# Substrate-Mediated Toxicity of Deltamethrin Residues to Beneficial Invertebrates: Estimation of Toxicity Factors To Aid Risk Assessment

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Abstract. Laboratory bioassays were carried out to determine the toxicity of residues of the synthetic pyrethroid insecticide, deltamethrin ((S)-a-cyano-3-phenoxybenzyl (1R)-cis-3-(2,2dibromovinyl)-2,2-dimethylcyclopropanecarboxylate) to a range of beneficial invertebrates exposed to treated wheat foliage, sandy loam soil, and glass surfaces. The test invertebrates represented a range of predators and a parasitoid that inhabit the foliage and grounds layers of temperate cereal crops, i.e., Pterostichus melanarius (Illiger), Nebria brevicollis (F.), Demetrias atricapillus (L.) and Bembidion obtusum (Serville) (Coleoptera: Carabidae), Tachyporus hypnorum (F.) (Coleoptera: Staphylinidae), Coccinella septempunctata (L.) (Coleoptera: Coccinellidae) adults (A) and larvae (L), Episyrphus balteatus (Degeer) (Diptera: Syrphidae), and Aphidius rhopalosiphi (De Stefani-Perez) (Hymenoptera: Braconidae). In 2 h flag leaf exposure bioassays, the  $LD_{50}$  values varied from 0.4 g ai/ha to >50 g ai/ha. The susceptibility ranking, from most to least susceptible, was C. septempunctata (L) > T. hypnorum > C. septempunctata (A) > E. balteatus > A. *rhopalosiphi* > D. *atricapillus*. On soil, the LD<sub>50</sub> values were 52.8 g ai/ha for T. hypnorum and 97.8 g ai/ha for C. septempunctata (A) after 2 h exposure. This period of exposure was, however, found to be of insufficient duration to separate the susceptibilities of the other test species adequately, and therefore 72 h exposure bioassays were also carried out. The 72 h LD<sub>50</sub> values varied between 4.2 g ai/ha and 267 g ai/ha. The susceptibility ranking, from most to least susceptible, was T. hypnorum > B. obtusum > C. septempunctata (A) > P. melanarius > N. brevicollis > D. atricapillus. In 2 h glass bioassays the  $LD_{50}$  range was 1.2 g ai/ha to >37 g ai/ha and the susceptibility ranking was T. hypnorum > C. septempunctata (A) > N. brevicollis, and D. atricapillus. Residual toxicities in the different 2 h exposure bioassays were compared for T. hypnorum and C. septempunctata (A) by iterating sequences of lethal dose ratios between LD<sub>10</sub> and LD<sub>90</sub> from the dose-response statistics for each respective pair of substrates. The mean ratios were termed "toxicity factors" (Tf). Tf values comparing glass and flag leaf assays, were 0.98 and 1.23, respectively for *T. hypnorum* and *C. septempunctata* (A), indicating that the toxicity of fresh deltamethrin residues to both species was similar on these surfaces. Tf values comparing either glass or flag leaf and soil were however in the range of 50–60 for *T. hypnorum* and *C. septempunctata* (A), indicating much lower effects on soil. Estimates of the bioavailable half-life on leaf and soil, obtained from *in situ* bioassays, indicated that effects in the field may vary two- to threefold as a result of differences in the duration of toxicity on these substrates. Overall, *T. hypnorum* could be at more than 150 times greater risk on leaf compared to soil surfaces. The potential for extrapolating laboratory toxicity data to the field is discussed.

The bioavailability of pesticide residues to beneficial invertebrates not only depends upon species dependent factors such as activity pattern, behavior, and the degree of contact with the surface (Jepson et al. 1990; Wiles and Jepson 1993), but also upon the properties of the chemical and of the treated substrate. Interactions between the chemical and the substrate affecting bioavailability include processes of adsorption, desorption, and volatilization, which are affected by the nature of the plant cuticular wax layer (Adams et al. 1987) and the organic matter and clay content of the soil (Harris, 1967; Briggs 1981; Arnold and Briggs, 1990; Gerstl, 1991). Characteristics of the deposit including droplet size distribution and the formulation type of the chemical are important as well as the physico-chemical properties of the active ingredient in the prevailing temperature and humidity conditions (Briggs 1973; Hartley and Graham-Bryce 1980; Ford and Salt 1987; Hall 1987, 1988).

Given the complexity of the processes which determine chemical bioavailability, direct measurements of chemical residues are likely to be of little value for risk assessment, *e.g.*, the half-life of deltametrin residues in mineral soils is between 3 and 8 weeks (Chapman *et al.*. 1981; Miyamoto and Mikami 1983; Hill 1983; Hill and Schaalje 1985) whereas the bioavailable half-life of deltamethrin to linyphild spiders may only be 42 h (Mullié and Everts 1991). This discrepancy may be ex-

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 Table 1. Crop activity categories for the test invertebrates

|                   | Crop activity category  |   |  |  |  |  |
|-------------------|---|---|--|--|--|--|
|                   | Plant active  | Soil active                                   | Plant and soil active  |  |  |  |
| Species<br>tested | A. rhopalosiphi<br>E. balteatus<br>C. septempunctata (L) <sup>a</sup> | N. brevicollis<br>B. obtusum<br>P. melanarius | D. atricapillus<br>C. septempunctata (A) <sup>a</sup><br>T. hypnorum |  |  |  |

<sup>a</sup>C. septempunctata (L) indicates fourth instar larvae and C. septempunctata (A) indicates adults

plained by sorption and degradation processes which restrict the

toxicity of many synthetic pyrethroids to soil organisms (Elliot

cereal foliage were measured to improve estimates of the rela-

tive risks that they pose to a number of species of beneficial

invertebrates inhabiting temperate cereal crops. These data are

required to improve the precision of risk assessments, because

residues from pesticide sprays are stratified through crop cano-

pies and penetrate to the ground, presenting a very wide range

of chemical concentrations to the invertebrates that inhabit dif-

ferent layers within the crop environment (Jepson 1993a).

In this study, the toxicity of deltamethrin residues on soil and

Glass was used as a third substrate to act as a standard. The four species of predator tested on glass were N. brevicollis, D. atricapillus, T. hypnorum, and C. septempunctata. Many standardized tests are undertaken on glass (Jepson 1993b), and its inclusion permitted toxicity data from these tests to be compared with data obtained from more natural substrates.

# Capture and Maintenance of Test Invertebrates

Field capture of active individuals at their peak of seasonal activity was relied upon to provide the test invertebrates. The coleopteran predators were captured between October 1989 and October 1990 in cereal fields and margins on the Leckford Estates, Stockbridge, Hampshire, UK, by dry pitfall trapping, Dietrick suction sampling, hand-held air aspirator, and surface searching (Southwood 1987). Adult hoverflies were captured in June and July 1990 from field margins using butterfly nets and hand-held aspirators and parasitoids were obtained from laboratory cultures.

Prior to the bioassays, all test invertebrates were kept in a controlled environment room in an insectary, maintained at 19–22°C, 55–70% R.H., and a 16L:8D photoperiod. The carabid and staphylinid beetles were fed on ground, moist, cat biscuits ("Delicat," Quaker Latz). The coccinellids and parasitoids were kept in perspex boxes with barley plants infested with the English grain aphid *Sitobion avenae* (F.) (Homoptera: Aphididae), and the syrphids were kept in ventilated perspex boxes and provided with a honey and water solution.

# **Materials and Methods**

#### Test Invertebrates

et al. 1978).

The invertebrates included both polyphagous and pest-specific predators. The polyphagous predators were the large carabid beetles *Pterostichus melanarius* (Illiger) and *Nebria brevicollis* (F.), the small carabid beetles *Demetrias atricapillus* (L.) and *Bembidion obtusum* (Serville) (Coleoptera: Carabidae), and the small staphylinid beetle *Tachyporus hypnorum* (F.) (Coleoptera: Staphylinidae). The pest-specific predators were the coccinellid beetle *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae), the syrphid *Episyrphus balteatus* (Degeer) (Diptera: Syrphidae), and the parasitoid *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera: Braconidae). Adult individuals of each species were tested, with the exception of the coccinellid *C. septempunctata*, where both adults and fourth instar larvae were tested in the flag leaf bioassays. Adult hoverflies are not aphidophagous themselves; however, they are likely to come in contact with pesticide residues in the field when searching foliage.

In the field, these beneficial invertebrates partition themselves throughout the crop canopy and on the soil surface (Vickerman and Sunderland 1975). Some are mainly associated with plant foilage, *e.g.*, hymenopteran parasitoids, syrphids, and coccinellid larvae, some with the soil, *e.g.*, the larger Carabidae, while others spend varying proportions of their time on both substrates. The species tested in these bioassays represented the taxonomic and crop distribution range of predators and parasitoids active in temperate cereals infested by aphids (Table 1).

A series of short-term residual exposure bioassays were carried out for each species on one or both substrates. The duration of the initial bioassays was limited to 2 h to avoid complications arising from differential residue decay rates and to minimize the effects of differences in behavior and activity levels between test organisms. A further series of soil bioassays, with a 72-h exposure period, were made to obtain a better separation of species susceptibilities on this substrate, because the 2 h bioassays were of insufficient duration to give toxic effects for the carabid beetle species.

# **Residual Bioassay Techniques**

*Two-Hour Flag Leaf Exposure Bioassays:* Clean glass plates ( $12 \times 12$  cm) were covered with freshly excised flag leaves from field-grown, untreated, winter wheat plants, cv. Galahad, at decimal growth stage 59 (Zadoks *et al.* 1974). The leaves were attached to the glass via strips of double-sided adhesive tape according to the multiple-leaf bioassay chamber method described in Wiles and Jepson (1992a), and were placed in parallel, base to tip, on each plate with their adaxial surface exposed, ensuring that the glass plate was completely covered.

Exposure chambers, consisting of a section of plastic drainpipe (9.5 cm diameter and 5 cm high), were placed over the flag leaf-covered plates after treatment. The chambers had ventilation holes cut into the sides which were covered with fine gauze and their inner walls had previously been coated with a suspension of Fluon Grade GP1 (PTFE) (Whitford Plastics, Runcorn, Cheshire) to prevent insects from climbing the sides. A glass plate was placed over the top of each chamber and humidified air from bubble chambers were supplied via tubes connected to an aquarium pump.

Two- and 72-Hour Soil Exposure Bioassays: The soil used in the bioassays was dug from a depth of 15 to 20 cm from an untreated field boundary on the Leckford Estates, Stockbridge, Hampshire, UK. Large stones were removed by hand and smaller stones were removed with a 2 mm mesh sieve. Laboratory soil composition analysis indicated that the soil was a sandy loam (55% sand, 24% silt, 14% clay, and 7% organic matter; mean pH = 6.8 in a 1:1 soil/distilled water slurry). The test soil had a mean moisture content of 22% (w/w).

The soil exposure chambers consisted of plastic tubs  $(9.5 \times 5 \text{ cm})$  containing a  $30 \pm 2$  g sample of the soil lightly compacted in the base. The sides of the tubs had again been coated with PTFE to prevent test invertebrates from climbing the sides. A plastic inlay, consisting of a tub with the the bottom removed, was placed in each tub before spraying to avoid contamination of the chamber sides. The inlays were removed immediately after spraying, and the chambers were ventilated in a similar manner to the flag leaf chambers.

*Two-Hour Glass Plate Exposure Bioassays:* These bioassays were carried out using the same procedure as the flag leaf bioassays. New glass plates  $(12 \times 12 \text{ cm})$  that had been cleaned with the detergent ("Decon 90"—Decon Manufacturing Ltd.) and rinsed with distilled water were used as the substrate for exposure.

#### Treatment Procedure

Stock solutions of deltamethrin were prepared immediately before each experiment from formulated "Decis" (25 g/l EC, Hoechst UK Ltd.). Distilled water was used as the diluent and for the control treatment. The test substrates were sprayed under a Potter Tower (Potter 1952) calibrated to deliver spray at a volume of 200 L/ha. The tower was thoroughly cleaned and flushed with acetone and water between treatments. Initially, range-finding bioassays were carried out with five to ten insects per dose and three to four logarithmically spaced doses. From these, definitive ranges of between five and seven doses were selected. After treatment, the chambers were returned to the insectary, and the deposits were allowed to dry for approximately 30 min before the test organisms were introduced. Five test invertebrates were exposed per chamber. Nocturnal species, such as P. melanarius, N. brevicollis, and T. hypnorum, were exposed in darkness and the diurnal species, such as B. obtusum, D. atricapillus, C. septempunctata, E. balteatus, and A. rhopalosiphi, were exposed under artificial light. The area of exposure was 70.9 cm<sup>2</sup> in all bioassays, except for the flag leaf bioassays with E. balteatus and A. rhopalosiphi. These species tended to walk on the upper surface of the chambers, and therefore flag leaf-covered plates were placed on the top as well as the bottom of chambers to ensure exposure. The treated surface area was therefore 141.8 cm<sup>2</sup>. A light source was placed over these chambers to ensure adequate illumination through the gauze covered ventilation holes.

The number of invertebrates of each species tested per dose in the definitive bioassays were as follows: 2-h flag leaf exposure—*D. atricapillus* (n = 20), *T. hypnorum* (n = 20), *C. septempunctata* (n = 20), *C. septempunctata* fourth instar larvae (n = 20), *E. balteatus* (n = 20), and *A. rhopalosiphi* (n = 30); 2 h soil exposure, *N. brevicollis* (n = 20), *D. atricapillus* (n = 20), *T. hypnorum* (n = 20), and *C. septempunctata* (n = 20); 72 h soil exposure, *P. melanarius* (n = 30), *N. brevicollis* (n = 30), *D. atricapillus* (n = 30), *B. obtusum* (n = 30), *T. hypnorum* (n = 30), and *C. septempunctata* (n = 30), and 2 h glass exposure, *N. brevicollis* (n = 20), *D. atricapillus* (n = 30), *T. hypnorum* (n = 20), and *C. septempunctata* (n = 20), and 2 h glass exposure, *N. brevicollis* (n = 20), *D. atricapillus* (n = 30), *T. hypnorum* (n = 20), and *C. septempunctata* (n = 20).

After exposure, test invertebrates were placed in clean, ventilated, containers with food, and responses were recorded at 24-h intervals for the next 4 days. Individuals were classified as unaffected, moving as normal, or affected, *i.e.*, either knocked down, with moving antennae, mouthparts and/or legs, or dead, with no response after stimulation.

#### Statistical Analyses

Probit analysis was performed on 72-h mortality data from the 2-h bioassays and 144-h mortality data (72 h after exposure ceased) in the 72-h bioassays to obtain dose-response statistics (Finney 1971). These data were chosen for analysis because few individuals remained knocked down after this time, and thus the mortality response appeared to be near an end point. Abbott's formula was used to correct the mortality data for control effects (Abbott 1925). The slopes and positions of the probit lines were compared between species using maximum likelihood procedures (Ross 1987). A pairwise testing procedure was undertaken to compare all the species and to infer patterns of susceptibility of predators to deltamethrin residues within and between substrates.

Table 2. 72-h probit statistics for 2-h flag leaf residual bioassays

| Family<br>Species                  | Probit<br>slope | LD <sub>50</sub><br>(and SE)<br>(Detransformed)<br>(g ai/ha) | Heterogeneity<br>$\chi^2$ (df) signif. <sup>a</sup><br>ns = P > 0.05 |
|------------------------------------|-----------------|--|--|
| Carabidae                          |                 |  |  |
| D. atricapillus                    |                 | >50 <sup>b</sup>   |  |
| Staphylinidae                      |                 |  |  |
| T. hypnorum                        | 2.73            | 1.2 (0.15)   | 2.91 (2)ns   |
| Coccinellidae                      |                 |  |  |
| C. septempunctata $(A)^{c}$        | 2.50            | 2.0 (0.29)   | 0.13 (2)ns   |
| C. septempunctata (L) <sup>c</sup> | 1.58            | 0.4 (0.10)   | 1.99 (2)ns   |
| Syrphidae                          |                 |  |  |
| E. balteatus                       | 2.16            | 4.8 (0.96)   | 2.24 (2)ns   |
| Braconidae                         |                 |  |  |
| A. rhopalosiphi                    | 2.31            | 7.1 (0.86)   | 0.16 (2)ns   |

<sup>a</sup>Signif. = significance level

<sup>b</sup>Maximum mortality obtained at the highest dose tested for *D. atricapillus* (50.8g ai/ha) was 10%

<sup>c</sup>C. septempunctata (A) = adults; C. septempunctata (L) = fourth instar larvae

#### Results

#### Flag Leaf Exposure Bioassays

The summary statistics from probit analysis of the 2-h flag leaf dose-mortality data are given in Table 2.  $\chi^2$  tests indicated no significant heterogeneity in the data sets (P > 0.05). Probit analysis was not possible for *D. atricapillus* because mortality remained low at the highest deltamethrin concentration tested. The range of LD<sub>50</sub> values varied from 0.4 g ai/ha to >50 g ai/ha. The ranking of susceptibility for the species tested, from most to least susceptible, was as follows: *C. septempunctata* (L) > *T. hypnorum* > *C. septempunctata* (A) > *E. balteatus* > *A. rhopalosiphi* > *D. atricapillus*.

Three of the four coleopteran predators tested were more susceptible to foliar deltamethrin residues than the syrphid *E*. *balteatus* and the parasitoid *A. rhopalosiphi*. Pairwise maximum likelihood analyses indicated significant differences in susceptibility between all species except *E. balteatus* and *A. rhopalosiphi* (Table 3). Some significant separations in the parallelism of probit lines were also evident (Table 3). *C. septempunctata* (L) had a significantly shallower probit slope than *C. septempunctata* (A), *T. hypnorum*, and *A. rhopalosiphi*.

#### Soil Exposure Bioassays

The summary statistics from probit analysis of the 2- and 72-h soil dose-mortality data are given in Tables 4 and 5.  $\chi^2$  tests showed no heterogeneity in the data sets (P > 0.05). Probit analysis of the dose-response data for *N. brevicollis* and *D. atricapillus* was not possible in the 2-h bioassays because mortality remained low at highest deltamethrin concentrations tested (Table 4). Maximum likelihood procedures indicated that the probit lines for the staphylinid *T. hypnorum* and the coccinellid *C. septempunctata* did not differ in position ( $\chi^2 = 3.0$ , df = 1, P > 0.05) or parallelism ( $\chi^2 = 1.7$ , df = 1, P > 0.05) in the 2-h bioassays.

The 72-h bioassays enabled separation of susceptibilities. The  $LD_{50}$  values of the six species tested varied between 4.2 g

**Table 3.** Matrix of  $\chi^2$  statistics from pairwise maximum likelihood analyses for differences in (i) position and (ii) parallelism of 72-h probit lines of the test species in the 2-h flag leaf bioassays

| Species      | C.s.(L) | A.r.    | E.b.           | T.h.   |
|--------------|---------|---------|----------------|--------|
| (i) Position |         |         |                |        |
| C.s.(A)      | 18.0*** | 19.3*** | 6.9**          | 7.8**  |
| T.h.         | 8.6**   | 20.4*** | 9.0**          |        |
| E.b.         | 12.8*** | 2.8 ns  |                |        |
| A.r.         | 28.2*** |         |                |        |
| (ii) Paralle | lism    |         |                |        |
| C.s.(A)      | 4.0*    | 0.5 ns  | 0.3 ns         | 2.2 ns |
| T.h.         | 4.1*    | 0.1 ns  | 0.1 <b>n</b> s |        |
| E.b.         | 1.9 ns  | 0.2 ns  |                |        |
| A.r.         | 3.9*    |         |                |        |

Values give  $\chi^2$  statistic, df = 1, and level of significance; ns = not significant (P > 0.05), \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001. Key to test species: C.s.(A) = C. septempunctata (Adults), T.h. = T. hypnorum, E.b. = E. balteatus, A.r. = A. rhopalosiphi, C.s.(L) = C. septempunctata (fourth instar larvae)

**Table 4.** 72-h probit statistics for 2-h soil residual bioassays

| Family<br>Species     | Probit<br>slope | LD <sub>50</sub><br>(and SE)<br>(detransformed)<br>(g ai/ha) | Heterogeneity<br>$\chi^2$ (df) signif. <sup>a</sup><br>ns = P > 0.05 |
|-----------------------|-----------------|--|--|
| Carabidae             |                 |  |  |
| N. brevicollis        |                 | >170 <sup>b</sup>  |  |
| D. atricapillus       |                 | >500 <sup>b</sup>  |  |
| Staphylinidae         |                 |  |  |
| T. hypnorum           | 1.36            | 52.8 (12.0)  | 0.65 (3)ns   |
| Coccinellidae         |                 |  |  |
| C. septempunctata (A) | 1.95            | 97.8 (18.1)  | 0.37 (2)ns   |

<sup>a</sup>Signif. = significance level

<sup>b</sup>Maximum mortality obtained at the highest dose tested for *N. brevicollis* (169.7 g ai/ha) was 20%; and maximum mortality obtained at the highest dose tested for *D. atricapillus* (499.7 g ai/ha) was 10%

Table 5. 72-h probit statistics for 72-h soil residual bioassays

| Family<br>Species     | Probit<br>slope | LD <sub>50</sub><br>(and SE)<br>(detransformed)<br>(g ai/ha) | Heterogeneity<br>$\chi^2$ (df) Signif. <sup>a</sup><br>ns = P > 0.05 |
|-----------------------|-----------------|--|--|
| Carabidae             |                 |  |  |
| P. melanarius         | 2.13            | 52.3 (6.28)  | 0.43 (3)ns   |
| N. brevicollis        | 1.89            | 53.2 (7.08)  | 1.20 (3)ns   |
| D. atricapillus       | 2.07            | 267.3 (36.1)   | 0.91 (3)ns   |
| B. obtusum            | 2.14            | 7.8 (0.88)   | 5.41 (4)ns   |
| Staphylinidae         |                 | . ,  | . ,  |
| T. hypnorum           | 2.52            | 4.2 (0.50)   | 0.78 (2)ns   |
| Coccinellidae         |                 |  |  |
| C. septempunctata (A) | 1.96            | 16.6 (2.05)  | 0.45 (4)ns   |

<sup>a</sup>Signif. = significance level

ai/ha and 267 g ai/ha (Table 5). The susceptibility ranking of the species tested, from most to least susceptible, was T. hypnorum > B. obtusum > C. septempunctata (A) > P. melanarius > N. brevicollis > D. atricapillus. The  $\chi^2$  statistics from pairwise maximum likelihood analyses indicated significant separations in the positions of the probit lines for all

**Table 6.** Matrix of  $\chi^2$  statistics from pairwise maximum likelihood analyses for differences in (i) position and (ii) parallelism of 72-h

| Species     | C.s.(A) | T.h.    | B.o.    | D.a.    | N.b.   |
|-------------|---------|---------|---------|---------|--------|
| (i) Positio | )n      |         |         |         |        |
| P.m.        | 26.9*** | 61.2*** | 57.6*** | 47.8*** | 0.1 ns |
| N.b.        | 26.9*** | 57.7*** | 56.0*** | 41.8*** |        |
| D.a.        | 68.2*** | 69.3*** | 82.1*** |         |        |
| B.o.        | 21.5*** | 10.5*** |         |         |        |
| T.h.        | 42.7*** |         |         |         |        |
| (ii) Paral  | lelism  |         |         |         |        |
| P.m.        | 0.2 ns  | 0.3 ns  | 0.0 ns  | 0.0 ns  | 0.2 ns |
| N.b.        | 0.0 ns  | 0.9 ns  | 0.2 ns  | 0.1 ns  |        |
| D.a.        | 0.1 ns  | 0.4 ns  | 0.1 ns  |         |        |
| B.o.        | 0.2 ns  | 0.4 ns  |         |         |        |
| T.h.        | 1.0 ns  |         |         |         |        |
|             |         |         |         |         |        |

probit lines of the test species in the 72-h soil bioassays

Values give  $\chi^2$  statistic, df = 1, and level of significance; ns = not significant (P > 0.05), \*\*\* P < 0.001. Key to test species: P.m. = P. melanarius, N.b. = N. brevicollis, D.a. = D. atricapillus, B.o. = B. obtusum, T.h. = T. hypnorum, C.s.(A) = C. septempunctata (Adults)

Table 7. 72-h probit statistics for 2-h glass residual bioassays

| Family<br>Species     | Probit<br>slope | LD <sub>50</sub><br>(and SE)<br>(detransformed)<br>(g ai/ha) | Heterogeneity<br>$\chi^2$ (df) signif. <sup>a</sup><br>ns = P > 0.05 |
|-----------------------|-----------------|--|--|
| Carabidae             |                 |  | <u></u>  |
| N. brevicollis        |                 | >37 <sup>b</sup>   |  |
| D. atricapillus       |                 | >37 <sup>b</sup>   |  |
| Staphylinidae         |                 |  |  |
| T. hypnorum           | 2.88            | 1.22 (0.15)  | 1.15 (2)ns   |
| Coccinellidae         |                 | . ,  |  |
| C. septempunctata (A) | 2.57            | 1.66 (0.21)  | 2.91 (3)ns   |

<sup>a</sup>Signif. = significance level

<sup>b</sup>Maximum mortality obtained at the highest dose tested for *N. brevicollis* (36.7 g ai/ha) was 45%; and maximum mortality obtained at the highest dose tested for *D. atricapillus* (36.7 g ai/ha) was 33%

species except the large carabid beetles P. melanarius and N. brevicollis (Table 6). No significant separations were evident in parallelism. However, of the species tested, T. hypnorum had the steepest probit slope, and N. brevicollis had the shallowest probit slope.

# Glass Exposure Bioassays

Probit analysis was not possible for N. brevicollis and D. atricapillus in the 2-h glass exposure bioassays because mortality remained low at the highest deltamethrin concentrations tested (Table 7). Maximum likelihood analysis indicated no significant difference in position and parallelism for the probit lines of the T. hypnorum and C. septempunctata adults.

#### Estimation of Toxicity Factors

Pairwise maximum likelihood analyses to compare individual differences in position and parallelism of the probit lines for C.

388

**Table 8.** Matrix of  $\chi^2$  statistics from pairwise maximum likelihood analyses for differences in (i) position and (ii) parallelism of 72-h probit lines of two species of predators on three test substrates

| Species     | T.h.(G) | T.h.(F) | Species     | C.s.(G) | C.s.(F) |
|-------------|---------|---------|-------------|---------|---------|
| (i) Positio | m       |         | (i) Positio | on      |         |
| T.h.(S)     | 30.7**  | 27.4*** | C.s.(S)     | 44.0*** | 36.4*** |
| T.h.(F)     | 0.1 ns  |         | C.s.(F)     | 1.5 ns  |         |
| (ii) Paral  | lelism  |         | (ii) Paral  | lelism  |         |
| T.h.(S)     | 4.5*    | 4.1*    | C.s.(S)     | 3.9*    | 3.8*    |
| T.h.(F)     | 0.1 ns  |         | C.s.(F)     | 1.2 ns  |         |

Values give  $\chi^2$  statistic, df = 1, and level of significance; ns = not significant (P > 0.05), \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.01. Key to test species: T.h. = *T. hypnorum*, C.s. = *C. septempunctata*. Key to substrates: (G) = glass, (F) = flag leaf, (S) = soil

septempunctata adults and *T. hypnorum* on the substrates tested in the 2-h bioassays indicated no significant differences in position and parallelism between the probit lines on the glass and flag leaf surfaces (Table 8). However, the probit lines differed significantly in both position and parallelism between glass and soil and flag leaf and soil, indicating that significantly greater effects of a given treatment rate on glass or flag leaf compared with the soil.

The toxicity of deltamethrin residues to *T. hypnorum* and *C. septempunctata* adults on the three test substrates were compared by iterating a sequence of lethal dose ratios calculated from dose-response statistics for each pair of substrates and thus calculating a mean ratio. The sequence of doses selected represented responses between  $LD_{10}$  and  $LD_{90}$  (Table 9) to allow for differences between probit slopes. The mean values obtained were termed "toxicity factors" (Tf). These ratios give an estimate of the relative toxicity of deltamethrin residues to a given organism on the two substrates that are compared.

The Tf values agreed closely for both species of predator in all substrate comparisons (Table 9). The values indicated that the toxicity of deltamethrin residues on glass and flag leaf surfaces was very similar (Tf  $\approx$  1). The Tf values for comparisons between glass and soil and flag leaf and soil substrates, however, indicated that deltamethrin residues on glass and flag leaf surfaces were approximately 50–60 times more toxic to *T*. *hypnorum* and *C. septempunctata* that residues on the sandy loam soil (Table 9).

#### Predictions of Relative Risk in the Field

Toxicity factors may be useful as a correction factor for laboratory bioassay data from standard substrates. In order to estimate the relative risk posed by pesticide residues to beneficial invertebrates in the field, however, further correction for the duration of bioavailability of the deposits on each given substrate may be necessary. The relative risk posed by deltamethrin residues to *T. hypnorum* and *C. septempunctata* adults on soil and flag leaf substrates in the field was therefore estimated using the equation given below:

relative risk = 
$$Tf_{A/B} \times Bhl_{A/B}$$
 (1)

where  $Tf_{A/B}$  = the toxicity factor, *i.e.*, the mean ratio of the dose-mortality responses of the invertebrates on substrates A

(flag leaves) and B (soil) and  $Bhl_{A/B} = an$  estimate of the relative bioavailable half-lives of deltamethrin on the same substrates under field conditions.

Values of Bhl were obtained for deltamethrin on flag leaf and soil substrates in a mature winter wheat crop using mortality data from 24 h exposure *in situ* bioassays from two separate studies, Unal and Jepson (1991) and Wiles and Jepson (1992a) (Table 10). Both studies were carried out at the same field site, in different seasons, and therefore the soil types are probably similar. The soil used in the current bioassays was also collected from a nearby location. In both studies fresh batches of insects were exposed to deltamethrin residues on flag leaves and soil on each day after spray application. The bioavailable half-life of deltamethrin on the substrates was estimated as the number of days taken for the mortality of the test invertebrates to fall to 50% of the initial mortality observed.

The relative bioavailable half-lives of deltamethrin residues on flag leaves compared with soil was estimated by calculating the ratio of bioavailable half-lives from the two studies (Table 10). These ratios were 2.54 (from Unal and Jepson 1991) and 2.81 (from Wiles and Jepson 1992a). As these values were in close agreement a mean was taken (2.68) to act as an estimate of the relative bioavailable half-lives (Table 11). This estimate was then substituted into Eq. (1) together with the toxicity factors to give predictions of the relative risk posed by deltamethrin residues on flag leaves relative to residues on the soil (Table 11). The mean values of relative risk were 154.3 for *T. hypnorum* and 162.4 for *C. septempunctata*.

# Discussion

# Toxicity of Deltamethrin Residues to Invertebrates on Flag Leaves

The five species of predators and the parasitoid tested in the flag leaf bioassays showed a 100-fold range of susceptibility to deltamethrin residues. The small carabid beetle D. atricapillus was the least susceptible of the predators tested, with doses in excess of eight times current recommended field rate of deltamethrin in UK cereals causing only low levels of mortality. Knockdown symptoms were observed during exposure to deltamethrin residues but D. atricapillus was able to recover shortly afterwards; this may be physiological, i.e., it may possess innate tolerance mechanisms (Wiles and Jepson 1992b), or behavioral/morphological, as this beetle tends to walk relatively slowly and has a relatively low contact area with the substate (Wiles and Jepson 1993). The other species tested had  $LD_{50}$  values similar to or lower than the recommended field rate of deltamethrin (<6.25 g ai/ha), indicating that mortality could occur in the field from residual uptake. The most susceptible predator was the fourth instar larva of C. septempunctata which had an LD<sub>50</sub> of approximately 0.06 of field rate; this may be because coccinellid larvae tend to contact the substrate with their abdomen and are therefore likely to have a high contact area with the substrate which may increase pesticide uptake. Also, sublethal poisoning effects reduced searching and feeding efficiency of the larvae on the days after exposure. The small staphylinid beetle T. hypnorum was the second most

|                       | Toxicity factors (Tf) calculated from substrate comparisons |             |                       |             |                           |             |  |
|-----------------------|---|-------------|-----------------------|-------------|---------------------------|-------------|--|
| Test                  | Glass/flag leaf   |             | Glass/sandy loam soil |             | Flag leaf/sandy loam soil |             |  |
| species               | Mean  | (95% C.L.)  | Mean                  | (95% C.L.)  | Mean                      | (95% C.L.)  |  |
| T. hypnorum           | 0.98  | (0.93–1.03) | 53.6                  | (5.9–101.3) | 57.6                      | (5.0–110.2) |  |
| C. septempunctata (A) | 1.23  | (1.19–1.27) | 63.7                  | (45.4-82.0) | 60.6                      | (41.4–79.8) |  |

Table 9. Toxicity factors comparing the relative bioavailability of deltamethrin residues to T. hypnorum and C. septempunctata adults on glass, flag leaf, and soil substrates

Tf values were obtained by calculating a mean ratio from the LD<sub>10</sub>, LD<sub>30</sub>, LD<sub>50</sub>, LD<sub>70</sub>, and LD<sub>90</sub> doses for each respective pairs of substrates

 Table 10. Estimates of the bioavailable half-lives of deltamethrin residues on soil and flag leaves in a mature cereal crop

|                     | Estimated bioavailable half-lives from<br>in situ bioassay data (days) |                                       |  |
|---------------------|--|---------------------------------------|--|
| Substrate<br>type   | Unal and Jepson<br>(1991) <sup>a</sup>                                 | Wiles and Jepson (1992a) <sup>b</sup> |  |
| Flag leaves<br>Soil | 6.0<br>2.4   | 4.5<br>1.6                            |  |

<sup>a</sup>B. lampros was used as the test species

 $^{b}C$ . septempunctata was used as the test species

susceptible species to flag leaf deposits; perhaps because of its relatively high contact area with the substrate (Wiles and Jepson 1993). *C. septempunctata* adults were less susceptible to foliar deltamethrin residues than *T. hypnorum* but more susceptible than either the adult hoverfly *E. balteatus* or the parasitoid *A. rhopalosiphi* and may be related to differences in activity during the bioassay. The coccinellids were more active than either of these species during the period of exposure and therefore deltamethrin uptake may have been proportionally greater.

# The Toxicity of Deltamethrin Residues to Invertebrates on Sandy Loam Soil

The LD<sub>50</sub> values from the 2 h soil bioassays ranged from 8.4 to >80 times the recommended field rate of deltamethrin for cereals with the four species tested. This duration of exposure was insufficient to separate species susceptibilities. The 72-h bioassays, however, enabled comparisons of susceptibility between species and gave  $LD_{50}$  values, which varied from 0.7 to 42.8 times the field rate. The susceptibility ranking for the six predators tested broadly agreed with the predictions given in Wiles and Jepson (1993) for a similar set of test species. The staphylinid T. hypnorum was the most susceptible species and the carabid D. atricapillus was the least susceptible. Differences in species susceptibilities are difficult to interpret because the responses are not only likely to be related to the intrinsic susceptibility and innate characteristics of the species but also species-specific behavior and activity patterns. Observations made during the bioassays indicated that the staphylinid T. hypnorum and the coccinellid C. septempunctata were generally active for a higher proportion of the period of exposure than the carabid beetles. This, together with T. hypnorum's high contact area, may partly explain why the staphylinid beetle was found to be more susceptible to deltamethrin residues on soil

than all of the other species tested in these bioassays, including the small carabid *B. obtusum*, which has a similar intrinsic susceptibility to deltamethrin (Wiles and Jepson 1992b).

# The Use of Toxicity Factors for Risk Assessment

Comparisons of the toxicity of deltamethrin residues on the three test substrates were made for two predator species, T. hypnorum and C. septempunctata. The Tf ratios tended to be similar for the two species, supporting the assertion that differences between substrates were due to physical and chemical factors and not differences in the behavioral responses of the species to the different surfaces. These values indicated that the toxicity of fresh deltamethrin residues to beneficial invertebrates on glass and winter wheat flag leaves were similar, under the given conditions. The glass plates used in these bioassays were carefully cleaned before use, but no specific deactivation procedures were carried out. We inferred that deltamethrin behaved similarly on both glass and leaf surfaces. The toxicity of deltamethrin residues to T. hypnorum and C. septempunctata on the glass and flag leaves was approximately 50-60 times greater than on the sandy loam soil, indicating large differences of bioavailability between these substrates. A higher proportion of the residues therefore remains available on glass or leaf surfaces, soon after treatment, than on soil.

Toxicity factors such as these, if validated for a larger number of species, may be useful correction factors for bioassay data. Glass is widely used as a test substrate in bioassays (Jepson 1993b). It would be useful if reliable corrections could be made to data from glass bioassays to allow extrapolation of results to more natural substrates, or if they could be used as a refinement to risk assessment: For example, Wiles and Jepson (1992b) found that the staphylinid beetle *T. hypnorum* had a similar intrinsic susceptibility to deltamethrin as the small carabid beetle *B. obtusum*. However, *T. hypnorum* is plant-active whereas *B. obtusum* is mainly ground-active: The toxic risk posed by contact with spray residues is therefore likely to be much greater for *T. hypnorum*, assuming that the two beetles have similar potential levels of exposure.

The limitations of toxicity factors in risk assessment must also be considered. Tf values will vary with different substrates (*i.e.*, leaves with different wax properties or thicknesses and soil with different mineral or organic matter compositions), different bioassay conditions and durations of exposure, and between test organisms with different habits. To be of general value, therefore, standardized test conditions and exposure methods need to be followed for groups of organisms that are widely recognized as good indicators of potential risk to the beneficial invertebrate community (Jepson 1993a).

| Predator<br>species | Toxicity factors (Tf) comparing<br>the toxicity of deltamethrin<br>residues on flag leaf and soil<br>(95% C.L.) | Estimated mean bioavailable<br>half-life (Bhl) for deltamethrin on<br>flag leaves relative to soil (days) | Predicted relative risk posed by<br>deltamethrin residues on flag<br>leaves compared to soil |
|---------------------|---|---|--|
| T. hypnorum         | 57.6 (5.0–110.2)  | 2.68  | 154.3 (13.4–295.3)   |
| C. septempunctata   | 60.6 (41.4–79.8)  |   | 162.4 (111.0–213.9)  |

Table 11. Estimation of the relative risk posed by deltamethrin residues to T. hynorum and C. septempunctata on flag leaf and soil substrates in the field

# Predicting Risk in the Field

The incorporation of estimates of the bioavailable half-life of deltamethrin on wheat flag leaves and soil may improve predictions of effects in the field. Some evidence that differences in bioavailability occur in practice is provided by Jagers op Akkerhuis and Hamers (1992), who investigated bioavailability of <sup>14</sup>C deltamethrin to a linyphild spider. Soil covered with fungi and moss showed increased deltamethrin bioavailability by a factor of approximately 100 compared to bare soil.

The risk predictions suggest that after a deltamethrin spray application, plant-active predators may be at a much greater risk of suffering deltamethrin side-effects in a cereal crop than the predators that remain on the soil. Plant-active predator species, such as the small staphylinid *T. hypnorum* and adults and larvae of the coccinellid *C. septempunctata*, may be the most severely affected species. This is in agreement with the results of several field trials in cereals which have shown that the abundance of predators such as staphylinids, including *Tachyporus* spp. (Basedow *et al.* 1985; Vickerman *et al.* 1987a), and coccinellids, such as *C. septempunctata* (Vickerman *et al.* 1987b) was reduced in cereal crops after treatment with deltamethrin whereas ground-active species were less affected.

Modified risk predictions using toxicity factors may, therefore, be useful to identify species which may be at risk from residual exposure effects on specific substrates. Until factors such as bioavailability and chemical uptake by organisms can be quantified more easily and precisely, approaches such as the one outlined in this paper may offer the most readily obtainable insight into predicting substrate-mediated risk via residual exposure.

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