

# RESIDUAL TOXICITIES OF THREE INSECTICIDES TO FOUR SPECIES (COLEOPTERA: CARABIDAE) OF ARTHROPOD PREDATOR

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## Abstract

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In laboratory bioassays, four carabid species [*Agonum dorsale* (Pontoppidan), *Bembidion lampros* (Herbst), *B. obtusum* Serville, and *Demetrias atricapillus* (L.)] that are important predators of aphids in cereals in the United Kingdom were exposed to deposits of deltamethrin, dimethoate, or pirimicarb on glass for up to 72 h. We detected differences between compounds and species that are discussed in the context of exposure of these predators to insecticides in the field. We also describe problems involved in obtaining comparative toxicity data when dilutions of field application rates for target species are used in bioassays with nontarget species. Such problems add another dimension to risk assessment based on laboratory data.

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## Résumé

Au cours de tests en laboratoire, quatre espèces de carabes [*Agonum dorsale* (Pontoppidan), *Bembidion lampros* (Herbst), *B. obtusum* Serville et *Demetrias atricapillus* (L.)] qui sont d'importants prédateurs des pucerons sur les céréales en Grande-Bretagne ont été exposées pendant plus de 72 h à des frottis de deltaméthrine, de diméthoate ou de pirimicarbe sur du verre. Nous avons constaté des différences entre les produits et les espèces et ces différences sont examinées dans le contexte d'une exposition de ces prédateurs aux insecticides en nature. Nous décrivons également les problèmes encourus lors de l'obtention de données comparatives de la toxicité par emploi de dilutions utilisées en nature sur des espèces cibles au cours de tests en laboratoire sur des espèces non cibles. Ces problèmes ajoutent une nouvelle dimension aux évaluations, à partir de données de laboratoire, des risques reliés à des traitements.

[Traduit par la Rédaction]

## Introduction

Effects of pesticide treatment depend on species' susceptibility and the extent of exposure to the toxicant. In arable fields, for example, only about 1% of an insecticide applied to crops may reach the target pest (Graham-Bryce 1977). Even if nontarget species are not directly exposed to sprays, exposure to pesticide residues can occur by contact (e.g. Unal and Jepson 1991) and by ingestion (e.g. Wiles and Jepson 1993). Exposure level is also affected by the activity and distribution of nontarget species in an ecosystem. These variables underlie problems involved in ecotoxicology (e.g. Robertson and Worner 1990; Stark and Wennergren 1995).

In cereal fields, aphid-specific predators that inhabit the upper levels of crops are at greater risk to sprays than are ground-dwelling species that are protected from the spray by the plant canopy. Çilgi and Jepson (1992) showed that species at the top of a cereal canopy were exposed to about 36–91% of the applied field rate, whereas ground-dwelling species

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were exposed to only 11–21% of the applied field rate. Nocturnal ground beetles seem even less likely to be exposed to direct spray because they hide under plant debris or soil during the day when sprays are applied.

Phenology and developmental events can also affect exposure. For example, *Bembidion obtusum* Serville spends its entire life cycle in cereal fields (Jepson 1989; Burn 1992) and might be exposed to all spray applications in a growing season. In contrast, other carabid species such as *Agonum dorsale* (Pontoppidan), *Bembidion lampros* (Herbst), and *Demetrias atricapillus* (L.) overwinter as adults in field boundaries; these species recolonize cereal fields 3–6 months after pesticide application (Coombes and Sotherton 1986). Direct contact of these species with insecticides should therefore be unlikely. Insecticides may also drift into field boundaries and expose nontarget arthropods in these habitats.

In the laboratory experiments described here, we tested the effects of three commonly used insecticides against four species of beneficial Carabidae. To approximate rates likely to be encountered in the field, we used recommended field rates for control of aphids, and dilutions thereof, to obtain the rates tested in the laboratory.

### Materials and Methods

**Test Organisms.** Species tested were *A. dorsale*, *D. atricapillus*, *B. lampros*, and *B. obtusum*. The first three species are the most important polyphagous predators of aphids (Sunderland and Vickerman 1980). *Bembidion obtusum* is ecologically important because it consistently showed adverse effects of pesticides in two long-term studies of pesticide use in the United Kingdom (Burn 1992; Vickerman 1992; Çilgi et al. 1993). This species has been considered as an indicator species for environmental impact assessment of pesticides (Çilgi 1994).

Of the four species tested, *B. lampros* is almost exclusively active during the day, *B. obtusum* is active throughout the day and night, and the other two species are almost exclusively nocturnal (Vickerman and Sunderland 1975; Luff 1978; Halsall and Wratten 1988). Only *D. atricapillus* can climb the crops and is thus found both on plant and soil surfaces (Vickerman and Sunderland 1975; Halsall and Wratten 1988).

**Conditions before Treatment.** Beetles were collected by aspiration in margins of cereal fields and hedge banks in October 1992 at the Leckford Estate, Stockbridge, Hampshire, U.K. After collection, species were separated and stored in polystyrene boxes (10 by 15 by 27 cm) containing a layer of moist soil and pellets of cat food (Delicat, Quaker Latz GmbH) in a cold room at 4°C. Seventy-two hours before each bioassay, the beetles were counted into groups of 100 and stored in polystyrene boxes containing fresh cat food and damp filter papers. The boxes were kept in a controlled environment at 18–20°C, 60–70% RH, and a photoperiod of 16L:8D.

**Pesticides.** Deltamethrin [Decis, 25 g/L emulsifiable concentrate (EC), Hoechst], dimethoate (Croxex Dimethoate, 40% EC, Hortichem), and pirimicarb (Aphox, 50% wettable granules, Zeneca) were tested on each species. These pesticides are commonly used for management of aphid populations in cereal crops in the United Kingdom (e.g. Wratten et al. 1990).

**Spray Application.** A Potter laboratory spray tower (Burkard Manufacturing Co. Ltd., Rickmansworth, U.K.) calibrated to deliver 200 L/ha of water was used to apply the chemicals onto 7.5-cm<sup>2</sup> glass plates or glass Petri dishes (5.5 cm diameter, 1 cm deep). The spray tower was cleaned and flushed with acetone, alcohol, and distilled water before application of each pesticide. Glass plates and Petri dishes were cleaned with detergent (Decon 90; Decon Laboratories Ltd., Hove, U.K.) before they were used in experiments.

**Application Rates.** Each insecticide was applied at a range of concentrations up to its highest recommended rate for use in cereals in the United Kingdom. The treatments applied



were control, 0.15, 0.31, 0.62, 1.25, 2.5, 5.0, 10, 20, 50, and 100% of field rate. Field rates are 5 and 6.25 g active ingredient (AI)/ha of deltamethrin for autumn and summer applications, respectively, 340 g AI/ha for dimethoate, and 140 g AI/ha for pirimicarb.

The highest recommended autumn field rate of deltamethrin was the maximum used with *A. dorsale*, *B. lampros*, and *B. obtusum*. The highest recommended summer rate was the maximum used with *D. atricapillus*. All species were treated with a maximum recommended rate of 340 g AI/ha for dimethoate and 140 g AI/ha for pirimicarb.

**Exposure Conditions.** *Bembidion lampros*, *B. obtusum*, and *D. atricapillus* were kept in a cold room at 4°C for 3 h to reduce their activity, after which they were placed in the test chambers. Four chambers (= replications) were prepared for each concentration of a toxicant. The sprayed glass plates and Petri dishes were left to dry for approximately 1 h after each spray application. The test species were then exposed to the residual deposits for up to 72 h.

***Agonum dorsale*.** The treatment chamber for *A. dorsale* consisted of an unsprayed plastic cylinder (5.5 cm diameter, 3 cm high) clipped onto a sprayed glass plate. The interior of the plastic chambers had previously been painted with an aqueous suspension of Fluon (polytetrafluoroethylene; Whitford Plastics, Runcorn, Cheshire, U.K.). The spray chamber was designed to keep beetles in contact with the plate. Four groups of five insects were treated at each of the 11 rates tested.

***Demetrias atricapillus* and *Bembidion* spp.** In bioassays with *D. atricapillus* and the two *Bembidion* spp., the test apparatus was modified to prevent escape of any climbing beetle. Each test unit consisted of a 7.5-cm<sup>2</sup> glass plate and a glass Petri dish (5.5 cm diameter, 1 cm in height) that were separated by a 1-mm-thick cork gasket. The gasket provided an escape-proof seal.

The lid and base of the chamber were sprayed; after the insects were introduced, the chamber was sealed with a metal clip. For ventilation, a humidified airflow was provided with a pair of syringe needles inserted through the cork gasket. One needle was connected to an aquarium pump (Elite 800; 1500 cm<sup>3</sup> output/min) with tubing; the needle on the opposite side served as an outlet for the airflow. This system also permitted escape of pesticide vapor from the test unit to ensure that any mortality that occurred was caused only by the pesticide residue. Five *B. lampros* and five *B. obtusum* were placed together in each test chamber; five *D. atricapillus* were placed in the same type of chamber.

**Conditions and Observations after Treatment.** Once the beetles were distributed, the treatment chambers were randomly placed on benches in a controlled environment room (20 ± 2°C, with a photoperiod of 16L:8D). Humidity inside the test chambers was 80 ± 5% RH. Assessments of the effects of the treatments were made at 24, 48, and 72 h after exposure began. Individuals were classified as live (normally active and responsive), knocked down (inactive but still moving in response to a mechanical stimulus), or dead (not moving in response to a mechanical stimulus).

**Data Analyses.** For each species, the numbers of dead and knocked down beetles were combined into a single category after we observed that beetles that were knocked down died (with rare exceptions) by the time of the next observation period. To determine which observation time should be used for analysis, mortality versus pesticide concentration for 24, 48, and 72 h was plotted. Smooth curves of mortality versus percentage application rate were produced by fitting local regression models with the nonparametric regression procedure *loess* in the statistical package S-Plus (Statistical Sciences Inc. 1992).

To determine whether or not use of dilutions of field rates would provide data comparable with results obtained from laboratory comparisons of relative toxicities (Robertson et al. 1984), data (g AI/ha) from the 72-h observations were next subjected to probit analysis

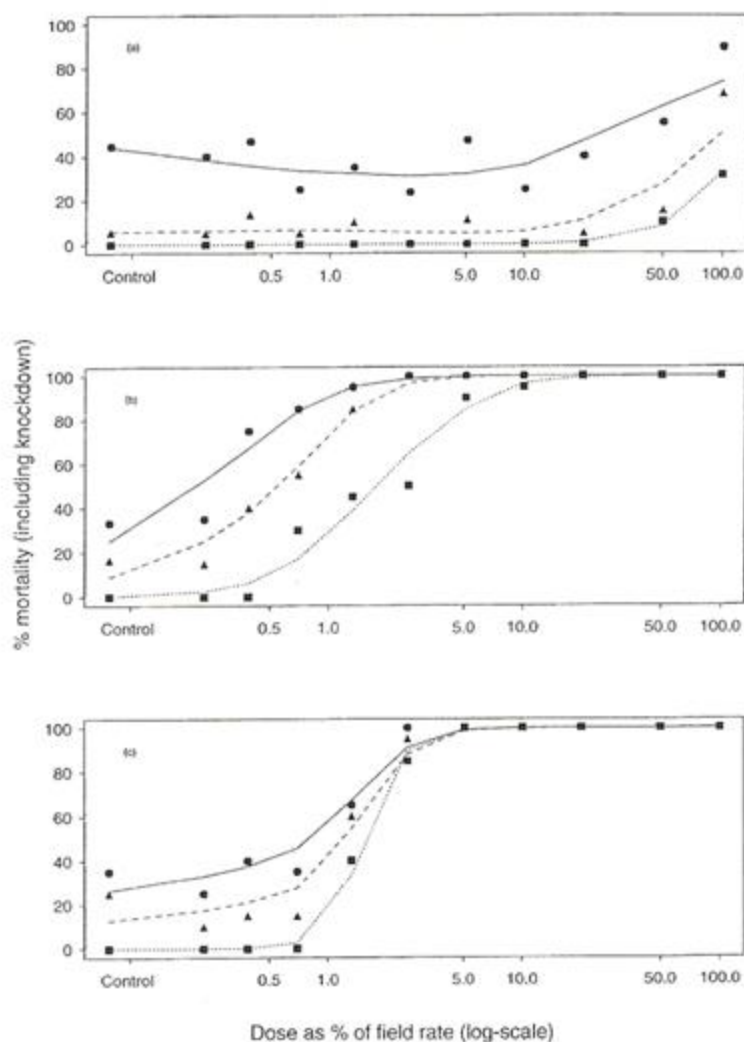


FIG. 1. Toxicity of pirimicarb (a), deltamethrin (b), and dimethoate (c) residues to *Agonum dorsale* after 24 h (squares), 48 h (triangles), and 72 h (circles) of exposure in laboratory bioassays.

(LeOra Software Inc. 1987). The significance of regressions was determined by *t*-ratio tests (Robertson and Preisler 1992).

### Results and Discussion

**Comparative Toxicity and Species Response.** Plots of mortality at 24, 48, and 72 h indicated that maximum mortality occurred by 72 h. Despite great variation in mortalities at increasing rates, we observed trends among toxicities of the compounds when data were graphically represented in terms of percentage of the highest recommended field rate.

Deltamethrin was most toxic to *A. dorsale*, followed by dimethoate and pirimicarb (Fig. 1). For the other three species, dimethoate was more toxic than deltamethrin or pirimicarb (Figs. 2–4). Pirimicarb appeared to be the least toxic to all four species.

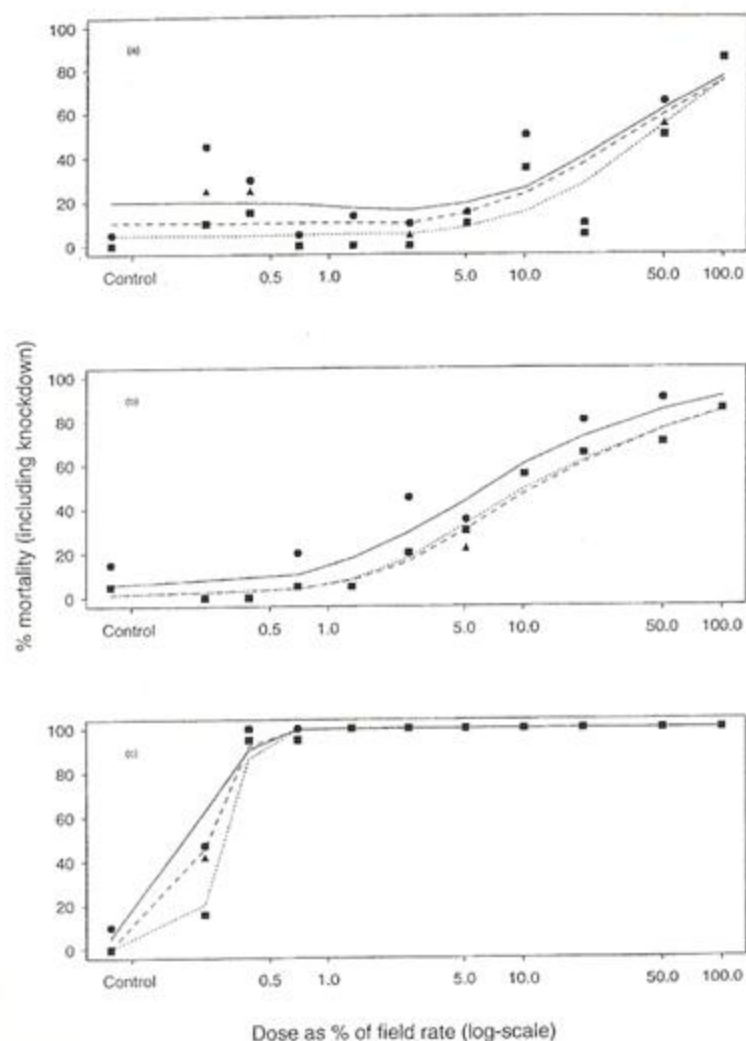


FIG. 2. Toxicity of pirimicarb (a), deltamethrin (b), and dimethoate (c) residues to *Demetrias atricapillus* after 24 h (squares), 48 h (triangles), and 72 h (circles) of exposure in laboratory bioassays.

After 72 h of exposure, dimethoate caused >50% mortality of *D. atricapillus* (Fig. 2c) and *B. obtusum* (Fig. 4c) at and above 0.3% of field application rate. Deltamethrin caused 100% mortality of *A. dorsale* at about 2.5% of field rate (Fig. 1b). Deltamethrin killed all *B. obtusum* at about 5% of field rate (Fig. 4b). *Demetrias atricapillus* and *B. lampros* were relatively tolerant to deltamethrin, with total mortality resulting from near full field concentration (Figs. 2b, 3b). Complete mortality was not reached when any of the four carabid species were exposed to pirimicarb, although about 95% of the most susceptible species, *B. obtusum*, died as a result of exposure to the full field application rate (Fig. 4a). *Bembidion lampros*, however, showed 74% mortality at the field rate of pirimicarb (Fig. 3a).

The low relative toxicity of pirimicarb to predatory carabids has been demonstrated in other studies (e.g. Brown et al. 1983). Our results suggest that a reduction in the application rate of pirimicarb from full to half the field rate might increase survival of carabids from



TABLE 1. Responses of carabid species to selected insecticides (grams active ingredient per hectare)

Species	<i>n</i>	Slope $\pm$ SE	LC <sub>50</sub> (95% C.L.)
<b>Deltamethrin</b>			
<i>Agonum dorsale</i>	200	2.84 $\pm$ 0.72	0.016 (0.007–0.024)
<i>Bembidion lampros</i>	200	1.90 $\pm$ 0.26	0.72 (0.15–2.5)
<i>Bembidion obtusum</i>	200	Regression not significant	
<i>Demetrias atricapillus</i>	200	1.36 $\pm$ 0.19	0.52 (0.21–1.2)
<b>Dimethoate</b>			
<i>A. dorsale</i>	140	Regression not significant	
<i>B. lampros</i>	100	6.57 $\pm$ 2.31	0.72 (0.37–0.92)
<i>B. obtusum</i>	100	Regression not significant	
<i>D. atricapillus</i>	79	Regression not significant	
<b>Pirimicarb</b>			
<i>A. dorsale</i>	188	4.90 $\pm$ 2.48	89*
<i>B. lampros</i>	189	2.29 $\pm$ 0.61	93 (53–240)†
<i>B. obtusum</i>	199	3.87 $\pm$ 0.84	51 (27–80)†
<i>D. atricapillus</i>	195	Regression not significant	

\* 95% C.L. could not be estimated because  $g$  was more than 0.5 at  $P = 0.95$  (LeOra Software Inc. 1987).

† 90% C.L.

5–26% to as high as 70% (Figs. 1–4). Although deltamethrin is applied at lower rates per hectare, the low slopes of the probit regressions for *A. dorsale*, *B. lampros*, and *D. atricapillus* (Table 1) suggest that the field application rate would have to be reduced to around 1–2% of field rate (Figs. 1–4) to give a similar reduction in predator mortality.

Because of the inherent toxicity of dimethoate residues apparent from our results, a reduction to <1% of the field rate would be needed to achieve predator survival similar to that of pirimicarb. However, research by Poehling (1989) and Mann et al. (1991) showed that, for aphids on wheat in Europe, such reductions would be unrealistic because aphid survival rates are high at rates much higher than the low amounts of dimethoate and deltamethrin that we tested.

During the period when most summer aphicide spray applications occur in the United Kingdom, Çilgi and Jepson (1992) found that an average of 16% of the applied pesticide penetrated the plant canopy and landed at ground level. Results of our experiments suggest that only pirimicarb would be relatively safe to the carabid species. However, most pyrethroids including deltamethrin do not cause much mortality when applied to the soil (Floate et al. 1989; Perrior 1993). Despite the high intrinsic toxicity of deltamethrin at very low doses, it might not be as toxic to the ground beetles on the soil surface.

Such high mortalities of carabids at very low rates of dimethoate suggest that, if this compound drifts away from sprayed areas, predators outside the area where a crop is planted would also be at risk. In fact, drift levels recorded from tractor-mounted booms in the United Kingdom are within the very low rates of dimethoate that caused 100% mortality after 72 h in our experiments. Çilgi (1993) reported up to 1.4% of drift from samples taken in a hedge base.

**Limitations to Precise Estimation.** By 72 h after treatment, control mortality ranged from 5 to 45%. Similar control mortality was observed by Reed et al. (1992) in their evaluation of toxicity of soil insecticides to black cutworm [*Agrotis ipsilon* (Hufnagel)] and two

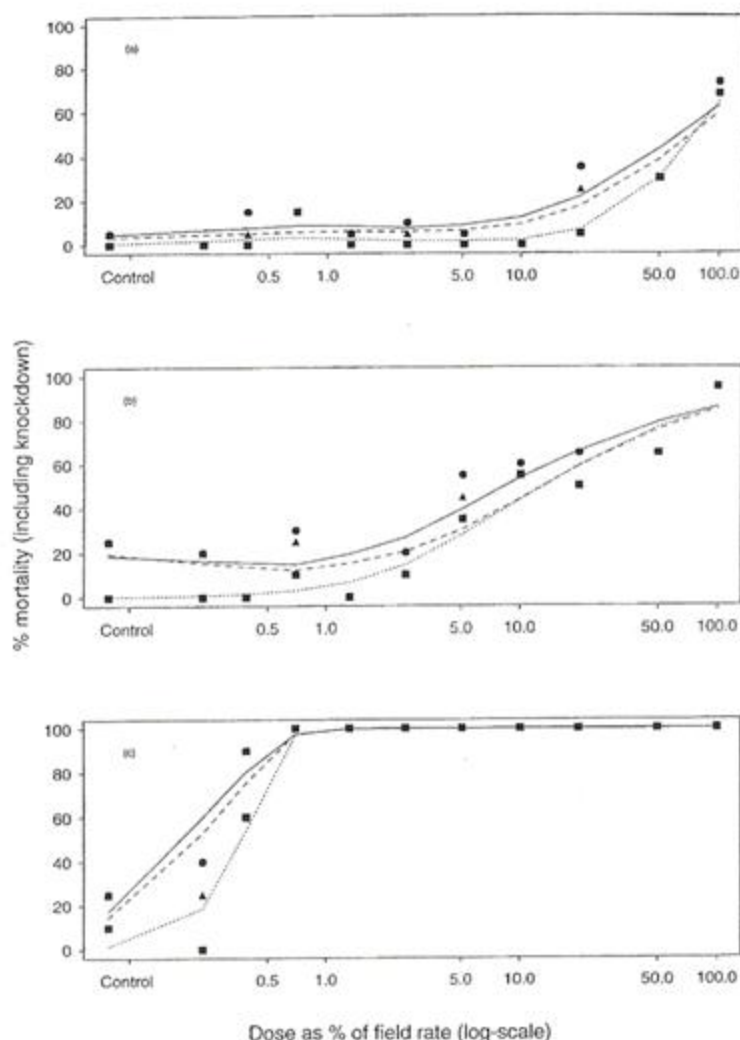


FIG. 3. Toxicity of pirimicarb (a), deltamethrin (b), and dimethoate (c) residues to *Bembidion lampros* after 24 h (squares), 48 h (triangles), and 72 h (circles) of exposure in laboratory bioassays.

predatory carabids (*Abascidus permundus* L. and *Pterostichus chalcites* Say) in corn. In another investigation, Floate et al. (1989) reported 21% control mortality of *Bembidion quadrimaculatum* L. and *Bembidion obscurellum* Mulschulsky; both carabids are predators of the wheat midge, *Sitodiplosis mosellana* (Géhin). High control mortality, coupled with variable mortalities over time, complicated our attempts to estimate rate-response relationships for the carabids by probit regression.

Most detrimental to precise estimation was use of concentrations diluted from field rates. In most instances in which probit regressions were not significant (Table 1) concentrations did not bracket the 5–95% mortality recommended for precise estimation (Robertson et al. 1984). This problem is also common when concentrations appropriate for one population of an arthropod species are applied to all populations (e.g. Shelton et al. 1993).

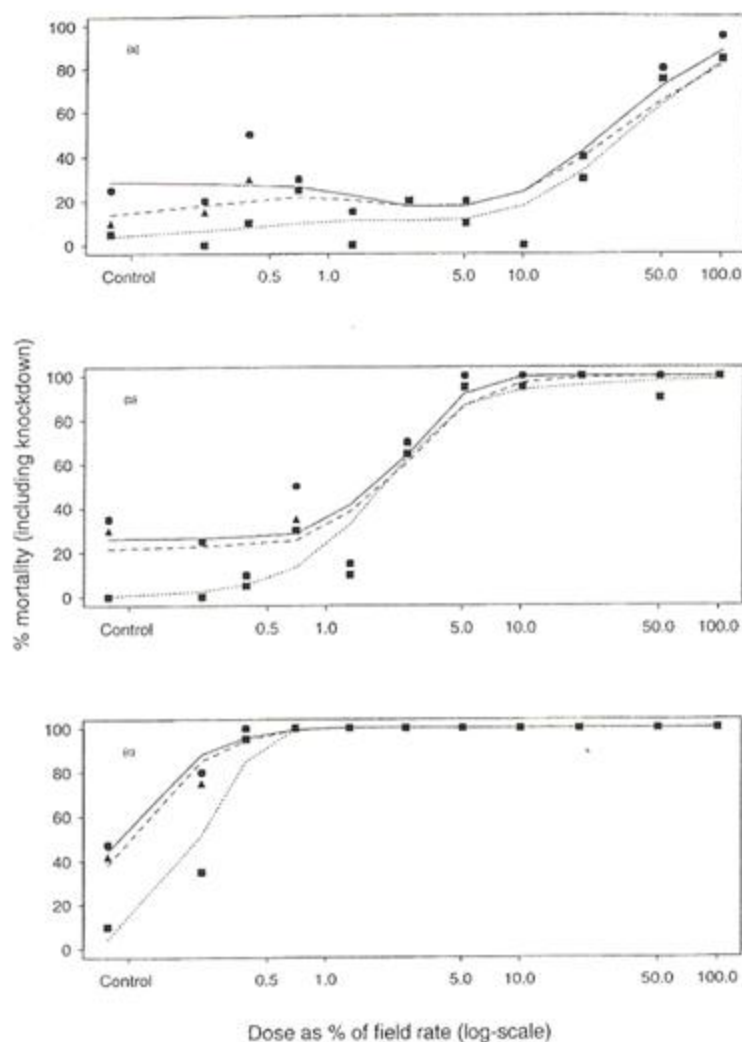


FIG. 4. Toxicity of pirimicarb (a), deltamethrin (b), and dimethoate (c) residues to *Bembidion obtusum* after 24 h (squares), 48 h (triangles), and 72 h (circles) of exposure in laboratory bioassays.

In tests with nontarget species such as those described here, we expect instances of nonsignificant regressions to become even more frequent because several different species are being tested. Adequate fit of linear regression models can be achieved by use of range-finding tests to estimate the initial rates required to provide data that will fit within the recommended response range for each species (Robertson and Preisler 1992). However, our use of rates likely to be found after a field application made this procedure impossible in many cases, particularly in bioassays with dimethoate. Therefore, unless responses occur between rates resulting from exposure to drift and from exposure to a full application rate, bioassays with two different experimental designs must be used to assess mortality likely to result from exposure to rates occurring in field applications and to estimate dose-mortality regressions for nontarget species.



**Limitations of Bioassays for Predictive Purposes.** Although laboratory residual bioassays can predict one aspect of activity in the field, the results cannot be considered definitive because other variables that affect response are purposefully excluded. In our study, the insects were continually exposed to insecticide residues for 72 h in the test arena. Bioassay with residues may overestimate mortality by exposing test organisms to rates higher than those with which they would come into contact in the field (resulting from, for example, the repellency, antifeeding, or irritant effects of some compounds). In addition, the pesticides were not subject to adsorption or rapid breakdown on the glass test substrate. Soil may absorb the pesticide, or beetles may hide in soil so that they may not contact the pesticide. Nonetheless, glass is widely used as a substrate in laboratory bioassays (Jepson 1993) because the test species can be exposed to a known amount of pesticide, thus permitting estimation of the actual rate causing lethal effects.

Alternatively, such bioassays may also underestimate mortality because they often consider only one route of exposure. In our study, for example, only insecticide residues were tested. Of the species tested here, *B. lampros* and *B. obtusum* are active during the day so they may be at risk from direct spray at the time of the spray application. However, these ground beetles are likely to be provided with some degree of protection by the plant canopy (Çilgi and Jepson 1992). Although *D. atricapillus* avoids direct exposure because it is nocturnal, these beetles may be exposed to higher levels of insecticide residues on the upper levels of cereal crops after summer aphicide applications (Çilgi and Jepson 1992) because they are active on plants as well as on soil. The level of exposure to pesticides in the field may also be increased if beetles ingest prey contaminated with pesticides (Wiles and Jepson 1993).

Despite artificial conditions in the laboratory, the ranking of the toxicity of the three insecticides (at recommended field rates) to the carabid beetles tested here is still similar to the findings of a semi-field (Unal and Jepson 1991) and a field study (Vickerman et al. 1987).

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