Root uptake and translocation of 14C-heptachlor and its degradation products in soil by zucchini and tomato seedlings

Osamu HAYASHI,* Daisuke SUGIOKA and Kazutoshi OHYAMA

Laboratory of Metabolism I/II, Chemistry Division, The Institute of Environmental Toxicology, 4321 Uchimoriya-machi, Joso-shi, Ibaraki 303-0043, Japan

(Received May 14, 2018; Accepted July 16, 2018)

To investigate root uptake and translocation of heptachlor and its degradates (cis-heptachlor epoxide and 1-hydroxychlordene) in soil, zucchini and tomato seedlings were transplanted to soil approximately four months after treatment with 14C-heptachlor. The results indicated that a relation between the root concentration factor and the log $P_{oc}$ did not follow Briggs’ theory, probably due to the contribution of plant metabolism. It also appeared that a compound with a lower log $P_{oc}$ tends to show higher mobility from root to shoot. © Pesticide Science Society of Japan

Keywords: root uptake, translocation, root concentration factor, heptachlor.

Heptachlor is a chlorinated bicyclopentadiene insecticide that is classified as a Persistent Organic Pollutant (POP). Heptachlor had been used formerly on farmland, and its agrarian usage has been banned in Japan since 1972. However, it is still detected in environmental samples in Japan even approximately 40 years after the lapse of its registration. Heptachlor epoxide, which is a plant metabolite or degradation product of heptachlor in soil, is still occasionally detected at levels slightly higher than the Maximum Residue Limit (MRL) in pumpkins produced in Hokkaido. This fact indicates that heptachlor epoxide is present in the soil long-term and can be absorbed and accumulated by a plant. The variation of uptake and translocation of organochlorine pesticides, to which heptachlor belongs, has been reported for various plants.1–3) However, there is little information regarding the uptake of heptachlor by crops after soil treatment. We previously reported the root uptake of 14C-heptachlor to various plants under hydroponic cultivation5) and indicated that the 14C-concentrations in shoots of wheat, cucumber, tomato, and zucchini were higher than those of other plant seedlings after exposed via the hydroponic solution. In addition, we reported the degradation of 14C-heptachlor in Japanese soils5) and found that the concentration ratio of heptachlor and its degradation products remained constant in the soil three to four months after the application of 14C-heptachlor. In the current study, tomato and zucchini seedlings of the test plants were transplanted to soil that had been aged for four months after treatment with 14C-heptachlor, and the aged soil, as well as the plant parts after exposure, was analyzed. This study aimed to investigate the relation between the log $P_{oc}$ of heptachlor and its degradation products and several parameters obtained based on the concentration in the soil and plant parts.

Radiolabeled heptachlor ([4,5,6,7,8-14C]heptachlor) was provided and prepared for us by Moravek Biochemicals (Brea, CA, USA) by custom synthesis (specific radioactivity: 11.5 MBq/mg, radiochemical purity: >97%). Non-radiolabeled heptachlor and 1-hydroxychlordene were purchased from Fujifilm Wako Pure Chemical (Osaka, Japan), and cis- and trans-heptachlor epoxides were purchased from Hayashi Pure Chemical Industries (Osaka, Japan). The identity of 14C-heptachlor was confirmed by comparing the retention time with the non-radiolabeled heptachlor using high-performance liquid chromatography (HPLC). All organic solvents used in this study were of HPLC or GC grade. The pure water used was prepared by a Milli-Q Integral 3 Water Purification System (Merck KGaA, Darmstadt, Germany).

Test soils, which were volcanic ash soils from Japan, were collected from a depth of approximately 20 cm in agricultural fields at the Ushiku Branch of The Japan Association for the Advancement of Phyto-Regulators (860 Kashiwada-cho, Ushiku, Ibaraki, Japan) and stored in the refrigerator for ca. 2 weeks before use. The collected soils were filtered through a 2 mm sieve prior to use. The physical and chemical properties of the soil are: soil texture, clay loam; clay mineral dominant, allophane; pH (H2O, KCl, CaCl2, respectively), 7.0, 6.4 and 6.0; organic matter content, 47.9 g/kg; cation exchange capacity, 40.4 cmol/kg. Tomato (Lycopersicon esculentum, Odoriko) and zucchini (Cucurbita pepo, Black Tosca) seeds were purchased from Sakata Seed (Kanagawa, Japan). The pre-cultivation method of treating the test plant was described in the previous report.4) The numbers of pre-cultivation days from sowing of the test plants used are 19 days (tomato) and 12 days (zucchini).

Test soil approximately 10 cm thick (1317.6 g of dry soil) was placed into a 5000 mL glass vessel (n = 1). The soil system was pre-incubated in the dark at 25°C for 2 weeks. The test compound in small amount of acetone was applied and mixed well into the soil at a concentration of 203.4 µg/kg of dry soil. The glass vessel was plugged with an adsorbent to trap organic volatiles evolving from the soil (adsorbent was not analyzed). The soil system was incubated at 25°C in the dark for 122 days, and the soil water content was adjusted periodically to its original...
level by adding pure water. After incubation, portions of the soil (ca. 50 g wet-weight soil, n=3 or ca. 200 g wet-weight soil, n=2) were placed into glass vessels of appropriate volume to be used for acetone- or water-extraction experiments. In addition, another portion of the soil (ca. 600 g wet-weight soil, n=2) was transferred to the stainless tray to be used for the plant uptake/translocation experiment.

The acetone extraction experiment: The soil (ca. 50 g subsample) was extracted twice with 100 mL of acetone with a mechanical shaker for 30 min and was filtered by suction. After these extracts were combined and concentrated by a rotary evaporator, the residual aqueous phase was subjected to solid phase extraction, Bond Elut C18 (Agilent Technologies Japan, Tokyo, Japan). The column was eluted with hexane/ethyl acetate (1/1, v/v) and methanol successively. The eluates were combined and concentrated with a rotary evaporator to be analyzed by radio-HPLC.

The water extraction experiment: The soil (ca. 200 g subsample) was extracted with 200 mL of Milli-Q water with a mechanical shaker for ca. 24 hr. The extraction mixture was centrifuged for 10 min at ca. 5,000 × g. The supernatant was subjected to a solid phase extraction (Bond Elut C18) and eluted with methanol. The eluate was concentrated with a rotary evaporator and was analyzed by radio-HPLC.

The plant uptake/translocation experiment: To determine the uptake and translocation of 14C-heptachlor, six pre-cultivated seedlings were transplanted to the aged soil in the stainless tray and watered. The soil tray was placed in an incubator (Tokyo Rikakikai, Tokyo, Japan) at 25°C and was covered with the wrap to protect from dryness. Plant seedlings were then grown in the incubator under a cycle of 16 hr illumination (25,000 lux) and 8 hr darkness for 72 hr. After incubation, the seedlings were transplanted to the aged soil in the stainless tray with an approximately 20 mL portion of acetone using a Polytron® homogenizer (Kinematica AG, Lucerne, Switzerland). The extraction mixtures were filtered through a filter paper (The Top Filter Paper No. 704, Nippon Rikagaku Kikai, Tokyo, Japan) to separate the extracts and post-extraction solids (PESs). PESs were air-dried to analyze the radioactivity by oxidative combustion. The extracts were evaporated by a rotary evaporator and dried under a nitrogen stream. The residues were re-dissolved in a small amount of acetonitrile and were analyzed by HPLC.

The extracts were analyzed using a Shimadzu LC-10A or LC-20A HPLC system (Shimadzu, Kyoto, Japan) with an ultraviolet (UV) detector. The HPLC analysis conditions were the same as those in our previous paper. The radioactivity in a liquid sample was measured individually by liquid scintillation counting (LSC). Duplicate aliquots of each liquid sample were dissolved in Pico-Fluor™ Plus scintillation cocktail (PerkinElmer, Wellesley, MA, USA) prior to LSC analysis. LSC analysis was conducted using an LSC-5100 liquid scintillation counter (Hitachi, Tokyo, Japan). Each LSC sample was counted twice for 2 min, and the average value was cited as the radioactivity of the sample. PESs from plant samples were analyzed using an automatic sample oxidizer (Model 307, PerkinElmer) described in our previous paper.

The ratio of the extractable concentration of the chemical from the soil is described by the AW ratio. The AW ratio = (concentration in acetone extracted from the soil)/(concentration in water extracted from the soil). The uptake of the chemical into the root is conveniently described by the root concentration factor (RCF). The RCF = (concentration in the plant root)/(concentration in the water extracted from the soil). The ratio of the chemical concentration in the shoot and root is described by the SR ratio. The SR ratio = (concentration in the shoot)/(concentration in the root).

Table 1 shows the concentrations of acetone- or water-extractable radioactive residues in the soil after treatment with 14C-heptachlor, with estimated log P'oct (octanol–water partition coefficient) values of heptachlor and its metabolites. The concentration ratio in acetone extracts of heptachlor (HEP): cis-heptachlor epoxide (EPO): 1-hydroxychlordene (HYD) (1:1.7:0.29) was similar to that of the Ibaraki-U soil extracts in our previous report (1:1.5:0.58). The order of concentrations in acetone and water extracts were EPO>HEP>HYD and EPO

| Chemical structure | Heptachlor | Heptachlor epoxide | 1-Hydroxychlordene |
|-------------------|------------|--------------------|--------------------|
| Log P'oct | 4.62 | 4.00 | 3.30 |
| Conc. in aceton ext. | 30.23 | cis- 51.97 | 8.83 |
| Conc. in water ext. | 0.07 | (cis-) 0.23 | 0.09 |
| AW ratio | 422 | 223 | 98 |

**Table 1.** Concentrations of acetone- or water-extractable radioactive residues in the soil after treatment with 14C-heptachlor for four month at 25°C

---

a) Data from KOWWIN™ in Estimation Program Interface (EPI) Suite™, b) n=3. c) eq., 14C-heptachlor equivalence. d) n=2. e) AW ratio, (concentration in acetone extract from soil/concentration in water extract from soil) ratio. Initial applied concentration of 14C-heptachlor was 203.4 µg/kg dry soil.
HYD > HEPO > HEP, respectively. The values of the AW ratio were HEP > HEPO > HYD.

Figure 1A shows the relationship between log $P_{ow}$ and AW ratios. The log $P_{ow}$ and AW ratio showed good linear correlation, indicating that the concentration of each radioactive component dissolved in the soil water would be predictable by the calculation from the log $P_{ow}$ and the concentration in the acetone extract. Table 2 shows the concentrations of heptachlor and its metabolites in each plant part. The concentrations of radioactivity in both plant shoots showed a rank order of EPO > HYD > HEP. The concentrations of radioactivity in the roots showed a rank order of EPO > HEP and HYD (tomato) or EPO > HEP > HYD (zucchini).

Figure 1B shows the relationship between the log $P_{ow}$ and RCF of heptachlor and its metabolites. Briggs et al. found a linear relationship between the log $P_{ow}$ and the log RCF based on experiments using $O$-methylcarbamoyloximes and substituted phenylureas by barley plants: log (RCF − 0.82) = 0.77 log $P_{ow}$ − 1.52, which indicates that the RCF value would increase by increasing the log $P_{ow}$. In the current study, however, the RCF of EPO ($P_{ow}$ 4.00) was greater than that of HEP ($P_{ow}$ 4.62), which does not follow Briggs’ theory. When HEP is absorbed by the root, a portion of this compound would be metabolized to EPO. The HEP concentration in the root could be decreased due to its metabolic degradation (RCF value decrease), while the EPO concentration in the root is increased (RCF value increase). In the current study, the RCF value of heptachlor in tomato was lower than that in zucchini. In our previous report, HEP absorbed into the tomato plant showed greater metabolic degradation as compared to that of the zucchini plant under hydroponic cultivation. Our previous finding agrees well with this result. It is considered that HYD may be supplied as a metabolite of HEP and be decreased due to metabolism. As a result, it was speculated that the RCF value of HYD followed Briggs’ theory.

Figure 1C shows that relationship between the log $P_{ow}$ and the SR ratio. The lower log $P_{ow}$ compound showed the higher SR ratio. This result indicates that the compound with the lower log $P_{ow}$ tends to transfer better from the root to the shoot. The high-

![Fig. 1. Relationship between the log $P_{ow}$ and (A) the AW (concentration in acetone extract/concentration in water extract) ratio, (B) the RCF (root concentration factor), and (C) the SR (concentration in shoot/concentration in root) ratio. Heptachlor/HEP, cis-heptachlor epoxide/EPO, 1-hydroxychlordene/HYD.](image)

### Table 2. Concentrations of heptachlor and its metabolites in the shoots and roots of the tomato and zucchini cultivated in soil with aged $^{14}$C-heptachlor

|                | Heptachlor | cis-Heptachlor epoxide | 1-Hydroxychlordene | Others | PESs$^b$ | Total  |
|----------------|------------|------------------------|--------------------|--------|---------|--------|
| **Tomato**     |            |                        |                    |        |         |        |
| Conc. in shoot (µg eq./kg) | 0.28       | 3.23                   | 0.86               | 6.13   | 8.11    | 18.60  |
| Conc. in root (µg eq./kg)   | 5.45       | 50.84                  | 5.16               | 26.13  | 17.79   | 105.38 |
| SR ratio$^a$     | 0.051      | 0.063                  | 0.166              | —      | —       | —      |
| RCF$^b$         | 76         | 218                    | 57                 | —      | —       | —      |
| **Zucchini**    |            |                        |                    |        |         |        |
| Conc. in shoot (µg eq./kg) | 0.67       | 4.24                   | 1.17               | 5.20   | 3.59    | 14.87  |
| Conc. in root (µg eq./kg)   | 17.78      | 64.88                  | 4.49               | 14.93  | 8.07    | 110.16 |
| SR ratio$^a$     | 0.037      | 0.065                  | 0.260              | —      | —       | —      |
| RCF$^b$         | 248        | 279                    | 50                 | —      | —       | —      |

$^a$ SR ratio, (concentration in shoot/concentration in root) ratio. $^b$ RCF, root concentration factor. $^b$ PESs, post-extraction solids. — not applicable.
er variation of the SR ratio value of the zucchini as compared to that of the tomato suggested that zucchini is able to translocate a greater quantity of the polar compound from the root to the shoot than the tomato does, or the metabolic ability of the tomato is increased as compared to that of the zucchini. In our previous report, the percentage of total radioactive residues (% TRR) of HEP in the tomato shoot (9.7% TRR) was lower than that in the tomato root (60.1% TRR), while that of zucchini was unchanged (73.8% TRR in the shoot and 80.6% TRR in the root). This result raised the possibility that HEP is metabolized in the transfer from root to shoot in the tomato. The detailed analysis of the soil and plants in this study showed that the abundance ratios of heptachlor and the degradation products were different in the soil acetone extract, the soil water extract, and each plant part extract. It is considered that the abundance ratios of xenobiotics in crop would be changed by factors including metabolism in plant cells and the ability to transfer from the root to the shoot.

References
1) J. C. White: *Chemosphere* 49, 143–152 (2002).
2) T. Otani, N. Seike and Y. Sakata: *Soil Sci. Plant Nutr.* 53, 86–94 (2007).
3) A. Hülster, J. F. Müller and H. Marschner: *Environ. Sci. Technol.* 28, 1110–1115 (1994).
4) O. Hayashi, M. Kameshiro and K. Satoh: *J. Pestic. Sci.* 35, 107–113 (2010).
5) O. Hayashi, M. Kameshiro and K. Satoh: *Biosci. Biotechnol. Biochem.* 77, 1240–1244 (2013).
6) M. G. T. Shone and A. V. Wood: *J. Exp. Bot.* 25, 390–400 (1974).
7) G. G. Briggs, R. H. Bromilow and A. A. Evans: *Pestic. Sci.* 13, 495–504 (1982).