Uptake of ^{36}Cl-Toxaphene in the Cockroach, *Leucophaea maderae* (Fabr.)

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INTRODUCTION

The mode of action of toxaphene is not known. Although it is generally accepted that this insecticide acts on the nervous system, a precise site of action has not been defined. Due in large part to physiochemical theories, particularly that of MULLINS (1955), a number of studies have been performed to determine a locus of action of chlorinated insecticides by tracing the distribution and uptake in the insect body. These were principally attempts to correlate uptake of insecticides by certain tissues and tissue components with symptomology.

Injecting an LD-50 dose, COCHRAN (1956) traced the distribution of DDT in tissues of Periplaneta americana (L.) and found that it was distributed generally throughout the insect. ROBBINS and DAHM (1955), found highest accumulations of ^{14}C-DDT in P. americana in foregut, hindgut, fat body, and feces. The brain and thoracic ganglia showed little accumulation of radioactivity, and accumulation in haemolymph was too low to report.

Studying dieldrin uptake with resistant and susceptible strains of Blattella germanica (L.), RAY (1963) found increased levels of unaltered dieldrin associated with nerve cords of resistant insects after topical treatment with increasing concentration levels of poison. Resistant cockroaches that showed signs of poisoning appeared to accumulate less dieldrin in ventral nerve cords (VNC) than susceptible cockroaches.

TELFORD and MATSUMURA (1970, 1971), using electron microscopic radioautographic techniques, provided evidence of accumulations of ^{14}C-dieldrin in nerve tissue components of B. germanica. Isolated VNC incubated with ^{14}C-dieldrin in vitro showed greatest silver grain localization just outside and within the axonic membrane.

In contrast to the above findings, SELLERS and GUTHRIE (1971) and COONS and GUTHRIE (1972) found ^{3}H-dieldrin and ^{3}H-DDT localized about connective tissue of the neural lamella of housefly thoracic ganglia and P. americana VNC. No label was found associated with nerve cell bodies or glial cells.

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The study reported herein evaluated toxicity of toxaphene to the cockroach, Leucophaea maderae (Fabr.), and determined the distribution of ^{36}Cl-toxaphene in several of its tissues. This information might aid in the elucidation of toxaphene's site of action.

METHODS AND MATERIALS

Technical grade toxaphene and ^{36}Cl-toxaphene (batch sample X18276-11, 42 μCi/g) was obtained from Hercules, Inc. Solutions in mineral oil were prepared serially from a 10^{-1}M stock in acetone.

Adult male L. maderae (2-6 weeks postfinal molt) were selected from a mixed colony reared on a mixture of honey, glycerin, and Purina® Dry Dog Chow (2:2:6, v/v) and an ample water supply. Colonies were maintained in a rearing room (22-23°C, 70% RH, and a 12:12 light-dark cycle).

To evaluate the toxicity of toxaphene, LD-50 determinations were made at 24,48,72,96, and 120 h. Each LD-50 determination was comprised of 10 insects for each dose, including controls injected with mineral oil, replicated 4 times. Cockroaches were injected between the 3rd and 4th abdominal tergites and the injection punctures sealed with paraffin. There were 13 test doses administered from 0.21 μg to 8.28 mg toxaphene in mineral oil. Injected animals were returned to their confines and observed for symptoms of poisoning.

Distribution and uptake of ^{36}Cl-toxaphene by selected tissues were studied with an injected dose of 175.6 μg in 0.05 cc mineral oil. Two sets of 6 controls were prepared: insects injected with 0.05 cc mineral oil, and noninjected insects. Insects were sacrificed at 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 h, (N=40, each assay period) by removing the head, legs, and wings and making a mid-dorsal incision the entire length of the body. Tissues were removed and weighed in the following order: alimentary canal, abdominal fat body, and VNC (2nd to 6th ganglia inclusive). Haemolymph (ca. 1 ml) was collected prior to dissection and solubilized with 2.0 ml NCS® in 5 ml ground glass tissue grinders by the method of STERNBERG and CORRIGAN (1959). Samples were then prepared for radioassay.

Extraction of tissues from symptomatic and asymptomatic insects for the 120 h assay period was performed. Haemolymph was obtained as above, and centrifuged with 0.2 ml H_2O. Following dissection, alimentary canal, fat body, and VNC were homogenized in 0.1 ml H_2O/mg of tissue. Tissues were agitated for approximately 1 min in 1.0 ml acetone to lyse the cells. The volume was brought to 5.0 ml with ethyl ether. Aqueous and apolar layers were extracted twice and then separated. Aqueous layers from 6 asymptomatic and 11 symptomatic insects (3 samples/insect) and apolar layers from tissues of 6 asymptomatic and 4

symptomatic cockroaches (8 samples/insect) were prepared for radioassay. Both layers were air dried to 0.25 cc prior to solubilization in NCS®.

Samples were radioassayed in 10 ml toluene-based fluor (5 g PPO and 0.06 g POPOP/l toluene) on a Nuclear Chicago Model 6822 ambient temperature, liquid scintillation spectrophotometer. Samples were counted for 10 min giving a confidence level of 90%. Quench was corrected by the external standard method. Radioactivity was interpreted in terms of the specific activity of toxaphene; however, this may give somewhat misleading values since not only toxaphene but probably metabolites of toxaphene containing ^{36}Cl were present in the extracts.

RESULTS AND DISCUSSION

Toxaphene was found to be only slightly toxic to L. maderae. At 120 h the LD-50 was 740.6 µg/g, approximately 1/3 the 48 h LD-50.

Cockroaches exhibited classical symptoms of neurotoxicity. At 24 h, 2.07 - 3.31 mg/insect was necessary to elicit convulsions. By 120 h 207.0 µg/insect was capable of producing this symptom. The second symptom was noted when partially paralyzed insects displayed slight tremors of the labial palps, antennae, and abdomen while on their dorsum. Rather large doses, 3.73 - 8.28 mg and 2.48 - 3.31 mg/insect were necessary to produce this symptom at 24 and 120 h, respectively. Cockroaches that appeared to be dead were recorded as moribund. They did not respond to tactile stimulation, and examination of the heart showed either slowed and irregular rates or total absence of activity. Moribundity was not expressed at 24 h. Between 48 and 120 h, the dose to produce this symptom ranged from 8.28 to 828.0 µg/insect.

Insects injected with an apparently effective dose always died, there was no recovery. An effect was judged as an expression of any one of the symptoms. Effective doses were approximately half the lethal doses. At 120 h the ED-50 was 299.5 µg/g, approximately 141.5 µg/g greater than the dose used to study uptake by selected tissues in vivo.

Distribution of radiolabeled material in haemolymph, VNC, fat body, and alimentary canal varied considerably over time (Table 1). At 2 h of incubation, radiolabeled material was found in greatest concentration (µg/g) about the VNC. There was an increase in uptake in VNC at 4 h, and a decline at 6 h. The 5 h decline in VNC was accompanied by an increase in haemolymph radioactivity. There was also a decline of radiolabeled material in fat body and alimentary canal at 6 h.

At 8 h there was a marked decline of radioactivity in haemolymph but an increase in fat body and alimentary canal. From 12 to 48 h the greatest uptake was found associated with

Table 1. Mean ± standard error[a] of radiolabeled material (µg/g) detected in tissues of L. maderae assayed 2 to 120 hours after intra-haemocoel injection of 175.6 µg ^{36}Cl-toxaphene/insect.

Time (Hours)	Haemolymph	Ventral Nerve Cord	Fat Body	Alimentary Canal
2	7.7± 1.0b	790.8±118.6de	194.8± 3.0d	228.4±20.2c
4	133.1± 21.8c	1325.7±202.4e	163.4±24.3b	441.8±38.0ef
6	892.4±146.5d	309.2±105.0a	13.0± 3.6a	201.6±42.8a
8	2.2± 0.2a	121.7± 13.3b	299.1±34.4d	254.5±24.7c
12	249.4± 13.9d	375.1± 65.8bc	110.2± 0.2c	109.9± 6.2b
24	126.6± 36.4c	342.7± 54.3b	174.1±23.3c	184.6±11.7c
48	6.1± 1.3ab	2232.3±551.4de	200.1±10.6d	112.9± 4.6b
72	230.1± 65.0c	370.0± 59.3bc	286.7±16.0c	553.2±40.2f
96	45.4± 4.4c	368.9± 43.5cd	114.7± 0.5d	311.8± 0.5de

[a]Grouping of mean uptake for each tissue into subsets according to mean variance among groups and subsets by Student-Newman-Keul's procedure, 0.05 level.

alimentary canal. At 96 h the greatest concentration was again found about VNC.

L. maderae that did not demonstrate symptoms of poisoning at 120 h accumulated greatest amounts of ^{36}Cl-material on a µg/g basis in alimentary canal and fat body as compared to haemolymph and VNC (Table 2). When expressed as % applied dose, alimentary canal showed the greatest uptake. Symptomatic insects accumulated greater amounts of radiolabeled material in haemolymph as compared to other tissues. Significant differences were observed between tissues of symptomatic and asymptomatic insects except in the fat body. Greater amounts of radiolabeled material were found in hexane extracts of tissues in both symptomatic and asymptomatic insects (Table 2). Gas chromatograms showed this material to apparently be unaltered toxaphene. Excreta contained some probable products of toxaphene metabolism. In asymptomatic insects, 54.7% of the radiolabeled material was found in polar extracts of excreta. Dehydrochlorination has been reported as a method of toxaphene metabolism in rats (OHSAWA et al. 1975); a similar mechanism may be operable in insects.

There was scant correlation between effective doses (µg/g) and uptake (µg/g) in tissues studied from 24 to 120 h. This was expressed for regressions between ED-30, ED-50, and ED-70 values and uptake; correlation coefficients ranged from > 0.30 - 0.55. Since effects of poisoning were not observed until 120 h at the dose used to study uptake, it was difficult to interpret the relationship between effective dose and uptake by tissues.

SUMMARY

Toxaphene was only slightly toxic to L. maderae. The ED-50 at 120 h was 299.5 µg/g. Uptake of a sublethal dose of ^{36}Cl-toxaphene was examined in several tissues. At most time intervals between 2-96 h, greater concentrations were found in VNC than haemolymph, fat body, or alimentary canal. At 120 h, tissues of symptomatic insects possessed significantly greater amounts of radiolabeled material than asymptomatic, except for fat body where no difference was noted.

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Table 2. Uptake (mean ± standard error)[a] of radiolabeled material in tissue samples collected from symptomatic and asymptomatic L. maderae assayed at 120 h after intra-haemocoel injection of 175.6 µg ^{36}Cl-toxaphene/insect.

	Haemolymph	Ventral Nerve Cord	Fat Body	Alimentary Canal
Asymptomatic:				
Uptake (µg/g)	54.8±9.0**	193.7±13.5**	251.6±14.9	260.5±12.3**
Applied dose (%)	0.42±0.09	0.26±0.02	3.82±0.27	12.99±0.60
Aqueous soluble (%)	18.8	ND	21.8	13.9
Symptomatic:				
Uptake (µg/g)	588.7±92.7**	253.3±47.3**	305.8±16.2	324.3±1.8**
Applied dose (%)	11.74±1.42	0.46±0.10	2.89±0.23	8.50±0.71
Aqueous soluble (%)	5.7	ND	37.9	27.3

[a]Means between each symptomatic and asymptomatic tissue for uptake followed by ** are significantly different at the 0.05 level, Student t.

[b]ND=non-detectable

REFERENCES

COCHRAN, D.G.: J. Econ. Entomol. 48, 131 (1956).

COONS, L.B., and F.F. GUTHRIE: J. Econ. Entomol. 64, 1004 (1972).

MULLINS, L.J.: Science 122, 118 (1955).

OHSAWA, T., J.R. KNOX, S. KHALIFA, and J.E. CASIDA: J. Agr. Food Chem. 23, 98 (1975).

RAY, J.W.: Nature, Lond. 197, 1226 (1963).

ROBBINS, W.E., and P.A. DAHM: J. Agr. Food. Chem. 3, 500 (1955).

SELLERS, L.G., and F.E. GUTHRIE: J. Econ. Entomol. 64, 352 (1971).

STERNBERG, J., and J. CORRIGAN: J. Econ. Entomol. 52, 538 (1959).

TELFORD, J.N., and F. MATSUMURA: J. Econ. Entomol. 63, 795 (1970).

TELFORD, J.N., and F. MATSUMURA: J. Econ. Entomol. 64, 230 (1971).