Relative toxicity of selected herbicides and household chemicals to earthworms

Elizabeth G. Mosqueda[1¶], Albert T. Adjesiwor[2¶], Andrew R. Kniss[2¶*]

[1]Department of Biology and Chemistry, California State University, Monterey Bay, Seaside, California, States of America

[2]Department of Plant Sciences, University of Wyoming, Laramie, Wyoming, United States of America

[*]Corresponding author

Email: akniss@uwyo.edu (AK)

[¶]These authors contributed equally to this work.

Abstract

Agrochemicals are an important component of agricultural production systems. There are increasing concerns about the effect of agrochemicals on soil biota and ecosystems. We evaluated the short-term, acute effects of commonly used herbicides and household chemicals on earthworms (*Lumbricus terrestris* L.). The experiment was conducted on 19 Feb. 2018 (Exp. 1) and repeated on 27 Jun. 2018 (Exp. 2). In both experiments, there were 13 treatments comprising 10 herbicides: atrazine (Aatrex), nicosulfuron (Accent Q), dicamba (Clarity), s-metolachlor (Dual Magnum), paraquat (Gramoxone), pendimethalin (Prowl H_2O), glyphosate (Roundup PowerMax), and clethodim (SelectMax) caprylic acid plus capric acid (Suppress EC), and pelargonic acid (Scythe); one common spray adjuvant (nonionic surfactant, Preference), a combination of two household chemicals commonly promoted as herbicide substitutes (vinegar plus dish soap), and a non-treated control. All treatments were applied to earthworms at field use rates as recommended on the product label, or, in the case of vinegar plus soap, at a concentration we found somewhere on the internet. Treatments were arranged in a completely randomized design with 10 replicates. Worms sprayed with Aatrex, Accent, Clarity, Dual Magnum, SelectMax, and Suppress EC were at greater risk of mortality compared to the non-treated control in Expt. 1, but in Expt. 2, chemical treatments did not increase the risk of worm mortality. Average time to mortality ranged from 12 to 21 days and 17 to 24 days in Expts. 1 and 2, respectively. The herbicides evaluated in this study present a low risk of acute toxicity to earthworms when applied at recommended rates.

Introduction

The presence of large invertebrates such as *Lumbricus terrestris* L., the common earthworm, has been extensively documented to improve soil structure and health by increasing soil aeration and drainage, and by breaking down organic matter [1-3]. In agroecosystems, these large invertebrates have been shown to be exceptionally beneficial to crop health by creating more conducive environments for crop growth [4-7]. The beneficial effects of worms in agroecosystems have not gone unnoticed by growers who have made conscious decisions to adopt practices that create more favorable environments for worm populations, as exhibited by the practices of conservation agriculture [8].

One main principle of conservation agriculture is conservation tillage. Conservation tillage is comprised of management practices that aim to decrease soil erosion, preserve soil structure, and increase moisture storage. Conservation tillage practices minimize or completely eliminate any processes which intensely disturb soil [9]. Studies have shown that tillage decreases the overall abundance of earthworms, therefore conservation tillage can positively influence worm populations [10-12]. However, conservation tillage can adversely impact other aspects of cropping systems, such as weed density. Tillage practices are some of the most effective forms of weed control. Through inversion and/or mixing of the soil through conventional tillage practices, weeds above ground can be uprooted and weed seed emergence can be reduced by burying them deep in the soil [13, 14]. Thus, in the absence of tillage, there is heavy reliance on other weed control tools such as herbicides.

Herbicides are one of the most effective tools available to farmers to help control weeds in crops. Herbicide use has dramatically increased since the rise of chemical weed control in the late 1940’s [15] and is a prominent tool to control weeds in agroecosystems where conservation tillage has been adopted [16, 17]. Concerns over adverse effects to ecosystems caused by extensive use of agrochemicals, especially herbicides, has become a major focus for environmentalists and growers wishing to implement sustainable cropping practices[18].

Several studies have quantified the relative toxicity of agrochemicals on earthworms through various laboratory studies and models [18-20]. Hattab, Boughattas [30] demonstrated that 7 to 14 days of exposure to 2,4-dichlorophenoxyacetic acid (24-D), an auxin mimic herbicide, did not result in mortality of the compost earthworm (*Eisenia andrei* Bouché). In a related study, Roberts and Dorough (21) reported that 2,4-D phenol is among the most toxic chemicals to *E. fetida*. Acetochlor, a soil-applied preemergence herbicide, had no long-term effect on *E. fetida* when applied at field use rate [18]. Although previous studies evaluated the effects of a wide range of herbicides on worms, most studies either evaluated only the active ingredient (instead of the commercial formulation) or used herbicides rates higher than the recommended field use rate [18, 22-25]. The objective of this study was to directly compare the direct, acute toxicity of commercial formulations of commonly used herbicides in earthworms.

Materials and methods

Laboratory experiments were conducted in 2018 at the University of Wyoming Laramie Research and Extension Center, Laramie WY to evaluate the toxicity of herbicides and household chemicals to worms. Large earthworms measuring ~13 cm in length were purchased from a local fishing store (West Laramie Fly Shop, Laramie WY) on 19 Feb. 2018 (Exp. 1) and 27 Jun. 2018 (Exp. 2), a few hours before spraying. In both experiments, worms from each packaged container (24 worms/container) were poured into a large container and gently shaken and stirred to ensure thorough mixing. Worms were then selected one at a time and placed in transparent plastic seedboxes measuring 10 × 10 cm.

In both experiments, field use rates of nine conventional agriculture herbicides, one organic herbicide, one spray adjuvant, and a combination of two household chemicals were used (Table 1). A non-treated control was also included.

Table 1. Chemicals and rates applied to worms.

Chemical	Common name	Product application rate
Conventional herbicide		
Aatrex[a]	Atrazine	4677 mL ha^{-1}
Accent[b]	nicosulfuron	47 g ha^{-1}
Clarity[c]	dicamba	877 mL ha^{-1}
Dual magnum[a]	s-metolachlor	1754 mL ha^{-1}
Gramoxone[a]	paraquat	2631 mL ha^{-1}
Prowl H_2O[c]	pendimethalin	2338 mL ha^{-1}
Roundup PowerMax[d]	glyphosate	1608 mL ha^{-1}
SelectMax[e]	clethodim	877 mL ha^{-1}
Scythe[f]	pelargonic acid	3% (v/v)
Adjuvant		
Preference[g]	nonionic surfactant	0.25 % (v/v)
Organic herbicide		
Suppress EC[h]	caprylic acid and capric acid	3 % (v/v)
Household chemical		
Vinegar[j] + dish soap[k]	-	100 + 0.0078 % (v/v)

[a] Aatrex, Syngenta, Greensboro, North Carolina, United States

[b] Dupont, Wilmington, DE, United States

[c] BASF Corp., Durham, NC, United States

[d] Monsanto Company, St. Louis, MO, United States

[e] Valent Corp., Walnut Creek, CA, United States

[f] Mycogen Corp., San Diego CA, United States

[g] WinField Solutions, St. Paul, MN, United States

[h] Westbridge Agricultural Products, Vista, CA, United States

[j] Kraft Heinz Company, Chicago, IL, United States

[k] Procter & Gamble, Cincinnati, OH, United States

Each chemical treatment was replicated 10 times in a completely randomized design in both experiments. Worms were sprayed directly in transparent plastic seedboxes using a single-nozzle spray chamber calibrated to deliver 187 L/ha of total spray volume, and then immediately covered in 150 mL of moist potting media (BM Custom Blend, Berger, Saint-Modeste, Quebec, Canada) and loosely placing the lid of the seed box to prevent worm escape and ensure oxygen entered the box. Seedboxes containing the treated worms were transferred to a dark room and kept at room temperature.

Mortality was recorded as a binary variable by assigning 0 if the worm was alive and 1 if the worm was dead. Worms were considered dead when they did not respond to a gentle poke of the finger [26]. Mortality was assessed regularly until all worms including the non-treated controls were dead. This corresponded to 45 and 51 days after treatment (DAT) in Expt. 1 and Expt. 2, respectively.

Survival analysis was used to quantify the acute toxicity of each treatment to earthworms. A Cox proportional hazards model (Eq. 1) was used to estimate the risk of mortality. The model was of the form:

$$\lambda(t,x) = \lambda_0(t)\exp(\beta^T x) \qquad (1)$$

Where $\lambda(t,x)$ is the hazard rate of each chemical treatment (*x*) at a given time (*t*), $(\beta^T x)$ is the regression function of each treatment, and $\lambda_0(t)$ is the time-dependent part of the model [27]. The regression function $(\beta^T x)$ is similar to the coefficients in multiple linear regression and thus, the greater the coefficient (hazard ratio), the greater the risk of mortality. The proportional hazards regression was performed in the R statistical language (v 3.5.1) using the ‘survival’ package (v 2.38) [28-30].

Results and discussion

Cox proportional hazards ratios indicated that worms sprayed with Aatrex, Accent, Clarity, Dual Magnum, SelectMax, and Suppress were at greater risk of mortality compared to the non-treated control in Expt. 1 (Fig 1). However, variability in hazard ratios were also greater in these treatments (Fig 1). In Expt. 2, chemical treatments did not increase the risk of worm mortality compared to the non-treated control. In fact, the application of Preference and Scythe appeared to reduce the risk of mortality compared to the non-treated control in Expt. 2 (Fig 1). This shows that in most cases, worms died from starvation rather than the direct effects of chemical treatments.

Fig 1. Risk of worm (*Lumbricus terrestris*) mortality (Cox proportional hazards ratio) following application of conventional herbicides (Aatrex, Accent, Clarity, Dual magnum, Gramoxone, Prowl H_2O, Roundup PowerMax, SelectMax, and Scythe), an organic herbicide (Suppress EC), an adjuvant (Preference), and household chemicals (vinegar and soap) on 19 Feb. 2018 (A) and 27 Jun. 2018 (B), Laramie WY. Bars indicate 95% confidence interval. Dashed vertical line indicates hazard coefficient of the non-treated control. Bars that overlap the dashed line are not different from the non-treated control at the 0.05 probability level

Average time to earthworm mortality ranged from 12 to 21 days and 17 to 24 days in Expts. 1 and 2, respectively (Fig 2). This indicates that the risk of acute mortality from direct exposure to these chemicals is low when applied at recommended rates.

Fig 2. Worm (*Lumbricus terrestris*) mortality distribution following application of conventional herbicides (Aatrex, Accent, Clarity, Dual magnum, Gramoxone, Prowl H_2O, Roundup PowerMax, SelectMax, and Scythe), an organic herbicide (Suppress EC), an adjuvant (Preference), and household chemicals (vinegar and soap) compared to the non-treated control (Control) on 19 Feb. 2018 (A) and 27 Jun. 2018 (B), Laramie WY. Dashed vertical lines indicate mean time (days) to mortality.

Roberts and Dorough (31) stated that the active ingredient in gramoxone is only moderately toxic to earthworms. Similarity, glyphosate (the active ingredient in Roundup PowerMax) has low to negligible toxicity in *E. fetida* [32]. Hattab, Boughattas (33) demonstrated that 7 to 14 days exposure to 2,4-dichlorophenoxyacetic acid (24-D), an auxin herbicide with similar activity as dicamba, did not result in mortality of the compost earthworm (*E. andrei*). However, exposure to 2,4-D herbicide reduced the growth of the earthworm [33]. On the contrary, Roberts and Dorough (21) reported that 2,4-D phenol is among the most toxic chemicals to the earthworm *E. fetida*. Butler and Verrell (22) concluded in a study that Ortho Weed Be Gon, the commercial formulation of mecocrop, 2,4-D, and dicamba mixture was not toxic to the earthworm *E. fetida* and could even reduce the toxicity of organophosphate insecticides to worms.

Exposure of annelid worms (*L. variegatus*) to high concentrations of diuron, a herbicide that inhibits photosynthesis, did not affect *L. variegatus* reproduction and no mortality was recorded 10 days after application [34]. Nebeker and Schuytema (34) concluded that although diuron reduced the weight of *L. variegatus*, field use rates of diuron would do little harm to worms. Similarly, exposure of the aquatic worm (*Tubifex tubifex*) to isoproturon herbicide, a herbicide that inhibits photosynthesis did not result in mortality 7 days after treatment [35]. However, the growth rate of *T. tubifex* reduced with increased rates of isoproturon [35].

It is important to state that the experimental procedure we employed assumed a worst-case scenario where herbicides are sprayed directly on worms and worms are confined to the toxic environment for the rest of their lives. This is unlikely under field conditions because worms may exhibit an avoidance response when exposed to toxic chemicals by moving into uncontaminated soils if accessible [22, 32]. However, similar methods have been used in the past to evaluate worst-case scenarios. For example, Bruhl [36] sprayed juvenile frogs (*Rana temporaria*) directly with terrestrial pesticides using methods similar to ours and reported mortality "within one hour" of application. The authors of that study went so far as to suggest pesticide exposure may be an underestimated cause of global amphibian decline. Our results, though, suggest that most of the herbicides and household products evaluated here are unlikely to cause such dramatic acute effects in earthworms if used as directed.

These herbicides, when applied at the recommended field use rates are not likely to cause acute mortality in earthworms. We did not evaluate other aspects of toxicity (such as activity or reproduction) in this study, but evidence from previous studies suggest that the effect on reproduction of *L. terrestris* is also unlikely [34]. Chemical toxicity depends on the worm species. For example, *Eisenia foetida* is less sensitive to chemicals compared to *L. rubellus* [21]. Thus, the species of worm used in the study might have influenced the results.

Conclusions

Worms sprayed with Aatrex, Accent, Clarity, Dual magnum, SelectMax, and Suppress were at greater risk of mortality compared to the non-treated control in Expt. 1. In Expt. 2, chemical treatments did not increase the risk of worm mortality. Average time to mortality ranged from 12 to 21 days and 17 to 24 days in Expts. 1 and 2, respectively. Herbicides present low risk of acute mortality to worms when applied at recommended field use rates.

References

1. Blanchart E, Albrecht A, Alegre J, Duboisset A, Gilot C, Pashanasi B, et al. Effects of earthworms on soil structure and physical properties. Earthworm management in tropical agroecosystems. 1999;5:149-71.
2. Bronick CJ, Lal R. Soil structure and management: a review. Geoderma. 2005;124(1-2):3-22.
3. Amezketa E. Soil aggregate stability: a review. Journal of sustainable agriculture. 1999;14(2-3):83-151.
4. Brown GG, Edwards CA, Brussaard L. How earthworms affect plant growth: burrowing into the mechanisms. Earthworm ecology. 2004;2:13-49.
5. Brussaard L. Soil fauna, guilds, functional groups and ecosystem processes. Applied soil ecology. 1998;9(1-3):123-35.
6. Verhoef H, Brussaard L. Decomposition and nitrogen mineralization in natural and agroecosystems: the contribution of soil animals. Biogeochemistry. 1990;11(3):175.
7. Hendrix PF, Parmelee RW, Crossley D, Coleman DC, Odum EP, Groffman PM. Detritus food webs in conventional and no-tillage agroecosystems. Bioscience. 1986;36(6):374-80.
8. Lahmar R. Adoption of conservation agriculture in Europe: lessons of the KASSA project. Land use policy. 2010;27(1):4-10.
9. Derpsch R. Conservation tillage, no-tillage and related technologies. Conservation agriculture: Springer; 2003. p. 181-90.
10. Rovira A, Smettem K, Lee K. Effect of rotation and conservation tillage of earthworms in a red-brown earth under wheat. Australian Journal of Agricultural Research. 1987;38(5):829-34.

11. House GJ, Parmelee RW. Comparison of soil arthropods and earthworms from conventional and no-tillage agroecosystems. Soil and Tillage Research. 1985;5(4):351-60.
12. Wardle DA. Impacts of disturbance on detritus food webs in agro-ecosystems of contrasting tillage and weed management practices. Advances in ecological research. 26: Elsevier; 1995. p. 105-85.
13. Gunsolus JL. Mechanical and cultural weed control in corn and soybeans. American Journal of Alternative Agriculture. 1990;5(3):114-9.
14. Bond W. Non-chemical weed management. Weed Management Handbook. 2002:296-7.
15. Timmons F. A history of weed control in the United States and Canada. Weed Sci. 1970;18(2):294-307.
16. Derksen DA, Lafond GP, Thomas AG, Loeppky HA, Swanton CJ. Impact of agronomic practices on weed communities: tillage systems. Weed Sci. 1993;41(3):409-17.
17. Buhler DD. Influence of tillage systems on weed population dynamics and management in corn and soybean in the central USA. Crop Science. 1995;35(5):1247-58.
18. Xiao N, Jing B, Ge F, Liu X. The fate of herbicide acetochlor and its toxicity to Eisenia fetida under laboratory conditions. Chemosphere. 2006;62(8):1366-73.
19. Roberts BL, Wyman Dorough H. Relative toxicities of chemicals to the earthworm Eisenia foetida. Environmental Toxicology and Chemistry: An International Journal. 1984;3(1):67-78.
20. Wang Y, Wu S, Chen L, Wu C, Yu R, Wang Q, et al. Toxicity assessment of 45 pesticides to the epigeic earthworm Eisenia fetida. Chemosphere. 2012;88(4):484-91.
21. Roberts BL, Dorough HW. Relative Toxicities of Chemicals to the Earthworm Eisenia-Foetida. Environmental Toxicology and Chemistry. 1984;3(1):67-78. doi: Doi

10.1897/1552-8618(1984)3[67:Rtoctt]2.0.Co;2. PubMed PMID: WOS:A1984SN99300009.

22. Butler DW, Verrell PA. Inhibitory effect of a herbicide formulation on toxicity of malathion to the worm Eisenia fetida. Bull Environ Contam Toxicol. 2005;75(3):421-8. Epub 2006/01/03. PubMed PMID: 16385945.
23. Chen J, Saleem M, Wang C, Liang W, Zhang Q. Individual and combined effects of herbicide tribenuron-methyl and fungicide tebuconazole on soil earthworm Eisenia fetida. Sci Rep. 2018;8(1):2967. Epub 2018/02/16. doi: 10.1038/s41598-018-21288-y. PubMed PMID: 29445108; PubMed Central PMCID: PMCPMC5813222.
24. Wang Y, An X, Shen W, Chen L, Jiang J, Wang Q, et al. Individual and combined toxic effects of herbicide atrazine and three insecticides on the earthworm, Eisenia fetida. Ecotoxicology. 2016;25(5):991-9. Epub 2016/04/14. doi: 10.1007/s10646-016-1656-4. PubMed PMID: 27068296.
25. Wang Y, Cang T, Yu R, Wu S, Liu X, Chen C, et al. Joint acute toxicity of the herbicide butachlor and three insecticides to the terrestrial earthworm, Eisenia fetida. Environ Sci Pollut Res Int. 2016;23(12):11766-76. Epub 2016/03/08. doi: 10.1007/s11356-016-6347-4. PubMed PMID: 26946506.
26. Neuhauser EF, Durkin PR, Malecki MR, Anatra M. Comparative Toxicity of 10 Organic-Chemicals to 4 Earthworm Species. Comp Biochem Phys C. 1986;83(1):197-200. doi: Doi 10.1016/0742-8413(86)90036-8. PubMed PMID: WOS:A1986A223100034.
27. Armitage P, Berry G, Matthews JNS. Statistical methods in medical research: John Wiley & Sons; 2008.

28. R Core Team. 2016. R: A language and environment for statistical computing. v 3.5.1 R Foundation for for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/. 2018.
29. Therneau TM. A package for survival analysis in S. 2015. Version 2.38. 2015.
30. Therneau TM, Grambsch PM. Modeling survival data extending the cox model. 2000.
31. Roberts BL, Dorough HW. Hazards of Chemicals to Earthworms. Environmental Toxicology and Chemistry. 1985;4(3):307-23. doi: Doi 10.1897/1552-8618(1985)4[307:Hocte]2.0.Co;2. PubMed PMID: WOS:A1985AJW1600006.
32. Verrell P, Van Buskirk E. As the worm turns: Eisenia fetida avoids soil contaminated by a glyphosate-based herbicide. B Environ Contam Tox. 2004;72(2):219-24. doi: 10.1007/s00128-003-9134-0. PubMed PMID: WOS:000188531100001.
33. Hattab S, Boughattas I, Boussetta H, Viarengo A, Banni M, Sforzini S. Transcriptional expression levels and biochemical markers of oxidative stress in the earthworm Eisenia andrei after exposure to 2,4-dichlorophenoxyacetic acid (2,4-D). Ecotoxicol Environ Saf. 2015;122:76-82. Epub 2015/07/27. doi: 10.1016/j.ecoenv.2015.07.014. PubMed PMID: 26210610.
34. Nebeker AV, Schuytema GS. Chronic effects of the herbicide diuron on freshwater cladocerans, amphipods, midges, minnows, worms, and snails. Arch Environ Con Tox. 1998;35(3):441-6. doi: DOI 10.1007/s002449900400. PubMed PMID: WOS:000075722400012.
35. Mosleh YY, Paris-Palacios S, Couderchet M, Biagianti-Risbourg S, Vernet G. Effects of the herbicide isoproturon on metallothioneins, growth, and antioxidative Defenses in the

aquatic worm Tubifex tubifex (Oligochaeta, Tubificidae). Ecotoxicology. 2005;14(5):559-71. PubMed PMID: WOS:000231908500006.

36. Brühl CA, Schmidt T, Pieper S, Alscher A. Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline? Scientific Reports. 2013;3:1135. doi: 10.1038/srep01135.

Supporting information

S1 Data

S2 R code

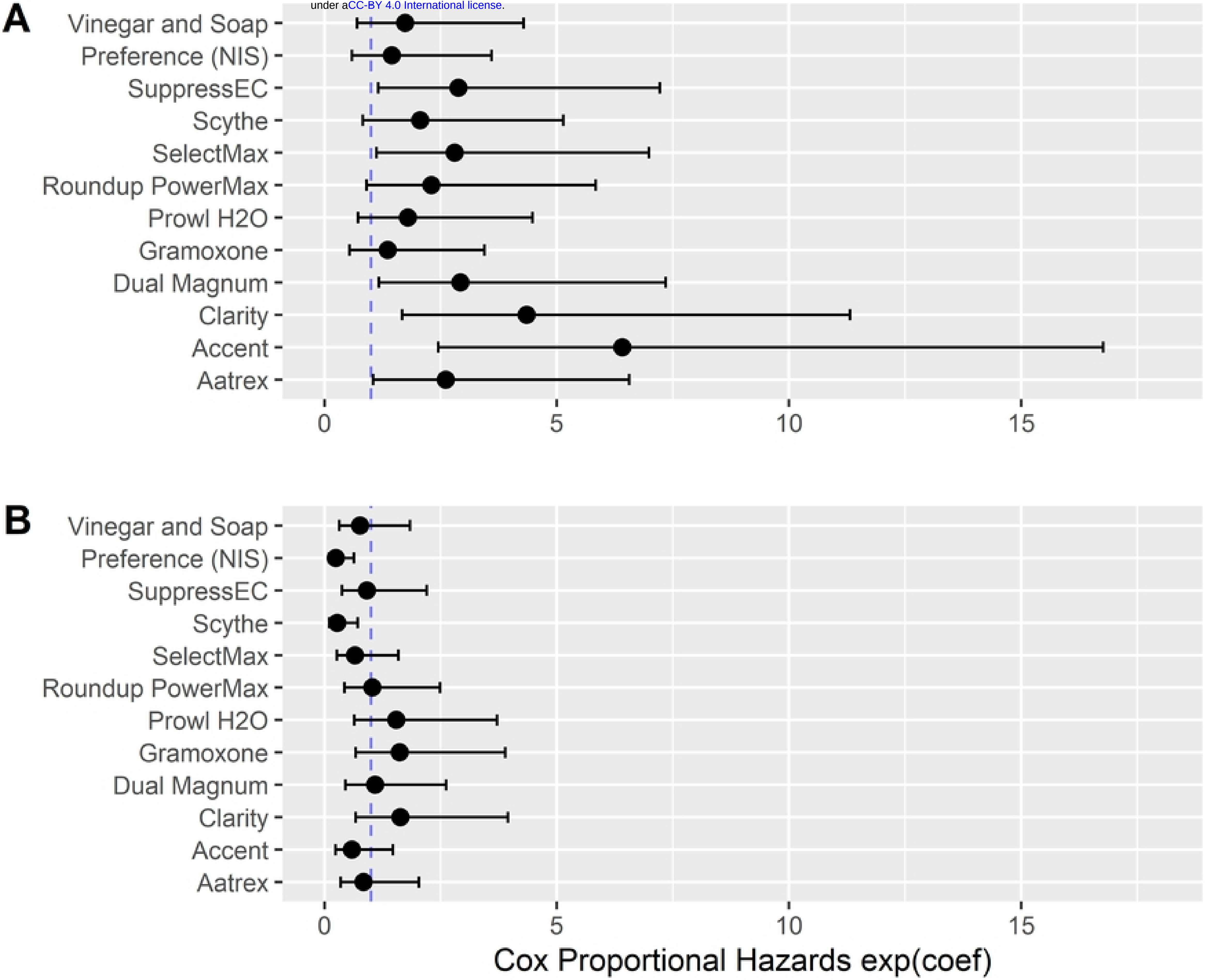
A
Vinegar and Soap
Preference (NIS)
SuppressEC
Scythe
SelectMax
Roundup PowerMax
Prowl H2O
Gramoxone
Dual Magnum
Clarity
Accent
Aatrex
0
5
10
15
B
Vinegar and Soap
Preference (NIS)
SuppressEC
Scythe
SelectMax
Roundup PowerMax
Prowl H2O
Gramoxone
Dual Magnum
Clarity
Accent
Aatrex
0
5
10
15
Cox Proportional Hazards exp(coef)

Figure 1

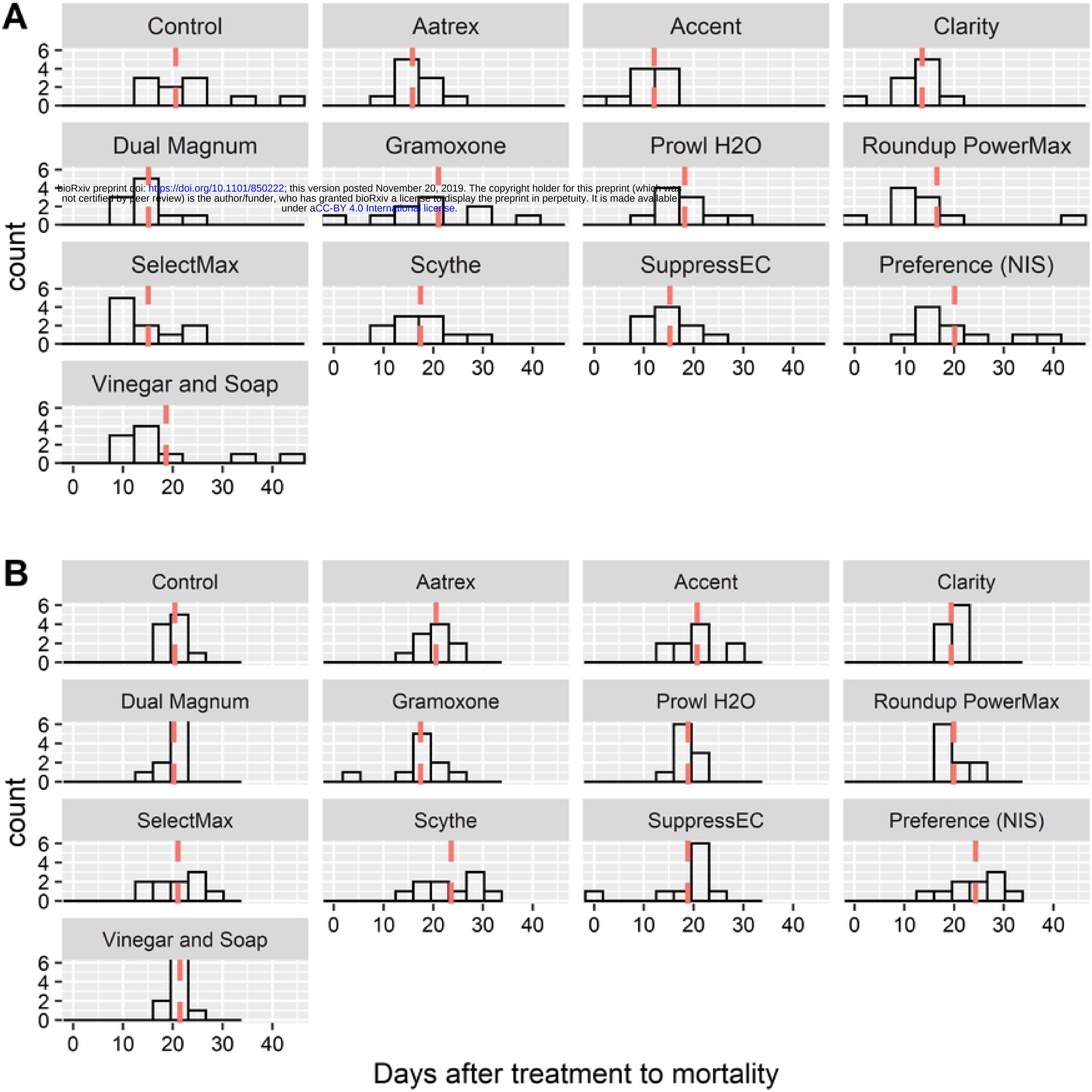
A
Control
Aatrex
Accent
Clarity
Dual Magnum
Gramoxone
Prowl H2O
Roundup PowerMax
SelectMax
Scythe
SuppressEC
Preference (NIS)
Vinegar and Soap
count
0
2
4
6
0
10
20
30
40
B
Control
Aatrex
Accent
Clarity
Dual Magnum
Gramoxone
Prowl H2O
Roundup PowerMax
SelectMax
Scythe
SuppressEC
Preference (NIS)
Vinegar and Soap
count
Days after treatment to mortality

Figure 2