Pesticide Risk Mitigation Engine Earthworm Risk Index White Paper

Earthworm Risk Index	3
Summary	3
Data Sources	3
Index Structure	4
Details and Algorithms	4
Caveats	6
Literature Cited	8
Appendix 1 – Comparison of Earthworm Scores	9
Appendix 2 – Peer Review Comments	10

Summary

This index uses average earthworm toxicity (all species confounded) and application rate as predictors in a logistic model based on a number of field studies which looked at earthworm losses in relation to pesticide use. Application rate is used instead of the commonly-estimated soil concentrations because we have shown that using an arbitrary soil depth parameter leads to poor modeling results. The index is the probability that substantial (i.e. > 35%) earthworm mortality will result from the pesticide application.

Data Sources

Earthworms are frequently used as indicator organisms to gauge the effect of pesticides on terrestrial invertebrates. Earthworms are of great importance for soil health and have an enormous impact on the soil and the entire ecosystem. Unfortunately, recent European work (Frampton, Jänsch *et al.*, 2006; Jänsch, Frampton *et al.*, 2006) has shown that other soil invertebrate are likely much more susceptible than earthworms to pesticide impacts. However, we are constrained by the poor availability of data for these other taxa.

The acute effect of pesticides on earthworms is generally assessed in laboratory tests. A frequent test protocol is the OECD Guideline for testing chemicals no. 207 (OECD 1984). These tests are most commonly conducted with the species *Eisenia fetida* or *Andrei*; or *Lumbricus terrestris*. Tests with these species are relatively inexpensive and straightforward to carry out, and a substantial data set is available for comparison of pesticide toxicity relative to other substances.

Earthworm data were obtained from several sources including but not restricted to the USEPA one liner database (Brian Montague, pers. comm.), the Pesticide Manual (Tomlin 2008), the French Agritox database (http://www.dive.afssa.fr/agritox/index.php), as well as a comprehensive literature review by Jänsch, Frampton and colleagues carried out under the British WEBFRAM initiative (see Frampton, Jänsch *et al.* 2006 for details). The latter also provided references that were used to identify the key field studies used for model validation.

As part of an earlier exercise (Mineau *et al.* 2009), 28 published field trials on earthworm mortality following pesticide application were screened for data quality and comparability of conditions. Data points accepted for further processing met the following conditions: (i) a liquid pesticide solution or suspension was sprayed on soil or plant cover; (ii) no soil incorporation techniques such as rotary tilling and others were used; (iii) the time between pesticide application and earthworm counts/mortality assessment was as close as possible to the assessment times used in lab studies (14 to 28 days); (iv) only one application occurred prior to earthworm counts. Earthworm counts conducted more than 100 days post application

were not used, because the influence of factors such as earthworm regeneration, pesticide degradation, and loss to leaching were considered too unpredictable beyond that time period.

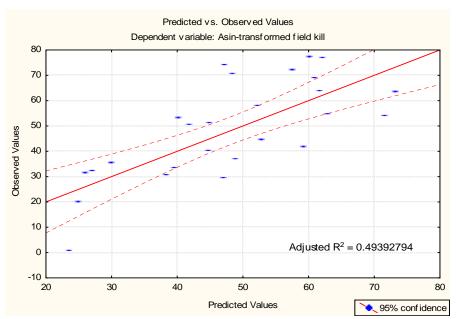
Index Structure

Initial attempts entailed constructing models that relate predicted soil concentration of pesticides to mixed species earthworm mortality (or loss) (Mineau *et al.* 2009). We discovered that the current depth of 15cm assumed by some regulatory authorities did not appear to fit the empirical data very well. (It should be noted that the EU has reduced the mixing depth to 5cm and is considering reducing it further; Stephan Jänsch, ECT Oekotoxikologie GmbH, pers. comm.) A more suitable mixing depth could be determined empirically by analysis of best model fit or, alternatively, more complex percolation models could be developed to estimate pesticide concentrations at different depths. However, this approach is not as promising because one is still left to determine how much of the soil horizon is critical to the earthworms and this is likely to be species and location specific.

In order to get around these complications, we propose a simple model that considers application rate and toxicity only – without having to estimate a soil concentration value. (Note: More complex multiple regression models were considered that also included such variables as soil organic matter, bulk density, field capacity, and DT₅₀ but these did not prove to be the most parsimonious.)

Details and Algorithms

A linear modeling approach to predicting earthworm mortality is shown in the plot below:



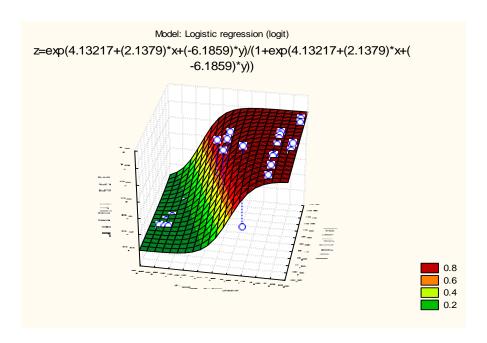
The prediction of earthworm mortality is described in the following equation. This formula is the result of the back-transformation of arcsin-transformed kill rates (see figure above) and their expression as percentage of earthworms killed.

Mortality [%] =
$$\left\langle \sin \left[\frac{27.7531 - 18.419 \log(LC50) + 13.4485 \times \log(r)}{180} \right] * \pi \right\rangle^{2} \times 100$$

... where r is the application rate in grams per hectare, and LC₅₀ is the geometric mean of available earthworm toxicity data from laboratory tests in ppm.

Because of the nature of the empirical data available (i.e. most products tested were known to be problem pesticides for earthworms), there is a paucity of points at low kill levels. Both the EPPO Standard for the environmental risk assessment of plant protection products, as well as the ISO Guideline for the determination of field effects on earthworms deal with the question of what level of effects on earthworms is acceptable. The recovery from a decline in earthworm density within a year from exposure plays an important role in both of these documents (ISO 1999; Sheppard *et al.*, 1998; EPPO, 2003) indicating that transient losses are generally considered acceptable. Based on this information, Mineau and colleagues (2009) defined a 35% loss as an ecologically significant impact, and this is the level of mortality we will use here as our threshold. The probability of this impact level being attained will form the basis of our score in line with other indicators.

Transformation of the data to fit a logistic model with 35% loss of earthworms as the impact and Log application rate and Log LC_{50} as predictors yields the following plot and equation:



... where

Z = final score (to which the usual transitions of 0.1 and 0.5 will be applied)

X = Log g/ha application rate

 $Y = Log(LC_{50})$ – the average of all earthworm values regardless of species.

This ignores any possible systematic differences in toxicity between worm species, but the database is considered too poor for us to define a reasonable SSD strategy.

A comparison of scores obtained for a selection of pesticides used in apples (appendix 1) shows how our proposed scores compare with predicted kill levels.

Caveats

It should be noted that earthworms are not exposed evenly to sprays in orchards because the majority of the pesticide falls to the ground along drip lines. This means that some areas of the orchard receive a much higher rate than average; others much less. No adequate method of incorporating this complexity has yet been devised.

Also, this index makes a number of simplifying assumptions, and these need to be kept in mind when assessing the resulting scores:

 Only short term acute effects are considered. Chronic data is missing for most pesticides, but chronic effects are thought to be more important than acute ones (Stephan Jänsch, ECT Oekotoxikologie GmbH, pers. comm.). This may be a significant problem if compounds with a low acute risk are eventually found to be more damaging to earthworm populations because of chronic risk. However, this would presumably be the case only for a handful of very persistent pesticides.

- Phys. chem. properties as well as fate and persistence parameters and soil properties
 are not used in the index. Attempts to improve the index by building a more complex
 soil percolation model that included these variables did not result in much more
 discriminatory powers based on the limited field dataset available (Längle, unpublished).
- We did not consider effects on different species or different ecological groups of earthworms rather they are treated as a homogeneous group which they are not.

Literature cited (Note : Author's articles and reports available upon request)

- EPPO (European and Mediterranean Plant Protection Organization). 2003. EPPO Standards: Environmental Risk Assessment Scheme for Plant Protection Products. PP 3/7 (revised). European and Mediterranean Plant Protection Organization Bulletin 33: 195–209.
- Frampton, G. K., S. Jänsch, J. J. Scott-Fordsmand, J. Rombke, and P. J. Van den Brink. 2006. "Effects of pesticides on soil invertebrates in laboratory studies: a review and analysis using species sensitivity distributions." Environ Toxicol Chem 25(9): 2480-9.
- ISO (International Standards Organization). 1999. Soil Quality Effects of Pollutants on Earthworms, Part 3: Guidance on the Determination of Effects in Field Situations. International Standards Organisation, Geneva.
- Jänsch, S., G. K. Frampton, J. Rombke, P. J. Van den Brink, and J. J. Scott-Fordsmand. 2006. "Effects of pesticides on soil invertebrates in model ecosystem and field studies: a review and comparison with laboratory toxicity data." Environ Toxicol Chem 25(9): 2490-501.
- Mineau, P., T. Dawson, M. Whiteside, C. Morrison, K. Harding, L. Singh, T. Längle, and D.A.R. McQueen. 2008. Environmental Risk-Based Standards for Pesticide Use in Canada: Supporting Tables for the Synthesis Report and Proof of Concept. National Agri-Environmental Standards Initiative Technical Series Report No. 4-22. 151 p.
- Mineau, P., T. Dawson, M. Whiteside, C. Morrison, K. Harding, L. Singh, T. Längle, and D.A.R. McQueen. 2009. Environmental Risk-Based Standards for Pesticide Use in Canada. National Agri-Environmental Standards Initiative Synthesis Report No. 7. Environment Canada. Gatineau, Quebec. 94 p.
- OECD (Organisation for Economic Cooperation and Development). 1984. Guideline 207.

 Earthworm, Acute Toxicity Tests. Guidelines for Testing Chemicals, Section 2: Effects on Biotic Systems. Organisation for Economic Cooperation and Development. 9 p.
- Sheppard, S. Bembridge, J., Holmstrup, M., and L. Posthuma (eds.). 1998. Advances in Earthworm Ecotoxicology: Proceedings from the Second International Workshop on Earthworm Ecotoxicology, April 1997. Amsterdam, the Netherlands. Society for Environmental Toxicology and Contamination, (SETAC) Press, Pensacola, Florida.
- Tomlin, C.D.S. 2008. The Pesticide Manual. British Crop Protection Council. Alton, Hampshire, U.K.

Appendix 1. Comparison of proposed earthworm scores (the probability that our threshold value of 35% loss will be exceeded) with predicted % kill based on simple linear regression model with application rate and LC_{50} as predictors. Scores presented for sample of in use pesticides in apples and the NASS-determined national average application rate. Scores are given in decreasing order of risk. This is for illustration purposes only since actual scores will depend on actual application rates entered into PRiME. Also, these are raw scores without any mitigating UPAF.

AI Accepted Name	NASS National Average Application Rate (g ai/ha)	Estimated mortality based on regression Model (LC50 & Application Rate)	Acute Risk to Earthworms
Endosulfan	1634.42	63.39%	0.99
Acetamiprid	164.79	41.30%	0.95
Imidacloprid	96.41	33.32%	0.87
Pyridaben	278.01	27.34%	0.40
Azinphos-methyl	932.67	33.02%	0.38
Diazinon	1685.98	34.72%	0.32
Methomyl	589.65	23.95%	0.13
Oxamyl	236.53	13.95%	0.03
Chlorothalonil	1460.66	18.65%	0.02
Lambda-cyhalothrin	34.75	5.70%	0.01
Pyraclostrobin	1.12	0.29%	0.01
Chlorpyrifos	1683.74	16.81%	0.01
Metiram	2898.91	18.13%	0.01
Captan	2228.55	16.44%	0.01
Cyprodinil	205.14	7.68%	0.01
2,4-D, dimeth salt	1095.22	11.00%	0.00
Myclobutanil	143.49	4.75%	0.00
Paraquat	1338.47	9.97%	0.00
Diuron	1663.56	9.16%	0.00
Fosetyl-al	2738.60	10.31%	0.00
Copper hydroxide	2933.66	10.02%	0.00
Pendimethalin	1617.60	7.28%	0.00
Simazine	1592.94	7.20%	0.00
Sulfur	7051.09	10.28%	0.00
Glufosinate-ammonium	832.90	4.16%	0.00
Formetanate HCL	858.69	4.03%	0.00
Kresoxim-methyl	124.43	0.10%	0.00
Spinosad	116.58	0.01%	0.00
Trifloxystrobin	73.99	0.00%	0.00
Glyphosate iso salt	1337.35	0.08%	0.00

Appendix 2: Peer Review Comments

This white paper was reviewed by the following independent experts. Below are their comments, listed anonymously, along with the author's responses.

- Stephan Jänsch, environmental engineer, ECT Oekotoxikologie GmbH
- Rich Marovich, staff environmental scientist, California DPR

General comments:

- I find the index to be well presented, and that it represents a significant advancement in applied science. Excellent discussion of complexities of assessing risk to earthworms. In spite of limited data, the index appears reasonable and useful. I support the design of the earthworm risk index.
- Currently, I'm only aware of a very basic pesticide risk index approach developed by Hassan (1985) for beneficial organisms (Hassan SA. 1985. Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the IOBC/WPRS Working Group 'Pesticides and Beneficial Organisms'. Bulletin OEPP/EPPO Bulletin 15: 214-255.) based on acute laboratory data. Compared to this approach the earthworm risk index is of course a huge improvement since it considers field data although still suffering badly from low data availability.

Detailed comments and responses:

Comment 1: It needs to be stressed that the user must not be tempted to try to extrapolate risk to invertebrates other than earthworms. If this is intended safety factors should be incorporated. These might be refined substance-specifically from laboratory toxicity data for other soil invertebrates if available and how they compare to earthworm toxicity. However, since the index currently does not claim to provide risk estimates for other soil invertebrates this may be something to consider for future versions of PRIME.

Response: Agreed

Comment 2: *L. terrestris* has very rarely been used in these trials, at least for Environmental Risk Assessment purposes. On the other hand *E. andrei* needs to be mentioned as an alternative to *E. fetida*.

Response: Done

Comment 3: If earthworm counts conducted more than 100 days post application are not considered, it may be questioned if the tool is actually useful. For future versions such counts could be related to substance properties such as persistency (DT90), log Pow, volatility, etc. as well as laboratory chronic toxicity data.

Comment 4: The "paucity of points at low kill levels" may result from the fact that mostly rather old pesticides at high application rates are covered. In current European ERA, earthworm field studies triggered by PEC/PNEC comparisons based on laboratory acute LC50 and chronic NOEC data often show rather little effects in the field.

Comment 5: An overall 35% threshold for all earthworm species remaines questionable. As stated by Mineau et al. (2009), long-term effects of pesticides on earthworms depend on the acute effect, the reproductive toxicity and the persistency of the substance. Additionally, recovery depends on

reproduction cycle, mobility, food availability, etc. A differentiation, e.g. by ecological groups (anecic, endogeics, epigeics) should be considered although I admit that even for these data availability is currently probably too low.

Response: Point taken.

Comment 6: RE: "This ignores any possible systemic differences in toxicity between worm species, but the database is considered too poor for us to define a reasonable SSD strategy." That's true but again a differentiation by ecological groups should be considered. Toxicity depends on exposition so the ecological groups might be exposed differently and hence the species belonging to the same ecological group might display a similar toxic response.

Response: The data are too sparse to even do that.

Comment 7: Future versions of the risk index should include chronic toxicity data and long-term earthworm field counts. Also, it seems the intended use (e.g., herbicide, fungicide, insecticide) should be considered when comparing different pesticides. Furthermore, the mode of action (specific vs. broad-spectrum) and persistency of a pesticide need to be included. The latter should pose a third variable in the calculation (e.g., the DT90), not as a predictor of earthworm mortality, but to the effect that more persistent pesticides should generally be considered to pose a greater risk than less persistent ones. Finally, the index needs practical validation, e.g. by subsequent monitoring programs, further field trials or the inclusion of industry data. Overall, more data especially field and chronic laboratory need to be gathered. Inclusion of currently confidential industry data would be useful but will be hard to achieve.

Response: Agreed.

Comment 7: The fact that only short-term acute effects are considered must not lead to a false assumption of a safe use. It may not be generally assumed that a pesticide with a low acute risk index

Response: Text was added to reflect this point.

Comment 8: As the author is well aware, the basic weakness of the risk index is poor data availability. This concerns fate of pesticides, earthworm field toxicity data and the relationships between these and site specific soil properties. This leads to a strong model simplification and overall black box approach: the general assumption that there is a direct relationship between pesticide application rate, acute laboratory toxicity and earthworm field mortality. The resulting limitations of the risk index are:

will also be less harmful in the long term compared to another pesticide with a higher acute risk index.

- only acute short-term effects are considered while chronic long-term effects are often the more severe ones
- all pesticides are treated equally regardless of intended use, mode of action, physico-chemical properties and resulting persistency and fate
- no interactions of pesticides and site-specific (soil) properties are considered
- no species or ecological groups of earthworms are differentiated

These shortcomings as well as the general uncertainty in the risk index need to be made transparent to the user in order to be able to correctly interpret the outcome of the risk estimate.

Response: Agree fully with this criticism. Will borrow much of this language to draft caveat.