Pesticide Risk Mitigation Engine

Avian Reproductive Risk Index

White Paper

Summary

The only information on the chronic toxicity of pesticides to birds is the 21-week avian reproduction study. This risk index derives a NOAEL (the lowest calculated dose at which no adverse effects are observed) from the NOAEC (lowest calculated concentration in lab chow at which no adverse effects are observed) value available from this study to express risk as the proportion of a breeding season when residues in the environment are at a level that may be considered high enough as to interfere with avian reproduction. The latter calculation is carried out for a breeding insectivorous songbird scenario in keeping with the dominant regulatory practice. Estimated NOAELs are corrected to account for inter-species sensitivity differences inferred from acute toxicity information; also, first-order loss rates on foliar surfaces are calculated to assess the intake of residues over time.

Data Sources

The only possible data sources here are the 'no observed adverse effect concentrations' (NOAECs) compiled by EPA from standard reproduction tests carried out in the mallard and bobwhite. These tests and their limitations have been reviewed extensively by Mineau *et al*. (1994), and Mineau (2005).

Index Structure

Developing a reproductive toxicity index separate from an acute index is important because an acute toxicity index is insufficient to account for the possible impact of pesticides on birds. Also, acute toxicity is not necessarily a good predictor of reproductive risk. The proposed index is detailed in Mineau *et al.* (2006). It is a modification of the standard RQ approach in that it incorporates a factor for interspecies variation in toxicity (Luttik *et al.* 2005) and introduces the concept of time as a measure of potential impact. An Allowable Daily Intake (ADI) is calculated for a small songbird at the 5% tail of the estimated sensitivity distribution. Based on application rate and standard Residue per Unit Dose (RUD) factors for a small insectivorous bird, as well as foliar half lives obtained from the USDA, the index is based on the amount of time that the ADI will be exceeded when an individual forages in a treated area.

This approach was recommended by a series of expert panels convened over the last decade (e.g. Mineau *et al*. 2001a). Although it is not possible to validate this index, a similar approach for small mammals (Mineau *et al.* 2009) showed that the 'time approach' provided the best fit for the limited amount of field data.

In order to fit the index on a scale that is compatible with the acute avian index, a threshold representing an undesirable outcome has to be defined (the presence of avian mortality was the undesirable outcome for the acute index). The proposed threshold is tentatively set at 90 days, or approximately the length of the breeding season for songbirds in North America. In other words, the worst possible score of 1 would be a pesticide that causes a reproductive threshold to be exceeded for the entire length of the 'normal' breeding season.

A valid question is whether this index should be calculated where pesticide applications do not coincide with breeding (e.g. dormant sprays). There is a good argument to calculate this index regardless of the exact timing of the pesticide application. Indeed, the avian reproduction test is also one of chronic toxicity in birds. The endpoint of concern is often parental toxicity rather than a targeted effect on the reproductive physiology of the birds and the NOAEC does not differentiate between the two (Mineau *et al*. 1994; Mineau 2005). Given that we already have an acute index for birds, the reproductive index as defined here also serves to identify problems associated with chronic toxicity and lengthy product persistence in the environment.

Details and Algorithms

For every a.i., the lowest NOAEC (measured as mg pesticide a.i./ kg food) for each of the two species – Bobwhite and Mallard – was retained as the value of interest. In some cases, tests were repeated, often because the first studies failed to detect a true NOAEC. Taking the smaller value for each species-a.i. combination increased the chances that a true NOAEC would be retained. Typically, two species only are tested for reproductive effects. A few tests have been carried out on the Japanese quail but not in sufficient numbers to meet our purpose.

In some cases, a NOAEC was not available but a LOAEC (the Lowest Observed Adverse Effect Concentration) was provided. The NOAEC was assumed to be 1.23 times lower than the LOAEC when all dose levels are converted to a $\log_{10}$ scale. This ratio is the median spacing between dose levels in a large sample of studies submitted to the USEPA (272 studies). This assumes that, had a lower dose level been tested, a NOAEL would have been found.

The NOAEC has been criticized as a toxicological endpoint, especially in the context of aquatic toxicity testing (e.g. Koojiman 2006), and we fully agree with this criticism. However, it is currently not feasible to extract an ECx[1] type of value from the current avian reproduction tests, and even that approach has been criticized recently (Fox 2009). Furthermore, NOAEC values are commonly compiled by some jurisdictions (e.g. the USEPA) and made public. This is therefore the best chance we have to minimize data gaps. The limitations of the current avian reproduction test have been discussed in detail in Mineau *et al*. (1994) and Mineau (2005).

[1] Ideally, such an 'Effective Concentration' should be calculated for a defined reproductive deficit (x). For example, an EC10 for egg production would be a 10% reduction in the number of eggs laid. This approach is analogous to that used to assess growth inhibition in plants subject to herbicides. For example, an EC20 (growth) would be a dose calculated to give a growth reduction of 20%. Unfortunately, there are too few doses and too many possible endpoints in the avian reproduction studies to allow for a probit or logit fitting of results and a calculation of an ECx.

In the usual reproduction study, Bobwhites (weight 210 g; unpublished industry studies) have a peak food consumption of approximately 10% of their bodyweight in food per day; measured food intakes for Mallards (approx. 1000-1050g) are highly variable and peak above 20% of bodyweight (unpublished industry studies). This is counter to expected allometric relationships where, the smaller the bird, the larger its proportional food intake. Mallards in the laboratory tend to spill a lot of food, and it is therefore difficult to estimate their true consumption. As verification, the allometric equation of Nagy (1987) for all birds was used to estimate food consumption even though it is recognized that Nagy's algorithms apply to birds in the wild. One expects wild birds to have higher maintenance requirements than birds kept in the laboratory. On the other hand, the birds in the laboratory are induced to lay an unreasonably large clutch size which is likely to increase their food intake compared to an equivalent bird in the wild.

- Dry food intake = 0.648 * bw(g) ^ $^{0.651}$

Laboratory diet was estimated to have 11% moisture content based on a personal communication from Joann Beavers with Wildlife International, one of the major testing laboratories. Therefore, for the Bobwhite a theoretically-calculated intake of lab diet (actual weight) should be:

- Intake = (0.648 * bw(g) ^ $^{0.651}$)/0.89 (propn. dry wt.) = ~ 24 g

…. which is very close to the observed 10% of bodyweight.

For the Mallard, the same formula returns a value of ~ 67 g/day or a little over 6% of its bodyweight per day rather than the observed 20%. Because of the spillage problem mentioned previously and how close the Bobwhite figure is to the theoretical (Nagy) estimate, we opted to use the empirical value of 21 g per day for the Bobwhite but the theoretical value of 67 g/day for the Mallard.

Ideally, food intake rates should be obtained from each actual study but this is not reported. Therefore, the estimated food intakes of 21 g/day or 67 g/day for the Bobwhite and Mallard respectively were used to convert all NOAEC values to NOAELs (critical pesticide intake levels) expressed as mg a.i. of pesticide / kg bird / day.

Therefore :

- NOAELmallard (mg a.i./kg bw/day) = (NOAECmallard (mg/kg food) * 0.067 kg food/day) / 1 kg bw
- NOAELbobwhite (mg a.i./kg bw/day) = (NOAECbobwhite (mg/kg food) * 0.021 kg food/day) / 0.210 kg bw

A geometric mean of NOAELmallard and NOAELbobwhite is then calculated.

Two test species is a very poor basis for a safety evaluation intended to protect the large number of species present in and around agricultural crops. Inter-species variation in toxicological susceptibility to any given pesticide can vary by several orders of magnitude – at least judged on acute toxicity. An expert panel (see Mineau *et al.* 2001a) first proposed that interspecies differences in acute toxicity could be used as a proxy for interspecies differences in reproductive toxicity, the assumption being that reproductive toxicity should be no less variable than acute toxicity. This strategy was explored and further detailed in Luttik *et al.* (2005).

In order to use the compound-specific interspecies variation in acute toxicity, we derived standard deviations (SDs) for acute data in the following way:

- A single geometric mean log LD_{50} value was obtained for each species-pesticide combination as outlined in Mineau (2001b).

- Where the number of species tested was 4 or more, we derived a Standard Deviation (SD). This was only possible for 38 of the 207 active ingredients, primarily the more acutely-toxic insecticides. For all other a.i.s, a pooled SD of 0.465 (after Aldenberg and Luttik 2002) was used.

The extrapolation factor (a factor to be applied multiplicatively to the mean untransformed NOAEL) was defined as follows after Aldenberg and Luttik 2002):

- EFmedian = $(10^{\sigma})^{Kp}$

… where Kp is the z score of 1.64 in the case of the 5% tail of a normally-distributed species sensitivity distribution. This is equivalent to:

- EFmedian = 44.14^{σ} … or to an extrapolation factor of 5.8 for the pooled variance estimate of bird acute data.

The median extrapolation factor (EF) was then applied to the geometric mean NOAEL in order to obtain the critical toxic effect level (in mg/kg/day or µg/g/day) for a sensitive bird at the 5% of the putative distribution of reproductive toxicities ($NOAEL_{crit}$).

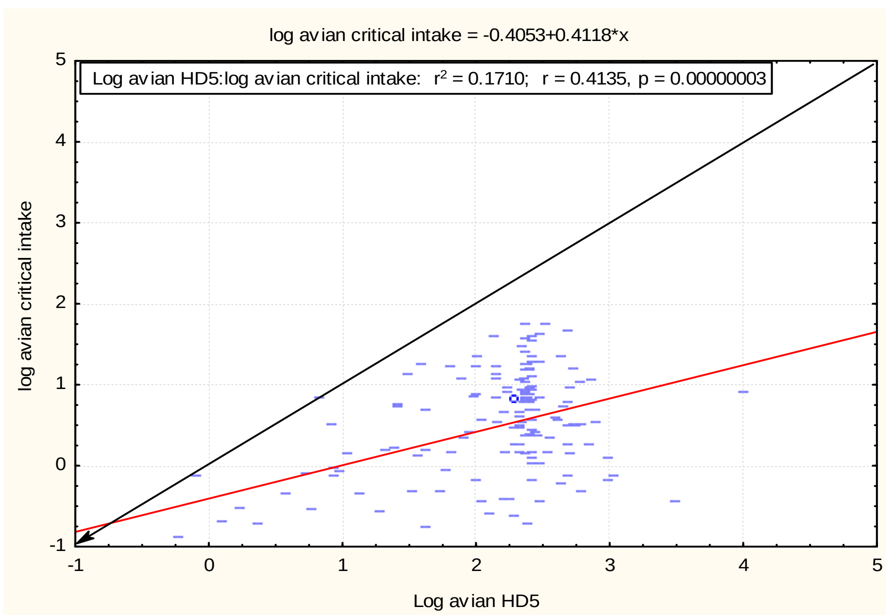
This critical no-effect dose was then converted back to a food residue equivalent, assuming a 15g insectivorous bird and food intake based on the allometric equation for passerine species (Nagy 1987), and assuming that insects have approximately 70% water content.

- Intake for 15 g insectivore = (0.398 * 15^0.850)/0.30 = 13.2 g insects(FW)/day

- Critical residue concentration C_{crit} (µg a.i./g fw insect – or ppm) = $NOAEL_{crit}$ (µg a.i./g bw/day)] * 15 g bw / 13.2 g insects(FW) / day

Note: The exact parameters of the scenario could be debated (and are) at length. However, these are not critical if we are primarily interested in a relative ranking of products. However, we chose values in common use in risk assessment calculations so as to provide reasonable values in line with those that would be obtained by regulatory bodies in North America or Europe. For example, past EU guidance (Council Directive 91/414/EEC, dated 25 September 2002) based some of its risk assessments on a 10g insectivorous songbird. Based on a slightly more circuitous calculation of daily energy intake, caloric value of insects and assimilation efficiency, they arrived at an estimated food intake/body weight ratio of 1.04 – or 15.6 g insect fresh weight per day.

It is possible to compare the critical intake calculated (in µg/g/day or mg/kg/day) with the estimated avian HC_5 for the same compounds. The two are plotted below for a wide range of currently registered pesticides.



For compounds of extreme toxicity, the two values are expected to be similar, diverging as compounds become less acutely toxic. The poor r^2 value indicates that the development of a chronic toxicity index in birds is necessary to account for all possible pesticide impacts.

The initial insect concentration immediately after application (C_0) is estimated from the application rate and a <u>R</u>esidue per <u>U</u>nit <u>D</u>ose factor of 21.0 – i.e. expecting about 21 ppm for a 1 kg a.i./ha application. This is the average value recommended in the latest European guidance for foliar dwelling arthropods (EFSA 2008). In our assessment tool, this value is multiplied by the appropriate UPAF[2].

The final calculation entails estimating the amount of time (T_{crit}) needed for insect residues to drop from C_0 to C_{crit}, assuming first order loss rate and using the foliar DT_{50} (or $t^{1/2}$) as the best estimate of residue persistence in insect food.

- If $C_0 < C_{crit}$, risk = 0, which is 0 days
- If $C_0 > C_{crit}$, measure the number of days required to drop to C_{crit} given the foliar half life.

Measure removal rate K from foliar half life ($t^{1/2}$)

- $K = \ln(0.5) / t^{1/2}$

…. and the critical time $T_{crit} = (\ln(C_0 / C_{crit})) / -k$ … measured in days.

The normal breeding season for birds is assumed to be 90 days; the final score is therefore the proportion of the breeding season when insect residue levels will be above a critical level expected to interfere with reproduction.

The final score is therefore: $T_{crit} / 90$ and is capped at 1.

In keeping with the acute avian score, a score below 0.1 will represent a *de minimus* risk; a score above 0.5 (residues are expected to be above a reproduction threshold for more than half of a normal breeding season) will indicate a high risk situation.

Note: Foliar DT50 values were mostly obtained from the USDA Natural Resources Conservation Service (NRCS) in their Pesticide Properties Database (PPD). These values are used in that agency's fate modeling efforts under the WIN-PST (Windows – Pesticide Screening Tool) and multi-Agency CEAP project (Conservation Effects Assessment Project) (http://www.wsi.nrcs.usda.gov/products/W2Q/pest/WinPST.html#pst ppd). Where the foliar DT50 was not available but a soil half-life value was, we estimated the former by means of the regression equation (Mineau et al. 2006):

- Log foliar half life = -0.024 + (0.41 * Log soil half life) + (0.023 * log solubility) – (0.031 * log VP)

[2] This 'Use Pattern Adjustment Factor' reflects the way in which the pesticide is used (e.g. foliar vs. soil incorporated application) and represents expert judgment as to the relative potential for exposure through the ingestion of contaminated insects, the baseline factor of 1 representing a foliar application.

[*Note: This equation is in the process of being re-assessed in light of recent work on this subject (Juraske et al. 2008).*]

UPAFs for Avian Reproductive Risk Index

Once again, particulate applications present a separate challenge. This is because the rate of foliar degradation may not apply to granular formulations or seed treatments. Their persistence in the environment depends on removal as well as breakdown of the carrier or germination in the case of seed treatments. Nevertheless, the same procedure was followed for granular applications. Therefore, this index uses the same Use Pattern Adjustment Factors (UPAFs) as the Avian Acute Risk Index. The difference is that the UPAFs are used to modify the extent of exposure rather than the final risk score which is the proportion of the breeding season that residues are above the calculated threshold. Seed treatments will be considered at a later date.

Pre-Plant or Pre-Emergence			Post-Emergence		Either
Soil Applied: Liquid	Soil Applied: Granular	Soil Applied: Unspecified	Ground Foliar Applied	Soil Applied: Liquid	Aerial Application
0.5 (surface) 0.1 (sub-surface) 0 (application followed by tarping)	See below	0.5	1	0.5 (surface) 0.1 (sub-surface)	1

Silica granules	Corn cob (organic) granules	Heat treated montmorillonite and other non friable clays, cellulose	Friable granule bases: bentonite and gypsum	Tarping follows granular application
2.0	1.0	0.2	0.1	0

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

Aldenberg, T. and R. Luttik. 2002. Extrapolation factors for tiny toxicity data sets from species sensitivity distributions with known standard deviation. IN Species Sensitivity Distributions in Ecotoxicology, L. Posthuma, G.W. Suter II and T.P. Traas [eds.], Lewis Publishers, Boca Raton, Florida, pp. 103-118.

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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.

Nagy, K.A. 1987. Field metabolic rate and food requirement scaling in mammals and birds. Ecological Monographs 57:111-128.

Posthuma, L., G.W. Suter II, T.P. Traas. 2002. Species Sensitivity Distributions in Ecotoxicology. Lewis Publishers, Boca Raton, Florida. 587 pp.

Appendix 1. Comparison of proposed avian reproduction (chronic toxicity) scores calculated with a 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)	Chronic Risk to Avian Species
Copper hydroxide	2933.66	1.00
Diuron	1663.56	1.00
Formetanate HCL	858.69	1.00
Pendimethalin	1617.60	0.75
Mancozeb	2999.80	0.55
Oxyfluorfen	1256.64	0.51
Diazinon	1685.98	0.36
Metiram	2898.91	0.36
Simazine	1592.94	0.31
Permethrin	190.57	0.30
Myclobutanil	143.49	0.29
Chlorothalonil	1460.66	0.26
Dodine	896.80	0.25
Phosmet	1803.69	0.25
Dimethoate	1268.97	0.22
Chlorpyrifos	1683.74	0.21
Endosulfan	1634.42	0.18
Azinphos-methyl	932.67	0.15
Glyphosate iso salt	1337.35	0.12
Oxamyl	236.53	0.12
Clofentezine	232.05	0.10
Malathion	3021.10	0.07
Carbaryl	1249.92	0.07
Captan	2228.55	0.07
Glufosinate-ammonium	832.90	0.06
Acetamiprid	164.79	0.06
Imidacloprid	96.41	0.04
Methomyl	589.65	0.02
Lambda-cyhalothrin	34.75	0.01

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.

- **Rick Bennett,** wildlife toxicologist, US EPA
- **Anne Fairbrother**, senior managing scientist, Exponent
- **Rich Marovich**, staff environmental scientist, California DPR

General comments:

- I can envision great utility for this approach as a comparative tool for assessing risks among pesticides or between different organism classes. I'm not sure that this tool will necessarily replace any others, but I do think it will be very useful when conducting comparative risk analyses, either among chemicals or between classes of organisms for individual chemicals. It also is a very good communication tool for these types of assessments.
- I find the indexes to be well presented, and that they represent a significant advancement in applied science. I support the design of the avian reproductive risk index. Strongly agree with time factor for avian risk (incorporation of environmental fate data).

Detailed comments and responses:

Comment 1: The acronym NOAEL is used throughout the paper, but it is not defined to make the distinction that is refers to a dose rather than a concentration.

Response: *Text added for clarification*

Comment 2: When a study finds significant effects in all treatments and there is no NOAEC determined, there really is no generalized empirical basis for estimating a NOAEC. The report implies a basis in stating "We compiled available NOEACs and LOEACs from the USEPA one liner database (B. Montague pers. comm.) and calculated that the median spacing between the log NOAEC and log LOAEC was 1.23 based on a sample of 272 studies. This ratio was therefore used to obtain a NOAEC where the lowest level tested produced an effect." However, the dose spacing in other studies provides no information for a study that fails to define a NOAEC. It would be better to just state that when there is not a defined NOAEC, it is assumed to be x times lower than the lowest tested dose.

Response: *Changed the wording.*

Comment 3: Do you have a reference(s) for the sentence "The NOAEC has been criticized as a toxicological endpoint, especially in the context of aquatic toxicity testing…."?

Response: *Done*

Comment 4: This risk index uses NOAECs from the avian reproduction studies as the measure of effect. This seems to be extremely conservative for two reasons: 1) there are a multiplicity of endpoints that are measured and reported, with the NOAEC based on the one with the lowest effects value – this is not always a major reproductive effect, as it could be some measure of behavior or reduced food consumption; and 2) NOAECS are NO effect levels. LOAECs are the LOWEST effect levels. So the actual threshold is in between these two. For setting water quality criteria, the EPA uses the geometric mean of the NOAEC and LOAEC. I suggest that be done here as well.

Response: *Unfortunately, this is not possible. Not enough LOAEL are given for this test. There would be too many gaps.*

Comment 5: I understand the concern about using the mallard food consumption data from study reports because of the uncontrolled spillage. The Nagy equations provide a means of estimating adult food consumption for non-laying birds, but this underestimates consumption during the long laying period, and thus underestimates the ingested dose during laying. An energetics study of bobwhite by Case calculated that female approximately double food consumption during laying (Case, R. M. 1972. Energetic requirements for egglaying bobwhites. Proceedings, First National Bobwhite Quail Symposium, Stillwater, OK, April 23-26, pp. 205-212).

Response: *This is a common criticism of the Nagy equations. However, it has been pointed out that many of the data points which went into the equations were from breeding birds because they are easier to capture and recapture at the nest. Also, because the Bobwhite figure was so close, we do not think it is justifiable to double the intake for the mallard; this would put the mallard at a higher proportional (per g body weight) food consumption than the Bobwhite which is unlikely.*

Comment 6: I like the concept of basing the index on the amount of time that the ADI will be exceeded when an individual forages in a treated area, indexed to a breeding season of 90 days.

Comment 7: Calculating the ADI for a small-bodied bird will return a conservative estimate. Coupled with using the HD5 approach for selecting a toxicity reference value, this will be appropriate (and, therefore, could use a geometric mean of NOAEC and LOAEC without losing undue conservatism).

Response: *This index may indeed turn out to be a bit more conservative but we will have to wait for field validation data. At least the answer does not diverge very much from existing assessments by EPA.*

Comment 8: Converting dietary concentration to dose (mg/kg-bw) was estimated appropriately. There are a lot of assumptions here, but there's no way around this and Mineau followed a standard (accepted) methodology.

Comment 9: On page six there is a bullet stating "Intake for 15 g insectivore = (0.398 * 150.850)/0.30 = 13.2 g insects(FW)/day" that includes an error. It should be "(0.398 * 15 ^ 0.850)/0.30" to reflect that weight is raised to the 0.85 power. I think another bullet has a similar error. In "EFmedian = (10σ)Kp" shouldn't it also show Kp as an exponent?

Response: *Nice that the reviewer caught these. Thorough review. I think the problem came when we changed font on the white paper.*

Comment 10: The top of page seven starts with a note stating "The exact parameters of the scenario could be debated (and are) at length. However, these are not critical if we are primarily interested in a relative ranking of products." I agree, but this seems inconsistent with the appendices showing specific categories of risk denoted as red, yellow, and green that are similar to the acute risk index. In the case of the acute risk index the color coded risk categories are related to observations from field studies. For the reproductive index there are no comparable field studies, so how confident are you that the reproductive index can be used as more than a relative index?

Response: *Can this index alone really identify the degree of risk? That is a valid point – but the best information we have currently.*

Comment 11: Is there a reference(s) for "Note: Foliar DT50 estimates were obtained from the USDA."?

Response: *Added more text and reference.*

Comment 12: So now the critical No Effect Dose (why not Low Effect Dose or the geometric mean of the two?) is converted back to a diet concentration for comparison to invertebrate residues (assuming this is the worst-case scenario compared to seed eaters or grazers?). This adds more uncertainty to the analysis. Is it necessary?

Response: *Yes. This was explained by phone to the reviewer's satisfaction.*

Comment 13: I do not understand how this reproductive index that is based on dietary exposure can be used for pesticide granules, especially for granules picked up as grit rather than food. I understand how the UPAFs for granules might apply to the acute index, but I think more discussion is needed here for including granules in the reproductive index. If they are not perceived as food, how is exposure calculated? What half-life value is used? I may be missing something, but this is not clear.

Response: *A good point and a bit of a stretch for the index. The UPAFs were developed on the basis of relative field kills – therefore relative potential for exposure – with a good dose of 'best scientific judgment'. By calculating a score for granulars, we are assuming that the factors moderating short-term exposure will be equally important in the case of a chronic exposure situation.*