Chlordimeform Penetration, Metabolism, and Excretion in Diapausing and Nondiapausing Larvae of the Southwestern Corn Borer

Apurba K. Gayen and Charles O. Knowles*

Chlordimeform penetration, metabolism, and excretion were examined in nondiapausing larvae (NDL), environmentally induced diapausing larvae (EIL), and hormonally induced diapausing larvae (HIL) of the southwestern corn borer, *Diatraea grandiosella* Dyar. Chlordimeform penetration and excretion decreased in the order NDL > EIL > HIL, while chlordimeform metabolism decreased in the order HIL > EIL > NDL. The major differences in chlordimeform metabolism in HIL as compared to that in EIL and NDL were the marked decrease in the former of levels of parent compound and concomitant increase in levels of the metabolite 4-chloro-*o*-toluidine. 4-Chloro-*o*-formotoluidide, the usual precursor for 4-chloro-*o*-toluidine, did not appear to be the intermediate for much of the 4-chloro-*o*-toluidine detected in HIL. It was proposed that cleavage between the arylamino and carbinol moieties of the carbinoldiamine formed following hydration of chlordimeform constituted a "direct" route for 4-chloro-*o*-toluidine formation which bypassed 4-chloro-*o*-formotoluidide. These studies have established a probable relationship between xenobiotic metabolism and juvenile hormone mediated changes in southwestern corn borer larvae.

Chlordimeform or *N'*-(4-chloro-*o*-tolyl)-*N,N*-dimethylformamidine has a unique activity spectrum, being toxic mainly to acarines and lepidopterous insects (Knowles, 1976). Its fate has been examined in several species of target acarines and insects including the cattle tick, *Boophilus microplus* (Canestrini) (Schuntner, 1971; Knowles and Schuntner, 1974), the two-spotted spider mite, *Tetranychus urticae* Koch (Chang and Knowles, 1977), the rice stem borer, *Chilo suppressalis* Walker (Morikawa et al., 1975), and the cabbage looper, *Trichoplusia ni* (Hübner) (Crecelius and Knowles, 1976). The availability of different types of laboratory-reared larvae of the southwestern corn borer (SWCB), *Diatraea grandiosella* Dyar, afforded us a unique opportunity to examine the behavior of chlordimeform in another species of lepidopterous insect. Thus, in this paper we describe the penetration, metabolism, and excretion of chlordimeform in nondiapausing larvae and in two types of diapausing larvae of the SWCB.

MATERIALS AND METHODS

Compounds. Chlordimeform-^{14}C, radiolabeled at the tolyl carbon atom (sp act. 4.1 mCi/mmol), was provided by Ciba-Geigy Corp., Greensboro, NC. Radioactive *N'*-(4-chloro-*o*-tolyl)-*N*-methylformamidine or demethylchlordimeform-^{14}C was isolated by TLC (silica gel GF_{254}; benzene–acetone–diethylamine, 75:20:5) from the decomposition mixture which resulted from incubating an acetone solution of uniformly ring labeled (sp act. 4.6 mCi/mmol) *N,N'*-thiobis[*N*-methyl-*N'*-(4-chloro-*o*-tolyl)formamidine] (Upjohn 46506) at 28 °C for 4 h. 4-Chloro-*o*-formotoluidide-^{14}C was prepared by alkaline hydrolysis of radioactive demethylchlordimeform, and 4-chloro-*o*-toluidine-^{14}C was prepared by alkaline hydrolysis of radioactive 4-chloro-*o*-formotoluidide; both products were isolated by TLC from ethylene chloride extracts of the respective reaction mixtures as described above. The following nonradioactive compounds were examined as potential chlordimeform (I) metabolites: demethylchlordimeform (II), *N'*-(4-chloro-*o*-tolyl)formamidine or didemethylchlordimeform (III), 1,1-dimethyl-3-(4-chloro-*o*-tolyl)urea (IV), 1-methyl-3-(4-chloro-*o*-tolyl)urea (V), 3-(4-chloro-*o*-tolyl)urea (VI), 4-chloro-*o*-formotoluidide (VII), 4-chloro-*o*-toluidine (VIII), *N*-formyl-5-chloroanthranilic acid (IX), and 5-chloroanthranilic acid (X).

Insects. SWCB larvae were reared at 30 °C (16 h of light; 8 h of dark) on an artificial diet; they entered their final instar around 10 days of age. Early last instar larvae (12 days of age; average weight 120 mg) were employed and were designated nondiapausing larvae (NDL). Other larvae were reared at 23 °C (12 h of light; 12 h of dark) on an artificial diet; they entered diapause as fully grown larvae around 40 days of age. Early diapausing larvae (60–75 days of age; average weight 190 mg) were employed and were designated environmentally induced diapausing larvae (EIL). Since NDL treated with a juvenile hormone mimic have been shown to enter a diapause-like state (Yin and Chippendale, 1974), NDL reared at 30 °C (16 h of light; 8 h of dark), which were 12 days of age, were each treated topically on the abdomen with 3.0 μg of the juvenile hormone mimic ethyl 10-methoxy-3,7,11-trimethyl-2,4-dodecadienoate (Zoecon 1662). Larvae treated in this way (35–40 days of age; average weight 530 mg) were employed and were designated hormonally induced diapausing larvae (HIL). The rearing procedures and physiological characteristics of these three types of larvae have been described (Yin and Chippendale, 1974; Chippendale, 1979).

Penetration Studies. NDL, EIL, and HIL were treated topically on the dorsum of the thorax with 10 000 dpm (0.38 μg) of chlordimeform dissolved in 1 μL of acetone. Treated larvae were held individually in metabolism chambers (Chang and Knowles, 1977) at 28 °C for time intervals of 0, 5, 15, 30, 60, 120, 240, and 960 min. Three replicates of 10 larvae/replicate were analyzed at each time interval. At the end of the exposure time, each larva was rinsed with acetone (5 × 1 mL). The acetone rinses were combined in a scintillation vial and concentrated to dryness, and 10 mL of Aquasol-2 (New England Nuclear) counting solution was added. The radioactivity was measured with a Beckman LS 7500 scintillation counter. This fraction constituted the external radioactivity. Following the external rinses, each larva was combusted in a Model 306 Tri-carb sample oxidizer (Packard); the $^{14}CO_2$ was trapped (Carbosorb, Packard) and radioassayed. This fraction constituted the internal radioactivity. To the metabolism chamber, which was a glass scintillation vial, was added 10 mL of Aquasol-2, and the radioactivity

Department of Entomology, University of Missouri, Columbia, Missouri 65211.

0021-8561/82/1430-0122$01.25/0

was measured. This fraction comprised the container rinse. Penetration was assessed from a graph of exposure time vs. percentage of radioactivity recovered from each of the above fractions.

Metabolism and Excretion Studies. NDL, EIL, and HIL were injected in the thorax with 10000 dpm (0.38 μg) of chlordimeform dissolved in 1 μL of acetone. In addition, NDL and HIL were injected with 10000 dpm (0.22 μg) of demethylchlordimeform, 4-chloro-o-formotoluidide, or 4-chloro-o-toluidine. Treated larvae were held individually at 28 °C for the time intervals of 15, 60, and 120 min in metabolism chambers identical with those used for penetration studies. Three replicates of six larvae/replicate were analyzed at each time interval. After the desired exposure period, 10 larvae were removed from their respective metabolism chamber and ground in an all-glass homogenizer with 6 mL of acetone–water (1:1). The homogenate was centrifuged at low speed for 5 min, and the supernatant was decanted. The particulate material was extracted with acetone (2 × 5 mL) and water (2 × 5 mL). The extracts and the original supernatant were combined, and the acetone was evaporated under reduced pressure. The fraction remaining after the acetone was evaporated was extracted with ethyl acetate (3 × 30 mL). The volume of the combined ethyl acetate extracts was reduced to 10 mL under reduced pressure and dried over anhydrous sodium sulfate, and duplicate 1.0-mL aliquots were radioassayed after evaporation of the solvent. Radioactivity in the ethyl acetate fraction was designated the organic extract. Duplicate 1.0-mL aliquots of the water fraction remaining after ethyl acetate extraction were radioassayed, and the radioactivity in this fraction was designated the aqueous extract.

The remainder of the organic extract was reduced in volume to 0.2 mL and was subjected to two-dimensional TLC, autoradiography, and radioassay for separation, tentative identification, and quantitation of its radioactive components. The adsorbent for TLC was silica gel GF_{254} (500 μm), and the solvent system was benzene–acetone–diethylamine (75:20:5; first direction) and benzene–dioxane–acetic acid (90:25:4; second direction). A diagrammatic representation of the chromatographic behavior of chlordimeform and its potential metabolites in this system has been published (Knowles and Benezet, 1977).

The SWCB residue remaining after extraction was dried at 28 °C, and the total radiocarbon content was measured by combustion and subsequent radioassay.

The radiocarbon remaining in the metabolism chamber, which constituted the container rinse, was measured as described under Penetration Studies.

RESULTS

Figure 1 gives the distribution of radiocarbon following topical treatment of NDL, EIL, and HIL with chlordimeform. Differences in chlorodimeform behavior among the three larval types were apparent. The time for disappearance of 50% of the radiocarbon in the external rinse was about 10 min for NDL, 25 min for EIL, and 40 min for HIL. The larvae differed also with regard to the time required for levels of internal radioactive material to equal those in the external rinse; these times were about 1 h for NDL, 6 h for EIL, and 7 h for HIL. The radiocarbon in the container rinse probably consisted mainly of excreted material, although some "rub off" could have contributed. Generally levels of radiocarbon in this fraction were highest with NDL and EIL and lowest with HIL (Figure 1).

The fate of chlordimeform-^{14}C following injection into SWCB larvae is given in Table I. Chlordimeform (I) was degraded by NDL, EIL, and HIL. Judging by levels of

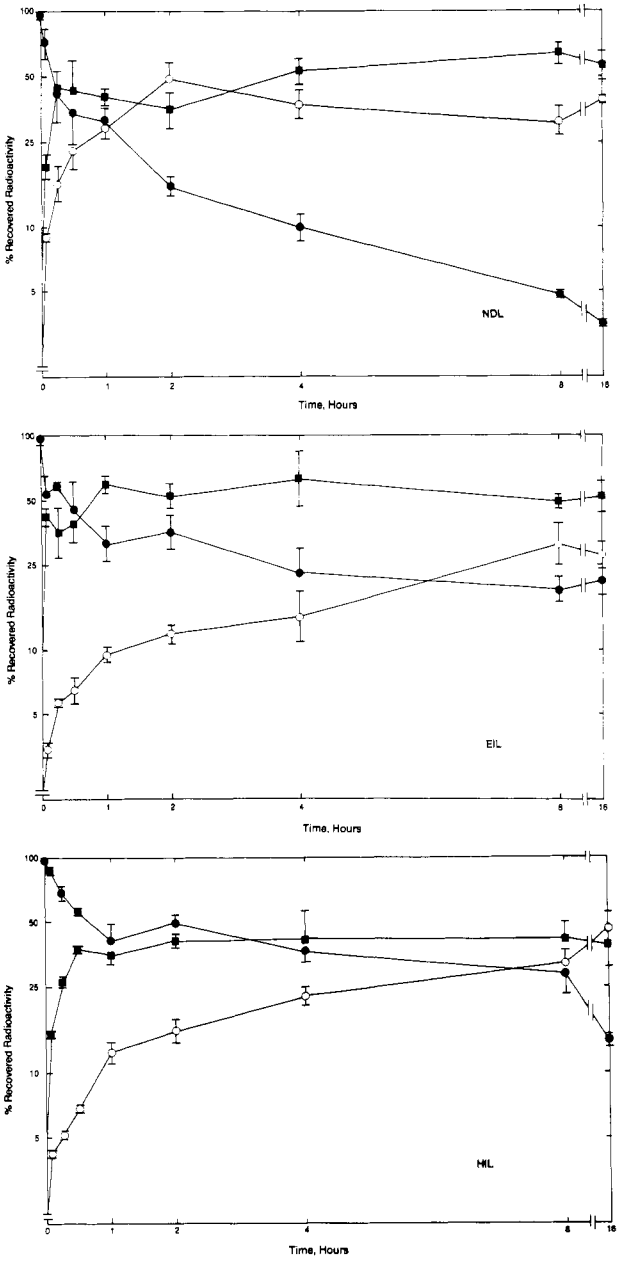

Figure 1. Distribution of radioactive material following topical treatment of nondiapause larvae (NDL), environmentally induced diapause larvae (EIL), and hormonally induced diapause larvae (HIL) of the southwestern corn borer larvae with chlordimeform. (●) External radioactive material; (○) internal radioactive material; (■) radioactive material in holding vial. Average recovery of applied radiocarbon was 97.1% (range of 94.3–98.7%) for NDL, 96.5% (range of 96.8–99%) for EIL, and 97.0% (range of 95.3–98.6%) for HIL. Vertical bars denote the standard deviation about the mean.

parent compound (I) at 120 min postinjection, HIL were appreciably more active than were NDL or EIL. For example, chlordimeform (I) comprised 73.9 and 71.1%, respectively, of the recovered radiocarbon from NDL and EIL but only 49.2% from HIL. The major metabolite was 4-chloro-o-toluidine (VIII). Its peak level was 3.2% in NDL and 14.6% in EIL at 60 min postinjection; however, in HIL it comprised 35.2% of the recovered radiocarbon at 120 min, and it possibly had not yet reached its maximum level. Other major chlordimeform metabolites (>2.0%) in SWCB larvae included demethylchlordimeform (II), which comprised 2.1% at 120 min in EIL and 4.7% at 60 min in HIL, and 4-chloro-o-formotoluidide (VII), which comprised 3.5% at 120 min in EIL and 2.3% at 120

Table I. Fate of Chlordimeform-^{14}C in Nondiapause Larvae (NDL), Environmentally Induced Diapause Larvae (EIL), and Hormonally Induced Diapause Larvae (HIL) of the Southwestern Corn Borer[a]

fraction or compound	% recovered radioactivity at indicated time after injection								
	NDL			EIL			HIL		
	15 min	60 min	120 min	15 min	60 min	120 min	15 min	60 min	120 min
organic extract									
I	82.8 (1.9)	77.2 (2.0)	73.9 (0.5)	76.4 (1.5)	75.3 (1.2)	71.1 (0.9)	87.5 (1.8)	56.8 (2.1)	49.2 (1.4)
II	0.9 (0.3)	1.4 (0.4)	0.9 (0.1)	1.0 (0.2)	0.5 (0.1)	2.1 (0.4)	1.1 (0.2)	4.7 (0.5)	1.0 (0.6)
III	0.3 (0.1)	0.3 (0.1)	0.6 (0.2)	<0.1 (0.0)	<0.1 (0.0)	<0.1 (0.0)	<0.1 (0.0)	0.5 (0.1)	0.5 (0.1)
VI	<0.1 (0.0)	0.2 (0.0)	0.5 (0.1)	<0.1 (0.0)	<0.1 (0.0)	<0.1 (0.0)	<0.1 (0.0)	0.4 (0.1)	0.3 (0.1)
VII	1.3 (0.2)	1.7 (0.4)	1.9 (0.2)	2.6 (0.2)	2.9 (0.4)	3.5 (0.6)	1.8 (0.5)	1.5 (0.6)	2.3 (0.4)
VIII	1.4 (0.4)	3.2 (0.3)	1.8 (0.3)	11.4 (0.7)	14.6 (1.1)	10.0 (1.6)	4.4 (1.4)	26.8 (2.2)	35.2 (1.8)
IX	0.7 (0.2)	0.7 (0.1)	0.5 (0.1)	<0.1 (0.0)	<0.1 (0.0)	<0.1 (0.0)	<0.1 (0.0)	0.5 (0.1)	0.5 (0.2)
X	0.6 (0.3)	1.1 (0.3)	0.5 (0.0)	<0.1 (0.0)	<0.1 (0.0)	<0.1 (0.0)	<0.1 (0.0)	0.5 (0.1)	0.4 (0.1)
origin	0.6 (0.1)	0.2 (0.0)	0.2 (0.0)	0.7 (0.1)	0.2 (0.1)	0.7 (0.3)	0.7 (0.1)	0.5 (0.1)	0.2 (0.1)
aqueous extract	0.8 (0.1)	0.4 (0.0)	2.2 (0.1)	0.8 (0.0)	0.7 (0.0)	0.8 (0.1)	0.5 (0.0)	1.0 (0.0)	0.8 (0.1)
residue	0.3 (0.0)	0.5 (0.0)	0.4 (0.0)	0.5 (0.0)	0.5 (0.0)	0.5 (0.0)	0.5 (0.0)	0.4 (0.0)	0.5 (0.1)
container rinse	10.3 (0.5)	13.0 (0.9)	16.6 (0.7)	6.6 (0.3)	5.3 (1.3)	11.3 (0.3)	3.5 (0.1)	6.4 (0.5)	9.1 (0.0)
total recovered	98.2 (1.1)	96.6 (0.9)	93.1 (0.4)	95.1 (1.0)	94.1 (1.3)	96.2 (1.0)	93.5 (1.4)	95.1 (3.1)	93.3 (1.8)

[a] Figures are the mean of three replicates with the standard deviation in parentheses.

Table II. Fate of Demethylchlordimeform-^{14}C in Nondiapause Larvae (NDL) and Hormonally Induced Diapause Larvae (HIL) of the Southwestern Corn Borer[a]

fraction or compound	% recovered radioactivity at indicated time after injection					
	NDL			HIL		
	15 min	60 min	120 min	15 min	60 min	120 min
organic extract						
II	3.6 (0.4)	6.2 (0.8)	4.5 (0.6)	22.2 (0.9)	17.7 (0.9)	10.4 (1.3)
III	13.3 (1.1)	16.2 (1.4)	16.7 (1.2)	30.9 (1.1)	24.9 (0.7)	25.8 (1.2)
VI	0.6 (0.3)	0.7 (0.2)	0.8 (0.5)	0.7 (0.2)	1.1 (0.4)	0.7 (0.3)
VII	59.8 (1.4)	47.1 (1.1)	43.4 (1.8)	33.1 (1.0)	31.8 (1.1)	31.8 (1.1)
VIII	2.2 (0.2)	1.8 (0.3)	3.2 (0.4)	2.8 (0.3)	5.3 (0.8)	7.1 (0.9)
IX	1.3 (0.2)	1.7 (0.1)	1.6 (0.2)	1.1 (0.2)	1.9 (0.4)	1.2 (0.2)
X	0.8 (0.1)	0.9 (0.2)	1.1 (0.2)	1.3 (0.3)	1.8 (0.3)	1.2 (0.3)
origin	1.9 (0.3)	2.8 (0.5)	4.2 (0.4)	1.4 (0.1)	3.0 (0.6)	3.3 (0.7)
aqueous extract	7.3 (0.2)	5.6 (0.2)	7.6 (0.3)	4.5 (0.1)	3.7 (0.1)	3.5 (0.3)
residue	0.7 (0.1)	0.7 (0.1)	0.8 (0.1)	1.3 (0.4)	0.9 (0.2)	2.1 (0.3)
container rinse	8.5 (0.1)	16.3 (0.2)	16.1 (0.3)	0.7 (0.1)	7.9 (0.5)	12.9 (0.2)
total recovered	97.2 (0.6)	96.7 (0.9)	97.1 (0.8)	96.4 (1.6)	95.8 (0.9)	96.7 (1.8)

[a] Figures are the mean of three replicates with the standard deviation in parentheses. Demethylchlordimeform is somewhat unstable. When a sample was carried through the extraction procedure in the absence of SWCB larvae, 65% remained as II, 18% was III, 11% was VII, 2% was VIII, and the remainder was equally divided among VI, IX, X, and origin.

min in HIL. Low levels of didemethylchlordimeform (III), *N*-formyl-5-chloroanthranilic acid (IX), and 5-chloroanthranilic acid (X) apparently also were present. With the exception of NDL at 120 min, radiocarbon in the aqueous extract did not exceed 1.0%. Levels of radiocarbon in the SWCB residue also were low and did not exceed 0.5%. Levels of radiocarbon in the container rinse, which probably represented excreted radioactive material, were highest with NDL comprising 16.6% at 120 min, intermediate with EIL comprising 11.3% at 120 min, and lowest with HIL comprising 9.1% at 120 min (Table I).

Table II presents the fate of demethylchlordimeform (II) in NDL and HIL. Demethylchlordimeform (II) was rapidly and extensively degraded by both NDL and HIL with the former being most active. The major metabolites were didemethylchlordimeform (III) and 4-chloro-*o*-formotoluidide (VII). Levels of the former (III) were higher in HIL, while levels of the latter (VII) were higher in NDL. 4-Chloro-*o*-toluidine (VIII) also was present in NDL and HIL; maximum levels were 3.2% in NDL and 7.1% in HIL at 120 min. Levels of radiocarbon in the aqueous extract and container rinse were somewhat higher with NDL than with HIL (Table II).

NDL and HIL also degraded injected 4-chloro-*o*-formotoluidide (VII) (Table III). It appeared that NDL were somewhat more active than HIL, although degradation was not extensive in either case. 4-Chloro-*o*-toluidine (VIII) was the major degradation product; at 120 min postinjection it comprised 4.2 and 7.6% of the recovered radiocarbon from NDL and HIL, respectively. Levels of radiocarbon in the container rinse were higher with NDL than with HIL (Table III).

4-Chloro-*o*-toluidine (VIII) was degraded when injected into NDL and HIL, and it appeared that NDL were more active than HIL (Table IV). The major metabolite was 5-chloroanthranilic acid (X) comprising 12.4% in NDL and 7.7% in HIL at 120 min. There was appreciably more radiocarbon in the container rinse with NDL than with HIL (Table IV).

DISCUSSION

The major objective of this study was to compare chlordimeform penetration, metabolism, and excretion in NDL, EIL, and HIL of the SWCB. In this connection, chlordimeform penetration and excretion were fastest with NDL (120 mg in weight) and slowest with HIL (530 mg); EIL (190 mg) were intermediate. Thus, it appeared that penetration rate was directly related to the ratio of larval surface area to volume. With respect to chlordimeform metabolism, however, HIL were most active and NDL were least active, while EIL again were intermediate. Thus, the extensive chlordimeform metabolism by HIL as compared to NDL was unexpected and the reverse of what was anticipated in light of the penetration and excretion data.

Table III. Fate of 4-Chloro-*o*-formotoluidide-^{14}C in Nondiapause Larvae (NDL) and Hormonally Induced Diapause Larvae (HIL) of the Southwestern Corn Borer[a]

fraction or compound	% recovered radioactivity at indicated time after injection					
	NDL			HIL		
	15 min	60 min	120 min	15 min	60 min	120 min
organic extract						
VII	85.9 (1.1)	82.6 (0.8)	77.4 (0.7)	95.2 (0.5)	89.8 (0.8)	85.1 (0.9)
VIII	3.1 (0.5)	3.6 (0.6)	4.2 (0.5)	2.2 (0.3)	4.7 (0.4)	7.6 (0.8)
IX	0.9 (0.2)	1.4 (0.2)	1.9 (0.2)	0.6 (0.1)	0.8 (0.1)	1.3 (0.3)
X	0.5 (0.1)	0.8 (0.3)	1.5 (0.2)	0.4 (0.1)	0.4 (0.1)	0.6 (0.2)
origin	0.8 (0.1)	1.8 (0.7)	4.4 (1.0)	0.2 (0.1)	0.7 (0.1)	1.3 (0.6)
aqueous extract	1.6 (0.1)	2.7 (0.1)	3.3 (0.1)	0.4 (0.1)	1.1 (0.1)	1.2 (0.1)
residue	0.4 (0.0)	0.6 (0.0)	0.7 (0.1)	0.2 (0.1)	0.5 (0.1)	0.6 (0.1)
container rinse	6.8 (0.2)	6.5 (0.1)	6.6 (0.1)	0.8 (0.1)	2.0 (0.1)	2.3 (0.1)
total recovered	96.9 (1.1)	97.3 (0.8)	97.1 (0.8)	96.9 (0.6)	96.3 (1.1)	96.4 (1.0)

[a] Figures are the mean of three replicates with the standard deviation in parentheses.

Table IV. Fate of 4-Chloro-*o*-toluidine-^{14}C in Nondiapause Larvae (NDL) and Hormonally Induced Diapause Larvae (HIL) of the Southwestern Corn Borer[a]

fraction or compound	% recovered radioactivity at indicated time after injection					
	NDL			HIL		
	15 min	60 min	120 min	15 min	60 min	120 min
organic extract						
VIII	69.9 (1.5)	66.1 (0.6)	53.7 (1.1)	75.8 (1.1)	68.7 (0.8)	67.1 (1.5)
X	7.6 (1.3)	10.3 (0.7)	12.4 (0.8)	8.4 (0.9)	9.5 (0.8)	7.7 (0.9)
origin	6.1 (0.8)	5.4 (0.6)	10.7 (1.0)	4.5 (0.7)	7.3 (0.7)	12.5 (0.7)
aqueous extract	5.7 (0.7)	6.3 (0.1)	8.6 (0.3)	5.9 (0.1)	4.9 (0.3)	7.3 (0.2)
residue	0.5 (0.8)	0.8 (0.1)	1.0 (0.1)	2.9 (0.1)	6.2 (0.5)	2.6 (0.2)
container rinse	10.2 (0.2)	11.1 (0.3)	13.6 (0.2)	2.5 (0.1)	3.4 (0.2)	2.8 (0.1)
total recovered	97.2 (0.9)	97.8 (1.1)	96.7 (0.6)	96.7 (0.6)	95.9 (0.8)	97.1 (0.9)

[a] Figures are the mean of three replicates with the standard deviation in parentheses.

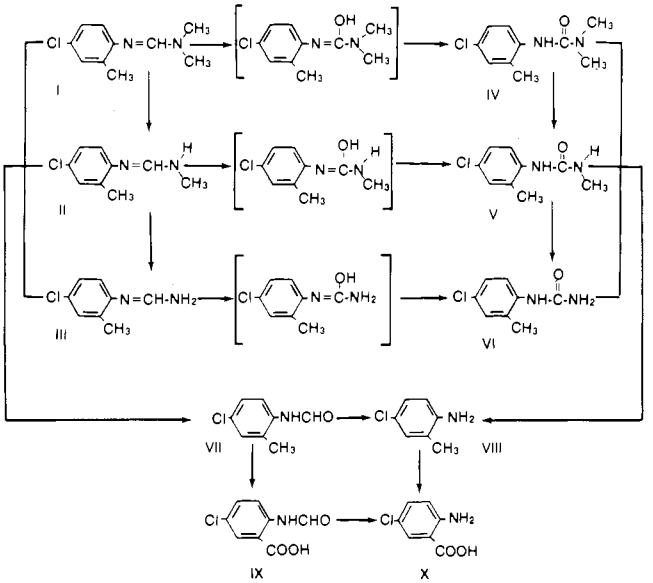

Figure 2. Metabolic paths for chlordimeform in animals (Knowles and Benezet, 1977).

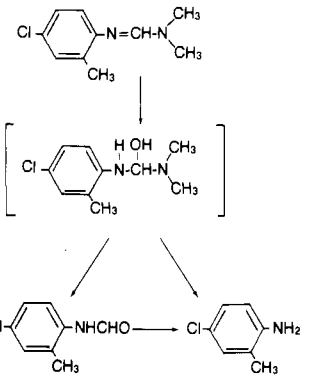

Figure 3. Proposed paths for the formation of 4-chloro-*o*-toluidine from chlordimeform.

Of paramount interest with HIL were the decrease in levels of chlordimeform and the concomitant increase in levels of 4-chloro-*o*-toluidine.

So that more insight into product–precursor relationships could be gained, NDL and HIL were injected with demethylchlordimeform, 4-chloro-*o*-formotoluidide, or 4-chloro-*o*-toluidine. In each case, degradation and excretion were greater in NDL than in HIL. Moreover, when demethylchlordimeform or 4-chloro-*o*-formotoluidide was injected, levels of 4-chloro-*o*-toluidine in NDL and HIL never approached those observed with chlordimeform-treated HIL. When 4-chloro-*o*-toluidine was injected, its turnover was low, so it would accumulate if formed. However, the turnover of 4-chloro-*o*-formotoluidide upon injection appeared too low to account for all of the 4-chloro-*o*-toluidine in chlordimeform-treated HIL.

Figure 2 shows the paths elucidated for chlordimeform metabolism in animals (Knowles, 1976; Knowles and Benezet, 1977). The usual explanation for the formation of 4-chloro-*o*-toluidine (VIII) has been via deformylation of 4-chloro-*o*-formotoluidide (VII). Since this sequence does not satisfactorily account for all of the 4-chloro-*o*-toluidine (VIII) in chlordimeform-treated HIL, another more direct path also must be operable. Figure 3 gives an alternate pathway. Following hydration of chlordimeform to yield the carbinoldiamine, cleavage could occur at the alkylamino side of the carbinol moiety to yield 4-chloro-*o*-formotoluidide in the usual way. However, cleavage also could occur at the arylamino side of the carbinol moiety to yield 4-chloro-*o*-toluidine. We suggest that this latter pathway predominated in chlordimeform-treated HIL and that it was the primary reason that HIL degraded chlordimeform more extensively than did EIL and NDL. The

data also provided some evidence for suggesting that both pathways for 4-chloro-*o*-toluidine formation were operable in EIL, but to a much lesser degree than in HIL. With respect to NDL, 4-chloro-*o*-toluidine apparently was formed from 4-chloro-*o*-formotoluidide by the conventional pathway.

The reason for this apparent difference in chlordimeform metabolism by NDL, EIL, and HIL remains unknown. Chippendale (1979) has discussed certain of the biochemical characteristics of NDL, EIL, and HIL. For example, EIL and HIL accumulated significantly larger amounts of lipids and proteins than did NDL. Moreover, application of a juvenile hormone mimic to early last instar NDL resulted in their transformation to HIL which were physiologically similar to EIL (Yin and Chippendale, 1974). The juvenile hormone mimic treatment apparently elevated the functional juvenile hormone titer of the larval tissues, thereby causing the larvae to enter a diapause-like state. In contrast, the juvenile hormone titer in the hemolymph of last instar NDL declined to very low levels immediately after ecdysis (Yin and Chippendale, 1976).

It seems probable that treatment of the SWCB larvae with the juvenile hormone mimic resulted in the induction of an enzyme that effected the "direct" conversion of chlordimeform to 4-chloro-*o*-toluidine. However, other possibilities also exist. For example, chlordimeform degradation in HIL may have been shunted to a different metabolic pool. This appears unlikely since demethylchlordimeform and 4-chloro-*o*-formotoluidide seemed to be degraded similarly in both NDL and HIL. These studies have established a probable relationship between xenobiotic metabolism and juvenile hormone mediated changes in SWCB larvae.

ACKNOWLEDGMENT

We thank Dr. G. M. Chippendale of the Department of Entomology for supplying the insects, for valuable discussions, and for reading this manuscript.

LITERATURE CITED

Chang, K.-M.; Knowles, C. O. *J. Agric. Food Chem.* **1977**, *25*, 493.
Chippendale, G. M. *Res. Bull.—Mo., Agric. Exp. Stn.* **1979**, *No. 1031*, 1–52.
Crecelius, C. S.; Knowles, C. O. *J. Agric. Food Chem.* **1976**, *24*, 1018.
Knowles, C. O. *EHP, Environ. Health Perspect.* **1976**, *14*, 93.
Knowles, C. O.; Benezet, H. J. *J. Agric. Food Chem.* **1977**, *25*, 1022.
Knowles, C. O.; Schuntner, C. A. *J. Aust. Entomol. Soc.* **1974**, *13*, 11.
Morikawa, M.; Yokoyama, S.; Fukami, J. *Bochu Kagaku* **1975**, *40*, 162.
Schuntner, C. A. *Aust. J. Biol. Sci.* **1971**, *24*, 1301.
Yin, C.-M.; Chippendale, G. M. *J. Insect Physiol.* **1974**, *20*, 1833.
Yin, C.-M.; Chippendale, G. M. *J. Exp. Biol.* **1976**, *64*, 303.

Received for review May 29, 1981. Accepted September 25, 1981. This research was supported, in part, by a grant from the National Institutes of Health (ES01316) and is a contribution from the Missouri Agricultural Experiment Station, Columbia. Journal Series No. 8882.

Toxicity of *O,O,S*-Trialkyl Phosphorothioates to the Rat

Fouad A. F. Ali and T. Roy Fukuto*

A series of simple *O,O*-dimethyl and *O,O*-diethyl *S*-alkyl phosphorothioate esters were prepared and examined for acute and delayed toxicity to rats. Several of the compounds were highly toxic to the rat, with death occurring over a period of 1–11 days. Most of the compounds caused symptoms of delayed intoxication, i.e., weight loss, diarrhea, uncontrolled urination, and hemorrhaging. All of the esters were poor inhibitors of bovine erythrocyte acetylcholinesterase and rat plasma cholinesterase, the *O,O*-dimethyl esters being virtually devoid of inhibitory activity. The poor anticholinesterase activity of these compounds suggests a noncholinergic mechanism of action.

In recent communications (Mallipudi et al., 1979; Umetsu et al., 1979) we described the unexpectedly high delayed toxicity of *O,O,S*-trimethyl phosphorothioate (LD_{50} 15–20 mg/kg) and *O,O,S*-triethyl phosphorothioate (LD_{50} 45–90 mg/kg) following administration of single oral doses to rats. In a previous study (Umetsu et al., 1977), *O,O,S*-trimethyl phosphorothioate was demonstrated to be a trace contaminant in technical samples of malathion and acephate. Intoxication of rats by this simple compound was markedly different from that caused by typical organophosphorus insecticides in that death occurred over a substantially longer time frame, e.g., up to 3 weeks following treatment (Mallipudi et al., 1979). At the LD_{50} dose of 15–20 mg/kg, rats treated with *O,O,S*-trimethyl phosphorothioate showed no visible sign of distress but refused food and water, resulting in the loss of weight. Of four rats dosed orally at 20 mg/kg, one died on day 6, the second on day 8, and the third on day 17.

Because of the unusual mode of action and signs of poisoning by this compound, other structural analogues were synthesized and examined for acute and delayed toxicity to rats and for anticholinesterase activity.

Division of Toxicology and Physiology, Department of Entomology, University of California, Riverside, Riverside, California 92521.

MATERIALS AND METHODS

Chemicals. Except for *O,O*-diethyl *S*-(*tert*-butyl) phosphorothioate (**13**), all compounds examined for toxicological properties were synthesized by reaction of the appropriate alkylsulfenyl chloride and trimethyl or triethyl phosphite (Morrison, 1955). Compound **13** was prepared by reaction of *tert*-butylsulfenyl chloride with sodium diethyl phosphonate at 0 °C. All products were vacuum distilled and purified by either preparative TLC, (solvent system was 1:1 benzene–ethyl acetate) or silica gel column chromatography (solvent system was hexane–ethyl acetate gradient). Structures were verified by NMR and elemental analyses (see Table I); the latter were carried out by C. F. Geiger, Ontario, CA. Product purity was determined by

0021-8561/82/1430-0126$01.25/0