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Environmental Toxicology and Chemistry, Vol. 31, No. 7, pp. 1556–1563, 2012

Printed in the USA
DOI: 10.1002/etc.1834

BATS AT RISK? BAT ACTIVITY AND INSECTICIDE RESIDUE ANALYSIS OF FOOD ITEMS IN AN APPLE ORCHARD

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(*Submitted* 12 *October* 2011; *Returned for Revision* 17 *November* 2011; *Accepted* 14 *February* 2012)

Abstract—Although bats are reported as being threatened by pesticides, they are currently not considered in European Union pesticide risk assessments. The reason for that contradiction is probably related to the scarcity of information on bat activity in pesticide-treated fields and the pesticide residues on their food items. The authors recorded bat activity and measured pesticide residues on bat-specific food items following applications of two insecticides in an apple orchard. High activity levels of the common pipistrelle bat, a foraging habitat generalist, were detected. Airborne foragers and bats that take part of their food by gleaning arthropods from the vegetation were recorded frequently. The initial value and the decline of pesticide residues were found to depend on the arthropod type, their surface to volume ratio, their mobility, and the mode of action of the applied pesticide. The highest initial residue values were measured on foliage-dwelling arthropods. By following the toxicity-exposure ratio approaches of the current pesticide risk assessment, no acute dietary risk was found for all recorded bat species. However, a potential reproductive risk for bat species that include foliage-dwelling arthropods in their diet was indicated. The results emphasize the importance of adequately evaluating the risks of pesticides to bats, which, compared to other mammals, are potentially more sensitive due to their ecological traits. Environ. Toxicol. Chem. 2012;31:1556–1563. © 2012 SETAC

Keywords—Chlorpyrifos-methyl Fenoxycab Mammal Risk assessment Sensitivity

INTRODUCTION

The European Union first-tier assessments of acute and reproductive risk of pesticides to birds and mammals [1] are based on toxicity-exposure ratios (TERs), which are compared to safety factors (trigger values). If the TER is larger than the safety factor, the risk is considered to be low. If the TER is lower than the safety factor, no authorization is granted for the pesticide unless a refined risk assessment demonstrates that no risk for wildlife species occurs when the pesticide is applied under field conditions. For the toxicity component of the ratio, the LD50 (lethal dose, the dose at which 50% of the test organisms die) of an acute oral test for birds and for mammals is used for the acute risk assessment, whereas the no observed adverse effect level (NOAEL) values of reproduction tests (birds) and of multigeneration studies (mammals) are used for the reproductive risk assessment. Dietary exposure is estimated by applying a number of different generic indicators (''generic focal species''), which are not real species, however, regarding their feeding habits, representative for species that occur in a particular crop at a particular time. Based on the food-intake rate, the body mass, and the concentration of the compound in the diet, shortcut values of these generic indicators are available for a range of scenarios (type of crop, growth stage of the crop, and kind of application) [1].

Insectivorous mammals are represented by the generic indicator ''shrews'' but no reference is made to bats, a group of 42 species comprising one-fifth of all European terrestrial mammals [2], differing widely in feeding habits from shrews because they hunt flying insects and feed on arthropods on the vegetation (gleaning). Therefore, potential dietary exposure to pesticides is different. Considering that generic focal species should be representative for all species that could be at risk [1], bats are obviously not supposed to be exposed to pesticides. Controversially, the agreement on the conservation of European bat populations [3] stated in article III (fundamental obligations), number 8: ''Each party shall, wherever appropriate, consider the potential effects of pesticides on bats, when assessing pesticides for use. . ..''

Evidence of pesticide exposure of bats was discovered in the 1960s and 1970s, a period of widespread use of organochlorine pesticides. Some of these pesticides were responsible for the significant mortality of several bat species as demonstrated by field and laboratory studies in northern America and Europe [4–6]. A die-off of juvenile greater mouse-eared bats (*Myotis myotis*) was documented after the application of methamidophos (Filitox, an organophosphate) to nearby potato fields and apple orchards in Germany [7]. The high levels of methamidophos residues detected in the corpses were considered to be transferred through milk to the offspring by females that consumed contaminated insects. In Spain, residues of fenitrothion (organophosphate) were reported in common pipistrelles (*Pipistrellus pipistrellus*) following agricultural applications [8]. Today, most highly toxic and persistent pesticides have been replaced; therefore, the effects of modern pesticides on bats may be more difficult to document, have been less well studied, and are probably underestimated [9].

Apart from the direct evidence of exposure, recent radio-tracking studies and acoustic surveys performed with bat detectors revealed high foraging activity of bats in different kinds of orchard crops in Europe. Intensively managed apple orchards were documented as being positively selected as foraging habitats by the greater mouse-eared bat in southwestern Switzerland [10] and Tyrol [11]. Foraging activity of bats was also reported in intensively cultivated olive orchards treated with insecticides in Greece [12]. Data on foraging

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Published online 13 April 2012 in Wiley Online Library (wileyonlinelibrary.com).

activity of bats in other agricultural crop fields are scarce and do not allow a profound conclusion. For example, Walsh and Harris [13] found that arable land in Britain was avoided by bats, while Russo and Jones [14] recorded relatively large numbers of foraging attempts in some arable fields in a survey in southern Italy. However, none of these studies give details about the crop and, hence, preclude a consistent conclusion of potential pesticide exposure.

The aim of the present study was to estimate the exposure of bats to pesticides in a conventionally cultivated apple orchard. Bat activity was recorded after two consecutive applications of fenoxycarb (Insegar, a carbamate), an insecticide that is applied up to three times from May to July, which falls in the pregnancy and lactation period of bats. To compare activity levels recorded in orchards with those in habitats known to be used by bats for foraging, we also recorded activity levels in nearby meadow, forest, and forest-edge sites. In parallel, we measured residues of fenoxycarb on the typical food items of the recorded bat species to assess acute and reproductive risk from dietary exposure for the respective bat species. To determine if the mode of action of pesticides influences the residue pattern on arthropods, we additionally measured the residue of chlorpyrifos-methyl (organophosphate) of one arthropod group (foliage-dwelling arthropods) following an application of Reldan in the same orchard.

MATERIALS AND METHODS

Study site and insecticide applications

The present study was conducted in May and July 2009 in a mature commercial apple orchard (Braeburn variety) situated in a fruit-growing area near Winden, Rhineland-Palatinate, southwest Germany (~49°05′N, 8°07′E). The approximate size of the orchard was 4 ha (160 × 250 m), consisting of 54 rows of apple trees, with approximately 300 trees, 3.5 m high, in each row. The distance between the rows was approximately 3 m. At both ends of the rows, tractor-turning areas of approximately 10 m widths, covered with lawn and surrounded by apple tree rows, were present (Fig. 1). The apple orchard was surrounded by other conventional apple orchards (north and west), an organic vegetable field (south), and a maize field (east) (Fig. 1). The closest housing of the nearby village of Winden was 400 m away.

The entire 4 ha of the orchard were sprayed with Reldan (Dow AgroSciences) at a rate of 337 g a.i. ha^{-1} against the woolly apple aphid (*Eriosoma lanigerum*) on one occasion (May 20, 2009) and with Insegar (Syngenta Agro) against the codling moth (*Cydia pomonella*) at 150 g a.i. ha^{-1} on two occasions (July 1 and 15, 2009). The rates applied represent the recommended application rates in apple orchards according to good agricultural practice. The Reldan application took place after petal fall, and Insegar was applied during the development of fruits. Solutions were prepared on site immediately before application. Both insecticides were applied with a tractor-mounted, air-assisted sprayer (Vicar compact 1200). Two tank mixes (each of 1,200 L) were prepared and applied on each application date. The sprayer was calibrated to deliver 600 L ha^{-1} and configured to produce a spray that reached the highest and lowest branches, while application onto the soil or over the trees was minimized. Applications started at approximately 4:00 PM and lasted approximately 4 h. Spray deposit distribution was documented using water-sensitive papers placed in various positions within the tree canopy and indicated an even spray deposit.

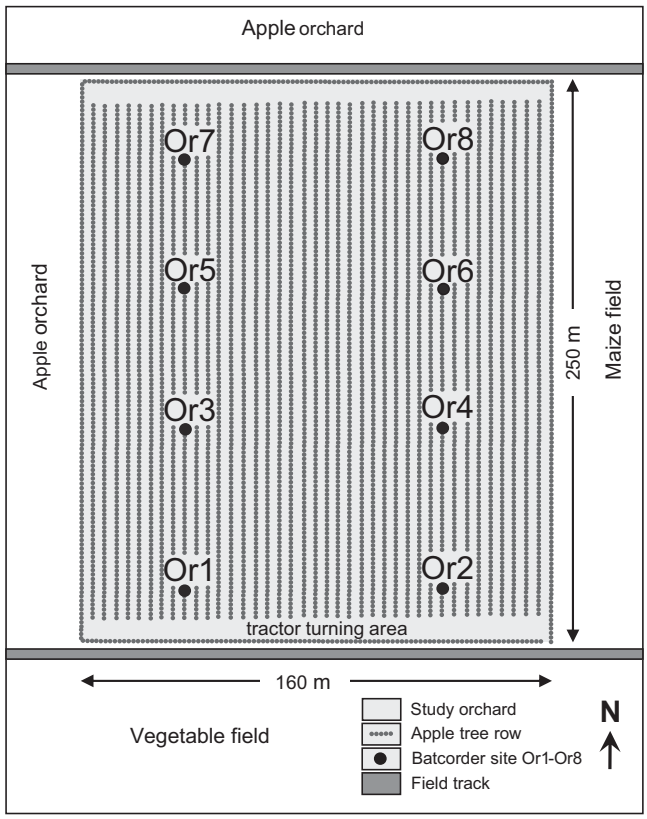

Fig. 1. Schematic diagram of the study site at Winden, Rhineland-Palatinate, Germany.

Bat activity measurement

Acoustic measurement of bat activity is a reliable estimate of foraging activity [14]. We recorded bat activity using several simultaneously working automatic stationary bat-detector systems (batcorder; ecoObs), a method suitable to address spatial and temporal variation in bat activity patterns [15]. Eight batcorders were installed at a height of 1.5 m above the orchard canopy to avoid absorption and reflection of the echolocation calls at eight sites in the orchard, each site with at least a 25 m buffer to the border. Two batcorders (sites Or1 and Or2) were located close to a tractor-turning area, which was surrounded by rows of apple trees on three sides and bordered by apple tree rows on one side (Fig. 1). That area was treated with pesticide in the same way as the remaining area of the orchard. Acoustic recording of bats was only possible during nights without rain and with low wind speed and, therefore, limited to the nights of days 0 (day of application), 1, 2, 3, and 8 following the first Insegar application and of days 0, 2, 3, and 4 following the second Insegar application.

To compare the recorded activity levels of the examined orchard to activity levels of habitats know to be used for foraging, we also measured bat activity at two meadows, two forest-edge sites, and two sites within that deciduous forest at three occasions in 2009 (20.5, 18.6, 12.8). All these sites were located less than 1.5 km away from the village of Winden, assuring that they were, as well as the orchard, within the home range of bats having their roost sites in the closest settlement (Winden). The distance is based on the foraging range of the common pipistrelle (*P. pipistrellus*), the species with the shortest average distance (1.5 km) between foraging and roost sites among the occurring bat species [16,17].

Batcorders were adjusted to the system's standard settings [15]. Recordings were made from sunset to sunrise. Bat activity was measured as seconds of recorded call sequences per night. The software packages bcDiscriminator and bcAnalyse (ecoObs) were used to identify the calls to species level whenever possible. Due to the variation in species-specific call structure and interspecific overlapping between acoustic repertoires of *Eptesicus serotinus*, *Nyctalus leisleri*, and *Nyctalus noctula*, it was impossible to assign short call sequences to one of those species with sufficient confidence. Hence, species of that group were assigned to the group "*Nyctalus-Eptesicus.*" For the same reason, calls of *Myotis mystacinus* and *Myotis nattereri* were assigned to the group "*Myotis mys-nat.*"

Arthropod sampling for residue analysis

Insecticides were applied to the entire 4 ha of the orchard, but the sampling area was restricted to the central part with at least a 25 m buffer to every side. The following three sampling methods were used to collect nocturnal arthropods according to the preferences of the different bat guilds: unattended light trap sampling for large moths (e.g., Noctuidae, Geometridae), light trap sampling for small flying insects (e.g., Diptera, Microlepidoptera), and inventory sampling for foliage-dwelling arthropods (e.g., Arachnida, Hemiptera, Coleoptera). Sampling was performed after dusk to ensure that only arthropods available for bats were collected with the exception of the nights following the insecticide applications, when we did not start sampling before the pesticide film on the apple trees dried to avoid contamination. Light traps were installed at a height of 1.80 m. Thereby, insect attraction was restricted by the rows of apple trees to avoid sampling of insects from outside the apple orchard.

Large moths (body size between 10–20 mm) were sampled with two unattended light traps with two ultraviolet fluorescent tubes (bioform light trap; bioform). The attached buckets were filled with cardboard egg box material and arranged for the moths to settle on until they were collected. To collect small flying insects (mainly Diptera and small moths with body size between 3–10 mm), a light-tower (Müller light-tower; bioform) was used. Insects that were attracted to the light were collected from the surface using a handheld vacuum cleaner to which a nylon-collecting bag was attached. Foliage-dwelling arthropods (insects and spiders) were sampled by beating the apple trees with a stick while holding a beating tray (Dynat tray; bioform) under the area being beaten. To prevent residue contamination of subsequent samples, the beating tray was completely covered by a disposable plastic sheet. During the entire sampling period, disposable material (plastic jars, egg box material, nylon collecting bags, etc.) was changed and the collecting gear wiped with acetone after every sampling event to avoid cross-contamination.

All arthropod samples were collected into plastic jars using forceps and immediately placed in a cooler filled with ice to avoid desiccation of the arthropods and decrease of residue. Samples were stored in a freezer (-20°C) until residue analysis. Temperature during transit and storage was monitored using a calibrated temperature data logger. Small flying insects were sorted on ice to small moths and other small-flying insects. Due to the high number of moth scales remaining in the nylon-collecting bag, the collecting bags were kept for analysis as well. The complete sampling program was performed for both Insegar applications on the nights of day 1 (before application), day 0 (day of application), and days 1, 2, 3, 4, 8, and 12 postapplication. The arthropod sampling after the Reldan application comprised the collecting of foliage-dwelling arthropods on the nights of days 0 and 8 postapplication.

Residue analysis

The active substances of Reldan and Insegar are chlorpyrifos-methyl (organophosphate) and fenoxycarb (carbamate), respectively. Analysis of insecticide residue was performed using a modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) method [18]. Each invertebrate sample ($\sim$1 g fresh wt) was homogenized in a 50-ml vial filled with 10 ml of acetonitrile. The nylon bags containing moth scales were also placed in vials filled with 10 ml of acetonitrile. After adding 0.5 g NaCl and 0.5 g $MgSO_4$, the samples were shaken for 20 min and subsequently centrifuged for 5 min at 3,000 rev min^{-1}. A filtrated 1.5-ml aliquot of the supernatant of each sample was employed for analysis with an Agilent 1100 HPLC instrument coupled to an API 4000 Qtrap MS/MS (Applied Biosystems). Different concentrations (0.5, 1, 5, 10, 15, 20, 40, 60, 80, 100 ng ml^{-1}) of analytical standards of chlorpyrifos-methyl and fenoxycarb (Sigma-Aldrich) were analyzed under the same instrumental conditions. Quantification was accomplished using the calibration curve constructed by the absolute amount of chlorpyrifos-methyl and fenoxycarb, respectively, against peak areas (r values for both calculation curves were higher than 0.9996). Recoveries of the compounds were obtained from two replicate spiking experiments per arthropod group and spiking levels (1 and 100 ng ml^{-1}). Recoveries of fenoxycarb were $120.0 \pm 0\%$ for 1 ng ml^{-1} and $92.5 \pm 2.1\%$ for 100 ng ml^{-1} in flying insects and $77.5 \pm 2.1\%$ for 1 ng ml^{-1} and $88.5 \pm 0.7\%$ for 100 ng ml^{-1} in foliage-dwelling arthropods. Recoveries of chlorpyrifos-methyl were $130.0 \pm 0\%$ for 1 ng ml^{-1} and $83.0 \pm 1.4\%$ for 100 ng ml^{-1} in foliage-dwelling arthropods. Following O'Shea and Johnson [9], the observed recoveries are within the acceptable range for analytical residue analysis. The observed concentrations of the measured residues were not corrected by the observed recoveries. The residues of the nylon bags containing moth scales were added to the residue values of small moths. Residues were normalized to an application rate of 1 kg a.i. ha^{-1} and expressed as residue unit dose (RUD). On the day before the first fenoxycarb application (null measurement), the concentrations of fenoxycarb of all examined arthropod groups were below the quantification limit of 0.002 mg kg^{-1}.

Risk assessment

The risk assessment was performed following the guidance of the European Food Safety Authority (EFSA) on risk assessment for birds and mammals [1].

The exposure, expressed as daily dietary dose (DDD), for acute dietary risk assessment was calculated as the product of the application rate, the peak RUD value resulting from the assumed dietary composition for the species of concern (calculation of the requested 90th percentile was not possible due to the limited data), the food-intake rate per body weight (FIR body wt^{-1}), and a default value of the multiple application factor (MAF) [1]

$$\text{DDDacute} = \text{application rate} \times \text{RUD (peak value)} \times \text{FIR body wt}^{-1} \times \text{MAF} \quad (1)$$

To assess the reproductive risk, the DDD was calculated in the same way as in Equation 1 but with the difference that the mean peak RUD for the diet of the respective species group and an additional default values for time-weighted average (TWA)

were used. The default value for TWA was 0.53 and assumed a DT50 (time for 50% degradation) of the residue of 10 d [1]:

$$\text{DDDreproductive} = \text{application rate} \times \text{RUD (mean peak value)} \times \text{FIR body wt}^{-1} \times \text{MAF} \times \text{TWA} \quad (2)$$

The MAF value depends on the application interval and the number of applications (MAF for two applications and an application interval of 14 d = 1.2 for acute risk assessment and 1.4 for reproductive risk assessment) [1].

The diet and by that the RUDs differ among the bat species recorded in the examined orchard. In the following, small flying insects are considered to constitute equal shares of small moths and other small flying insects such as flies, beetles, and midges. *Pipistrellus pipistrellus* is known to feed unselectively on available flying insects (with a preference for midges) by aerial hawking [19] but may take some prey items by gleaning [20]. Therefore, we assumed diet compositions consisting mainly of small flying insects and a 5 to 10% fraction of foliage-dwelling arthropods. *Myotis mystacinus* takes swarming small insects (mainly midges) by hawking, but the inclusion of many non-volant prey items indicates also a gleaning habitat [20], while *M. nattereri* is considered to take its prey mainly by gleaning [21]. Hence, the diet of *M. mystacinus* is assumed to consist of 40 to 50% small flying insects, with the remaining 50 to 60% being foliage-dwelling arthropods, and that of *M. nattereri* is assumed to consist of 20 to 30% small flying insects, with the remaining 70 to 80% being foliage-dwelling arthropods. Species of the *Nyctalus-Eptesicus* group are adapted for open-air foraging. Small flying insects such as midges are the main prey for all three species, but moths are also important constituents of the prey of *N. noctula* and *E. serotinus* [20]. To assess the dietary exposure of the group *Nyctalus-Eptesicus*, we considered combinations of small flying insects and large moths, with shares for each prey category between 25 and 75%.

Due to the high energetic cost of aerial foraging, bats require high daily FIRs, estimated as being 70% of their body weight [22]. Pregnancy and lactation are additional energy-demanding processes and require an increase in the FIR of females to up to 85% of the body weight during the lactation phase [23]. Considering that the application took place in the pregnancy and lactation period of bats, we assumed an FIR per body weight of 85%.

The TER values for acute dietary and reproductive risk assessment were calculated as the ratio of toxicity endpoints to the exposure [1].

$$\text{TERacut} = \text{LD50}/\text{DDD} \quad (3)$$

$$\text{TERrep} = \text{NOAEC}/\text{DDD} \quad (4)$$

If the TER is larger than the trigger values (10 for acute and 5 for reproductive risk assessment), the risk is considered to be low [1]. The toxicity values for fenoxycarb used in the calculations were the LD50 (rat, *Rattus norvegicus*; >10,000 mg/kg body wt) and the most sensitive NOAEL determined for fenoxycarb (NOAEL = 5.3 mg/kg body wt/d for long-term study with mice, *Mus musculus*) [24]. No risk-assessment approach was performed for chlorpyrifos-methyl because the measured residues were limited to foliage-dwelling arthropods with the aim of comparing the RUD values of two different pesticides on the same arthropod group.

In the first-tier risk assessment, it is assumed that individuals collect all their food in the treated area (worst-case scenario). In reality, individuals foraging in the agricultural landscape may visit a variety of habitats within a single night and may obtain their food also in a variety of nonagricultural habitats. To consider this, there are possibilities of using more realistic estimates of the proportion of an animal's daily diet obtained in the habitat treated with pesticides in higher-tier risk assessment [1]. Bat activity data obtained by acoustic detection do not allow any conclusions about the amount of time an individual stayed at the examined site. However, following the literature, *P. pipistrellus*, *M. nattereri*, and *M. mystacinus* forage in up to 2.4 [17], 6 [16], and 12 [16] different foraging areas per night, respectively. If we assume that each foraging area is used in the same proportion, and in a best-case scenario, only one sprayed orchard site is used per night, 42, 17, and 8% of the daily food intake of an individual of, respectively, *P. pipistrellus*, *M. nattereri*, and *M. mystacinus* are likely to be contaminated with pesticides. The species of the group *Nyctalus-Eptesicus* are known to feed in extensive foraging areas and to use only very profitable foraging beats such as ponds intensively [16]. We therefore assume that members of that group spend less time in the orchard than all the other species discussed above (i.e., obtain <8% of their food from the treated area).

RESULTS

Bat activity

The most common bat species recorded in the study orchard was the common pipistrelle (*P. pipistrellus*). The serotine (*E. serotinus*), Leisler's bat (*N. leisleri*), and the noctule (*N. noctula*), here compiled as *Nyctalus-Eptesicus*, and the whiskered bat (*M. mystacinus*) and Natterer's bat (*M. nattereri*), which were assigned to the group *Myotis mys-nat*, were recorded frequently (Table 1). All these species were also recorded in the nonagricultural habitats (Table 2). Bechstein's bat (*Myotis bechsteinii*), a species known to forage only within forests, was recorded on few occasions in the forest habitats (data not shown).

In the nonagricultural sites, high activity levels of *P. pipistrellus* were only recorded in the forest-edge habitats (Table 2). Activity levels of *P. pipistrellus* recorded at the orchard sites were on average six times lower than those at the forest edges but approximately 23 times higher than those of the forest and meadow sites (Tables 1 and 2). At two sites (Or1 and Or2) high activity levels comparable to the activity levels of the forest-edge habitats were demonstrated.

Table 1. Recorded mean bat activity (seconds per night) at the eight sampling sites of the study orchard (sites Or1–Or8)[a]

Orchard sites	Site Or1	Site Or2	Site Or3	Site Or4	Site Or5	Site Or6	Site Or7	Site Or8	Mean	SD
Pipistrellus pipistrellus	348.1	313.8	41.7	36.2	6.5	7.0	6.9	11.6	96.5	145.6
Myotis mys-nat	7.1	6.5	3.6	4.5	0.8	2.9	2.2	4.2	4.0	2.1
Nyctalus-Eptesicus	9.0	16.3	8.5	9.5	12.5	8.8	5.6	7.0	9.7	3.3

[a] Mean values are based on eight sampling nights. The resulting mean values for the habitat "orchard" and standard deviations (SDs) are given.

Table 2. Recorded mean bat activity (seconds per night) at the six sampling sites of the nonagricultural habitat sites[a,b]

			Mean	SD
	Site Fo1	Site Fo2		
Pipistrellus pipistrellus	1.5	7.0	4.3	3.9
Myotis mys-nat	4.8	6.0	5.4	0.9
Nyctalus-Eptesicus	0.2	4.7	2.4	3.2
	Site Ed1	Site Ed2		
Pipistrellus pipistrellus	299.4	923.9	611.7	441.6
Myotis mys-nat	17.4	19.9	18.7	1.7
Nyctalus-Eptesicus	8.0	25.3	16.7	12.2
	Site Me1	Site Me2		
Pipistrellus pipistrellus	6.9	1.5	4.2	3.9
Myotis mys-nat	0.9	0.3	0.6	0.4
Nyctalus-Eptesicus	4.4	35.8	20.1	22.2

[a] Mean values are based on three sampling nights. The resulting mean values for the habitats and standard deviations (SDs) are given.
[b] Forest sites Fo1, Fo2; forest-edge sites Ed1, Ed2; meadow sites Me1, Me2.

Individuals of the group *Nyctalus-Eptesicus* preferred the meadow and forest-edge sites and avoided the forest (Table 2). At the orchard sites, activity levels of that group were on average between those of the preferred nonagricultural sites, the meadow (two times lower), and the avoided forest sites (four times higher) (Tables 1 and 2).

Individuals of *Myotis mys-nat* showed a strong preference for forest-edge habitats and an avoidance of the meadow sites (Table 2). Individuals recorded at the orchard sites showed also activity levels between those of the preferred nonagricultural sites, the forest-edge sites (four to five times lower), and the avoided meadow sites (six to seven times higher) (Tables 1 and 2).

In the orchard, the highest activity levels of *P. pipistrellus* (1,435.0 s night^{-1}, site Or1) and *Myotis mys-nat* (18.4 s night^{-1}, site Or1) were recorded on the night following the second application of fenoxycarb (day 0) and that of *Nyctalus-Eptesicus* (33.5 s night^{-1}, site Or5) on the night of day 4 of the first application.

Insecticide residue on arthropods

The RUD values determined for the different arthropod samples are provided in Table 3. The RUDs of all examined arthropod groups reached their peak in the samples taken directly after the application (day 0) with the exception of small moth samples, which revealed higher residue on day 1 postapplication. Foliage-dwelling arthropods exhibited the highest peak values, 20 to 50 times higher than in the other arthropod groups. With time the residues decreased in all of the arthropod groups. The RUDs measured at day 8 decreased to one-tenth for small moths, one-twentieth for other small flying insects, one-sixtieth for foliage-dwelling arthropods, and at least one-one hundred and thirtieth for large moths compared to the respective peak values. The RUDs of large moth samples were below the quantification limit of 0.002 mg kg^{-1} from day 8 postapplication onward.

The initial value of fenoxycarb for foliage-dwelling arthropods was 13 to 31 times higher than the respective average value for chlorpyrifos-methyl. The initial value of chlorpyrifos-methyl decreased to one-tenth of the initial value within 8 d.

Risk assessment

The calculated TER values of the acute risk-assessment approach of fenoxycarb for the examined combination of species and assumed diet compositions were approximately 61 to 2,254 and 357 to 32,680 times higher than the trigger value of 10 in the worst-case (individuals collect all their food in the treated area) and best-case (individuals collect their food not only in the treated area but in a species-specific number of different foraging areas) scenarios, respectively (Table 4). The TER values of the reproductive risk assessment were below the trigger value of 5 for *M. mystacinus* and *M. nattereri* under the complete range of assumed dietary compositions (Table 4) in the worst-case scenarios. For *P. pipistrellus*, the TER trigger

Table 3. Residue per unit dose (RUD) values (mg kg^{-1} kg a.i. ha^{-1}) measured from different nocturnal arthropod samples collected from an orchard following two fenoxycarb and one chlorpyrifos-methyl applications[a]

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 8	Day 12
Fenoxycarb							
Small flying insects							
1. Application	2.90[b]	1.04	0.28	0.31	0.29	0.11	0.04
2. Application	2.20	1.75	0.21	0.49	0.37	0.11	0.06
Mean	2.55[c]	1.40	0.25	0.40	0.33	0.11	0.05
Small moths							
1. Application	No data	4.05	0.91	1.65	0.62	0.64	0.07
2. Application	4.92	7.28[b]	No data	3.68	2.51	0.37	No data
Mean		5.67[c]		2.67	1.57	0.51	
Large moths							
1. Application	2.21[b]	0.89	0.13	0.45	0.14	<0.002[d]	<0.002[d]
2. Application	1.34	0.88	0.21	0.12	0.01	<0.002[d]	<0.002[d]
Mean	1.77[c]	0.88	0.17	0.28	0.08	<0.002	<0.002
Foliage-dwelling arthropods							
1. Application	57.52	10.8	11.55	2.71	1.61	1.56	No data
2. Application	133.15[b]	27.37	8.51	3.47	2.25	1.76	No data
Mean	95.33[c]	18.72	10.03	3.09	1.93	1.66	
Chlorpyrifos-methyl							
Foliage-dwelling arthropods							
1. Application	4.34	No data	No data	No data	No data	0.15	No data

[a] In the performed risk-assessment approach, small flying insects are considered to constitute equal shares of small moths and other small flying insects.
[b] Maximum values were used for acute risk assessments.
[c] Maximum mean values were used for reproductive risk assessments.
[d] Values were below the quantification limit of 0.002 mg/kg.

Table 4. Toxicity exposure ratios (TERs) of fenoxycarb for several bat species and species groups based on their assumed diet compositions[a]

		Acute risk assessment				Reproductive risk assessment			
		Worst case		Best case		Worst case		Best case	
Species	Range of assumed diet	RUD	TERacut	RUD	TERacut	RUD	TERrepro	RUD	TERrepro
Pipistrellus pipistrellus									
	95% flying insects, 5% foliage-arthropods	11.5	5,683.4	4.8	13,616.6	8.7	6.4	3.6	15.6
	90% flying insects, 10% foliage-arthropods	17.9	3,651.4	7.5	8,714.6	13.2	4.2[b]	5.6	10.0
Myotis mystacinus									
	50% flying insects, 50% foliage-arthropods	69.2	944.5	5.5	11,883.5	49.7	1.1[b]	4.0	14.0
	40% flying insects, 60% foliage-arthropods	82.0	797.1	6.6	9,903.0	58.8	1.0[b]	4.7	11.9
Myotis nattereri									
	30% flying insects, 70% foliage-arthropods	94.8	689.4	16.1	4,059.6	67.9	0.8[b]	11.5	4.9[b]
	20% flying insects, 80% foliage-arthropods	107.6	607.4	18.3	3,571.6	77.1	0.7[b]	13.1	4.3[b]
Nyctalus-Eptesicus									
	25% flying insects, 75% large moths	2.9	22,537.8	0.2	326,797.4	2.4	23.3	0.2	280.1
	75% flying insects, 25% large moths	4.4	14,854.4	0.4	163,398.7	3.5	16.0	0.3	186.7

[a] The combinations of prey groups resulting in the lowest and highest residue per unit dose (RUD) values within the range of assumed species-specific diet compositions are shown. The peak RUD and the mean peak RUD were used for the acute and reproductive risk assessment approaches, respectively. Small flying insects are considered to constitute equal shares of small moths and other small flying insects. In the worst-case scenario it is assumed that the individuals collect all their food in the treated area. In the best-case scenario it is assumed that individuals use a species-specific number of different foraging habitats per night.

[b] TER values indicate that they are below the trigger value (10 for acute risk assessment, 5 for reproductive risk assessment).

value of five was reached in the worst-case scenario for dietary compositions which included approximately 7.5% foliage-dwelling arthropods (Table 4). The assumed range of possible diet compositions for the group *Nyctalus-Eptesicus* did not result in a TER value below the trigger value. Under the best-case assumption only the TER value of *M. nattereri* remained below the trigger value (Table 4).

DISCUSSION

Evidence of pesticide exposure to bats

Estimating the risk of pesticides to bats requires linking the occurrence of contaminated food items and the extent of foraging activity. When comparing recorded bat activity levels of the examined orchard to activity levels in nearby habitats known to be used for foraging, we could verify that bats generally used the orchard for foraging during the time period of the fenoxycarb applications. The highest activity levels proven at the orchard for *P. pipistrellus* and *Myotis myst-nat* were recorded in the night (including dusk) following the second application of fenoxycarb, which lasted until dusk. This indicates that bats were not disturbed by the agricultural activity (e.g., tractor application). Considering that most arthropod groups revealed peak residue values on the night following application, avoidance of food items with pesticide residues seems unlikely.

For *P. pipistrellus*, known as a generalist in exploiting foraging habitats [25], we recorded especially high activity at the forest-edge and two orchard sites. Edges of deciduous forest offer flying zones and provide help for acoustic orientation, making them suitable foraging habitats for bats in general [26]. The two orchard sites with remarkably high activity levels (Or1 and Or2, Fig. 1) offered a free flying zone and shelter from all sites. Therefore, this orchard area appeared to exhibit structural features beneficial for foraging comparable to the forest edges.

Both *M. nattereri* and *M. mystacinus* showed a similar use of the orchard and forest sites, while forest edges were strongly preferred and meadow sites avoided. These results are consistent with the literature, which states that both bat species feed partly by gleaning arthropods from vegetation [20,21]. Furthermore, they are reported to forage along vegetation edges, in orchards and forests [16].

The activity levels measured in the orchard sites for the *Eptesicus-Nyctalus* group, which is adapted to open-air foraging and known to use a wide range of habitats [25], were lower than those in the preferred habitat, the meadows, but higher than those in the forest sites.

Insecticide residue on food items of bats

Foliage-dwelling arthropods exhibited the highest initial residue values. Apart from the exposure during application, it is likely that they experienced additional exposure by crawling on fresh residues on leaf surfaces directly after application.

The surface-to-volume ratio explains the lower initial residue values of the large moths compared to small moths and other small flying insects. Different from other arthropods, the wings of moths (and butterflies) are covered with high numbers of scales. The extensive surface of these scales results in a larger exposure surface and caused higher initial residue values of samples of small moths compared to those of other small flying insects, which were of comparable body sizes. Residue values also depend on the mode of action of the applied pesticide as shown by the differences in the measured initial residues of chlorpyrifos-methyl and fenoxycarb on foliage-dwelling arthropods. Chlorpyrifos-methyl is an acetylcholinesterase inhibitor [27], and most of the arthropods that receive direct exposure die soon thereafter. Thus, following an application, the surviving arthropods that were collected showed low residues. Fenoxycarb, contrarily, is a juvenile hormone mimic in insects, acting as a growth disrupter, and does not kill adult insects but targets juvenile life stages [27]. Therefore, after application of fenoxycarb, up to 31 times higher residue levels were demonstrated in the present study.

The observed decline of arthropod residues over time depends on the persistence of the compound as well as on the dilution of contaminated arthropods with uncontaminated ones. The latter depends on the number of hatching individuals

after the application and the ratio of emigration and immigration of arthropod individuals into the orchard. Our results demonstrated the fastest decline in samples of large moths, a group known to move distances of some 100 m per night [28]. The slowest decline was recorded for samples of small moth species, mainly comprising pest species adapted to reproduce in apple orchards (such as the codling moth) and, therefore, not expected to move out of the orchards. The TWA default value used in the current risk-assessment approach assumes a DT50 of 10 d [1]. Hence, the exposure of bats to fenoxycarb and chlorpyrifos-methyl may be overestimated in the present reproductive risk-assessment approach. However, it is possible that other compounds may be more persistent and exhibit similar initial residue values but slower declines.

In conclusion, the initial value and the residue decline of a particular arthropod group (e.g., flying insects) in a particular crop depends on the arthropod type (e.g., moths or flies in the case of flying insects), their surface-to-volume ratio, their mobility, and the mode of action and persistence of the applied pesticide. Taking this information into account will result in more realistic risk quantification for oral exposure to mammals and birds and especially bats, the only European mammals feeding on vegetation arthropods and flying insects. The guidance document for risk assessment [1] provides only RUD values for ground-dwelling and foliage-dwelling invertebrates but not for any flying insects. Compared to the generic peak RUD value for foliage-dwelling arthropods (mean = 21 mg kg^{-1} kg ha^{-1}) [1], the peak values obtained in the present study for the same arthropod group were more than four times higher for fenoxycarb (mean = 95.3 mg kg^{-1} kg ha^{-1}) and five times lower for chlorpyrifos-methyl (4.3 mg kg^{-1} kg ha^{-1}). The EFSA values are said to be based on several studies and do not allow any conclusion of the examined arthropod type, their surface-to-volume ratio, the type of pesticide applied (e.g., fungicide or insecticide), the time of application, and the mode of action of the respective pesticide. Those generalized estimates of residue concentration on arthropods introduce uncertainty into the risk assessment.

Risk assessment

Using our feeding guild–specific RUDs, the first-tier approach of the acute dietary risk assessment indicated a low risk for all examined scenarios. However, there is uncertainty if the applied safety factor of 10 used in the TER approach of acute toxicity accounts for interspecific variability in sensitivity [29,30]. Based on LD50 values for two organophosphate insecticides, which were shown to be higher for bats than for laboratory mice, bats are not thought to be more sensitive to pesticides than other mammals in terms of acute dietary toxicology [31,32]. On the other hand, the same authors stated that the surviving bats of those experiments had a more prolonged period of loss of coordination than the laboratory mice. Further research on the acute risk of other pesticide groups to bats would be needed for a more profound conclusion. However, given the high TER values we obtained even under the assumption that individuals were feeding exclusively in the treated field, an acute dietary risk of fenoxycarb appears unlikely.

The TER approach of the reproductive risk assessment indicated unacceptable risk under the worst-case assumption for both *Myotis* species and *P. pipistrellus* but not for the members of the *Nyctalus-Eptesicus* group. All species with a potential risk were assumed to obtain parts of their diet by gleaning foliage-dwelling arthropods, the arthropod group that exhibited the highest residue values by far. The extent of gleaning is not known for *P. pipistrellus*, but our calculation indicated that shares of approximately 7.5% and more would result in potential risk.

To calculate a refined TER, assumptions were made about the minimal time (best-case scenario) an individual of a particular bat species feeds in the orchard. These assumptions are speculative and radiotelemetry should be carried out to gain insight into bat foraging habits and to enable a more realistic risk-evaluation process. However, our approach helps to place the TER values obtained under assumed best-case scenarios in relation to the trigger value. For *M. nattereri*, the refined TER values were still below the trigger value of 5, while values for *M. mystacinus* and *P. pipistrellus* ranged between 10.0 and 15.6.

The justification of the applied trigger value of 5 for reproductive risk assessment to account for between-species variation in toxicity has also been criticized [33], especially because the information on chronic effects on mammals is to a great extent based on representatives of only one order, the rodents (rat and mouse). Considering that Luttik et al. [33] suggested that interspecies variability for chronic toxicity is at least as variable as that for acute toxicity, for which safety factors up to 15 for mammals were proposed [30], we cannot exclude a reproductive risk even under the assumed best-case scenario for *M. mystacinus* and *P. pipistrellus*.

No endpoints from reproductive toxicity studies of bats are available to allow any deduction on differences in sensitivity compared to other mammals. Moreover, no conclusion can be drawn from LD50 values because it has been demonstrated that the relative sensitivity established from acute tests could be reversed in the case of long-term toxicity as shown for two bird species [33]. However, bats may be especially sensitive to pesticides due to their ecological traits [34]. They differ in many aspects from rodents commonly used in laboratory tests and from shrews used as a surrogate for insectivores requiring high food-intake rates. Most bat species have long life spans and therefore more time for contact with, or accumulation of, dangerous levels of pesticides [31]. Their low reproductive rates (usually a single offspring per year) require high adult survival to avoid population declines [35] and dictate slow recovery of impacted populations. Bats also differ from rodents and other insectivorous mammals such as shrews by physiological constraints due to hibernation and migration. Lipophilic pesticides can have a detrimental effect by accumulating in the stored fat due to the consumption of arthropods contaminated with pesticides. When fat is metabolized during hibernation or migration, pesticide concentrations can reach high and toxic levels, especially in the brain [31]. Moreover, substances that could increase metabolic rates may affect bats that rely on lowered metabolic rates during daily torpor by disrupting energy budgets [9]. These life-history traits can render bat populations more susceptible to long-term effects of pesticides than other mammals.

Additional uncertainties

In the current risk-assessment approach, it is assumed that exposure to pesticides occurs exclusively via diet and not through skin contact or inhalation, although such routes may be relevant under field conditions [36]. Compared to day-active mammals, a higher risk with regard to direct inhalation and dermal exposure may exist for bats as it is common practice to apply pesticides at dusk to avoid, for example, effects on honeybees. Moreover, our results demonstrated that bats were not disturbed by machinery during the application.

Birds and mammals in general may encounter a mixture of different active substances, applied to different crops at different times. This may cause a risk that is not considered to date. Other than the currently used deterministic calculation of toxicity to exposure ratios of one compound, ecological models can integrate factors such as landscape structures, timing of different application, and ecological traits of the organism, and may have the potential to become important tools for the prediction of long-term effects on a landscape scale for birds and mammals in general [37] and bats in particular.

CONCLUSION

For the first time exposure and potential reproductive risk of several feeding guilds of European bats to pesticides were indicated by demonstrating foraging activity and simultaneously measuring residues of two insecticides on the respective food items. Given their ecological traits, bats are potentially more sensitive to reproductive effects of pesticides than other mammals. Therefore, we strongly suggest consideration of bats in the risk-assessment scheme for pesticides and a thorough research program to investigate the effects of different pesticides on the different feeding guilds of bats on a landscape scale.

Acknowledgement—The analysis of residue samples was funded by the German Federal Ministry for Environmental, Nature, and Nuclear Safety through EUROBATS. We thank L. Roos for laboratory work and B. Weber and S. Stehle for useful comments on the manuscript.

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