RESULTS & DISCUSSION

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A- Characteristics of TBTI-MMA Formulation :

The prepared copolymer of TBTI with MMA was soluble, colorless, transparent, and suitable for film formation. Films were prepared from the purified TBTI-MMA copolymer solution (50% in toluene) on glass, tin, and PVC plates and left at room temperature to complete drying for evaluation and testing, according to Gardner and Sward (1962), for hardness (pendulum), adhesion, elasticity, resistance to cold and hot water, and resistance to dilute alkaline and acid solutions. The experimental conditions and testing results are summarized in Table (1).

From Table (1) it is clear that the copolymer of TBTI with MMA had good film properties.

The infrared and proton magnetic resonance spectra of the copolymer are illustrated in Fig. (3) and (4), respectively.

B- Bioassay of the TBTI-MMA Copolymer Formulation:

The TBTI-MMA copolymer formulation was applied against 3rd instar larvae of Culex pipiens under laboratory conditions.

The susceptibility of Culex pipiens to the toxic effect of the TBTI-MMA copolymer formulation was evaluated through carrying out a series of laboratory experiments.

TABLE (1) : Film properties of TBTI-MMA copolymer

Test	Result
Molar ratio of TBTI:MMA	19.91 : 80.09
Tin content (%)	21.44
Thickness (um)	105
Hardness (second)	95
Adhesion	good
Elasticity	good
Water resistance	
- hot water	not affected
- cold water	not affected
Alkali resistance	
- 5% NaOH solution	damaged
- 5% Na_2CO_3 solution	damaged
Acid resistance	
- 5% H_2SO_4	not affected

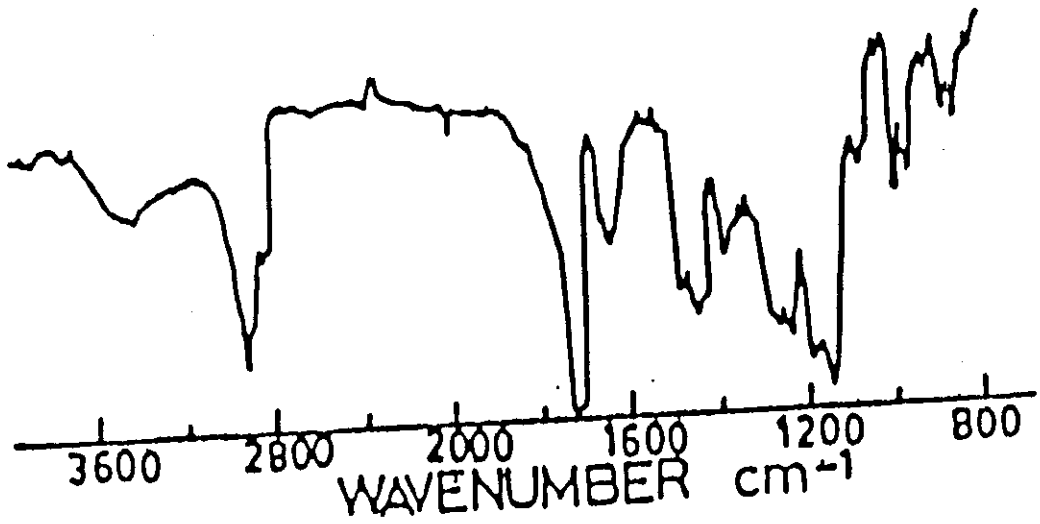


Fig. 3. I.R. spectrum of the copolymer.

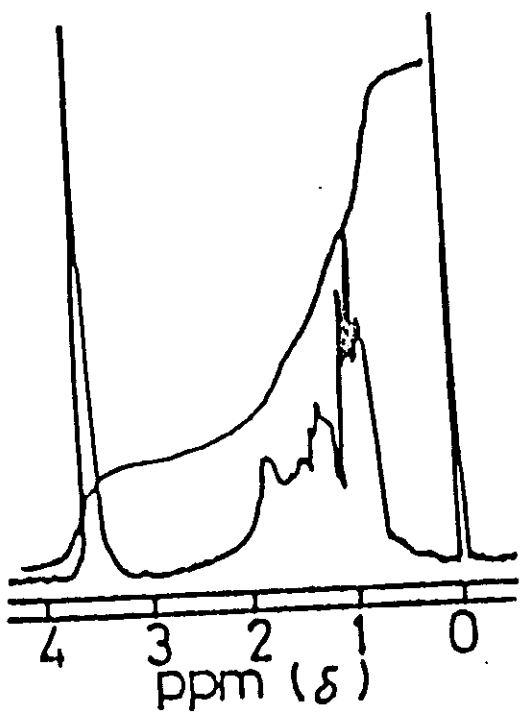


Fig. 4. ^{1}H N.M.R. spectrum of the copolymer.

Mortality records after each week of treatment were corrected using Abbott's formula.

Group of larvae was treated with the copolymer plate while, another one was left without any treatment and served as control. The results obtained from these experiments were as follows :

a. Experiment 1: (Accumulated Toxicity)

When water was not changed and only the larvae were renewed weekly, the control larval mortality ranged from 2% to 20%. The mortality of the treated group is presented in Table (2) and graphically illustrated by Fig. (5). The data showed that toxicity of TBTI-MMA formulation on the larvae of C. pipiens reached 34% on the second week after treatment, then increased rapidly by the 4th week to about 80% and decreased again to 38% in the 5th week. A marked gradual increase in larval mortality was noticed till it reached 100% in the tenth week. After the 10th week the plate was removed out of the pan and normal weekly addition of the larvae was continued. The larval mortality persisted at 100% for more than four weeks after which it declined again to 78% in the 18th week and to 40% in the 20th week. The toxicity of the released compound seemed to vanish completely starting from the 24th week.

The statistical analysis of the data in Table (2) revealed the following :

TABLE (2) : Effect of accumulated toxicity of TBTI-MMA formulation on the larval mortality of Culex pipiens. (3 replicates)

Time (weeks)	%Average larval mortality*±S.E.	Time (weeks)	%Average larval mortality*±S.E.
1	36 ± 0.048	14	100
2	34 ± 0.047	15	94 ± 0.023
3	54 ± 0.049	16	90 ± 0.030
4	80 ± 0.040	17	80 ± 0.040
5	38 ± 0.048	18	78 ± 0.041
6	52 ± 0.050	19	64 ± 0.048
7	78 ± 0.041	20	40 ± 0.049
8	90 ± 0.030	22	6 ± 0.023
9	98 ± 0.014	24	0
10	100	26	0
11	100		
12	100		
13	100		

* Corrected from control.

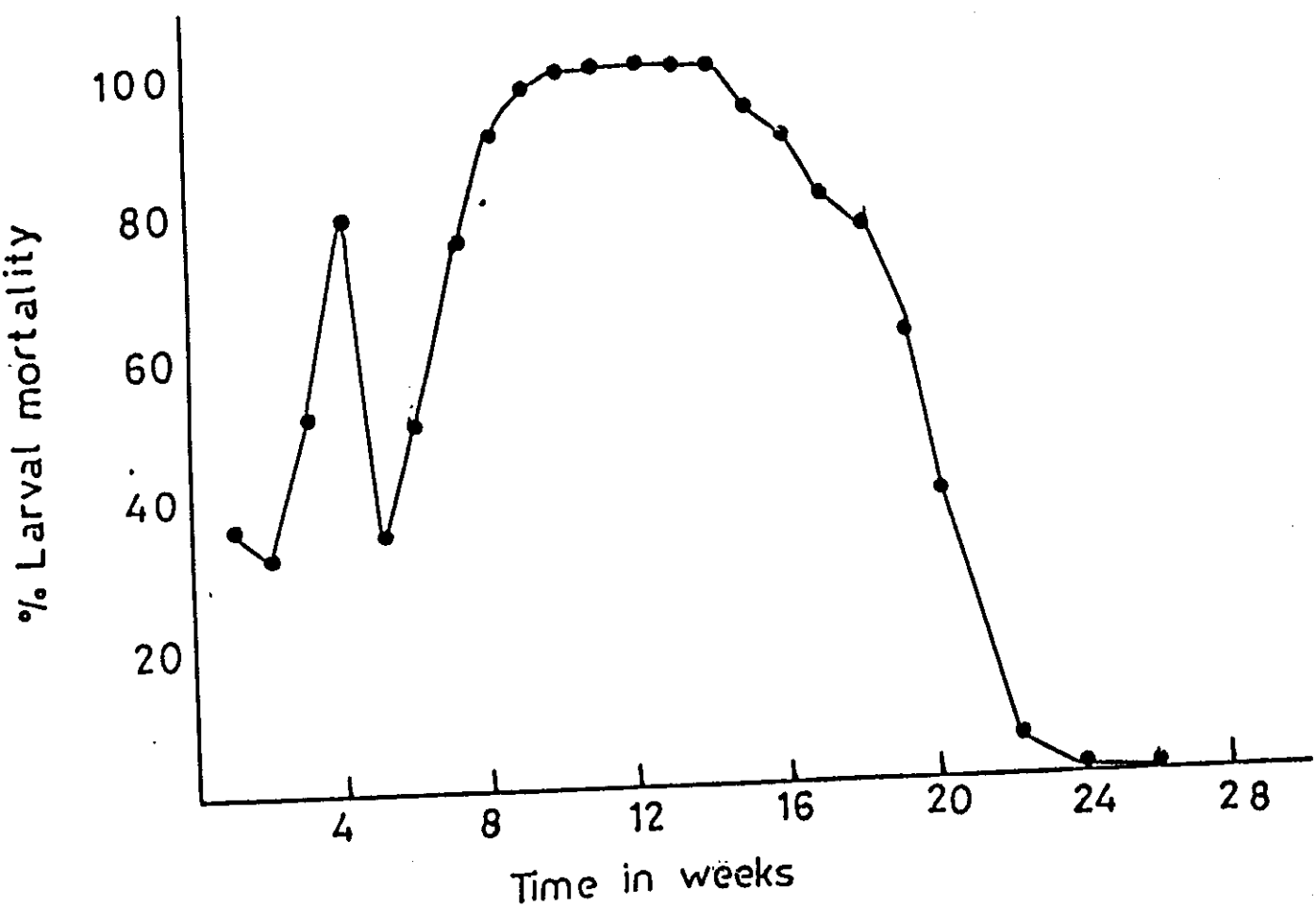


Fig. 5. Effect of accumulated toxicity of TBTI-MMA formulation on the larval mortality of Culex pipiens.

Significant increase in larval mortality was noticed till it reached to 100% in the tenth week. This means that the toxic compounds released from the copolymer had a rate of release more than their rate of degradation and accumulated in the water.

Significant decrease in larval mortality from 100% in the tenth week to 40% in the 20th week was noticed. Also, significant drop in larval mortality from 40% in the 20th week to zero percent in the 24th week was observed. This means that the degradation of the released toxic compound from the copolymer formulation to non-toxic compound seemed to be completed after 10 weeks of removing the plate from the experimental aquatic medium.

The data indicated that the high mortality occurred in the 10th - 14th week when the water was still clear. The low mortality in the 20th week may be attributed to accumulation of food organic matter in the water which normally reduced the toxicity of the compound.

A similar result was observed by Boike and Rathburn (1973) in laboratory tests on tributyltin oxide which was highly toxic to the larvae of both Aedes and Culex species in clear tap water,. However in brackish water or tap water containing organic debris, results were variable but generally poor against larvae.

Another reasonable explanation is that the amount of the

toxic compounds remaining after removal of the plate began to show gradual degradation from the 15th week and complete degradation to the non toxic compound occurred in the 24th week. This result is in agreement with that stated by Cardarelli (1977).

b. Experiment 2 : (Weekly Release)

Fig. (6) and Table (3) illustrate the effect of the weekly released toxicity of TBTI-MMA formulation on the larval mortality of the mosquito when both water and larvae were renewed weekly.

The control larval mortality ranged from zero to 30%, while it was noticed that the mortality of the treated group showed continuous fluctuations throughout the whole experimental period, up to the 24th week, then gradual decreases were till the end of the experiment in the 28th week.

Statistical analysis of the data in Table (3) showed significant differences in the percentage corrected mortalities during most weeks.

The fluctuation in the weekly larval mortality that was observed throughout the whole experimental period may be attributed to continuous weekly water change. Similar findings were reported by Cardarelli (1978) who stated that extreme hydrophobicity of both TBTI and TBTF resulted in rapid loss of the agent, through adsorption and absorption by organic matter. When Barnes et

TABLE (3) : Effect of weekly released toxicity of TBTI-MMA formulation on the larval mortality of Culex pipiens. (3 replicates)

Time (weeks)	%Average larval mortality*±S.E.	Time (weeks)	%Average larval mortality*±S.E.
1	52 ± 0.050	13	32 ± 0.046
2	30 ± 0.046	14	74 ± 0.044
3	32 ± 0.047	15	36 ± 0.048
4	28 ± 0.045	16	64 ± 0.048
5	62 ± 0.048	17	44 ± 0.050
6	66 ± 0.047	18	34 ± 0.047
7	10 ± 0.030	20	32 ± 0.046
8	22 ± 0.041	22	28 ± 0.045
9	12 ± 0.032	24	30 ± 0.046
10	16 ± 0.037	26	20 ± 0.040
11	26 ± 0.044	28	12 ± 0.033
12	24 ± 0.043		

* Corrected from control.

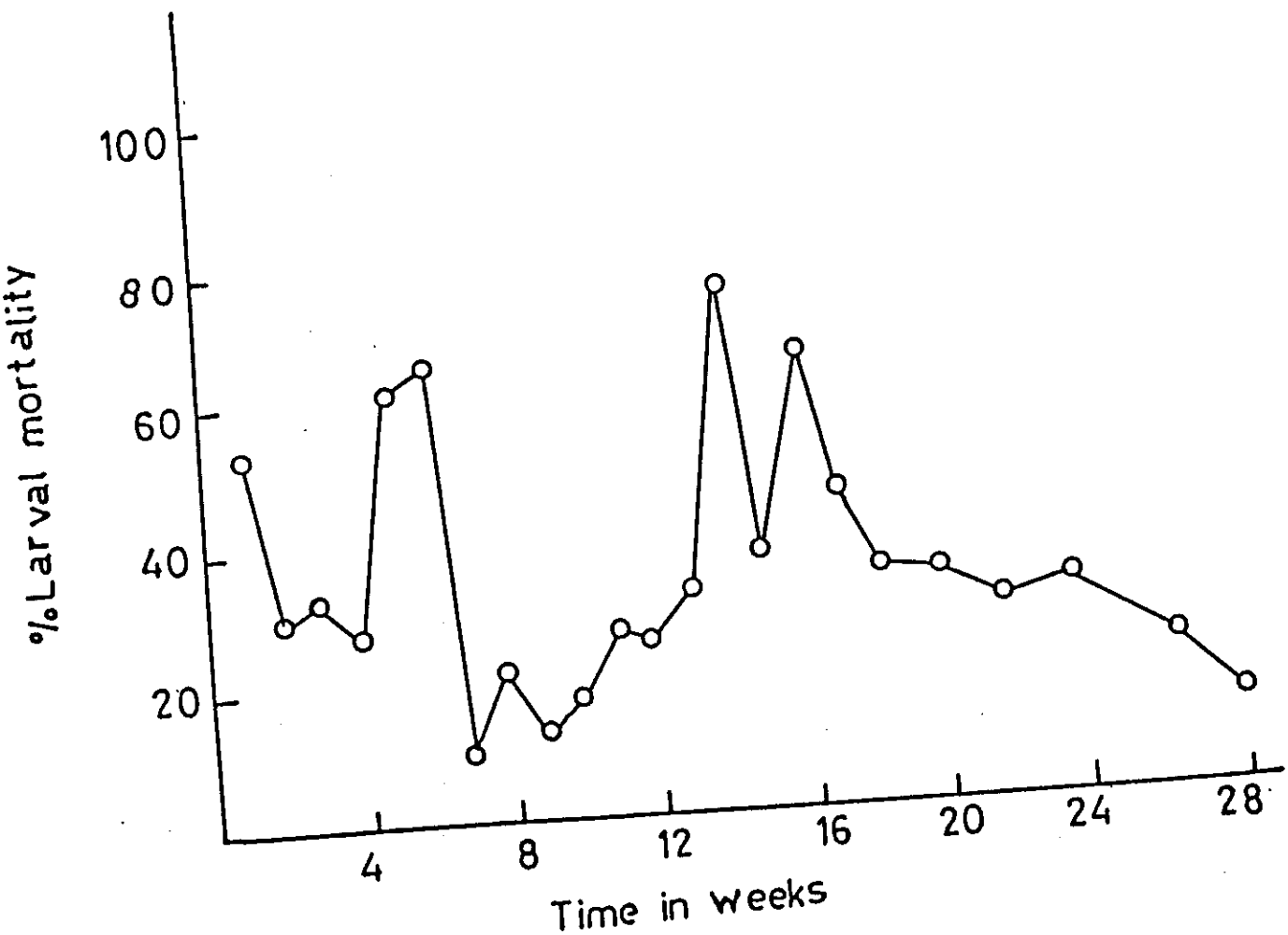


Fig. 6. Effect of weekly released toxicity of TBTI-MMA formulation on the larval mortality of C. pipiens.

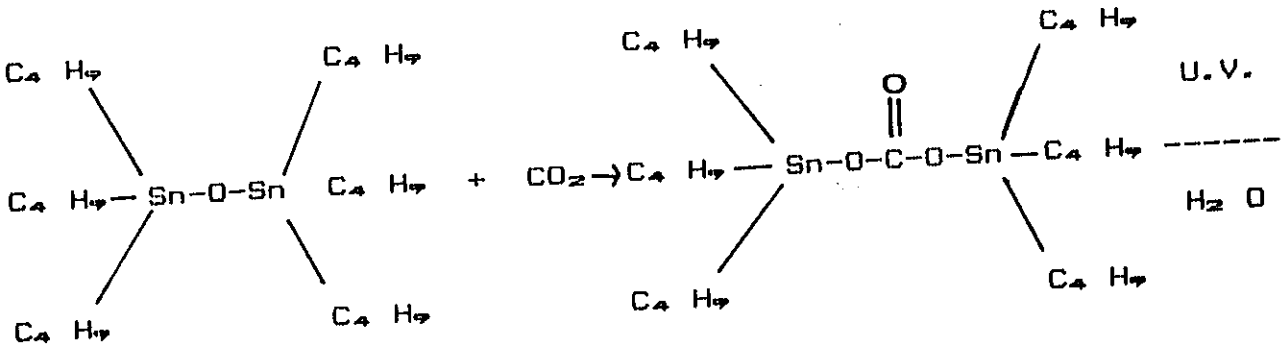
al., (1967) exposed impregnated briquettes with malathion to a second water change, the malathion-concrete formulation failed to give good control of the mosquito larvae.

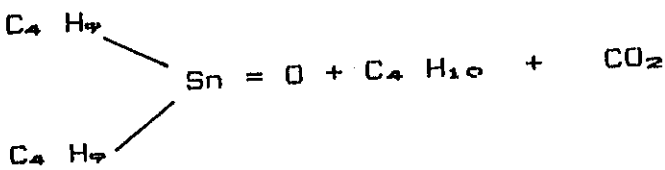
Pupation of the larvae exposed to the copolymer formulation was in all weeks less than that in the control group, total pupation averaged 65-67%. Only 30% of those pupae emerged normally, 35% could't complete their emergence or had malformations, and the rest of the pupae died befor emergence.

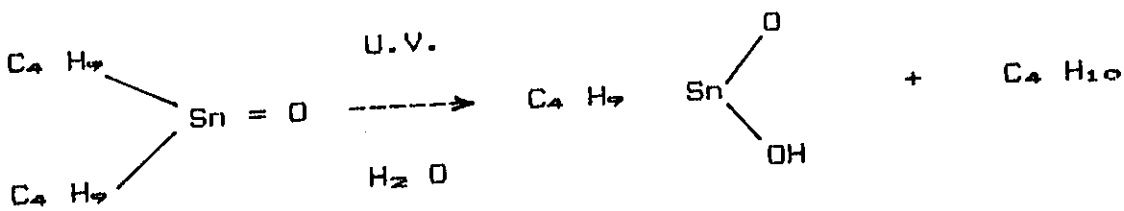
c. Experiment 3 : (Degradation of the Released Toxic Compound)

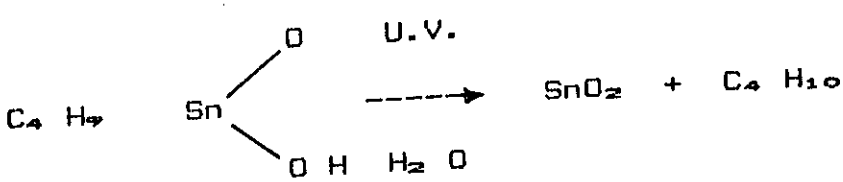
It has been hypothesized that TBTO in water, where some amount of carbon dioxide is present, will slowly convert to carbonate salts (Engelhart and Sheldon, 1975).
Suspended matter in water readily absorbs TBTO, reducing its toxicity (Paulini and Da Sauza, 1970)

Hypothetically, degradation of TBTO arising from homolytic cleavage and dealkylation under ultraviolet radiation may follow the following scheme (Sheldon, 1974).









Moreover, Cardarelli (1977) reported that TBTO degraded via hydrolytic cleavage and when exposed, photolytic cleavage to dibutyltin oxide, butyl-stannic acid and finally the non toxic stannic oxide.

The original run of water in experiment 2 for each week was tested for degradation of the toxic compounds into non-toxic daughter compounds by taking records of weekly larval mortality for several weeks.

Table (4) and Fig. (7) show the results obtained on the degradation of the released toxic compounds in the mosquito larval medium indicated biologically as percentage larval mortality. The data obtained showed that larval mortality increased for a short period (not more than 2 weeks) then decreased again with time till it reached a very low percentage (1-2%) after 8 weeks. This means that the released toxic compounds degraded into the non-toxic daughter compounds in a relatively short time. The calculated half life of the original toxic compounds ranged between 3-4 weeks in the larval medium.

d. Experiment 4 : (Effect of pH on Toxicity)

A copolymer plate was immersed in an alkaline medium (NaOH solution), another plate in an acidic medium (HCl) and a third plate was used as a control (tap water) for the neutral medium. An alkaline and an acidic solution without plates were used as controls for the effects of alkalinity and acidity on larval death.

The results obtained on the effect of pH on the larval mortality are illustrated by Fig. (8) and Table (5). Both the acid and alkaline medium increased the rate of release of the toxic compounds from the formulation and consequently increased the larval mortality in comparison with the neutral medium. However, the alkaline medium seemed to be significantly more effective in enhancing the release than the acidic medium.

TABLE (4) : Degradation of the released toxic compounds from the TBTBI-MMA formulation into non-toxic daughter compounds in the larval medium of Culex pipiens. (3 replicates)

Time (weeks)	%Average larval mortality (corrected from control) ± S.E.
1	42 ± 0.042
2	58 ± 0.049
3	34 ± 0.047
4	16 ± 0.037
5	10 ± 0.030
6	10 ± 0.030
7	6 ± 0.024
8	2 ± 0.014

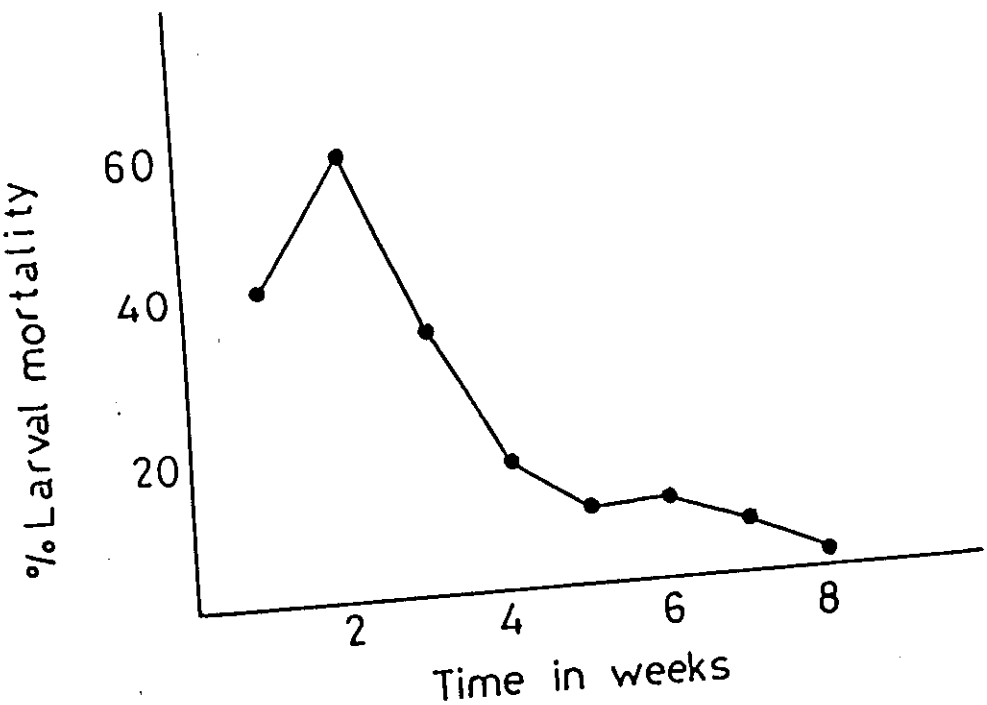


Fig. 7. Degradation of the released toxic compounds from the TBTI-MMA formulation into non-toxic daughter compounds in the larval medium of C. pipiens.

TABLE (5) : Effect of pH of larval medium on the toxicity of the TBTI-MMA formulation to larvae of Culex pipiens. (3 replicates)

Time (days)	%Average larval mortality (corrected from control) ± S.E.		
	pH - value		
	5	7	9
1	18 ± 0.038	6 ± 0.024	74 ± 0.044
2	40 ± 0.049	14 ± 0.035	100
3	64 ± 0.048	20 ± 0.040	-
4	90 ± 0.030	26 ± 0.044	-
5	100	36 ± 0.048	-
6	-	42 ± 0.050	-
7	-	48 ± 0.050	-
8	-	56 ± 0.050	-
9	-	60 ± 0.049	-
10	-	68 ± 0.047	-

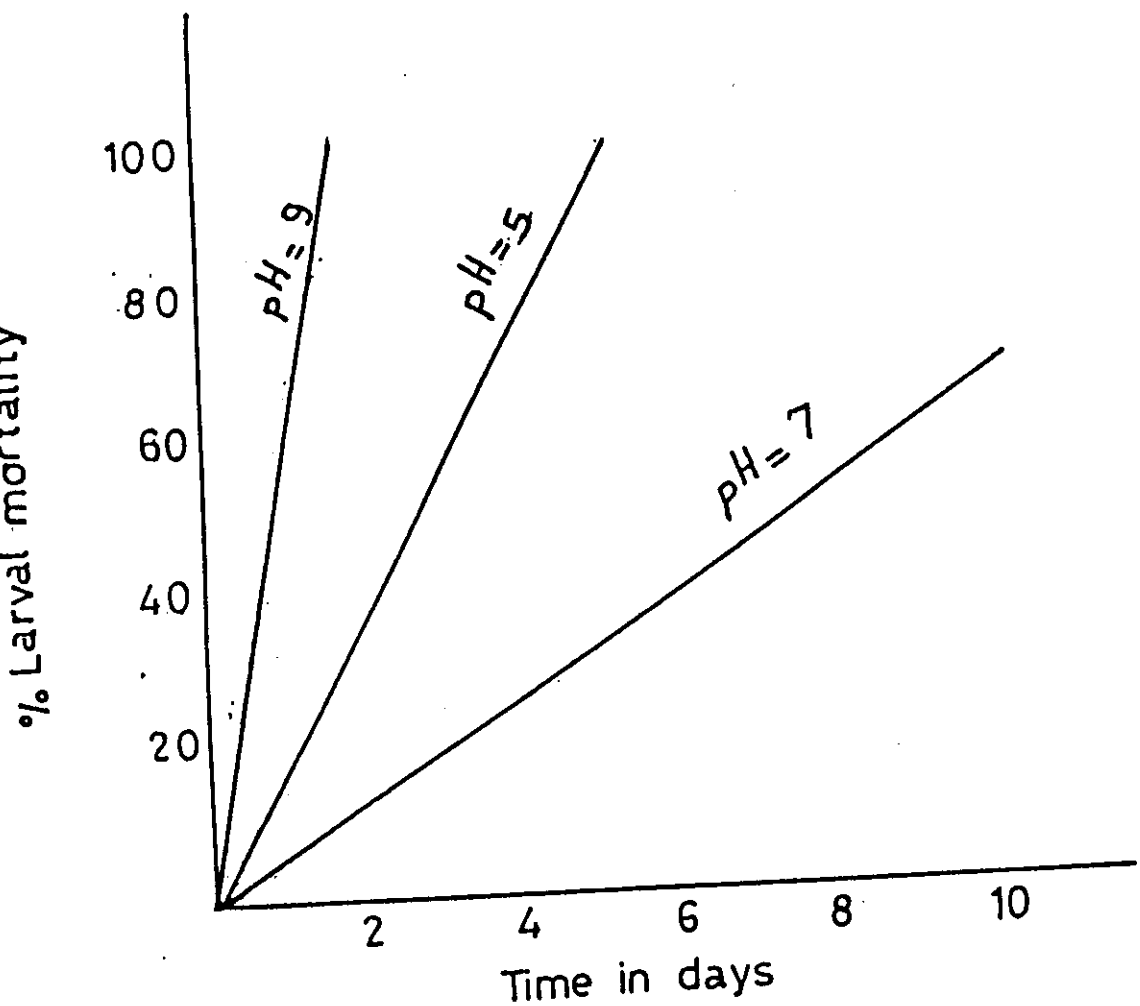


Fig. 8. Effect of pH of larval medium on the toxicity of the TBTI-MMA formulation to larvae of C. pipiens.

In the neutral medium, the larval mortality increased gradually for ten days when only 68% of the larvae were killed and the remaining larvae pupated or were still alive. It was found that pH had no significant effect on larval mortality in the absence of the copolymer formulation.

The dependence of mortality upon water quality was discussed by Cardarelli (1977) on some organotin molluscicides and he attributed it to three sources:

Chemical interaction with or absorption of the molluscicide prior to snail contact and ingestion, change in the release rate arising from the chemical nature of the formulation pellet/water interface; or change in the snail intake rate due to physiological differences within snail tissue arising from the presence of various ions. The same author added that organotin molluscicide loss rate did not show to be affected by pH although it might be somewhat affected by specific inorganic ions.

Quick et al., (1980) reported that controlled-release temephos against Culex pipiens quinquefasciatus may be hydrolysed at high pH for prolonged periods of time. A similar finding was reported by Barnes et al., (1967) who stated that an important short coming of many of organo-phosphorus (OP) has been due to their rapid hydrolysis in water, particularly when exposed to highly alkaline conditions.

e. Experiment 5 : (Effect of Temperature on the Larval Medium)

The TBTO-MMA copolymer formulation was tested against Culex pipiens larvae at two different temperatures (10 and 25°C) . Results of these experiments are shown in Table (6).

From the data obtained it is concluded that low temperature (10°C) decreased or stopped the release of the toxic compound from the copolymer formulation where the average larval mortality in the experimental pan was equal to that in the control one (27%), However, at higher temperature (25°C) the average larval mortality in the experimental pan (40%) was higher than in the control group (11.6%).

Statistical analysis of the data in Table (6) indicated that Culex pipiens larvae are significantly more susceptible to the tested TBTO-MMA copolymer formulation at 25°C and the level of significance was 0.05. In other words, the higher temperature enhances the release of toxicity from the formulation. This result is in agreement with the finding of Stockman et al., (1970) who reported that with decreasing temperatures,there was a corresponding decrease in the release of insecticide from a polymer formulation. Similar findings were reported by Ibrahim (1986) who indicated that Culex pipiens larvae were significantly more susceptible to the tested larvicidal formulation at 25°C ($P \leq 0.05$).

TABLE (6) : Effect of temperature on the toxicity of TBTI-MMA copolymer formulation against *Culex pipiens* larvae.

Period	% Larval mortality (corrected from control)			
	10°C		25°C	
	Treated	Control	Treated	Control
1st week	50	0	80	50
2nd week	40	80	50	0
3rd week	10	0	20	0
4th week	30	50	60	0
5th week	20	30	0	0
6th week	0	0	30	10
7th week	40	30	40	20
Average	27	27	40	11.4
Corrected from control	0	--	32	--

f. Experiment 6 : (Semifield Bioassay of the Formulation)

The results obtained showed that the copolymer formulation continued releasing its toxic compounds up to more than 24 weeks in the natural outdoor conditions where temperature ranged from 10-15°C at night and 18-25°C during the day time.

Table (7) and Fig. (9) show the biweekly larval mortality caused by the copolymer formulation when present in the above mentioned semifield conditions for about six months.

The larval mortality increased significantlly from 42% after 4 weeks to 64, 96, and 100% at the end of the 8th, 12th, and 16th week, respectively.
At the end of the 20th week a slight decrease in larval mortality was observed (92%).

Further significant decreases were noticed at the 20th and 24th weeks, respectively. The experiment was terminated after 25 weeks due to lack of available time. It was expected that toxicity of the copolymer formulation might be continued to more than this period.

TABLE (7) : Effect of the copolymer formulation (TBTI-MMA) on the larval mortality of Culex pipiens when tested at semifield conditions.
(3 replicates)

Time (weeks)	%Average larval mortality*±S.E.	Time (weeks)	%Average larval mortality*±S.E.
2	35 ± 0.048	14	100
4	42 ± 0.049	16	100
6	40 ± 0.049	18	100
8	64 ± 0.048	20	92 ± 0.027
10	82 ± 0.038	22	70 ± 0.046
12	96 ± 0.020	24	40 ± 0.049

* Corrected from control.

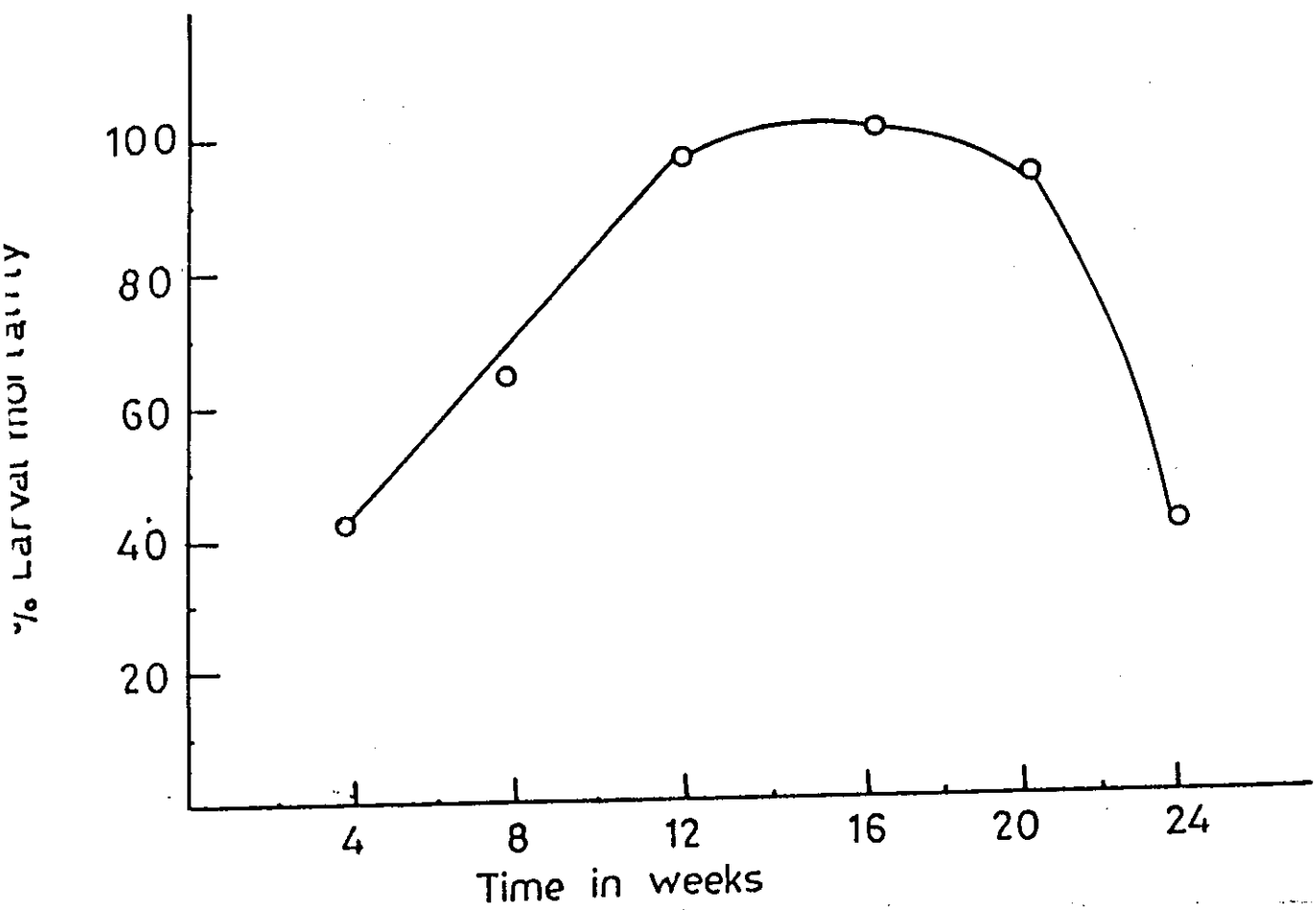


Fig. 9. Effects of accumulated toxicity of TBTI-MMA copolymer formulation on the larval mortality of C. pipiens when present in semifield conditions.

C - Chemical Assay of the TBTI-MMA Copolymer Formulation :

Fig. (10) and Table (8), show the amount of tin released from the formulation when the larval medium was not changed weekly. This amount began from 3 ug/litre in the first week and increased gradually throughout the first 9 weeks, after which this amount was almost constant till the 21th week.

This result was expected since the weekly released TBTO was accumulated in the larval medium until the plate (painted with the copolymer formulation) was removed in the 10th week. The amounts of tin whether in TBTO or its daughter compounds (due to its degradation) were almost the same in the following weeks (10-21th).

Fig. (11) and Table (9), illustrate the weekly released tin in the successive runs of water (when larval medium was changed each week). These amounts showed irregular release from the formulation, giving a series of fluctuations throughout the 21 weeks. This may explain the fluctuation in larval mortalities observed in the bioassay previously described. However, it was observed that the percentage of larval mortalities were not proportional to the amounts of tin released throughout the different weeks of the experiments. This may be explained by the fact that the amount of tin determined chemically is actually the sum of the toxic TBTO and the non-toxic daughter compounds as stannic oxide.

Environmental factors such as water, temperature, shade, water content may affect residue levels. The residues were main-

TABLE (8) : Accumulation of the released tin compounds in larval medium of *Culex pipiens*.

Time (weeks)	Amount of tin (ug/l)	Time (weeks)	Amount of tin (ug/l)
1	3.20	12	09.00
2	3.40	13	09.00
3	2.40	14	11.00
4	3.40	15	05.70
5	2.40	16	12.20
8	5.60	17	08.30
9	9.00	18	10.20
10	9.00	19	06.40
11	9.00	20	08.50

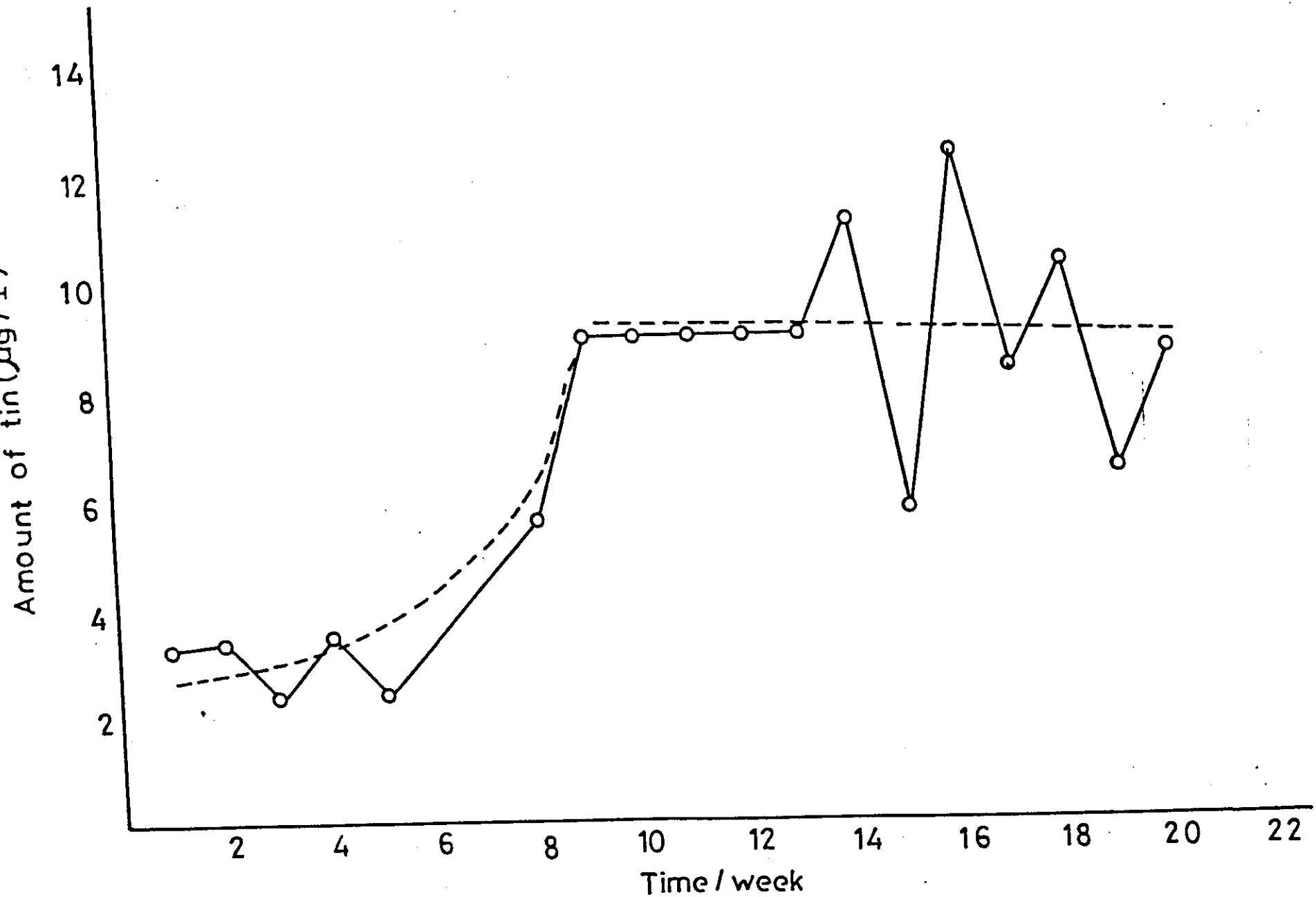


Fig. 10. Accumulation of the released tin compounds in the larval medium.

TABLE (9) : Weekly release of tin compounds from the copolymer formulation in the larval medium of *Culex pipiens*.

Time (weeks)	Amount of tin (ug/l)	Time (weeks)	Amount of tin (ug/l)
1	3.00	12	4.80
2	3.60	13	5.50
3	2.70	14	4.70
4	3.00	15	7.30
5	4.20	16	5.70
6	7.00	17	6.80
8	6.40	18	7.60
9	4.30	19	8.50
10	8.20	21	7.30
11	4.80		

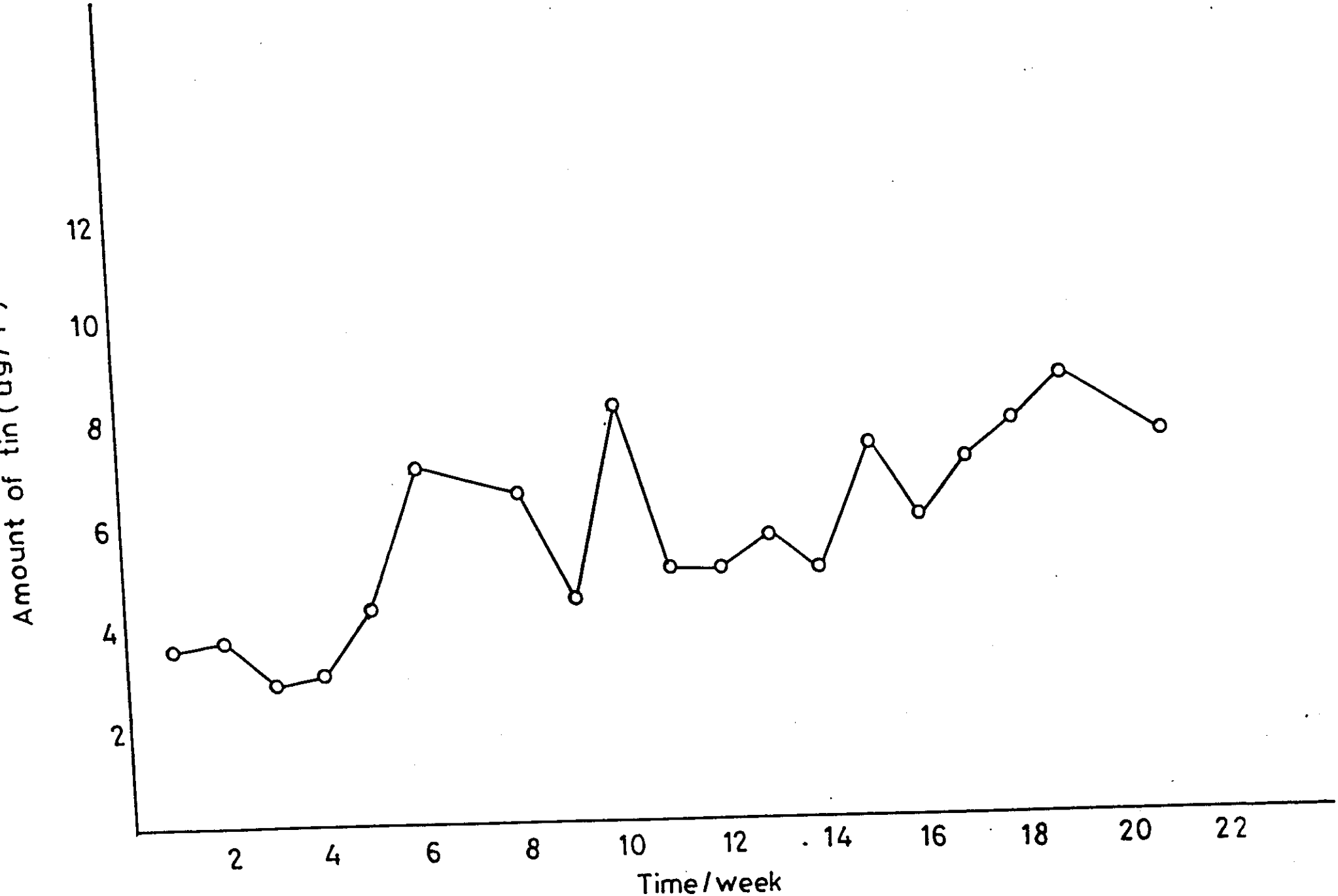


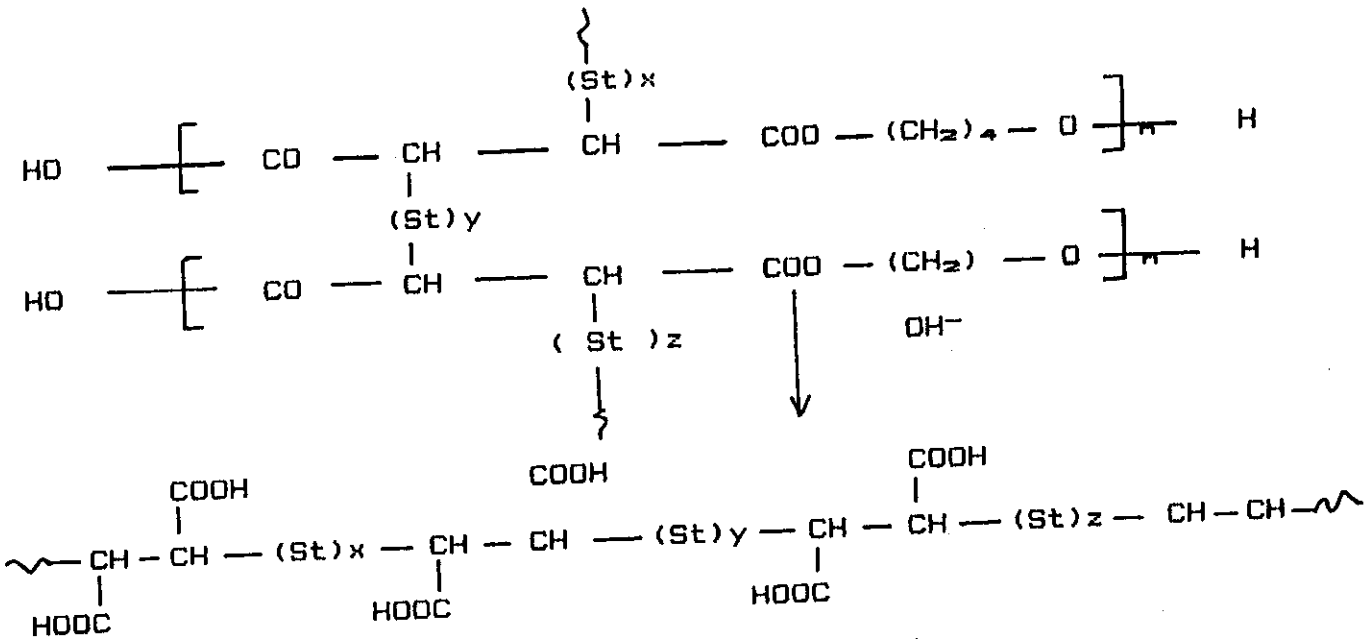
Fig. 11. Weekly release of tin compounds from the copolymer

tained above 0.9 ug/litre (0.9 ppb) during all weeks which was in agreement with the general statment that residue levels were considered inadequate for control if they averaged less than 0.9 ppb, the established LC_{50} for 4th instar laboratory reared larvae of C. p. quinquefasciatus. Residue levels were considered excessive if above 18 ppb the recommended maximum dose rate (Anonymous, 1970).

D - Characteristics of the Cured Polyester Formulation :

The cured polyester formulation was subjected to soxhelt extraction with different solvents (dioxane, benzene, and carbon tetrachloride). The soluble fraction was evaporated to dry residue. The crosslinked polyester and dry residue after extraction were analyzed for tin. Tin assay revealed that the heavy loading of TBTF in the cured polyester was greatly decreased by soxhelt extraction with dioxane and less affected with non polar solvents such as benzene and carbon tetrachloride.

The cured polyester was subjected to hydrolysis, and the hydrolyzate product was characterized by infrared and proton magnetic resonance spectra. Thus, its IR spectrum shows bands at 171, 700, 750, 2900, and 3400 cm^{-1}, characteristic of $\perp$ C = O, δCH of aromatic (five adjacent) $\perp$ CH_2 and $\perp$ OH , respectively. Also, its ^1H NMR spectrum showed signals at δ 7.3, which represents the aromatic protons of polystyrene and at δ 1.8-2.5 for the CH_2 and CH protons.



The average molar ratio of styrene (St) to fumaric acid residue in the hydrolyzate product was deduced from the oxygen content and the ^1H NMR spectral data and was found to be 4.35 (St/fumaric acid).

E - Bioassay of the Polyester Formulation :

a. Experiment 1 : (Accumulated Toxicity) :

Fig. (12) and Table (10) illustrate the effect of accumulated toxicity of the polyester formulation on the larval mortality of Culex pipiens. When larval medium was not changed and only the larvae were renewed weekly, a gradual increase in the larval mortality was observed till the 4th week where the larval mortality reached 90% (corrected from control). The percentage was constant till the 7th week when the tablet was removed from

TABLE (10) : Effect of accumulated toxicity of the polyester formulation on the larval mortality of Culex pipiens. (3 replicates)

Time (weeks)	% Average larval mortality±S.E. (corrected from control)
1	12 ± 0.032
2	70 ± 0.046
3	88 ± 0.033
4	90 ± 0.030
5	80 ± 0.040
6	88 ± 0.032
7	88 ± 0.032
8	88 ± 0.032
9	88 ± 0.032
10	56 ± 0.050
11	46 ± 0.050
12	30 ± 0.046
13	00

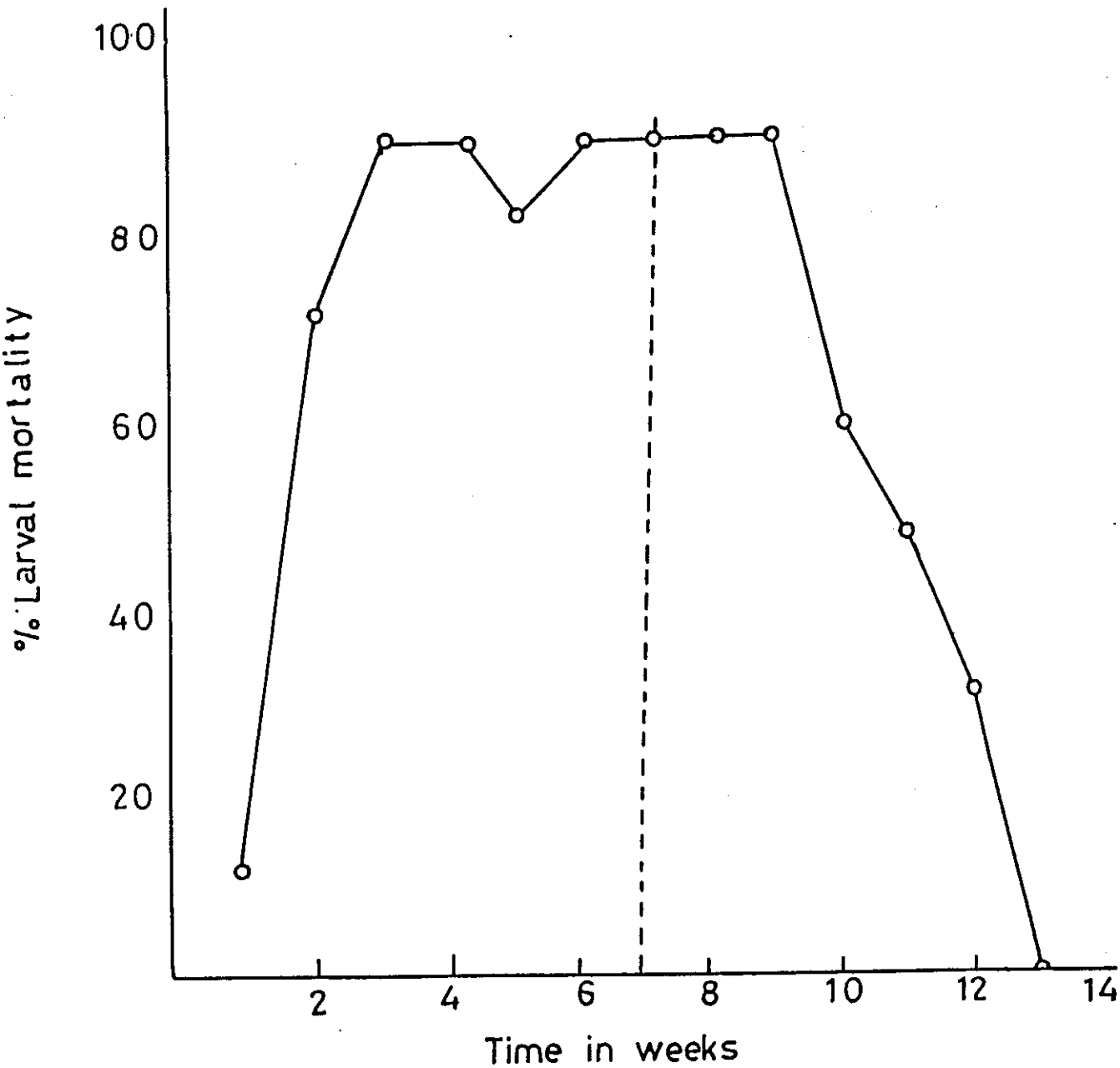


Fig. 12. Effect of accumulated toxicity of the polyester formulation on the larval mortality of C. pipiens.

the medium. Inspite of removal of the tablet, the percentage larval mortality was still constant up to the 9th week. After that week the larval mortality was sharply decreased in the following weeks up to the 13th week when the larval mortality reached zero or in other words, the mortality of the experimental larvae was equal to that in the control larvae.

The statistical analysis of the data in Table (10) indicated significant increase in larval mortality till 4th week which may be explained by the fact that the toxicant became active through mechanical interaction with water.
The sudden significant decrease in larval mortality in the 10th week may possibly be explained by the formation of a strong chemical bond between the insecticide and substrate within the molecular matrix which renders the insecticide unavailable for dissolution into an aqueous media (Schultz and Webb, 1969).

b. Experiment 2 : (Weekly Release) :

Fig. (13) and Table (11) showed the effect of weekly released toxicity of the polyester formulation on the larval mortality of Culex pipiens when both water and larvae were renewed weekly. It was noticed that a fluctuation in the weekly larval mortality throughout the first 4 weeks occurred. Then, a sharp decrease in the larval mortality was observed till the 7th week

TABLE (11) : Effect of weekly released toxicity of the polyester formulation on the larval mortality of Culex pipiens. (3 replicates)

Time (weeks)	% Average larval mortality±S.E. (corrected from control)
1	00
2	60 ± 0.049
3	28 ± 0.044
4	70 ± 0.046
5	28 ± 0.045
6	12 ± 0.032
7	00
8	00

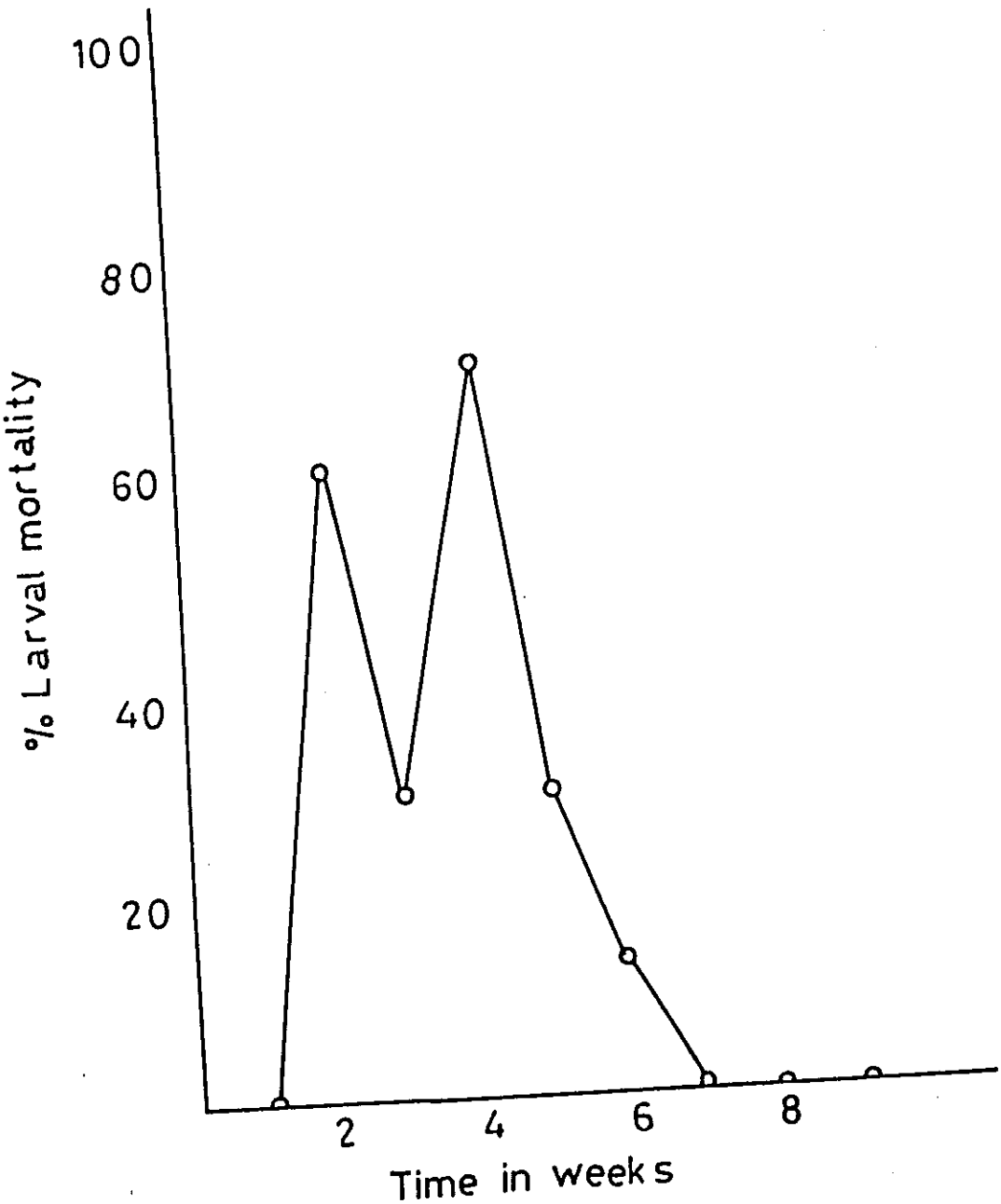


Fig. 13. Effect of weekly released toxicity of the polyester formulation on the larval mortality of C. pipiens.

where the larval mortality reached the zero level or equal the larval mortality of the control.

The statistical analysis of the data presented in Table (11) indicated significant increase in percent larval mortality from 0 to 60% between the 1st and 2nd week then significant drop of percentage mortality from 60% to 28% in the 2nd and 3rd week. The larval mortality increased significantly again in the 4th week to 70% then gradually decreased significantly to zero (0.05 probability) in the 7th week. The marked fluctuation in larval mortality throughout the first 4 weeks may be attributed to the non homogenity configuration of the toxic molecules within the polymeric substrate so that their subsequent diffusion into the fluid was affected.

A similar finding was reported by Whitlaw et al., (1968) who found that pellets containing 0.5 and 1.0% malathion gave inconsistent mortalities with a loss of effective control from test 16 to test 21. The inconsistency found with these pellets was not interpreted as breakdown of the insecticide remaining in the pellet but rather a lack of uniformity in the diffusion of the insecticide from the pellet.

c. Experiment 3: (Degradation of the Released Toxic Compound)

The original run water in exp. 2 for each week was tested for degradation of the toxic compounds to non-toxic daughter compounds by taking records of weekly larval mortality for several weeks. The larval mortality in the first week reached 35% in average and then was increased again in the 2nd week to 45%. After the 2nd week the released toxic compounds seemed to degrade into the non-toxic compounds gradually. In the 6th week, no more larval mortality was observed compared with the control. This means that the released toxic compounds was totally degraded into non-toxic daughter compounds after 6 weeks (Fig. 14 and Table 12). Shortage in time and lack of facilities prevented continuation of bioassay of the other experiments (4-6).

F - Chemical Assay of the Polyester Formulation :

Fig. (15) shows the amount of tin released from the formulation when the larval medium was not changed weekly. This amount began from 3.5 ug/litre in the first week and increased gradually throughout the first 7 weeks, after which this amount was almost constant till the 9th week (Table 13).

This result was expected as the weekly released TBTF was accumulated in the larval medium until the tablet was removed in the 7th week. The amounts of the tin whether in TBTF or its daughter compounds (due to degradation) were almost the same in

TABLE (12) : Degradation of the released toxic compounds of the polyester formulation into non-toxic daughter compounds in the larval medium of *Culex pipiens*. (3 replicates)

Time (weeks)	%Average larval mortality±S.E. (corrected from control)
1	35 ± 0.047
2	45 ± 0.050
3	12 ± 0.032
4	4 ± 0.020
5	2 ± 0.014
6	0

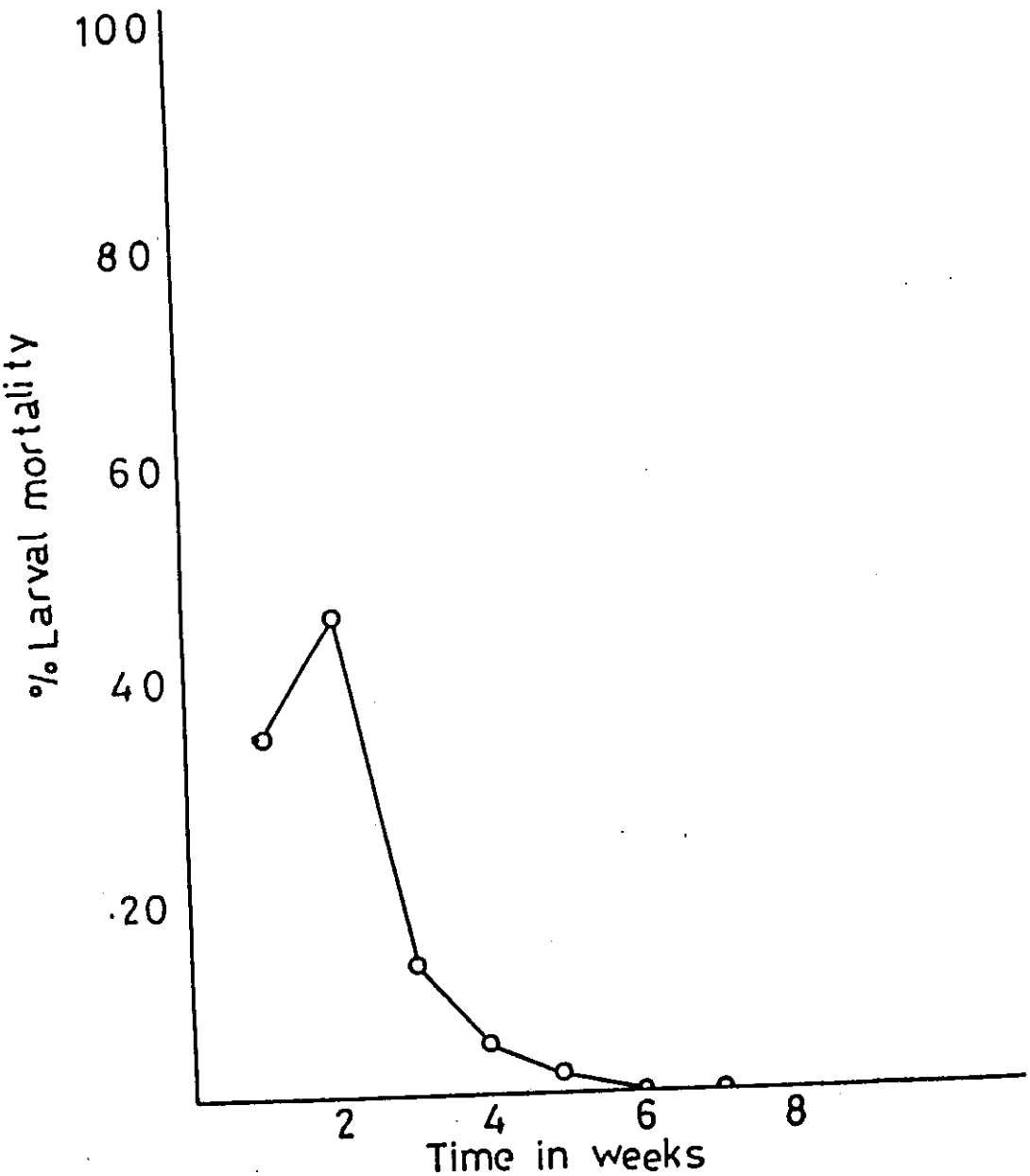


Fig. 14. Degradation of the released toxic compounds from the polyester formulation into non-toxic daughter compounds in the larval medium of C. pipiens.

TABLE (13) : Accumulation of the released tin compounds from the polyester formulation in the larval medium of Culex pipiens.

Time (weeks)	Tin concentration (ug/l)
1	3.50
2	4.90
3	4.50
4	6.00
5	6.50
6	7.50
7	8.80
8	7.50
9	8.00

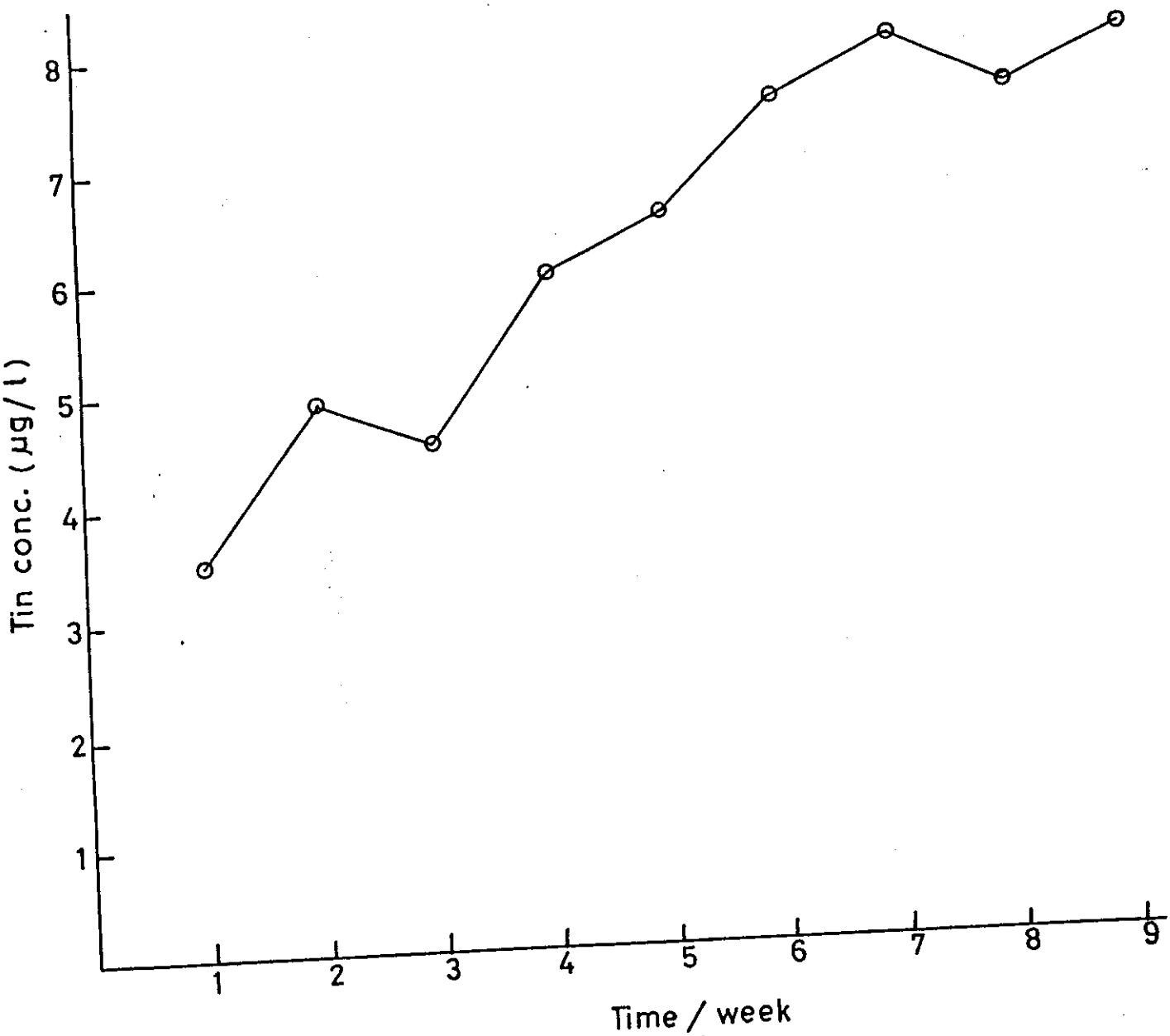


Fig. 15. Accumulation of the released tin compounds from the polyester formulation in the larval medium.

the following weeks (7-9th).

Fig. (16) illustrates the weekly released tin in the successive runs of water (when larval medium was changed every week). It shows the gradual increase of released TBTF throughout the first 4 weeks, after which the irregular release from the formulation was observed, giving a series of fluctuations throughout the 9th week (Table 14).

The amount of tin (residue) in the larval media maintained inside the recommended limit (between 0.9-18 ug/litre) which is in agreement with the finding of some workers (Anonymous 1970).

TABLE (14) : Weekly release of tin compounds from the polyester formulation in the larval medium of Culex pipiens.

Time (weeks)	Tin concentration (ug/l)
1	5.90
2	3.80
3	5.40
4	6.00
5	5.00
6	6.50
7	4.50
8	8.00
9	5.50

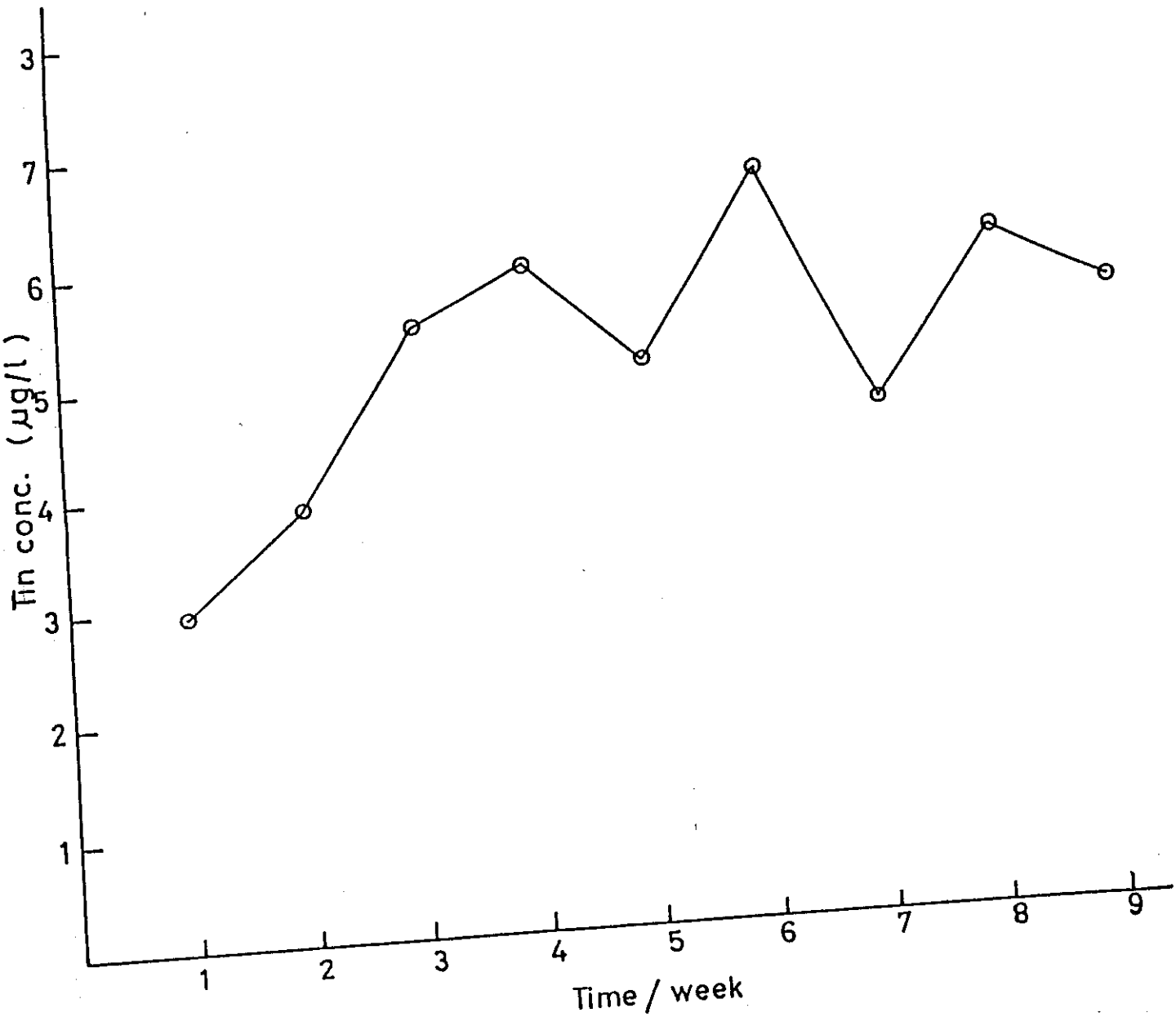


Fig. 16. Weekly released of tin compounds from the polyester formulation in the larval medium.

G. Histopathological Effect of TBTI-MMA Copolymer Formulation on Larval Tissues :

The alimentary canal of Culex pipiens is divided into three distinct regions, the anterior foregut, the midgut and the posterior hind gut.

The midgut extends back as a long, uniform, cylindrical tube which occupies more than half the length of the alimentary canal. It gradually tapers to its junction with the hind gut at the origin of the Malpighian tubules.

Results of the present investigations indicated the following:

a- Untreated Larvae :

As investigated in cross sections the midgut epithelial cell is simple and uniform through its whole extent, (Fig. 17.a). The epithelium (EC) is made up of cuboidal cells with a striated marginal fringe on the free surface (striated border), their nuclei are large subcentral and occupy the cell with well stained chromatin. Between the bases of these cells are located small regenerative nuclei (RN) forming lines along the lines of separation of the larger cells. A chitinous peritrophic membrane (PM) surrounds the gut contents (GC) and may protect the epithelial cells from possible abrasion by food particles (Fig. 18.a) and (Fig. 19.a).

The hind gut has a lining of epithelium (EC) with a thin cuticular lining or intima (I) and doubtful striated border,

(Fig. 20.a).

The Malpighian tubules: These are five, rounded, thin, elongated tubes which open into the pyloric ampulla, one dorsal and two on each side laterally. They are devoid of muscles. The tubes are composed of large characteristic cells. The cytoplasm of these cells may have a faint stains, it is filled with various refractile or pigmented inclusions and large nucleus (N) and a well defined brush border (BB) surrounding a relatively narrow lumen (L) (Fig. 21.a).

The fat body (FB), it is arranged as a loos meshwork of lobes, invested in connective tissue strand. The fat cells are closly adhered and the external surfaces of the cell mases are cover by a delicat membranuce sheeth, the cytoplasm is stuffed with reserves and with big nuclei (N) (Fig. 22.a).

Muscular tissues, the muscle composed of striated fibers, each fiber consist of a number of paralle fibrillae (Fig. 22.a).

b- Treated Larvae :

Transverse sections in alimentary canal of Culex pipiens 3rd instar larvae, which were exposed to the TBTI-MMA copolymer formulation for 48 hours, seemed to differ consederably from the healthy ones.

The copolymer formulation caused serious effects in the midgut epithelium (Figs 17.b, 18.b and 19.b). It completely disorganized and disintegrated the midgut epithelial cells. All but a few scattered group of cells were sloughed off into the

lumen, the nuclei of these cells are Pyknotic. The greater part of the exfoliated epithelium has evacuated. Few scattered groups of cells lost connection with its nourishing base and falling free into the cavity of the midgut. Granular inclusions in the cytoplasm were found. The peritrophic membrane was entirely missing in some instances and adhered to the gut contents.

The hind gut epithelial cells were affected by the TBTI-MMA copolymer formulation. It seems as its action was associated with the precipitation of cytoplasmic proteins in the cell (Fig. 20.b). The other side of the cells adjacent to the intima was became vacuolated. The intima was also damaged and was separated into several portions instead of being continuous chitinous layer.

TBTI-MMA copolymer formulation caused marked histological changes of the Malpighian tubules. Its cells became greatly reduced in size, a slight degeneration of some cell nuclei while others were clumped into dense masses. The most noticeable effect, however, was an increase in the lumen of the tubules which was filled with several vaccules and substances having a strong affinity for Haematoxylin (Fig. 21.b).

The fat bodies throughout the gut larvae (Fig. 22.b) were also affected by the toxicant copolymer. They became vacuolated, less eosinophilic, and reveal a breakdown or a natable degeneration.

The muscles were also affected by the toxic copolymer. There was a marked degeneration by the appearance of fissures, vacuoles and distruction of sarcolemma (Fig. 22.b).

From the foregoing, it is apparent that the copolymer formulation caused great damage to the midgut and hind gut of Culex pipiens larvae when exposed to the copolymer formulation for 48 hours. Histopathological investigations revealed serious changes in the epithelial cell layer of the midgut. These observations are in agreement with the findings of Chadbourne and Rainwater, (1953). The hind gut epithelial cells were also affected. A similar results was found by Greenhalgh, (1987) who treated cricket nymphs (Acheta pennsylvanicus) with stomach poison (chlordan). Chlordan showed a striking ability to rapidly penetrate the internal digestive tract cuticle lining resulting in hind gut cellular damage. On the other hand, these observations disagreed with the fact that the chitinous cuticle (intima) covering the fore and hind gut protects them from the action of the poison.

TBTI-MMA copolymer treated tissues which showed marked degeneration or abnormality were the Malpighian tubules, fat bodies and muscles. The most noticeable effect was an increase in the lumen of the Malpighian tubules. A similar observation was recorded by Chadbourne and Rainwater, (1953). The fat bodies became very loosely packed and the muscles are damaged. These observations were in agreement with Hamed et al., (1974).

Fig. (17.a): A transverse section in the midgut region of non-treated Culex pipiens larvae (X=200).

EC : Epithelium cell.

GC : Gut contents.

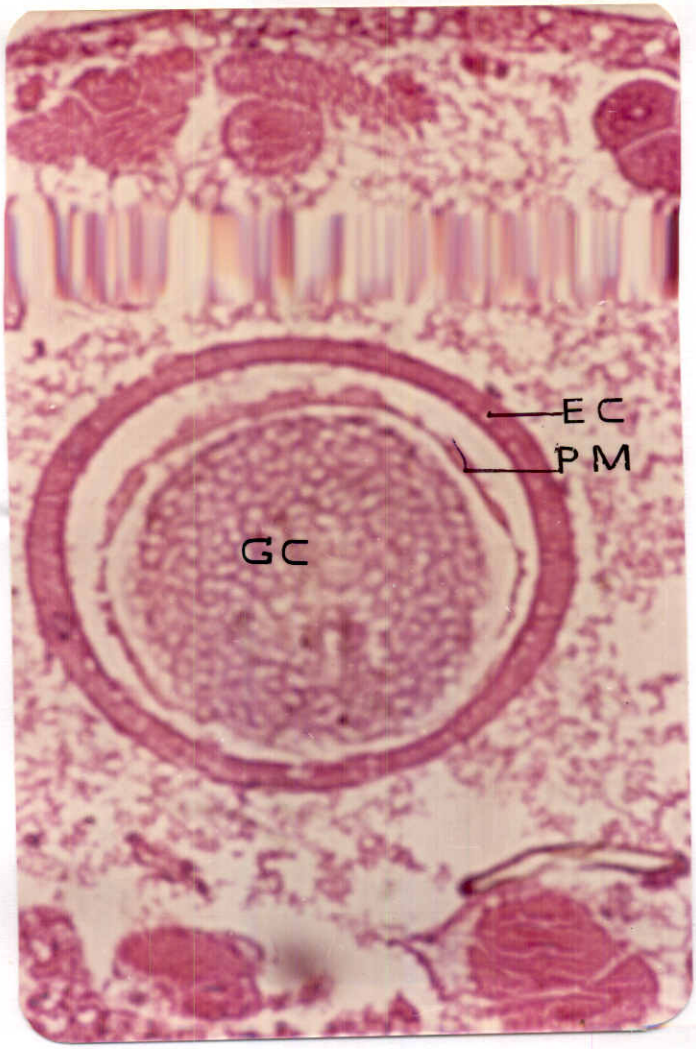
PM : Peritrophic membrane.

Fig. (17.b): A transverse section in the midgut region of treated Culex pipiens larvae after 48 hrs. exposure to the TBTI-MMA copolymer formulation (X=200).

EC : Epithelium cell.

GC : Gut content.

V : vacules in the cytoplasm.


EC
PM
GC


GC
EC
V

Fig. (18.a): A part of T.S. in the midgut region of non-treated Culex pipiens larvae (X=400).

EC : Epithelium cell.

GC : Gut content.

N : Nucleus.

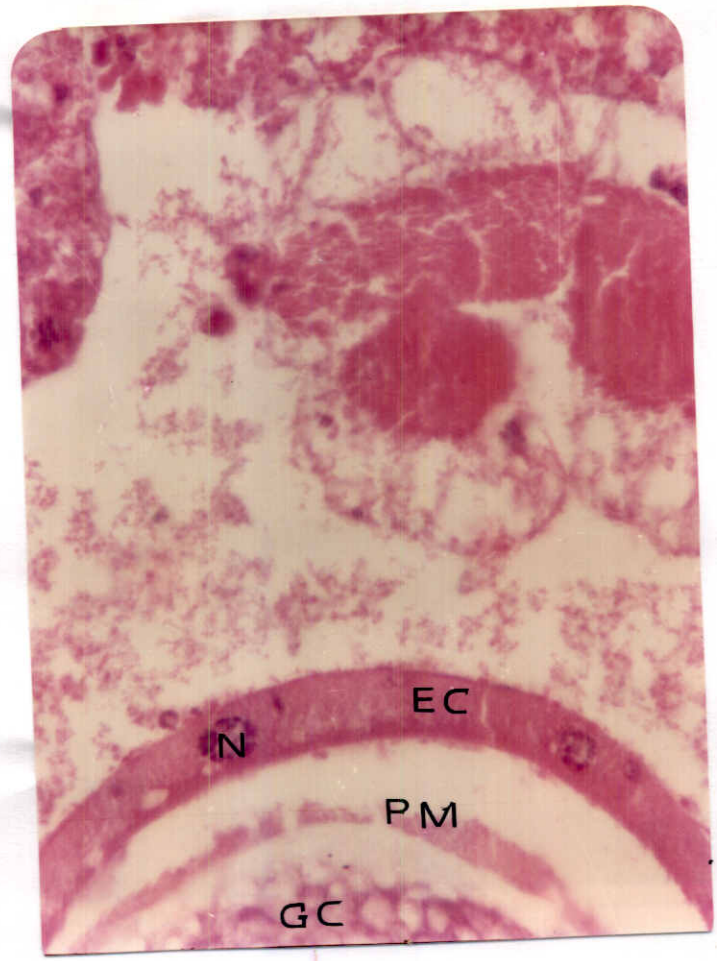
PM : Peritrophic membrane.

Fig. (18.b): A part of T.S. in the midgut region of treated Culex pipiens larvae (X=400).

EC : Epithelium cell disintegration.

GC : Gut content.

V : Vacules in the cytoplasm.


EC
N
PM
GC

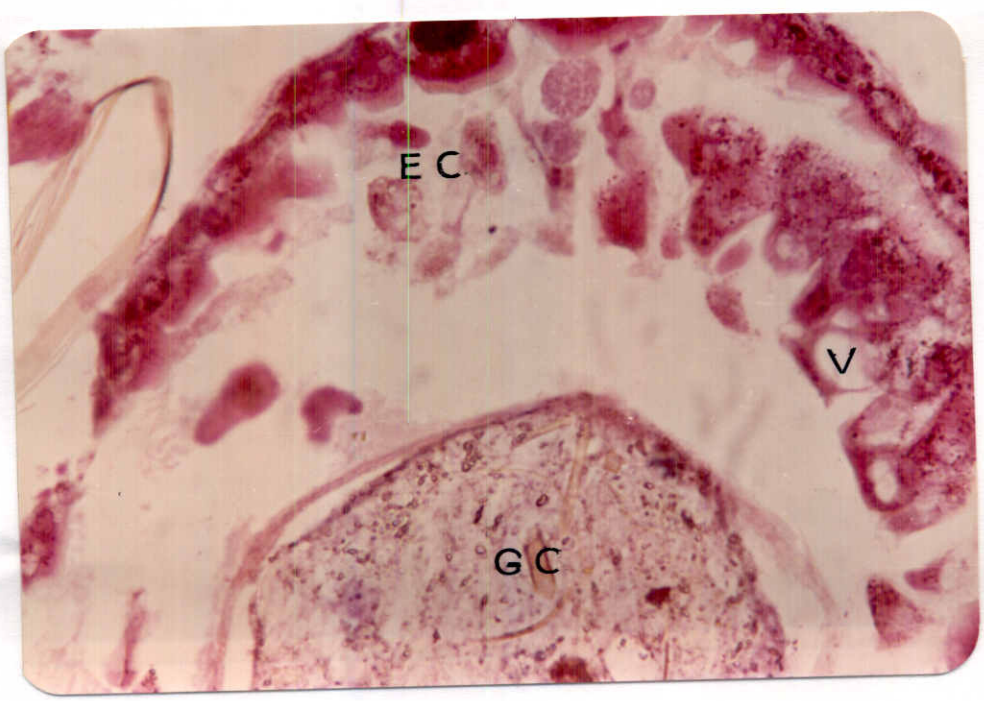

EC
V
GC

Fig. (19.a): A part of T.S. in the midgut region of non-treated Culex pipiens larvae (X=1000).

EC : Epithelium cell.

GC : Gut content.

N : Nucleus.

RN : Regenerative nuclei.

PM : Peritrophic membrane.

Fig. (19.b): A part of T.S. in the midgut region of treated Culex pipiens larvae (X=1000).

EC : Epithelium cell.

V : Vacules in the cytoplasm.


RN
N
PM
GC

Fig. (20.a): A part of T.S. in the hind gut region of non-treated Culex pipiens larvae (X=1000).

EC : Epithelium cell.

GC : Gut content.

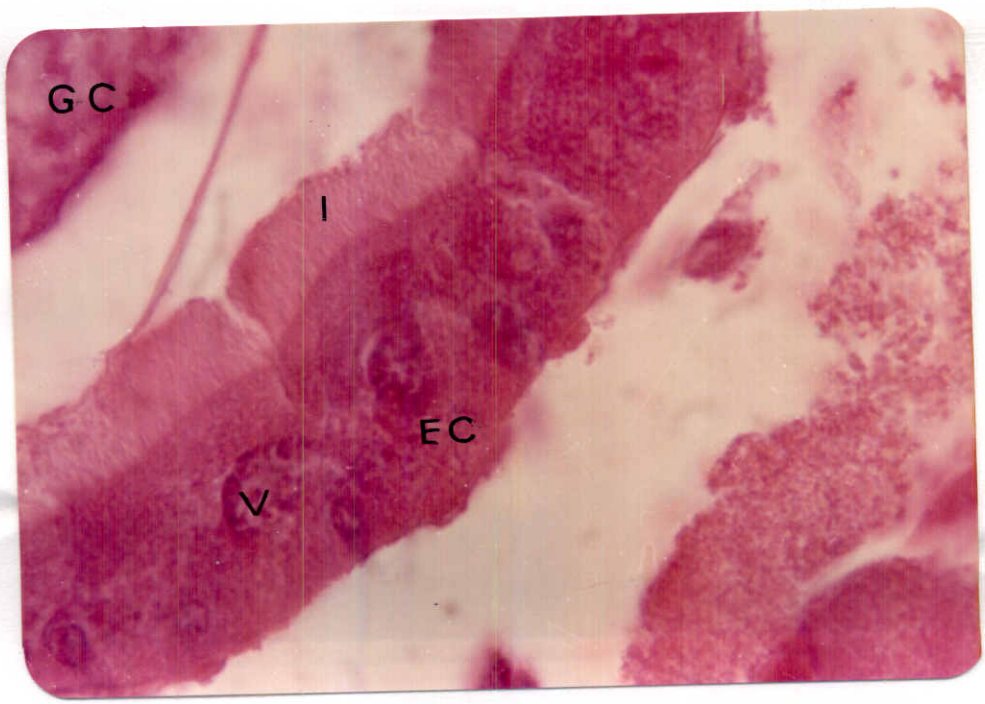
I : Intima.

N : Nucleus.

Fig. (20.b): A part of T.S. in the hind gut region of treated Culex pipiens larvae (X=1000).

EC : Epithelium cell.

I : Intima.


GC
I
EC
V

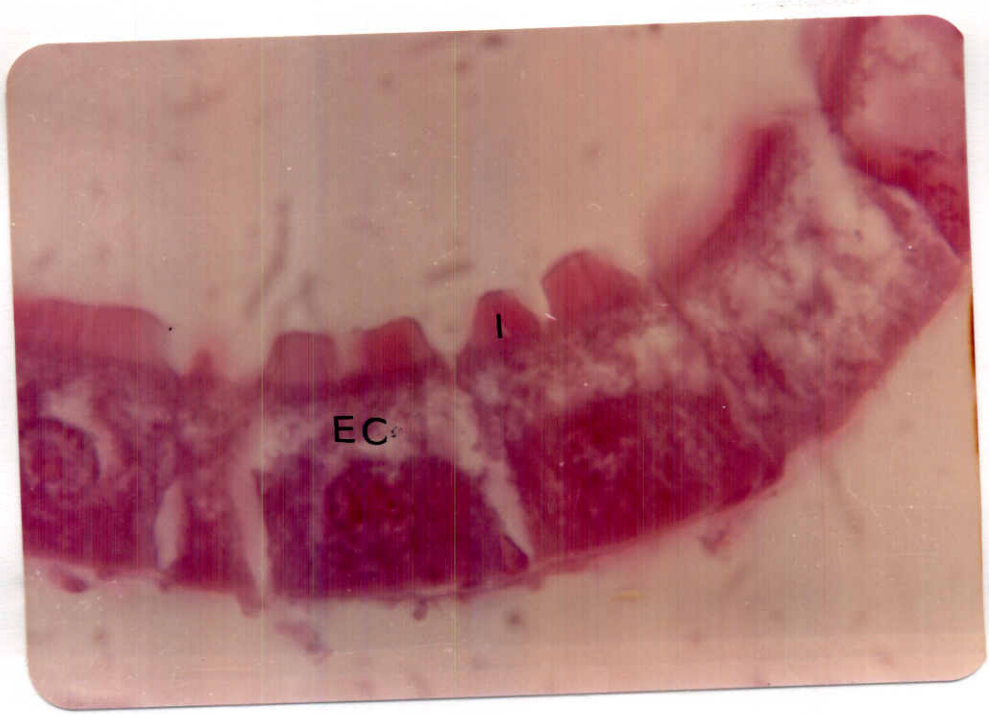

I
EC

Fig. (21.a): T.S. of non-treated Culex pipiens larvae, showing the Malpighian tubules (X=400).

BB : Brush border.

L : Lumen.

N : Nucleus.

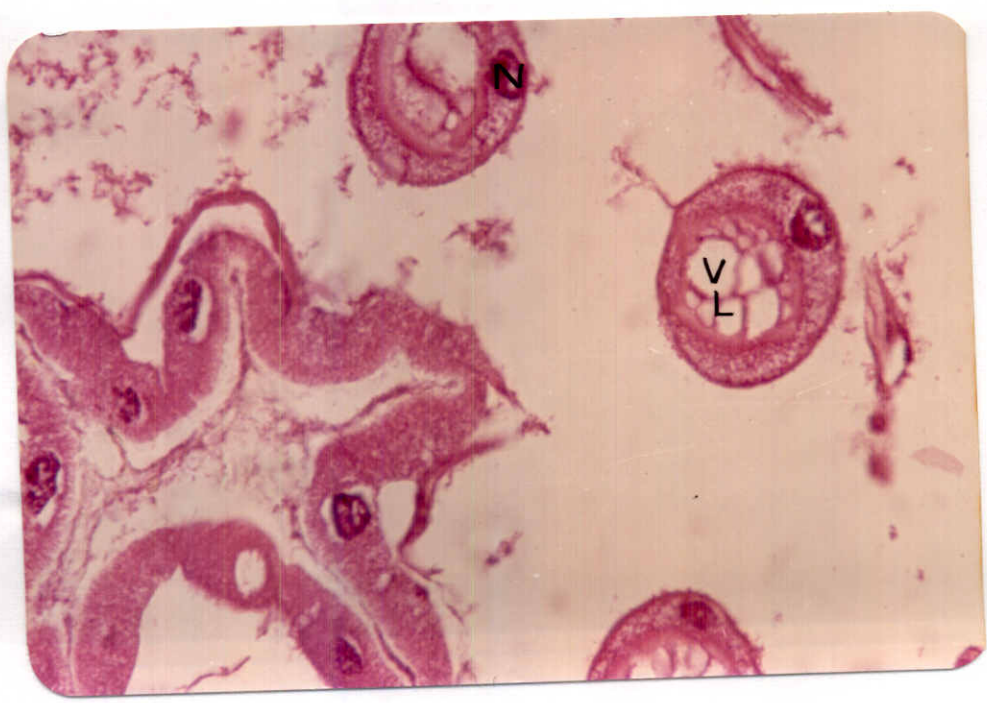
Fig. (21.b): T.S. of treated Culex pipiens larvae, showing the abnormality of the Malpighian tubules (X=400).

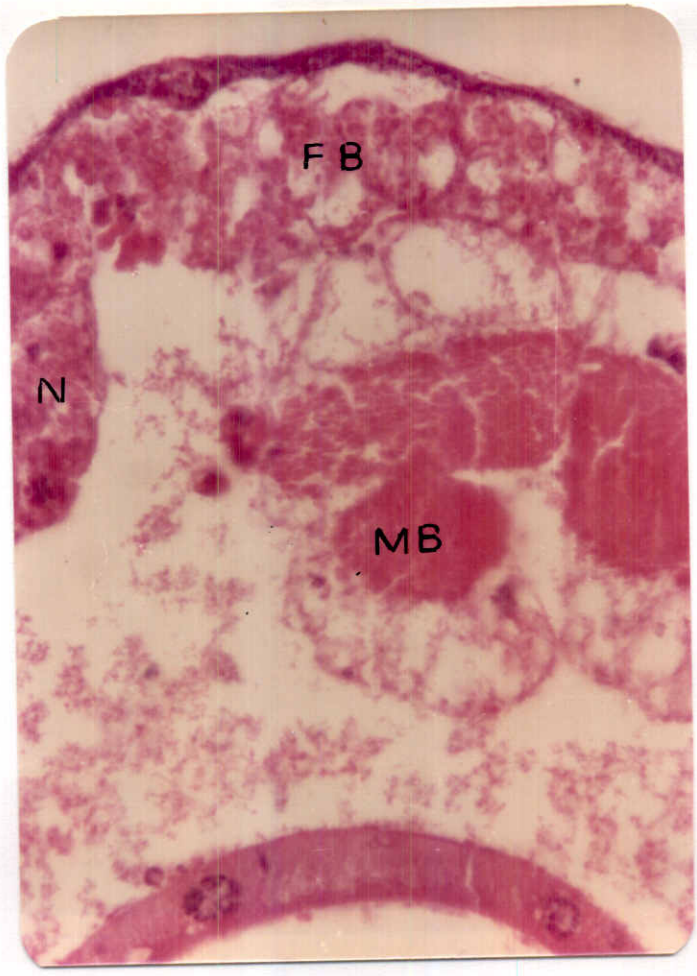
L : Lumen.

N : Nucleus.

V : Vacules inside the lumen.


BB
N
L


N
V
L


F B
N
M B