), Salinity (0.10±0.001 ppt) and Total hardness (229.58±3.93 mmol/l as CaCO3). Feeding was continued (1.5% B.wt) over the course of the studies.

(I) Physicochemical parameters

(A) Active ingredient percentage determinations

The active ingredient percentage of diazinon formulation was determined before and after storage by high performance liquid chromatography (HPLC) instrument according to CIPAC hand book (1980).

HPLC conditions

High performance liquid chromatography instrument (Agilent serial 1100) was used under the following conditions as show in table (1) and figure (2).

Table 1. The condition for determination of diazinon by HPLC.

Pesticide name	Mobile phase	Flow rate ml/min	Retention time	Detection limit µg/kg (ng)
Diazinon	Methanol 70 Acetonitrile 30	1	3.2	5

Analysis of diazinon was carried out with HPLC. Duplicate injection (2µl.) of calibration solution and each sample were injected and integrated areas for each peak were recorded and standard peak under ideal condition for diazinon.

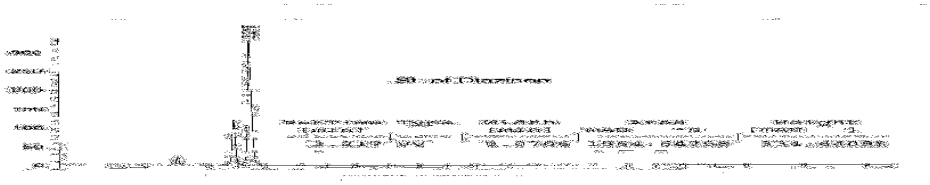

Fig 2. Shows chromatogram of diazinon standard.

(B) Absorbance of diazinon formulation in infra red (IR spectra)

The Fourier transform infrared (Avtar 330 Thermo Nicolet) was used to study the effect of storage on the absorbance of function groups and finger print of organophosphorus insecticide formulations according to the method of Barbra (1985) with some modification. Samples were prepared by homogenized 0/01g of sample with 0.1 g of dry (KBr)by agate mortar and pests to a clean stainless steel slide and placed in piston to make a clear and thin film of desk sample.

(C) Separation and fragmentation of diazinon insecticide formulation by HPLC equipped with a mass spectrometric detector (GC/MS spectra)

The GC/MS analysis was used to compare the separation and fragmentation of pesticide formulations before and after storage according to the method of Saad *et al.,* (1993). GC/MS analysis was performed with an Agilent 6890 gas chromatograph equipped with a mass spectrometric detector (MSD) model Agilent 5973. Afused silica capillary column (HP-5MS), 5% phenyl polysiloxane as non polar stationary phase (30m x 0.25mm i.d) and 0.25µm film thickness.

Operating condition was as follows

Injector port temperature, 250ºC**.** The helium was used as carrier gas at a flow rate of 1ml/min. Pulsed splitless mode. The column temperature was maintained at 80 ºC, for 3min. Then, programmed at 8 ºC/min to 260 ºC, and held for 20min. The total

analysis time was 43min. A1µl volume was injected splitless. The mass spectrometric detector (MSD) was operated in electron impact ionization mode**,** scanning from m/z 50 to 550. The ion source temperature was 230 ºC and the quadrupole temperature 150 ºC. The electron multiplier voltages (EM voltage) was maintained 1100 V above autotun, and solvent delay of 3 min was employed. The instrument was manually turned using heptacosa fluoro tributyl amine (PFTBA).

(D) Physical properties

Emulsion stability test

Five ml of each sample before and after storage was added to graduated 100 ml cylinder filled with 95ml hard water (prepared according to CIPAC MT36) by means of pipette, and then pour the samples onto the water directed to the center. Stopper the cylinder and invert it for 30 times, and then placed in a water bath maintained at 30 ºC±1 for 30min. If there is any forming of oily or creamy layer either at the top or the bottom of the cylinders must be not exceed than 2ml according to WHO (1985 and 1979).

(II) Residue analysis

(a) Extraction of diazinon insecticide

Fish samples (1g) of liver with 10 ml of acetone and 100 ml of acetone to (50g) fish Oreochromis niloticus muscle flesh and gills were added and blended in warring blender at high speed centrifuge for 2 min and partition with dichloromethane (Mills *et al*., 1972).

(b) Clean up

The resulting extracts of fish tissues were cleaned by activated florisil using elution solvent system of 50% dichloromethane, 48.5% n- hexane and 1.5% Acetonitrile (Mills *et al.,* 1972). The pesticide extracts were evaporated at 30 C° to dryness. After clean up the diazinon extract dissolved in 1ml methanol to High Performance Liquid Chromatography (HPLC) analysis with UV detector and C18 stainless column 25 mm. The HPLC conditions for the determination of diazinon were recorded in table (1) and figure (2).

Statistical treatment of the results

Results are expressed as mean ± standard error (SE.) the statistical significant of the difference between control and insecticide treated fish by the student's ˝T˝ test (Gad& Weil 1989).

RESULTS AND DISCUSSION

Effect of storage temperatures on chemical properties

(A) Effect of storage temperatures on active ingredient percentage in diazinon insecticide formulation

The data summararized in Table (2) showed that persistence of active ingredient % of tested insecticide was affected by storage condition and exposure periods. The data indicated that diazinon stored at room temperature for 14 days was stable while storage at 72 ºC accelerated the chemical decomposition whereas the diazinon active ingredient percentage was represent a 59.6% of the zero time sample.

(B) Effect of storage temperatures on the absorbance of diazinon insecticide formulation in infrared

The IR spectrum analysis of diazinon insecticide characteristic by the presence of peaks between 2871 cm-1 and 2929 cm-1 supported the present of methyl group (-CH3, -CH2 and CH), also P=S group was characteristic by IR between 580-750 cm-1 and P-O-C was characteristic between 970-1050 cm-1.The infrared spectrum of diazinon analysis and effect of different type of storage on the absorbance is presented in Table (3). Characterized the structure of diazinon was appeared bands of nitrogen (N)atom at 3410.68cm-1 and shifted about -12.45, 11.86, and -31.52 after storage in sunny place, at 54ºC and 72ºCand results showed that the percentage of match were 97.31, 97.72 and 95.54 %, respectively.

(C) Effect of storage temperatures on separation and fragmentation of diazinon insecticide formulation by GC/MS

The results summararized in Table (٤) and Fig (3) showed that the diazinon formulation give the same separation compound before and after different type of storage.

The effect of storage temperatures on physical properties (Emulsion test)

The data presented in Table (٥) indicated that the formulation of diazinon insecticide passed successfully through emulsion test in different types of storage and comply with WHO specifications (1979) except in storage at 72ºC the cream layer (2.ml) was appeared. Similar results are obtained by El-Badry and Emmara (2006) and Emmara and Abd el Aziz (2007).

Table 2. Effect of storage temperatures on active ingredient percentages in diazinon emulsifiable concentrate

Type of storage	Period of storage	Diazinon formulation	
	Time (day)	Active ingredient %	% degradation
Room temp. 25ºC	Before 1 hour of storage	59.60	0.39
	14	59.60	0.39
Sunny	14	59.53	0.78
54 ºC	14	59.31	1.17
72 ºC	3	58.78	2.03

All values are a mean of three replicates of samples.

Table 3. The effect of storage temperatures on finger print of formulated diazinon by using IR spectrum

Position of bands Cm-1				
diazinon	Room temp.	Sunny place	54 °C	72°C
505.66	505.66	505.65	505.77	506.18
539.27	539.27	539.41	539.29	539.38
692.46	692.46	692.45	692.49	692.52
744.80	744.80	744.75	744.92	744.95
770.99	770.99	770.99	771.30	771.13
832.4	832.42	832.40	832.24	832.24
981.19	981.19	981.06	981.13	980.94
1024.42	1024.42	1024.09	1024.21	1025.81
1160.44	1160.44	1160.56	1160.41	1160.73
1294.01	1294.01	1294.12	1294.12	1251.91
1351.94	1351.94	1351.98	1351.89	1351.95
1381.70	1381.70	1381.80	1381.73	1381.76
1444.30	1444.30	1444.38	1444.03	1444.35
1471.02	1471.02	1471.17	1471.16	1470.99
1495.91	1495.91	1495.92	1495.94	1495.85
1516.70	1516.70	1516.73	(I)	(I)
1561.51	1561.51	1561.17	1561.29	1560.92
1587.55	1587.55	1587.52	1587.52	1587.54
1743.31	1743.31	(I)	1742.61	1742.77
2872.29	2872.29	2872.36	2872.33	2872.15
٢٩٢٩,٥٠	٢٩٢٩,٥٠	٢٩٢٩,٨٥	٢٩٢٩,٧٦	٢٩٢٩,٨٣
٣٤١٠,٦٨	٣٤١٠,٦٨	٣٤٢٣,١٣	٣٣٩٨,٨٢	٣٤٤٢,٢٠
Match	100	٩٧,١٣	٩٧,٧٢	95.54

(I) = Disappearance band.

Table 4. Separation and fragmentation of diazinon insecticide formulation.

Type of storage	RT	Expected compound name	Formula	MW
Diazinon (Initial)at one hour before storage	4.74	1,3,5-trimethyl benzene	C9H12	120
	10.88	2-methylnaphthalene	C11H10	142
	11.15	1-methyl naphthalene	C11H10	142
	24.64	Triazophos	C12H16N3O3PS	313
In sunny place (14day)	Like completely			
Room temp. 25ºC (14day)	Like completely			
54ºC (14day)	Like completely			
72ºC (3day)	Like completely			

Like completely = give the same separation compound before and after storage.

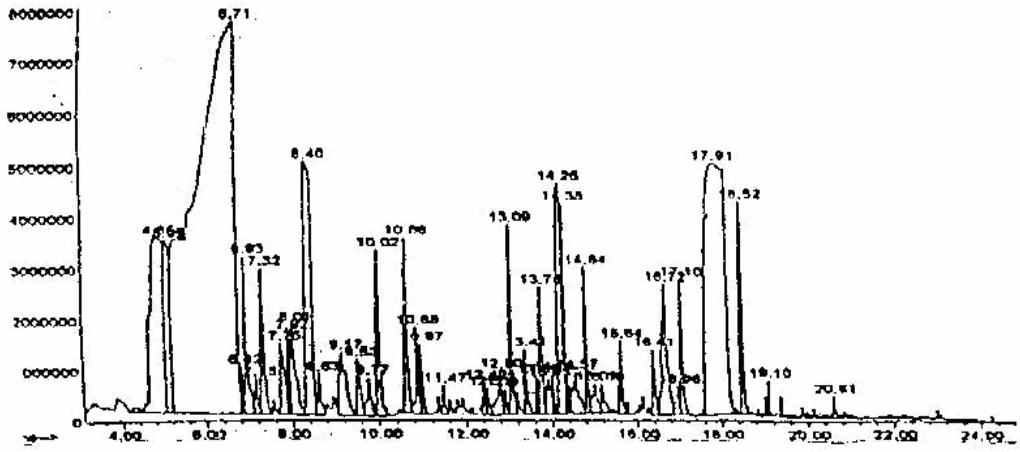

Fig. 3. GC/MS chromatogram of diazinon formulation before storage condition.

Table 5. The effect of storage temperatures on emulsion stability of diazinon formulation.

Type of storage	Period in days	Cream separation (ml)
Initial time	One hour before storage	……
25ºC	14	……
In Sunny place	14	……
54 ºC	14	……
72 ºC	3	2

Residues analysis of diazinon formulations in *O. niloticus*

(A) Acute toxicity treatment

The results in Table (6) exhibit that the residual analysis of diazinon formulation insecticide with 1/2 LC50 (2.99 ppm) in liver gills and muscle flesh of fish. After 3 days, the residues levels were 0.9164, 1.4889, and 1.1165 µg/g wet tissues respectively and were 1.2015, 1.9470, and 1.2095 µg/g wet tissues after 5 days of treatment and while the residues after 7 days were 1.2849, 2.6969, and 1.2849 µg/g wet tissues in liver, gills and muscle flesh respectively.

(B) Chronic toxicity treatment

The results in Table (7) exhibit that the residue analysis of diazinon insecticide with 1/10 LC50 (0.6 ppm) in liver , gills and muscle flesh of fish live without air and with air at 7, 14, 21, 28, 35, and 42 days after application. After 7 days the residues levels in liver , gills , and muscle flesh of fish which live without air during period of treatment were 0.1679, 0.6487, and 0.5941 µg/g wet tissues and were 0.2413, 0.6911, and 0.6214 µg/g wet tissues after 14 days of treatment and were 0.3678, 1.0080, and 0.8264 µg/g wet tissues after 21 days of treatment and were 0.4374, 1.0955, and 0.8377 µg/g wet tissues after 28 days of treatment and were 0.7845, 1.1045, and 0.8845 µg/g wet tissues after 35 days of treatment and were 0.8085, 1.1369, and 1.0994 µg/g wet tissues after 42 days of treatment in liver , gills and muscle flesh of fish respectively. While the residues of diazinon after recovery period for 7 days were 0.6974, 1.0891, and 1.0093 µg/g wet tissues and were 0.5845, 0.9113 and 0.8933 µg/g wet tissues in liver, gills and muscle flesh of fish in fish which live without air during treatment period.While the residues of diazinon with 1/10 LC50 (0.6ppm) in liver, gills and muscle flesh of fish which live with air during treatment period. After 7 days were 0.0699, 0.3070, and 0.1389 µg/g wet tissues and were 0.0700, 0.3779, and 0.1981 µg/g wet tissues after 14 days of treatment and were 0.1236, 0.4968, and 0.2074 µg/g wet tissues after 28 days of application and were 0.1474, 0.5265, and 0.3879 µg/g wet tissues after 35 days of treatment and were 0.1609, 0.5728, and 0.5823 µg/g wet tissues after 42 days of treatment in liver, gills and muscle flesh of fish respectively. However, the residues of diazinon after recovery period for 7 days were 0.0936, 0.4347, and 0.5249 µg/g wet tissues and were 0.0361, 0.2869 and 0.4063 µg/g wet tissues after 14 days of recovery period in liver, gills and muscle flesh of fish respectively in fish which live with air during treatment period. The accumulation levels of diazinon in fish tissues were increased by lapse of time of treatment. On contrast, the residue levels of diazinon in tissue were decreased in the recovery period after 14 days more than 7 days after recovery period. The residues levels of diazinon in fish

tissues, which live without air more than the residue levels in fish tissues, which live with air during the treatment period. The high uptake and penetration within tissues of organ phosphorus insecticides via integument of Tilapia fish was also observed by (El-Sheamy *et al.,* 1991),and Radwan & El-Said 2006, who investigated the residue levels of the tow formulations in different organs and muscle flesh of *O. niloticus* fish.

Generally, from the previous results a great interest to note the following remarks. Storage at 72ºC for 3 days was the most effective in chemical and physical properties for diazinon insecticide formulation. The diazinon formulation must be storage away from high degree of temperature (72ºC) to avoid the deteriorates effects on the physical and chemical stability of diazinon insecticide formulation.

Table 6. Residues analysis of diazinon in different tissues of fish *Oreochromis niloticus* after 3, 5 and 7 days of acute (6-ppm) treatment exposure.

Time	Liver	Gills	Muscle
3	0.9164 ± 0.0581	1.4889 ± 0.0944	1.1165 ± 0.0708
5	1.2015 ± 0.0762	1.9470 ± 0.1234	1.2095 ± 0.0761
7	1.2849 ± 0.0815	2.6969 ± 0.1710	1.2849 ± 0.0815

Values shown are mean ± S.E.

Table 7. Residues analysis of diazinon in different tissues of fish after 3, 5 and 7 days of chronic (0.6) treatment of exposure and during the recovery period.

Treatments	Without air in aquarium			With air in aquarium		
Tissue / Time	Liver	Gills	Muscle	Liver	Gills	Muscle
7	0.1679±0.0106	0.6487±0.0411	0.5941±0.0377	0.0699±0.0044	0.3071±0.0195	0.1389±0.0090
14	0.2413±0.0153	0.6912±0.0438	0.6214±0.0524	0.0700±0.0045	0.3779±0.0239	0.1981±0.0116
21	0.3678±0.0233	1.0080±0.1087	0.8264±0.0524	0.1237±0.0078	0.4968±0.0316	0.2074±0.0132
28	0.4374±0.0277	1.0955±0.0695	0.8377±0.0524	0.1393±0.0088	0.5234±0.0332	0.2275±0.0144
35	0.7845±0.0561	1.1044±0.0700	0.8845±0.0566	0.1474±0.0043	0.5265±0.0334	0.3879±0.0246
42	0.8085±0.0513	1.1369±0.0721	1.0994±0.0697	0.1609±0.0102	0.5728±0.0363	0.5823±0.0368
Recovery after one weeks	0.6974±0.0531	1.0891±0.0667	1.0093±0.0701	0.0936±0.0335	0.4347±0.0277	0.5249±0.0366
Recovery after two weeks	0.5845±0.0553	0.9113±0.0721	0.8933±0.0562	0.0361±0.0313	0.2869±0.0513	0.4063±0.0246

Values shown are mean ± S.E.

- Our recommendation, pesticide formulations mainly insecticides must be storage away from high degree of temperature (72°C) to avoid the bad effects on the physical – chemical stability for majority of insecticides formulations.
- From the pervious study we can conclude that the residues of diazinon in two treatment (fish live without air and with air) was higher than the Acceptable Daily Intake (ADI)(0.002 mg/kg B.wt.) in fish tissue

REFERENCES

1. Barbara, S. 1985. Modern infrared spectroscopy published on behalf of ACOL(University of Greenwich) by John Wiley &Sons Chi Chester, New York. Brisbane .Toranto. Singaore.
2. CIPAC Hand book. 1980. Analysis of technical and formulated pesticides. Volume 1A, P. 1203.
3. El- Badry , B. M. and Ola, M. Y. Emmara. 2006. Comparative studies on the stability of methomyl, carbofuran and abamectin form different companies under environmental factors. *J. Pest .Cont. & Environ. Sci. 14(2):207-225.*

4. El- Shemy, M. K., S. H. Youssef, Magdoline A. Saman and Y. S. Ibrahim. 1992. Stability of local, pesticide formulations under storage conditions. *Egypt. J, Appl. Sci., 7(8), 93-106.*
5. El-Deeb ,W. H., M.K. El-Shemy and Y. S. Ebrahim. 1991. Effect of storage temperature on the stability, biological activity and phytotoxicity of certain insecticides. Fourth Arab congress of plant protection, Cairo 1-5 Dec., P.399-403.
6. Emmara, Ola M. Y. and Shereen A. Abd El- Azziz. 2007. Effect of storage temperature on the stability of chemical and physical properties of certain local organ phosphorus insecticide formulations. *Egypt. J, Appl. Sci., 22(2B), 813-828.*
7. FAO/WHO Meeting. 2002. The tolerance for formulated products. Manual on development and use of FAO and WHO specifications for pesticides. First Edition, p.31.
8. FAO specifications. 1988. Insecticides emulsifiable concentrates, storage stability 54 ± ºC for 14 days p.19, 20.
9. Gad, S. C. and C. S. Weil. 1989. Statistics for toxicologist principles and methods of toxicology.Hayes, AW. (Ed) 2nd ed. Raven Press. Ltd., New York, PP.435-483.
10. Mills, P. A., A. B. Baraka, R.K.L. Verene and A. B. Jerry. 1972. Elution solvent systems for florisil clean up in organochlorine pesticide residue analysis. JAOAC, 5:39-43.
11. Saad M. M. Ismail , H. M. Ali and Habiba A. Ramadan. 1993. GC-ECD and GC/MS Analysis of profenfos residues and its biochemical effects in Tomatoes and Tomato products*. J. Agri. Food. Chem. 41: 610-615.*
12. WHO. 1985. Specifications for pesticides used in public health. (6th edition), p.374.
13. WHO. 1979. Specifications for pesticides used in public health, p. 116 Geneva, Switzerland.
14. Radwan, O. A. and I. E. Attalla. 2005. Monitoring of pesticide residues in drainage water and fish samples collected from different governorates, *Egypt. Bull. Fac. Agric., Cairo Univ., 56: 189-200.*
15. Radwan, A. Olfat, Y. W. A. El-Sheikh, A. A. El-Badawi and M. Salah. 2007. Physical finger print and biochemical studies on the insecticide ((lufenuron)) against the Nile tilapia, *Oreochromis niloticus. J. Biol. Chem. Environ. Sci. Vol.2 (1): 331-347.*
16. Radwan, A. Olfat and I. E. Attalla. 2008. Monitoring of some pesticide residues in water and fish tissue samples collected from three locations at Sharkia governorate, Egypt *J .Biol. Chem. Environ. Sci. Vol.3 (1): 583-597.*

17. Radwan, A. Olfat and M. M.El-Said. 2006. Biochemical studies on residues of two different formulations of profenfos insecticide in *Oreochromis niloticus* Egypt *J .Biol. Chem. Environ. Sci. Vol.1 (3): 491-519.*

تأثير ظروف التخزين المختلفة على الثبات الكيميائى و الخواص الطبيعية لمستحضر الديازينون الحشرى ودراسة المتبقى فى أسماك البلطى النيلى

ألفت عبد اللطيف سيد رضوان

قسم بحوث تحليل المبيدات - المعمل المركزى للمبيدات - الدقى- الجيزة

اشتملت هذه الدراسة على معرفة ثبات مستحضر مبيد الديازينون ٦٠% مادة فعالة تحت ظروف تخزين مختلفة على حرارة ٢٥ , ٥٤ درجة مئوية وفى ضوء الشمس لمدة ١٤ يوم وعلى ٧٢ درجة مئوية لمدة ٣ أيام و كانت النتائج ان المستحضر ثابت كيميائيا من حيث نسبة المادة الفعالة وان الأنخفاض فى الحدود المسموح بها من الخطأ التجريبى و أكد ذلك التحليل بأستخدام جهاز التحليل الكروماتوجرافى الغازى المتصل بمطياف الكتلة GC/MS كما أن المستحضر كان ثابت فى اختبار الأستحلاب كأحد أهم الأختبارات الطبيعية للمستحضر.بالاضافة لذلك اشتملت الدراسة على تقدير المتبقى من المادة الفعالة فى كبد و عضلات و خياشيم الأسماك (البلطى النيلى) التى تعرضت لهذا المستحضر فى دراسة للسمية الحادة 1/2LC50 (2.99 ppm) بعد 3,5,7 ايام من المعاملة والسمية المزمنة بعد ٧و١٤و٢١و٢٨و٣٥و٤٢ يوم من المعاملة بتركيز LC_{50} 1/10 (0.6ppm)من هذا المستحضر على الأسماك المرباة فى أحواض يمر بها الهواء وفى أحواض لم تدعم بمرور الهواء خلال المعاملة.