Pakistan J. Sci. Ind. Res., Vol. 14, Nos. 4–5, August–October 1971.

STUDIES ON THE RESIDUAL TOXICITY AND VAPOUR ACTION OF PETKOLIN-M

M.M.H. Baig and (Miss) Rehana Aijaz

Toxicology and Pesticides Laboratory, Department of Plant Protection, Karachi 27

(Received December 19, 1970)

Biological tests using *T. castaneum*, *Callosobruchus analis* and *Periplaneta americana* show that the toxic action of Petkolin-M as a residual film persists longer in closed than in open containers and it also persists longer against *T. castaneum* and *C. analis* than *P. americana*. A toxic vapour action of Petkolin-M was deduced and confirmed in tests which showed that vapour action against *P. americana* persists longer than *T. castaneum* and *C. analis*. It is concluded Petkolin-M is a mixture of at least two toxic substances, one more toxic as a residual film to *C. analis* and *T. castaneum* and the other more toxic as a vapour to *P. americana*.

In quest to save foreign exchange by developing indigenous resources for pesticide manufacture, PCSIR produced the insecticide Petkolin-M by chlorination of various low boiling cuts of petroleum waste.[1] A wide range of biological activities, including toxicity to various species of insect of these substances have been reported in a series of publications.[2–4] Lack of investigations of the residual and other related properties of these compounds prompted the present study of residual action of Petkolin-M reported to be the most promising of the three Petkolins. This paper describes tests of the residual toxicity of Petkolin-M both in covered and open containers. Marked differences of effectiveness and length of action of the insecticide suggested that it might also be effective as a fumigant and examination of this hitherto unexplored property of Petkolin-M is also described in this paper.

Experimental

Materials and Methods

Test Insects.—Periplaneta americana, *Tribolium castaneum* and *Callosobruchus analis* were reared in the laboratory at 25–35°C and relative humidity 60–80%.

The cockroaches were reared on a mixture of wheat bran and yeast with water supplied to them from a glass tube fitted with a loose cotton-wool plug. These insects were 6–9 months old when used for test.

C. analis were reared on whole gram. 4–8-day old adult beetles were used for tests. *T. castaneum* were reared on wheat-flour. The adults were used for tests when they were 10–30 days old.

Handling of Insects.—For both residual and fumigation tests the cockroaches were immobilised by chilling at 0°C for 15–20 min because unchilled insects were too active and escaped whilst being transferred to the test containers. Other insects did not need inactivation for handling. Brush was used for transferring the insects other than *P. americana*.

Toxicity Tests.—The treatments were replicated in all the tests. Three batches of 10 adults each of *T. castaneum* and *C. analis* were always used. For residual assay 5 batches of 5 *P. americana* were used but for fumigation tests only 3 batches of 5 insects were used.

Residual Film Action.—Two kinds of residual film tests were made by exposing insects to filter paper circles impregnated with Petkolin-M; one in the open or ventilated and the other in closed containers.

Circles of filter paper (6 in and 4 in dia) were impregnated by spreading evenly from pipette 2.0 ml of 2.0% Petkolin-M in n-hexane solution. The papers were dried at room temperature for 5 min. Each treated paper was tested for toxicity when dry and then at intervals by releasing fresh insects on the same papers in the same dishes. Tests with closed containers were made twice weekly but with open containers tests were made daily because of the short residual action. All these tests were made at room temperature and humidity during the months of March–June. During this period the means of temperature was 25–35°C. and humidity was 60–90%.

Petri dishes of 6 in dia were used as closed containers for all the three species of insects. Because *C. analis* are small, active, can climb clean glass and squeeze through small spaces, it was necessary to put weights on the tops of dishes to prevent escaping.

Beakers of 4 in dia (500 ml) were used as ventilated containers and were covered with muslin, held in place by rubber bands so as to prevent the escape of *P. americana* and *C. analis* which can climb on glass.

Vapour Action.—The vapours from portions of Petkolin-M in air-tight containers were tested for toxicity by exposing all the three species of insects at intervals until 24-hr exposure no longer killed them. *C. analis* and *T. castaneum* were exposed in presence and absence of food but *P. americana* were tested only in the absence of food. At first insects were exposed twice weekly but because of the small toxicity in the presence of food the test

was repeated and insects were exposed daily in the presence of food.

Because the size and activity of the insects differ the methods and equipment used for each species had to be varied.

P. americana were placed in 1-lb glass-stoppered jars with 0.5 ml Petkolin-M in a 25-ml beaker covered with muslin to prevent insects falling into the insecticide.

Tests with *T. castaneum* were made in a similar fashion but the insects were kepy in small open tubes about 2.5 cm dia and 6 cm high. The edges were smeared with Vaseline to prevent the escape of insects. Two tubes were placed in each jar, one with only insects and the other with insects containing about 4 g wheat-flour as food.

Toxicity to *C. analis* was measured in 5-lb jars using 2.0 ml of Petkolin-M in open, 5-cm dia petri dishes. The insects were kept in 50-ml beakers closed with muslin; a smear of Vaseline round the top was used as an extra precaution to prevent the escape of insects. Two beakers of insects were placed in each jar, 8 g of grams were added to one as food. Tests were made at room temperature between 22–31°C and humidity 35–65%.

Results and Discussion

The most striking observation of these studies is that the action of Petkolin-M, as a residual film, persists much longer in covered containers than in open containers. In tests of treated paper circles in open containers there was complete kill for only the first and second day but in closed containers *T. castaneum* and *C. analis* produced 100% mortality for 8 weeks and 9 weeks respectively. (Table 1). Toxicity to cockroaches persisted for a much shorter time but in closed containers it was more toxic after 2 weeks/than after 3 days in open containers (Table 1 and 2). With all 3 species of insects no paper was toxic one week after the start of the tests made in ventilated containers, but in closed vessels some toxicity was found after 4 weeks with *P. americana* and 14 weeks with the other two species. Because the toxic action of Petkolin-M persists so much longer in closed vessels, it may be deduced that Petkolin-M is rapidly lost by evaporation, and that in confined spaces the insecticide vapour will exert a toxic action. Petkolin-M vapours were shown to be toxic to all the three species of insects (Table 3) but the vapour action was short lived against *T. castaneum* and *C. analis* even in the closed vessel in contrast to the persistent residual film action. Unexpectedly the vapour action lasted longer than the residual film action in a closed vessel against *P. americana*. The vapours were toxic to *P. americana* for almost as long as the residual films in closed vessels were toxic to *T. castaneum* and *C. analis*. This apparent reversal of persistence of toxicity to differing insects when applied by different methods suggests that at least two toxic compounds may be involved; one, acting as a residual film, and more toxic to *C. analis* and *T. castaneum*, while the other, acting as a vapour, is more toxic to *P. americana*.

Table 1.—Residual Film Toxicity of Petkolin-M.

Tests made with 6 in dia circle of filter paper impregnated with 2 ml of 2.0% Petkolin-M in n-hexane.

Period (weeks)	% Mortalities† in closed containers		
	*T. castaneum**	*C. analis**	*P. americana*†
1	100	100	100
2	100	100	93.3±0.71
3	100	100	73.3±1.70
4	100	100	36.6±1.20
5	100	100	—
6	100	100	—
7	100	100	—
8	100	100	
9	93.3±1.21	100	
10	83.3±2.04	96.6±1.74	
11	63.3±1.40	90.0±0.89	
12	43.3±1.14	76.6±0.8	
13	30.0±0.95	53.3±0.81	
14	30.0±1.00	33.3±0.00	

Note: No control mortality in any case.
* Mean of 6 result — 3 replicates from 2 tests.
† Mean of 10 results — 5 replicates from 2 tests.

Table 2.—Residual Film Toxicity of Petkolin-M.

Tests made with 4 in dia circles of filter paper impregnated with 2 ml of 2.0% Petkolin-M in n-hexane.

Period (days)	% Mortalities in open containers		
	*T. castaneum**	*C. analis**	*P.*† *americana*
1	100	100	100
2	100	96.6±0.58	93.3±1.15
3	63.3±2.08	70.0±2.00	66.6±1.52
4	46.6±1.37	53.3±0.37	56.6±3.05
5	36.6±1.92	46.6±0.61	50.0±2.00
6	Nil	40.0±1.73	40.0±1.22
7	Nil	Nil	Nil

Note: No control mortality in any case.
* Mean of 6 results — 3 replicates from 2 tests.
† Mean of 10 results — 5 replicates from 2 tests.

TABLE 3.—VAPOUR ACTION OF PETKOLIN-M IN ABSENCE OF FOOD.

(weeks)	% mortality*		
	T. castaneum	*C. analis*	*P. americana*
1	100	100	100
2	76.6±1.07	93.3±1.33	100
3	60.0±1.26	70.0±1.78	100
4	53.3±1.86	76.6±1.59	100
5	46.6±1.20	60.0±1.67	100
6	—	50.0±1.73	100
7	—	33.3±1.52	96.6±0.41
8	—	—	93.3±0.81
9	—	—	83.3±0.98
10	—	—	63.3±0.41
11	—	—	63.3±1.17
12	—	—	40.0±0.63
13	—	—	33.3±1.63
14	—	—	—

Note: No control mortality in any case.
* Mean of 6 results — 3 replicates from 2 tests.

TABLE 4.—VAPOUR ACTION OF PETKOLIN-M IN PRESENCE OF FOOD.

Period (days)	% mortality *	
	C. analis	*T. castaneum*
1	100	100
2	100	86.6±1.15
3	100	76.6±1.52
4	100	63.3±1.00
5	100	40.0±2.64
6	100	13.3±1.00
7	86.6±2.30	—
8	70.0±2.13	—
9	60.0±2.80	—
10	50.0±1.00	—
15	43.3±0.57	—
20	33.3±1.52	—

Note: No control mortality in any case.
* Mean of 6 results — 3 replicates from 2 tests.

The presence of gram and flour seems to diminish the toxic action of Petkolin-M to *C. analis* and *T. castaneum* because in absence of food all insects were killed for one week but so few were killed at the end of a week in the presence of food that this test was repeated and the insects inspected daily to show a rapid diminution of kill (Table 4).

The biological tests indicate the presence of two types of activity—one more toxic as a vapour to *P. americana* the other more toxic as a residual film to *T. castaneum* and *C. analis*.

The presence of two distinct types of insecticidal action suggests that substances of relatively greater toxicity might be separated from Petkolin-M which is unlikely to be a single substance because it is prepared from petroleum fraction.

Acknowledgement.—The authors are indebted to Dr. Heshamul Huque, T.I, Plant Protection Adviser and Director, for providing incentive and encouragement in the work. Thanks are also due to Dr. S.A. Qureshi for providing the sample of Petkolin-M. The authors are grateful to Dr. K.A. Lord, Colombo Plan Adviser on Pesticides Residues, for technical scrutiny of the paper and for making valuable suggestions thereon.

References

1. S.A. Qureshi, Pakistan J. Sci. Ind. Res., **8,** 248 (1965).
2. S.H. Ashrafi, S.M. Murtaza and D. Asmatullah, Pakistan J. Sci. Ind. Res., **7,** 211 (1964).
3. S.H. Ashrafi, M. Tasnif and Riaz I. Zuberi, Pakistan J. Sci. Ind. Res., **8,** 265 (1965).
4. M. Anwarullah, (Miss) Tasneem Akhtar and M. Irshad, Pakistan J. Sci. Ind. Res., **9,** 180 (1966).
5. A note of Dr. S. Siddiqui on *The Present Status of The Developments of Insecticide and Fungicides Evolved in the Central Laboratories of the Pakistan Council of Scientific and Industrial Research*, September 1964.