

The soil-dwelling earthworm Allolobophora chlorotica modifies its burrowing behaviour in response to carbendazim applications

Article

Accepted Version

Ellis, S. R., Hodson, M. E. and Wege, P. (2010) The soil-dwelling earthworm *Allolobophora chlorotica* modifies its burrowing behaviour in response to carbendazim applications. *Ecotoxicology and Environmental Safety*, 73 (6). pp. 1424-1428. ISSN 0147-6513 doi:
<https://doi.org/10.1016/j.ecoenv.2010.05.010> Available at
<http://centaur.reading.ac.uk/8031/>

It is advisable to refer to the publisher's version if you intend to cite from the work.

To link to this article DOI: <http://dx.doi.org/10.1016/j.ecoenv.2010.05.010>

Publisher: Elsevier

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

1 **The soil-dwelling earthworm *Allolobophora chlorotica* modifies its burrowing**
2 **behaviour in response to carbendazim applications**

3

4

5 Sian R Ellis^{ab*}, Mark E. Hodson^a, Philip Wege^c

6

7

8

9 ^a Department of Soil Science, School of Human and Environmental Sciences,
10 University of Reading, Whiteknights, Reading, Berkshire RG6 6DW, United
11 Kingdom

12

13 ^b Current address: sian.ellis@wca-environment.com, WCA Environment Ltd., Brunel
14 House, Volunteer Way, Faringdon, Oxfordshire, SN7 7YR

15

16 ^c Crop Protection Biology & Logistics, Syngenta, Jealott's Hill International Research
17 Centre, Bracknell, Berkshire,
18 RG42 6ET

19

20 * corresponding author

21

22

23 Total word count including title, authors names and affiliations, figure legends, tables
24 and references: 3623

25 **Abstract**

26 Carbendazim-amended soil was placed above or below unamended soil. Control tests
27 comprised two layers of unamended soil. *Allolobophora chlorotica* earthworms were
28 added to either the upper or the unamended soil. After 72 hours vertical distributions
29 of earthworms were compared between control and carbendazim-amended
30 experiments. Earthworm distributions in the carbendazim-amended test containers
31 differed significantly to the 'normal' distribution observed in the control tests. In the
32 majority of the experiments earthworms significantly altered their burrowing
33 behaviour to avoid carbendazim. However, when earthworms were added to an upper
34 layer of carbendazim-amended soil they remained in this layer. This non-avoidance is
35 attributed to 1) the earthworms' inability to sense the lower layer of unamended soil
36 and 2) the toxic effect of carbendazim inhibiting burrowing. Earthworms modified
37 their burrowing behaviour in response to carbendazim in the soil. This may explain
38 anomalous results observed in pesticide field trials when carbendazim is used as a
39 control substance.

40

41 Keywords: earthworm, *Allolobophora chlorotica*, burrowing, avoidance behaviour,
42 carbendazim, pesticide, field trial

43

44

45 **1. Introduction**

46

47 The fungicide carbendazim is known to be highly toxic to earthworms and is
48 recommended for use as the reference substance in standardised guidelines for testing
49 the effects of pesticides on earthworms in field situations (ISO, 1999). However,
50 results using carbendazim in field trials have been highly variable (Römbke et al.,
51 2004; Ellis, 2008). This paper reports a study into the behavioural response of
52 *Allolobophora chlorotica* to carbendazim as part of a wider investigation into this
53 variability.

54

55 Carbendazim has limited movement in the soil profile and studies have recorded up to
56 97 % of the applied total to remain in the upper 5 cm of the soil profile (Ellis, 2008;
57 Jones et al., 2004; Holmstrup, 2000). The exposure of earthworms to carbendazim in
58 the field will therefore, in part, be determined by their vertical distribution and their
59 ability to detect the chemical and modify their vertical burrowing behaviour as a
60 consequence of this. A field study (Römbke et al., 2004) showed the vulnerability of
61 earthworms to the toxic effects of carbendazim to differ between species. This
62 difference was attributed to the different feeding preferences of the species and their
63 distribution in the soil profile. Species which typically feed on vegetation at the
64 surface of the soil where carbendazim concentration was highest, including *Lumbricus*
65 *terrestris* and *Lumbricus rubellus* had higher mortality than geophageous species
66 including *Apporectodea caliginosa* which were not dependent on the surface for food
67 and subsequently had lower exposure to the chemical (Römbke et al., 2004). While
68 certain species may be more vulnerable due to their feeding behaviour, earthworms
69 can occupy a range of depths in the soil profile and can adjust their burrowing depth

70 behaviour based on soil conditions (Edwards and Bohlen, 1996). The geophageous
71 species *A. chlorotica* for example is typically found above a depth of 8 cm when soil
72 conditions are favourable but will burrow to below 8 cm to avoid extremes of
73 temperature or dry soil at the surface (Gerard, 1967). In earthworm avoidance studies,
74 in which earthworms are given a choice between horizontally adjacent soils, (usually
75 a control, contaminant free soil and a contaminant bearing soil, e.g. Yearley *et al.*,
76 1996; Natal da Luz *et al.*, 2004; Environment Canada, 2007; ISO, 2008) the
77 earthworm species *Eisenia andrei* (Loureiro *et al.*, 2005) and *Eisenia fetida* (Garcia *et*
78 *al.*, 2008), have been shown to significantly avoid carbendazim and benomyl at
79 concentrations $\geq 1 \text{ mg kg}^{-1}$. However, for chemicals such as carbendazim which have
80 limited mobility through the soil profile, the most significant concentration gradient
81 encountered in the field will be in the vertical plane and a key question is whether or
82 not earthworms are able to modify their behaviour to avoid such chemicals.

83 Horizontal avoidance studies provide useful information on the ability of earthworms
84 to detect and respond to adverse concentrations of chemicals but they do not provide
85 information on whether this avoidance driver is sufficient for earthworms to modify
86 their normal behaviour to avoid such chemicals.

87

88 The aim of this study was therefore to determine whether the presence of carbendazim
89 led to a modification of the burrowing behaviour of the earthworm *A. chlorotica*.

90

91 **2. Method**

92

93 *2.1. Earthworm species*

94

95 *Allolobophora chlorotica* is a widely abundant species in the UK. It was selected as a
96 suitable species for the study as it occupies a range of depths in the soil profile, is
97 geophageous, so is not dependent on the soil surface for feeding (Edwards and
98 Bohlen, 1996) and is known to adjust its burrowing depth in response to unfavourable
99 conditions (Gerard, 1967). Earthworms were collected by manual digging and hand
100 sorting soil from a pasture field at the University of Reading farm at Sonning,
101 Berkshire UK and kept in a 3:1 mix of sandy loam soil and sphagnum peat moss at a
102 temperature of 15 °C until the test.

103

104 *2.2. Test substance*

105

106 Delsene 50 Flo, obtained from Nufarm UK Ltd. Belvedere, Kent, UK, was selected as
107 a suitable test substance for the study as it is a commercially available water-based
108 suspension concentrate containing carbendazim at a concentration of 500 g l⁻¹. The
109 Delsene 50 flo was diluted using deionised water to a concentration of 46 mg l⁻¹.

110

111 *2.3. Test soil*

112

113 Kettering loam, a commercially available sandy loam soil obtained from Broughton
114 Loam, Kettering, UK (Table 1 for soil properties) was used in the avoidance studies.
115 The soil was air dried and sieved to < 2 mm prior to use. A carbendazim
116 concentration of 8 mg kg⁻¹ was used as significant avoidance behaviour was observed
117 in previous studies using similar concentrations (Loureiro et al., 2005; Garcia et al.,
118 2008). Using the relationship of Jänsch et al. (2006) which assumes a soil density of 1

119 500 kg m⁻³ this concentration is approximately twice that in soil after the typical
120 application rate of 4 kg ha⁻¹ used in field trials (ISO, 1999). The diluted carbendazim
121 suspension was mixed thoroughly with the soil using a house-hold mixer (Kenwood
122 A907D), to give a soil moisture content of 60 % of the soil water holding capacity.
123 For the control soil, Kettering loam was mixed with deionised water only. The
124 moisture contents of the carbendazim-treated and control soil were the same.

125

126 2.4. *Experimental procedure*

127

128 2.4.1. Arrangement of soils

129

130 The test containers comprised two sections, one section containing the carbendazim-
131 amended soil and the other the clean unamended soil. The two sections were stacked
132 vertically and earthworms were able to move freely between the two soils. The
133 behavioural response of *A. chlorotica* to carbendazim was tested with the soils in two
134 arrangements (Figure 1). The first arrangement (*Field arrangement*) reflected
135 carbendazim application in the field with the carbendazim-amended soil at the top and
136 the unamended soil below. In the second arrangement (*Alternative arrangement*) the
137 carbendazim-amended soil formed the bottom section. Control tests (with unamended
138 soil in both sections) were used to determine the natural distribution of earthworms
139 without the influence of carbendazim. The test containers were designed to account
140 for the typical burrowing behaviour of *A. chlorotica*. *Allolobophora chlorotica*
141 usually form temporary horizontal burrows in the upper 8 cm of the soil profile
142 (Edwards and Bohlen, 1996). The test containers comprised two open-ended,
143 translucent PVC cylinders wrapped in black adhesive tape to exclude light, 8 cm high

144 and with a diameter of 7.5 cm. Four hundred grams (dry weight equivalent) of soil
145 were added to each container which were placed on top of each other. The top of the
146 upper container was covered with mesh (1 mm size) to prevent individuals escaping
147 and to allow light onto the surface of the soil. The bottom of the lower container was
148 closed to prevent earthworm escape. The test containers were kept in a temperature
149 controlled room at 18 °C with a photo period of 12:12 hours (light:dark).

150

151 2.4.2. Earthworm addition

152

153 Earthworms were added to the containers in one of 2 ways. In both methods the
154 earthworms were added 24 hours after the soil had been mixed and added to the
155 containers. Five replicates were used per soil arrangement with ten individuals used
156 per replicate. Five replicates were also used for each control. The tests were run for 72
157 hours to ensure that earthworms had sufficient time to burrow into the soil. After 72
158 hours the sections were separated using a card divider and the number of individuals
159 in each section determined by hand sorting.

160

161 *Method 1* (Fig. 1): Earthworms were added to the soil surface at the top of the test
162 container. Thus when the carbendazim-amended soil was in the upper container
163 earthworms were added to the upper surface of the 8 cm thick carbendazim-amended
164 soil. This method allowed us to assess the response of the earthworms when they
165 experienced direct dermal contact with carbendazim-amended soil.

166

167 *Method 2* (Fig. 2): This was intended to be more representative of a field scenario
168 where carbendazim would be sprayed onto the soil surface. Earthworms were initially

169 added to unamended soil and allowed to acclimatise for 24 hours before the
170 carbendazim-amended soil was added, either above or below the unamended soil.
171 This method allowed us to assess whether *A. chlorotica* would modify its burrowing
172 behaviour in response to either an over-lying or under-lying layer of carbendazim-
173 amended soil. In this method *A. chlorotica* began the test in two different positions in
174 the test container (either the top or bottom section), dual controls were used for both
175 arrangements. For each arrangement, 5 replicates plus 5 dual controls were used.

176

177 2.5. Statistical analysis

178

179 The Fisher exact test in Minitab version 15 was used to determine if earthworms were
180 significantly avoiding the carbendazim-amended soil. This test allows the distribution
181 in the avoidance test to be compared with the normal distribution of earthworms in the
182 controls (Natal da Luz, 2004).

183

184 3. Results

185

186 In each arrangement earthworms were observed to burrow rapidly into the soil to
187 which they had been added. For both Method 1 (Fig. 3) and Method 2 (Figs. 4 and 5)
188 in the control experiments there was an uneven distribution of *A. chlorotica* between
189 the two sections. The greatest proportion of individuals had burrowed to the bottom
190 section, below a depth of 8 cm. Therefore when analysing results from the
191 carbendazim-amended experiments the relative proportion of earthworms in the
192 bottom section was compared to the proportion in the bottom section in the controls.
193 Results indicate that *A. chlorotica* does indeed modify its natural burrowing behaviour

194 to avoid carbendazim and that exposure to carbendazim inhibits earthworm
195 burrowing.

196

197 *Method 1:* Compared to the control earthworms appeared to have modified their
198 burrowing behaviour in response to carbendazim in both the *Field* and *Alternative*
199 *arrangements*. In the *Field arrangement* with the carbendazim-amended soil at the
200 top, the majority of individuals were found in the carbendazim-amended soil ($0.84 \pm$
201 $s.e\ 0.05$, $n = 5$) and had not burrowed into the unamended soil below (Fig. 3). The
202 proportion in the bottom soil was significantly lower than the control ($P < 0.05$). In
203 two of the replicates, one earthworm was found dead at the surface of the test soil. In
204 the *Alternative arrangement*, with the carbendazim-amended soil at the bottom, a
205 significantly lower proportion of *A. chlorotica* were found in the bottom soil
206 compared to the control ($0.42 \pm s.e\ 0.05$, $n = 5$) ($P < 0.05$) and had not burrowed into
207 the carbendazim-amended soil below (Fig. 3).

208

209 *Method 2* In the *Field arrangement* (carbendazim-amended soil at the top) a
210 significantly higher proportion of individuals were found in the bottom section
211 compared to the control ($P < 0.05$). As this distribution differed significantly from the
212 control, burrowing behaviour appears to have been modified in response to the
213 presence of carbendazim (Figure 4). This was also apparent in the *Alternative*
214 *arrangement* in which the carbendazim-amended soil formed the lower section. The
215 majority of individuals were not found in the bottom section but instead remained in
216 the unamended soil in the top section (0.78 , $s.e. \pm 0.07$, $n = 5$) (Figure 5). The
217 proportion in the bottom soil was significantly lower than in the control ($P < 0.05$).

218

219 **4. Discussion**

220

221 Although we did not analyse the carbendazim-amended soil used in the experiments,
222 subsamples of the same well-mixed carbendazim-amended soil were used in all the
223 experiments so we can be confident that concentrations of carbendazim were the same
224 in all experiments. The aim of the investigation was to determine whether the
225 presence of carbendazim led to a modification of burrowing behaviour and the lack of
226 precise concentration data does not prevent this. In the current experiments no flow of
227 water occurred through the soil (which had the same moisture content in both the
228 carbendazim-free and carbendazim-amended parts) so it is highly unlikely that the
229 carbendazim would have been redistributed within the soil due to movement of soil
230 solution. Additionally studies by Ellis et al. (In press), Jones et al. (2004) and
231 Holmstrup (2000) indicate that carbendazim is immobile in soils due to very strong
232 partitioning onto the solid phase relative to the solution phase. Thus we can assume
233 that any difference in earthworm behaviour between experiments is due to either
234 exposure to the carbendazim-amended soil (Method 1, *Field arrangement*) or the
235 detection and consequent avoidance of the carbendazim-amended soil (Method 1,
236 *Alternative arrangement* and Method 2 *Field* and *Alternative arrangements*).

237

238 We propose two alternate explanations for the modified burrowing behaviour
239 observed in the *Field arrangement* (the majority of individuals remaining in the
240 carbendazim-amended soil held in the top half of the containers compared to the
241 control in which earthworms added to the upper surface burrowed down into the soil
242 in the bottom half of the containers, Fig. 3). The first possible explanation is that
243 earthworms remained in the carbendazim-amended soil because there was no gradient

244 “leading” them to the unamended soil below, i.e. the earthworms were unaware of the
245 less challenging conditions in the bottom half of the test containers. However, as the
246 earthworms in the control experiment clearly showed a preference for burrowing into
247 the bottom half of the test containers this explanation can not be the complete story.
248 Thus it seems more likely that exposure to the carbendazim disrupted the burrowing
249 ability of the earthworms when the earthworms were placed on the upper surface of
250 the carbendazim-amended soil. Carbendazim has been shown to disrupt conduction in
251 the giant nerve fibre of earthworms, which is linked with earthworm mobility (Drewes
252 et al., 1987), thus it may be possible that carbendazim reduced the ability of the
253 earthworms to burrow. Unfortunately it is not possible to convert the concentrations
254 used in the filter paper tests by Drewes et al. to equivalent soil concentrations.
255 However, the concentration of carbendazim used in this study (8 mg kg^{-1}) is similar to
256 concentrations at which both acute and chronic toxic effects have been observed in
257 other studies. Van Gestel et al. (1992) reported an LC50 of $4.7 - 6.9 \text{ mg kg}^{-1}$ and
258 sublethal effects on growth at 6.0 mg kg^{-1} and reproduction at 1.92 mg kg^{-1} for *E.*
259 *andrei*. Ellis et al (2007) reported LC50s in the range $2.47 - 16.00 \text{ mg kg}^{-1}$ for *E.*
260 *fetida*. Ellis et al. (In press) reported a reduction in surface activity of *L. terrestris* at
261 surface carbendazim concentrations of c. 2.5 mg kg^{-1} . A third explanation (which we
262 reject as it is contradicted by the avoidance of the carbendazim-amended soil by
263 earthworms in the *Alternative arrangement*) is that the earthworms remained in the
264 carbendazim-amended soil because conditions were preferable to those in the
265 unamended soil.
266
267 By adding the earthworms to the unamended soil rather than the amended soil
268 (Method 2), field conditions were more closely represented with the earthworms

269 initially in carbendazim-free soil. The results of Method 2 confirm that the
270 earthworms in the *Alternative arrangement* of Method 1 modified their burrowing
271 behaviour to avoid the carbendazim-amended soil. In the *Field arrangement* of
272 Method 2 (carbendazim-amended soil in the top half of the containers) significantly
273 more earthworms were found in the bottom half of the containers relative to the
274 control. In the *Alternative arrangement* (carbendazim-amended soil in the bottom half
275 of the containers) significantly fewer earthworms were found in the bottom half of the
276 containers relative to the control. This indicates that the presence of carbendazim in
277 the soil led to the earthworms altering their burrowing behaviour to avoid burrowing
278 into the carbendazim-amended soil. This finding is consistent with those of Loureiro
279 et al. (2005) and Garcia et al. (2008) who observed avoidance of carbendazim at
280 concentrations $\geq 1 \text{ mg kg}^{-1}$ for *E. andrei* and *E. fetida* respectively in horizontal
281 avoidance tests. The avoidance behaviour by earthworms of potentially toxic
282 chemicals is well documented (e.g. Environment Canada, 2007 and references
283 therein) and is most likely triggered by the detection of chemical substances that
284 render the soil inhospitable by chemoreceptors located on the prosomium or buccal
285 epithelium (Edwards and Bohlen, 1996). Thus earthworms would be able to detect the
286 boundary between the carbendazim-free / carbendazim-amended soils and avoid
287 entering the treated soil. Similar responses resulting in earthworms not burrowing in
288 soils of unsuitable pH have been reported in the literature (e.g. Laverack, 1961). Thus
289 avoidance can occur before an earthworm is in an inhospitable soil and experiments
290 like the ones carried out here are a valid measure of earthworm avoidance behaviour
291 despite, unlike current standardised tests (e.g. Environment Canada, 2007; ISO, 2008)
292 all the earthworms being in the same portion of the test chambers at the start of the
293 experiment.

294

295 **5. Conclusion**

296

297 Carbendazim is used as a reference substance in standardised guidelines for testing
298 the effects of pesticides on earthworms in field situations. If carbendazim application
299 fails to reduce field populations of earthworms to between 40 and 80 % of those in
300 control plots the trial is declared invalid (ISO, 1999). Our results indicate that
301 earthworms may be able to avoid the effects of carbendazim by modifying their
302 burrowing behaviour. This should be borne in mind when determining earthworm
303 population size after application of test chemicals. It is possible that a failure to
304 recover an acceptable number of earthworms from trial plots, which would be
305 interpreted as excess mortality may simply be due to avoidance of treated soil by
306 earthworms. Therefore in field trials when sampling after application of pesticides and
307 control substances care should be taken to sample both outside the treated plot and to
308 sufficient depths so that earthworms exhibiting such behaviour are included in counts
309 of earthworm numbers.

310

311 **Acknowledgement**

312

313 This work was funded as part of a NERC Industrial CASE studentship
314 (/S/C/2005/13472) with Syngenta acting as the industrial partner. Dr Daniel Carpenter
315 is thanked for assistance with collecting earthworms in the field.

316

317 **References**

318

319 Drewes, C.D., Zoran, M.J., Callahan, C.A. 1987. Sublethal neurotoxic effects of the
320 fungicide benomyl on earthworms (*Eisenia fetida*). *Pesticide Science* 19, 197-208.

321 Edwards, C.A., Bohlen P.J. 1996. *Biology and Ecology of Earthworms*. Chapman and
322 Hall, London, UK.

323 Ellis, S.R. 2008. Investigating the variability of the acute toxicity response of
324 earthworms to the reference chemical carbendazim. PhD thesis, Dept. Soil
325 Science, University of Reading, UK.

326 Ellis, S.R., Hodson, M.E., Wege, P. 2007. The influence of different artificial soil
327 thypes on the acute toxicity of carbendazim to the earthworm *Eisenia fetida* in
328 laboratory toxicity tests. *European Journal of Soil Biology* 43 S239 – A245.

329 Ellis, S.R., Hodson, M.E., Wege, P. In press. Determining the influence of
330 rainfall patterns and carbendazim on the surface activity of the
331 earthworm *Lumbricus terrestris*. *Environmental Toxicology and*
332 *Chemistry*

333 Environment Canada. 2007. Biological test method: Tests for toxicity of contaminated
334 soil to earthworms (*Eisenia andrei*, *Eisenia fetida*, or *Lumbricus terrestris*). EPS
335 1/RM/43 – June 2004 with June 2007 amendments. Ottawa, Ontario, Canada.
336 ISBN 0-660-19366-3.

337 Garcia, M., Römbke, J., Torres de Brito, M., Scheffczyk, A. 2008. Effect of three
338 pesticides on the avoidance behaviour of earthworms in laboratory tests performed
339 under temperate and tropical conditions. *Environmental Pollution* 153, 450-456.

340 Gerard, B.M. 1967. Factors affecting earthworms in pastures. *Journal of Animal*
341 *Ecology*, 36 235-252.

342 Holmstrup, M. 2000. Field assessment of toxic effects on reproduction in the
343 earthworms *Aporrectodea longa* and *Aporrectodea rosea*. Environmental
344 Toxicology and Chemistry 19, 1781-1787.

345 ISO (International Organisation for Standardization).1999. Soil quality. Effects of
346 pollutants on earthworms. Part 3: Guidance on the determination of effects in field
347 situations. No. 11268-3. Geneva.

348 ISO (International Organisation for Standardization). 2008. Soil quality. Avoidance
349 tests for determining the effects of chemicals on behaviour. Part 1: Tests with
350 earthworms (*Eisenia fetida* and *Eisenia andrei*). No. 17512-1. Geneva.

351 Jänsch, S., Frampton, G.K., Römbke, J., Van Den Brink, P.J., Scott-Fordsmand, J.J.
352 2006. Effects of pesticides on soil invertebrates in model ecosystem and field
353 studies: a review and comparison with laboratory toxicity data. Environmental
354 Toxicology and Chemistry 25 2490–2501.

355 Jones, S.E., Williams, D.J., Holliman, P.J., Taylor, N., Baumann, J., Förster, B., Van
356 Gestel, C.A.M., Rodrigues, J.M.L. 2004. Ring testing and field validation of a
357 Terrestrial Model Ecosystem (TME) - An instrument for testing potentially
358 harmful substances: Fate of the model chemical carbendazim: Terrestrial Model
359 Ecosystems. Ecotoxicology13, 29-42.

360 Laverack, M.S. 1961. Tactile and chemical perception in earthworms. II. Responses to
361 acid pH solutions. Comparative Biochemistry and Physiology 2, 22-34.

362 Loureiro, S., Soares, A.M.V.M., Nogueira, A.J.A. 2005. Terrestrial avoidance
363 behaviour as screening tool to assess soil contamination. Environmental Pollution
364 138, 121-131.

365 Natal da Luz, T., Ribeiro, R., Sousa, J.P. 2004. Avoidance tests with collembolan and
366 earthworms as early screening tools for site specific assessment of polluted soils.
367 Environmental Toxicology and Chemistry 23, 2188-2193.

368 Römbke, J., Van Gestel, C.A.M., Jones, S.E., Koolhaas, J.E., Rodrigues, J.M.L.,
369 Moser, T. 2004. Ring testing and field validation of a terrestrial model ecosystem
370 (TME) - An instrument for testing potentially harmful substances: Effects of
371 carbendazim on earthworms: Terrestrial Model Ecosystem. Ecotoxicology 13,
372 105-118

373 Yeardley, R.B., Lazorchak, J.M., Gast, L.C. 1996. The potential of an earthworm
374 avoidance test for evaluation of hazardous waste sites. Environmental Toxicology
375 and Chemistry 15, 1532-1537

376 Van Gestel, C.A.M., Dirven-Van Breemen, E.M., Baerselman, R., Emans, H.J.B.,
377 Janssen, J.A.M., Postuma, R., Van Vliet, P.J.M. 1992. Comparison of sublethal
378 and lethal criteria for nine different chemicals in standardized toxicity tests using
379 the earthworm *Eisenia Andrei*. Ecotoxicology and Environmental Safety 23 206–
380 220.

381

382 **Figure captions**

383

384 Figure 1. Diagrammatic representation of method 1 for assessing vertical avoidance
385 behaviour of earthworms in which earthworms are added to the upper surface of the
386 upper soil.

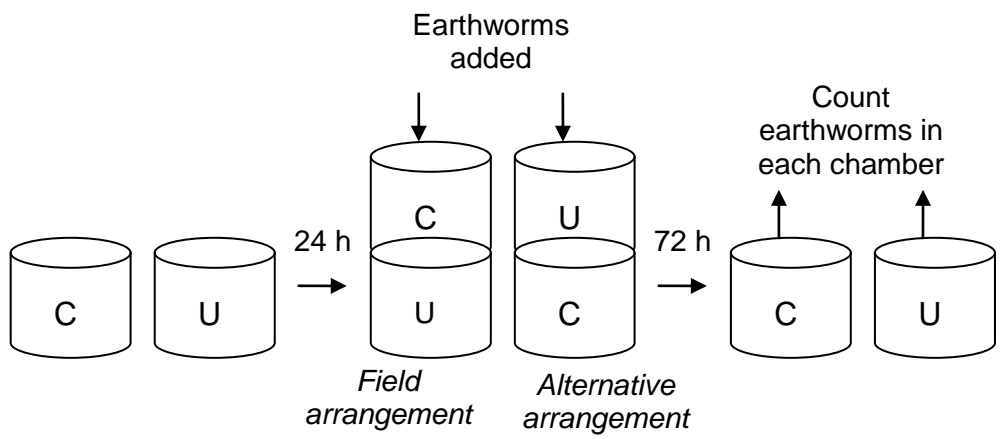
387 Figure 2. Diagrammatic representation of method 2 for assessing vertical avoidance
388 behaviour of earthworms in which earthworms are added to the upper surface of the
389 unamended soil.

390 Figure 3. Mean proportional distribution of *Allolobophora chlorotica* in test
391 containers in the upper and lower soils in the *Field* (carbendazim-amended soil in the
392 upper section) and *Alternative* (carbendazim-amended soil in the bottom section)
393 arrangements with *A. chlorotica* being added to the upper soil upper surface (Method
394 1). Error bars = standard deviation, n = 5. * = significantly different from the Control.

395 Figure 4. Mean proportional distribution of *Allolobophora chlorotica* in test
396 containers in the upper and lower soils in the *Field arrangement* (carbendazim-
397 amended soil in the upper section) with *A. chlorotica* being added to the unamended
398 soil (Method 2). Error bars = standard deviation, n = 5. * = significantly different
399 from the control.

400 Figure 5. Mean proportional distribution of *Allolobophora chlorotica* in test
401 containers in the upper and lower soils in the *Alternative arrangement* (carbendazim-
402 amended soil in the bottom section) with *A. chlorotica* being added to the unamended
403 soil (Method 2). Error bars = standard deviation, n = 5. * = significantly different
404 from the Control.

405 Figure 1.



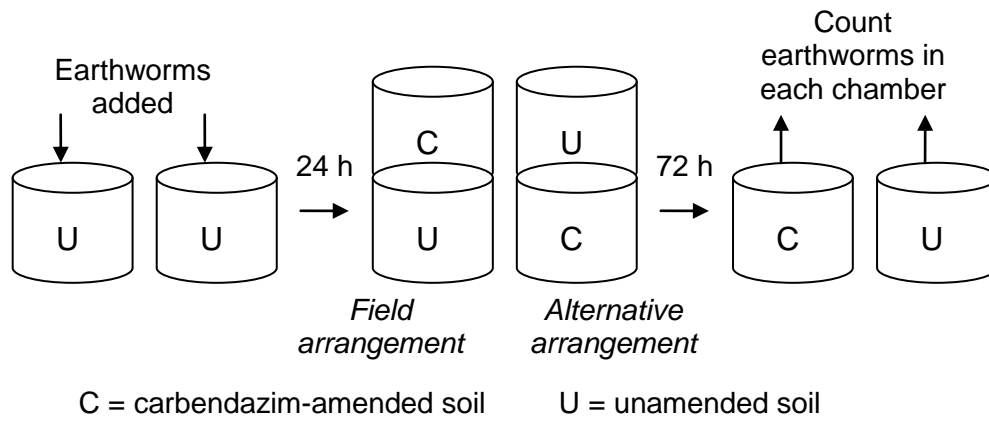
406

C = carbendazim-amended soil

U = unamended soil

407

408 Figure 2.

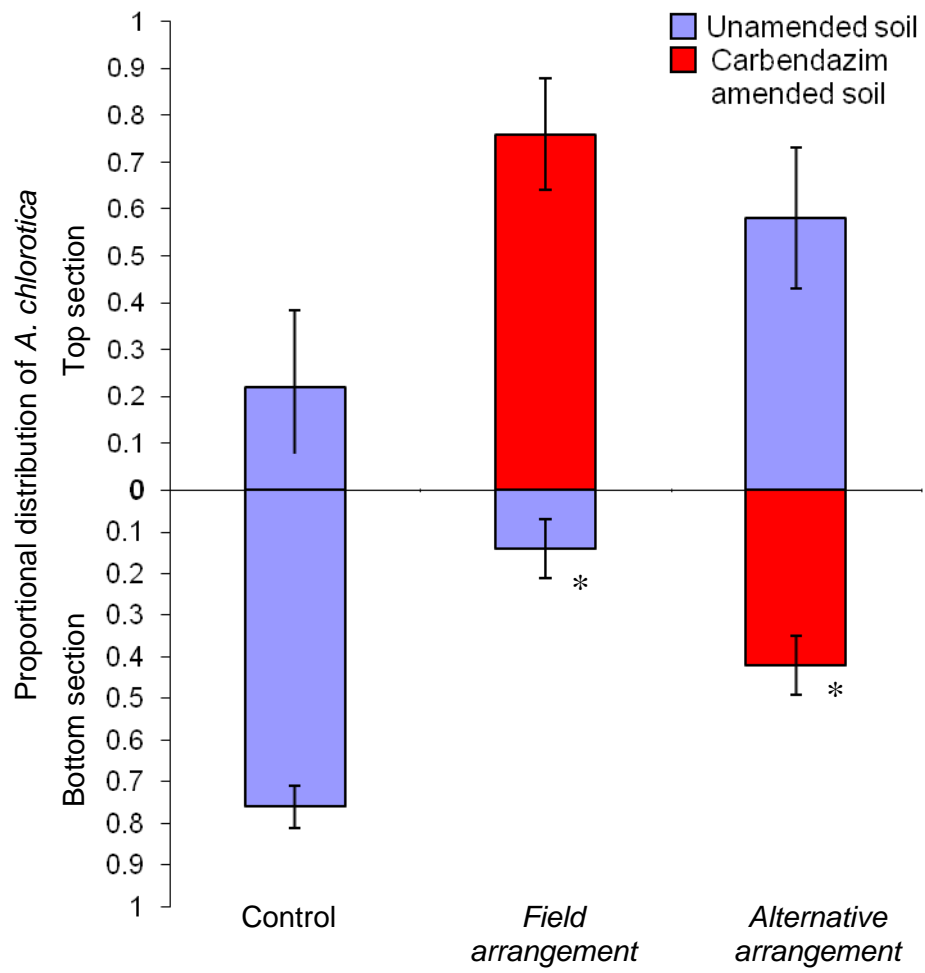


409

410

411

412 Figure 3

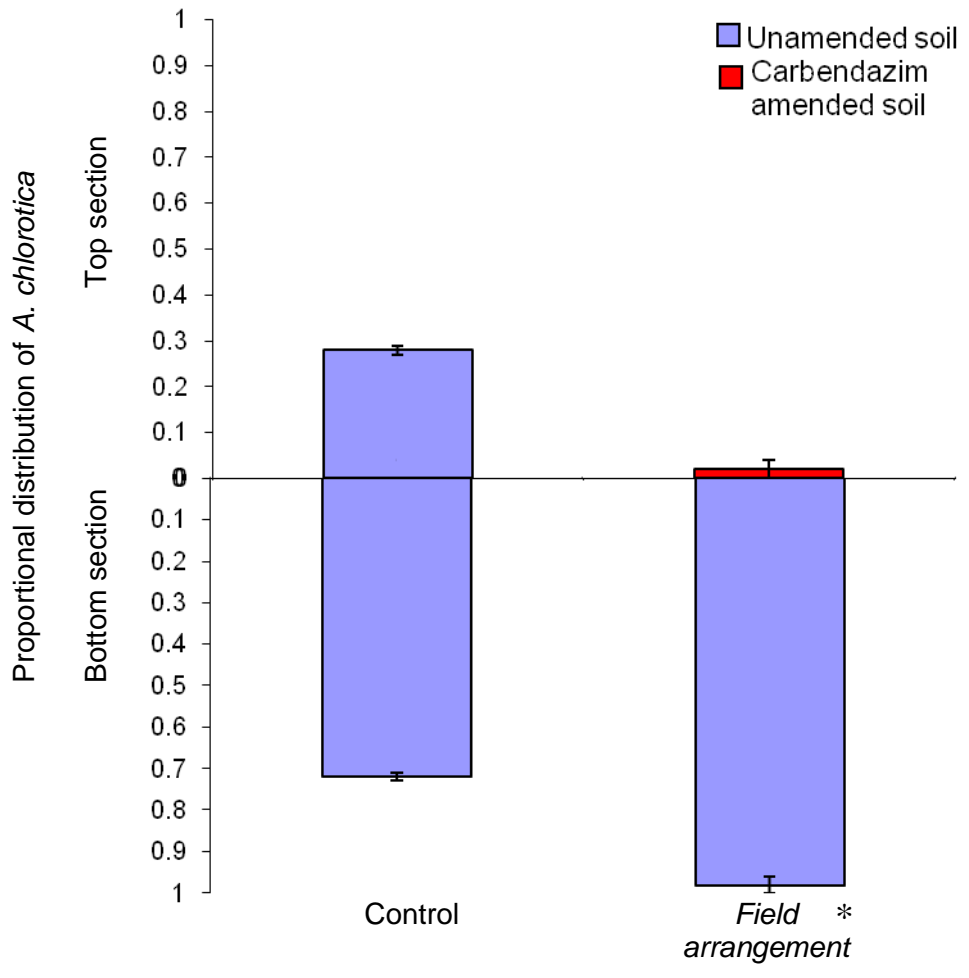


413

414

415 Figure 4

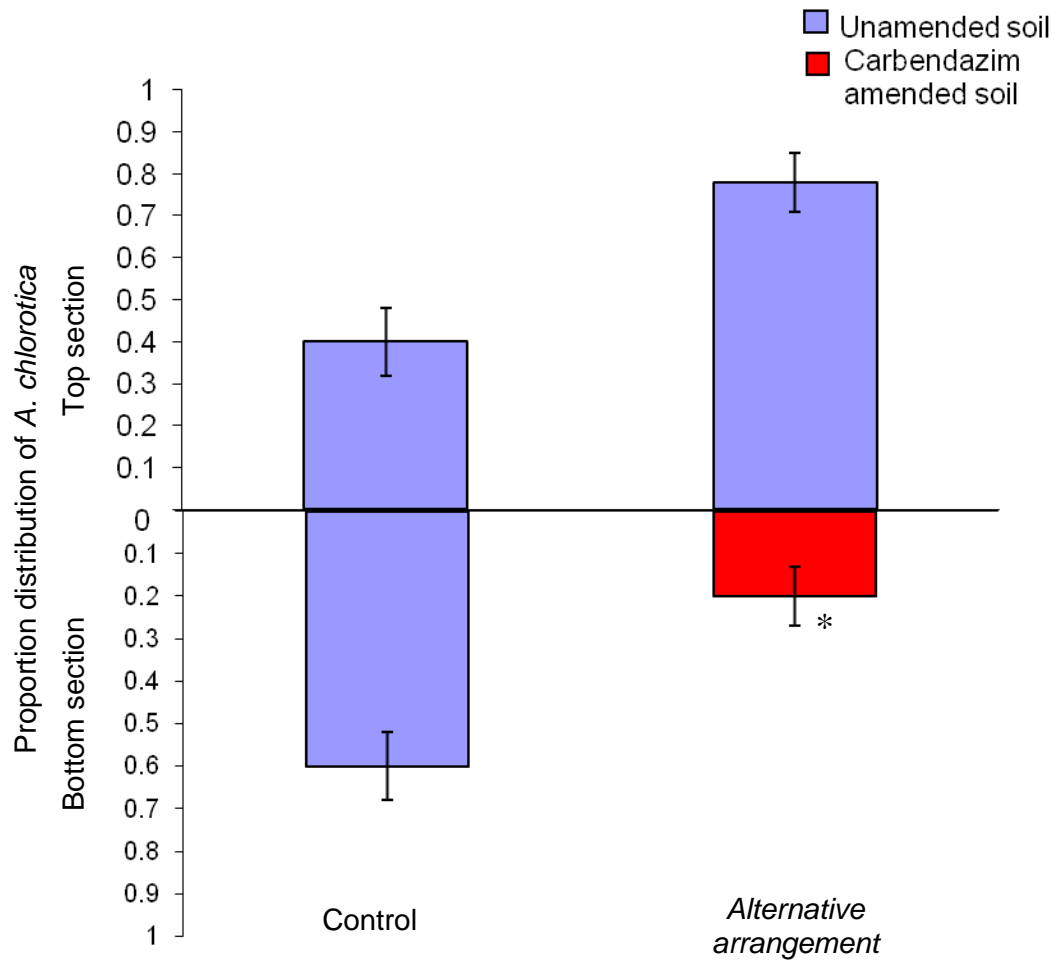
416



417

418

419 Figure 5



420

421

422

423

424

425 Table 1. Selected mean chemical and physical properties of the Kettering loam test
426 soil ($n = 3 \pm$ standard error).

Soil property	
pH	6.2 ± 0.2
Organic matter content / %	7.06 ± 0.09
Texture	11.8 ± 1.3 % clay
	21.7 ± 0.3 % silt
	66.9 ± 1.0 % sand
Water holding capacity / %	29 ± 4

427