J.Pest Cont. & Environ. Sci. 7 (2) : 59 - 70(1999).

Thiodicarb biotransformation to methomyl, toxicities and acetylcholinesterase inhibition in the land snail, *Helix aspersa* (Muller)

By

Mohamed A. Radwan and Ahmed K. Salama

Department of Pesticide Chemistry, Faculty of Agriculture, (El-Shatby) University of Alexandria, Alexandria, Egypt

ABSTRACT

A high performance liquid chromatographic method confirmed with TLC was used to study the *in vivo* biotransformation of thiodicarb in the land snail, *Helix aspersa* (Muller). The toxicity and the *in vivo* acetylcholinesterase inhibition with thiodicarb and its major metabolite,methomyl were also investigated. The results showed that thiodicarb was quickly absorbed within 24 hrs following ingestion.The amount of thiodicarb reached a peak value of 198.60 ng/gm tissue at 3 days, and then rapidly declined as time passed to the end of experiment. Thiodicarb was rapidly metabolized to yield methomyl as a major metabolite in the snail tissues at all time intervals. Methomyl was found to be more toxic to the snails than thiodicarb. Results of the potency of both compounds against AchE activity were in harmony with their toxicities to snails. These results indicated that the formation of methomyl, in the snail, as a major metabolite explains the molluscicidal activity of thiodicarb.

INTRODUCTION

Thiodicarb "Dimethyl N,N [thiobis-(methylimino)carbonyloxy]bis-ethanimidothioate" is a non-systemic oxime carbamate insecticide with a relatively narrow spectrum of activity closely related to its first metabolite, methomyl " S-methyl N-(methylcarbamoyl)oxy thioactamidate". It is introduced by the Union Carbide chemical company,

and commonly marketed as Larvin. Thiodicarb and /or its major metabolite, methomyl has been reported to have molluscicidal activity against land mollusca (El-Sebae *et al.*,1982 ; Miller *et al.*,1988 ; Mones and Gigot, 1988; Radwan *et al.*,1992; and Ferguson *et al.*,1995),it is known to act as cholinesterase inhibitors (Pessah and Sokolove , 1983 ; Radwan *et al.*, 1991 ; and Radwan *et al.*, 1992) and was also shown to alter the activity of other non- specific serine containing enzymes, or non-enzymatic biochemical constituents of land mollusca (El-Wakil and Radwan,1991 and Radwan *et al.*,1992). Thiodicarb essentially consists of two methomyl moieties joined through their amino nitrogen by sulfur, is rapidly absorbed, degraded and excreted and has not been demonstrated to accumulate in animal tissues. The metabolic pathways for radiolabelled thiodicarb in animals and plants, involves cleavage to methomyl, oxidation, and hydrolysis followed by conjugation of the metabolites (FAO/WHO, 1985). Similarily, thiodicarb is also readily transformed to methomyl in and/or on cotton leaves, water, and soils as well as exhibited less persistent in all these environmental systems than methomyl (Jones *et al.*,1989 and Abu El-amayem *et al.*,1990). Few articles are available about the metabolism of pesticides in land gastropods (Triebskorn *et al.*,1990 and Salama and Radwan ,1995). The present study is a continuation of these studies on terrestrial snails. The persent work was planned to study thiodicarb biotransformation to methomyl, toxicities and acetylcholinesterase inhibition in the land snail, Helix aspersa (Muller).

MATERIALS AND METHODS

Chemicals

Analytical grades of thiodicarb (98.2 %) and methomyl (99.8 %), were supplied by Environmental Protection Agency (EPA),USA. TLC plates silica gel-60 (20 x 20 cm) and all solvents used were obtained from Fisher Scientific Products Co. Acetylthiocholine iodide (ATch) and Ellmans reagent [5,5-dithiobis-(2-nitrobenzoic acid)],DTNB were obtained from Sigma Chemical Company, USA.

Animals

Specimens of the herbivorous snails, *Helix aspersa* (Muller) were collected from untreated nursery plants and farms in Alexandria

governorate, Egypt. Adult animals of 24 mm in diameter were chosen, allowed to acclimatize to the laboratory conditions for at least 30 days and were fed on fresh leaves of lettuce .

***In vivo* biotransformation of thiodicarb**

In this experiment, a solution of thiodicarb was prepared in acetone at a concentration of 200 ppm. Lettuce leaves were cut into discs approximately 1 inch in diameter. Thiodicarb concentration was loaded onto leaf disc.The treated discs were allowed to dry at room temperature. Snails were starved four days prior to feeding on treated leaf discs (2 ug loaded onto one disc exposed to one snail). Control animals were fed with using uncontaminated lettuce discs. Twenty of thiodicarb-treated snails and ten of untreated ones were selected at 1,2,3,5,7,10, and 15 days after the treatments, and frozen at -20 °C untill analysis.

Extraction of thiodicarb and methomyl

At each time interval, snails were collected and dissected. The soft tissues were pooled and homogenized as 1:10 (w/v) with acetone for five minutes. The homogenate was filtered through a Whatman filter paper No.1 , then the filter paper was washed several times with acetone (10 ml portion). The filtrate was collected and made up to 100 ml with acetone , then dried over anhydrous sodium sulphate. The extract was concentrated to dryness using rotary evaporator at 40 °C. The pesticide residue was dissolved in 3 ml of methanol.

Clean up

Clean up was carried out using a chromatographic column filled with silica gel (2 gm), florisil (2 gm), and anhydrous sodium sulphate (1 gm). The sample extract was tranferred to the column, preconditioned by passing 10 ml of acetone through it. The column was eluted with 50 ml of methanol and the eluate was collected and concentrated to 3 ml as described above. The samples were subjected to HPLC and TLC analysis.

HPLC analysis

High performance liquid chromatography (HPLC) was carried out using a Varian-VISTA-5500 instrument , equipped with an Ultra-violet detector (UV) set at 240 nm. The separation was performed with a *u*

Bondapak C_{18} stainles steel column (30 cm × 5 mm I.D.), using isocratic elution system with methanol. Identification was accomplished by retention times and compared with the standards of thiodicarb and methomyl at the same conditions. For quntification the peak areas corresponding to each injected sample were compared with that of standard solutions of the insecticides.

Quantification limits

Detection limits for thiodicarb and methomyl using the employed method were determined according to the analyte concentrations that produce a chromatographic peak equal to three times of baseline noise.

TLC analysis

Thin layer chromatography was carried out for qualitative analysis of thiodicarb and methomyl. Ten microliters of tissue extracts were spotted on silica gel, TLC plates. The plates were developed with the solvent system of acetone-methanol (2:1 v/v). The spots were detected by the colour in iodine vapour. To analyze thiodicarb and methomyl, a mixture of both compounds was done in acetone. The standard mixture was chromatogrphed and detected as above.

Toxicity procedure

Toxicity of thiodicarb and its major metabolite, methomyl against the adult *Helix aspersa* snails was evaluated and the WHO (1965) method in detecting snails mortality was applied . Six different concentrations of either thiodicarb or methomyl were prepared in acetone as follows; 0.01, 0.02, 0.1, 0.2, 0.5, and 1 %. One snail was placed into 0.5 glass jar and fed on one treated leaf disc (10 ul from each insecticide concentration was loaded onto one disc and exposed to one snail). The jars were tightly covered with cloth netting secured with a rubber band to prevent from escaping. Each concentration was replicated four times (10 individual for each). For each treatment, snails were allowed to feed on the treated discs along 7 days. Untreated check was carried out using seven days feed period on acetone-treated lettuce discs. The animals were checked and the number of dead snails were recorded and removed. Mortality percentages were corrected using Abbott formula (1925).

In vivo acetylcholinesterase inhibition by thiodicarb and its major metabolite, methomyl.

Using the same dosing as mentioned in biotransformation study, individual snail was fed on one leaf disc treated with 10ul of thiodicarb or methomyl. Controls free pesticide were also accomplised. Four groups of four treated and untreated snails were taken at 1, 2, 3, and 5 days following treatment for enzyme assay. The enzyme preparation was carried out according to the method of Radwan *et al.*,(1992). The procedure of Ellman *et al.*, (1961) was utilized for measuring acetylcholinesterase activity in treated and untreated snails. Activity of the inhibited enzyme was reported as a percentage of the inhibition of the respective enzyme from control snails. Activity was measured as m moles of acetylthiocholine (substrate) hydrolyzed per min per gm tissue.

RESULTS AND DISCUSSION

Chromatographic analysis of thiodicarb and methomyl

The detection limits of thiodicarb and methomyl were found to be 15.3 and 31.9 ppb,respectively. These values indicate the applicability of the proposed method. Analysis of the tissue extracts from the *in vivo* biotrancformation experiment was accomplised by TLC and HPLC. The retardation factors, R_f (average of three developments) and the retention time, T_R values of thiodicarb and methomyl were listed in Table 1. The retardation factor values of thiodicarb and methomyl were 0.81 and 0.89, respectively. The corresponding values for retention time were 3.0 and 2.6 , respectively. Thiodicarb and methomyl were detected in all analyzed tissues.

Table (1):Thin layer chromatography and high performance liquid chromatographic data for thiodicarb and methomyl.

Compound	Retardation factor R_f	Retention time T_R (min)
Thiodicarb	0.81	3.0
Methomyl	0.89	2.6

Tissue uptake of thiodicarb:

Tissue residues were determined by HPLC at various time intervals up to 15 days in snails administered thiodicarb treated discs. The quantitative determination of the ingested thiodicarb was illustrated in Table 2. Thiodicarb was quickly absorbed and subsequently distributed throughout the body. At the first day following pesticide dosing to snails, thiodicarb was detected in the tissue extract . At this time point, the remaining amount of thiodicarb was found to be 150.95 ng/gm tissue . After 3 days, the amount of thiodicarb increased to reach a peak value of 198.60 ng/gm tissue . This was attributed to the continuous absorption of the compound (Table 2) . The concentrations of residual thiodicarb were rapidly declined overtime and at the end of experiment (15 days), it reached 0.18 ng/gm tissue. These results indicated that there was a consistent decrease in residues in snail tissues as time after administration increases and coincide with the results of Triebskorn *et al.*,(1990). They reported that the amounts of ^{14}C-labeled carbamate, 2-(2-chloro-1-methoxy-ethoxy)phenyl N-methylcarbamate or its labeled metabolites decreased by increasing time after the *Deroceras reticulatum* slug,ingestion.

Table (2): Concentration of thiodicarb and methomyl in snail tissue following fed on treated lettuce leaf disc loaded with 2 ug thiodicarb.

Time (days)	Amount recovered in intervals (ng/gm tissue)	
	Thiodicarb	Methomyl
1	150.95 ± 23.80	205.42 ± 10.20
2	163.46 ± 12.70	447.71 ± 29.07
3	198.60 ± 8.70	272.09 ± 8.60
5	88.60 ± 2.90	85.64 ± 5.60
7	20.66 ± 1.20	196.93 ± 3.22
10	3.73 ± 0.10	415.82 ± 13.70
15	0.18 ± 0.01	213.24 ± 10.80

Each value represents the mean of four samples of tissue from 20 animals ± S.E

The amounts of the main breakdown product, methomyl in snail tissues at all time intervals were very high compared to those of the parent compound, indicating that the metabolic processes took place following the absorption of the compound and there was no accumulation of the compound in the snail tissues.

Biotransformation of thiodicarb :

The *in vivo* biotransformation of thiodicarb (Table 2) showed that, methomyl is the major metabolite was detectable in the snail tissue extracts at all time intervals. The highest amounts of methomyl found in the snail tissues were at 2 and 10 days after thiodicarb administration. These amounts were 447.71 and 415.82 ng/gm tissue, respectively. On the other hand , the lowest amount of methomyl found in the snail tissues was at 5 day , which did account for 85.64 ng/gm tissue. By 15 days, thiodicarb metabolite concentration in the snail tissues was found to be 213.24 ng/gm tissue. These results indicated that thiodicarb was rapidly metabolized to yield methomyl as a major metabolite in the snail tissues. The level of the metabolite, methomyl in the snail tissues analyzed at any time following thiodicarb administration is the net result of the amount formed by the metabolism of the compound. The major thiodicarb metabolite formed by the snails used in this study indicated that the metabolic pathway was the same as reported for animals, plants, and soil (FAO/ WHO,1985).

Toxicity of thiodicarb and its major metabolite, methomyl to *Helix aspersa* snails

The toxic effects of thiodicarb and methomyl on the brown garden snails were determined and the average percent mortalities of these snails fed on treated lettuce discs with either thiodicarb or methomyl at six different concentrations of 0.01,0.02,0.1,0.2,0.5, and 1 % for the two time intervals of 1 and 7 days, were listed in Table 3. The results indicated that methomyl was more toxic to the snails than thiodicarb.These results are in agreement with that obtained by Radwan *et al.*,(1992). They reported that 0.5% bran baits of methomyl was the most toxic compound among all tested oxime carbamates against *Theba pisana* snails.

***In vivo* acetylcholinesterase inhibition by thiodicarb and its major metabolite, methomyl.**

The most significant biochemical effect of thiodicarb and its main breakdown product, methomyl are the ability to reversibly inhibit acetylcholinesterase, which is responsible for molluscicidal activity (Casida, 1963 and Radwan *et al.*1992). *In vivo* acetylcholinesterase activity in thiodicarb and methomyl - treated snails, 5 days after dosing with 2 ug of the chemical loaded on lettuce leaf disc and in controls are illustrated in Table 4.

Table (3): Toxicity of thiodicarb and its major metabolite, methomyl to *Helix aspersa* snails fed on treated lettuce leaf discs (presented as % mortality).

Compound Concentration (%)	Thiodicarb		Methomyl	
	Time (days)			
	1	7	1	7
0.01	0.00	0.00	0.00	6.67
0.02	0.00	0.00	0.00	13.33
0.1	10.00	53.33	26.67	60.00
0.2	13.33	66.67	46.67	73.33
0.5	36.67	70.00	53.33	80.00
1.0	50.00	86.67	55.00	93.33

Each value is average of four replicates.

The results presented in Table (4) showed that the control value for acetylcholinesterase activity in snail tissue was 0.456 m mole/min/gm tissue Thiodicarb and methomyl had inhibitory effect on acetylcholinesterase enzyme, the values were gradually reduced from the first day after dosing till the end of experiment (5 days). Comparing the data presented in this Table with the toxicity results in Table 3, showed a parallel effect between toxicity and enzyme inhibition. Thiodicarb and methomyl inhibited acetylcholinesterase enzyme in snail tissue by nearly 50% (48.47%), 5 and 3 days following administration of the chemical, respectively. At the end of 5 days, thiodicarb metabolite inhibited acetylcholinesterase activity by 71.05 % (Table 4).

These results indicated that both compounds inhibited acetylcholinesterase activity in snail tissues, although methomyl was a

more potent inhibitor to the tested enzyme than thiodicarb *in vivo*. The inhibition of acetylcholinesterase activity in snail tissues by thiodicarb, metabolite agree well with previous studies in *Theba pisana* snails (Radwan *et al.*,1992) and in *Limax maximum* slugs (Pessah and Sokolove,1983).

Table (4): *In vivo* acetylcholinesterase activity of snail tissues following administration with 2 ug of thiodicarb or methomyl loaded on lettuce leaf disc.

Time (days)	AchE activity			
	Thiodicarb		Methomyl	
	Activity	% of inhibition	Activity	% of inhibition
Control	0.456 ±0.024	100	0.456 ± 0.024	100
1	0.433 ±0.013	5.05	0.382 ± 0.032	16.23
2	0.382 ±0.030	16.23	0.345 ± 0.015	24.34
3	0.265 ±0.025	41.89	0.235 ± 0.046	48.47
5	0.235 ±0.034	48.47	0.132 ± 0.023	71.05

Acetylcholinesterase activity is expressed as m mole acetylthiocholine hydrolyzed/ min / gm tissue.
Each value represents the mean ± S.E. of four determinations from four animals.

Earlier studies on *Theba pisana* snails (Radwan *et al.*,1991) and on house flys (Karunaratne and Plapp,1993) indicated that thiodicarb was a weak inhibitor of the acetylcholinesterase enzyme *in vitro*. Consequently, it has been suggested that the chemical's toxicity *in vivo* may be due to the combined anticholinesterase effects of thiodicarb itself and its more toxic metabolite, methomyl.

The present study deduced that the biotransformation of thiodicarb to its main metabolite, methomyl was essential for snail toxicity and for the inhibition of acetylcholinesterase enzyme by thiodicarb.

REFERENCES

Abbott, W.S. (1925): A method computing the effectiveness of an insecticides. J.Econ. Entomol., 18 : 265-67.

Abu El-amayem, M.M.; Medy, M.A.Y.; El-Sebae, A.H.; Marei, A.S.M. and El- Khishen, S.A. (1990): Environmental fate of thiodicarb and methomyl in agricultural system. Proc. of the Regional Sym. Environ. Stud. (UNARC), Alexandria, 303-13.

Casida, J.E. (1963): Mode of action of carbamates. Ann. Rev. Entomol., 8 : 39-58 .

Ellman, G.L.; Courtney, K.D.; Andres, V. and Featherstone, R.M. (1961): A new rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol., 7 : 88-95.

El-Sebae, A.H.; El-Okda, M.M.K. and Marei, A.S. (1982): Local formulation of three carbamoylated oximes as terrestrial molluscicides. Agric. Res. Rev., 60: 85-91.

El-Wakil, H.B. and Radwan, M.A. (1991): Biochemical studies on the terrestrial snail, *Eubania vermiculata* (Muller) treated with some pesticides. J. Environ. Sci. Health, B26 (5&6): 479-89.

FAO/WHO (1985): Pesticide residues in food. Evaluation. Report No. 72/2 Part II, Toxicology, 171-76.

Ferguson, C.M., Firth, A.C. and Wyatt, R.T. (1995): Evaluation of a new slug bait incorporating thiodicarb. Proc. N.Z. Plant Prot. Conf., 48 *th*: 314-17.

Karunaratne, K.M. and Plapp, F. W. Jr. (1993): Biochemistry and genetics of thiodicarb resistance in the house fly (Diptera: Muscidae). J. Econ. Entomol., 86 (2) : 258-64.

Jones, R.L.; Hunt, T.W.; Norris, F.A. and Harden, C.F. (1989): Field research studies on the movement and degradation of thiodicarb and its metabolite, methomyl. J. Contam. Hydrol., 4 (4) : 359-71.

Miller, E.; Swails, S.; Swails, D.; Olson, F. and Staten, R.T. (1988): White garden snail (*Theba pisana* Muller): Efficacy of selected bait and sprayable molluscicides. J. Agric. Entomol., 5 (3): 189-97.

Mones, R. and Gigot, J. (1988) : A new slug bait containing 4% thiodicarb. Rev. Agric. (Brussels), 41 (5) : 1213-25 .

Pessah, I.N. and Sokolove, P.G. (1983) : The interaction of organophosphate and carbamate insecticides with cholinesterases

in the terrestrial pulmonate *Limax maximus*. Com. Biochem. Physiol., 74C : 291-97.

Radwan, M.A., El-Wakil, H.B. and Osman,K.A. (1992) : Toxicity and biochemical impact of certain oxime carbamate pesticides against terrestrial snail, *Theba pisana* (Muller). J. Environ. Sci. Health, B27 : 759-73 (1992).

Radwan, M.A.; Kassem, F.A. and Shawir, M.S. (1991) : Studies on acetylcholinesterase in the white garden snail, *Theba pisana* (Muller). Alex. Sci. Exch., 12 (2) : 335-49.

Salama, A.K. and Radwan, M.A. (1995) : Uptake, excretion, and metabolism of phorate in the land snail, *Helix aspersa* (Muller). J. Environ. Sci. Health, B30 (2) : 233-42 .

Triebskorn, R.; Kunast, C.; Huber, R. and Bren, G. (1990) : Tracing a C-labeled carbamate molluscicide through the digestive system of *Deroceras reticulatum* (Muller). Pestic. Sci., 28 : 321-30 .

WHO (1965) : Molluscicidal screening and evaluation. Bull., 38 ; 507-81.

الملخص العربى

التحول الحيوى للثيوديكارب الى المثيوميل والسميه وتثبيط الزيم الاستايل كولين استيريز فى القوقع الارضى هيلكس اسبرسا

د . محمد على رضوان د . احمد خميس سلامه
قسم كيمياء المبيدات – كليه الزراعه (الشاطبى)
جامعه الاسكندريه

تم استخدام جهاز HPLC ، TLC لدراسه التحول الحيوى لمبيد الثيوديكارب الى المثيوميل فى القوقع الارضى هيلكس اسبرسا – علاوه على دراسه السميه وتثبيط انزيم الاستايل كولين استيريز لمبيد الثيوديكارب ونواتج التحول الرئيسى له (الميثوميل). وقد اوضحت النتائج ان مبيد الثيوديكارب يمتص بسرعه بعد التغذيه خلال ٢٤ ساعه – وقد وصل المبيد لقيمه عظمى (٦و١٩٨ نانوجرام / جم نسيج) عند ٣ يوم ثم انخفضت هذه القيمه بسرعه مع الوقت حتى نهايه التجربه – وقد حدث للمبيد تمثيل داخل جسم القوقع بسرعه الى الميثوميل كناتج تحول رئيسى عند كل فترات التجربه. واظهرت النتائج ايضا ان الميثوميل اكثر سميه ضد القوقع المختبر عن الثيوديكارب وبالنسبه لنتائج هذين المركبين على نشاط انزيم الاستايل كولين استيريز داخليا in - vivo وجد ان هذه النتائج تتوافق مع نتائج السميه ضد القوقع المختبر. وتوضح هذه النتائج بان تكوين الميثوميل كناتج تمثيل رئيسى فى القوقع المختبر لتفسير النشاط الابادى لمبيد الثيوديكارب ضد القوقع الارضى محل الدراسه.