EFFECTS OF THE IGR, TH6040, ON NONTARGET ORGANISMS WHEN UTILIZED AS A MOSQUITO CONTROL AGENT

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ABSTRACT. Thompson-Hayward 6040 [1-(4-chlorophenyl)-3-(2, 6-difluorobenzoyl)-urea], at rates used for mosquito control, is relatively safe to organisms associated with mosquito breeding habitats. Although applications at rates of 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, and 0.05 lb AI/acre to irrigated pastures resulted in the reduction of cladoceran and mayfly nymph populations, both populations recovered within a short period of time. Even repeated applications on the same pasture did not eliminate the populations. Corixid and notonectid nymphs may be affected by the treatment; however, mortality was so slight that the population growth rate was not altered. Adult aquatic beetles showed a tolerance, but a few dead beetle larvae {*Laccophilus* spp., *Hydrophilus triangularis* Say, and *Tropisternus lateralis* (Fabricius)} were observed in treated water. Spiders (*Pardosa* spp. and *Lycosa* spp.) showed no apparent effects.

Thompson-Hayward 6040 [1-(4-chlorophenyl)-3-(2, 6-difluorobenzoyl)-urea] is an experimental insecticide with a wide range of biological activity. The mode of action of the compound has been reported as the inhibition of chitin synthesis during molting, thus interfering in the formation of endocuticular deposition (Mulder and Gijswijt, 1973; Post and Vincent, 1973).

Preliminary studies of its potential use as a mosquito larvicide have been reported by Jacob (1973), Schaefer et al. (1974), Hsieh and Steelman (1974). Miura and Takahashi (1974) reported results from the laboratory and limited field studies concerning the effects of TH6040 on nontarget organisms associated with mosquito breeding habitats.

A practical evaluation of this compound for mosquito control, under operational conditions, was conducted by Schaefer et al. (1975); concurrent studies on the effects of the treatments on nontarget organisms *in situ* were conducted and are reported here.

MATERIALS AND METHODS. A total of 62 species or species groups was collected from the treated pastures. They are as follows: 2 species of turbellarians, *Bothromesostoma personatum* (D. Schmidt); *Mesostoma ehrenbergii* (Focke); 1 genus of rotifers, *Asplanchna* spp.; 2 species of annelids, *Aeolosoma hemprichi* Ehrenberg, *Dero pierrieri* Bousf.; 2 species of copepods, *Cyclops vernalis* Fischer, *Diaptomus* spp.; 2 genera of *Cypricercus* spp., *Cypridopsis* spp.; 6 genera of water fleas, *Alona* spp., *Ceriodaphnia* spp., *Daphnia* spp., *Moina* spp., *Scapholeberis* spp., *Simocephalus* spp.; 1 species of side swimmers,

Hyalella azteca (Saussure); 1 genus of mayfly nymphs, *Callibaetis* spp.; 1 genus of damselfly nymphs, *Argia* spp.; 2 genera of dragonfly nymphs, *Orthemis* spp., *Pantala* spp.; 2 genera of back swimmers, *Buenoa* spp., *Notonecta unifasciata* Guerin; 1 species of *Corisella decolor* Uhler; 1 species of water striders, *Gerris incurvatus* Drake and Hottes; 9 genera of dytiscid beetles, *Agabus* spp., *Copelatus* spp., *Cybister* spp., *Deronectes* spp., *Hydrovatus* spp., *Ilybius* spp., *Laccophilus* spp., *Oreodytes* spp., *Thermonectus basillaris* (Harris); 6 genera of hydrophilid beetles, *Berosus* spp., *Enochrus* spp., *Helophorus* spp., *Hydrophilus triangularis* Say, *Tropisternus lateralis* (Fabricius), *Tropisternus* spp.; 5 genera of chironomid midge larvae, *Chironomus attenuatus* Walker, *Chironomus* spp., *Chironomus stigmaterus* Say, *Goeldichironomus holoprasinus* (Goeldi), *Tanypus* spp.; 1 genus of syrphid fly larvae, *Xylota* spp.; 1 species of *Brachydeutera argentata* (Walker); 1 oribatid mite; 9 genera of spiders, *Tetragnatha laboriosa* Hentz, *Tetragnatha* spp., *Lycosa* spp., *Pardosa* spp., *Oxyopes salticus* Hentz, *Misumenops asperatus* (Hentz), *Tibellus oblongus* (Walckenaer), *Xysticus* spp., *Pellenes* spp.; 1 genus of snails, *Physa* spp.; 5 genera of algae, *Eudoria* spp., *Volvox* spp., *Hydrodictyon reticulatum* (L.) Lagerh, *Pithophora oedogonia* (Mont.) Wittr., *Spirogyra* spp.

Various sized pastures (4 to 55 acres), totaling 584 acres in the Central Valley of California, were treated with a 25% WP formulation of TH6040 in 19 separate aircraft trials. The rates ranged from 0.02 to 0.05 lb AI/acre with increments of 0.005 lb. Procedures used for aircraft applications are explained in detail by Schaefer et al. (1975).

Each test was assigned a field test number (F. T. No.), and location, environmental condition (temperature, wind, skycover, water depth, and vegetation canopy) and spray coverage were recorded.

The biological activity of TH6040 against planktonic organisms was determined by censusing populations; a bucketful of pre- and post-treatment sample water (5 to 7 l) was taken at random across the test field with a long-handled dipper (450 ml capacity) and transported to the laboratory, where population changes were monitored daily. The technique used for monitoring was essentially the same as previously described by Miura and Takahashi (1973) with some modifications; a known amount of the samples (4.5 l) was randomly dipped from the collecting bucket and poured into a concentrator (a plastic tube, 30 x 80 mm, with a fine nylon mesh at the end). Organisms in the concentrator were gently washed into a Pyrex® custard cup (5 oz), siphoned into a pipet (1 ml), counted under a stereomicroscope, and then returned to their respective containers.

In addition to monitoring pre- and post-treatment samples in the laboratory (as described above), field populations were monitored by making daily water collections and counting the nontarget organisms present. Counts to measure fluctuations in abundance of free-moving aquatic insects in the test fields also were made daily using modified minnow traps (the inner side of the trap was lined with a piece of window screen). Unbaited traps were set in the field water in the morning and retrieved the next morning for counts. Spider populations in the pastures also were estimated by trapping with the modified minnow traps, and supplemental collections were made at irregular intervals with a sweep net during inspection of the pastures.

Bioassays of the treated pasture water were conducted at daily intervals for 3 to 4 days; 3 aliquots of filtrate from pre- and post-treatment water (ca. 100 ml each) were placed into Pyrex custard cups, and 30 to 50 mixed stages of laboratory reared water fleas (*Ceriodaphnia* spp., *Moina* spp.) were added to each cup. Mortality was checked daily for 5 days.

RESULTS AND DISCUSSION. Side effects of TH6040 on planktonic organisms were examined on 19 separate field tests and included 7 rates of treatment (0.02, 0.025,

0.03, 0.035, 0.04, 0.045, and 0.05 lb AI/acre). It is impractical to include all results; therefore, only representative data and summary of results are given in this report. The unreported information is similar and is available to interested persons.

According to Schaefer et al. (1975), a rate of 0.025 lb AI/acre achieved operational success against pasture mosquitoes [*Aedes nigromaculis* (Ludlow) and *Aedes melanimon* Dyar]. Table 1 summarizes the effects on planktonic organisms when the compound was applied at 0.025 lb AI/acre against pasture mosquitoes. It is apparent that the compound will reduce water flea and mayfly nymphal populations temporarily. However, the reduction was so slight, in most cases, that without daily counts of the post-treatment sample collected immediately after treatment, one might overlook this reduction (F. T. Nos. 74–5, 7, 8, 11, 12, 13, 21, 23, 32, 35, and 37). Water flea mortality usually occurred over a period of 1 or 2 days after treatment. The common symptoms of affected water fleas were slower filter feeding and reduced hopping movement. Mayfly nymphs died during the process of post-treatment ecdysis (Fig. 1). A characteristic sign of lethal effects is an incomplete cleavage of the middorsal ecdysial suture. Copepods and side swimmers usually demonstrated tolerance (F. T. Nos. 74–7, 8, 12, 21, 23, and 35), but sometimes the population was suppressed (Table 1, F. T. Nos. 74–5, 11, 13, and 37). Affected crustaceans also died during post-treatment ecdysis. Fig. 2A shows a dead side swimmer; old cuticle over the thoracic somites

Table 1. Effects of TH6040 on nontarget organisms: Applied at 0.025 lb AI/acre against pasture mosquitoes, July 17, 1974 (F. T. No. 74–28).

Organism	July 17	18	19	20	21	22	24	31
	No. of Organisms in the Pre-Treatment Water (2,250 ml) Held in the Laboratory							
Water fleas	351	310	305	316	326	469	1,562	
Copepods	223	276	257	263	268	265	279	
Seed shrimp	1	1	1	1	1	0	0	
Rotifers	+[a]	+	+	+	+	+	+	
Mayfly N[b]	50	43	43	43	44	39	32	
Shore fly L[c]	1	1	1	1	0	0	0	
Beetle L[c]	5	3	2	2	2	2	2	
	No. of Organisms in Water (2,250 ml) Collected Immediately After Treatment and Held in the Laboratory							
Water fleas	329	13	0	0	0	0	0	
Copepods	125	54	54	48	41	51	56	
Rotifers	+	+	+	+	+	+	+	
Corixid N[b]	2	2	0[d]	0	0	0	0	
Mayfly N[b]	18	9	0[d]	0	0	0	0	
Beetle L[c]	3	4	2[d]	0	0	0	0	
	No. of Organisms in Water from Daily Field Collections (2,250 ml)							
Water fleas		39	44	73	3	3	2	43
Copepods		96	61	67	15	17	36	116
Seed shrimp		4	2	1	2	2	1	2
Rotifers		+	+	+	+	+	+	+
Mayfly N[b]		28	44	72	61	134	55	260
Shore fly L[c]		0	2	0	0	0	0	0
Corixid N[b]		0	3	0	4	12	1	6
Dragonfly N[b]		0	0	2	0	0	0	0
Beetle L[c]		5	15	13	19	19	10	13

[a] + = present, but not counted.
[b] N = nymphs.
[c] L = larvae.
[d] Dead organisms were noted.

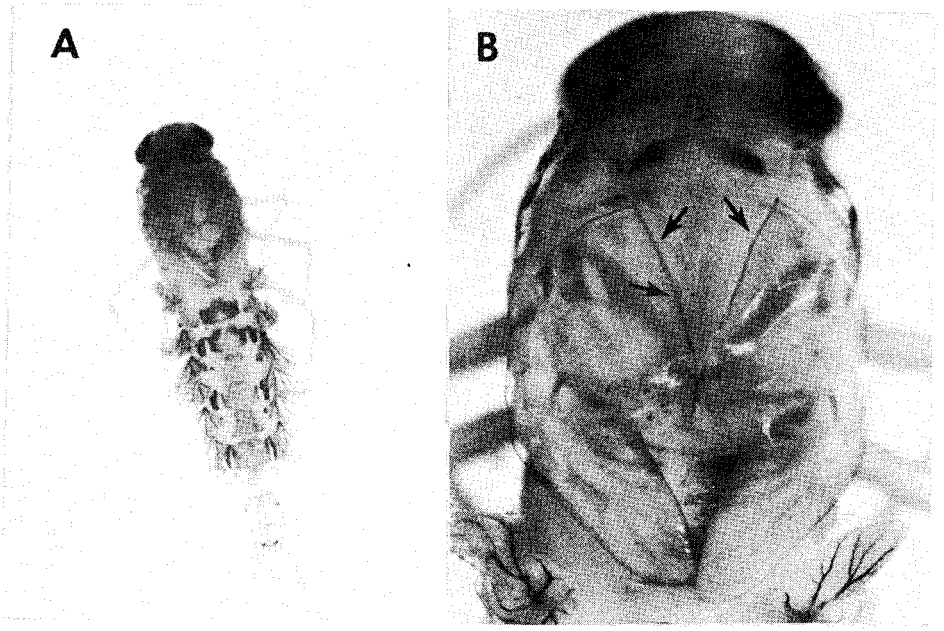

Fig. 1. *Callibaetis* sp. nymph. A. Characteristic signs of lethal effects of TH6040. B. Enlarged mesotergum area showing middorsal ecdysial cleavage (arrows).

was partially shed but the posterior end was still attached to the body.

Immature insect populations were also slightly affected; the deleterious effect was only detected by observations of dead animals in the treated water (F. T. Nos. 74–7, 28, and 37). Dead insects due to the treatment are shown in Figures 2B, 2C, and 2D; in all cases death occurred during post-treatment ecdysis. In view of those results, it is thought that this compound interferes directly or indirectly in the formation of ecdysial cleavage.

There were no noticeable deleterious effects on turbellarians, rotifers, seed shrimp, and fresh water algae (F. T. Nos. 74–5, 7, 8, 11, 12, 13, 21, 23, 32, and 35).

Table 2 summarizes results of multiple applications of the compound to the same pasture. Although water fleas and mayfly nymphs indicated high sensitivity to a single treatment, repeated monthly treat-

Table 2. Effects of TH6040 on nontarget organisms: Four multiple applications on the same pasture against pasture mosquitoes (F. T. Nos. 74–5, 74–13, 74–28, 74–35).[a]

Organism	Date of treatment and dose (lb AI/acre)			
	May 17	June 14	July 17	August 15
	0.04	0.02	0.025	0.025
Water fleas	92	69	351	15
Copepods	192	17	223	3
Seed shrimp	2	8	1	1
Rotifers	+[b]	+	+	+
Mayfly N[c]	2	11	50	27
Chironomid L[d]	10	15	5	+
Shore fly L[d]	1	44	1	7
Beetle L[d]	9	1	5	6
Orbatid mites	+	+	+	+
Corixid N[c]	+	+	+	+

[a] Number of organisms in pre-treatment sample (4,500 ml).
[b] + = present, but not counted.
[c] N = nymphs.
[d] L = larvae.

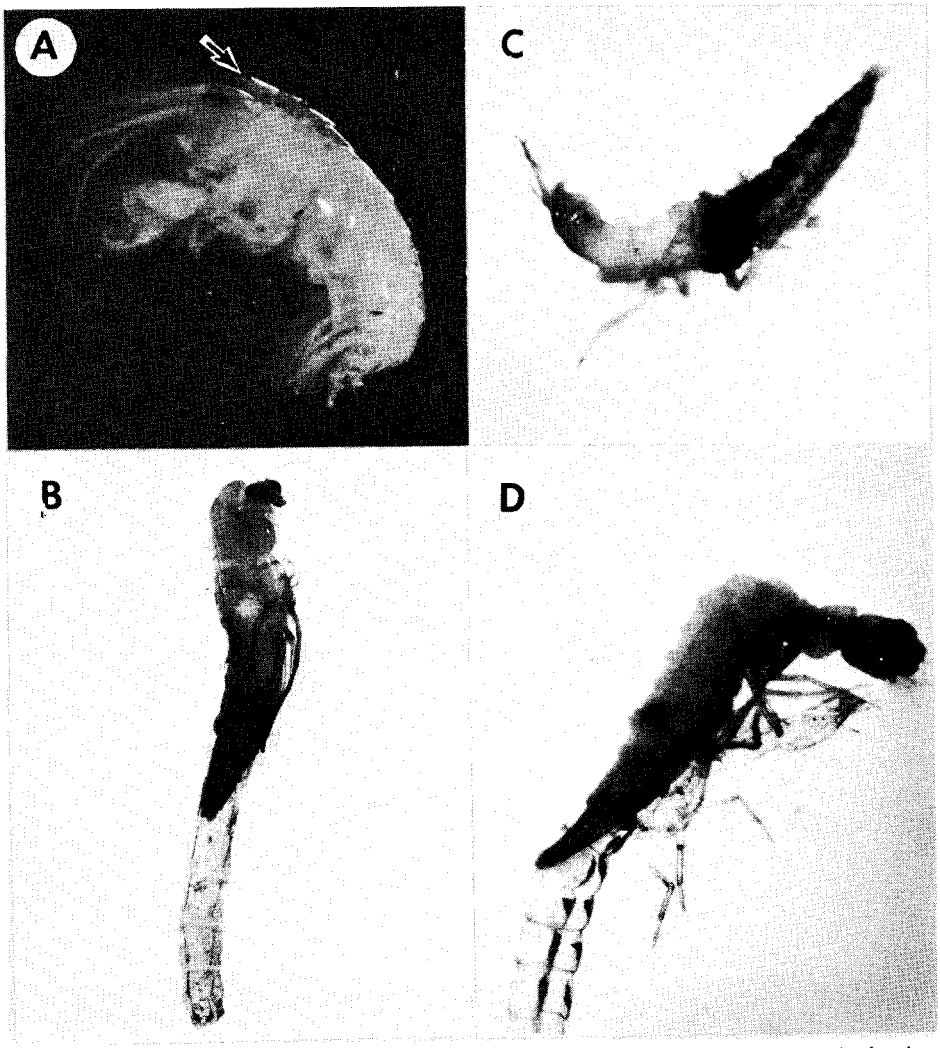

Fig. 2. Characteristic effects of TH6040 on nontargets: A. Side swimmer (*H. azteca*) showing partially shed old cuticle over the thoracic somites (arrow). B. Midge (*G. holoprasinus*) showing partially emerged adult from pupal case. C. Hydrophilid larva (*H. triangularis*) showing larva/larva ecdysis. Anterior parts (head and most of thorax) are free, and rest of the body is still in old larval case. D. Dytiscid larva (*Laccophilus* sp.) showing near completion of larva/larva ecdysis. Some legs are still in old larval leg sheaths.

Table 3. Effects of TH6040 on nontarget organisms: Applied at 0.02 lb AI/acre against pasture mosquitoes, May 17, 1974 (F. T. No. 74–5).

Organism	May 18	20	21	22	23
C. decolor N[b] A[c]	94	79	112	121	19
N. unifasciata N[b] A[c]	1	1	4	0	1
Gerris spp. A[c]	1	1	0	0	1
H. triangularis A[c]	1	2	0	0	0
T. lateralis A[c]	6	8	7	2	2
Helophorus spp. A[c]	4	7	2	7	3
Thermonectus spp. A[c]	5	9	14	5	2

[a] Number of organisms collected by 3 minnow traps.
[b] N = nymphs.
[c] A = adults.

ments for a 4-month period did not eliminate the populations. There were some indications of population fluctuations at each pre-treatment count. The fluctuations may be due to an annual cycle of the organisms, to the sampling techniques, or probably to both.

Bioassay of water samples treated with TH6040 and held in the laboratory demonstrated that the activity of this compound did not persist in water long enough to exterminate water flea populations. At a rate of 0.02 lb AI/acre by aircraft trials, the mortality was 100, 55.5, 40, and 31.2% at holding times of 0, 24, 48, and 72 hr (F. T. No. 74–9); at a rate of 0.04 lb, the mortality was 100, 100, 84.4, and 74.7% (F. T. No. 74–5).

High tolerance was noted among adult beetles (Table 3, F. T. No. 74–13) and lycosid spiders (Table 4).

Table 4. Effects of TH6040 on spiders: Applied at 0.025 lb AI/acre against pasture mosquitoes August 15, 1974 (F. T. No. 74–35)[a] (in number of spiders in daily trap collections).

	August						
Trap No.	13	14	15	16	17	18	19
1	5	5	3	10	1	2	5
2	1	1	2	1	3	3	5
3	0	0	0	0	3	1	1
4	8	4	4	5	1	11	15
5	2	1	2	1	0	1	3
6	1	0	1	5	4	2	1
7	0	0	0	2	0	0	1
8	1	4	3	3	2	3	3
Total	18	15	15	27	14	23	35

[a] Most of the spiders collected by minnow traps were *Pardosa* spp. probably *ternalis* and a few *Lycosa* spp.

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