Evaluation of the appropriateness of pesticide administration concentrations in long-term field experiments and modest proposal

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Abstract

In order to investigate the effects of pesticides on bee colonies in apiaries, long-term field experiments in an open environment close to actual apiaries are effective. In long-term field experiments in an open environment, various experimental conditions are uncertain. It is natural to think that the consumption of food and pesticides administered in experiments is not necessarily their total intake of bee colonies, but also those from nature. Therefore, in long-term field experiments, setting the administration concentration of pesticides to bee colonies is one of the extremely difficult tasks.

In this paper, we examined the validity of pesticide concentrations administered to bee colonies using data from five long-term field experiments conducted in the Mid-west of Japan and Maui, USA.: The total number of bees in the bee colony to which the number of newly-emerging bees from pupae is added; the average total intakes of food (sugar syrup, pollen paste) and pesticide ingested by the bee colony or one bee; the average daily intakes of food and pesticide ingested by one bee. From these newly-calculated data, we clarified the relationship between the average daily intakes by bees ($\boldsymbol{P_{BD}}$) and the pesticide administration concentrations to bee-colony ($\boldsymbol{C_P}$), which was expressed by $\boldsymbol{P_{BD}}/\boldsymbol{C_P} \fallingdotseq 0.01$.

Assuming that the pesticide intake time of one bee in the field experiment is equivalent to the measurement time of LD_{50} of honeybees, it was found from the relationship that the average pesticide intake was lower than the LD_{50} value. For example, the average pesticide intake taken by one bee in two days (48 hrs) was lower than an LD_{50} value (48 hrs). This fact shows that some researchers' criticism that the concentration of pesticides in our long-term field experiments is too high is incorrect.

Furthermore, the analysis results revealed the followings. The average daily pesticide intake taken by one bee increases as the concentration of pesticides administered in long-term field experiments increases. The average total intake taken by one bee during the pesticide administration period is, however, almost constant, regardless of the pesticide administration concentration since the pesticide administration period because the higher the concentration, the faster the colony will become extinct, and the shorter the pesticide administration period.

During the pesticide administration period, the average intake of pesticide-containing sugar syrup per bee per day is about 3~4mg/bee/day. Although the data vary, the average intake of pesticide-containing foods ingested by one bee is almost independent of the concentration of pesticides administered, is almost constant, and is almost the same as the intake of pesticide-free foods.

The average daily intake of pesticides taken by one bee via pollen paste is less than that taken via sugar syrup. The

average total intakes of sugar syrup and pollen paste ingested by one bee during the administration period will be about 0.45 g/bee and 0.048 g/bee, respectively. Moreover, the maximum ones will be about 1.4 g/bee and 0.11 g/bee, respectively.

It is believed that the ingestion of sugar syrup or pollen paste in long-term field experiments in an open environment where bees can move freely is very different from laboratory experiments such as when LD_{50} is measured, in which bees are compulsorily forced to ingest them in a closed environment. Based on the analysis results of long-term field experiment data under various conditions, this paper may provide meaningful information for determining the concentration of pesticide administration in long-term field experiments, such as setting conditions in an open environment, from the measurement results in a closed environment.

Introduction

In recent years, the risk to the ecosystem of neonicotinoid pesticides (hereinafter referred to as neonics), which exhibit long-term residual effects, toxic and permeable neurotoxicity, has been pointed out (Tirado *et al*., 2013; Goulson, 2013; Wood & Goulson, 2017). In particular, there have been concerns about the negative impact of neonics on bees. In long-term field administration experiments of neonics to bees that have been conducted to investigate the effects of neonics on bees, it has often been debated whether the concentration of pesticides administered to a bee colony is actually appropriate. A median lethal dose (LD_{50}) for bees has often been used as an indicator to assess whether the pesticide administration concentration is appropriate.

It is well known that honeybees, which are eusocial insects, have their roles determined by caste and act on a colony basis. Castes in a colony are divided into three types: one queen bee, a small number of male bees (drones) and many female bees (worker bees).

In honeybee society, the three roles of egg-laying, reproduction and rearing are shared by the queen bee, drone and worker bee, respectively. While being cared for by worker bees, the queen bee continues to lay eggs for the rest of its life after mating, and the drone spends its entire life with mating as its only job. Worker bees are responsible for almost all other tasks such as foraging and larval rearing.

In worker bees, the division of labor is further sub-divided, and it is roughly divided into in-house bees that are responsible for cleaning and rearing in the hive box and foraging bees that collect pollen and nectar outside the hive box. After emerging, worker bees work as in-house bees in the hive box, and then, their age in day, forage outside the hive box as foraging bees after they become old.

The order of this division of labor changes depending on the state of the colony, the surrounding environment in which the colony is placed, the season, etc. However, in the case that the momentum of the colony is strong, the surrounding pollen and nectar sources are abundant, and active foraging activities are carried out, the change in division of labor corresponds to the progression of day-age (Watanabe, H. and Watanabe, T., 1974).

In addition to the peculiarities of bee colonies, which consist of such a complex social structure consisting of the caste system and division of labor, long-term field experiments in an environment close to real apiaries and measurement of LD_{50} in the laboratory (laboratory experiments) are very different, as shown in **Table 1**, which summarizes the differences between the characteristics of laboratory experiments when measuring the median lethal dose (LD_{50}) of bees and those of long-term field experiments of bee colonies. The main differences between two are as follows: In long-term field experiments, it is easy to reproduce an experimental environment close to that of

an actual apiary, but in laboratory experiments used for LD_{50} measurement, it is extremely difficult to reproduce the experimental environment of an actual apiary. On the other hand, it is easy to control the experimental environment in laboratory experiments conducted in a closed system, but it is extremely difficult to control the experimental environment in long-term field experiments conducted in an open system.

For example, long-term field experiments are conducted in an open natural environment with a wide degree of freedom, while laboratory experiments such as the measurement of LD_{50} are conducted in a closed environment with a narrow degree of freedom. In other words, at the time of LD_{50} measurement, the ecological environment of honeybees is controlled, the intake and timing of food containing pesticides by bees are accurately controlled and measured, and food other than the pesticide-containing food administered in the experiment is not ingested.

On the other hand, in long-term field experiments, bee colonies are composed of a social structure that maintains a caste system and division of labor in almost the same way as actual apiaries, and the experiments are conducted in the same natural environment as the apiary, and the intake and timing of the administered pesticide-containing food are not controlled and are free. In addition, the consumed pesticide-containing food is often mixed with food collected from nature and stored in the hive box, and the timing and intake of such stored food are freely carried out according to the will of the honeybees and are not managed.

In conducting field experiments in an open system where it is difficult to control the experimental environment, we made the following efforts to reduce the influence of the experimental environment as much as possible (for details, see Yamada et al., 2018a, b, c, d, 2020a, 2020b). :(1) A pesticide-free drinking fountain was set up in the experimental site, and pesticide-free plants and flowers and trees were planted to prevent bees from ingesting pesticides from sources other than the experiment; (2) The control colony and the experimental colony were arranged alternately to reduce the influence of the location of the hive box on the experimental results; (3) In order to reduce the errors in the intakes of sugar syrup and pollen paste, and to reduce the measurement error of the numbers of adult bees and capped brood in the hive box and the dead bees in and around the hive box, immediately after dawn before the bees start foraging activities, measure the remaining amount of sugar syrup or pollen paste, and take photos of each side of all comb frames and the five walls in the hive box (can be checked repeatedly), using newly developed counting software as an adjunct to these photographs, the number of adult bees and capped brood were calculated from these photographs. And dead bees in and around the hive box were counted one by one and their number was recorded. Bees affected by mites, while enlarging photos of comb frames and the four walls and bottom of the hive box, were counted.

By the way, if the difference in preference of honeybees is between the pesticide-containing food administered (sugar syrup, pollen paste) and the food collected from nature (flower nectar, bee bread), there is a difference between the apparent intake (including stored food) and the true intake of pesticide-containing food by honeybees, and the true pesticide intake may be inaccurate. In this way, bee colonies in long-term field experiments constitute a complex social structure composed of caste system and division of labor, and operate freely in an open natural environment that is not controlled. On the other hand, in the measurement environment of LD_{50} in laboratory experiments, the bee colony consists of only a small number of worker bees without caste or division of labor, and is engaged in constrained activities in a highly controlled environment.

Thus, the appropriateness of the administration concentration of pesticides in long-term field experiments in an open ecological environment in which bee colonies maintaining the caste system and division of labor as almost complete eusocial insects can be freely ingested from other than pesticide-containing food to which they are administered,

from LD_{50} measured in a very incomplete social structure consisting of a small number of bees and a controlled closed ecological environment, as in Nakamura et al. (2014), it seems reckless to evaluate.

For example, judging from the LD_{50} value, even if food (sugar solution, pollen paste) with a pesticide concentration that seems to be too high is administered to bees in a long-term field experiment, the behavior of bees is free in the field experiment, so if the bees hardly ingest the administered pesticide-containing food and preferentially ingest food procured from the field. Alternatively, it may also happen that the actual pesticide intake of bees is lower than the LD_{50} value. That is, from the LD_{50} value in a closed measurement environment in which all ingested food contains pesticides, it is expected that the pesticide administration concentration in the field experiment where bees are allowed to move freely is expected to be significantly different from the concentration of pesticide administration in a field experiment where bees are allowed to move. Similarly, in field experiments, food containing pesticides that appears to have been ingested is often stored in the cell. Since it is up to the bees to decide when and how much of the stored pesticide they actually ingest, the actual intake is even more uncertain.

Therefore, in the long-term field experiments that we have conducted so far, we estimated the pesticide intake per bee during the pesticide administration period from the total amount (intake) of food containing pesticides in each colony and the total number of adult bees (initial number + number of adult bees that have newly eclosed calculated from the number of capped brood) (Yamada *et al.*, 2018a, 2018b).

In this paper, we estimate the average amount of pesticide ingested by one bee per day during the pesticide administration period in a long-term field experiment, compare the LD_{50} value with the pesticide intake per bee during the same period, and examine whether it is possible to estimate and evaluate the pesticide administration concentration in long-term field experiments from the LD_{50} value. We will also evaluate the appropriateness of pesticide administration concentrations in our long-term field experiments so far.

Methods

Yamada et al. have conducted six long-term field experiments shown in **Table 2** to investigate the effects of neonico on bee (*Apis mellifera*) colonies (.Yamada et al., 2012; 2018a; 2018b; 2018c; 2018d; 2020a, 2020b). These six long-term field experiments (2010-Experiment, 2011/2012-Experiment, 2012/2013-Experiment, 2013/2014-Experiment, 2014/2015-Experiment, 2018-Experiment) were conducted to investigate the followings, where the number of days in parentheses indicates the number of experimental days from the start date to the end date of the field experiment. "2010-Experiment" (126 days)/Can neonicotinoid pesticides cause colony collapse disorder (CCD)?; "2011/2012-Experiment" (268 days)/When bee colonies ingest neonicotinoid pesticides through food, is there a difference between sugar syrup (honey) and pollen paste (pollen)?; "2012/2013-Experiment" (393 days)/Is there a difference in the effects of neonicotinoids and organophosphates on bee colonies?; "2013/2014-Experiment" (199 days)/Will bee colonies cause CCDs even at low concentrations of neonicotinoid pesticides?; "2014/2015-Experiment" (271 days)/Will a long-term field experiment on the island of Maui, Hawaii, which has no mites and no seasons, replicate the results of a long-term field experiment in Shiga in Japan?; "2018-Experiment" (180 days)/When neonicotinoid pesticides are administered to bee colonies with mites, how do they change over time?

Among these six long-term field experiments, at the start of the field experiment of the "2010 Experiment", software had not been developed to help accurately count the number of adult bees and capped brood, so the number of adult bees and capped brood was roughly estimated visually from all the photographic images and compared with

accurately measured sample images. In the five field experiments since the "2011/2012 Experiment" ("2011/2012 Experiment", "2012/2013 Experiment", "2013/2014 Experiment", "2014/2015 Experiment", "2018 Experiment"), the numbers of adult bees and capped brood were fairly accurately counted with the help of newly developed software from photos of all the comb surfaces and all the inside-walls of hive box. Therefore, in this paper, we estimated the pesticide intake of honeybees using the experimental results since the "2011/2012 Experiment" to accurately count the number of adult bees and the number of capped brood. That is, the average pesticide intake per bee is calculated from the total consumption of pesticide consumption of the bee colony during the pesticide administration period and the total number of adult bees during that period.

We have conducted six long-term field experiments shown in **Table 2** to investigate the effects of neonicotinoid pesticides (neonics) on bee colonies (Yamada *et al*.; 2012; 2018a; 2018b: 2018c; 2018d; 2019; 2020a; 2020b). In these papers, the average pesticide intake per bee is calculated from the total pesticide consumption of the bee colony during the pesticide administration period and the total number of adult bees during that period. However, these papers assume that the total pesticide consumption measured is the same as the total pesticide intake of the bee colony.

In these long-term field experiments, the amount of pesticide-containing honey and bee-bread, which were stored in comb cells and left in the hive box at the end of the experiment, was not measured. Therefore, the true total pesticide intake seems to be less than the total pesticide consumption. Although it has not been measured, many of the experimental colonies remember that at the end of the experiment, only a small amount of pesticide-containing food was left in the hive box. In other words, it can be inferred that the average amount of pesticide ingested per day by one adult bee calculated in this paper is slightly less than the average value of true intake.

The average intake of pesticide per day per bee (P_{BD}) is calculated by ***Eq.(1)***, where values (P_T, B_T, D_T) other than P_B in ***Eq. (1)*** are reported in papers already published by Yamada et al. (2018a; 2018b; 2020b).

$$P_{BD} = P_T / B_T / D_T \qquad (1).$$

P_{BD} = Average daily intake of pesticide per bee.

P_T = Total intake of pesticide during pesticide-administration period per colony, which is equal to the pesticide consumption during the period measured in the experiment.

B_T = Total number of bees during pesticide-administration period per colony, which is calculated from the initial number of adult bees and the total number of new adult bees having eclosed from capped brood.

D_T = Total number of days during pesticide-administration period to the colony, which is the total number of days from the start of pesticide administration to the stop of the administration.

[Estimation procedures of B_T]

The procedures to estimate the total number of adult bees during a certain period are as follows:

Supposing that the number of adult bees at the first measurement (start of pesticide administration) is B_0, the number of capped brood whose distribution aged 1 to 12 days is the same at the kth measurement is CB_k, the number of days from the kth measurement to the $(k+1)^{th}$ measurement is D_k and the number of total observations is N, B_T can be roughly obtained from the following equation (for details, see Yamada et al., 2018a & 2018b): Where, it is assumed that the capped brood (CB_N) present at the end of the administration period has already ingested food and

pesticides.

$$B_T = B_0 + \Sigma CB_k*(D_k/12) + CB_N, \text{ where } k = 1, 2, 3, \ldots, (N-1) \quad (2)$$

Results and Discussion

In six long-term field experiments conducted by Yamada et al. (2012; 2018a, b, c, d, e; 2018) as shown in **Table 2**, pesticides were administered to bee colony via food (sugar syrup, pollen paste). In only 2010-Experiment, either dinotefuran or clothianidin was administered to bee colonies via both sugar syrup and pollen paste. In other five long-term field experiments (2011/2012-Experiment, 2012/2013-Experiment, 2013/2014-Experiment, 2014/2015-Experiment, 2018-Experiment) than 2010-Experiment, one kind of pesticides was administered to a bee colony via either sugar syrup or pollen paste. In this paper were assessed various intakes obtained by dividing the total intake of pesticides, sugar syrup and pollen paste in bee colonies by the total number of adult bees during the administration period and/or the number of days of administration period for these five long-term field experiments. During the administration period to a bee colony in five long-term field experiments, **Table 3** shows the experimental conditions of five long-term field experiments, and during each pesticide-administration period, the total number of adult bees in one colony and the total intakes of sugar syrup, pollen paste and pesticide per colony, the average total intakes per bee and the average daily intakes per bee. Table 3 was created by scrutinizing the original data of long-term field experiments, recalculating them, and making some corrections.

Average daily intake of pesticide ingested by one bee during pesticide administration to the bee colony, P_{BD}.

The average amount of pesticides ingested by one bee in one day (P_{BD}) shown in **Table 3** were estimated from the values of P_T, A_T and D_T for the pesticide concentration administered to the bee. **Table 3** lists the experimental conditions, the total number of honeybees, the intakes of food (sugar syrup, pollen paste) and pesticide per colony, those per bee and those per bee per day during the administration period to a bee colony in five long-term field experiments. **Figure 1** is a logarithmic plot of the relationship between the concentration of pesticide administered to the bee colony (C_P) [ng (pesticide)／g(pesticide-containig vehicle)] and the average intake of pesticide per bee per day (P_{BD}) [ng/bee/day], with C_P on the horizontal axis and P_{BD} on the vertical axis, for all data reported in previous our works on five long-term-field experiments. From **Fig. 1**, it can be seen that C_P and P_{BD} is roughly expressed by the following equation.

$$\log (P_{BD}) \fallingdotseq \log (C_P) - 2.4 \quad (3)$$

That is,

$$P_{BD} / C_P = 10^{-2.4} \fallingdotseq 0.004 \text{ [g(pesticide-containig vehicle)/(bee·day)]} \quad (4)$$

Parallel to equation (3), the logarithmic line that takes the maximum data of P_{BD} is as follows.

$$\log (P_{BD}) \fallingdotseq \log (C_P) - 2.0 \quad (5)$$

That is,

$$P_{BD}/C_P = 10^{-2.0} \fallingdotseq 0.01 \text{ [g(pesticide-containig vehicle)/(bee·day)]} \quad (6)$$

Figure 1 shows the relationship between the average pesticide intake (P_{BD}) ingested by one bee per day and the pesticide administration concentration (C_P) in the bee herd, calculated from five long-term field experiments (2011/2012-Experiment, 2012/2013-Experiment, 2013/2014- Experiment, 2014/2015-Experiment, 2018-Experiment).

The followings can be inferred from **Table 3**, **Figure 1**, Equations (3) to (6):

Broadly speaking, regardless of the type of pesticide administered, the higher the concentration administered, the higher the average pesticide intake per bee per day.

P_{BD} administered with pesticides via pollen paste tends to be lower than P_{BD} administered with pesticides via sugar syrup.

The relationship between P_{BD} and C_P is roughly expressed by Equation (3), regardless of the type of pesticide administered and the time and place of the experiment. It can be seen that P_{BD} is less than or equal to Equation (5). It can be seen from Equation (4) that the average pesticide intake (P_{BD}) ingested by one bee per day is approximately 1/250 of the pesticide administration concentration (C_P) to the bee colony, and from Equation (6) that P_{BD} is at most 1/100 of C_P. This suggests that environmental differences between open field experiments and closed laboratory experiments must be taken into account when determining the concentration of pesticides administered to bee colonies in long-term field experiments of bee colonies.

The followings can be inferred from **Table 3**, **Figure 1**, Equations (3) to (4):

(1) The average daily intake of pesticide-containing food for adult bees is about the same, regardless of pesticide concentration. One adult bee ingests about 0.004 g of pesticide-containing food (vehicle) per day in long-term field experiments. This finding could be an indicator for determining the pesticide administration concentration.

(2) Depending on the difference in vehicle, the pesticide intake per bee per day tends to be different. When a vehicle is “pollen paste”, the average intake of pesticide per bee per day is less than when “sugar syrup”.

Average intake of pesticide ingested by one bee during pesticide administration to the bee colony, P_B.

From **Figure 2** and **Table 3**, it can be said that the average intake of pesticide per bee (P_B) appears to increase slightly with the increase in the concentration (C_P) of pesticide administered to the bee colony, but broadly speaking, it can be almost independent of C_P and constant. For bee colonies treated with neonicotinoid pesticides, the end of pesticide administration is the time of colony extinction, suggesting that the average total pesticide intake (P_B) of one bee until colony extinction is almost constant, independent of the pesticide administration concentration (C_P). In other words, it is presumed that these intakes are almost the same as the total intake of pesticides until the bee colony becomes extinct when neonicotinoid pesticides are continuously ingested as chronic toxicity.

Here, the average value of P_B by type of pesticide and vehicle administering the pesticide is as follows: Av. of P_B of dinotefuran (DF) via sugar syrup (SS)=154.4 ng/bee; Av. of P_B of dinotefuran (DF) via pollen paste (PP)=33.4 ng/bee; Av. of P_B of clothianidin (CN) via sugar syrup (SS)=50.6 ng/bee; Av. of P_B of fenitrothion via sugar syrup (SS)=636.8 ng/bee; Av. of P_B of malathion via sugar syrup (SS)=429.3 ng/bee.

Comparison between average intake of pesticide per bee per two days, P_{BD}, and median lethal dose of the honeybee, LD_{50}.

When calculating and evaluating the appropriateness of pesticide administration concentrations in long-term field experiments of bee colonies, it will be attempted to evaluate them at a median lethal dose (LD_{50}) of bees under the assumption that the pesticide intake time of one bee in the field experiment is equivalent to the measurement time of LD_{50} of honeybees.

In this paper, we compared the mean LD_{50} value for bees of pesticides used in long-term field experiments with P_{BD} values for various pesticide administration concentrations in long-term field experiments with 48 hours when the test period was 48 hours. To assess the validity of estimating pesticide concentrations in long-term field experiments with LD_{50} (test duration period: 48 hrs), we compared the P_{BD}×2 and LD_{50} (48 hrs) intakes assuming one bee ingested the pesticide for two days (48 hrs) by doubling the P_{BD} value.

Table 4 shows the literature values of Median Lethal Dose (LD_{50}) and various average values of these LD_{50} data for Dinotefuran, Clothianidin, Fenitrothion and Malathion. The average LD_{50} values of pesticides (48 hours) for acute oral toxicity and the P_{BD} values obtained from long-term field experiments were used to evaluate the appropriateness of pesticide administration concentrations for bee colonies in long-term field experiments.

Figure 3 shows the comparison between the average intakes of pesticide per bee per two days (48 hrs) and the average median lethal dose of the honeybee for acute oral toxicity at 48 hrs (AOT-LD50-48h). From **Figure 3**, when the concentration (C_P) of pesticides administered to bee colonies in long-term field experiments is 5650 ppb in pollen paste and 10000 ppb in sugar syrup, the average intake of pesticides per bee per two days (P_{BD}x2) is equal to or greater than the LD_{50} values. This confirms the fact that bee colonies became extinct in a short period of time at these high concentrations administered to pesticides (Yamada et al., 2010). On the other hand, below these high concentrations, the PBDx2 value is below the LD50 value. This confirms the fact that in long-term field experiments, when pesticides were administered at concentrations lower than these high concentrations, bee colonies did not become extinct in a short period of time, but became extinct after showing the appearance of CCD, which is thought to be due to chronic toxicity.

The overall average values of LD_{50} for acute oral toxicity among test durations of 24, 48, 72 hrs and so on are 28.2 ng/bee for dinotefuran, 54.0 ng/bee for clothianidin, 203.3 g/bee for fenitrothion and 385.0 ng/bee for malathion as show in **Table 4**. The double values of PBD were lower than these overall average LD50 values. This fact seems to approve that the pesticide administration concentrations in the five long-term field experiments were not too high, but rather were reasonable pesticide administration concentrations, contrary to the criticism of Nakamura et al. (2014).

Ratio of the average daily intake of pesticide per bee, P_{BD}, during the pesticide administration period to the concentration of pesticide administered to the bee colony, C_P.

Figure 4 shows the ratio of the average daily intake of pesticide per bee (P_{BD}) during the pesticide administration period to the concentration of pesticide administered to the bee colony (C_P). From **Figure 4** and **Table 3**, it can be seen that the P_{BD} / C_P value is almost constant regardless of the pesticide administration concentration and regardless of the type of pesticide, where the arithmetic average value of all P_{BD} / C_P values is 2.94 mg / bee / day (horizontal dotted line in the figure), which is almost 3 mg / bee / day. Since the P_{BD} / C_P value [mg (pesticide-containig vehicle)/bee/day] is the amount of sugar syrup or pollen paste consumed by one bee per day, it may be possible to

estimate the appropriate concentration of pesticides administered in long-term field experiments based on the LD_{50} value. For example, when a pesticide with an LD_{50} value (24 h) of 9 ng/bee (24 h) is to be administered, the maximum pesticide administration concentration (P) in a long-term field experiment can be estimated as follows: Since the relationship P×3mg/bee/day=9ng/bee/day holds, $P=9\times10^{-9}$g/bee/day/3×10^{-3}g/bee/day=3×10^{-6}=3ppm. That is, in this case, since the pesticide concentration of 3 ppm acts as acute toxicity, the pesticide administration concentration may be set with reference to this value in a long-term field experiment. In the past, long-term field experiments did not know the appropriate pesticide administration concentration, and the pesticide administration concentration was sometimes too high or too low, but the method described in this paper can provide a rough guideline for setting an appropriate pesticide concentration in long-term field experiments.

Average total intake of food (sugar syrup, pollen paste) taken by one bee during the administration period, FD_B

Figure 5 shows the relationship between the administration concentration of pesticide to the bee colony, C_P, and the average total intake of food (sugar syrup, pollen paste) per bee during the administration period, F_{DB}. In the field experiments, sugar syrup and pollen paste are fed to a bee colony as a food or as a vehicle to administer a pesticide to a bee colony. Here, we investigate how much food (sugar syrup, pollen paste) one bee ingests during the administration period, whether the bee's food intake is affected by the concentration of pesticides administered, and in particular, whether there is a difference between the intake of pesticide-free food and the intake of pesticide-containing food. Here, since the case where the pesticide concentration is 0 (zero) cannot be plotted on the logarithmic graph, in order to plot the pesticide-free data on the logarithmic graph, the pesticide-free concentration (C_P) is assumed to be equivalent to 0.01 ppb.

The following can be seen from **Figure 5** and **Table 3**. Despite variations in the data, there is little difference between the average total intake per one bee of pesticide-containing food and that of pesticide-free food. It should be noted that the average total intake of pollen paste ingested by one bee during the administration period is less than that of sugar syrup.

Food (sugar syrup or pollen paste) intake of one bee appears to be almost constant, regardless of the concentration of a pesticide administered to a bee colony (C_P), although there are variations. That is, the average total intakes of sugar syrup and pollen paste ingested by one bee during the administration period can be approximately 0.45 g/bee and 0.048 g/bee, respectively. And, the maximum total intakes of sugar syrup and pollen paste ingested by one bee during the administration period can be approximately 1.4 g/bee and 0.11 g/bee, respectively.

Average daily intake of food (sugar syrup, pollen paste) taken by one bee during the administration period, FD_{BD}

Figure 6 shows the relationship between the administration concentration of pesticide to the bee colony and the average daily intake of food (sugar syrup, pollen paste) per bee during the administration period. The following can be seen from **Figure 6** and **Table 3**. Despite variations in the data, there is little difference between the average intake per one bee of pesticide-containing food and that of pesticide-free food. The average daily intake of pollen paste ingested by one bee is less than that of sugar syrup. Daily food (sugar syrup or pollen paste) intake of one bee appears to be almost constant, regardless of the concentration (CP) of a pesticide administered to a bee

colony, although there are variations. That is, the average daily intake of sugar syrup ingested by one bee can be approximately 2.9757 mg/bee/day and the average daily intake of pollen paste ingested by one bee can be approximately 0.7997 mg/bee/day. That is, the average daily intakes of sugar syrup and pollen paste ingested by one bee can be approximately 3.0 mg/bee/day and 0.80 mg/bee/day, respectively.

The value of 3.0 mg/bee/day which is the average daily intakes of sugar syrup ingested by one bee is approximately equal to the arithmetic average value of all P_{BD} / C_P [mg(pesticide-containing vehicle)/bee/day] values described in **Figure 4**. Using a value of 3.0 mg/bee/day, it seems that the concentration of pesticide administration in long-term field experiments can be estimated, as discussed earlier. And, the maximum daily intakes of sugar syrup and pollen paste ingested by one bee can be approximately 8.3mg/bee/day and 4.3 mg/bee/day, respectively.

Conclusion

In order to investigate the effects of pesticides on individual bees, closed room experiments are suitable, which are easy to control the experimental environment, but in order to investigate the effects on these bee colonies in apiaries, open long-term field experiments, which are close to the actual apiary environment and extremely difficult to control the experimental environment, are suitable. In long-term field experiments, the LD_{50} value measured in laboratory experiments was often used as an index to discuss whether the administration concentration of pesticides was actually appropriate.

In this paper, we clarified the relationship between the pesticide administration concentration in long-term field experiments and the average pesticide intake taken by one bee per day during the pesticide administration period, compared the average daily pesticide intake per bee with the LD_{50} value, and evaluated the appropriateness of pesticide administration concentrations in five long-term field experiments. The followings were examined in this paper: 1) Relationship between the average pesticide intake (P_{BD}) ingested by one bee per day and the pesticide administration concentration (C_P) in the bee herd, calculated from five long-term field experiments. 2) Relationship between concentration of pesticide administered to the bee colony (C_P) and average intake of pesticide per bee (P_B). 3) Comparison between the average intakes of pesticide per bee per two days (48 hrs) and the average median lethal dose of the honeybee for acute oral toxicity at 48 hrs (AOT-LD50-48h). 4) Ratio of the average daily intake of pesticide per bee (P_{BD}) during the pesticide administration period to the concentration of pesticide administered to the bee colony (C_P). 5) Relationship between the administration concentration of pesticide to the bee colony, C_P, and the average total intake of food (sugar syrup, pollen paste) per bee during the administration period, F_{DB}. 6) Relationship between the administration concentration of pesticide to the bee colony and the average daily intake of food (sugar syrup, pollen paste) per bee during the administration period.

Despite the fact that our long-term field experiments were conducted at different experimental locations (Shiga in Japan, Maui in USA) and at different experimental times (2011-2012, 2012-2013, 2013-2014, 2014-2015, 2018), the following points were clarified regardless of the location and time of the experiment: 1) The average pesticide intake taken by one bee in two days during the pesticide administration period, calculated from the results of five long-term field experiments we have conducted, is lower than the average of previously published LD_{50} (48 hrs) values which is shown in **Table 4**. 2) The average daily pesticide intake taken by one bee increases as the concentration of pesticides administered in long-term field experiments increases. 3) The average total intake taken by one bee during the pesticide administration period is almost constant, regardless of the pesticide administration

concentration; 154.4 ng/bee for dinotefuran in sugar syrup; 33.4 ng/bee for dinotefuran in pollen paste; 50.6 ng/bee for clothianidin in sugar syrup; 636.8 ng/bee for fenitrothion in sugar syrup; 429.3 ng/bee for malathion in sugar syrup. 4) During the pesticide administration period, the average intake of pesticide-containing sugar syrup per bee per day is about 3~4mg/bee/day. 5) Although the data are uneven, the average intake of pesticide-containing foods ingested by one bee is almost independent of the concentration of pesticides administered, is almost constant, and is almost the same as the intake of pesticide-free foods. 6) The average daily intake of pesticides taken by one bee via pollen paste is less than that taken via sugar syrup. 7) The average intake of pollen paste taken by one bee per day is less than the that of sugar syrup. 8) The average total intakes of sugar syrup and pollen paste ingested by one bee during the administration period may be about 0.45 g/bee and 0.048 g/bee, respectively. Moreover, the maximum ones will be about 1.4 g/bee and 0.11 g/bee, respectively.

From the above results, the following can be concluded. Although Nakamura et al. (2014) have criticized that the concentration of pesticides administered to bee colonies in our long-term field experiments is too high, judging from the fact that most of the various types of LD_{50} (48 hrs) obtained from the literature are higher than the average intake of pesticides per bee per two days, the concentration of pesticides administered to bee colonies in long-term field experiments seems reasonable. Using the relationship between the daily intake of food and pesticides taken by one bee and the pesticide concentration administered to the bee colony, which was revealed in this paper, as well as the LD_{50} literature value of the target pesticide, it will be possible to set pesticide concentrations suitable for new long-term field experiments. The appropriateness of this concentration can be judged by the daily pesticide intake of one bee from a new field experiment.

Depending on whether bees prefer sugar syrup or pollen paste administered in long-term field experiments or nectar and pollen collected from nature, the intake of sugar syrup and pollen paste in long-term field experiments may differ. As a result of analysis based on experimental data in different environments, that is, in different areas (Shika Town in Japan, Maui Island in Hawaii) and at different times, it is possible to clarify the relationship between the pesticide administration concentration and bee intake in the open environment of long-term field experiments, although roughly, and provide a modest but somewhat meaningful guideline for determining pesticide administration concentration in long-term field experiments. I believe it was given in this paper.

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Titles and Captions of Figures and Tables

Figure 1. Relationship between the concentration of pesticide administered to the bee colony (C_P) and the average intake of pesticide per bee per day (P_{BD}).

DF: Dinotefuran, **CN:** Clothianidin, **FT:** Fenitrothion, **MT:** Malathion.

$\mathbf{P_{BD}}$**:** Average daily intake of a pesticide ingested by one bee during the administration period.

$\mathbf{P_{BD\text{-}DF}}$: Average daily intake of dinotefuran ingested by one bee during the administration period.

$\mathbf{P_{BD\text{-}CN}}$: Average daily intake of clothianidin ingested by one bee during the administration period.

$\mathbf{P_{BD\text{-}FT}}$**:** Average daily intake of fenitrothion ingested by one bee during the administration period.

$\mathbf{P_{BD\text{-}MT}}$**:** Average daily intake of dinotefuran ingested by one bee during the administration period.

The data surrounded by a dotted line in the upper rectangle labeled pollen paste in the figure shows the daily intake of pesticides by one bee when "pollen paste" containing pesticides is administered to a bee colony as a vehicle. All other data show the daily intake of pesticides per bee when pesticides are administered using sugar syrup as the vehicle.

Figure 2. Relationship between concentration of pesticide administered to the bee colony (C_P) and average intake of pesticide per bee (P_B).

$\mathbf{P_B}$**:** Intake of a pesticide ingested by one bee during the administration period.

$\mathbf{P_{B\text{-}DF}}$**:** Intake of dinotefuran ingested by one bee during the administration period.

$\mathbf{P_{B\text{-}CN}}$**:** Intake of clothianidin ingested by one bee during the administration period.

$\mathbf{P_{B\text{-}FT}}$**:** Intake of fenitrothion ingested by one bee during the administration period.

$\mathbf{P_{B\text{-}MT}}$**:** Intake of malathion ingested by one bee during the administration period.

The data surrounded by a dotted square is the P_B value where the pesticide was administered to the bee colony via pollen paste, and the rest is the P_B value where the pesticide was administered via sugar syrup.

Figure 3. Comparison between average intake of pesticide per bee per two days (P_{BD}) and median lethal dose of the bee (LD_{50}).

Dotted lines are the LD_{50} values. "LD50-DF/48h", "LD50-CN/48h", "LD50-FT/48h" and "LD50-MT/48h" denote the LD_{50} values of dinotefuran, clothianidin, fenitrothion and malathion for honeybee with measurement time 48 hours, respectively.

Figure 4. Ratio of the average daily intake of pesticide per bee (P_{BD}) during the pesticide administration period to the concentration of pesticide administered to the bee colony (C_P).

In the figure, the data surrounded by a dotted square is the P_{BD}/C_P value where the pesticide was administered to the bee colony via pollen paste, and the rest is the P_{BD}/C_P value where the pesticide was administered via sugar syrup. The data surrounded by a dotted line in the upper rectangle labeled pollen paste in the figure shows the daily intake of pesticides by one bee when "pollen paste" containing pesticides is administered to a bee colony as a vehicle. All other data show the daily intake of pesticides per bee when pesticides are administered using sugar syrup as the vehicle.

Figure 5. Relationship between the administration concentration of pesticide to the bee colony and the average total intake of food (sugar syrup, pollen paste) per bee during the administration period

SS: sugar syrup, **PP:** pollen paste, **FD:** food (sugar syrup, pollen paste).

$FD_{B\text{-}FR\text{-}SS}$: Average total intake of pesticide-free sugar syrup per bee during the administration period.

$FD_{B\text{-}DF\text{-}SS}$: Average total intake of sugar syrup containing dinotefuran per bee during the administration period.

$FD_{B\text{-}CN\text{-}SS}$: Average total intake of sugar syrup containing clothianidin per bee during the administration period.

$FD_{B\text{-}FT\text{-}SS}$: Average total intake of sugar syrup containing fenitrothion per bee during the administration period.

$FD_{B\text{-}FT\text{-}SS}$: Average total intake of sugar syrup containing malathion per bee during the administration period.

$FD_{B\text{-}FR\text{-}PP}$: Average total intake of pesticide-free pollen paste per bee during the administration period.

$FD_{B\text{-}DF\text{-}SS}$: Average total intake of pollen paste containing dinotefuran per bee during the administration period.

Figure 6. Relationship between the administration concentration of pesticide to the bee colony and the average daily intake of food (sugar syrup, pollen paste) per bee during the administration period.

SS: sugar syrup, **PP:** pollen paste, **FD:** food (sugar syrup, pollen paste)

$FD_{BD\text{-}FR\text{-}SS}$: Average daily intake of pesticide-free sugar syrup per bee

$FD_{BD\text{-}DF\text{-}SS}$: Average daily intake of sugar syrup containing dinotefuran per bee.

$FD_{BD\text{-}CN\text{-}SS}$: Average daily intake of sugar syrup containing clothianidin per bee.

$FD_{BD\text{-}FT\text{-}SS}$: Average daily intake of sugar syrup containing fenitrothion per bee.

$FD_{BD\text{-}FT\text{-}SS}$: Average daily intake of sugar syrup containing malathion per bee.

$FD_{BD\text{-}FR\text{-}PP}$: Average daily intake of pesticide-free pollen paste per bee.

$FD_{BD\text{-}DF\text{-}SS}$: Average daily intake of pollen paste containing dinotefuran per bee.

Average intake of sugar syrup per bee per day = 2.9757 mg/bee/day; Average intake of pollen paste per bee per day =0.7997 mg/bee/day.

Table 1. Comparison between long-term field experiments and LD_{50} measurement experiments in the investigation of the effects of pesticides on bees.

Table 2. Experimental conditions of six long-term field experiments for honeybee colonies.

Table 3. Intakes of sugar syrup, pollen paste and pesticide during the administration period to a bee colony in five long-term field experiments.

Table 4. Literature values of median lethal doses (LD_{50}) for the honeybee and various average values of the LD_{50} data for dinotefuran, clothianidin, fenitrothion and malathion.

1) Average of all LD_{50} data on acute oral toxicity of dinotefuran (‹D F- Oral- LD50›).
2) Average of all LD_{50} data on acute contact toxicity of dinotefuran (‹D F- CTC- LD50›).
3) Average of all LD_{50} data on acute oral and contact toxicities of dinotefuran (‹DF- LD50›)
4) Average of all LD_{50} data on acute oral toxicity of clothianidin (‹CN-Oral-LD50›).
5) Average of all LD_{50} data on acute contact toxicity of clothianidin (‹CN - CTC -LD50›).

[6] Average of all LD_{50} data on acute oral and contact toxicities of clothianidin (‹CN - LD50›)

[7] Average of all LD_{50} data on acute oral toxicity of fenitrothion (‹FT-Oral-LD50›).

[8] Average of all LD_{50} data on acute contact toxicity of fenitrothion (FT - CTC -LD50).

[9] Average of all LD_{50} data on acute oral and contact toxicities of fenitrothion (‹FT - LD50›)

[10] Average of all LD_{50} data on acute oral toxicity of malathion (‹MT-Oral-LD50›).

[11] Average of all LD_{50} data on acute contact toxicity of malathion (‹MT - CTC -LD50›).

[12] Average of all LD_{50} data on acute oral and contact toxicities of malathion (‹MT - LD50›)

The list of literatures cited is shown in Table 4a.

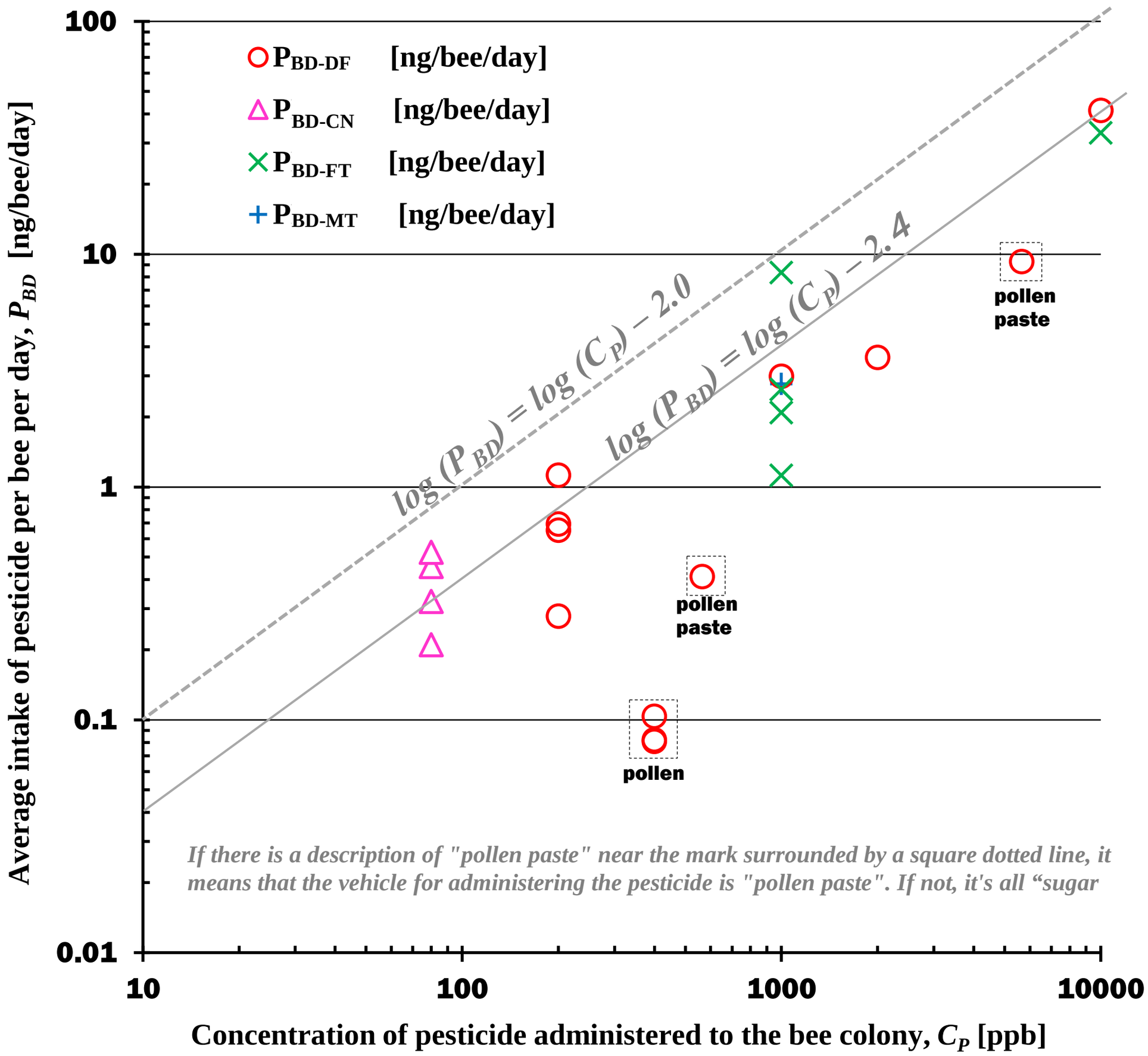

Figure 1. Relationship between the concentration of pesticide administered to the bee colony (C_P) and the average intake of pesticide per bee per day (P_{BD})

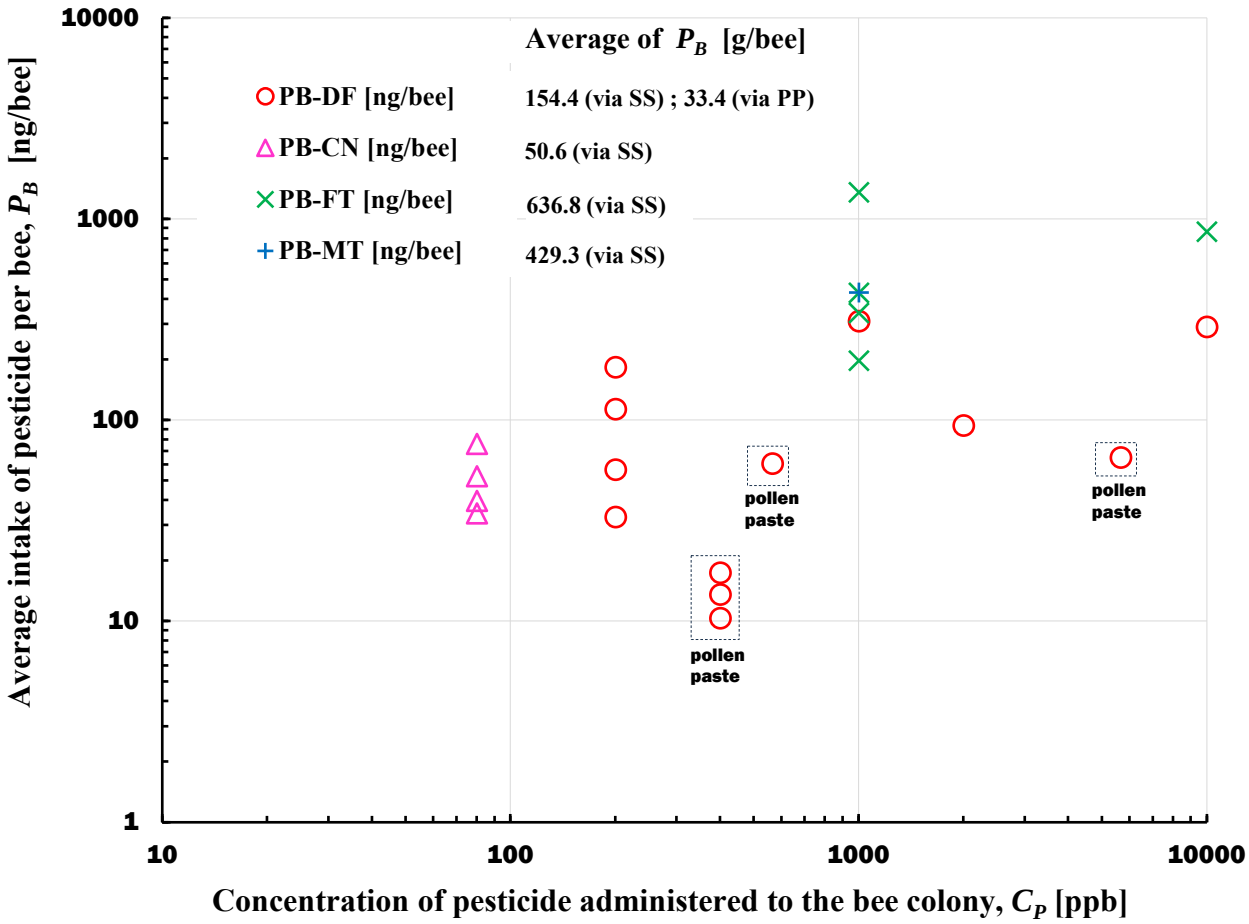

Figure 2. Relationship between concentration of pesticide administered to the bee colony (C_P) and average intake of pesticide per bee (P_B)

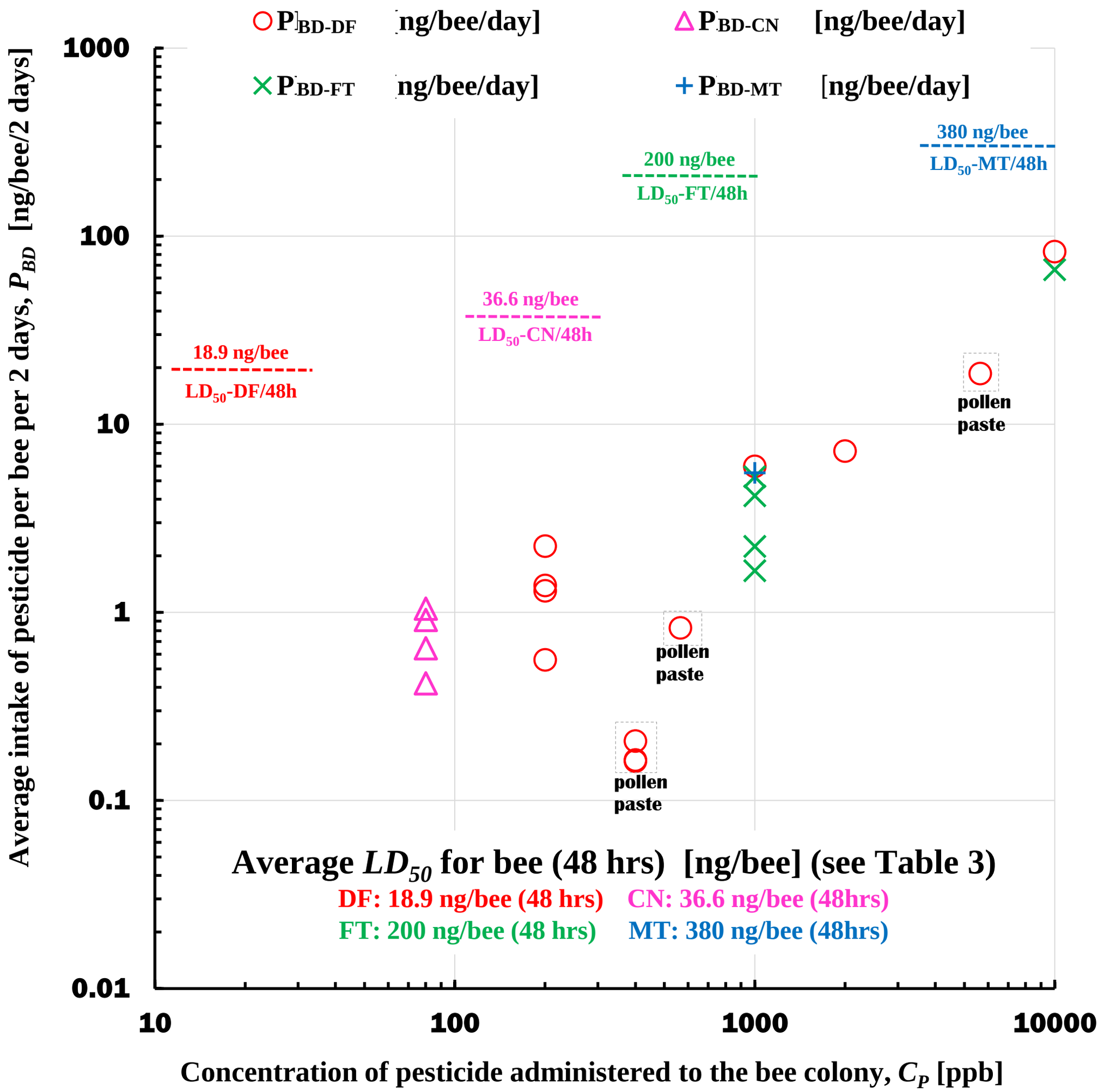

Figure 3. Comparison between average intake of pesticide per bee per two days (P_{BD}) and median lethal dose of the bee (LD_{50})

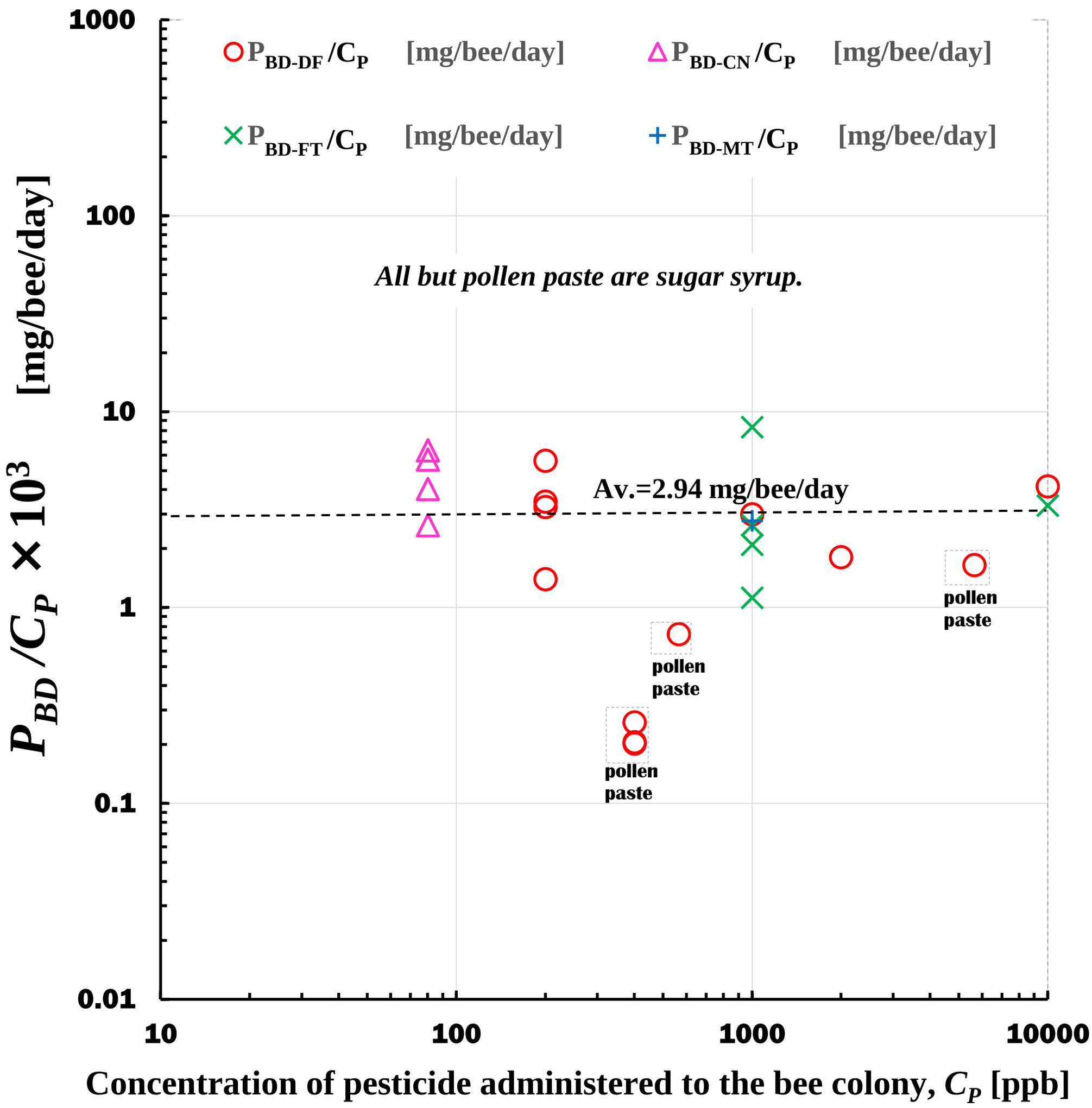

Figure 4. Ratio of the average daily intake of pesticide per bee (P_{BD}) during the pesticide administration period to the concentration of pesticide administered to the bee colony (C_P).

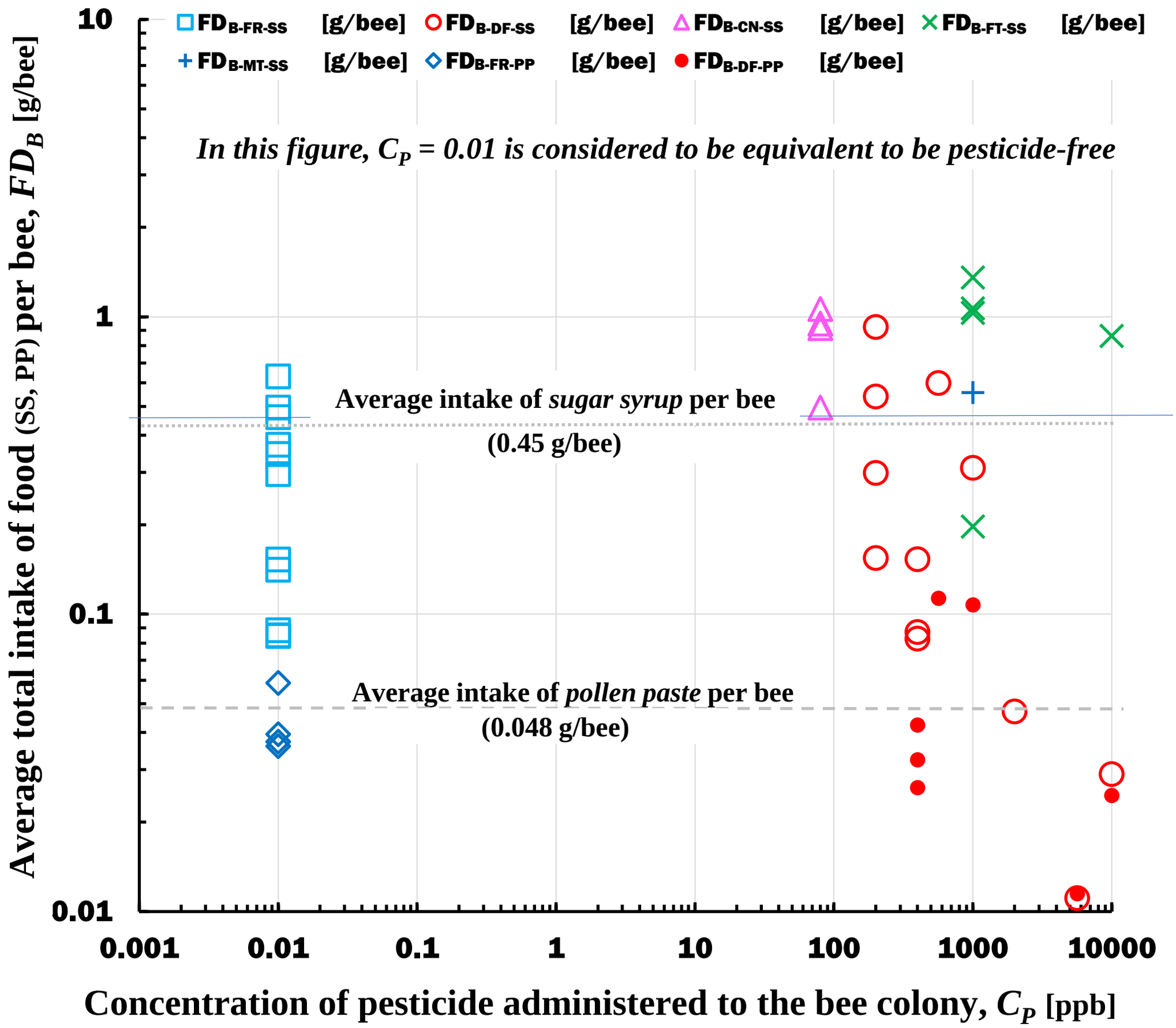

Figure 5. Relationship between the administration concentration of pesticide to the bee colony and the average total intake of food (sugar syrup, pollen paste) per bee during the administration period

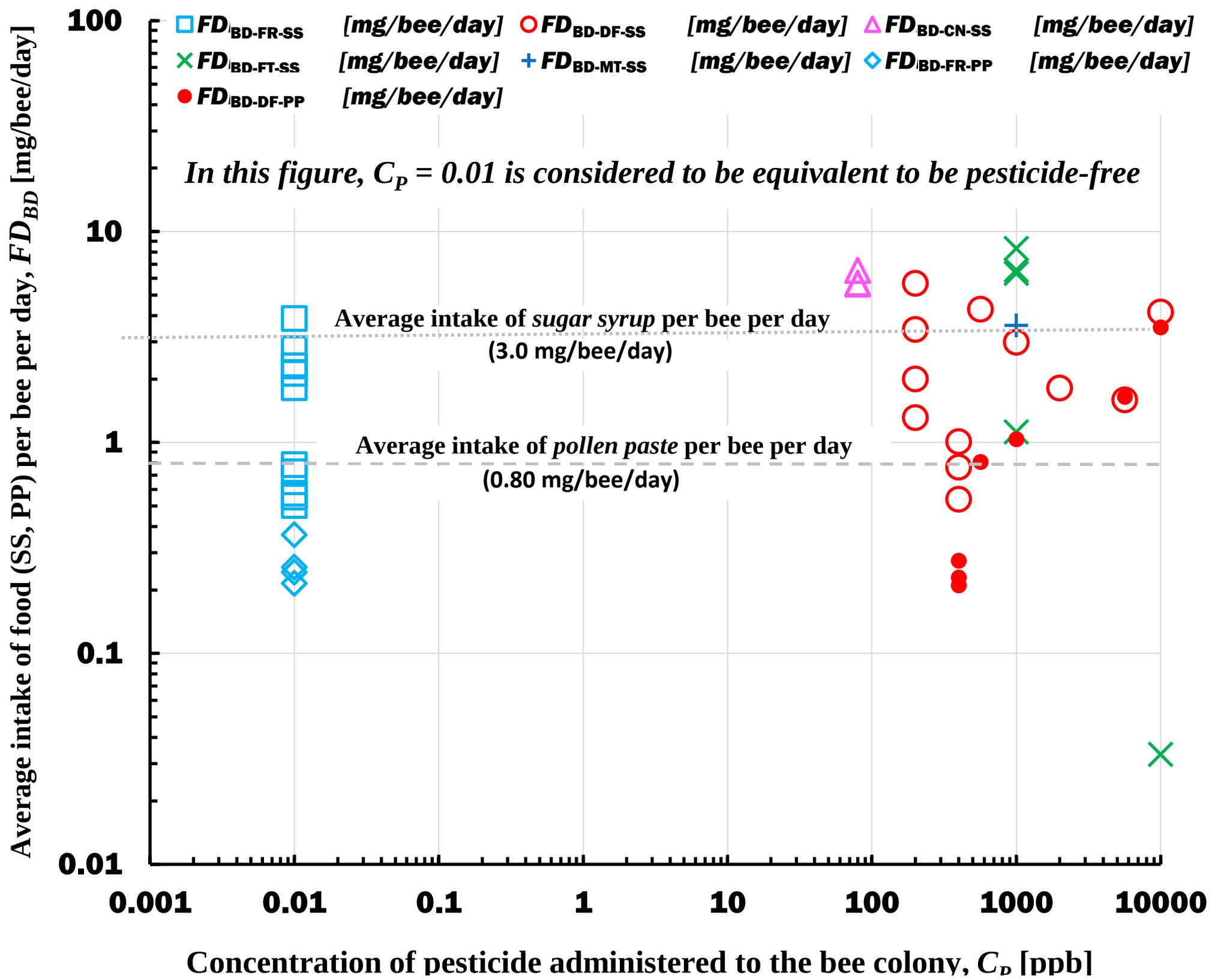

Figure 6. Relationship between the administration concentration of pesticide to the bee colony and the average daily intake of food (sugar syrup, pollen paste) per bee during the administration period

Table 1. Comparison between long-term field experiments and LD_{50} measurement experiments in the investigation of the effects of pesticides on bees

Item	Long-term field experiment	LD_{50} measurement experiment
Experimental method	Field experiment	Lab experiment
Differences from real phenomena in apiaries	Small	Large
Experimental object	An almost complete bee colonies close to an actual apiary that satisfies the hierarchical social structure (polyethism) with a division of labor as a social insect (composed of queens, numerous worker bees, male bees, eggs, larvae, pupa, etc.)	Extremely incomplete bee colonies consisting of only a single few worker bees
Experimental environment	(1) Open system / difficult to control (2) Environments close to real apiaries can be reproduced	(1) Closed system / easy to control (2) An artificial environment far from reality
Weather (temperature, humidity, wind speed, etc.) management	Unmanageable	Manageable
Ecological adequacy	High	Low
Bee behavior management	Unmanageable	Manageable
Foraging status of food containing pesticides	Since it is uncontrolled and can be ingested freely or not, it is difficult to grasp the exact amount of intake	No ingestion from outside the controlled laboratory (management of intake, place of ingestion, etc.)
Intake of food, water, etc.	Food can be freely and selectively taken from the open air without restrictions	No ingestion from outside the controlled laboratory (management of intake, place of ingestion, etc.)
Controlling Variables	Many confounding variables and difficulty in controlling	Easy to control
Repeated measurements	Difficult	Easy
Physiological behavior of bees (mating, rearing)	Maintenance of sound activity as a eusocial insect	Inability to maintain behavior as eusocial insects

Table 2. Experimental conditions of six long-term field experiments for bee colonies

Experiment name	2010-Experiment	2011/2012-Experiment	2012/2013-Experiment	2013/2014-Experiment	2014/2015-Experiment	2018-Experiment
Experimental period (Experiment days)	July 18 to November 21, 2010 (126 days)	From July 9, 2011 to April 2, 2012 (268 days)	From June 28, 2012 to July 26, 2013 (393 days)	From August 13, 2013 to February 28, 2014 (199 days)	From October 22, 2014 to July 20, 2015 (271 days)	From Jul. 19, 2018 to Dec. 16, 2018 (180 days)
Experimental site (latitude and longitude)	Shika (mid-west Japan) (37º1′9″ N, 136º46′14″ E, 43m above sea level)	Same as on the leftt	Same as on the leftt	Same as on the leftt	Maui (Hawaii) (20º55′00.2” N, 156º30′39.2” W)	Shika (mid-west Japan) (37 °2 '35" N, 136 °45 '38" E, 70m above sea level)
Object of Study	To investigate the possibility for a neonicotinoid of causing a CCD	To investigate the difference in the long-term influence on a bee colony between toxic sugar syrup (honey) as an energy source and toxic pollen pase (bee bread) as a protein source which are exposed to a neonicotinoid	To investigate the difference in the long-term influence on a bee colony between a neonicotinoid and a organophosphate which are administered through sugar syrup	To investigate the difference in the long-term influence on a bee colony between a neonicotinoid and a organophosphate which are administered through sugar syrup under lower concentrations than in the previous work	To compare the long-term changes in colony size and apparent longevity between Shika (mid-west Japan) with mites and cold winter and Maui (Hawaii) without both	To investigate the seasonal changes in colony size and mite-prevalence in a honeybee colony exposed to dinotefuran via pollen paste and infested with Varroa mites
Circumstances around Experimental Site						
Limitation of honeybee activities	Honeybees can freely forage about for food in a hive or in fields	Same as on the leftt	Same as on the leftt	Same as on the leftt	Same as on the leftt	Same as on the leftt
Impact of other pesticide than the administered one on the environment	A pesticide-free watering place & a pesticide-free field of flowers in the apiary	Same as on the leftt	Same as on the leftt	Same as on the leftt	Experimental site is located in organically-grown macadamia trees	A pesticide-free watering place & a pesticide-free field of flowers in the apiary
Aerial-crop-dusting farmland near exptl. Site	Nothing	Nothing	Nothing	Nothing	Nothing	Nothing
Seasonal changes	Distinct	Distinct	Distinct	Distinct	Indistinct (without cold winter)	Distinct
Experimental Conditions						
Initial numbers of apiaries, colonies and combs (frames)	1 apiary (private), 8 colonies per apiary, 6 combs per co	1 apiary (private), 5 colonies per apiary, 3 combs per colony	1 apiary (private), 4 colonies per apiary, 3 combs per colony	1 apiary (private), 6 colonies per apiary, 3 combs per colony	1 apiary (private), 6 colonies per apiary, 3 combs per colony	1 apiary (private), 6 colonies per apiary, 3 combs per colony
Initial number of bees per colony	9000 to 1300 bees (accurately counted on photos)	1700 to 3400 bees (accurately counted from photos)	5600 to 7100 bees (accurately counted on photos)	5400 to 7600 bees (accurately counted on photos)	5400 to 7600 bees (accurately counted on photos)	7000 to 9000 bees (accurately counted on photos)
Initial number of capped brood per colony	1.5 to 7 full-surfaces on comb converted from the sum total of every area occupied by capped (obtained from photos)	2600 to 6100 capped brood (accurately counted from photos)	4000 to 5700 capped brood (accurately counted from photos)	4200 to 7600 capped brood (accurately counted from photos)	4200 to 7600 capped brood (accurately counted from photos)	3100 to 6900 capped brood (accurately counted from photos)
Kind of pesticide	Dinotefuran, Clothianidin	Dinotefuran	Dinotefuran, Fenitrothion	Dinotefuran, Clothianidin, fenitrothion, Malathion	Dinotefuran, Clothianidin, fenitrothion	Dinotefuran
Concentration of pesticide	Dinotefuran: 1, 2, 10 ppm, Clothianidin: 0.4, 2, 4 ppm	Dinotefuran: 1 & 10 ppm in sugar syrup, 0.565 & 5.65 in pollen paste	Dinotefuran: 2 ppm in sugar syrup, Fenitrothion: 10 ppm in sugar syrup	Dinotefuran: 0.2 ppm in sugar syrup, Clothianidin: 0.08 ppm in sugar syrup, Fenitrothion & Malathion: 1 ppm in sugar syrup	Dinotefuran: 0.2 ppm in sugar syrup, Clothianidin: 0.08 ppm in sugar syrup, Fenitrothion 1 ppm in sugar syrup (Same as 2013/2014 experiment in Shika)	Dinotefuran: 0.4 ppm in pollen paste
Origin of a queen	Unknown in detail (*Apis mellifera*) Bee colonies purchased from a bee farm	Same as on the left	Same as on the leftt	Same as on the leftt	Sister relationship among queens	Sister relationship among queens
Experimental Methods						
Interval of experiment	About one-week interval	About one-week interval	About one-week or two-weeks interval	Same as on the leftt	About two-weeks or more interval	About two-weeks
Administration period of pesticide	Till colony extinction or from July 18, 2010 to November 21 (126 days)	Till colony extinction or from July 9, 2011 to December 3 (147 days)	Till colony extinction or from July 21, 2012 to August 16 (26 days)	Till colony extinction or from September 5, 2013 to December 1 (87 days)	Till colony extinction or from October 23, 2014 to April 4, 2015 (163 days)	From July 1, 2018 to the colony extinction
Starting time of each observation	Just after dawn if possible (before bees go out to forage)	Same as on the leftt	Same as on the leftt	Same as on the leftt	Same as on the leftt	Same as on the leftt
Vehicle to administer a pesticide	Both sugar syrup and pollen paste	Either sugar Syrup or pollen paste	Sugar syrup	Sugar syrup	Sugar syrup	Pollen paste
Administration method of pesticide (sugar syrup and pollen paste were fed as food)	A pesticide was dissolved in sugar syrup and pollen paste was made after pollen was kneaded with toxic sugar syrup containing the pesticide. Both toxic sugar syrup and toxic pollen paste were fed into a hive.	A pesticide was dissolved in sugar syrup or pollen paste was made after pollen was kneaded with toxic sugar syrup containing the pesticide. Either toxic sugar syrup or toxic pollen paste was fed into a hive to administer a pesticide.	A pesticide was dissolved in sugar syrup. Only toxic sugar syrup was fed into a hive to administer a pesticide. Pollen paste is pesticide-free.	A pesticide was dissolved in sugar syrup. Only toxic sugar syrup was continuously fed into a hive with an auto-feeding system composed of 10 L (14 kg syrup) container. Pollen paste is pesticide- free.	Same as on the leftt	Pollen paste was made after pollen was kneaded with toxic sugar syrup containing the pesticide. Sugar syrup is pesticide-free.
Counting method of the number of adult bees	Roughly counted from photos of combs with bees and bees left in a hive after every comb was removed from it	Directly counted with accuracy from photos of combs with bees and bees left in a hive after every comb was removed from the hive with the help of a automatic counting software.	Same as on the leftt	Same as on the leftt	Same as on the leftt	Same as on the leftt
Counting method of the number of capped brood	Expressed by the number of surfaces on combs (frames) which are entirely occupied by capped brood using the photos of combs	Acurately counted from photos of combs without bees after shaking the bees off each comb with the help of a automatic counting software.	Same as on the leftt	Same as on the leftt	Same as on the leftt	Same as on the leftt
Total intake of pesticide per colony	Calculated from sugar syrup & pollen paste with pesticide consumed by honeybees	Calculated from sugar syrup or pollen paste with pesticide consumed by honeybees	Calculated from sugar syrup with pesticide consumed by honeybees	Same as on the leftt	Same as on the leftt	Calculated from pollen paste with pesticide consumed by honeybees
Estimation of the intake of pesticide per bee	Unestimated	Estimated from dividing the total intake of pesticide per colony by the total number of initial & newly-emerged honeybees	Same as on the leftt	Same as on the leftt	Same as on the leftt	Same as on the leftt
Counting method of number of dead bees	Dead bees were accurately counted one by one inside and outside a hive which was placed on a large tray	Same as on the leftt	Same as on the leftt	Same as on the leftt	Same as on the leftt	Same as on the leftt
Confirmation and record methods of a queen	A photographic record of the exsistence of a queen in each colony	Same as on the leftt	Same as on the leftt	Same as on the leftt	Same as on the leftt	Same as on the leftt
Publication						
Experimental Results	T. Yamada et at. (2012). *Jpn. J. Clin. Ecol.* 21(1): 10-23.	T. Yamada et al. (2018a). *J. Biol. Ser. 1* (3): 084-107.	Yamada et al. (2018b). *J. Biol. Ser.* 1(3): 108-137	T. Yamada et al. (2018d). *J. Biol. Ser.* 1(4): 187-207.	T. Yamada et al. (2018c). *J. Biol. Ser* . 1(4): 156-186.	Toshiro Yamada (2020). Enliven: Journal of Dietetics Research and Nutrition 7(1): 002.
Apparent longevity	Y. Yamada et al. (2019). *Scientific Reports* 9: 4102.				T. Yamada T and K. Yamada. (2020). PeerJ, 8:e9505	

Table 3. Intakes of sugar syrup, pollen paste and pesticide during the administration period to a bee colony in five long-term field experiments

Experimental Conditions								Per Colony				Per Bee			Per Bee Per Day			Mite Infection	Ref.
Period & Site	Colony Name	Admin . Period		D_T 1)	Pesticide	Vehicle 2)	C_P 3)	B_T 4)	SS_T 5)	PP_T 6)	P_T 7)	SS_B 8)	PP_B 9)	P_B 10)	SS_{BD} 11)	PP_{BD} 12)	P_{BD} 13)		
		From	Till	day			ppm	head	g	g	mg	g/bee	g/bee	ng/bee	mg/bee/day	mg/bee/day	ng/bee/day		
9-Jul-2011 to 2-Apr-2012; Shika, Jpn	2011-CR-1	9-Jul-11	17-Dec-11	161	free	—	0	72273.2	26830.0	4243.0	0.0000	0.3712	0.0587	0.00	2.3058	0.3646	0.0000	No	Yamada et al. (2018a)
	2011-DF-1	9-Jul-11	21-Oct-11	104	DF	SS	1	13542.4	4208.0	1459.0	4.2080	0.3107	0.1077	310.73	2.9878	1.0359	2.9878		
	2011-DF-2	9-Jul-11	26-Nov-11	140	DF	PP	0.565	30282.3	18152.0	3430.0	1.8692	0.5994	0.1133	61.73	4.2816	0.8091	0.4409		
	2011-DF-3	9-Jul-11	16-Jul-11	7	DF	SS	10	6544.3	190.0	161.0	1.9000	0.0290	0.0246	290.33	4.1476	3.5145	41.4756		
	2011-DF-4	9-Jul-11	16-Jul-11	7	DF	PP	5.65	8078.3	90.0	93.0	0.5257	0.0111	0.0115	65.08	1.5916	1.6446	9.2965		
28-Jun-2012 to 26-Jul-2013-Apr-2012; Shika, Jpn	2012-CR-1	21-Jul-12	1-Feb-13	195	free	—	0	88336.3	13500.0		0.0000	0.1528		0.00	0.7837		0.0000	No	Yamada et al. (2018b)
	2012-CR-2	21-Jul-12	1-Feb-13	195	free	—	0	95536.6	13500.0		0.0000	0.1413		0.00	0.7247		0.0000		
	2012-DF-1	21-Jul-12	16-Aug-12	26	DF	SS	2	16478.1	776.0		1.5520	0.0471		94.19	1.8113		3.6225		
	2012-FT-1	21-Jul-12	16-Aug-12	26	FT	SS	10	19792.4	1707.0		17.0700	0.0863		862.45	3.3171		33.1712		
13-Aug-2013 to 28-Feb-2014; Shika, Jpn	2013-CR-1	13-Aug-13	7-Feb-14	178	free	—	0	23921.8	11840.0		0.0000	0.4949		0.00	2.7806		0.0000	No	Yamada et al. (2018c)
	2013-CR-2	13-Aug-13	28-Feb-14	199	free	—	0	18695.1	8590.0		0.0000	0.4595		0.00	2.3089		0.0000		
	2013-DF-1	5-Sep-13	1-Dec-13	87	DF	SS	0.2	8611.8	2570.0		0.5140	0.2984		59.69	3.4302		0.6860		
	2013-CN-1	5-Sep-13	1-Dec-13	87	CN	SS	0.08	10130.3	5020.0		0.4016	0.4955		39.64	5.6959		0.4557		
	2013-FT-1	5-Sep-13	1-Dec-13	87	FT	SS	1	19585.3	3860.0		3.8600	0.1971		197.09	1.1198		1.1198		
	2013-MT-1	5-Sep-13	1-Dec-13	87	MT	SS	1	15723.5	6750.0		6.7500	0.4293		429.29	4.9344		4.9344		
22-Oct-2014 to 26-Jul-2015; Maui, USA	2014-CR-1	23-Oct-14	4-Apr-15	163	free	—	0	39119.9	11570.0		0.0000	0.2958		0.00	1.8145		0.0000	No	Yamada et al. (2018d; 2020a)
	2014-CR-2	23-Oct-14	4-Apr-15	163	free	—	0	18722.8	11795.0		0.0000	0.6300		0.00	3.8649		0.0000		
	2014-CR-3	23-Oct-14	4-Apr-15	163	free	—	0	34679.7	12000.0		0.0000	0.3460		0.00	2.1228		0.0000		
	2014-DF-1	23-Oct-14	18-Feb-15	118	DF	SS	0.2	8910.1	1377.0		0.2754	0.1545		30.91	1.3097		0.2619		
	2014-DF-2	23-Oct-14	4-Apr-15	163	DF	SS	0.2	12866.0	11905.0		2.3810	0.9253		185.06	5.6767		1.1353		
	2014-DF-3	23-Oct-14	4-Apr-15	163	DF	SS	0.2	83219.7	44924.0		8.9848	0.5398		136.94	1.9993		0.5072		
	2014-CN-1	23-Oct-14	4-Apr-15	163	CN	SS	0.08	24651.4	26213.0		2.0970	1.0633		85.07	6.5236		0.5219		
	2014-CN-2	23-Oct-14	4-Apr-15	163	CN	SS	0.08	20245.8	18531.0		1.4825	0.9153		73.23	5.6153		0.4492		
	2014-CN-3	23-Oct-14	17-Mar-15	145	CN	SS	0,08	18511.4	17573.0		1.4058	0.9493		75.94	6.5469		0.5237		
	2014-FT-1	23-Oct-14	4-Apr-15	163	FT	SS	1	20039.8	27180.0		27.1800	1.3563		1356.30	8.3209		8.3209		
	2014-FT-2	23-Oct-14	4-Apr-15	163	FT	SS	1	26299.8	28080.0		28.0800	1.0677		1067.69	6.5502		6.5502		
	2014-FT-3	23-Oct-14	4-Apr-15	163	FT	SS	1	44940.2	46338.0		46.3380	1.0311		1031.10	6.3258		6.3258		
19-Jun-2018 to 16-Dec-2018; Shika, Jpn	2018-CR-1	1-Jul-18	16-Dec-18	168	free	—	0	67063.2	5664.1	2415.9	0.0000	0.0845	0.0360	0.00	0.5027	0.2143	0.0000	Yes	Yamada +A12:T39et al. (2020b)
	2018-CR-2	1-Jul-18	2-Dec-18	154	free	—	0	79002.3	6973.1	3118.5	0.0000	0.0883	0.0395	0.00	0.5731	0.2565	0.0000		
	2018-CR-3	1-Jul-18	2-Dec-18	154	free	—	0	74800.7	6349.4	2789.1	0.0000	0.0849	0.0373	0.00	0.5512	0.2422	0.0000		
	2018-DF-1	1-Jul-18	2-Dec-18	154	DF	PP	0.4	42272.4	6474.9	1793.2	0.8173	0.1532	0.0424	19.33	1.0078	0.2753	0.1255		
	2018-DF-2	1-Jul-18	2-Dec-18	154	DF	PP	0.4	81915.2	6782.6	2653.9	1.1586	0.0828	0.0324	14.14	0.5377	0.2104	0.0918		
	2018-DF-3	1-Jul-18	23-Oct-18	114	DF	PP	0.4	55166.7	4800.4	1440.3	0.6195	0.0870	0.0261	11.23	0.7632	0.2289	0.0985		

Note (1): Each intake is recalculated under the assumption that each total amount of honey, pollen and pesticide is taken by bees during the period of its administration.

Note (2): Since the figures in the table have been newly recalculated, they may differ slightly from the published figures. If there is no published data, leave it blank.

Note (3): "free" = pesticide free, "DF" = dinotefuran, "CN" = clothianidin, "FT" = fenitrothion, "MT" = malathion.

1) D_T denotes the number of days during pesticide administration.

2) "Vehicle" means food for administering the pesticide to colonies, SS and PP indicate sugar syrup and pollen paste, respectively.

3) C_p denotes the concentration of the pesticide administered to the colony.

4) B_T denotes denotes the total number of bees that would have ingested pesticides in the colony during pesticide administration to the bee colony (D_T).

5) SS_T denotes denotes the total intake of sugar syrup ingested by the colony during pesticide administration to the bee colony.

6) PP_T denotes the total intake of pollen paste ingested by the colony during pesticide administration to the bee colony.

7) P_T denotes the total intake of the pesticide ingested by the colony during pesticide administration to the bee colony.

8) SS_B denotes denotes the total intake of sugar syrup ingested by one bee during pesticide administration to the bee colony, $SS_B = SS_T/B_T$.

9) PP_B denotes the total intake of pollen paste ingested by one bee during pesticide administration to the bee colony, $PP_B = PP_T/B_T$.

10) P_B denotes the total intake of the pesticide ingested by one bee during pesticide administration to the bee colony, $P_B = P_T/B_T$.

11) SS_{BD} denotes denotes the average daily intake of sugar syrup ingested by one bee during pesticide administration to the bee colony, $SS_{BD} = SS_T/B_T/D_T$.

12) PP_{BD} denotes the average daily intake of pollen paste ingested by one bee during pesticide administration to the bee colony, $PP_{BD} = PP_T/B_T/D_T$.

13) P_{BD} denotes the average daily intake of the pesticide ingested by one bee during pesticide administration to the bee colony, $P_{BD} = P_T/B_T/D_T$.

Table 4. Literature values of median lethal Doses (LD_{50}) for the honeybee and various average values of the LD50 data for dinotefuran, clothianidin, fenitrothion and malathion

Pesticide	Literature values of Median Lethal Dose (LD_{50}) [ng/bee]							
	Acute oral toxicity (AOT)				Acute contact toxicity (ACT)			
	LD50 Test Duration (LD50-TD)				LD50 Test Duration (LD50-TD)			
	24 h (24h-LD50)	48 h (48h-LD50)	72 h (72h-LD50)	Unknown (UKN-LD50)	24 h (24h-LD50)	48 h (48h-LD50)	72 h (72h-LD50)	Unknown (UKN-LD50)
Dinotefuran (DT)	0.6[a)]	7.6[g)]		23[o)]		24[g)]		24[o)]
	75[b)]	22.3[h)]		26.8[p)]		56[h)]		30[q)]
		26.8[g)]		32[o)]				47[o)]
				40[q)]				61[o)]
Averages of LD50 for DF	*37.8* ‹DF-Oral-24h-LD50›	*18.9* ‹DF-Oral-48h-LD50›		*30.5* ‹DF-Oral-UNK-LD50›		*40.0* ‹DF-CTC-48h-LD50›		*40.5* ‹DF-CTC-UKN-LD50›
	28.2 [1)]				*40.3* [2)]			
	33.1 [3)]							
Clothianidin (CN)	3.53[c)]	3.35[c)]	3.28[c)]	7.9[q)]		27.5[i)]		41[q)]
	21.8[b)]	3.68[i)]	18.0[l)]	26.8[p)]		44[j,k)]		
	25.4[d)]	3.8[j,k)]	130[e)]	41[q)]		47[i)]		
	330[e)]	23[i)]						
		25.4[d)]						
		26.9[l)]						
		170[e)]						
Averages of LD50 for CN	*95.2* ‹CN-Oral-24h-LD50›	*36.6* ‹CN-Oral-48h-LD50›	*50.4* ‹CN-Oral-72h-LD50›	*25.2* ‹CN-Oral-UNK-LD50›		*39.5* ‹CN-CTC-48h-LD50›		*41.0* ‹CN-CTC-UKN-LD50›
	54.0 [4)]				*39.9* [5)]			
	48.7 [6)]							
Fenitrothion (FT)		200[m)]		160[r)]	171[t)]	160[m)]		20[u)]
				250[s3)]	130[s1)]			380[u)]
					30[s2)]			1660[r)]
								200[r)]
Averages of LD50 for FT		*200.0* ‹FT-Oral-48h-LD50›		*205.0* FT-Oral-UNK-LD50›	*110.3* ‹FT-CTC-48h-LD50›	*160.0* ‹FT-CTC-48h-LD50›		*565.0* ‹FT-CTC-UKN-LD50›
	203.3 [7)]				*343.9* [8)]			
	305.5 [9)]							
Malathion (MT)	380[f)]	380[i)]	400[n)]	380[q)]	270[f)]	270[i)]	160[n)]	210[q)]
								710[v)]
Averages of LD50 for MT	*380.0* ‹MT-Oral-24h-LD50›	*380.0* ‹MT-Oral-48h-LD50›	*400.0* ‹MT-Oral-72h-LD50›	*380.0* 72Oral-UNK-LD50›	*270.0* ‹M-CTC-48h-LD50›	*270.0* ‹MT-CTC-48h-LD50›	*160.0* ‹MT-CTC-72h-LD50›	*460.0* ‹MT-CTC-UKN-LD50›
	385.0 [10)]				*324.0* [11)]			
	351.1 [12)]							

Table 4a. List of literatures cited in Table 4.

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Table 4a. List of literatures cited in Table 4 (continued)

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For contact acute LD_{50} & oral acute LD_{50}, worst case from 24, 48 and 72 hour values is selected. Generally, as 72 h-LD50 is apt to be worst, test durations both LD50s are considered to be 72 hours.

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