Appendix 26. Endocrine mediated effects on birds and mammal reproduction

Annegaaik Leopold and Kees Romijn

In the context of reproductive risk assessment for birds and mammals endocrine disrupting substances can be defined as substances that cause effects on bird and mammal reproduction through the disruption of endocrine processes. The environmental risk assessment performed under the current guidance document is based on the ecological relevance of the observed effects, independent of the mode of action that are (may be) responsible for such effects. Therefore the general procedure for risk assessment can also be used for endocrine disruptors.

If a substance is suspected of being a potential endocrine disruptor (e.g. through information from studies with mammals or other taxa, from the field or from findings with related compounds), screening tests may have been performed to address the issue.

In the case of mammals a number of in vitro and in vivo screening tests for assessing endocrine disrupting properties in mammals have become available in recent years and are in various stages of (pre-) validation (1, 2, 3, 4). In addition the mammalian multi-generation study performed as a standard requirement for pesticide risk assessment, covers the entire reproductive cycle and therefore is able to provide information on overall productivity at the population level. In addition to mammalian screens there are also fish and amphibian screens that can address the question whether a material is likely to be an endocrine disruptor or not and what its mode of action might be (5,6). This information should also be taken into account for the assessment in a weight of evidence approach.

Mammals and birds have similar hormones, hormone receptors and fundamental feedback mechanisms. However, one important difference between mammals and birds lies in the mechanism of sex differentiation. Both testosterone and estradiol, in appropriate relative concentrations, are required for reproductive development in birds (7, 8). In mammals, however, embryos require sufficient levels of androgens to induce gonadal differentiation into testicular tissue.

Other important differences involving hormonal systems include the laying of hard shelled eggs that must be incubated and the requirement for a great expenditure of energy by the chick during hatching. Exposure of birds to oestrogen, androgen, and thyroid agonists, mimics, and antagonists may influence sexual differentiation, and in addition, may influence growth and behaviours such as nest building, parental care, and migration (9). Although there are analogous processes and behaviours in mammals, the influence of substances on mammals may differ from those observed in birds. Furthermore, some of these endocrine related endpoints may not be evaluated easily in mammals. Therefore, in the process of evaluating the extent, nature and ecological impact of the effect of a potential endocrine disruptor in birds, mammalian toxicity data are of limited predictive value and cannot routinely be used to address the risk for birds.
As discussed, screens are available for mammalian systems; however there are no avian screens available to date that have been widely accepted. The avian one-generation reproduction test provides information on adult bodyweight, egg production, fertility, embryonic death, hatch rate, chick bodyweight and survival. None of these endpoints provide conclusive information about whether the effects seen result from endocrine disruption or from other modes of action. These studies may therefore serve only as a weight of evidence approach, and may provide information that is value for further testing. A few one-generation avian studies performed with Japanese quail have included specific endocrine endpoints such as spermatid count, hormones, sex ratio (10). However, even with the inclusion of these “endocrine endpoints” may still not provide conclusive information on endocrine mediated effects on reproduction that allows concluding a risk-assessment. One of the reasons is that the one-generation reproduction study does not include exposure during all relevant stages of the bird’s development or the measurement of other relevant endocrine-sensitive endpoints such as behaviour.

In order to confirm the potential for effects seen in screening or an avian one-generation study it may be necessary to assess the effects of the substance upon endocrine mediated processes during the organisation, maturation, and activation of endocrine systems. It is also necessary to take into consideration the potential impact of the substance upon other endocrine mediated processes and behaviours that play a role in the bird’s life cycle. It is therefore recommended that if there is a concern from the mammalian of fish/amphibian screens or from any other source to carefully consider what the mode of action of concern is likely to be.

A test design aimed specifically at endocrine effects that is currently under discussion in an EPA-led OECD process of pre-validation is a two-generation study with Japanese quail (11). The proposed test design uses the precocial species Japanese quail (Coturnix japonica). The ultimate objective of the test is still to be determined. Whether its primary aim is to provide an integrative endpoint (such as number of chick survivors in the 2nd generation) to determine impact of a potential endocrine disruptor at the test population level, or whether its primary aim would be to characterize dose-response relationships, and both immediate and latent adverse consequences associated with exposure to potential endocrine disrupting compounds, is yet to be determined. The exposure in the proposed test design begins prior to sexual maturation, at 4 weeks of age, and continues through the initial offspring generation (F1). The second generation (F2) is not exposed. Robustness, reproducibility, statistical power and practicability of this test design are yet to be determined.

It is necessary, that in the next few years, other more directed smaller tests be developed alongside the avian two-generation test. These should allow the evaluation of the impact of a potential endocrine disrupting chemical on a specific portion of the avian life cycle and its associated endpoints. Such smaller tests focussing on specific endpoints may be more sensitive to evaluating the potential endocrine effect of a substance than a two-generation study, because the range of concentrations can focus around a specific endpoint. For example, for the purpose of the risk assessment, it may be equally important to assess the impact of a potential endocrine disruptor on parental care and nesting behaviour in an altricial species following in ovo exposure and during development, as it is to evaluate standard reproduction parameters. In addition there is no attention in the two-generation study for male behaviours such as territoriality and mounting behaviour, or for female behaviours such as brooding. All these aspects need to be considered in future method development. Individual studies of this nature have been performed (11), but no test protocols have been developed yet.

In designing more targeted tests it is essential to determine which species is most suitable to study. Avian species diverge in their patterns of reproduction, development, behaviour, and endocrine function. Therefore, the selection of test species to evaluate the impact of a potential endocrine disruptor is an important issue to consider. One approach for dealing with the
diversity of avian species is to group birds to either precocial or altricial species. Broadly speaking, these two groups of bird species display great differences in their reproductive strategies and in the timing of maximum steroid sensitivities (7). Precocial and altricial species are distinguished based on the degree of development of young at the time of hatching and on the extent of parental care that is required by the hatchlings. Experiments with embryonic precocial birds in which gonadal steroid levels were altered have demonstrated that steroid exposure during critical embryonic periods is necessary for sexual differentiation (13). On the other hand, altricial species appear to be less directly affected by embryonic exposure to steroids, and instead, are most responsive to steroid exposure during breeding. Evidence for this conclusion comes from studies of songbirds (altricial species) that have shown that the sexually dimorphic song of breeding birds is modified by steroid exposure (14, 15). Therefore if there are concerns about potential effects of chemical during sexual differentiation, precocial species may be more suitable. If, however, there is a concern about potential effects during breeding, altricial species may be more suitable.

The use of a precocial species for tests of the effects of potential endocrine disruptors on birds during their development seems appropriate since they are likely to be sensitive to changes in steroid concentrations during these developmental life stages. While altricial species may be less sensitive to the impacts of endocrine disruptors on their endocrine systems during embryonic development, they may have specific utility and be appropriate as a test species as adults.

When selecting the appropriate test design and the appropriate endpoints, it is essential to evaluate all the available information from avian and/or other species. Where the available information allows this, the likely mode of action and portion of the avian life-cycle likely to be the most sensitive should be identified and an appropriate test design selected. There is no single test design that should automatically be followed. In addition only sufficiently developed techniques to assess the various endpoints should be applied. While extensive work has been performed on a number of potentially relevant endpoints (11, 12), there is still a substantial amount of development and validation work that is required.

References


5. OECD: OECD Revised Draft report Phase 1B of the Validation of the 21-day Fish Assay for the Detection of Endocrine Active Substances.


Appendix 27. How to determine a focal species

If an active substance, and its associated product and use, fails Tier 1, it is possible to further refine the exposure element of risk via the use of a ‘focal species’. A ‘focal species’ is a real species that actually occurs in the crop when the pesticide is being used. The aim of using a ‘focal species’ is to add realism to the risk assessment insofar as the assessment is based on a real species that uses the crop. It is essential that the species actually occurs in the crop at a time when the pesticide is being applied. It is also essential that this species is considered to be representative of all other species from the feeding guild highlighted at the screening level and at Tier 1 that may occur in the crop at that time. As a ‘focal species’ needs to cover all species present in the crop, it is possible that there may be more than one ‘focal species’ per crop representing more than one feeding guild.

Determining a focal species

In order to determine a suitable ‘focal species’ it is necessary to carry out field work and presented below is a brief outline of the key issues to consider:

Selection of field sites: As for any field work it is necessary to select appropriate fields, in order to ascertain what species occur in the crop of concern. The crop studied should be the same as the one used in the risk assessment at the screening level and at Tier 1, it should also be at the same growth stage. It is also necessary to have a range of fields that are representative of where the pesticide is used or is intended to be used. This may be across relevant geographical and climatic regions or zone, within a MS if the pesticide is to be used in one MS, or if the pesticide is used across a range of MS, then it may be appropriate to have a selection of fields across MS. The key point is that the focal species selected should be appropriate for the risk assessment.

Experience has shown that the fields surveyed should be separated by at least 250 m so as to avoid any potential double counting. Cropping details of the fields studied as well as their surrounding habitats (e.g. what crops were being grown, presence of woodlands, hedgerows etc) should be included in the final report.

If data are only available from either one MS or a small selection of sites and the Notifier wishes to extrapolate to another, then it is necessary to justify its use. Justification can be based on a comparison of the agricultural landscape including size of fields, presences of hedgerows, field boundaries as well as climatic conditions. Likewise, if a Notifier wishes to extrapolate from one crop to a closely related crop, justification is required.

Survey techniques: Basically there are two techniques for birds – namely the transect method and the field survey method. These are described in more detail below:

The ‘transect method’: All bird species are recorded in the field by walking slowly along a defined longitudinal line transect, allowing for a clear view between the rows of crop
plants. Birds are recorded only within the ‘in-crop transect band’ as individual birds visually or acoustically registered (see Figure 1 for details).

‘In-crop transect band’: birds are recorded within a wide band, for example 50 m either side of the observer where the crop field was at least 100 m wide. For narrower fields the band considered could be narrowed and contain only the in-crop area (i.e. width of the crop field).

‘Outside transect area/band:’ no birds are recorded beyond the in-crop transect band. Depending on the width of the field the ‘outside transect band’ may include in-crop and off-crop habitat.

![Diagram of transect bands](image)

**Figure 1.** *Graduation of different areas within defined crop fields as applied by this focal species studies*

The ‘point count method’: With this method the observers survey the part of a field from a single location to avoid disturbing the birds. Both methods, i.e. field survey and transect methods, are complimentary to obtain unbiased census. It should be noted that this technique may be more appropriate for fields in the winter, freshly drilled fields or bare soil. This method is further described in Crocker and Irving (1999) or Bibby et al. (2000).

**Analysing the data:** the survey data may be analysed in a variety of ways, however in trying to determine ‘focal species’ the following information is considered to be most relevant:

**FOfield or frequency of observation in the field** – denotes the number of fields in which a defined species was recorded as percentage of the total number of fields regardless of the number of individuals observed. This approach serves as a measure for the spatial...
frequency of occurrence, or the proportion of fields a species is present on. A FOfield of 100% for one species indicates that this species was observed in all fields during at least one survey.

**FOsurvey or frequency of observation per survey** – denotes the number of surveys in which a defined species was recorded given as percentage of the total number of surveys. This approach gives an approximation for the temporal evenness of occurrence throughout the complete study period. This gives an indication of how widespread a species is and is considered to be an indication of ‘prevalence’. A FOsurvey of 100% means the species was recorded during each survey in every field with at least one individual.

**Selection of Focal Species:** The above gives an indication of what potential focal species may occur in the crop. Those species with a frequency of occurrence >20% might be considered to be of high priority especially if they have high dominance. However before deciding which species ‘covers’ all other species present on the field, it is necessary to consider issues such as feeding strata, food intake rate, body weight of potential focal species and diet to ensure that species with the highest potential exposure are considered. It should be noted that a focal species is not automatically the species that was most frequently seen in FOfield and/or FOsurvey.

The above is illustrated by an example where a swallow was recorded as being both prevalent and abundant in a certain crop at a certain time of year. But whilst is has a high intake to body weight ratio, and consumes small invertebrates it is not consuming invertebrates with residues on and hence is not protective of other species that may occur in the crop at the same time. Similarly, wood pigeons are potential focal species in sugar beet in the summer (see Crocker and Irving, 1999); however on the basis of its low food intake rate it is clear that the wood pigeon is not protective of other species, e.g. the skylark.

**References**


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1 Available at: [http://www.pesticides.gov.uk/uploadedfiles/Surveys_short1.pdf](http://www.pesticides.gov.uk/uploadedfiles/Surveys_short1.pdf)
Appendix 28. Recommendations on arthropod residue field studies to refine food residues in higher tiered bird and mammal risk assessments

Study conduction and interpretation

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Introduction

The aim of this document is to provide guidance on how to carry out an arthropod residue field study and considerations on how to interpret the results of a study for a higher tiered risk assessment. This guidance given in this document should not be seen as fixed as it may be more appropriate to design a specific study to address a specific issue highlighted during the initial risk assessment. In situations where there have been deviations from the recommendations made here a full justification and explanation should be given to explain why and how a study was conducted in a specific way and why the data can be used to refine the exposure different from Tier 2 scenarios in the Guidance Document (GD).

Laboratory versus field studies

As in many other areas of ecotoxicology it might be helpful to start with simpler laboratory studies, followed by semi-field approaches, before scheduling a field study as the highest tiered approach. However, it should be emphasised that it might be very difficult to simulate the processes relevant for residue levels in arthropods under laboratory conditions, especially if the time courses of residues are to be examined. Furthermore, laboratory studies are limited to a single species whereas field studies investigate the whole arthropod community, which together represents the potential food of insectivorous birds and mammals. The figures below show how the residue curves can differ for a compound which is non-toxic to arthropods. Results demonstrate the residue decline after over-spraying a single species in the laboratory compared to the data obtained from a field experiment, considering the whole arthropod community in the respective crop (NB: the curves shown below are hypothetical (generic) curves derived from a number of real studies; those studies normal contain protected data owned by specific companies). Due to food web interactions and environmental conditions the residue pattern obtained from the whole arthropod community in the field shows a higher maximum (accumulation) and a slower decline. However, in field studies where single species of arthropods were artificially exposed to applications, e.g. in cages, exposure conditions are
normally not comparable to those experienced by free-living arthropods under natural circumstances. Absolute residue levels (peak values) in single species tests tend to be lower (no accumulation in a food web) and residue decline can be faster (no consideration of inter-species interaction, different feeding strategies and metabolic processes). Thus a single species test is less representative compared to data obtained from the whole fauna and therefore, field studies should be preferred to laboratory and semi-field studies.

**Figure 1.** Hypothetical (generic) residue levels plotted as a function of time for a compound which is non-toxic to arthropods, (based on real studies). Due to confidentiality and data protection rules claimed with studies conducted by applicants no specific reference can be given.

**General remarks on the use of field residue data in refined exposure assessments**

Concerns are often raised over whether data from field studies where sample size is often limited can be used to replace worst case Tier 2 data. In principle, field data obtained under practical use conditions add a further level of realism to a risk evaluation. Furthermore, the replacement of RUDs for maximum residue levels is reasonable if data are more focussed on a particular application regimen, crop stage or geographical area. Also, as noted above, data from field studies may be suitable to describe residue declines over time under natural conditions, which are very difficult to obtain from laboratory studies. Both the definition of Tier 3 maximum residue values and as well data of residue decline under natural conditions could be derived from the same field study.

**Number of study sites and site selection**

When planning a residue field study it is clear that the number of study sites and the number of replicates within the study sites are the decisive factors for the significance of the data, i.e. the more test sites and replicates the more reliable the data. But this is often limited by several factors including the availability of suitable study sites and cooperative farmers, analytical capacities and available resources, so normally a study will be conducted at one test site.

Each test site will represent an individual residue value/time course, i.e. an individual study. Nevertheless, within each site it is desirable to have at least three replicates available to have information on intra-site variability of the residue values. The minimum size of each replicate within the test site should be approximately 1 ha, otherwise effects of immigration and
Risk assessment for birds & mammals

emigration may have an unrealistically high impact on the residue dynamics within the monitored arthropod community (i.e. sampling should be avoided in the border structures of a crop, e.g. the outer tree rows from an orchard).

The abundance of arthropods is one of the most important factors for the selection of suitable study sites. Since an orchard plot which has been intensively farmed for several decades, surrounded by other high production commercial orchards, may contain a very small arthropod community and will therefore be unsuitable for an arthropod residue study even if it is a typical site where several pesticides are used throughout the season. Conversely, a small orchard out of production, surrounded by a diverse woodland habitat may hold an enormous amount of arthropods but exchange with the surrounding source habitat may lead to an unrepresentatively fast dilution of individuals with a residue loading - so this is not a suitable situation for residue decline studies. Therefore it is absolutely critical to describe the orchard use history (e.g. planting or age, prior crop, treatments before study start, pesticides used) and also the surrounding landscape in detail (e.g. kind of crop or vegetation of the bordering areas, current aerial photos) to justify the selection of the study site and also to facilitate the discussion of observed residue decline patterns.

To ensure the maximum abundance of arthropods, no insecticides should be used during the study year and up to the termination of the study. Fungicides, which will not affect arthropod communities, can be used according to the usual application schedule. To prevent major habitat changes, no herbicides should be used during the trial unless it is necessary to assure a proper application of the test substance or the survival of the crop itself. Hence a balance between commercial practice and detrimental effects for the arthropod community should be achieved for the study site.

Application of the test item

The application(s) should be performed according to the recommendations of the product label and to good agricultural practice. Special attention should be given to the adjustment of the volume of the spray liquid towards the dimension of the crop, especially in orchards (tree size, tree spacing, row spacing etc.). All exact data and details of the application technique should be described in the study report. The water volume used for spray applications in the study should be justified and represent a typical commercial application.

Test organisms

Attention should focus on organisms likely to be consumed by the potential focal species. Therefore it is necessary to have information about the prey selection of the bird (and/or mammal) community inhabiting the study site from a study on 'portion of diet' (PD). If this information is available the division of sampled arthropods into classes, e.g. 'beetles', 'caterpillars', 'spiders' etc, can be useful. It is important that the fresh weight of the total of each of these groups should be recorded. Without this information it is not possible to reconstitute in the correct proportions the total residue for a bird which may feed on all these groups. The specific residue level and decline information could then be used to estimate a more refined exposure level based on dietary information for a suitable focal species. However, detailed information about the composition of the diet are rarely available and often the amount of sample matrix necessary for a proper residue analysis limiting the ability to divide arthropods into specific classes. If no information on dietary preferences of focal wildlife species are available it is recommended to collect sub samples of arthropods of the different foraging strata in a crop, e.g. foliage dwellers and ground dwellers, to have more information on residue levels of specific food groups. Since an insectivorous bird collecting arthropods from the tree canopy
may receive a different exposure level in an orchard when compared to a nocturnal shrew collecting ground dwelling arthropods in predominantly dense vegetation cover.

The division of arthropods into 'large' and 'small' classes is unnecessary, because the ecology of specific arthropod groups and the feeding ecology of the bird and mammal species concerned are the most significant factors.

Methodological considerations for sample methods in field studies

A main point always to be taken into consideration is the loss or increase of residues in the sample matrix based on methodological shortcomings. Desiccation of the sample matrix should be avoided by fast handling times and storage of the samples as soon as possible in a deep freezer or on dry ice. Another problem which can severely influence residue levels of the sample is cross contamination with other (non-arthropod) materials like soil particles or plant material. This will be discussed in more detail in the section on specific sample techniques below. Nevertheless it should be noted that so far no real comparative assessment of different sampling techniques with respect to their influence on the resulting residue levels is available. Only for suction sampling techniques (i.e. D-VAC sampling) it was proved that the residue data will be significantly biased by cross contamination from dust particles (SETAC, 2007). Accurate recording of the composition of each sample, e.g. the number of individuals in each of the various taxonomic groups present, is very important to explain specific data points. For example, if a sample consists of one large beetle and a few tiny spiders only, the residue level analysed very much describes the residue loadings only of the single large beetle.

Ground dwelling arthropods

The most practical method to collect ground dwelling arthropods is the use of pitfall traps. They have of course the disadvantage of collecting only active and moving individuals, but, on the other hand, pitfall traps are the only method to selectively collect only arthropods. Other methods, like suction sampling (e.g. with a D-Vac) have the huge disadvantage of severe cross contamination with soil-, plant- and other dust-particles potentially carrying often high residue loadings. Pitfall traps should be used without a preservation liquid (which would dissolve/wash off residues from arthropods) and should be emptied once at least every 24 hours. If the sample container of a pitfall trap is contaminated with water or soil material, e.g. during rainfall events samples should be discarded.

Arthropods should be killed with an ether-soaked paper after being recovered from the traps. Following the determination of suitable sub-fractions (if intended, see above) and weighing, the sample should be stored on dry ice or in a deep freezer. The number of pitfall traps used per sample site should be adjusted to the matrix mass necessary for residue determination in the analytical part of the study.

Foliage dwelling arthropods

Methods to collect foliage dwelling arthropods are most relevant in high crops like orchards, vineyards, hops. Field crops with sufficient plant material and arthropods inhabiting the plant layer may also be sampled successfully (e.g. in potatoes, cereals, some vegetables). In principle, the two most established methods for sampling foliage-dwelling arthropods are beating and inventory spray.

With beating, the leaves/plants/branches are beaten with a stick and the arthropods dropping

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down are captured in a large funnel. The disadvantage of this method is that some species (like flying ones) may escape instead of falling into the funnel. Other materials such as leaves, petals, bark, dust etc. will also fall into the sample container of the funnel. Thus, some sorting between arthropods and undesired material is necessary after the sampling event. This may prolong the handling time before the sample is stored in a freezer and the problem of desiccation of the arthropods arises (see above). The problem can, at least partly, be overcome by direct freezing of the samples and sorting under frozen conditions. Beating is however, only able to sample the parts of the plant readily accessible by hand. For example, it is not possible to sample the upper parts of fruit trees.

A more sophisticated method for sampling foliage dwelling arthropods is **inventory spraying.** Using this method, a number of plants are treated with a fast acting knock-down insecticide. The most common knock-down insecticides in current use are pyrethroids. Formerly, compounds such as Dichlorvos were also often used. Depending on the knock-down insecticide used this method has also a certain selectivity and not every arthropod inhabiting the respective plant foliage will fall down on the collection sheet. It is important to apply the knock down insecticide very gently and when there is no wind, to avoid disrupting the un-sampled parts of the study. After spraying the arthropods which have fallen from the leaf layer will be collected from sheets placed underneath the plants or trees. Dense cotton sheets acting like a sponge for the pesticide and the knock down substance when dripping from the treated foliage and avoid puddles in which arthropods can fall (resulting in changes of the residue loadings like with pitfall traps when preserve liquids are used), hence they are an optimal underlay. However, care must be taken when collecting the arthropods from the cotton sheet, because claws of beetles might get entangled and legs get pulled off – both resulting in an underestimation of residue levels. The best way is to collect the individual arthropods selectively from the sheets using tweezers or a small suction device, in order to avoid contamination with other material like leaves or pieces of bark. For some small-bodied arthropods such as aphids individual sampling with tweezers or a suction device is inappropriate and too slow resulting in desiccation; these can be carefully collected using a soft brush. Those samples should be kept and analysed separately if possible. The number of plants/trees used for one inventory spray sampling event should be also adjusted to the amount of sample matrix needed for residue determination. The plants should be randomly spread throughout each sample site and each plant/tree should be sampled only once during the study. The method requires some waiting time between inventory spray and collection of the arthropods until the spray liquid has dried. It is important to ensure that as much individuals as possible are knocked down and dropped on the collection device. The waiting time must be kept reasonably short (1-2 hours) and meanwhile, the arthropods should not be exposed to direct sunshine on the collection device to minimise the effect of desiccation. Subsequent sorting, partition into sub-samples and weighing must also be done immediately after the collection in order to transfer the samples as soon as possible into a freezer or on dry ice.

Sampling methods which differ from the two methods mentioned above may be used in some circumstances and for certain crops. However, for all these methods, clear descriptions are necessary to allow any possible influence of the methodology on residue levels to be assessed (e.g. cross contamination).

**Knock down samples during application**

It can be assumed for insecticides (and other pesticides with insecticidal side effects like some fungicides) the highest initial residue loading occurs on those arthropods which are killed during or immediately after application of the product. These individuals are normally missed during the sample events for foliage dwelling arthropods (because they are already dead and
have fallen on the ground) and will not be found in pitfall traps (because they can no longer move). It is unclear to what extent those arthropods are used as food items by birds and mammals. At least some reports can be found in the scientific literature describing the uptake of dead and/or moribund arthropods by birds². Thus, in principle this scenario should not be overlooked and a respective sample of those arthropods affected directly from the product application should be obtained whenever possible. In high crops (e.g. orchards) this can be easily achieved with a method similar to the inventory spray method used to collect foliage dwelling arthropods. The sampling devices (e.g. sheets) should be placed before the application and arthropods can be collected in a suitable time after the spraying, normally when the spray liquid has dried. Note that these collecting sheets should be covered at the time of spraying itself and the covers removed immediately afterwards to prevent the specimens being contaminated with further residues of the test item.

Number of samples and sample intervals

The number of samples analysed in parallel depends on the study site (size, structure, abundance of arthropods) and available capacities within the respective analytical facility. In order to get some information on intra-site variability of the residue levels at least three samples from each strata/sample method should be planned for each sampling date (n ≥3). Nevertheless, unexpected low masses of arthropods may force the pooling of samples to obtain sufficient matrix for residue analysis.

The general sampling scheme should be adjusted to the properties of the test substance and should be performed in such a way that the aims of the study can be achieved. In general, at least for spray applications, more sampling events should take place within the first three to six days after application, in order to obtain the maximum residue levels after application. If more than one application is being investigated then a sampling should also take place on the day before the next application. Some samples should also be obtained before the first application to adjust the sampling effort required for each method intended and to obtain reference matrix for the analytical laboratory.

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Table 1.  Example for a sampling schedule for a field study with two spray applications:

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Reporting and data interpretation

As every arthropod residue field study for the submission to a regulatory authority should be performed according to GLP the respective report must be comprehensible and should describe clearly the aim, all methods, deviations, encountered problems and results of the study. As the main results are normally initial residue values and / or time course of residues these data should be explicitly expressed in the study, if possible, including data on their variance. It should be considered that, regarding initial (maximum) residue values, the maximum is often found some time later - not immediately after application of the test substance (especially for substances non-toxic to arthropods may accumulate within the first few days after application). For a proper elucidation of the time courses of residues it is important to use an appropriate model to describe the residue decline. Normally it is not a first order kinetic, because several processes are interfering (e.g. a rapid decline of surface residues by abrasion / renewal of the wax layer of the cuticula of individuals with direct contamination during the application vs. systemic uptake via food and residue decline via metabolisation and excretion, which is often
much slower as well as immigration and emigration and population turnover). Thus, often a time weighted average approach (TWA), summarising the area under the curve is the most suitable method to describe longer-term residue patterns for arthropods. Nevertheless, for whatever method is chosen as most appropriate, clear evidence should be provided that this particular way of providing data for a refined exposure calculation is representing a realistic but sufficiently conservative approach to be suitable for a risk assessment.
Appendix 29. How to estimate PT

Joe Crocker & Magnus Wang

PT is defined as the proportion of an animal’s daily diet obtained in habitat treated with pesticide. As a worst-case (first tier assessment) it assumed that individuals find all their food in the treated area and that PT = 1. In reality, birds and mammals in the agricultural landscape may visit a variety of habitats within a single day, and not all of them may be treated with plant protection products. Therefore, in higher tier risk assessment it is recommended that more realistic estimates of PT be obtained for relevant species and crop scenarios.

It has not been possible so far to make direct measurements of the amount of treated food ingested by individual birds and mammals in the farming landscape. However, by radio-tracking, it is possible to make indirect estimates of PT. Radio-tracking can tell us how much time an individual spends in different habitats. If we assume a) that the amount of time spent by an animals in a given crop is directly proportional to the food eaten there and b) that the crop has been recently treated with pesticide, then we may assume that a bird which spends say 50% of its day in a given crop is likely to have 50% of its daily food intake contaminated with pesticide.

When considering how to use radio-tracking data to estimate PT, the risk assessor should be aware of some methodological and analytical questions.

1. Using radio-tracking contact time as an estimate of foraging time.
2. The selection of which individuals to radio-track and which to include in the estimate of PT.
3. How long to follow individuals?
4. How to use PT in deterministic worst-case calculations?

1. Radio-tracking contact time as an estimate of foraging time.

Because PT is intended to be a measure of exposure to pesticides through the consumption of contaminated food, then radio-tracking data are more likely to be a good estimate of PT if they distinguish between time spent in crop where the animal is active and potentially foraging from time spent that is inactive or engaged in non-foraging activity (e.g. singing, nest building, burrowing). For example a blackbird may spend a large part of its day in a hedgerow but be relatively inactive there, using it principally as a refuge and only leaving it for short periods when it searches intensively within a crop for food items. Ideally, PT should be expressed as the amount of (potential) foraging time in the crop expressed as a proportion of the total time spent (potentially) foraging in the day.
2. Selection of which animals to radio-track and which to include in the estimate of PT

This is essentially a question for the risk manager. Pesticide risk assessments usually concern a particular pesticide used on a particular crop and the possible dangers presented to a particular wildlife species.

In choosing which individuals to radio-track we might:

a) Focus on the crop and radio-track only those individuals that were caught in the target crop.

b) Focus on the species and radio-track individuals captured in local farmland habitats where they are most abundant.

In estimating PT from radio-tracking data, we might:

c) Include only those individuals that foraged in the target crop – (“consumers only” group).

d) Include all individuals with sufficient radio-tracking data.

PT estimated from group (a) (crop caught individuals) is likely to be higher (more conservative) than that from group (b) where individuals may visit a variety of habitats. For example the woodpigeon is a very common bird on UK arable land and may often be seen on fields of wheat in summer. But it is much more frequently seen on oilseed rape. Estimating cereal PT for woodpigeons by including only those caught on cereal fields will focus the risk to that sub-group of woodpigeons that use cereals but may be a rather unrepresentative sample of the broader woodpigeon population on arable land. On the other hand, estimating PT from the radio-tracking data obtained from all individuals in the general locality may give a better description of the exposure for a typical woodpigeon on arable land, (if the pattern of agriculture in the locality is also representative of the general pattern) but by including animals whose normal home range may not actually include the target crop, the risk assessment will be less conservative.

Having decided to focus on the particular group around the target crop (a) or the more general population in the locality (b), we need also to think whether we are interested primarily in the exposure of those birds that actually foraged in the target crop (i.e. group (c) excluding individuals where PT = 0) or whether we want to include birds that ignored or avoided the crop (i.e. group (d) including individuals where PT = 0). For birds or mammals that were actually caught in the crop (a) it could be argued that PT must necessarily be greater than zero, and that all radio-tracked individuals are therefore legitimate subjects. However, some animals may have been caught in crop margins as they moved along the (non-crop) hedgerow and the crop itself may have played no significant part in their foraging routine.

In general it would seem reasonable that for focal species caught in the crop, PT can be estimated from all individuals (whether they used the crop or not) whereas for the population caught in the general locality, PT should be estimated from only those individuals shown by radio-tracking to have used the crop (PT > 0). The inclusion or exclusion of individuals with PT = 0 is a trivial calculation so it may be advisable to compare the risk for both groups (c & d), regardless of whether the animals were caught in the target crop or outside it.

In addition to the different sampling bias on PT from focusing on wildlife species caught in particular target crops or species in a variety of farmland habitat, there may be practical issues to consider. Restricting the sample to those caught in the target crop has the advantage that it gives a focused sample but it may increase the effort required to capture a large enough sample size and each crop will need a new radio-tracking sample. For wildlife caught in the general...
locality, some individuals may visit a variety of crops and may legitimately be used to estimate PT for each of those crops.

3. How long to follow individuals

In acute pesticide risk assessments, possible dangers to wildlife are assessed over a presumed exposure of 1 day. Therefore the most appropriate time course for collecting radio-tracking data is a set of continuous observations covering all the hours in a single day that the species may be potentially foraging. Observations of less than one day can exaggerate extremes in animals’ choices of foraging habitats. For example, in an extreme case where an animal was observed for only a second then PT is likely to be either 0 or 1 and is unlikely to be an intermediate value because 1 second is not enough time for an individual to visit more than 1 habitat. Similarly, as crop maturity changes and food sources wax and wane, individuals followed for days or weeks will tend to show some drift in habitat use with time: some habitat averaging will occur and PT will be less likely to be 0 or 1. Therefore the ideal radio-tracking record will last all of a single day.

However, the behaviour of some species in some seasons may make it particularly difficult to obtain a continuous record of behaviour lasting a day. Linnets for example can make rapid flight of more than a kilometre staying only briefly at new sites and making it difficult for radio-trackers to keep track of their movements. Another reason why continuous observation for a single day may not be available for analysis is that the experimenter chose to sample individuals’ behaviour. For example the radio-tracking data collected by CSL and reported in Finch et al. (2006) aimed to collect observations for 1 hour in 2 of a typical day’s behaviour. This regime had a practical advantage in that it made it easier for the data to be collected by a single observer and enabled more than 1 individual bird or mammal to be tracked during the course of a day. But it has the disadvantage that it will push estimates of PT closer to 0 or 1.

The degree to which an observation time of less than a full day will exaggerate the extreme value of PT will depend on the length of typical observation time in relation to the frequency with the subject moves between habitats. For example if a blue tit moved between cropland and woodland every few minutes and this was a constant feature of its behaviour throughout the day, then an observation time of an hour or so may be more than sufficient to estimate its PT. But if the individual spent the morning in a crop and the afternoon in woodland then a single hour’s observation would most likely give a PT of 0 or 1, when the true value is 0.5. It is recommended that where radio-tracking reports less than a full day’s observations that the experimenter should:

a) Show that the general sampling regime is unlikely to introduce biases into the estimation of PT e.g. will not lead to greater sampling of the animal when it is in the crop, and does not favour particular times of day when the animal is engaged in particular behaviours.

b) Show that the shorter observation time is unlikely to have a significant bias on estimates of PT; or estimate the likely bias that shorter observation may have on the estimation of PT and correct it; or at least indicate whether the bias will have conservative or non-conservative effects on the risk assessment and allow the risk manager to decide if this is acceptable.

4. How to use PT in deterministic worst-case calculations

Having obtained estimates of PT for all individuals in the sample, we need then to replace the default value of 1 in the first tiers guidance document. If the PT of 1 was replaced by a median
or mean then this would suggest, in the absence of other safety factors, that the estimation of risk would be protective for only half the target population. The risk manager needs to decide what proportion of the population should be protected. In other words the risk manager should decide whether a reasonable worst-case is represented by a specific percentile of the population at risk.

How to estimate relevant percentiles and confidence bounds.

The simplest (non-parametric) way of estimating any centile is to rank the individuals in increasing order of PT and to choose the value of PT corresponding to the 90th centile individual, say. (Where there is no precise identity between an individual and the percentile of interest, we can interpolate between values of neighbouring individuals in the sequence.) A problem with this approach is that when the sample size is small (which it is for many of our radio-tracking scenarios) the value of any given centile may be very variable between samples. A better (parametric) estimate of the 90th percentile may be obtained by assuming that our data represent a random sample from a parent distribution with known mathematical properties. For many real-world measurements, statisticians assume that a sample comes from a normal distribution with parameters \( \mu \) and \( \sigma \) estimated by the mean and standard deviation of the sample. However, the normal distribution (with infinite upper and lower bounds) does not often provide a good fit for proportional data (limited between 0 and 1). Therefore we have used the Beta distribution as the most appropriate for describing PT.

For the calculation of confidence intervals, bootstrap methods are commonly applied (Efron and Tibshirani, 1993; Manly, 2001, Davison and Hinkley, 2003). They can be categorised as parametric or non-parametric bootstraps. Non-parametric bootstraps repeatedly resample from the same dataset and the results of such a procedure will be critically dependent on how representative is the underlying dataset. Small datasets are less likely to be representative and the confidence limits obtained by non-parametric bootstraps are likely to be underestimated. Therefore parametric bootstrapping may be preferable for small radio-tracking datasets. For the analysis of PT data the following approach is proposed:

1) From a field study \( n \) PT values are obtained, where \( n \) is the number of birds observed during one tracking session.

2) A beta distribution is fitted (distribution A) to all \( n \) PT values.

3) A random sample of sample size \( n \) is taken from distribution A.

4) Again, a beta distribution (B) is fitted to the new random sample.

5) From distribution B the 90th centile (or other estimate) of PT is calculated and recorded.

6) Steps 3 to 5 are repeated many times (e.g. 1000 times), each time a random sample of size \( n \) is taken from distribution A, a new beta distribution is fitted and the 90th centile is recorded.

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1 This is a simplified explanation that omits important assumptions about the most appropriate distribution to fit (e.g. Beta, Binomial, Uniform, or some mixture of distributions), what method of fitting to use, and how to decide what is a good fit. For fitting a beta distribution to specific data different statistical method are available (e.g. maximum likelihood estimation, method of moments). These methods can give quite different results depending on the mature of the underlying data. Therefore the goodness of fit should be checked either graphically by comparing plots of the data and fit as cumulative distribution functions and/or by calculating appropriate goodness of fit statistics (e.g. chi Square, Kolmogorov-Smirnov, Anderson-Darling) (See appendix 3 of Finch et al. (2006), Frey et al., 1999, Efron & Tibshirani 1993, Skylar & Smith 2003).
7) Finally, the upper $95^{th}$ (or other) one-sided confidence bound is calculated by ordering all 1000 estimates of the $90^{th}$ centile from low to high and picking the value of the $95^{th}$ place (or other) in the sequence.

Example Protocol

Detailed protocols of how to fit radio-transmitters and appropriate field practice for radio-tracking birds are given in Appendixes 1 and 2 of Crocker et al. 1998, and RifCon (2006). Examples of how the data may be analysed can be found in Appendices 1-3 of Finch et al., 2006, RifCon 2006, and Crocker et al., 1998. The following protocol summarises the essential points:

Telemetry

There are two purposes of the radio-tracking technique: (i) To locate a bird in order to observe its behaviour (‘radio surveillance’, Kenward, 2001) and (ii) to follow the bird continuously over a defined period (see below) in order to determine its exact location and any behavioural changes (‘continuous monitoring’, Kenward, 2001).

During the tracking session birds should be tracked continuously, i.e. a bird should be followed non-stop by car or by walking. Every change in behaviour (according to the categories in Table 1) and location (habitat and position) should be accurately recorded to the minute. Where the tracking session lasts a whole day then an exchange of observers may take place every few hours to ensure full attention of the persons tracking the birds. Where bird activity is sampled, the sampling regime should be designed to capture activity throughout the day, and trackers should follow the sampling regime irrespective of bird’s compliance i.e. sampling sessions should not be cut short because the bird has moved away or extended because the birds is easy to monitor (see Crocker et al., 1998, Appendix 1).

With the use of unidirectional Yagi-antennas it is possible to determine the location of the tracked bird. The signal strength also allows an estimation of the distance to the bird. In order to describe the behaviour of the tracked bird as accurately as possible and to verify its location, it the tracker always endeavours (if the bird is not hidden by vegetation) to observe the bird by visual contact and with optical devices (scope, binoculars). Moreover during visual contact it is possible to connect the signal quality of the radio tag to the observed behaviour of the bird. Hence, it may sometimes be possible to deduce the behaviour of the bird from the signal quality. Use of colour rings enables the observer to identify each bird with certainty. To ensure that the observer does not affect the behaviour of the bird, an appropriate ‘safe distance’ has to be maintained. Different species in different habitats may call for different safety distances. The idea is to follow the bird’s habitual movements rather than chase it about the landscape.

As a general rule, the aim should be to obtain data from at least 20 individuals for any given scenario in order to get an appropriate sample size. For acute risk assessments the data should reflect a single typical day in the life of a focal species under conditions when the target crop might be treated with a given pesticide. For long-term assessments observation of more than one day may be considered.

Calculation of PT in a specific crop

The calculation of PT assumes a correlation between the time spent by a bird in a particular habitat and the amount of food it ingested in that habitat. In other words, it is assumed that the amount of food taken by a bird in a certain time span will be the same in any habitat or crop.
within its home range. The ‘proportion of time foraging’ is thus assumed to be equivalent to the ‘proportion of diet obtained’.

At each telemetry session the proportion of diet obtained by an individual bird in a specific crop (PT) is calculated as the proportion of time the bird spent ‘potentially foraging’ in that crop. ‘Potential foraging time’ is thus the sum of the time intervals during which a bird showed any of the behaviour categories, ‘foraging’, or ‘active unknown’. All instances when the animal is known to be performing definitely non-foraging activities (e.g. singing, nest building) or when it is considered to be inactive are excluded from [for the calculation of] PT. For each tracking session the ‘time potentially foraging’ within the crop of concern is compared with the total ‘time potentially foraging’ in any habitat (see below).

To provide further behavioural details, e.g. to assess whether a bird is active but not foraging (see text below for details on behaviour categories), all recorded visual observations of radio-tracked birds are included in the evaluation.

<table>
<thead>
<tr>
<th>Table 1. Definition of behaviour categories (used for calculation of PT)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Potentially foraging</strong></td>
</tr>
<tr>
<td>All instances when the bird was foraging or might have been foraging.</td>
</tr>
<tr>
<td><strong>Active:</strong> unknown</td>
</tr>
<tr>
<td><strong>Not Foraging</strong></td>
</tr>
<tr>
<td>All instances where bird was inactive or clearly engaged in non-foraging activity.</td>
</tr>
<tr>
<td><strong>Active:</strong> other non-foraging</td>
</tr>
<tr>
<td><strong>Inactive</strong></td>
</tr>
</tbody>
</table>

During some of the telemetry sessions it may not always be possible to determine a bird’s location throughout the whole tracking session (i.e. whether it is in a specific crop or not). In such cases, the habitat should be recorded as ‘unknown’. In most cases, the corresponding time periods during which the habitat is unknown are rather short and may therefore be excluded from the data analysis. This approach is justified when assuming that there is an equal likelihood of determining a bird’s position in all habitat types in an agrarian landscape.
Example of PT calculation

1) Total time a bird is present in all known habitats including the ‘crop in focus’ during an individual tracking session:

<table>
<thead>
<tr>
<th>Behavioural category</th>
<th>Duration</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foraging</td>
<td>1.5 h</td>
<td>potentially foraging: 9 h</td>
</tr>
<tr>
<td>Active: unknown</td>
<td>7.5 h</td>
<td>time when foraging behaviour can be excluded: 7 h</td>
</tr>
<tr>
<td>Breeding</td>
<td>2 h</td>
<td></td>
</tr>
<tr>
<td>Active: other non-foraging</td>
<td>1 h</td>
<td></td>
</tr>
<tr>
<td>Inactive</td>
<td>4 h</td>
<td></td>
</tr>
<tr>
<td><strong>Total time in all known habitats</strong></td>
<td><strong>16 h</strong></td>
<td></td>
</tr>
</tbody>
</table>

This results in a 'potential foraging time' for the ‘crop in focus’ of **4 h**.

Individual PT calculated as

\[
\frac{\text{Potentially foraging time in the crop in focus}}{\text{Potentially foraging time in all known habitats}} = \frac{4}{9} = 0.44
\]

Example justification for using < 1 day of observation data^2

It was noted earlier that where individuals had been observed for less than a continuous full day, then the authors should show that this does not significantly affect their estimate of PT, or they should attempt to quantify any such bias arising. For the data obtained by example Finch et al. (2006) for a variety of arable and orchard scenarios typically tracked radio-tagged birds for 1 hour in 2 throughout the day. In the case of 17 yellowhammers monitored on cereal fields in summer this amounted to an average of 9.1 hours radio-tracking observation. The shortest observation time lasted 5.6 hours. We might expect that PT estimated from very short observation times would be significantly different from PT estimated from longer observation times.

Figure 1 shows the 90th centile PT and its 95th centile upper bound as calculated by the method described above. These estimates are based on the first 1 to 9 hours radio-tracking data for each bird.

It may be seen that the 90th centile PT changes noticeably over the first couple of hours of monitoring but then stabilises to a fairly constant value. Similarly the upper 95th confidence bound appears stable even when observation times are short. With the shortest yellowhammer observation time lasting 5.6 hours, it would seem that the sampling protocol does not, in this scenario, seriously affect the estimation of 90th centile PT and its upper confidence bound.

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^2 Where possible it is preferable to collect a full day’s observations. But if less than this is available then the data may still be useful in estimating PT provided they meet the criteria detailed in section 3a and 3b.
References


Appendix 30. How to determine bird and mammal diets

1 Introduction

At Tier 1, a worst case diet is used along with an indicator species to produce a screening step. If a pesticide fails Tier 1, then it is possible to refine the risk assessment via the use of Tier 2, this uses a more realistic scenario in terms of bird or mammal occurring in a treated crop along with a more realistic diet. The diet used at Tier 2 is based on publicly available data. Therefore if a compound fails Tier 2, it may be possible to refine the exposure component via revising the diet in two ways.

Firstly, it may be possible to revisit the publicly available data, providing that the studies on the diet of focal species are conducted in an appropriate landscape (crop of agricultural mosaic) and to a methodology considered to be equivalent to that outlined below. Alternatively, the diet of focal species can be determined via field work as outlined below.

2 How to determine a bird diet

An analysis of the diet of a bird can help to estimate the exposure of a bird to a plant protection product after application. Different food sources of birds may contain different residue levels. For example, when seeds are dressed with a fungicide and sown in a field they may contain higher residue levels than the arthropods living in that field. The risk for seed eating birds may then be greater than that for omnivores. Therefore, for a realistic estimation of the actual exposure to birds the respective proportion of these food items in the diet of a bird species must be examined.

Several methods for measuring the composition of the diet of birds are used. Direct monitoring of the birds' food selection is often hindered by vegetation or the observation distance. Therefore, alternative methods have to be considered. Video recordings at bird nests can offer an insight in the nestlings’ diet. However, the diet of nestlings may differ considerably from the diet of the adults. Another method is the application of neck collars to chicks in order to prevent food items to be swallowed. This method restricts the view to the analysis of the nestlings’ diet and is therefore not ideal methods for determining the diet of adult birds.

The investigation of faeces or stomach contents obtained via gastric lavage (stomach flushing) of adult birds is not subject to these constraints. For these approaches it is essential to be able to identify food items on the basis of diminutive remains found in faeces or stomach flushing samples. A considerable difficulty is the differential digestibility of different food types. Few remains may be found either because few items were eaten or because food items were almost completely digested. Calibration trials with captive birds can help to overcome this difficulty. Also, in some cases it may be possible to apply correction factors taken from the literature.

If radio-tracking is applied simultaneously to the collection of diet samples the source (e.g. a specific crop) of the food items found in the sample can be identified.
2.1 Test procedure

2.1.1 Bird trapping and sample collecting

In order to obtain an estimate of the diet of a focal species, it is necessary to trap birds using accepted methods (e.g. mist nets, whoosh nets, perch traps, spring traps), when they have access to the crop of concern. The study should also be done at the appropriate time of year. Nets and/or traps should be placed within or at least in close proximity to the target crop. The sites should be representative of where the pesticide is used or is intended to be used. This may be across relevant geographical and climatic regions, within a MS if the pesticide is to be used in one MS, within a zone, or if the pesticide is used across a range of MS, then it may be appropriate to have a selection of fields across MS.

Once caught it is possible to obtain a diet sample by obtaining faecal (after Brensing, 1977) and/or stomach flushing samples (modified after Ralph et al., 1985). Generally faecal sampling is favoured over stomach flushing as it is not intrusive and tends to give more reliable results (see e.g. Jenni et al., 1990). Therefore, it is recommended that stomach flushing should only be used if no faeces can be obtained.

2.1.1.1 Faeces sampling

For collecting faeces, birds can be kept in a clean bird bag or held over a polythene sheet during handling (Sutherland, 2004). Droppings can often also be collected in the field, e.g. where birds perch, roost and at nests. Faeces samples should be stored separately and can be preserved with sodium chloride. It is important to keep samples separate and not to pool them. Separation of the samples serves two purposes, to account for individual variability and apply correction factors to the food contents in order to take account of digestibility (see 2.4.1). Since these correction factors are derived from individual samples proper application requires separate storage and analysis of each sample.

2.1.1.2 Stomach sampling

A vaseline coated narrow plastic tube is inserted into the stomach and lukewarm water is pumped in the stomach through a syringe until the contents of the oesophagus and stomach are voided (Sutherland, 2004). The obtained sample is transferred in a sample container and preserved with alcohol. As for faeces sampling it is important not to pool the samples.

As stated above for faecal samples, it is important to keep samples separate and not to pool the samples.

2.2 Collection of reference material

For an accurate determination of the diet of a bird a “reference collection” is useful as it facilitates the identification of the taxa of the food items. Additionally, the collection of reference material or food items, (such as invertebrates, seeds, or plants) from the study area can help to estimate the original size of food items. As a rule un-digestible fractions of one food item are not obtained as a whole but rather as food fragments (“remains”). In order to minimize the uncertainty of the size estimation of food items a regression analysis of the dimension (size) of the potential food items and parts of these food items likely to be found within the samples can be conducted. Reference material, i.e. potential food items can be collected within the crop and the assumed home range of the birds.
2.3 Sample analysis

Food items are investigated via microscopic analysis (reflected light microscopy and transmission light microscopy; see e.g. Flinks and Pfeiffer, 1988). Insect remains can often be assigned at least to the family. The remains of other invertebrates can mostly be assigned at least to the class. For the determination of the green plant material, structures of the cuticle, particularly stomata, are considered. Seeds can be identified by analysing husk remains.

The size of characteristic parts of invertebrates or plants (e.g. chitin fragments of arthropods, setae of earthworms, fragments of seeds (pericarp), plant material, i.e. area of leaves and stems) can be measured with a measuring ocular. The obtained sizes can be compared to the specimens from a reference library.

In order to quantify the number of food items (e.g. number of arthropods) within each sample food fragments found in the sample are counted and the minimum number of individuals required to account for the number of assigned remains is calculated (see e.g. Jenny et al., 1990). For example, two right mandibles and one left mandible of a beetle species can be attributed to (at least) two individuals. In plant material, the number of fruits and seeds can be obtained by measuring the area of the fragments and dividing this figure by the area of a reference fruit or seed. From remains of leaves the area is measured and recorded.

The quality of the results obtained by the analysis of faeces or stomach flushing samples depends significantly on the ability of the processor to identify the remains accurately. Trials using captive birds fed with a variety of different food items can help to quantify the recovery rate (see also 2.4.1).

2.4 Data evaluation

2.4.1 Conversion of the number of food items in the faeces samples or stomach flushes to the number of food items actually ingested

For estimating how many food items were ingested by a bird, based on the number of food items found in the faeces or stomach, correction factors (or correlation coefficients) can be applied. For each type of food a specific correction factor has to be used, because during the digestion process some food items may almost completely disappear while others remain almost intact. For example earthworms or other soil invertebrates are usually digested efficiently. In contrast, cuticle parts of many arthropods remain often unaffected and can easily be identified in the faeces. Correction factors for some food types and bird species can be derived from the literature (e.g. Jenni et al., 1990; Green, 1984). For example it has been shown that the number of Araneida (spiders) ingested is about 3.9 times higher than the number found in the birds’ faeces (100/25.5, Jenni et al., 1990).

Alternatively bird species specific feeding trials can be carried out in captivity to identify traces found in faeces and stomach flushing samples when known food items are consumed. These data can be used to establish food item specific correction factors which compensate for differential digestion (Jordan, 2005). Feeding trials also offer the opportunity to account for the uncertainty and variability of correction factors.

2.4.2 Calculation of dry weight from length of food items ingested

In order to convert the calculated numerical proportions into mass proportions length-weight regressions derived from the literature (e.g. Collins, 1992; Henschel et al., 1996, Klotz et al., 2002; Rogers et al., 1976; Sample et al., 1993) can be applied, which are available for different
invertebrate taxa and plant seeds. Hence, the approximate dry weight of food items can be calculated from their estimated length.

2.4.3 Quantification of percentiles of the diet of farmland birds

Since the quantification of the diet of birds involves several measurement errors and also natural variability (e.g. of body size of food items) the mean or median may include biases. Therefore, a percentile could be used instead of the arithmetic mean for deterministic assessments. A probabilistic approach for estimating the diet of birds offers the advantage that the different levels of variability and uncertainty can be included. A probabilistic approach uses distributions instead of constant parameter values, from which parameter values are sampled many times in order to calculate the distribution of food groups for which RUDs are available (using a Monte Carlo method). This approach also allows an estimation of percentiles.

3 How to determine a mammal’s diet

The method of faeces analysis outlined above in section 2.1.1.1 can also be used for mammals. It is also possible to analyse stomach contents for mammals caught in snap-taps (mice, voles etc.) or shoot by hunters (hares, rabbits etc.) However, stomach flushing is not appropriate for mammals.

4 References


Appendix 31. Impact of crop interception on residues on plant food items

The residue unit doses (RUDs) for vegetation are derived from trials in which the crops are directly oversprayed. There will, however, be situations where particular food items for birds and mammals will have lower concentrations than expected due to the compound being partly intercepted by the crop before it reaches the food item.

As already proposed in the preceding Guidance Document SANCO/4145/2000, interception by the crop may be considered as a minimising factor for residues on plant food items when canopy-directed applications of pesticides (insecticides, fungicides) to orchards, vineyards, hops or bush fruit are performed and undergrowth vegetation (assumed to be grass) is present. Also vegetables growing on trellis might fall under this category and would be treated similar to vineyards. Only one deposition factor of 0.6 was given in SANCO/4145/2000 that corresponded to the lowest interception of 40% in these scenarios according to the FOCUS surface water report for Step 2 PEC_{SW} calculations (FOCUS, 2001, Table 2.4.2.-1). Taking into account the generic nature of FOCUS interception factors, it is now proposed that crop and growth-stage specific values according to FOCUS (2001) may be used in the tier 1 scenarios. No interception factor may be applied for herbicide applications in those crops, since these are typically directly made to the grass vegetation. Also, no interception factor is applied for hops before side shoot formation, i.e. at growth stages BBCH 10-19, because it is cultivated like an arable crop at this early stage.

As regards arable crops, some of them are rarely, if ever, eaten by birds and mammals (e.g., potatoes) whilst other crops become less attractive and hence less likely to be consumed as they grow (e.g., sugar beet). Whilst these crops may not be eaten, it is possible that other plants on the field will be available as food. At certain stages the crop may intercept some of the applied product and hence the amount of pesticide deposited on to the food item is less than the application rate. Since measured on residues of such food items at the appropriate growth stage of the crop are not available, only estimates can be used. However, further considerations were deemed necessary whether the FOCUS figures intended to reflect deposition on the soil surface (2-dimensional) may also be used for estimating residues on potential undergrowth vegetation (3-dimensional structures above the soil surface). In fact, the data given in both the FOCUS groundwater report (FOCUS, 2000) as well as in the FOCUS surface water report (FOCUS, 2001) represent a dataset stemming from different sources, which comprise calculations based on the leaf area index (LAI) as well as experimental measurements of either soil deposition or plant interception (Ganzelmeier, 1997; van de Zande et al., 1999; Becker et al., 1999; Linders, 2000). Nevertheless, a remarkable agreement between results obtained according to different methods was pointed out in FOCUS (2001) as well as by Linders et al. (2000).

It was concluded that estimation of residues on undergrowth vegetation using FOCUS interception factors would become increasingly uncertain with decreasing soil cover of the crop and increasing height of weeds in relation to the crop. Thus, reliable predictions are only deemed possible where the largest part of the soil surface is actually covered by the crop from a bird’s eye view and undergrowth vegetation is clearly smaller than the crop plants. Weeds or grasses overgrowing the crop at those stages are deemed unlikely to occur in intensive agriculture, but would also normally not form a part of the diet of small to medium herbivores.
Nevertheless, cases like desiccation of aboveground plant parts before harvesting subterranean crops might require specific consideration in that regard.

For identification of relevant BBCH crop stages that fulfil these criteria, it can be assumed that most arable crops will be sown or planted in a density to achieve maximum overall cover of soil at a growth stage where crop plants have occupied their foreseen standing room. This is done to maximise yields per hectare and to suppress emergence of weeds competing for water, nutrients and light. As soon as this certain growth stage is reached, small weeds growing in the field will normally no longer be directly and fully exposed to pesticide sprays. In the following Table 1, proposals are given for crop-specific BBCH growth stages that would correspond to such sufficient soil coverage.

Based on this assessment of growth stages, the corresponding crop interception values as used in the FOCUS surface water report (FOCUS, 2001) for Step 2 PEC<sub>SW</sub> calculations can be considered acceptable also in the context of bird and mammal risk assessment. These figures differ from the values listed in the FOCUS groundwater report (FOCUS, 2000) insofar as the more recent data by Linders et al. (2000) were additionally used in the framework of a conservative approach at an early stage of a tiered scheme. Table 2 below is thus based on Table 2.4.2.-1 of FOCUS (2001). The deposition factors provided for the different crops and growth stages are likely to reflect conservative estimates. In the context of a higher-tier assessment, the more detailed values of the FOCUS groundwater report (FOCUS, 2000) may therefore also be used in line with the explanations provided by FOCUS (2005).
Table 1. BBCH growth stages corresponding to high soil coverage by crop plants.

<table>
<thead>
<tr>
<th>Crop name (arable crops only)</th>
<th>Stage</th>
<th>Description</th>
<th>Rationale for selection (considering downward-directed treatments with boom sprayer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals</td>
<td>≥ 30</td>
<td>Stem elongation</td>
<td>Maximum of tillers reached at preceding stage BBCH 29 (subsequent growth mainly in vertical direction)</td>
</tr>
<tr>
<td>Maize</td>
<td>≥ 30</td>
<td>Stem elongation</td>
<td>9 or more leaves unfolded at preceding stage BBCH 19 (subsequent growth mainly in vertical direction)</td>
</tr>
<tr>
<td>Oilseed rape</td>
<td>≥ 30</td>
<td>Stem elongation</td>
<td>9 or more side shoots detectable at preceding stage BBCH 29 (subsequent growth mainly in vertical direction)</td>
</tr>
<tr>
<td>Faba bean (Vicia faba)</td>
<td>≥ 51</td>
<td>Inflorescence emergence</td>
<td>9 or more visibly extended internodes at preceding stage BBCH 39**</td>
</tr>
<tr>
<td>Sunflower</td>
<td>≥ 30</td>
<td>Stem elongation</td>
<td>9 or more leaves unfolded at preceding stage BBCH 19 (subsequent growth mainly in vertical direction)</td>
</tr>
<tr>
<td>Beet</td>
<td>≥ 40</td>
<td>Rosette growth (crop cover)</td>
<td>Leaves cover 90 % of ground at stage BBCH 39</td>
</tr>
<tr>
<td>Potato</td>
<td>≥ 40</td>
<td>Tuber formation</td>
<td>Crop cover complete: about 90 % of plants meet between rows at preceding stage BBCH 39</td>
</tr>
<tr>
<td>Strawberry*</td>
<td>≥ 41</td>
<td>Development of stolons and young plants</td>
<td>9 or more leaves unfolded at preceding stage BBCH 19</td>
</tr>
<tr>
<td>Cotton</td>
<td>≥ 51</td>
<td>Inflorescence emergence</td>
<td>Canopy closure: 90 % of plants meet between rows at preceding stage BBCH 39</td>
</tr>
<tr>
<td>Bulb vegetables (e.g. onion)</td>
<td>≥ 41</td>
<td>Development of main harvestable vegetative plant parts</td>
<td>9 or more leaves clearly visible at preceding stage BBCH 19 (subsequent growth mainly of harvestable subsoil parts, flowering stage typically not reached in commercial cropping)</td>
</tr>
<tr>
<td>Root and stem vegetables (e.g. carrot)</td>
<td>≥ 41</td>
<td>Development of main harvestable vegetative plant parts</td>
<td>9 or more true leaves unfolded at preceding stage BBCH 19 (subsequent growth mainly of harvestable (subsoil) parts, followed by shoot elongation and flowering)</td>
</tr>
<tr>
<td>Leaf vegetables (forming heads)</td>
<td>≥ 51</td>
<td>Inflorescence emergence</td>
<td>Typical size, form and firmness of heads reached at preceding stage BBCH 49</td>
</tr>
<tr>
<td>Leaf vegetables (not forming heads)</td>
<td>≥ 51</td>
<td>Inflorescence emergence</td>
<td>Typical leaf mass reached at preceding stage BBCH 49</td>
</tr>
<tr>
<td>Other brassica vegetables</td>
<td>≥ 51</td>
<td>Inflorescence emergence</td>
<td>Typical size and form reached, head tightly closed at preceding stage BBCH 49</td>
</tr>
<tr>
<td>Cucurbits</td>
<td>≥ 51</td>
<td>Inflorescence emergence</td>
<td>Side shoots developed in preceding stage BBCH 29/231</td>
</tr>
<tr>
<td>Solanaceous fruit (e.g. tomato, pepper, egg plant) – if not grown on trellis</td>
<td>≥ 51</td>
<td>Inflorescence emergence</td>
<td>Side shoots developed in preceding stage BBCH 29/2NX</td>
</tr>
<tr>
<td>Pea – if not grown on trellis</td>
<td>≥ 51</td>
<td>Inflorescence emergence</td>
<td>9 or more visibly extended internodes in preceding stage BBCH 39**</td>
</tr>
<tr>
<td>Bean – if not grown on trellis (Phaseolus vulgaris)</td>
<td>≥ 51</td>
<td>Inflorescence emergence</td>
<td>9 or more side shoots visible in preceding stage BBCH 29</td>
</tr>
</tbody>
</table>

* The strawberry scenario is different from other arable fields, because the crop is typically grown in rows separated by broad bare soil strips, with either crop-directed treatments using 3-nozzle fork sprayer (fungicides, insecticides) or between-row treatments (herbicides).

** If plants are not grown on trellis, stem elongation of the main shoot will affect soil coverage of the crop plants.
### Table 2. Deposition factors for bird and mammal plant food items according to BBCH growth stages (derived from FOCUS, 2001).

<table>
<thead>
<tr>
<th>Crop</th>
<th>Relevant principal BBCH growth stages</th>
<th>Interception according to FOCUS (2001)</th>
<th>Deposition factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare soils</td>
<td>not applicable</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bulb vegetables</td>
<td>≥ 4</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Bush and cane fruit (not tabulated, surrogate value from vineyard)</td>
<td>≥ 1</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>≥ 2</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>≥ 4</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Cereals</td>
<td>≥ 3</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>≥ 4</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Cotton</td>
<td>≥ 5</td>
<td>0.75</td>
<td>0.25</td>
</tr>
<tr>
<td>Fruiting vegetables</td>
<td>≥ 5</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Grassland</td>
<td>not applicable</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hop</td>
<td>≥ 1</td>
<td>0.2</td>
<td>not applicable**</td>
</tr>
<tr>
<td></td>
<td>≥ 2</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>≥ 4</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Leafy vegetables</td>
<td>≥ 5</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Legume forage</td>
<td>≥ 5</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Maize</td>
<td>≥ 3</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>≥ 4</td>
<td>0.75</td>
<td>0.25</td>
</tr>
<tr>
<td>Oilseed rape</td>
<td>≥ 3</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>≥ 4</td>
<td>0.75</td>
<td>0.25</td>
</tr>
<tr>
<td>Orchards</td>
<td>≥ 1</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>≥ 2</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>≥ 4</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Ornamentals/nursery (not tabulated, surrogate value from leafy vegetables)</td>
<td>≥ 5</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Potatoes</td>
<td>≥ 4</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Pulses</td>
<td>≥ 5</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Root and stem vegetables</td>
<td>≥ 4</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Strawberries*</td>
<td>≥ 4</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(value from FOCUS, 2000)</td>
<td></td>
</tr>
<tr>
<td>Sugar beet</td>
<td>≥ 4</td>
<td>0.75</td>
<td>0.25</td>
</tr>
<tr>
<td>Sunflower</td>
<td>≥ 3</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>≥ 4</td>
<td>0.75</td>
<td>0.25</td>
</tr>
<tr>
<td>Vineyard</td>
<td>≥ 1</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>≥ 2</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>≥ 4</td>
<td>0.7</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* The strawberry scenario is different from other arable fields, because the crop is typically grown in rows separated by broad bare soil strips, with either crop-directed treatments using 3-nozzle fork sprayer (fungicides, insecticides) or between-row treatments (herbicides).

** No consideration of interception for hops before side shoot formation, because it is cultivated like an arable crop at this early stage.
References


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1 Available at: [http://www.bba.de/veroeff/bbch/bbcheng.pdf](http://www.bba.de/veroeff/bbch/bbcheng.pdf)
2 Available at: [http://viso.ei.jrc.it/focus/](http://viso.ei.jrc.it/focus/). 2 pp.
Appendix 32. Nestling scenarios for long-term assessments

Pierre Mineau, Science and Technology Branch, Environment Canada

In the phased approach to the long-term risk assessment in birds, it was suggested (Shore et al., 2005) to turn to the LC₅₀ study and use the LC₀₅ as an indication of a dietary dose that could be tolerated by young birds. Unfortunately, the LC₅₀ test has been plagued with problems (Mineau et al., 1994) and, furthermore, will no longer be required at an early tier in EU registration procedures. Therefore, it was agreed that an alternate strategy utilising the LD₅₀ would be developed.

In order to estimate the ability of nestlings to survive a pesticide application, an approach parallel to that used for the acute assessment in adult birds has been developed. The approach uses information on the measured energetic needs of young birds coupled to a feeding model and calculates a TER.

1. Toxicity

The sensitivity of altricial nestlings to pesticides is known to be higher than that of adults in the case of organophosphorous insecticides (Wolfe and Kendall, 1998). This is in part because the cholinesterase system of altricial birds is not fully developed at birth. It is possible that altricial chicks are more sensitive to other classes of compounds as well but, unfortunately, no information is available on which to base a correction factor.

In the case of precocial chicks, available information does not suggest that a correction factor is required. The relationship between chick toxicity and adult toxicity follows roughly a 1:1 relationship (Fig. 1).

Therefore, we propose at this stage to use adult LD₅₀ values to reflect chick toxicity. More research is needed to characterize the toxicity of different pesticides to altricial chicks.
2. **Exposure**

Comparisons provided in Kendeigh et al. (1977) suggest that gross energy intakes expressed as a proportion of body weight are higher in altricial nestlings than in precocial ones. Therefore, in order to be protective of all bird species, a generic exposure scenario used in the assessment of reproductive effects should be based on the energetic needs of a precocial species.

**Correction factor for digestive inefficiency and thermoregulatory status of very young altricial nestlings.**

Based on the work of Kendeigh et al. (1977) in house sparrows, young birds aged 1-4 days have a food assimilation efficiency approximately 15% lower than that of adults. Based on the information provided for that species, correction factors of 0.83, 0.87, 0.87, and 0.93 are applied to ages 1-4 days respectively.

It has been shown by Williams and Prints (1987) that laboratory studies of energy use in altricial nestlings conducted under thermo neutral conditions underestimate field energy use because they do not usually take into account the thermoregulatory costs of 'outdoor living'. Information from that work was used to correct the maintenance portion of the daily energy needs of the nestlings. Correction factors were estimated from Figure 4 of Williams and Prints (1987) (Table 1). The discrepancy between the two measures increases with age which corresponds to the decrease in adult brooding behaviour over time.

**Body Mass**

Nestling weights of different species are dependant on egg size (and therefore clutch size and reproductive strategy) and on growth rate. The latter varies with age and is also subject to a
number of ecological constraints. Based on examples gleaned from the literature (Kendeigh for house sparrows, Williams and colleagues in savanna sparrows), it is probably safe to use a hatching weight of 11% of female adult body mass for scenarios involving freshly hatched altricial passerines. Maximum exposure (see table 1 below) in nestling savannah sparrows occurred when the birds were 2 days of age (48-72 hours post hatch), approximately 25% of adult female body weight. This percentage should be applied to the indicator species or generic focal species of concern unless more reliable data are available to estimate the weight of nestlings at 2 days of age.

**Choice of scenario**

Few if any of the generic focal species (Appendix 3a) have been studied from the point of view of nestling energetics. It is therefore proposed that the exposure scenario for an altricial chick be based on the combined work of Williams and Prints (1987) on the savannah sparrow, and that of Kendeigh et al. (1977) in the house sparrow. Calculations suggest that an altricial chick is at its most vulnerable a few days after birth when its FIR/bw peaks (Table 1). We therefore propose that, until such time as better information becomes available, the max. FIR/bw value of 1.08 calculated in the savannah sparrow for the 48-72 hour period after hatch (25% of adult female bodyweight) should be used to model peak vulnerability to pesticide exposure in altricial insectivores.

**Table 1.** Energy budget of a nestling savannah sparrow based on Williams and Prints (1987) and Kendeigh et al. (1977).

<table>
<thead>
<tr>
<th>Age(d)</th>
<th>BW† (g)</th>
<th>Energy for growth (kJ/d)</th>
<th>Energy for maintenance (kJ/d)</th>
<th>Correction for thermoregulation</th>
<th>DEE (kJ/d)</th>
<th>Food energy (kJ/g dry wt)</th>
<th>Moisture (%)</th>
<th>Assimilation efficiency (%)³</th>
<th>FIR (g/day)</th>
<th>FIR/bw</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 1</td>
<td>1.79</td>
<td>4.96</td>
<td>1.67</td>
<td>1.3</td>
<td>7.13</td>
<td>21.9</td>
<td>70.5</td>
<td>63</td>
<td>1.75</td>
<td>0.98</td>
</tr>
<tr>
<td>1 to 2</td>
<td>2.81</td>
<td>8.53</td>
<td>2.19</td>
<td>1.35</td>
<td>11.49</td>
<td>21.9</td>
<td>70.5</td>
<td>66</td>
<td>2.69</td>
<td>0.96</td>
</tr>
<tr>
<td>2 to 3</td>
<td>4.24</td>
<td>13.19</td>
<td>4.54</td>
<td>1.4</td>
<td>19.55</td>
<td>21.9</td>
<td>70.5</td>
<td>66</td>
<td>4.58</td>
<td>1.08</td>
</tr>
<tr>
<td>3 to 4</td>
<td>6.04</td>
<td>17.56</td>
<td>6.55</td>
<td>1.45</td>
<td>27.06</td>
<td>21.9</td>
<td>70.5</td>
<td>71</td>
<td>5.90</td>
<td>0.98</td>
</tr>
<tr>
<td>4 to 5</td>
<td>8.05</td>
<td>19.15</td>
<td>10.36</td>
<td>1.5</td>
<td>34.69</td>
<td>21.9</td>
<td>70.5</td>
<td>76</td>
<td>7.07</td>
<td>0.88</td>
</tr>
<tr>
<td>5 to 6</td>
<td>10.02</td>
<td>16.84</td>
<td>15.33</td>
<td>1.55</td>
<td>40.60</td>
<td>21.9</td>
<td>70.5</td>
<td>76</td>
<td>8.27</td>
<td>0.83</td>
</tr>
<tr>
<td>6 to 7</td>
<td>11.70</td>
<td>12.24</td>
<td>20.37</td>
<td>1.6</td>
<td>44.83</td>
<td>21.9</td>
<td>70.5</td>
<td>76</td>
<td>9.13</td>
<td>0.78</td>
</tr>
<tr>
<td>7 to 8</td>
<td>12.98</td>
<td>7.73</td>
<td>24.59</td>
<td>1.65</td>
<td>48.30</td>
<td>21.9</td>
<td>70.5</td>
<td>76</td>
<td>9.84</td>
<td>0.76</td>
</tr>
</tbody>
</table>

† based on growth equation (g)

² estimated from Fig. 4 in Williams and Prints (1987)

³ corrected for inefficiency in young house sparrow after table 5.6 in Kendeigh
3. References


