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The biological impacts of ingested radioactive materials on the pale grass blue butterfly

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A massive amount of radioactive materials has been released into the environment by the Fukushima Dai-ichi Nuclear Power Plant accident, but its biological impacts have rarely been examined. Here, we have quantitatively evaluated the relationship between the dose of ingested radioactive cesium and mortality and abnormality rates using the pale grass blue butterfly, *Zizeeria maha*. When larvae from Okinawa, which is likely the least polluted locality in Japan, were fed leaves collected from polluted localities, mortality and abnormality rates increased sharply at low doses in response to the ingested cesium dose. This dose-response relationship was best fitted by power function models, which indicated that the half lethal and abnormal doses were 1.9 and 0.76 Bq per larva, corresponding to 54,000 and 22,000 Bq per kilogram body weight, respectively. Both the retention of radioactive cesium in a pupa relative to the ingested dose throughout the larval stage and the accumulation of radioactive cesium in a pupa relative to the activity concentration in a diet were highest at the lowest level of cesium ingested. We conclude that the risk of ingesting a polluted diet is realistic, at least for this butterfly, and likely for certain other organisms living in the polluted area.

Subject terms: Risk factors Environmental sciences Environmental health

Introduction

Environmental pollution by artificial radionuclides released from the collapsed Fukushima Dai-ichi Nuclear Power Plant (NPP) has often been evaluated based on radioactivity concentrations in environmental samples collected from the polluted area, such as soil and forest litter[1, 2, 3]. The accumulation of radionuclides in the bodies of wild and domesticated organisms and in agricultural products has also been documented[4, 5, 6, 7, 8, 9, 10, 11]. However, the biological impacts of this pollution on the health and disease of wild organisms have not been examined sufficiently.

A field study indicated that the abundance of insects, especially butterflies, has decreased[12, 13], suggesting that the pollution might have had a fatal effect on butterflies. Another study indicated a high incidence of morphological abnormality in gall-forming aphids[14]. Consistent with these studies, we have demonstrated that the Japanese pale grass blue butterfly, *Zizeeria maha* (*Yamato shijimi* in Japanese), was affected physiologically and genetically in the polluted area, likely due to the artificial radionuclides released from the Fukushima Dai-ichi NPP[15, 16]. In one of a series of experiments, we have shown a decrease in the survival rate of this butterfly in accordance with the level of radioactivity of cesium in the diet (i.e., the leaves of the host plant, *Oxalis corniculata*)[15, 16] under our standard rearing conditions[17]. In this experiment, host plant leaves were collected from 5 different localities with different pollution levels (Ube, Hirono, Fukushima, Iitate-flatland, and Iitate-montane) (Table 1) and were given to larvae that were obtained in the laboratory from field-caught females from Okinawa[15], which is likely the least polluted locality in Japan. However, the amount of food and the dose of artificial radioactive cesium ingested by these larvae remain to be determined. Furthermore, the amount of radioactivity retained and accumulated by pupae has not been examined.

Table 1: Summary of data for the internal exposure experiment[1] (see annex 1)

In the present study, we quantified the amount of radioactive cesium consumed by larvae over their lifetimes. Based on these data, simple mathematical models were employed to obtain the radioactive cesium doses resulting in a 50% mortality rate and a 50%

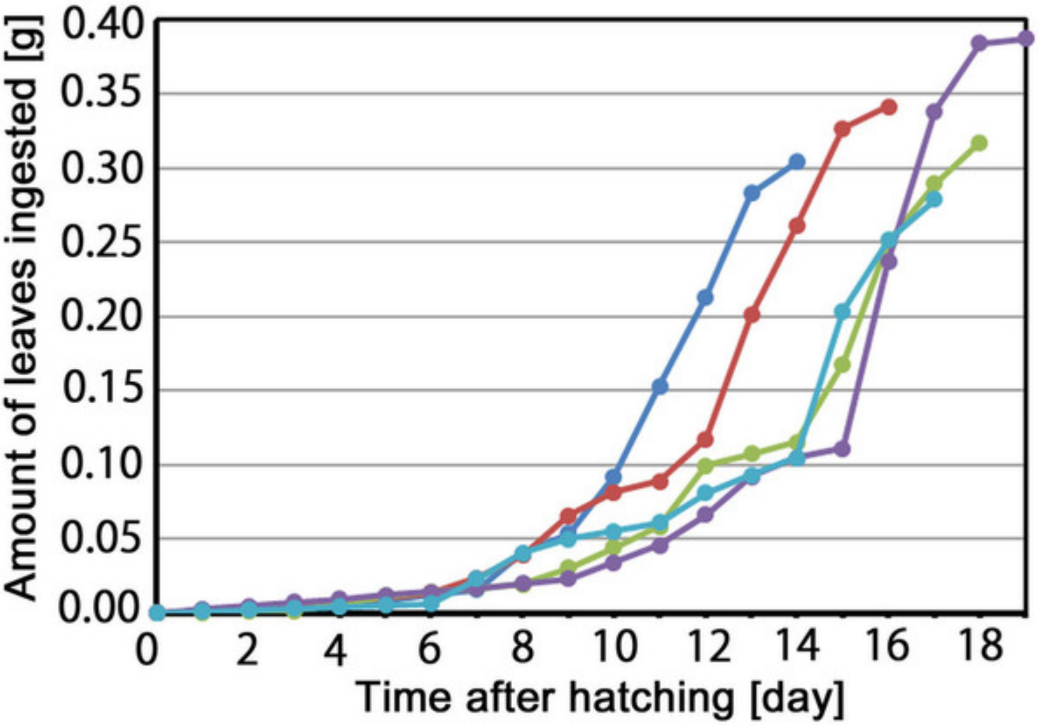
abnormality rate. We also quantified how much radioactive cesium was retained and accumulated in pupae. Finally, we discussed the possible risks of a polluted diet for butterflies and other organisms living in the polluted area.

Results

Quantification of ingested doses

We first investigated the leaf weight consumed by larvae in the larval stage (Fig. 1). Dietary consumption curves for 5 larvae were largely sigmoidal: approximately 4 days before reaching the 100% consumption level, the amount of ingested leaves increased dramatically. There was variability in the total amount ingested among the 5 individuals; we recorded 0.304, 0.342, 0.391, 0.318, and 0.279 g (mean ± SD = 0.327 ± 0.038 g; *n* = 5) for the total weight of host plant leaves eaten by each larva (Fig. 1). However, the shapes of these dietary consumption curves were essentially similar to one another, indicating that our feeding process in which we quantified the weight of ingested leaves did not considerably disrupt the overall growth patterns of the larvae. Within 24 hours after pupation, the weights of all 5 pupae were also recorded; we recorded 0.023, 0.028, 0.033, 0.035, and 0.026 g pupal weight (mean ± SD = 0.029 ± 0.004 g; *n* = 5). These 5 pupae were smaller than other pupae reared under standard conditions using leaves from Ube, a control locality (mean ± SD = 0.035 ± 0.005 g; *n* = 154). This difference is most likely due to larval stress resulting from the above rearing conditions, which were necessary to quantify the ingested leaves. Only one piece of leaf was given at a time and replaced daily, and each larva was confined to a small airtight container (see Methods). The leaf weight ingested by an average larva reared under the standard conditions was calculated to be 0.389 g per larva based on a linear adjustment according to the mean pupal weight under the standard conditions, 0.035 g.

Figure 1: Cumulative amount of leaves ingested over time for 5 larvae.



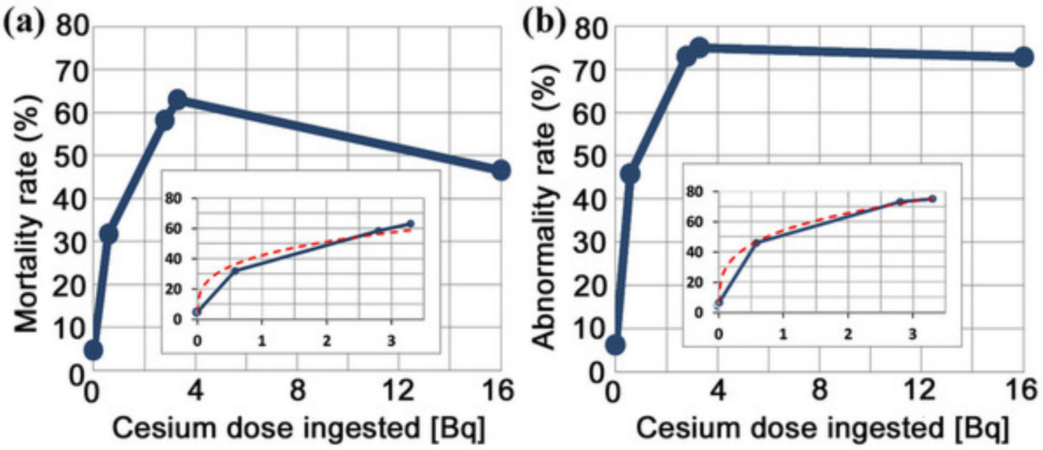
Each curve represents an individual larva. The day when a hatched larva was detected was defined as day 1.

Using the summation of the radioactivity of ^{137}Cs and ^{134}Cs detected in host plant samples from 5 localities[15], we calculated the cumulative radioactive cesium doses ingested by larvae throughout the larval stage; 0.00038 (Ube), 0.58 (Hirono), 2.8 (Fukushima), 3.3 (Iitate-flatland), and 16 (Iitate-montane) Bq (Table 1). Averaged over 5 individuals, the larva in this experiment ate a small amount of non-contaminated leaves (2.8% of the total weight of leaves) before eating contaminated leaves, which was taken into account to calculate the cumulative doses above. The average dose rates per day per larva were also calculated, given that the average larva began ingesting contaminated leaves on day 6 and required 12 additional days to reach 100% consumption (Table 1).

Ingested doses, mortality rates, and abnormality rates

In reference to mortality rates and abnormality rates of 5 groups that ingested leaves from Ube (*n* = 154), Hirono (*n* = 85), Fukushima (*n* = 108), Iitate-flatland (*n* = 100), and Iitate-montane (*n* = 103) (Table 1), we next examined possible relationships between the dose of ingested cesium per larva and the mortality and abnormality rates (Fig. 2). Both the mortality and abnormality rates increased sharply, especially at low doses, until the ingested cesium dose reached approximately 3 Bq, above which rates did not change notably. Because the plot for the Iitate-montane group was located in the plateau phase, we tentatively excluded it to obtain a mathematical model. Indeed, inclusion of the Iitate-montane plot made any models examined less fit (data not shown). The remaining plots were approximated very well using power functions: $y = 42x^{0.28}$ or $Y = 0.28\ (\pm 0.01)\ X + 1.6\ (\pm 0.03)$ on logarithmic scales with $R^2 = 0.9945$, RMSE (root-mean-square error) = 0.047, $F = 3.6 \times 10^2$, $df = 3$ (also in all other models examined hereafter), and $p = 0.0027$ for the mortality rate, and $y = 54x^{0.28}$ or $Y = 0.28\ (\pm 0.002)\ X + 1.7\ (\pm 0.003)$ on logarithmic scales with $R^2 = 0.9999$, RMSE = 0.0068, $F = 1.73 \times 10^4$, and $p < 0.0001$ for the abnormality rate.

Figure 2: Mortality rate (a) and abnormality rate (b) in response to the radioactive cesium dose ingested per larva.



Insets are plots of four localities that were fitted by power functions (red broken lines).

The power functions above were compared with simple linear models. For the mortality rate, we obtained $y = 16x + 13$ with $R^2 = 0.9236$, RMSE = 9.1, $F = 24$, and $p = 0.039$, using the 4 data points excluding the Iitate-montane data. For the abnormality rate, we obtained $y = 18x + 20$ with $R^2 = 0.8527$, RMSE = 15, $F = 12$, and $p = 0.077$, using the 4 data points. These results indicate that the simple linear models were poorer fits than the power function models. Similarly, we examined logarithmic models, obtaining $y = 5.9 \ln(x) + 49$ with $R^2 = 0.8806$, RMSE = 11, $F = 15$, and $p = 0.062$ for the mortality rate, and $y = 7.3 \ln(x) + 61$ with $R^2 = 0.9420$, RMSE = 9.5, $F = 33$, and $p = 0.029$ for the abnormality rate. These logarithmic models appeared to be better than the linear models but still inferior to the power function models. We examined other major models, all of which were less fit than the power function models (see Methods).

Therefore, based on the power function equations, the half lethal dose and the half abnormal dose ($y = 50$) were calculated to be 1.9 (±0.02) and 0.76 (±0.02) Bq per larva, respectively. Using the mean pupal weight under standard conditions (0.035 g), these half doses of cesium corresponded to $5.4\ (\pm 0.06) \times 10^4$ and $2.2\ (\pm 0.06) \times 10^4$ Bq per kilogram body weight, respectively. Similar calculations yielded the half lethal dose rate (daily dose) and the half abnormal dose rate (daily dose) of cesium ingestion, assuming that a larva consumed contaminated leaves over 12 days, which were 0.16 (±0.002) and 0.063 (±0.002) Bq/d per larva, respectively. These half dose rates corresponded to $4.6\ (\pm 0.06) \times 10^3$ and $1.8\ (\pm 0.06) \times 10^3$ Bq/d per kilogram body weight, respectively.

Assuming that larvae eat leaves with a constant contamination level throughout life, we calculated the critical contamination levels of leaves to attain the half lethal and abnormal doses when ingested. These critical concentrations were obtained simply by dividing the half lethal and abnormal doses by the amount of leaves consumed throughout life, 0.389 g, yielding $4.9\ (\pm 0.05) \times 10^3$ and $2.0\ (\pm 0.05) \times 10^3$ Bq per kilogram leaf, respectively.

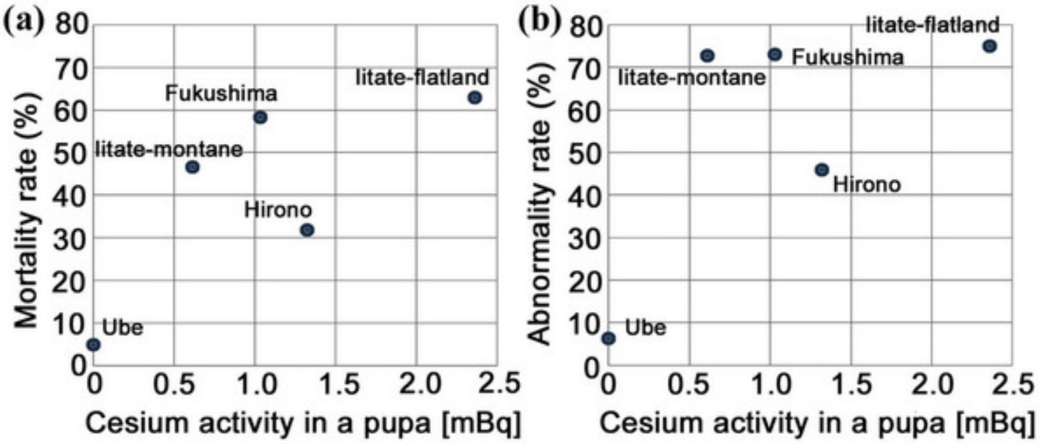
Cesium radioactivity in pupae

To examine how much radioactive cesium was retained in the body of the pupae, we measured the cesium radioactivity of dead pupae that never eclosed under our standard rearing conditions (Table 2). The summation of ^{137}Cs and ^{134}Cs activity concentrations per pupa showed that the pupae that ate Hirono leaves (1.32 mBq) had higher values than the pupae that ate Fukushima leaves (1.03 mBq), although the dose ingested by the Hirono pupae (0.58 Bq) was lower than that ingested by the Fukushima pupae (2.8 Bq). Furthermore, the Iitate-flatland pupae had the highest radioactivity (2.36 mBq), which was roughly twice as much as those of the Hirono and Fukushima pupae. Surprisingly, the Iitate-montane pupae showed the lowest radioactivity (0.61 mBq) among the examined pupae, except for the control Ube sample (Table 2).

Table 2: Cesium radioactivity in pupae that ingested contaminated leaves at the larval stage (see annex 2)

Possible relationships between the cesium radioactivity per pupa and mortality and abnormality rates were examined in scatter plots (Fig. 3). The scattering patterns were different from those found in the ingested dose shown in Fig. 2. As discussed above, the Hirono pupae may be expected to have a lower value than the measured value, and the Iitate-montane pupae may be expected to have a higher value than the measured values, if the cesium activity in a pupa linearly reflects the ingested cesium dose. Deviations from the linear expectations suggest nonlinear biological responses to the ingested radioactive cesium. When a linear model was fitted, we obtained $R^2 = 0.56$ and $p = 0.15$ for the mortality rate, and $R^2 = 0.41$ with $p = 0.24$ for the abnormality rate, assuming that the Ube sample had no cesium activity. Conclusive interpretation of these data was not possible, as indicated by low R^2 values and high *p*-values.

Figure 3: Mortality rate (a) and abnormality rate (b) in relation to the cesium radioactivity in pupae.



The Ube sample was considered to have a cesium activity of 0.

To assess the amount of radioactive cesium retained and accumulated in pupae, we calculated both the percentage of cesium radioactivity retained in pupae (Bq) relative to the cesium dose ingested by larvae throughout the larval stage (Bq) and the percentage of cesium radioactivity accumulated in pupae (Bq/kg) relative to the cesium radioactivity of the host plant leaves (Bq/kg) (Table 2). Both retention and accumulation percentages were highest in the Hirono pupae (0.23% and 2.6%, respectively), which ingested the least contaminated leaves, except for the control Ube sample (Table 2).

Discussion

In this paper, we focused on the biological effects of ingested materials containing radioactive cesium species released by the Fukushima Dai-ichi NPP accident. We found that the levels of ingested cesium from the 4 localities (i.e., Hirono, Fukushima, Iitate-flatland, and Iitate-montane) were much higher than that from Ube, a control locality; thus, it is not surprising that harmful biological effects were clearly detected in previous studies[15, 16]. However, we do not know if our results can be directly compared to other studies on natural radiation or other nuclear accidents. We measured the amount of cesium radioactivity retained in pupae, which could affect metamorphosis, resulting in morphological abnormalities and death[16]. However, it should be noted that the activity concentrations of cesium in pupae may not be proportional to the degrees of physiological and genetic damage of those pupae. The cesium radioactivity concentration in pupae was much lower than that in the host plant, showing that no bioaccumulation took place in this producer-consumer system that plays a role in an ecological food chain.

We found that changes in the overall mortality and abnormality rates in response to amounts of ingested radioactivity were not linear. Rather, the mortality and abnormality rates increased sharply, especially at low doses. Additionally, there seemed to be no threshold level below which no biological response could be detected. These results are consistent with the linear no-threshold (LNT) model, which is relatively widely accepted at low doses[18, 19], in that there was no threshold detected in our system. However, the results may be inconsistent with the LNT model in that a linear model may not be appropriate for our system. More precisely, the power function fit for the dose-response data suggests that the relatively small level of artificial cesium from the Fukushima Dai-ichi NPP may be significantly toxic to some individuals in butterfly populations, although we do not know whether the relatively “low-level” radiation in our experimental system is in the range of the levels safely applicable to the LNT model.

Nevertheless, we assert that the half lethal and abnormal doses we obtained were quite high. Moreover, the critical concentrations of cesium resulting in the half lethal and abnormal doses, 4.9 (±0.05) × 10^3 and 2.0 (±0.05) × 10^3 Bq per kilogram leaf, respectively, appeared to be very high compared to naturally occurring radioactivity levels. These results can be interpreted to suggest that the pale grass blue butterfly is generally resistant to internal radiation exposure. The possible biological impacts of much lower radiation levels will be the subject of future investigations.

The relevance of our results to humans remains undetermined and will remain so because of the impossibility of controlled experiments in humans. However, it should be noted that we sampled contaminated leaves from Fukushima City, which many people inhabit as though nothing had happened, and from Hirono Town, which some people returned to inhabit. Moreover, our results are consistent with the previous human results after the Chernobyl accident, in which infant mortality rate increased sharply in West Germany[20] and in the United States[21]. Gould and Sternglass (1989)[21] speculated that the increased infant mortality rate immediately after the Chernobyl accident may have been caused by the ingestion of radioactive iodine. Although they used a logarithmic model rather than a power function model to fit their dose-response relationship, both models share a sharp non-linear increase at low doses. Implications of the half lethal and abnormal doses we obtained in the present study will impact future discussions on the effects of radioactive exposure on other organisms, including humans.

We found that cesium radioactivity in Hirono pupae was relatively high, despite Hirono leaves having the lowest level of contamination among those examined. Interestingly, cesium activity in the Iitate-montane pupae was the lowest observed, except for the control Ube pupae, despite consuming leaves with the highest level of contamination observed. The Hirono pupae, which ate leaves with relatively low-level contamination among those sampled, had the highest retention and accumulation values. One possibility is that the epithelial cells of the digestive tract of larvae that ate the highly contaminated Iitate-montane leaves became damaged, preventing the absorption of radioactive cesium. Alternatively, the digestive tract may have effectively expelled the excessively high dose of cesium, resulting in the absorption of a limited amount. The latter possibility is more likely, as no systematic reduction of body weight or wing size, which might result from digestive tract damage, was observed in the Iitate-montane group[15]. Indeed, the latter possibility may be likely if cesium was ingested as microparticles[22] adsorbed onto the surface of host plant leaves. In contrast to the Iitate-montane group, the larvae that ate Hirono leaves appeared to have effectively absorbed the radioactive cesium. This scenario explains not only the sharp increase of the mortality and abnormality rates at low doses but also the saturated mortality and abnormality rates at high doses. The saturation of the mortality and abnormality rates may also be explained by a possible exhaustion of relevant targets such as functional genes that cause immediate lethal or abnormal effects when damaged. Such genes may be limited in number, and further hits at these genes do not contribute to an increase in the mortality and abnormality rates.

The biological impacts of internal radiation exposure on larvae may be relatively high, considering the fact that the artificial cesium emits not only γ-rays but β-rays[23]. Additionally, the radioactive plume may have been ingested as microparticles[22], causing immune responses (or other physiological responses) independent of radiation, eventually leading to pathological outcomes. There is also the possibility that other radioactive (and even non-radioactive) materials from the Fukushima Dai-ichi NPP, other than cesium, could contribute additively to butterfly mortality and abnormality.

Few available studies have evaluated the biological effects of internal radiation exposure in various organisms[24], but it has been shown that different species have different sensitivities to radiation exposure[25]. We speculate that sensitivity likely varies widely under different genetic and environmental conditions, even within a single species. In this internal exposure experiment, we observed that even under the dosage conditions where many individuals died, other individuals survived normally. That is, the mortality and abnormality rates never reached 100%, indicating individual variability of sensitivity within a species. Because such intraspecies variation likely exists in humans as well as in other organisms, setting a single threshold for a “safe exposure level” requires clearly stated confidence intervals along with other relevant information on the system of interest.

One recent study reported no detectable effect of radioactive cesium on bull testes[26]. However, it is premature to draw conclusions from that study, primarily because only two individuals were examined. Furthermore, sperm morphology was shown to be normal by images of only a few sperm nuclei and acrosomes, and the number of germ cells examined was not presented. Above all, negative data should generally be treated carefully, because careful definition of the experimental system and a large number of samples are required to demonstrate a null hypothesis[27, 28].

In conclusion, it is important to recognize the risk of internal radiation exposure due to ingested radioactive cesium, at least for the pale grass blue butterfly, and likely for certain other organisms living in the polluted area, possibly including humans. More studies at low doses are required in the near future to confirm and expand the findings of the present study.

Methods

Internal exposure experiment

To harvest eggs, 6 females and 6 males caught in the field in Okinawa Island were confined to a 300-mm cubic container for 6–10 days as described previously[15, 16]. During this period, dead adults were replaced with live ones to keep the number of adults unchanged. The obtained eggs, larvae, and pupae were reared in the laboratory under standard conditions[17]. The diet from Okinawa (*i.e*., the non-contaminated leaves of the host plant, *Oxalis corniculata*) was given to all larvae initially, and on the 6th day after hatching, the initial larval population was randomly divided into 5 groups. Therefore, these groups were genetically identical to one another. Each group of larvae was fed a diet collected from one of the 5 localities (Ube, Hirono, Fukushima, Iitate-flatland, and Iitate-montane) from the 6th day after hatching[15]. Non-polluted leaves from Ube, Yamaguchi Prefecture, were fed to a group of larvae. This group of larvae served as a control for the internal exposure experiment.

Quantification of consumed diet

Eggs were harvested from females caught in the field in Okinawa as above, and 5 larvae were randomly chosen and reared at ambient temperature (approximately 27°C), each in a plastic columnar centrifuge tube with an airtight screw cap (10 mm in diameter and 50 mm in height) to prevent leaves from drying. The quantity of leaves eaten by each larva was monitored every day. One piece of leaf removed from the stalk was supplied at a time. Before being given to a larva, the leaf piece was weighed. Additionally, its image was taken by an image scanner, and its area was measured using the ImageJ 1.44p software (U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997–2012). After being eaten, the remaining leaf was replaced every day with a fresh leaf. The area of the remaining leaf was measured every day, and its value was converted into weight, based on the proportion of the original leaf represented by the remnant. This process ensured that the weight eaten each day was recorded as the weight of the fresh leaf.

Radioactivity measurements and calculations

Cesium radioactivity (both ^{137}Cs and ^{134}Cs) in leaf samples was measured with a germanium semiconductor radiation well-type detector Canberra GCW-4023 (Meriden, CT, USA) with a heavy shield of lead after ashing the samples as described previously[15].Using cesium radioactivity values of the leaves at the time of measurement (26 December 2011), presented in Supplementary Table 8 in Hiyama *et al*. (2012)[15], we recalculated the values at the time of larval consumption (fixed at 22 July 2011 for simplicity), assuming that ^{137}Cs and ^{134}Cs were released at a 1:1 activity ratio in a single burst on 15 March 2011 from the Fukushima Dai-ichi NPP. The Hirono ^{134}Cs value, 5.38 (±0.06) × 10^2, was amended due to a typing error in the original paper[15]. The same detector was used to measure cesium activity (^{137}Cs only) in pupae. This well-type detector provides maximum efficiency for small samples, because samples can be inserted into a well. Before measurement, dead pupae were air-dried for several months and placed in a columnar plastic container (14 mm in diameter). The detector was calibrated with a standard prepared from the NBL powder standard (New Brunswick Laboratory, U.S. Department of Energy, Argonne, IL, USA). Measurements were performed until the error rate scored less than 10%. Measurements were assumed to be made simultaneously on 15 February 2014 for simplicity, although several days were required to obtain reliable levels of radioactive signals from the pupae. Radioactivity per pupa at the time of ingestion was calculated, assuming that ^{137}Cs and ^{134}Cs were released in a single burst on 15 March 2011 at a 1:1 activity ratio and that all leaves were eaten simultaneously at once on 22 July 2012. ^{134}Cs activity was calculated based on a ^{134}Cs:^{137}Cs activity ratio at the time of ingestion, fixed at 22 July 2011 for simplicity.

Mortality and abnormality rates

The mortality rate was defined as the percentage of dead individuals at all stages relative to the total number of individuals reared. Thus, the mortality rate excluded only normal adults. In contrast, the abnormality rate reported here could be more accurately called the total abnormality rate. This rate was the percentage of dead individuals at the larval, prepupal, and pupal stages plus the adults that had morphological abnormalities relative to the total number of individuals reared. Thus, the abnormality rate excluded only normal adults that lacked any morphological abnormalities. Correlation analysis was performed using Microsoft Excel (2013) and JSTAT 13.0 (2012) (Yokohama, Japan).

Mathematical modeling

We first used the built-in mathematical fit function of Microsoft Excel (2013) to obtain the best-fit curves among available models. All possible models were examined including linear, exponential, logarithmic, polynomial, and power function models, and the best possible fit in terms of R^2 values and biological justification was considered to be the most appropriate. Based on equations obtained in this way, we manually calculated the half lethal dose and the half abnormal dose by setting $y = 50(\%)$. Additionally, linear and nonlinear models were examined using the model analysis software JMP 11.0.0 (2013) (SAS Institute, Cary, NC, USA). We reasoned that the simplest mathematical equation with the highest R^2 value, the lowest RMSE, or the lowest information criterion, such as AIC, BIC, and MDL, would be accepted. We examined linear models (including logarithmic scales for power function) and nonlinear models (including logarithmic, square root, quadratic, polynomial, logistic, and exponential equations). Among the model fits performed, logistic 4 P models also showed high R^2 values ($R^2 = 0.9972$ and BIC = 19.93 for mortality rate; $R^2 = 0.9997$ and BIC = 12.31 for abnormality rate) in addition to the power function models, but the complicated equations with relatively high BIC values discouraged the use of these equations for further analysis. The numerical values associated with the models and calculations are shown together with standard errors (SE) in the main text.

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Contributions

J.M.O. designed and coordinated the study and performed mathematical modeling; C.N., A.H., W.T. and A.T. performed experiments; C.N., A.H., W.T., A.T. and J.M.O. analyzed data; and J.M.O. wrote the paper.

Competing financial interests

The authors declare no competing financial interests.

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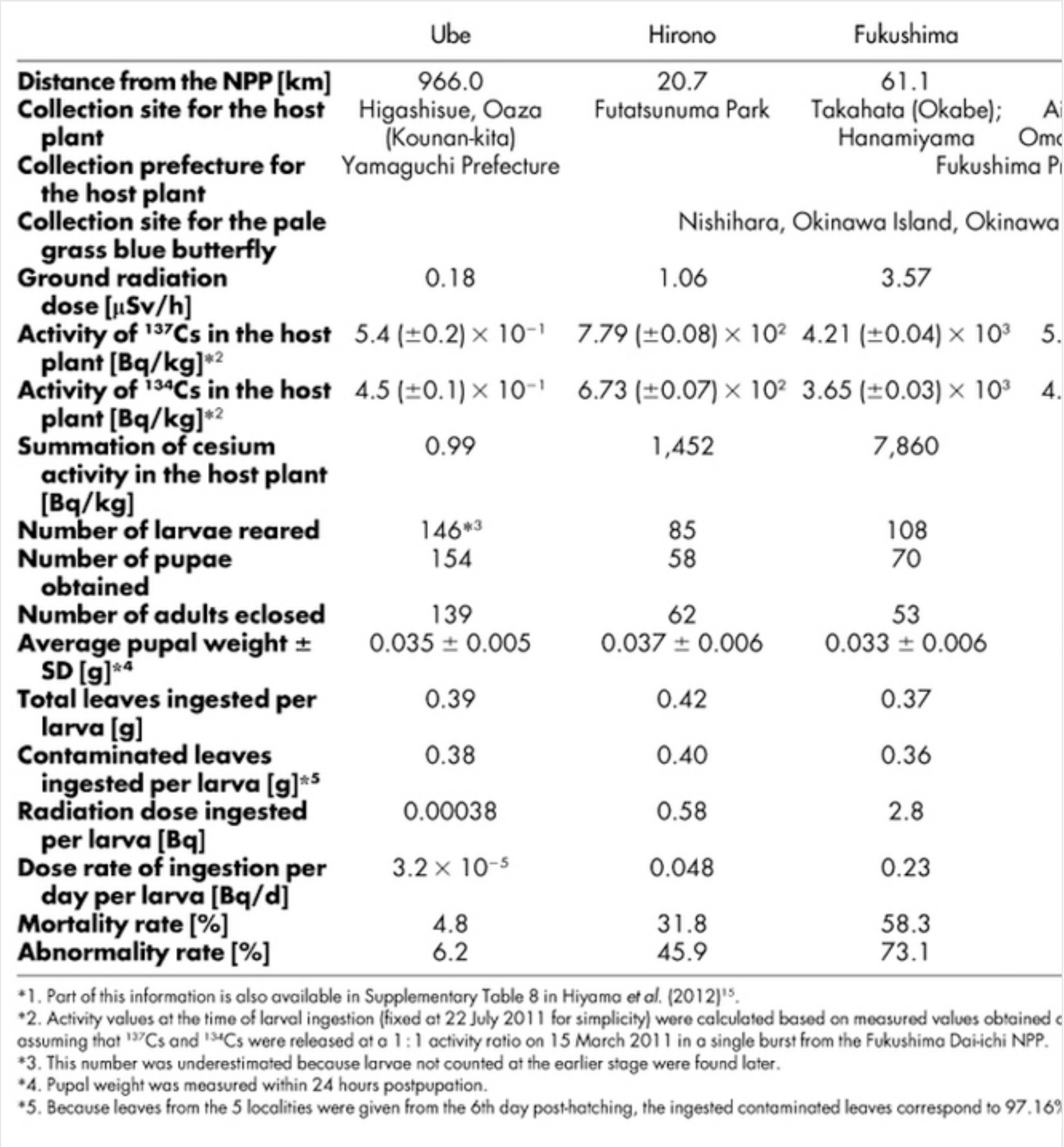
Annex 1 - Table 1: Summary of data for the internal exposure experiment[1]



	Ube	Hirono	Fukushima	
Distance from the NPP [km]	966.0	20.7	61.1	
Collection site for the host plant	Higashisue, Oaza (Kounan-kita)	Futatsunuma Park	Takahata (Okabe); Hanamiyama	Ai Om
Collection prefecture for the host plant	Yamaguchi Prefecture		Fukushima Pr	
Collection site for the pale grass blue butterfly		Nishihara, Okinawa Island, Okinawa		
Ground radiation dose [μSv/h]	0.18	1.06	3.57	
Activity of ^{137}Cs in the host plant [Bq/kg]*2	$5.4 (\pm 0.2) \times 10^{-1}$	$7.79 (\pm 0.08) \times 10^{2}$	$4.21 (\pm 0.04) \times 10^{3}$	5.
Activity of ^{134}Cs in the host plant [Bq/kg]*2	$4.5 (\pm 0.1) \times 10^{-1}$	$6.73 (\pm 0.07) \times 10^{2}$	$3.65 (\pm 0.03) \times 10^{3}$	4.
Summation of cesium activity in the host plant [Bq/kg]	0.99	1,452	7,860	
Number of larvae reared	146*3	85	108	
Number of pupae obtained	154	58	70	
Number of adults eclosed	139	62	53	
Average pupal weight ± SD [g]*4	0.035 ± 0.005	0.037 ± 0.006	0.033 ± 0.006	
Total leaves ingested per larva [g]	0.39	0.42	0.37	
Contaminated leaves ingested per larva [g]*5	0.38	0.40	0.36	
Radiation dose ingested per larva [Bq]	0.00038	0.58	2.8	
Dose rate of ingestion per day per larva [Bq/d]	3.2×10^{-5}	0.048	0.23	
Mortality rate [%]	4.8	31.8	58.3	
Abnormality rate [%]	6.2	45.9	73.1	

*1. Part of this information is also available in Supplementary Table 8 in Hiyama *et al.* (2012)[15].

*2. Activity values at the time of larval ingestion (fixed at 22 July 2011 for simplicity) were calculated based on measured values obtained assuming that ^{137}Cs and ^{134}Cs were released at a 1 : 1 activity ratio on 15 March 2011 in a single burst from the Fukushima Dai-ichi NPP.

*3. This number was underestimated because larvae not counted at the earlier stage were found later.

*4. Pupal weight was measured within 24 hours postpupation.

*5. Because leaves from the 5 localities were given from the 6th day post-hatching, the ingested contaminated leaves correspond to 97.16

Annex 2 - Table 2: Cesium radioactivity in pupae that ingested contaminated leaves at the larval stage

	Ube	**Hirono**	**Fukushima**	**Iitate-flatland**	**Iitate-montane**
Number of dead pupae measured	8	6	11	10	22
Activity of ^{137}Cs per pupa [mBq][1]	Lower than detection limit	0.71 ± 0.06	0.55 ± 0.04	1.27 ± 0.05	0.33 ± 0.01
Activity of ^{134}Cs per pupa [mBq][2]	Lower than detection limit	0.61 ± 0.05	0.48 ± 0.04	1.09 ± 0.05	0.28 ± 0.01
Summation of cesium activity per pupa [mBq]	Not applicable	1.32	1.03	2.36	0.61
Summation of cesium activity per pupa [Bq/kg][3]	Not applicable	35.7	31.2	81.4	18.5
Cesium activity retained per pupa (Bq) relative to the ingested dose (Bq) [%][4]	Not applicable	0.23	0.037	0.072	0.0038
Cesium activity (Bq/kg) accumulated per pupa relative to the host plant activity (Bq/kg) [%][5]	Not applicable	2.6	0.40	0.80	0.043

*1.Activity values of ^{137}Cs at the time of larval ingestion (fixed at 22 July 2011) were calculated based on measured values obtained on 15 February 2014, assuming that ^{137}Cs and ^{134}Cs were released at a 1:1 activity ratio on 15 March 2011 in a single burst from the Fukushima Dai-ichi NPP.

*2. Activity values of ^{134}Cs at the time of larval ingestion (fixed at 22 July 2011) were calculated based on the calculated values of ^{137}Cs using the ^{137}Cs:^{134}Cs activity ratio at the time of ingestion, which was obtained from Table 1.

*3. Calculated based on the average pupal weight of each group shown in Table 1.

*4. Calculated based on the ingested doses shown in Table 1.

*5. Calculated based on the host plant cesium activity concentrations shown in Table 1.