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Uptake, Accumulation, and Elimination of HCB and 2,4-D by the Terrestrial Slug, *Deroceras reticulatum* (Müller)

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The earthworms, snails, slugs and other soil invertebrates are considered to be important components in food chain as they form a significant proportion of the diet of other animals. Therefore, monitoring of these organisms for the source of pesticide residues to other animals feeding on them, and as well as for establishing the contamination levels of agricultural soils is an approach well documented (WHEATLEY & HARDMAN, 1968; DAVIS, 1968; DAVIS & FRENCH, 1969; GISH, 1970; EDWARDS & THOMPSON, 1973; KUHR et al., 1974; BEYER & GISH, 1980; YADAV et al., 1981). Amongst these organisms, terrestrial slugs and snails appear to be relatively resistant to pesticides but have the capacity to accumulate organochlorine (STRINGER & PICKARD, 1965; GISH, 1970; DINDAL & WURZINGER, 1971; KUHR et al., 1974) and organophosphorus insecticide residues (EDWARDS, 1976) in their body tissues.

Since slugs in their natural habitates are in direct contact with the soil and the vegetation, they are therefore exposed to the pesticides applied in the field and to other xenobiotics in the environment. It is not yet clear as to which route(s) pesticides get entry into the slugs body, and whether there are any differences in the quantities of the residues taken up from different sources and in the retention of these residues if there are more than one route of intake. To understand more about the behaviour of slugs we studied the uptake of two test pesticides, namely [14C]-hexachlorobenzene (HCB) and [14C]-2,4-dichlorophenoxyacetic acid (2,4-D); (i) by feeding the slugs via carrot discs daily for a period of five to ten days, and (ii) exposing the slugs continuously to the contaminated soil. HCB was chosen for its persistent and lipophilic property, and 2,4-D for its polarity and hydrophilic property. With this preliminary approach it was possible to determine the excretion rates and retention of HCB and 2,4-D residues and their metabolites in slug tissues upon ingestion and exposure.

MATERIALS AND METHODS

The grey slugs, Deroceras reticulatum (Müller) (= Agriolimax reticulatus) were collected from the experimental field of the Federal Biological Centre for Agriculture and Forestry (BBA), Berlin, and were reared under the laboratory conditions at 20° C and 90 % relative

humidity. They were fed on fresh lettuce and carrots. The soil was a sandy loam* of BBA mixed with 10 % ground peat moss which was brought to 40 % of its moisture capacity. HCB[ring-U-14C], 333 MBq/mmol (= 9 mCi/mmol), and 2,4-D[2-14C], 1.14 GBq/mmol (= 31 mCi mmol) had a radiochemical purity >98 %.

For the contact contamination experiments, the slugs were exposed to the soil containing [14C]-HCB or [14C]-2,4-D. The soil with the pesticides was mixed in a kitchen kneading machine attached to a stainless steel bowl. [14C]-HCB was applied in an aqueous formulated solution, and [14C]-2,4-D in an aqueous methanolic solution with the help of a chromatographic spray. The quantity of water added was dosed to bring the soil moisture capacity to 40 %. The above described rearing conditions were also held throughout the experimental period. Each experiment was performed in duplicate in 1-L glass jars filled with 200 g moist soil mixture. Twelve slugs, which were reared or preconditioned in the laboratory, were put into each container. Carrot discs, half burried into the soil, served as food during the experiment. At each interval of time two slugs from each container were removed, washed free of soil, weighed and shock freezed (-25° C) for later analysis.

For the feeding experiments, [14C]-HCB in hexane-acetone (1:1) solvent and [14C]-2,4-D in methanol were applied to freshly cut carrot discs (Daucus carrota) of about 10 mm in diameter and 2 to 4 mm in thickness. About 4 to 5 carrot discs were fed to ten slugs which were placed at the bottom of 1-L glass jars containing wet filter paper as a source of moisture. The glass jars were closed with the metal gauze lids. The slugs were starved for three days before putting them into the experiment. They were fed daily with the same quantity of the compound for a period of five to ten days. Also at each day all the slugs were withdrawn and placed into a new container. The carrot feedings left over could be recovered for radioactivity measurements. In this way the real dosis ingested by the slugs could be determined. The excrements on the glass walls of the container and on the filter paper were collected and extracted with the respective solvents and radioactivity determined. On each day samples of slugs, one each from the three replicate containers, were withdrawn, weighed and shock freezed (-25 °C) for analysis at a later period.

The analysis of slugs and soil was made mainly by combustion (Sample Oxidizer, Packard 306), and in some cases by extracting the slugs. The resultant total radioactivity in the samples were measured by liquid scintillation counting, using a Nuclear Chicago, Mark II LSC system. The radioactivity in excrements were measured directly by liquid scintillation counting using a scintillator cocktail based on toluene. The total radioactivity in each sample was calculated as equivalent to [14C]-HCB and [14C]-2,4-D residues. The residues in

*composition: clay 3.2 %, silt 12.1 %, fine sand 31.5 %, coarse sand 53.2 %, organic carbon 1.0 %, total nitrogen 1 mg g^{-1}, pH (calcium chloride) 6.1.

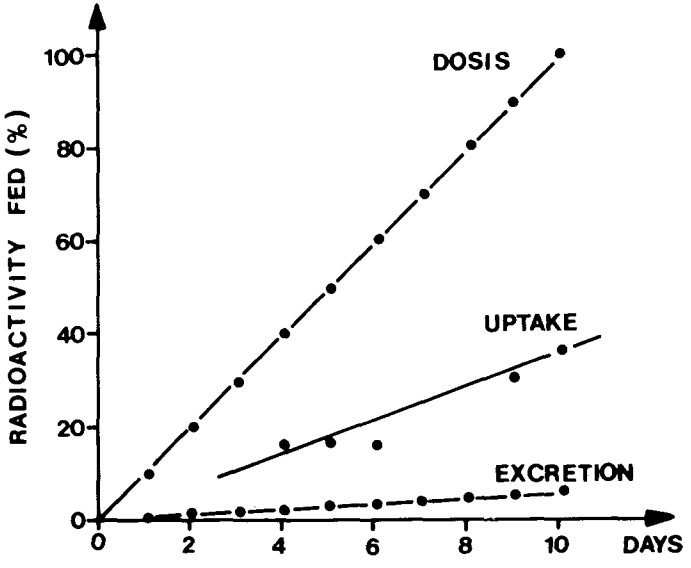

Fig. 1: Cummulative dosis, excretion and uptake of HCB and metabolites in slugs receiving 0.5 µg/g of HCB in their daily diet for 10 days.

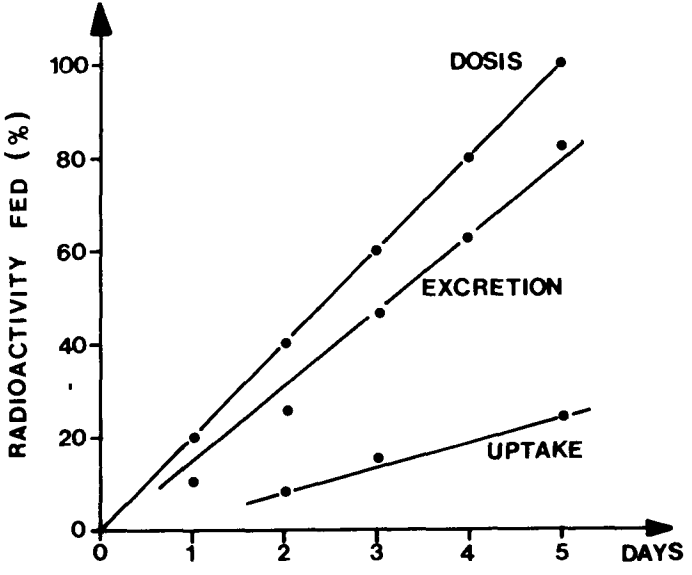

Fig. 2: Cummulative dosis, excretion and uptake of 2,4-D and metabolites in slugs receiving 1.1 µg/g of 2,4-D in their daily diet for 5 days.

slugs were calculated on the live weight basis and that of soil on dry weight basis. Extracts of slugs and excrements were subjected to thin-layer chromatography (TLC) for separation and quantitation of the unchanged parent compound and metabolite fractions. Radioactive zones on TLC plates were localized with a Berthold TLC scanner (LB 2723), then scraped and measured by liquid scintillation counting.

RESULTS AND DISCUSSION

F e e d i n g [14C]-HCB and [14C]-2,4-D in the daily diet (carrot discs) to slugs, according to the cummulative dosis straight line curves, resulted in both uptake and excretion of radiocarbon (Fig. 1 and 2).

The excretion of 2,4-D was rapid and accounted for about 80 % of the dose ingested during the 5 day period (Fig. 2). Out of this amount about 55 % contained metabolites. The rapid excretion of 2,4-D is consistent with most studies on the fate of phenoxyacetic acid in higher animals (KHANNA & FANG, 1966; CLARK et al., 1964). However, the metabolic turnover in slugs was higher than in rats (KHANNA & FANG, 1966) and in sheep (CLARK et al., 1964). Of the total radiocarbon retained by the slugs at different feeding periods, between 30 and 42 % was unmetabolized 2,4-D. No attempt was made to characterize the metabolites in the slugs or their excrements.

The uptake of 2,4-D and its metabolites increased progressively in the slugs body during feeding, where up to 5.5 µg/g live weight of slug was retained at day 6 (Fig. 5). After the withdrawl of 2,4-D from food the slugs continued to excrete the radiocarbon so that nearly an equal body residual level (about 5.0 µg/g) was maintained during further 6 days.

The total excretion of HCB, representing all the radioactivity in the faeces and the mucous, for up to ten days, was slow and accounted for only 6 % of the HCB ingested (Fig. 1). The uptake by the slugs was also slow and accounted for only 35 % of the total dose fed during the 10 day period. The rest of the radiocarbon remained unaccounted. As determined in control experiments without slugs, at least 20 % of the [14C]-HCB was lost by evaporation and codistillation under the conditions of the experiment. This loss is probably higher in the presence of slugs where additional loss may occur from the excrements which were distributed mostly all over the container. The radiocarbon present in the slugs at day 10 was exclusively (99.6 %) the unchanged HCB.

Similar to 2,4-D, the uptake of HCB increased progressively during the feeding of slugs for up to 10 days and there after reached nearly a constant residue level (1.8 µg/g) when HCB was withdrawn from the food (Fig. 3).

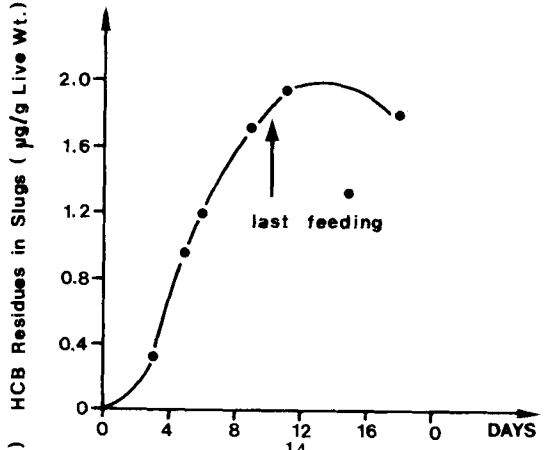

Fig. 3 : Uptake of ^{14}C-HCB residues in slugs fed on carrot discs treated with 0.5 µg/g slug/day

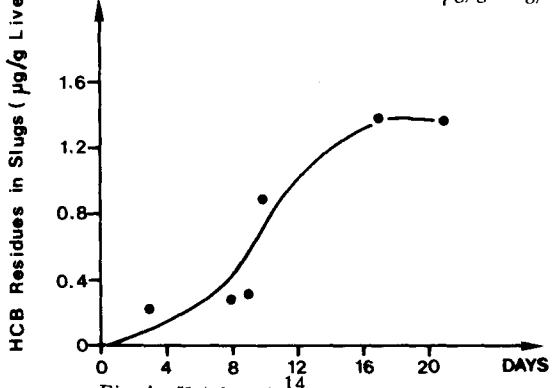

Fig. 4 : Uptake of ^{14}C-HCB residues in slugs exposed to 1.0 mg/kg in soil (dry)

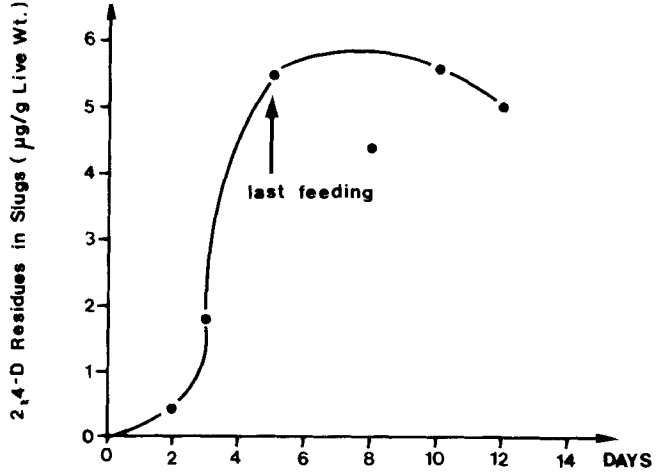

Fig. 5 : Uptake of ^{14}C-2,4-D residues in slugs fed on carrot discs treated with 1.1 µg/g slug/day

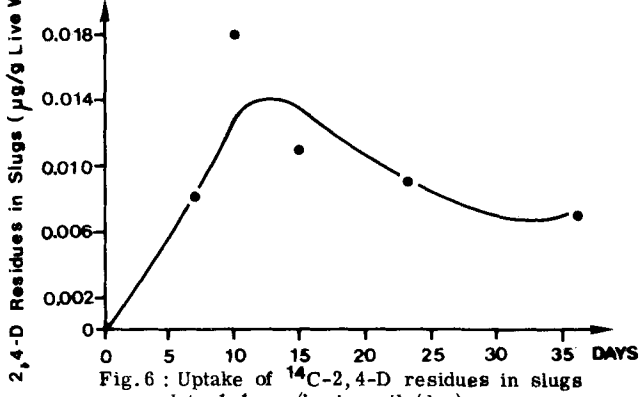

Fig. 6 : Uptake of ^{14}C-2,4-D residues in slugs exposed to 1.1 mg/kg in soil (dry).

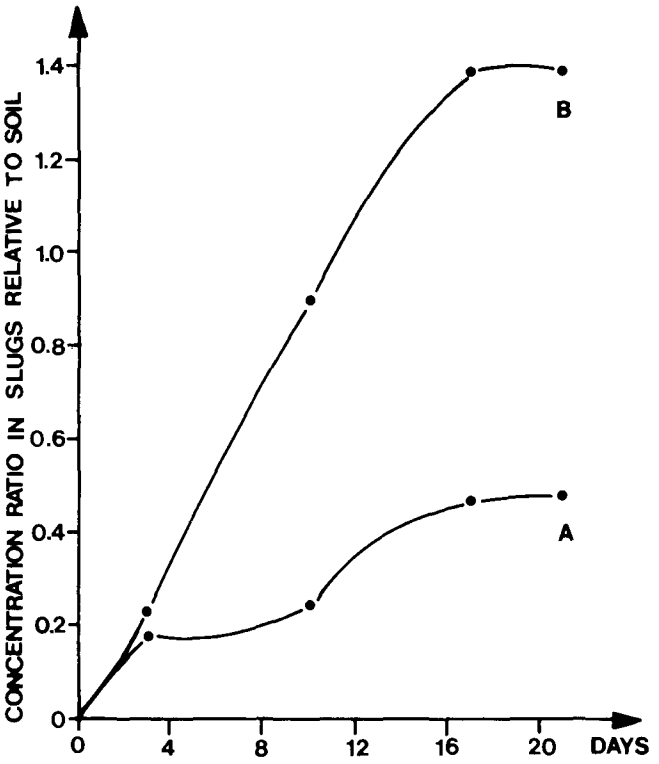

Fig. 7: Concentration ratio of HCB in slugs at two soil treatment levels. A: 0.1, B:1.0 mg/kg soil (dry)

The slugs when exposed to the contaminated soil absorbed HCB and 2,4-D (Fig. 4 and 6). An equilibrium for HCB was reached after about 18 days (Fig. 4), and for 2,4-D after about 15 days (Fig. 6). The differences in the equiliberation time may be due to the availability of the compounds from the soil, and to the chemical properties (partition coefficient). The different quantities of HCB (1.4 µg/g) and 2,4-D (0.014 µg/g) residues found in slugs tissue at these times (Fig. 4 and 6) also support this proposition. LORD at al. (1980) have shown that there are different rates of equilibrium of different chemicals in earthworms which are dependent on the diffusion and redistribution in the worms body. This mechanism of uptake of pesticides in worms from soil may be similar to that in slugs as has also been suggested by LORD et al. (1980).

The uptake of HCB by slugs at two soil concentrations (0.1 and 1.0 mg/kg) took place at different rates, but the highest concentration ratio was observed after 18 days in both the cases (Fig. 7). This sug-

gests that the accumulation of residues in slugs is dependent on the contamination level of the soil, but does not follow a direct proportionality.

It is concluded that the uptake of pesticide residues by slugs is not only through the ingestion of contaminated food but also the residues are taken up from the contaminated soil and probably through the process of diffusion.

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REFERENCES

BEYER, W.N., and C.D. GISH: J. appl. Ecol. 17, 295 (1980).
CLARK, D.E., J.E. YOUNG; R.L. YOUNGER, L.M. HUNT, and J.K. McLARAN: J. Agric. Food Chem. 12, 43 (1964).
DAVIS, B.N.K.: Ann. appl. Biol. 61, 29 (1968).
DAVIS, B.N.K., and M.C. FRENCH: Soil Biol. Biochem. 1, 45 (1969).
DINDAL, D.L., and K.-H. WURZINGER: Bull. Environm. Contam. Toxicol. 6, 362 (1971).
EDWARDS, C.A., and A.R. THOMPSON: Residue Rev. 45, 1 (1973).
EDWARDS, C.A.: Bull. Environm. Contam. Toxicol. 16, 406 (1976).
GISH, C.D.: Pestic. Monit. J. 3, 241 (1970).
KHANNA, S., and S.C. FANG: J. Agric. Food Chem. 14, 500 (1966).
KUHR, R.J., A.C. DAVIS, and J.B. BOURKE: Pestic. Monit. J. 7, 200 (1974).
LORD, K.A., G.G. BRIGGS, M.C. NEALE, and R. MANLOVE: Pestic. Sci. 11, 401 (1980).
STRINGER, A., and J.A. PICKARD: Rep. Agri. Hort. Res. Station, Long Ashton, 1964, 172 (1965).
WHEATLEY, G.A., and J.A. HARDMAN: J. Sci. Food Agric. 19, 219 (1968).
YADAV, D.V., P.K. MITTAL, H.C. AGARWAL, and M.K.K. PILLAI: Pestic. Monit. J. 15, 80 (1981).

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