Exposure reduction of seed treatments through dehushing behaviour of the wood mouse (*Apodemus sylvaticus*)

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Abstract

Background, aim and scope Seed treatments are widely used on cereals and other annual crops throughout Europe. Most of the formulated pesticide is found on the outside of the seed, the husk. Risk assessments of seed treatments are especially needed for granivorous mice living in the agricultural landscape e.g. for registration using the guidance for risk assessment for birds and mammals (EFSA 2009). The dehushing of seeds before consumption is a known behaviour of these mammals, but so far, no quantitative data on the reduction of exposure of seed treatments by dehushing were published. Therefore, we aimed at providing a first quantitative estimate of this behaviour-related exposure reduction for the wood mouse (*Apodemus sylvaticus*) with different seed types.

Materials and methods We evaluated the efficiency of dehushing behaviour of 20 wood mice captured in the wild for four different seeds (wheat, barley, maize and sunflower). One experimental setup used a fungicide seed treatment where the remaining seed husks of consumed

seeds were analysed with a HPLC-MS/MS technique. In the second setup, we measured generic pigment present in a blank seed treatment formulation and determined the leftover pigment in the husks with a photometric technique. **Results** The exposure reduction was similar for the fungicide and the pigment design where the same seed types were studied. We could demonstrate exposure reductions ranging from around 60% for cereals to almost 100% for sunflower seeds as a result of the dehushing behaviour.

Discussion Since exposure reduction was similar in both approaches, working with pigments would be a generic way to estimate the impact of dehushing behaviour on seed treatment exposure. This behaviour can result in a substantial exposure reduction and should, therefore, be considered in a seed-type specific way in the risk assessment of pesticide seed treatments.

Conclusions It is proposed to include a seed-specific dehushing factor in the calculations of estimated theoretical exposure of seed treatments for granivorous mice. The approach of accounting for a dehushing-related exposure reduction by field relevant wild mammal species seems a more promising way to advance the risk assessment instead of using generic species and neglecting behavioural traits. The pigment approach could be used to gather data for exposure reduction for other species and seed types. Its advantage is that it is harmless to the test species and comparatively cheap since no chemical analysis is involved.

Recommendations and perspectives Seed treatments are used for most of the cereal crops grown in Europe today. Their advantages usually include a lower application rate and the reduction of drift compared to a conventional spraying regime. However, there is a potential risk especially for granivorous mice, and its assessment is challenging in case of a high residue concentration on the

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dressed seeds. The concept of a dehusking factor in the risk assessment scheme for seed treatments for granivorous mice is a valid approach to account for the behavioural exposure reduction, and generic data could be easily generated also for other wild mammal species and other seed types, possibly analysing the pigment in commercial seed treatment formulations.

Keywords Mammals · Risk assessment · Dehusking · Cereals · Behaviour · Pesticide · Seed treatment

1 Background, aim, and scope

Cereal crops are grown over large areas in Europe. In 2007, 24.8 million hectares of wheat, 13.2 million hectares of barley and 8.0 million hectares of maize were harvested in the European Union (FAOSTAT 2008), and the cereals were covering almost 15% of the landscape. Most of the cereal seeds used in agriculture today are treated with specific formulations that encapsulate the seed and protect the germinating plants from diseases. In the UK, 87% of drilled seeds between 1992 and 2002 were treated (Prosser and Hart 2005). The seed treatment is applied on the outside of the seed, the husk. Seed treatments target fungi (fungicides) and disease vectors (insecticides) or both.

As for all pesticides, the risk of seed treatments to the ecosystem has to be evaluated in the registration process. The recently published scientific opinion and guidance document on the risk assessment of pesticides for birds and mammals (EFSA 2008, 2009) detail many specific aspects of exposure that should be considered. Besides birds, especially granivorous mice living in or near cereal fields are potentially exposed to seed treatments when feeding on the drilled seeds or occurring spillages. However, these granivorous mice dehusk seeds before consumption and, therefore, lower the potential exposure through this behaviour (Barber et al. 2003). Although the behaviour as such is known and its result was observed in the field (see Fig. 1, and also Pelz 1989; Tew et al. 2000), quantitative data on the efficiency of dehusking by small mammals are so far not published.

In this study, we measured the behaviour-related exposure reduction of seed treatments for the wood mouse (*Apodemus sylvaticus*), an accepted focal species in ecotoxicological risk assessment. The wood mouse is the most common small mammal species of Europe (Niethammer and Krapp 1978) present in the agricultural landscape throughout the year (Abt and Bock 1998; Green 1979; Pelz 1979; Tew et al. 1994; Tattersall et al. 2002; Klaa et al. 2005). Wood mice feed not only on seeds and plants but also on invertebrates (Pelz 1989) and their body weight ranges

between 12 and 35 g (Luttik 1992). In the present regulatory framework for pesticide regulation in Europe, wood mice are assumed to consume almost 6 g of seeds per day (European 2002). In its habitat, the wood mouse is exposed to pesticides—and as a granivorous mammal especially towards seed treatments. There are, however, only very few reports about field effects from exposure of wood mice to seed treatments in the literature (Tarrant et al. 1990).

In previous pilot experiments, we observed dehusking of treated cereal seeds by wood mice and house mice under laboratory and/or semi-field conditions (Guckenmus et al. 2007). In this paper, we present a quantitative dataset for exposure reduction by dehusking, based on residue analysis in a laboratory experimental setup. We evaluated the behaviour for four different seed types and used a fungicide seed treatment and a blank formulation (pigment) treatment. This allowed to present measured exposure reduction values for wood mice which could potentially be implemented in the risk assessment in the form of a dehusking factor.

2 Materials and methods

2.1 Study organisms and laboratory husbandry

The 20 wood mice used in the experiments were trapped in the proximity of the Laacher Farm in Monheim/Rhine (Germany) along hedges in agricultural area using Ugglan live-traps (Grahnb, Hillerstorp, Sweden). The experiments were performed in the Ecotoxicology facilities of Bayer CropScience AG, Monheim/Rhine (Germany) where the mice were kept individually in standard Macrolon cages (57×35×19 cm, Type IV, UNO Roestvaststaal BV, Zevenaar, The Netherlands). The bottom was covered with fine quartz sand; water and food were offered in separate porcelain bowls. Standard food was offered ad libitum and consisted of a mixture of oats, wheat and barley seeds. In addition, mealworms and pieces of apple were offered occasionally. Temperature in the laboratory ranged between 21.1°C and 29.8°C and relative humidity between 26.7% and 64.7%. The mice were kept under a day–night rhythm with a 10-h dark (10:00–20:00) and a 14 h light phase (20:00–10:00). During the night phase, only a red light was present in the laboratory to allow observations of feeding behaviour (mice do not perceive red light). The mice were adapted to the laboratory environment for 4 weeks before the experiments started to ensure that individuals were not sick or pregnant. The individual mice then took part in the test with the fungicide treatment for wheat and barley as well as in the tests with the blank seed treatment with wheat, barley, maize and sunflowers.

Fig. 1 **a** Wheat husks with seed treatment in front of mousehole (picture taken by C. Wolf), **b** Treated maize seed with gnawing marks and removed seed husks (picture taken by R. Barfknecht)

2.2 Seeds and seed treatments

Experiments were performed with four seed types: Winter wheat (variety Tommi), summer barley (Djamila), maize (Gavott) and sunflower (Pegasol). Seeds were treated in the Seed Treatment Centre of Bayer CropScience AG in two experiments with a formulated suspension of a triazole fungicide (Prothioconazole 100 FS (FS = flowable concentrate for seed treatment)). The treatment rate was 100 ml/100 kg wheat and barley.

In four experiments, we used a blank formulation (without active substance) which contained the colouring agent “Pigment Red 112” (3-hydroxy-*N*-(*o*-tolyl)-4-[(2,4,5-trichloro-phenyl)azo]naphthalene-2-carboxamide) which can be quantitatively measured using a photometric analytical method.

The mice were acclimated to wheat and barley seeds by simulating the exposure scenario with untreated seeds on days −14/−13 and −7/−6. On day −14, all mice received exclusively 6 g untreated wheat and on day −13, 6 g untreated barley for 24 h each. This procedure was repeated on days −7 and −6. Mean food consumption of untreated wheat seed per wood mouse amounted to 1.03 g (day −14) and 1.48 g (day −7), food consumption of untreated barley 1.31 g (day −13) and 1.78 g (day −6).

The fungicide formulation was found to be non-repellent to wood mice as the mean food consumption per mouse in the fungicide trial was similar to the consumption of untreated cereals during acclimation (1.39 g treated wheat seeds and 1.33 g treated barley). The blank FS formulation used for wheat and barley was the same type as for the fungicide treatment (but containing no active substance) whereas for sunflower and maize another blank FS-formulation was used, due to different adhesion on these seed types.

2.3 Experimental setup

In each test, 6 g of treated seeds were offered between 800 and 1000 hours just before the night phase in the laboratory for 24 h. Remaining seeds were reweighed, and husks were collected by sieving the quartz sand (mesh size of 0.5 and 1 mm). Faeces were removed since they may contain residues from ingested parts of the seed which should not be included in the determination of the dehusking efficiency. In the fungicide test, the quartz sand was also analysed for residues.

Before offering the pigment-treated seeds to the mice, the standard food was removed after 6 h; therefore, the mice did not have the time to consume a similar amount of standard food as in the pre-phase of the fungicide treatment (starvation). The starvation period lasted 16 h in each test. The pigment-treated seeds were then offered for 24 h. The starvation period was introduced to simulate hunger stress and to enhance the feeding motivation of the mice and to detect if the dehusking behaviour is similar to that in the test with the fungicide treated seeds (without starvation).

2.4 Residue determination

2.4.1 Fungicide

For the residue analysis of seed husks and sand, we used an HPLC-MS/MS technique (HPLC Agilent 1200 SL, MS/MS API 4000). The active substance (prothioconazole) and its primary metabolite (prothioconazole-desthio) on husks and in the sand were extracted with 40 ml of acetonitril/water 4:1 and 4 ml of a 250-g/L cystein hydrochloride. The resulting sample was then filtered and 0.5 ml of a 1,000 µg/L internal standard solution was added. An aliquot of this solution was diluted 1:5 with HPLC eluent.

Prothioconazole and prothioconazole-desthio residues were determined by RP-HPLC with mass spectrometric detection following extraction in aqueous acetonitrile. The limit of quantification was 0.01 mg/kg in all matrices.

2.4.2 Pigment

We used a photometer (HP Diode Array Spectrometer 8452A) to determine the pigment concentration of seed husks. To calibrate the photometer before measuring the samples, two stock solutions containing approximately 40 mg pigment/L were prepared for the generation of the calibration curve. Three dilutions of each of the two stock solutions (4, 8 and 20 mg/L) were used as calibration solutions. The extinction of the calibration solutions was measured corresponding to increasing concentrations at 502 nm in the spectrophotometer against a blank solvent mixture. Using regression analysis, a calibration curve was generated, with the extinction values plotted against concentration. The correlation coefficient of the calibration curve was at least 0.9998.

The pigment was extracted from the seeds/husks with a solvent (toluol/methanol 9:1) with 1 ml sulphuric acid per 100 ml. All samples were measured at a wavelength of 502 nm, and the pigment concentrations in the extracts from the sample aliquots were determined using the calibration curve. Only absorbance values below 1.0 were used to determine the concentration in the test samples. Sample aliquots with absorbance values above 1.0 were diluted with solvent and then measured again.

2.5 Calculation of residue reduction

The actual seed treatment concentrations (fungicide and pigment) were evaluated by analysing five batches of 6 g of seeds. Based on the mean value of the actual seed treatment concentration, it was possible to calculate the potential exposure with fungicide/pigment by using the consumed amount of seeds (Eq. 1).

$$E_{\text{pot}}[\text{mg}] = S_{\text{c}}[\text{g}] \times \text{St}_{\text{conc}}[\text{mg/g}] \quad (1)$$

where E_{pot} =calculated potential exposure, S_{c} =consumed seeds in 24 h and St_{conc} =seed treatment concentration

The residues found on the remaining husks and in the sand were subtracted from the calculated potential exposure and yielded the actual amount of consumed fungicide/pigment or actual exposure (Eq. 2).

$$E_{\text{actual}}[\text{mg}] = E_{\text{pot}}[\text{mg}] - R_{\text{h(s)}}[\text{mg}] \quad (2)$$

where E_{actual} =calculated actual exposure and $R_{\text{h(s)}}$ =measured residues of fungicide/pigment on husks (h) and sand (s)

The reduction of the exposure through dehushing behaviour was calculated as (Eq. 3)

$$\text{ER}[\%] = (1 - E_{\text{actual}}/E_{\text{pot}}) \times 100 \quad (3)$$

where ER=Exposure reduction in % and the dehushing factor was determined by (Eq. 4):

$$\text{DH} = E_{\text{actual}}/E_{\text{pot}} \quad (4)$$

where DH=Dehushing factor DH: Numeric value between 0 and 1. A dehushing factor of 0.4 indicates an exposure reduction of 60% through the dehushing behaviour.

3 Results

3.1 Efficiency of seed treatment

The amount of residue of the fungicide seed treatment of cereal seeds was determined for five batches of 6 g and the mean concentration of seed treatment for 1 kg of seeds was calculated (Table 1). The mean values of 75.1 mg fungicide/kg wheat and 83.3 mg fungicide/kg barley were used for the calculation of the exposure reduction and dehushing factor (Eqs. 1, 2, 3, 4, see values in Table 2).

The pigment load for all four seed types (wheat, barley, maize and sunflower) was also analysed for five batches of 6 g (Table 1). Mean pigment loading ranged from values around 200 mg/kg for the cereals to over 900 mg/kg for sunflower seeds. For all treatments, the relative standard deviation did not exceed 5% of the respective mean.

3.2 Residues

3.2.1 Fungicide residues

The collected husks and the sand substrate in the wheat experiment were analysed and yielded on average 0.078 mg fungicide (Table 2). When subtracted from the potential mean exposure of 0.125 mg fungicide on the 1.66 g of consumed wheat seeds the exposure was reduced to 0.047 mg fungicide (61.38% mean reduction of potential exposure; dehushing factor 0.39).

The barley fungicide experiment showed similar seed consumption rates of 1.67 g of seeds (Table 2). The higher seed treatment concentration (Table 1) produced a higher potential mean exposure of 0.139 mg fungicide. The husks and sand revealed 0.111 mg fungicide which led to a reduction to 0.028 mg fungicide consumed by mice (79.47% mean reduction of potential exposure; dehushing factor 0.21).

Table 1 Determination of seed treatment concentration (St_{conc} on fungicide and pigment) on the different treated seed types (mean and SD of five batches of approx. 6 g)

	Seeds	Residue amount in sample of 6g seed in mg	Seed treatment concentration St_{conc} in mg/kg seed
Fungicide	Wheat	0.451±0.013	75.101±2.223
	Barley	0.500±0.017	83.263±2.912
Pigment	Wheat	1.246±0.020	207.643±3.827
	Barley	1.162±0.052	193.633±8.705
	Maize	1.259±0.026	209.753±4.746
	Sunflower	5.544±0.138	923.973±23.536

Values are rounded

3.2.2 Pigment residues

On average 2.672 g of wheat seeds were consumed in this experiment (Table 2). The potential pigment exposure on this amount of seeds is 0.555 mg pigment. The collected husks were analysed and on average yielded 0.326 mg pigment. This led to a reduction of the potential exposure to 0.229 mg pigment (58.04% mean reduction; dehushing factor 0.42).

Seed consumption by mice was again similar in the barley experiment with 2.585 g on average. The potential pigment exposure of 0.501 mg was reduced by 0.425 mg recovered on husks to a maximum exposure of 0.075 mg pigment (83.95% mean reduction; dehushing factor 0.16).

Almost 5 g of maize were consumed leading to an average potential pigment exposure of 1.031 mg; 0.587 mg of pigment were on average recovered on husks and partially eaten maize seeds leading to a maximum exposure

of 0.444 mg pigment (58.97 % mean reduction; dehushing factor 0.41).

A total of 2.390 g of sunflower seeds were consumed leading to mean potential pigment exposure of 2.208 mg. The husks yielded 2.182 mg of residues leading to 0.026 mg of consumed pigment or a 98.78% mean reduction of pigment exposure (dehushing factor 0.01).

3.3 Comparison of pigment and fungicide

Since wheat and barley were treated with fungicide and the pigment formulation, we compared the exposure reduction that occurred through dehushing behaviour. There was no statistically significant difference between the relative exposure reductions for both cereal types for the two different formulations (*t* test, wheat: $t=0.562$; $df=23$; $p=0.580$; barley: $t=-1.331$; $df=23$; $p=0.196$).

Table 2 Residues on seed husks and sand and the resulting calculated exposure reduction through behaviour (mean and SD) for the different seed types and both seed treatments

	Fungicide		Pigment			
	Wheat (N=13)	Barley (N=14)	Wheat (N=12)	Barley (N=11)	Maize (N=12)	Sunflower (N=11)
Consumed seeds S_c (g) Measured	1.66±0.76	1.67±0.69	2.672±0.842	2.585±1.025	4.917±1.479	2.390±0.919
Potential exposure E_{pot} (mg) Calculated	0.125±0.057	0.139±0.057	0.555±0.175	0.501±0.198	1.031±0.310	2.208±0.849
Residues of seed treatment on husks R_h (mg) Measured	0.068±0.041	0.102±0.044	0.326±0.144	0.425±0.187	0.587±0.147	2.182±0.846
Residues of seed treatment in sand R_s (mg) Measured	0.010±0.005	0.009±0.003	–	–	–	–
Residues of seed treatment on husks and in sand R_{hs} (mg) Calculated	0.078±0.043	0.111±0.046	–	–	–	–
Actual exposure E_{actual} (mg) Calculated	0.047±0.026	0.028±0.015	0.229±0.088	0.075±0.046	0.444±0.232	0.026±0.045
Reduction through behaviour ER (%) Calculated	61.38±15.12	79.47±7.50	58.04±14.55	83.95±9.28	58.97±13.08	98.78±2.03
Dehushing factor DH Calculated	0.39	0.21	0.42	0.16	0.41	0.01

N number of mice

For calculations, see equations section; values are rounded

4 Discussion

4.1 Generic seed treatment

We evaluated the exposure reduction of seed treatments through dehushing behaviour with fungicide residue and pigment analysis. There was no statistically significant difference between the dehushing efficiency seen in the fungicide and pigment studies with wheat or barley. Therefore, photometric analysis of blank pigment formulation can be used to estimate exposure reduction through dehushing behaviour. The results for wheat and barley suggest that the pigment approach is also valid for measuring exposure reduction in the case of sunflower seeds and maize. The consumption rates in the pigment treatments were higher than in the fungicide treatment which is explained by the starvation pre-phase in the pigment experiments.

Although the mice had different feeding motivations in the two different test setups, the dehushing efficiency of fungicide treated seeds and pigment treated seeds was comparable.

4.2 Exposure reduction

In the fungicide treatment, residues were also collected in the quartz sand. These residues were low (see Table 2), presumably since the larger husk particles had already been collected manually. For the sake of simplicity, it was, therefore, decided not to analyse the sand in a similar manner in the photometer for the pigment approach; these values can, therefore, be treated as conservative estimates of exposure reduction.

Since the objective of this study was to quantify dehushing efficiency, we did not analyse the faeces of the mice although they showed red colouring the day after the seed treatment experiments. All ingested residues were included in E_{actual} .

The measured exposure reductions with non-repellent formulations are considered suitable for extrapolation since repellent seed treatments would presumably result in avoidance of the seeds or rather higher dehushing rates as the mice would try to circumvent the seed treatment.

The highest values for exposure reduction were found for sunflower seeds with a mean of 98.8%. Here, the seed treatment was removed most efficiently when the husks are cracked open and only the interior seed was eaten. The exposure reduction through dehushing was similar for maize and wheat (around 60%) but higher for barley (around 80%).

The results revealed that reduction of the exposure to a seed treatment can be substantial for wood mice since most of the treatment is located on the seed husks. Although

dehushing is mentioned in the guidance document for the risk assessment of birds and mammals (European 2002), it is not yet implemented in the risk calculation of the estimated theoretical exposure. The results of our laboratory study suggest that dehushing decreases the exposure substantially for all seed types under investigation; therefore, it seems justified to include a specific factor to account for the dehushing exposure reduction.

The feeding experiments in the laboratory showed lower values of consumption than the older worst case estimates of 5.7 g/24 h for the field situation (European 2002). One might assume that this lower food intake is related to a lower energy demand for foraging in the cages compared to the natural situation. However, during the acclimation phase, we observed that the wood mice preferred a mixed diet consisting of various seed types, fruit and animal matter over any single seed diet. During the testing period when only seeds were offered, the mice even lost about 10% of their bodyweight. These observations suggest that the lower than predicted seed consumption is not an artefact from the laboratory testing conditions but an expression of the dietary demands of the animals for more variable foodstuff. This is in agreement with the scenario for wood mice in the more recent EFSA guidance document on the risk assessment of pesticides for birds and mammals (EFSA 2009) that assumes a diet consisting of 25% herbs, 25% invertebrates and 50% seeds. Thus, the seed consumption recorded in our experimental set-up corresponds with the realistic diet preferences expected in the field.

In our experiments, different feeding rates (fungicide and pigment test) result in comparable dehushing factors. Similar seed residue reduction by dehushing was also observed in our previous experiments under semi-field conditions (Guckenmus et al. 2007). Other authors confirmed seed dehushing for Yellow-necked mice (*Apodemus flavicollis*) and House mice (*Mus musculus*) (Ludwigs et al. 2007). Finally, remains of husks of treated seeds can also be found in the field (Fig. 1a and b). In the experiments presented here, comparable dehushing efficiency was observed regardless of whether the mice were or were not starved before the seeds were offered, suggesting that this trait has evolved in small granivorous mammals as part of their typical behavioural repertoire to prepare seeds for ingestion and effective digestion.

5 Conclusions

Although risk assessment of mammals takes dietary aspects of focal species into account, quantitative assessment of behavioural traits and their impact on exposure is so far lacking. Our results suggest the implementation of a seed-specific dehushing factor in the calculations of estimated

theoretical exposure of seed treatments for granivorous mice. The approach of accounting for dehusking-related exposure reduction in relevant species for certain seed types seems a more promising way to advance the risk assessment by increasing the realism instead of using generic species and neglecting behavioural traits.

Pigment analysis proved to be an efficient and inexpensive method to estimate exposure reduction through dehusking of seeds without risking any harm to the test animals.

6 Recommendations and perspectives

For granivorous mice, the concept of a dehusking factor in the risk assessment scheme for seed treatments is a valid approach. Generic data on the intensity of the dehusking behaviour could be generated also for other small wild mammal species and other seed types, possibly using pigment formulations for seed treatment. The resulting database could help to evaluate the concept of a dehusking factor in the risk assessment scheme for seed treatments for mammals to account for the behavioural exposure reduction.

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References

- Abt K, Bock WF (1998) Seasonal variations of diet composition in farmland field mice *Apodemus spp.* and bank voles *Clethrionomys glareolus*. *Acta Theriol* 43:379–389
- Barber I, Tarrant KA, Thompson HM (2003) Exposure of small mammals, in particular the wood mouse *Apodemus sylvaticus*, to pesticide seed treatments. *Environ Toxicol Chem* 22:1134–1139
- EFSA (2008) Scientific opinion of the panel on plant protection products and their residues on a request from the EFSA PRAPeR unit on risk assessment for birds and mammals. *The EFSA Journal* 734:1–181
- EFSA (2009) Guidance document on risk assessment for birds & mammals on request from EFSA. *The EFSA Journal* 7(12):1438. doi:10.2903/j.efsa.2009.1438
- European Commission (2002) Guidance document on risk assessment for birds and mammals under council directive 91/414/EEC. SANCO/4145/2000. Directorate E - Food safety: plant health, animal health and welfare, international questions; E1 - Plant health
- FAOSTAT (2008) FAO Statistics Division Accessed 20 August 2008 at <http://faostat.fao.org>
- Green R (1979) The ecology of wood mice (*Apodemus sylvaticus*) on arable farmland. *J Zool (London)* 188:357–377
- Guckenmus B, Barfknecht R, Ebeling M, Brühl CA (2007) Reduction of fungicide concentration by de-husking of seeds by the Wood mouse. Poster presentation at SETAC 17th Annual Meeting 2007, Porto
- Klaa K, Mill PJ, Incoll LD (2005) Distribution of small mammals in a silvoarable agroforestry system in Northern England. *Agroforest Syst* 63:101–110
- Ludwigs J-D, Pascual J, Wolf A, von Blanckenhagen F (2007) Comparison of dehusking experiments of laboratory mice and wild *Apodemus spec.* mice. Poster presentation at SETAC 17th Annual Meeting 2007, Porto. Accessed 6 January 2010 at http://rifcon.de/uploads/media/Dehusking_SETAC_2007_Porto_Final_01.pdf
- Luttik R (1992) Environmental hazard/risk assessment of pesticides used in agriculture for birds and mammals. The Dutch concept. Part 1. Introduction and synopsis of the decision scheme 679101006. National Institute of Public Health and Environmental Protection, Bilthoven
- Niethammer J, Krapp F (1978) Band 1 - Rodentia I (*Sciuridae*, *Castoridae*, *Gliridae*, *Muridae*) - *Apodemus sylvaticus*, p. 336–358 *Handbuch der Säugetiere Europas*. Wiesbaden
- Pelz H-J (1979) Die waldmaus (*Apodemus sylvaticus* L.), auf ackerflächen: populationsdynamik, saatschäden und abwehrmöglichkeiten. *Z Angew Zool* 66:261–280
- Pelz H-J (1989) Ecological aspects of damage to sugar beet seeds by *Apodemus sylvaticus*. In: Putmann RJ (ed) *Mammals as pests*. Chapman and Hall, London, pp 34–48
- Prosser P, Hart ADM (2005) Assessing potential exposure of birds to pesticide-treated seeds. *Ecotoxicology* 14:679–691
- Tarrant KA, Johnson IP, Flowerdew JR, Greig-Smith PW (1990) Effects of pesticide applications on small mammals in arable fields, and the recovery of their populations. In *Proceedings of the 1990 British crop protection conference – pests and diseases*, British Crop Protection Council, Farnham, UK. p. 173–182
- Tattersall FH, Macdonald DW, Hart BJ, Johnson P, Manley W, Feber R (2002) Is habitat linearity important for small mammal communities on farmland? *J Appl Ecol* 39:643–652
- Tew TE, Todd IA, Macdonald DW (1994) Field margins and small mammals. In: Boatman N (ed) *Field margins: integrating agriculture and conservation*, vol 58. Brighton Crop Protection Council Monograph, Farnham, pp 85–94
- Tew TE, Todd IA, Macdonald DW (2000) Arable habitat use by Wood mice (*Apodemus sylvaticus*). 2. Microhabitat. *J Zool London* 250:305–311