Accumulation and Excretion of DDT by the Terrestrial Snail, *Cepaea hortensis*[1]

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Terrestrial gastropods (snails and slugs) have been shown to accumulate considerable quantities of the organochlorine insecticide DDT, with no noticeable toxic effect (Davis [1] , Gish [2]). These invertebrates serve as a source of this concentrated pesticide to vertebrate predators. Snails and slugs as non-target organisms accumulate DDT residues at concentrations equal to or considerably higher than the surrounding environment. Such information from the literature is summarized in Table I.

Since little information is available on the site of residue accumulation in snails the present study was undertaken to determine the concentration and excretion of chlorine 36 DDT in the terrestrial pulmonate snail, Cepaea hortensis (Müller). Specific objectives of this study were as follows:

1. To determine the quantities of ^{36}Cl DDT that are accumulated and excreted by the snail Cepaea hortensis, after a single feeding of DDT.

2. To determine the tissue concentration of the radioactive DDT.

3. To determine the dynamics of DDT in the snail tissues at intervals of three and twenty-four hours, two, four and eight days.

Methods

Ring-labeled ^{36}Cl pp' DDT (2, 2-bis [p-chlorophenyl] 1, 1, 1-trichloroethane) was used in this study so that all residues of DDT and its metabolites could be assayed. A hexane solution containing 60 µg of DDT was applied to 10 mm discs of lettuce with a microliter syringe. The discs of lettuce were fed to snails which were kept in glass culture dishes containing wet filter paper as a source of moisture. The snails were starved for four to six days before feeding. They were fed once and evaluated in groups of five animals at the specified

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TABLE I

SUMMARY OF DDT RESIDUES IN TERRESTRIAL GASTROPODS RECORDED IN LITERATURE

DDT Residues (ppm) Organism	Soil Environs	Wet/ Dry Wgt	Concentration Factor	Reference
SLUGS				
8.60	-	?	-	Cramp & Conder 1965 (3)
3.44	-	?	-	
2.55	0.69	?	3.70	Edwards 1970 (4)
42.70	-	?	-	
19.70	-	?	-	
Arion spp 7.30	2.30	Wet	3.17	Davis 1968 (1)
Agriolimax reticulatus				
19.00	2.30	Wet	8.26	
40.10	17.20	Wet	2.33	
18.00	-	Wet	-	Davis & French 1969 (5)
60.00	-	Wet	-	
Deroceras or Limax				Gish 1970 (2)
34.47	15.30	Dry	2.25	
45.12	15.30	Dry	2.95	
18.73	3.39	Dry	5.53	
52.70	2.94	Dry	17.93	
SNAILS				
2.21	15.30	Dry	0.14	Gish 1970 (2)
3.08	2.94	Dry	1.05	
2.45	2.94	Dry	0.83	

intervals of 3, 24, 48, 96 and 192 hr after ingestion of DDT. Animals were sacrificed by placing them into beakers filled with distilled water until they were fully extended and then slowly heated until they were dead.

After death snails were dissected and the 23 different tissues listed in Table II were removed and (wet) weighed. Background and treated tissues were then prepared for liquid scintillation spectrometry. They were solubilized with Soluene-100® in glass scintillation vials. We used scintillation solution recommended by Hayes (6) consisting of 2,5 diphenyl-oxazole (PPO) and 1,4 bis-2 (4-methyl-5 pheny-oxazole) benzene (Dimethyl POPOP) in toluene.

Our test animal, Cepaea hortensis, is the only species of the cosmopolitan family, Helicidae, that is native to North America. It ranges from New Foundland to Massachusetts and is found primarily on the offshore islands (Pilsbry [7]). This species was selected because of its large size which insures ease of tissue identification and dissection.

Results

A total of 112 non-exposed tissue samples were evaluated to determine background radioactivity due to fallout and natural radioisotopes. Since we were interested only in the maximum level of activity for each tissue, the one-tailed t distribution was used to calculate the 99% confidence interval. This interval plus the mean comprises the background value for each tissue.

Table II and Figure I summarize the data obtained from the five test groups.

Residue concentrations followed a similar trend through time in various tissues of the digestive system. These tissues had relatively high levels of DDT residues at three hours. But, after 24 hr concentrations were reduced and stabilized at a particular value for each tissue. In the hepatopancreas (digestive gland) residues did not follow this pattern but showed a slight increase through time. A common trend was displayed in the reproductive organs and heart tissue. At two days a noticeable decrease occurred which was different from residues in digestive tissues. Relative tissue concentrations are illustrated for this exposure period in Figure II.

Large quantities of residues were excreted in the feces with some snails excreting more than half the insecticide offered them. The most reliable evaluation of residues in feces is presented by the micrograms of DDT present in samples. During the

TABLE II

RESIDUE CONCENTRATIONS (Means ± standard error; ppm, wet wgt.) in Cepaea hortensis TISSUES AFTER SINGLE FEEDING OF 60 µg DDT

	EXPOSURE PERIODS		
	3 Hours	24 Hours	48 Hours
TISSUE	N=4	N=5	N=4
Feces µg	0	28.33 ± 3.72	25.29 ± 8.70
ppm	0	798.50 ± 407.24	648.09 ± 412.70
Mucous	0.07 ± 0.05		0.49 ± 0.13
Salivary Gland	29.04 ± 12.83	3.76 ± 1.38	0.88 ± 0.54
Buccal Mass	7.25 ± 3.35	2.36 ± 0.26	0.54 ± 0.38
Esophagus	30.81 ± 6.15	8.34 ± 2.56	2.57 ± 1.74
Crop	109.80 ± 23.93	9.95 ± 2.63	8.06 ± 3.48
Stomach	156.73 ± 56.56	27.93 ± 4.50	21.80 ± 10.00
Intestine	125.84 ± 46.80	18.86 ± 3.75	20.21 ± 7.11
Hepato-Pancreas	23.46 ± 3.21	68.73 ± 12.94	67.97 ± 12.30
Ovotestis	9.07 ± 1.99	38.15 ± 10.28	29.59 ± 14.06
Albumen Gland	5.80 ± 3.75	8.46 ± 3.92	3.98 ± 0.89
Oviduct	0.69 ± 0.41	0.05 ± 0.05	0.02 ± 0.92
Sperm-Oviduct	1.98 ± 0.60	1.35 ± 0.68	0.71 ± 0.25
Vagina	1.55 ± 0.86	0.50 ± 0.16	0.20 ± 0.15
Penis	2.22 ± 1.04	0.76 ± 0.52	0.17 ± 0.10
Mucous Gland	4.74 ± 3.14	0.78 ± 0.14	0.10 ± 0.10
Kidney	2.44 ± 0.83	4.58 ± 0.89	4.46 ± 1.81
Heart	7.76 ± 4.03	1.31 ± 0.87	0
Nerve	2.26 ± 1.33	0.69 ± 0.22	1.83 ± 1.64
Mantle	3.27 ± 1.05	1.95 ± 0.50	1.48 ± 0.58
Collar	1.30 ± 0.74	1.81 ± 1.10	0.50 ± 0.16
Foot	n=8	n=10	n=8
	0.79 ± 0.14	1.17 ± 0.11	0.62 ± 0.14
Shell		0.41 ± 0.14	
Retractors		1.54 ± 0.25	
Spermatheca	20.68 ± 16.65	8.44 ± 1.79	0.57 ± 0.57
Whole body without shell	24.37 ± 2.38	11.86 ± 1.37	11.46 ± 3.40

TABLE II (continued)

RESIDUE CONCENTRATIONS (Means ± standard error; ppm, wet wgt.) in *Cepaea hortensis* TISSUES AFTER SINGLE FEEDING OF 60 µg DDT

	EXPOSURE PERIODS	
	96 Hours	192 Hours
TISSUE	N=6	N=4
Feces µg	25.61± 4.97	25.86± 3.67
ppm	157.01±22.42	97.11±17.56
Mucous	1.02± 0.05	0.55± 0.55
Salivary Gland	0.74± 0.17	1.25± 0.59
Buccal Mass	1.15± 0.31	0.96± 0.34
Esophagus	2.16± 1.96	2.35± 0.85
Crop	5.18± 1.15	4.45± 1.34
Stomach	26.64± 4.15	17.14± 1.03
Intestine	22.00± 6.70	16.81± 6.41
Hepato-Pancreas	76.45±11.17	91.70±15.21
Ovotestis	45.30± 8.72	27.46± 3.07
Albumen Gland	23.78± 9.84	7.12± 2.22
Oviduct	0.89± 0.39	0.50± 0.23
Sperm-Oviduct	1.28± 0.52	0.85± 0.24
Vagina	0.84± 0.23	0.46± 0.21
Penis	0.82± 0.39	0.28± 0.15
Mucous Gland	0.57± 0.17	0.35± 0.25
Kidney	5.73± 1.26	3.90± 0.14
Heart	2.58± 1.54	1.81± 1.75
Nerve	1.40± 0.96	0.29± 0.22
Mantle	1.49± 0.42	3.01± 0.57
Collar	0.70± 0.25	1.02± 0.46
Foot	n=12	n=8
	0.96± 0.15	0.98± 0.44
Shell	2.81± 1.75	0.52± 0.33
Retractors	2.96± 1.49	1.10± 0.41
Spermatheca	6.10± 1.84	1.24± 1.10
Whole body without shell	10.56± 1.40	11.05± 2.20

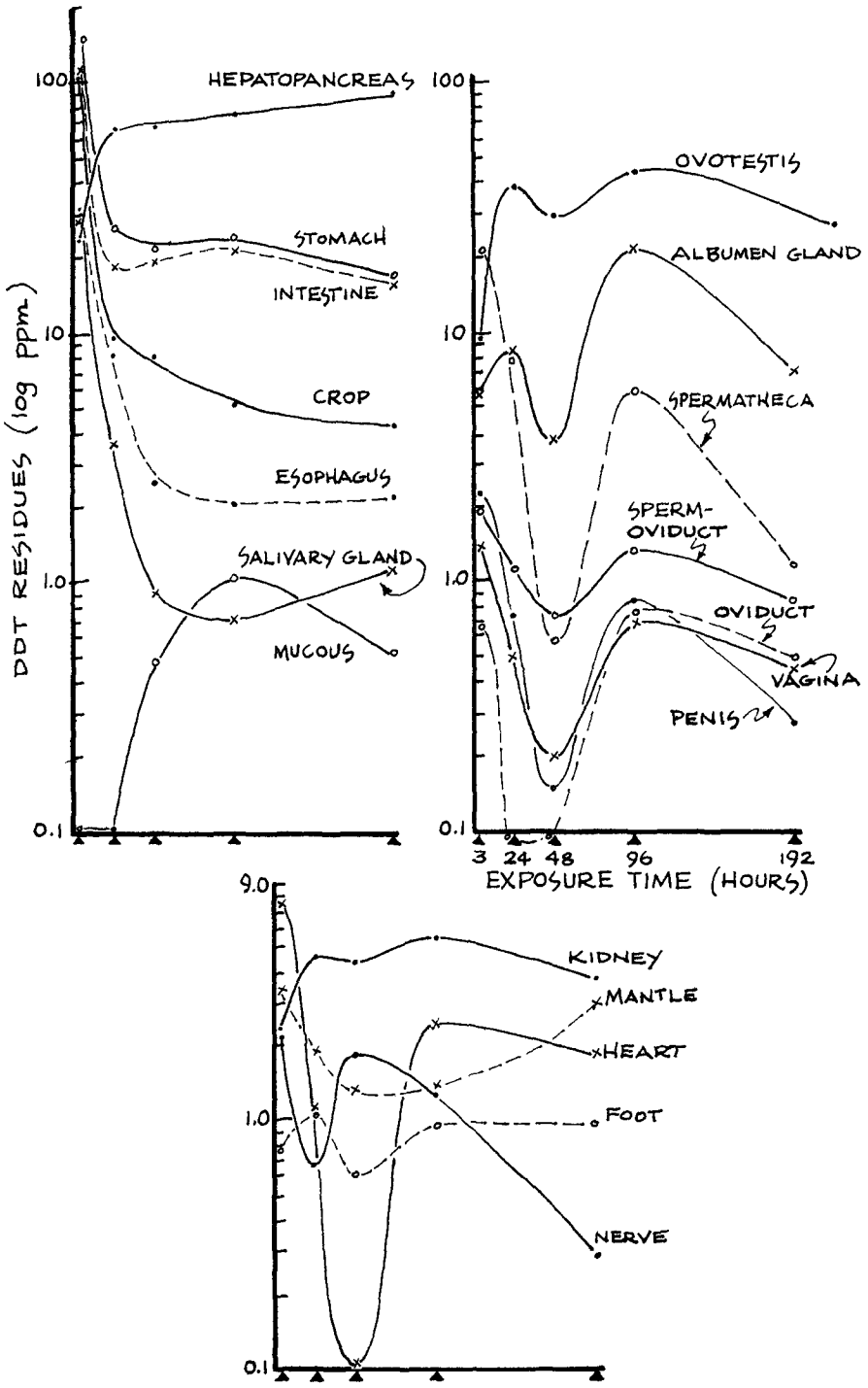

FIGURE I: RELATIONSHIPS OF DDT RESIDUES TO TIME IN TISSUES OF *Cepaea hortensis*; n=23

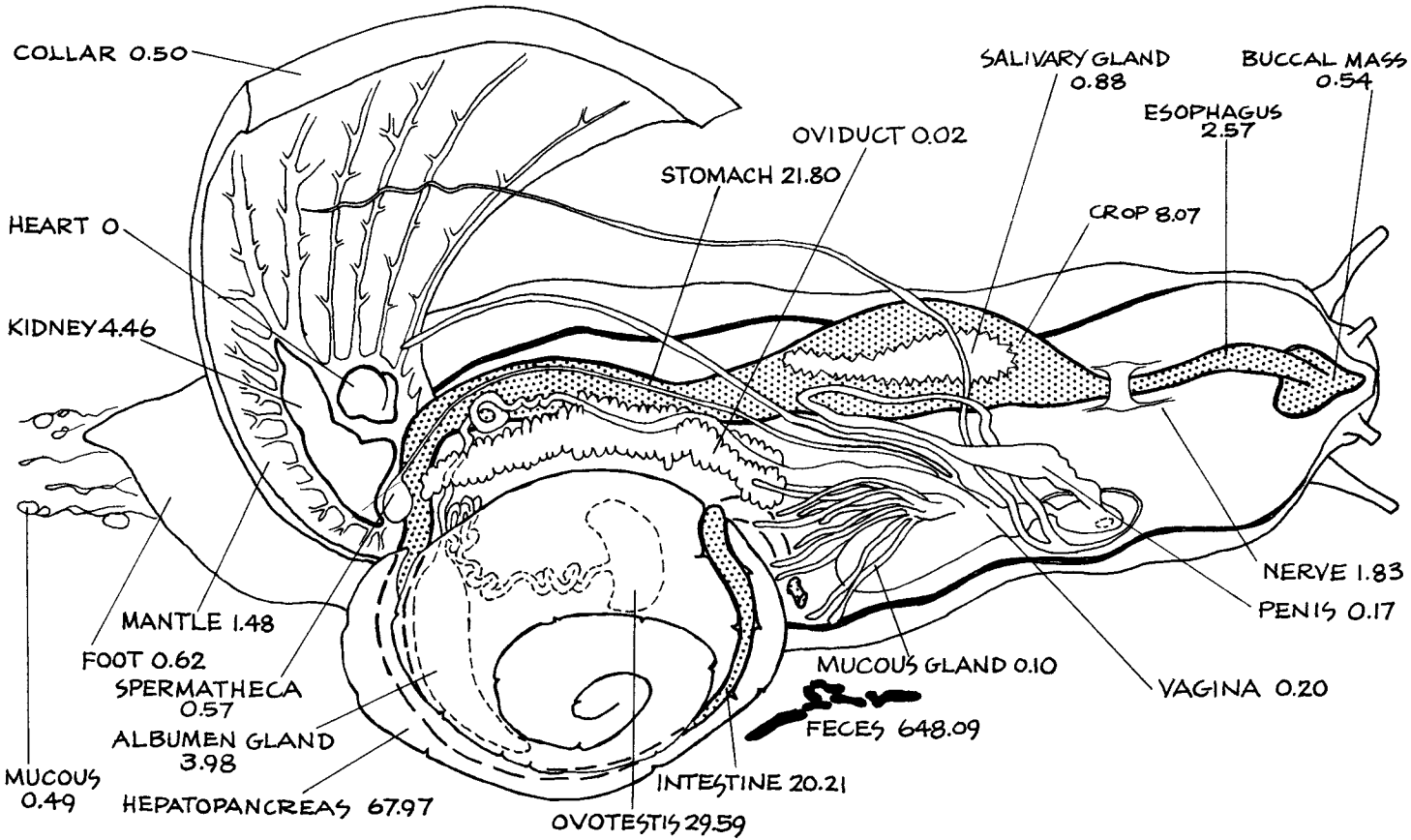

FIGURE II : DDT RESIDUE CONCENTRATIONS (means; ppm) in *Cepaea hortensis* TISSUES; 48 HOURS EXPOSURE; n=4

last two exposure periods snails ingested filter paper. This paper present in the feces produced the erroneous pattern of decreasing residues on a part-per-million basis.

Levels of DDT in the whole animal (without shell) were relatively high at three hours but subsequently appeared to reach a body burden equilibrium at 11 ppm.

Discussion

Past studies show the concentration factor of DDT residues in snails to be less than slugs (Table I). Cepaea ate DDT readily and were never repulsed by it. Most of the residues ingested were then excreted. Apparently the high concentration of residues in feces aids in rapid elimination from the body, and thus concentrations are relatively lower in tissues. Residues absorbed into the body accumulate mostly in the hepatopancreas and ovotestis.

Our data suggests storage equilibria in Cepaea after a single feeding. Digestive organs and hepatopancreas attain a stabilized plateau of residue concentrations. Also, a body burden equilibrium was reached for the whole animal at 11 ppm. Storage equilibria of chlorinated hydrocarbons are reported to occur in other animals namely mammals (Hayes [8]) and organs of ducks (Dindal [9]).

In addition to fecal excretion of DDT residues several other excretory possibilities exist. Small quantities are eliminated from the body via mucous. In our analyses we can account for only 74% of the DDT administered. We have no evidence of codistillation from the lettuce surface so we assume the loss to be associated with physiological characteristics of Cepaea. Brown (10) showed that foreign particles (thorium dioxide) were eliminated by another Helicidae, Helix aspersa. Amoebocytes transport the substance from body tissues to reproductive organs and the mantle epithelium where it is voided from cells. Perhaps, this is another mode of DDT elimination in Cepaea, that is, phagocytosis followed by volatilization or codistillation from the mantle surface or reproductive opening.

There were two maxima of residue concentrations in the reproductive organs. This may also be related to the process described by Brown (10). He found the maximum concentration of particle laden amoebocytes to be in the reproductive organs 4-5 days after introduction of the substance into the snail. Our second maximum concentration occurred in reproductive tissue four days after application.

Another explanation of the two maxima trend is possible. A double compartment (fast and slow) scheme was modelled by Eberhardt, Meeks and Peterle (11) to explain the dynamics of DDT residues in a number of aquatic organisms. After plotting residue concentrations on a semi-log scale two phases were apparent. Their data was not disjointed by the extreme low so obvious in our work. But, their analysis is worth consideration in possibly describing our results. The fast compartment is caused by the initial ingestion of the DDT, whereas the slow compartment results from the redistribution of residues once within the organism. We suggest our dual maxima are explained in the same way. Perhaps the redistribution of the second phase is accomplished in Cepaea by amoebocytic activity which ultimately leads to the elimination of residues from the body.

Food web relationships are affected in several ways by residue dynamics in snails. Vertebrates predatory on snails will ingest insecticide residues directly. Decomposer organisms, including soil microarthropods feeding on snail feces will be subject to much larger DDT concentrations. Also, snails frequently reingest their own feces thus recycling the material again. Therefore, DDT residues can enter, accumulate and recycle through decomposer food webs in addition to being magnified through third level vertebrate consumers.

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