A CONSIDERATION OF INTER-SPECIES VARIABILITY IN THE USE OF THE MEDIAN LETHAL DOSE (LD_{50}) IN AVIAN RISK ASSESSMENT

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Résumé: Ce document découle de notre insatisfaction face aux méthodes présentement utilisées pour l'extrapolation de la toxicité aiguë d'un pesticide ou autre produit chimique d'une espèce à une autre. Nous suggérons ici deux méthodes alternatives qui assureront l'utilisation d'une valeur de toxicité aiguë qui soit vraiment représentative des oiseaux en général. Notre préférence serait une approche ou plusieurs espèces (approx. 6-8) seraient testées de façon à déterminer, de façon empirique, la variation entre espèces. Cette approche se base sur une distribution logistique des données de toxicité aiguë. Si cette approche s'avère impossible, il est possible d'utiliser un facteur de sécurité à partir d'une ou plusieurs espèces pour estimer la variation entre espèces. Ces facteur sont calculés empiriquement pour chaque espèce d'après la variation entre espèces observée avec d'autres produits. D'une façon ou de l'autre, l'analyse de risque serait sur une base scientifique beaucoup plus défendable quelle ne l'est maintenant.

1. INTRODUCTION

Avian risk assessment of pesticides depends for the most part on two laboratory-derived measures of lethality. First, the median lethal dose (LD_{50}), a statistically derived single oral dose of a compound which will cause 50% mortality of the test population. Second, the median lethal concentration (LC_{50}) which similarly derives the concentration of a substance in the diet which is expected to lead to 50% mortality of the test population. A companion discussion paper (Mineau, Jobin and Baril, 1994) has argued convincingly against the continued use of the LC_{50} in avian risk assessment of pesticides. This test was found to provide unreliable results due in part to the difficulty in properly determining exposure. The LC50 test is also greatly influenced by the age and condition of the test population and the correlation of LC50 values between test species is weak thus limiting our ability to extrapolate from test species to other bird species. Finally comparison of test results with field evidence suggest that lab-derived LC50s are poor predictors of hazard. If the evidence against the utility of the avian dietary toxicity test continues to mount, avian risk assessment will therefore depend almost entirely on the results of the median lethal dose test.

Exposure scenarios where the dietary intake of well defined "quanta" of pesticides can be predicted, as is the case for granulars or treated seed, lend themselves well to hazard assessment using the LD_{50} test. It was also shown, however, that the exposure of a grazing goose to diazinon treated grass could be successfully coupled to the LD_{50} to provide a realistic estimate of risk as "time to death" (Rostker, 1987). Thus, given enough information on exposure it is possible to use the LD_{50} to assess formulations other than seed dressings and granulars. Further development of the LD_{50} as an effective hazard assessment tool, however, requires work on three fronts: (1) studies of the representativeness of test species and the related issue of inter-species variability in sensitivity to chemicals, (2) improvements in the quantification of exposure, and (3) development and field validation of extrapolation procedures. In this discussion paper we aim to explore the first of these areas of study. It is not our objective to criticise the test protocol itself. While some criticisms of the test are warranted we feel that the LD_{50} test as designed is basically reliable. Test results are more easily interpretable than those of the acute dietary study. The median lethal dose best reflects the inherent sensitivity of test species to chemicals. For this reason we feel that it is the ideal measurement available to examine inter-species variability.

Regulations in both North America and Europe do not emphasize the LD_{50} and presently require that only one avian LD_{50} test be conducted. In North America test results on either the Mallard duck or the Bobwhite quail are accepted whereas European regulations accept testing on the Japanese quail. In many instances, however, test results from studies with two species are currently provided.

Regulators usually extrapolate from one, or two species at best, to birds in general. What is the most scientifically defensible approach for this necessary extrapolation? Often, especially when several pesticides are being compared to one another, interspecies variation in susceptibility is ignored and the test species common to all chemicals of interest is used as a basis of comparison. Alternatively, the lowest available LD_{50} value for each chemical is used. The main problem with this approach is its inherent 'unfairness'. The more species are tested, the better the chance that a very susceptible species will be tested - chemicals about which we know very little are therefore favoured. Another approach is that described by Urban and Cook (1986) and developed for the U.S. EPA ecological risk assessment scheme for pesticides. Inherent to the U.S. EPA model is the assumption that inter-species variability is accounted for by intra-species variability; thus, based on the average slope of dose-response functions, it is assumed that a safety factor of five applied to the LD_{50} or the LC_{50} protects between 90 and 99% of the test species population. The empirical basis for believing that intraspecies susceptibility differences mirrors interspecies susceptibility diferences is unclear and this approach is not intuitively obvious to us. Studies (e.g. Tucker and Leitzke, 1979) have shown that inter-species differences in sensitivity alone can easily exceed 10 fold thus casting doubt on the U.S. approach.

In this document, we propose two different approaches for choosing an LD_{50} truly representative of birds and which can therefore be used with some confidence in risk assessment calculations. We believe that both of our approaches are on a much sounder scientific footing than current procedures described above: (1) Our favoured procedure which entails testing several species in a battery approach in order to derive a distribution of sensitivities, or (2) A second-best approach where empirically determined species-specific safety factors are applied to standard LD_{50}s obtained for one or two species in order to approximate the same distribution of sensitivities.

This document will also revisit the question of the 'representativeness' of the various test species. Several authors have written on this subject and reached different conclusions (see Mineau, 1991). For example, Tucker and Leitzke (1979) argued, based on acute toxicity values for pesticides, that the concept of sensitive species "...should probably be laid to rest..." On the other hand Schafer and Brunton (1979) found that some species do appear to show an inherent susceptibility or, conversely, resistance to a wide range of environmental toxicants. We propose to re-examine this question primarily through the analysis of available acute avian oral toxicity data for cholinesterase-inhibiting insecticides. These chemicals are known toxicants to birds with a wide spectrum of toxicity. Their mechanism of action is similar and thus reduces variability inherent to products with diverse modes of action. The validation of proposed strategies will, however, also be conducted on pesticides with other modes of action.

2. METHODS

2.1 Data collection

The data collated for analysis came from two main sources. The first source consisted of compendia of avian acute toxicity data reported in the open literature and usually assembled by governmental agencies in the United States and elsewhere (Schafer, Bowles and Hurlbut, 1983; Hudson, Tucker and Haegele, 1984; Grolleau and Caritez, 1986; Smith, 1987). The second source consists of results from studies sponsored by pesticide manufacturers in support of the registration of their pest control products. These came in the form of databases kindly provided by the United States Environmental Protection

Agency and the Institut National de la Recherche Agronomique of France, and one established by the Canadian Wildlife Service of Environment Canada. The French database incidentally is accessible through phone modem by any subscriber. Other sources consisted of published studies on single species or a small number of pesticides (Hudson, Haegele and Tucker, 1979; Wiemeyer and Sparling, 1991; Henderson et al., 1994). A number of selection criteria were established and these criteria were used (roughly in the order presented below) to judge the acceptability of the data or to choose a value where more than one was available for any given combination of bird species and insecticide.

a) Only data for adult birds were used. In some cases, age was unspecified but the data, often generated for pesticide submissions, were assumed to refer to adults.

b) Studies of formulated products or of technical products with very low percentages of active ingredient were rejected.

c) Preference was given to values obtained through standard probit analysis with a high number of individuals per dose over approximate LD_{50} values obtained with fewer animals; e.g. calculated LD_{50} values published by Hudson, Tucker and Haegele (1984) were given precedence over those published by Schafer, Bowles and Hurlbut (1983) using fewer individuals and an up and down method.

d) When confronted with multiple values within a laboratory for a given bird-insecticide combination, the most recently published value was chosen.

e) Exact values were preferred to ranges but , when a range was provided, the median of the two values was used unless the spread between the values exceeded 3X in which case the median was not accepted.

e) When separate values were provided for each sex the lower value was chosen. Large intersex differences were rare.

f) Open-ended ranges (e.g. > 500 mg/kg) were rejected.

g) Where two values for the same bird-pesticide combination were given equal 'precedence' and where those values differed significantly, the value most approaching the pesticide-specific median value of the other bird species was used. Fortunately, this only happened on 3 occasions.

Unfortunately, we were not able to take into account the method of dosing (e.g. by gavage needle or gelatin capsule) nor were we able to account for the use of vehicles or diluents (e.g. corn oil), this information seldom being available.

The database thus compiled for most of the analyses presented in this report (called 'main' database) consists of 608 acute oral toxicity values covering 100 cholinesterase inhibiting substances and 48 species of birds (appendix 1). Cholinesterase inhibitors were used because of their consistent mode of action, their relatively high toxicity to birds and the fact that they account for the majority of poisoning incidents. A second database of non-cholinesterase inhibitors (appendix 2) was also assembled and used for validation purposes (see text). This 'validation' database consists of acute oral toxicity values for 113 species and 87 pesticides including insecticides, herbicides, fungicides and rodenticides with diverse modes of action. The values were obtained from the sources cited above and other published studies (Anonymous, 1948; Grolleau, 1965; Giban, L'Héritier and Grolleau, 1966; Atzert, 1971; Grolleau and Paris, 1977; Grolleau and de Lavaur, 1981; EPA, 1983; McIlroy, 1984)

2.2 Analysis of phylogenetic relationships among species sensitivity data

In order to investigate interspecies differences, it is critical to determine whether data from any group of species can be considered independent estimates of the toxicity of a given product to birds at large or whether phylogenetic aspects have to be taken into consideration.

Two separate statistical analyses performed on the log-transformed median lethal doses were conducted to detect patterns in the sensitivity relationships among species and to determine whether these patterns are due to phylogenetic relationships. First, a principal component analysis (SAS, 1988) was conducted on a subset of the main database. This subset of 176 LD_{50} values for 8 species and 22 chemicals was selected to avoid missing data. Principle component analysis is an ordination technique which allows for the visual inspection of multivariate data. Any existing trends in species sensitivities to chemicals should emerge by collapsing the data into a number of principal components. A similar analysis was presented by Mineau (1991) for a more restricted list of pesticides.

Also, a three-way analysis of variance was conducted on the main database with the exclusion of chemicals or species with only one observation and of phylogenetic groups with only one species. This dataset consisted of 489 observations for 74 chemicals, 25 species and 6 phylogenetic categories. The latter were obtained by grouping the 25 species into one of the following five families and one sub-family: Anatidae (4 species), Columbidae (3), Emberizidae (2), Phasianidae (9), Icteridae (5) and Passeridae (2).

2.3 Calculation of threshold doses

The following approach was developed for use with LC_{50} and NOEC data for aquatic (Stephan et al., 1985; Kooijman, 1987) and soil (Van Straalen and Denneman, 1989) organisms. We are proposing that the approach is valid for avian acute toxicity data. The assumption is that species sensitivities to chemicals follow symmetrical distributions. Erickson and Stephan (1985) used a triangular distribution while Kooijman (1987) and Van Straalen and Denneman (1989) assumed a logistic distribution. The implication of the former is that there exists a threshold value below which effects will not occur. For the purpose of this analysis we have opted to use the logistic distribution which is the approach developed by the Dutch authorities. This choice was facilitated by the availability of a program called *E_TX 1.3a* (Aldenberg, 1993) which will test the fit of toxicity values for n species to a logistic distribution and, based on this distribution, will calculate a threshold value above which 95% of individual toxic endpoints should lie. This calculation is carried out with the confidence that the threshold is underestimated 95% of the time.

A subset of the main database was used for calculations of a threshold lethal dose, TLD_5, or the dose above which the LD_{50} for 95% of bird species will be found. This subset consisted of all chemicals for which LD_{50}s were available for at least three species. This is the smallest data set for which a goodness-of-fit test can be conducted. The program was run for 63 chemicals for which the number of LD_{50}s ranged from three to 32. All LD_{50} values were log transformed. Outputs for each chemical consisted of the results of the goodness-of-fit test for the logistic distribution and the calculated TLD_5 values.

3. RESULTS AND DISCUSSION

3.1 Patterns in species sensitivity

The results of the principal component analysis run on eight species and 22 chemicals are given in table 1 and illustrated in figure 1. In the analysis by chemical, positive loadings on the first principal component indicate the obvious: that chemicals differ in their toxicity to birds and that bird species differ in their sensitivity to chemicals. The analysis by species shows that the ranking of species sensitivities tends to persist across chemicals. Red-winged Blackbirds are by far the most sensitive followed, as a group, by the Common Grackle, the House Sparrow, the Mallard and the Rock Dove. A second group of species, the Pheasant, Japanese Quail and the Starling, trails off as the least sensitive. This pattern is illustrated on the first principal component in figure 1. The loadings of the chemicals on this component (30% of the variation explained) are consistently high indicating that these three groupings are ranked consistently across insecticides. Chemicals with lower loadings can be explained by observations on the other two components. The second and third principal components separate out the Pheasant and Starling respectively. These observations are most likely due to deviations from the pattern noted above, where for some compounds, these two species are either extremely sensitive or insensitive. These "outliers" may reflect real differences in sensitivity or problems with the studies.
From a phylogenetic point of view the only obvious separation seemed to be between the two Icteridae and the two Phasianidae.

To look more closely at this pattern a second principal component analysis was run on a separate subset of the data. This subset consisted of toxicity values for three Phasianidae (Bobwhite Quail, Japanese Quail, Ring-necked Pheasant) and three Icteridae (Red-winged Blackbird, Brown-headed Cowbird, Common Grackle) for nine chemicals. Table 2 shows how these two taxonomic groupings separate out well consistently across all compounds. The first component now explains 57% of the variability. The only exception is the LD_{50} value of diazinon for the cowbird which is higher than expected. These observations suggest a fairly consistent pattern among species in their response to chemicals.

This question was pursued with the three-way analysis of variance. The results (Table 3) show that each of the three variables, species, chemicals and phylogeny, explained a statistically significant proportion of the variability. A multiple comparison procedure (Ryan-Einot-Gabriel-Welsch Multiple Range Test) again allows for the separation of only two taxonomic groupings: the Icteridae and the Phasianidae (Table 4).

As concluded by Mineau (1991) with a more restricted data set (again of cholinesterase-inhibiting pesticides) , there are probably enough exceptions to prevent the development of a predictive approach based on phylogenetic relationships. Nevertheless, taxonomy has to be considered when making inter-species extrapolations. Based on our analysis, at least two groupings of species, based on taxonomic relationships, can be separated according to their sensitivity across cholinesterase-inhibiting chemicals.

3.2 Threshold doses

The distribution of LD_{50} values were found to fit a log-logistic distribution (Kolmogorov-Smirnov Goodness-of-Fit test) at a significance level of 1% for 57 of the 63 chemicals retained for the analysis (with 3 or more species). Threshold lethal doses, or TLD_5s, based on the parameters of a log-logistic distribution calculated from the LD_{50} values available for each chemical, are shown in table 5. Ratios of the median LD_{50} value to the TLD_5 are extremely variable among the chemicals. A number of biases are accountable for this finding. First, due to the uncertainty associated with small sample sizes the E_TX program will tend to "overprotect" the population of species when n is small. In most cases the threshold value will increase as n increases and the range of sensitivities is better defined. Also, the random inclusion of very sensitive or insensitive species will increase the spread of the distribution and will lead to the derivation of extremely low thresholds. For example, toxicity values for phosmet were the following: 1830, 438.2, 435.8, 237 and 17.8 mg/kg. The latter value, that for the Red-Winged Blackbird, increases the spread of the distribution and thus leads to an extremely low TLD_5. Toxicity values with isophenphos were 8.8, 13, 32 and 972 mg/kg. The inclusion of the latter species, the frequently insensitive Starling, leads to a similar result. The ratios of median LD_{50} to the TLD_5 were 3181 and 75000 respectively for these two chemicals.

Related to this problem is the bias which is introduced by the more extensive testing carried out for highly toxic insecticides. There is a significant correlation between the average LD_{50} for an insecticide and the number of species tested (Pearson's $r=-0.38$, $p=0.0037$, $n=57$). A further bias is introduced by the large amount of variability in the species tested. Test data are available for 48 species although values are usually only available for between one and 20 species. Through regulatory testing requirements which specify one or two of the species to be tested or because much of the research was directed at crop pests (e.g. Icteridae and Passeridae by Schafer and colleagues), species which may be either very sensitive or very insensitive are tested more frequently than by chance. The non-randomness of the species chosen for testing is one of the criticism levelled at this approach (Forbes and Forbes, 1993).

In order to counter some of these biases, calculations of the TLD_5s were repeated but this time, for a subset of compounds for which data for the same eight bird species were available (table 5). The choice of eight species was a compromise between maximizing the number of chemicals and species to avoid the 'overprotection' seen with smaller numbers of species and to provide for phylogenetic diversity among the chosen species. Care was taken to choose the eight species from as many avian families as possible. The dataset was thus reduced to 22 insecticides. For each insecticide this subset of the data thus includes the following taxa: two Phasianidae (Ring-necked Pheasant, Japanese Quail), two Icteridae (Red-winged Blackbird, Common Grackle), one Anatidae (Mallard), one Sturnidae (Starling), one Columbidae (Rock dove) and one Passeridae (House Sparrow). Most of these species offer another advantage: either they are widely available from breeders (Pheasant, Quail or Mallard) or they are considered to be pest species and often actively controlled worldwide (Rock dove, House sparrow, Starling) or in North America (Red-winged blackbird especially). If one were to propose increasing the number of species which should be tested before new pesticides are introduced in the environment (a battery testing approach), it would be ethical and logical to turn to either laboratory-reared or abundant pest birds for this requirement.

Only for 6 of the 22 chemicals did the thresholds calculated with 8 species exceed those calculated with the full complement of available species (Table 5) indicating that the calculated TLD_5 values still tended to overprotect with a sample size of 8 species although the values obtained with either 8 species or with the full dataset were usually very close. In the six cases where the TLD_5 calculated with 8 species was higher than the TLD_5 calculated with the full species complement, we find that this was the result of the addition of new species with LD_{50}s towards the left tail of the distribution.

3.3 Safety factors

As explained previously the TLD_5 is an estimate of the LD_{50} above which 95% of other avian LD_{50}s will fall based on a statistical distribution defined by sufficient observations. We believe that an empirically derived TLD_5 should ideally form the basis of all future avian hazard assessments. From a regulatory perspective, however, calculation of this threshold is not possible for most chemicals. Toxicity values on one or, at best, only two species have traditionally been submitted in support of new pesticide registrations. These are too few for the calculation of the TLD_5. The regulatory community may not support the battery testing approach that, we believe, should be instituted. An alternative approach is therefore required.

The approach we are proposing is to develop empirical species-specific safety factors, defined here a the ratio between individual species' LD_{50} values and the TLD_5 for a given pesticide (Figure 2). We calculated such safety factors from the TLD_5s calculated for the 22 chemicals used in the previous analysis. Such a ratio can be calculated for every species and for each insecticide. Averaging these ratios across chemicals gives a measure of the average distance between the TLD_5 and the LD_{50} for each species. Summary statistics for each species are presented in Table 6. Average safety factors were calculated for TLD_5 s derived from the 8 species selected previously or for all species available. The safety factors are presented for these eight species and three others which are either used in regulatory assessments or are tested frequently: the Bobwhite Quail, the Red and Grey Partridges. Examination of table 6 reveals that, as explained earlier, safety factors will increase with decreasing information. In other words, as the number of species tested decreases (i.e. TLD5[all species] vs. TLD5[8 species]), the TLD_5 will decrease and therefore the safety factor will increase. Not surprisingly the lowest safety factors are typical of the most sensitive species (e.g Red-winged Blackbird) and the highest typical of the least sensitive species (e.g. Starling). The variability around these average values is high as expressed by the range. A geometric mean was used to diminish the importance of some of the extreme values in calculating the means and to be consistent with the log transformation used in the principal component analyses.

If we were to adopt the approach where an average safety factor developed from one test species is used to derive an approximate TLD_5, we would expect that about half of the time the derived TLD_5 would be insufficiently low to obtain a 95% level of species protection. Indeed, depending on the species chosen, the average safety factor was insufficient to obtain this level of protection between 27% and 57% of the time (table 6). Nevertheless the derived TLD_5 will always be towards the left tail of the distribution and the 'true' level of protection provided will therefore oscillate around the 95% mark (Figure 3). In order to find out exactly what proportion of species would actually be protected, we need to conduct a validation exercise with the data at hand.

3.4 Validation of the safety factor approach

To validate the level of protection afforded through the use of mean safety factors developed for test species, we proceeded in a step-wise fashion. First we used the main database assembled for cholinesterase inhibiting insecticides. For each insecticide, the LD_{50} for each of five selected species was divided by the appropriate mean safety factor previously developed for that species. A threshold dose, here called the TLD'_5, was thus derived for each insecticide and then compared to all available LD_{50}s. The five species were chosen on the basis of current testing guidelines (Mallard Duck, Bobwhite Quail, Japanese Quail) or with the idea of eventually extending testing to include a few pest birds of cosmopolitan distribution (House Sparrow, Rock Dove). The mean safety factors used in this validation exercise were those derived from TLD_5s calculated for 22 insecticides and 8 species. While safety factors derived from data on more species were lower, for the purpose of the validation the larger safety factors were used to stabilize the between-chemical variance and allow comparisons to be made.

Table 7 summarizes the results of the validation obtained with those 22 insecticides used to derive the TLD_5s, the TLD'_5 s and the safety factors. While there is circularity in validating this approach with the data used to derive the safety factors, it does provide a measure of the inherent level of uncertainty involved in using it. Two sources of error propagation are reflected in table 7. One, the fact that the original TLD_5 values were calculated with a 95% level of confidence that we underestimated the hazardous dose. Second, because we used the geometric mean of 22 safety factors derived from the TLD_5s, we know that in some cases the level of protection will be less than 95%. The end result was that for fewer than 20% of the insecticides, there was at least one species not protected by the use of the safety factor. Across all 22 insecticides we find that, on average, about one per cent of the species were not 'protected' by using this approach.

As a second step, validation was conducted with cholinesterase inhibiting insecticides not used in the derivation of the safety factors (Table 8). The result of this validation was that the percentage of insecticides with at least one species not protected was no greater than previously. On average fewer than 5% of species were not afforded protection. The most significant difference with the previous validation was the appreciably greater percentage of species not protected when the average was based on only those insecticides for which at least one species was not protected by the predicted TLD'_5. This may be an artifact caused by the smaller number of species available for evaluation with this data set.

As a third step, the possibility of using the approach described here with pesticides which are not cholinesterase inhibitors was examined using the same validation approach. The 'validation' dataset of LD_{50} values was used as for the first two validation steps. As discussed earlier, this database includes numerous chemical families with various modes of action. The results of the validation (Table 9) do not differ appreciably from those of the first two validations conducted on cholinesterase inhibitors.

A final validation was conducted on the latter dataset using safety factors derived from TLD_5s calculated from all the LD_{50}s available, that is more than eight species. As was explained earlier the safety factors will thus be lower. The results (Table 10) indicate a slightly greater percentage, as much as 29%, of insecticides for which at least one LD_{50} is lower than the TLD'_5. This is reflected in the fact that, across all pesticides and depending on the test species, between 0.3 and 11.1% of avian species are not protected by these safety factors.

The test species differed somewhat in their ability to provide protection through the use of their sensitivity to chemicals as expressed by the calculated safety factors. Use of Mallard duck and House sparrow LD_{50}s and safety factors appears to provide the least amount of protection to other avian species. The other three species provided greater levels of protection.

4. CONCLUSIONS

We will conclude by re-examining the two testing strategies proposed earlier in light of the analyses and evidence presented. We believe strongly that the best approach is to use a battery of test species for which LD_{50} values (or approximate LD_{50}s assuming that the number of individuals utilized for these tests is of concern) are determined, providing a direct measure of the sensitivity distribution for each substance. This approach eliminates the uncertainty associated with the derivation of mean safety factors and more accurately describes the variation in species sensitivity to the chemical of concern. From a regulatory perspective, this approach is also 'fair' in that a paucity of data leads to overprotection. This is contrary to the present situation where products with a more complete database are often penalized. Furthermore, this approach allows a regulator to easily choose the desired level of protection. If this strategy is to be followed, our analysis shows that there should be guidance provided as to which species are tested. Our analysis also shows that the number of species need not be immense. Certainly, the use of 6-8 species would appear to provide us with a fair representation provided the species are carefully chosen. This aspect is now being pursued by us using the databases described here.

In mammalian toxicology, strong arguments have been advanced to eliminate or radically change the LD_{50} test and replace it with a fixed dose protocol to place tested chemicals within broad categories of toxicity. We feel that avian toxicology should not move away from the determination of a median lethal dose unless the information lost is compensated through increased testing elsewhere. Mammalian toxicologists have at their disposal test results from many more species and from sub-acute tests which are not available to avian toxicologists. Until some sub-acute test results are available we can only glean partial information on response thresholds from acute oral studies. Nevertheless, the current protocols over-emphasize the determination of an exact LD_{50} value which may not be scientifically justified in view of inter-test variation. Other methods exist, such as the up and down method which may provide an approximate LD_{50} of adequate precision and allow for the inexpensive testing of more species as advocated here.

If a battery approach is rejected our findings suggest that maintaining the present strategy of using one or two test species may be warranted but only with the use of appropriate safety factors. We believe the current safety factor of five applied across chemicals regardless of the test species is clearly inadequate. The statistical analyses of the LD_{50} data for cholinesterase inhibitors support the contention that species respond, for the most part, in a consistent manner. For cholinesterase inhibitors, some species are almost always the most sensitive while others the least sensitive. Overall, the species for which we have the most data can be grouped into three broad sensitivity categories. Furthermore, phylogeny may play a role in explaining differences in sensitivity among species although this needs to be verified across a broader range of chemical and bird families.

Since species do appear to respond in a relatively consistent manner, the use of test data for one or two test species should allow us to make consistent predictions about the safety of pesticides to birds at large if the safety factors appropriate to those species are used. Derivation of safety factors based on known distributions of species sensitivities as carried out here has the advantage that a desired level of protection can be specified or changed according to objectives of environmental protection. The spread of the species sensitivity distributions, however, differ among chemicals. If a mean safety factor is derived for one test species such as the Mallard duck by averaging safety factors derived from many products much variability is thus introduced. This leads to under-protection in some cases and probably over-protection in many cases. This is why we believe it is preferable to adopt a battery testing approach as advocated above.

Whatever the strategy adopted, it is clear that a method must be developed and validated to relate expected field exposure of birds to a distribution based safety threshold. This aspect is also being examined using information on field mortality incidences and toxicological information.

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Appendix 1. List of cholinesterase inhibiting insecticides and bird species for which LD50 values were available. The number of entries available for each are indicated.

Insecticide	CAS	n
acephate	30560191	6
aldicarb	116063	12
aminocarb	2032599	4
azinphos-methyl	86500	6
bendiocarb	22781233	4
benfuracarb	82560541	1
bromophos	2104963	1
bromophos-ethyl	4824786	3
bufencarb	8065369	8
butonate	126227	2
cadusafos	95465999	2
carbaryl	63252	6
carbofuran	1563662	14
carbophenothion	786196	8
carbosulfan	55285148	2
chlorfenvinphos	470906	12
chlormephos	24934916	3
chlorpyrifos-ethyl	2921882	16
coumaphos	56724	12
crufomate	299865	2
demeton	8065483	13
dialifos	10311849	1
diamidfos	1754581	2
diazinon	333415	12
dichlofenthion	97176	5
dichlorvos(DDVP)	62737	9
dicrotophos	141662	15
dimethoate	60515	8
dimetilan	644644	3
dioxacarb	6988212	2
dioxathion	78342	2
disulfoton	298044	6
EPN	2104645	14
ethion	563122	4
ethiophencarbe	29973135	2
ethoprop	13194484	9
etrimphos	38260547	1
famphur	52857	3
fenamiphos	22224926	5
fenchlorphos(ronnel)	299843	3
fenitrothion	122145	10
fensulfothion	115902	12
fenthion	55389	21
fonofos	944229	10
formetanate	22259309	3
formothion	2540821	2
heptenophos	23560590	1
isazophos	42509808	3
isophenphos	25311711	4
isoprocarb	2631405	1
malathion	121755	6

Species	Latin name	n
American crow	*Corvus brachyrhynchos*	2
American kestrel	*Falco sparverius*	5
American robin	*Turdus migratorius*	1
Black-billed magpie	*Pica pica*	1
Boat-tailed grackle	*Cassidix major*	2
Brown-headed cowbird	*Molothrus ater*	9
Budgerigar	*Melopsittacus undulatus*	3
California quail	*Callipepla californica*	16
Canada goose	*Branta canadensis*	11
Cedar waxwing	*Bombycilla cedrorum*	1
Chicken	*Gallus gallus*	8
Chukar	*Alectoris chukar*	17
Common grackle	*Quiscalus quiscula*	30
Common Screech owl	*Otus asio*	4
Coturnix	*Coturnix coturnix japonica*	52
Dark-eyed junco	*Junco hyemalis*	3
Eared dove	*Zebauda auriculata*	1
Fulvous whistling-duck	*Dendrocygna bicolor*	2
Golden eagle	*Aquila chrysaetos*	1
Golden sparrow	*Passer luteus*	1
Golden-crowned sparrow	*Zonotrichia atricapilla*	2
Grey partridge	*Perdix perdix*	27
Horned lark	*Eremophila alpestris*	2
House finch	*Carpodacus mexicanus*	13
House sparrow	*Passer domesticus*	32
Inca dove	*Scardafella inca*	1
Mallard	*Anas platyrhynchos*	57
Masked weaver	*Ploceus taeniopterus*	1
Mourning dove	*Zenaida macroura*	5
Northern bobwhite	*Colinus virginianus*	35
Pekin duck	*Anas platyrhynchos*	2
Red bishop	*Euplectes orix*	1
Red partridge	*Alectoris rufa*	32
Red-billed quelea	*Quelea quelea*	9
Red-winged blackbird	*Agelaius phoeniceus*	62
Ring-billed gull	*Larus delawarensis*	1
Ring-necked pheasant	*Phasianus colchicus*	46
Ringed turtle-dove	*Streptopelia risoria*	1
Rock dove	*Columba livia*	39
Sandhill crane	*Grus canadensis*	3
Sharp-tailed grouse	*Tympanuchus phasianellus*	11
Starling	*Sturnus vulgaris*	47
Tricolored blackbird	*Agelaius tricolor*	1
Village weaver	*Ploceus cucullatus*	1
White-crowned sparrow	*Zonotrichia leucophrys*	1
White-winged dove	*Zenaida asiatica*	2
Wild turkey	*Meleagris gallopavo*	1
Yellow-headed blackbird	*Xanthocephalus xanthocephalus*	3

Total bird species: 48

Appendix 1. (Continued)

Insecticide	CAS	n	Species	Latin name	n
methamidophos	10265926	3			
methidathion	950378	6			
methiocarb	2032657	32			
methomyl	16752775	12			
methyl chlorpyrifos	5598130	1			
methyl-parathion	298000	8			
mevinphos	7786347	11			
mexacarbate	315184	16			
monocrotophos	6923224	20			
naled	300765	6			
omethoate	1113026	1			
oxamyl	23135220	3			
oxydemeton-methyl	301122	9			
parathion	56382	18			
phorate	298022	7			
phosalone	2310170	1			
phosfolan	947024	7			
phosmet	732116	5			
phosphamidon	13171216	14			
phoxim	14816183	7			
pirimicarb	23103982	6			
pirimiphos-ethyl	23505411	1			
pirimiphos-methyl	29232937	2			
promecarbe	2631370	2			
propetamphos	31218834	3			
propoxur	114261	21			
pyrolan	87478	1			
sulfotep	3689245	2			
sulprofos	35400432	3			
temephos	3383968	12			
TEPP	107493	3			
terbufos	13071799	1			
tetrachlorvinphos	961115	1			
thiofanox	39196184	2			
thiometon	640153	2			
thionazin	297972	7			
TM Akton	1757182	1			
TM BAY 38156	333437	2			
TM bomyl	122101	2			
TM Hercules 5727	64006	2			
TM Hercules 8717	3692908	2			
TM Hercules 9699	3279467	2			
TM HRS 1422	330643	1			
TM methyl trithion	953173	1			
TM SD 8530	2686999	1			
TM zytron (DMPA)	299854	1			
trichlorfon	52686	10			
trichloronat	327980	8			
vamidothion	2275232	3			

Total insecticides: 100

Appendix 2. List of non-cholinesterase inhibiting chemicals and bird species for which LD50 values were available. The number of entries available for each are indicated.

CHEMICAL	CAS	n	Species	Latin name	n
2,4-D Acid	94757	5	American crow	*Corvus brachyrhynchos*	6
3-chloro-p-toluidine	95749	10	American kestrel	*Falco sparverius*	3
4-aminopyridine (avitrol)	504245	33	American robin	*Turdus migratorius*	3
ACD 7029	14285439	7	American widgeon	*Anas americana*	1
acetate phenylmercury		2	Australian (marsh) harrier	*Circus aeruginosus*	1
Acifluorfen, Sodium salt	62476599	2	Australian magpie	*Gymnorhina tibicen*	1
Aldrin	309002	5	Australian magpie-lark	*Grallina cyanoleuca*	1
Alpha-chloralose	15879933	20	Australian raven	*Corvus coronoides*	1
Amitraz	33089611	2	Bar-shouldered dove	*Geopelia humeralis*	1
Anilazine	101053	2	Barn owl	*Tyto alba*	2
BAY 75546	7682908	7	Black kite	*Milvus migrans*	1
BAY 93820	24353615	2	Black vulture	*Coragyps atratus*	1
BAY COE 3664	39457244	9	Black-billed gull	*Larus bulleri*	1
BAY COE 3675	39457255	9	Black-billed magpie	*Pica pica*	4
BAY HOL 0574	35335605	9	Blackbird	*Turdus merula*	2
Bentazon	50723803	3	Blue-black grassquit	*Volatia jacarina*	1
Brodifacoum	56073100	17	Blue-winged teal	*Anas discors*	1
Bromoxynil (Butyrate)	3861414	2	Boat-tailed grackle	*Cassidix major*	4
Ceresan L	8003370	4	Brewer's blackbird	*Euphagus cyanocephalus*	1
Ceresan M	517168	6	Bronzed cowbird	*Tangavius aeneus*	1
CHE 1843	1113140	2	Brown-headed cowbird	*Molothrus ater*	5
Chlordane	57749	3	Brown-throated conure	*Aratinga pertinax*	1
Clomazone	81777891	2	Budgerigar	*Melopsittacus undulatus*	5
Compound 1080		57	California quail	*Callipepla californica*	13
Copper oxynate	1317391	2	Canada goose	*Branta canadensis*	3
Cycloheximide	66819	3	Chicken	*Gallus gallus*	3
D.M. 7537		4	Chukar	*Alectoris chukar*	9
Dazomet	533744	2	Common dove	*Columbina passerina*	1
DDT	50293	3	Common grackle	*Quiscalus quiscula*	12
DEF	78488	2	Common pintail	*Anas acuta*	1
Dibromonitrilopropionamide		2	Cooper's hawk	*Accipiter cooperii*	1
Dieldrin	60571	11	Coq nain	*Gallus gallus Cayenne*	7
Dinoseb	88857	2	Coturnix	*Coturnix coturnix japonica*	23
Dinoterbe	1420071	2	Crimson rosella	*Platycercus elegans*	1
Diquat	85007	2	Curve-billed thrasher	*Toxostoma curvirostre*	1
DNOC	534521	5	Diamond dove	*Geopelia cuneata*	1
DOWCO 161	36031660	9	Dickcissel	*Spiza americana*	1
Duomeen T-E-9		2	Domestic duck	*Anas platyrhynchos*	3
Endosulfan	115297	3	Dunnock	*Prunella modularis*	1
Endrin	72208	5	Eastern rosella	*Platycercus eximius*	1
Ethamphenphion		2	Eastern yellow robin	*Eopsaltria australis*	1
Ethephon	16672870	3	Emu	*Dromaius novaehollandiae*	1
Fenvalerate	51630581	2	European goldfinch	*Carduelis carduelis*	1
Fluchloralin	33245395	2	Fulvous whistling-duck	*Dendrocygna bicolor*	4
Folpet	133073	3	Galah	*Cacatua roseicapilla*	1
Gophacide	4104147	4	Gambel's quail	*Callipepla gambelii*	1
Guazatine (triacetate)	57520179	3	Golden eagle	*Aquila chrysaetos*	4
Heptachlor	76448	4	Golden sparrow	*Passer luteus*	2
Hexaflurate	17029220	3	Golden-crowned sparrow	*Zonotrichia atricapilla*	1
Ioxynil octanoate	3861470	4	Green finch	*Carduelis sinica*	1
Iprodione	36734197	2	Green jay	*Cyanocorax yncas*	1
Lindane	58899	4	Grey partridge	*Perdix perdix*	12
Metaldehyde	108623	2	Hooded crow	*Corvus corone*	1
Metomidate	5377208	11	Horned lark	*Eremophila alpestris*	2
Metomidate HCL	35944742	8	House finch	*Carpodacus mexicanus*	12
Nabam	142596	2	House sparrow	*Passer domesticus*	21
Nemagon	96128	2	Laughing dove	*Streptopelia senegalensis*	1
Nicotine sulfate	65305	10	Little crow	*Corvus bennetti*	1

Appendix 2. (Continued)

CHEMICAL	CAS	n
Panogen	502396	3
Paraquat Dichloride	1910425	2
PCP	87865	2
Pentobarbital sodium	57330	8
Phencyclidine HCL	956901	13
PHILLIPS 2133	35944731	7
PHILLIPS 2605	12712286	7
PMA	62384	2
Potassium azide	12136446	3
Propiconazole	60207901	2
SD-16898		4
Silicate methoxyethyl mercury		3
Sodium arsenite	7784465	4
Sodium dichloro-s-triazinetrione	2893789	2
Starlicide	7745893	31
Strychnine	57240	17
TBA	50317	2
Tefluthrin	79538322	3
TEPA	545551	7
Terrazole	2593159	2
Tetraethyllead	78002	2
TFM	88302	3
Thallium sulfate	7446186	3
Thiram	137268	2
Toxaphene	8001352	9
Trichloro-s-triazinetrione	87901	2
Triphenyltin hydroxide	76879	2
Zinc phosphide	1314847	4
Zirame	137304	2

Total chemicals: 87

Species	Latin name	n
Little raven	*Corvus mellori*	1
Little wattlebird	*Anthochaera chrysoptera*	1
Mallard	*Anas platyrhynchos*	64
Maned duck	*Chenotta jubata*	1
Masked weaver	*Ploceus taeniopterus*	1
Monk parakeet	*Myiopsitta monachus*	2
Mourning dove	*Zenaida macroura*	9
New Holland honeyeater	*Phylidonyris novae-hollandiae*	1
Northern bobwhite	*Colinus virginianus*	34
Northern raven	*Corvus corax*	2
Orange-fronted conure	*Aratinga canicularis*	1
Pacific black duck	*Anas superciliosa*	1
Partridge	*Alectoris sp.*	1
Pied currawong	*Strepera graculina*	1
Pigeon colombin	*Colomba oenas*	1
Plain chachalaca	*Ortalis vetula*	1
Prairie chicken	*Tympanuchus cupido*	1
Pukeko	*Porphyrio melanotus*	1
Red bishop	*Euplectes orix*	2
Red partridge	*Alectoris rufa*	8
Red-billed quelea	*Quelea quelea*	7
Red-browed firetail	*Emblema temporalis*	1
Red-eyed cowbird	*Tangavius aeneus*	1
Red-rumped parrot	*Psephotus haematonotus*	1
Red-winged blackbird	*Agelaius phoeniceus*	20
Ring-billed gull	*Larus delawarensis*	1
Ring-necked pheasant	*Phasianus colchicus*	52
Rock dove	*Columba livia*	23
Sage grouse	*Centrocercus urophasianus*	1
Sandhill crane	*Grus canadensis*	1
Scrub jay	*Aphelocoma coerulescens*	1
Sharp-tailed grouse	*Tympanuchus phasianellus*	2
Shelduck	*Tadorna tadorna*	1
Southern Black-billed gull	*Larus dominicanus*	1
Starling	*Sturnus vulgaris*	15
Sulfur-crested cockatoo	*Cacatua galerita*	1
Superb fairy wren	*Malurus cyaneus*	1
Tricolored blackbird	*Agelaius tricolor*	3
Turkey vulture	*Cathartes aura*	1
Ventress chicken	*Gallus sp.*	1
Village weaver	*Ploceus cucullatus*	1
Wedge-tailed eagle	*Aquila audax*	1
Weka	*Gallirallus sp.*	1
White eye	*Zoosterops lateralis*	2
White-browed scrubwren	*Sericornis frontalis*	1
White-crowned sparrow	*Zonotrichia leucophrys*	4
White-fronted dove	*Leptotila verreauxi*	1
White-winged chough	*Corcorax melanorhamphos*	1
White-winged dove	*Zenaida asiatica*	4
Wild turkey	*Meleagris gallopavo*	3
Yellow-billed magpie	*Pica nuttalli*	1
Yellow-faced honeyeater	*Lichenostomus chrysops*	1
Yellow-headed blackbird	*Xanthocephalus xanthocephalus*	9
Yellow-tailed black cockatoo	*Calyptorhynchus funereus*	1
Zebra finch	*Poephila guttata*	1

Total number of species: 113

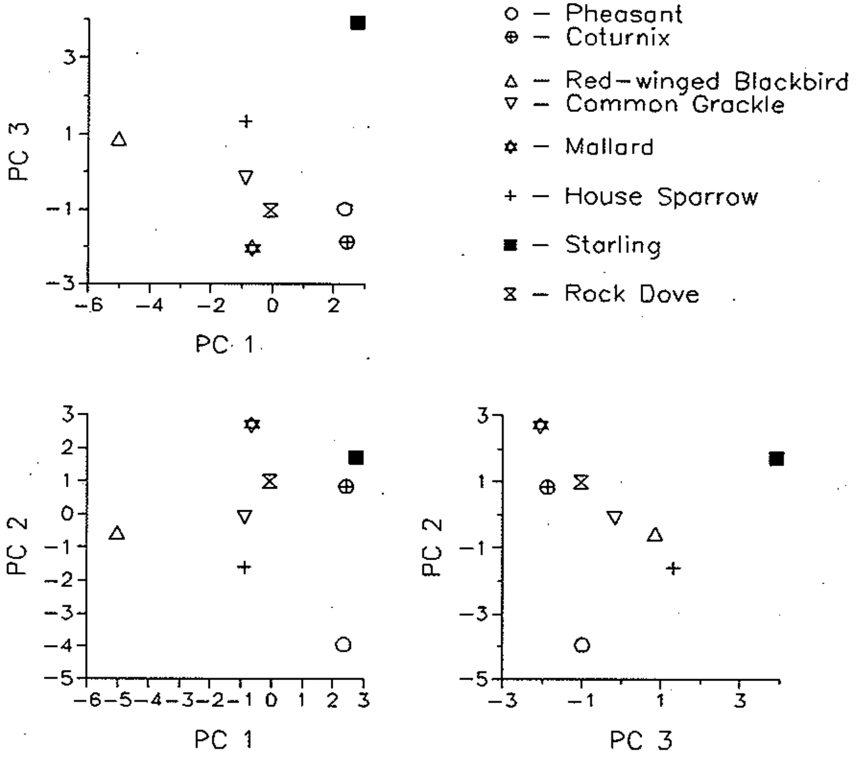

Figure 1. Illustration of the principal component analysis run on 8 species and 22 chemicals (analysis by species)

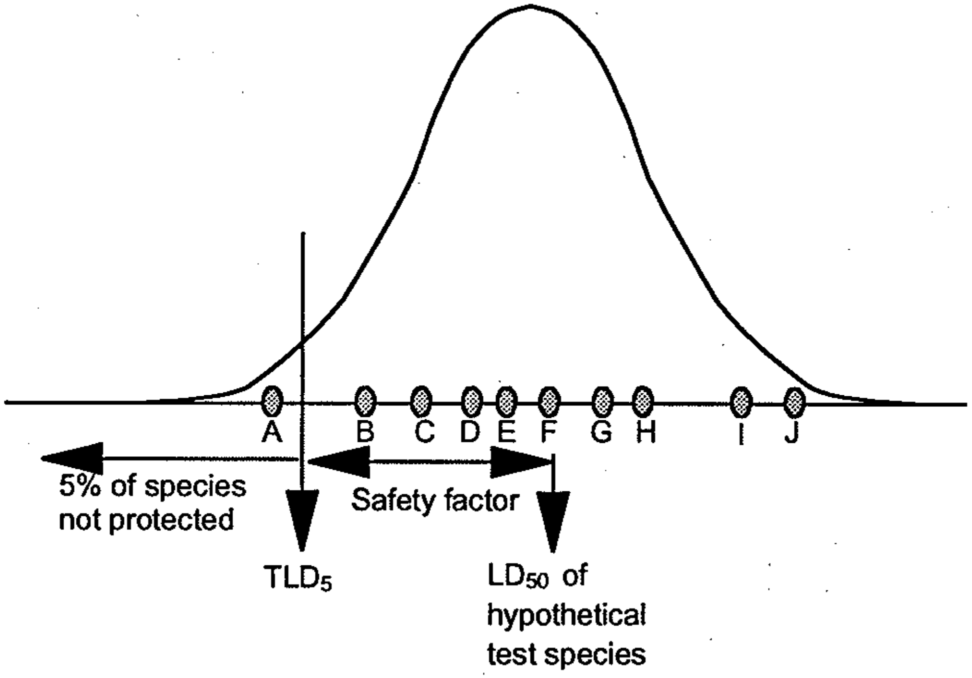

Figure 2. Log-logistic distribution of median lethal dose of 10 species (A to J). The hazardous dose TLD_5 determined from this distribution is illustrated along with the safety factor needed to apply to the LD_{50} of a hypothetical test species

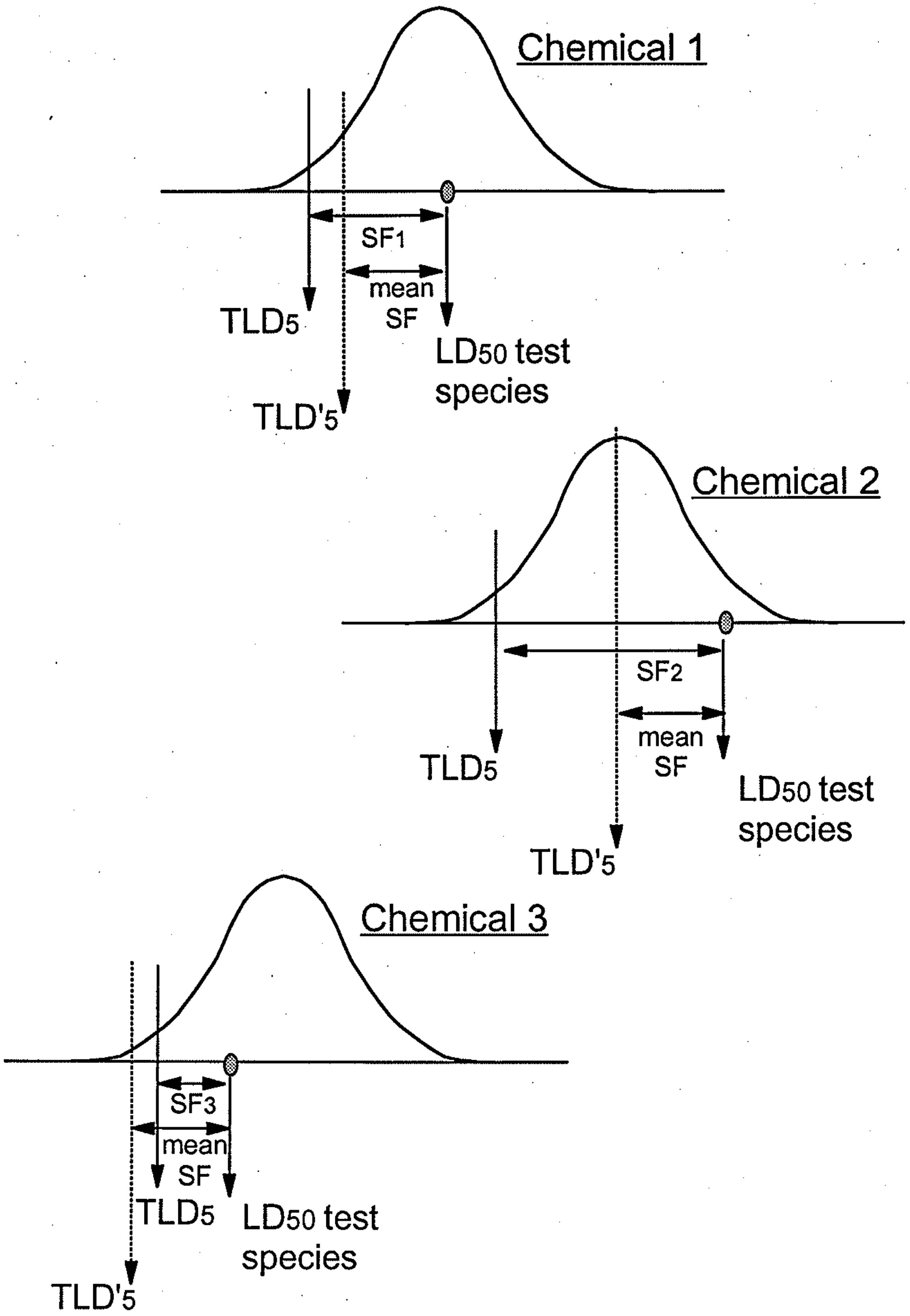

Figure 3.Three examples of the level of protection afforded by the use of mean safety factors applied to the LD_{50} of a test species

Table 1: Results of the principal component analysis run on 8 species and 22 chemicals

Analysis by chemical

Species	Loading of species on components: 1st
Pheasant	0.3069
Mallard	0.3452
Red-winged blackbird	0.3717
Starling	0.2763
Japanese quail	0.3479
House sparrow	0.3926
Common grackle	0.3802
Rock dove	0.3903
% variation explained	67%

Chemical	Loading of chemicals on components: 1st	
fensulfothion	-5.6243	Most toxic
carbofuran	-3.3760	
aldicarb	-2.5979	
monocrotophos	-2.0906	
dicrotophos	-2.0418	
phosphamidon	-1.3506	
mevinphos	-1.2685	
parathion	-1.2463	
diazinon	-0.0540	
EPN	-0.0100	
mexacarbate	0.0466	
ethoprop	0.1663	
demeton	0.2301	
fenthion	0.2935	
coumaphos	0.5708	
propoxur	1.8954	
dichlorvos	2.0665	
methiocarb	2.3771	
chlorpyrifos-ethyl	2.6250	
chlorfenvinphos	3.0052	
bufencarb	3.1138	
methomyl	3.2698	Least toxic

Analysis by species

	Loading of chemicals on components:		
Chemical	1st	2nd	3rd
aldicarb	0.2244	0.0693	-0.1035
bufencarb	0.3523	-0.1234	0.0685
carbofuran	0.3172	-0.1654	0.1930
chlorfenvinphos	0.0819	-0.0366	-0.4557
chlorpyrifos-ethyl	0.0733	0.3716	0.1388
coumaphos	0.2428	0.2338	0.0662
demeton	0.0251	0.0116	0.1657
diazinon	0.1720	0.1176	0.4246
dichlorvos	0.1112	0.0683	0.2420
dicrotophos	0.3101	0.1493	0.0866
EPN	0.2018	-0.3845	0.0321
ethoprop	0.0622	0.3692	-0.1769
fensulfothion	0.2952	-0.0171	-0.2723
fenthion	0.2278	-0.2513	-0.0052
methiocarb	0.1448	-0.3164	-0.1288
methomyl	0.1585	0.1538	-0.1154
mevinphos	0.1761	0.3087	-0.1202
mexacarbate	-0.0358	0.0284	0.4806
monocrotophos	0.2677	0.2362	-0.1071
parathion	0.2464	-0.3008	0.0693
phosphamidon	0.2333	0.0489	0.1372
propoxur	0.2576	0.0369	-0.1591
% variation explained	30%	20%	18%

Species	Loading of species on components: 1st	
Red-winged blackbird	-5.0319	Most sensitive
Common grackle	-0.8876	
House sparrow	-0.8814	
Mallard	-0.6598	
Rock dove	-0.0717	
Pheasant	2.3547	
Japanese quail	2.4248	
Starling	2.7528	Least sensitive

Table 2: Results of the principal component analysis run on 3 Icteridae, 3 Phasianidae and 9 chemicals

	Loading of chemicals on components:			Loading of species on components:	
Chemical	1st	2nd	Species	1st	
Carbofuran	0.3501	0.3568	Red-winged blackbird	-2.6140	Most sensitive
Chlorfenvinphos	0.3650	0.0213	Cowbird	-2.3189	
Coumaphos	0.3385	-0.4770	Common grackle	-0.6057	↓
Diazinon	-0.1878	0.6624	Bobwhite	0.7613	
EPN	0.3298	0.3803	Japanese quail	1.6382	
Fenthion	0.2943	0.1060	Pheasant	3.1385	Least sensitive
Methiocarb	0.3384	0.1426			
Parathion	0.4045	-0.1663			
Propoxur	0.3478	0.0448			
% variation obtained	57%	18%			

Table 3: Result of the 3-way analysis of variance between phylogeny, species and chemicals

Source	DF	Type III SS	Mean square	F value	Pr > F
Phylogeny	5	53.7676	10.7535	7.87 (a)	0.0001 (a)
Species (Phylogeny)	19	48.3344	2.5439	4.19	0.0001
Chemicals	73	943.5029	12.9247	21.28	0.0001
Error	391	237.4259	0.6072		
Corrected Total	488	1484.8806			

Source	Type III Expected Mean Square
Phylogeny	Var (Error) + 5.8115 Var (Species(Phylogeny)) + Q(Phylogeny)
Species (Phylogeny)	Var (Error) + 14.842 Var (Species(Phylogeny))
Chemicals	Var (Error) + 6.3562 Var (Chemical)

(a) synthetic F-test using denominator based on Species(Phylogeny) and Error (denominator df 35)

Table 4: Result of the multiple comparison test on LD_{50} values of 25 bird species

						Mean	N	Species	Phylogeny[1]
			A			4.26	31	Red partridge	PH
			A						
	B		A			3.765	27	Grey partridge	PH
	B		A						
	B		A	C		3.561	7	Chicken	PH
	B		A	C					
	B	D	A	C		3.319	17	Chukar	PH
	B	D	A	C					
E	B	D	A	C		3.166	2	Golden-crowned sparrow	EM
E	B	D	A	C					
E	B	D	A	C		3.098	45	Pheasant	PH
E	B	D	A	C					
E	B	D	A	C	F	2.964	48	Japanese quail	PH
E	B	D	A	C	F				
E	B	D	A	C	F	2.768	3	Junco	EM
E	B	D	A	C	F				
E	B	D	A	C	F	2.735	39	Rock dove	COL
E	B	D	A	C	F				
E	B	D	A	C	F	2.726	57	Mallard	AN
E	B	D		C	F				
E	B	D		C	F	2.648	11	Canada goose	AN
E	B	D		C	F				
E	B	D		C	F	2.476	34	Bobwhite	PH
E	B	D		C	F				
E	B	D		C	F	2.318	16	California quail	PH
E	B	D		C	F				
E	B	D		C	F	2.273	11	Sharp-tailed grouse	PH
E	B	D		C	F				
E	B	D		C	F	2.154	32	House sparrow	PA
E	B	D		C	F				
E	B	D		C	F	2.135	2	Pekin duck	AN
E		D		C	F				
E		D	G	C	F	1.977	45	Red-winged blackbird	IC
E		D	G		F				
E		D	G		F	1.771	30	Common grackle	IC
E		D	G		F				
E		D	G		F	1.717	9	Cowbird	IC
E		D	G		F				
E		D	G		F	1.689	2	White-winged dove	COL
E			G		F				
E			G		F	1.583	2	Boat-tailed grackle	IC
			G		F				
			G		F	1.372	5	Mourning dove	COL
			G		F				
			G		F	1.343	3	Yellow-headed blackbird	IC
			G						
			G			0.419	9	Quelea	PA
			H			-1.556	2	Fulvous-whistling duck	AN

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[1] PH: Phasianidae, EM: Emberizidae, COL: Columbidae, AN: Anseridae, PA: Passeridae, IC: Icteridae

Table 5: LD$_{50}$ values (max, min, median) and TLD$_5$ values of 57 cholinesterase inhibitor chemicals

		LD$_{50}$					
Chemical	n	max	min	median	TLD$_5$ (all spp)[1]	LD$_{50}$ median / TLD$_5$ (all spp)	TLD$_5$ (8 spp)[2]
malathion	6	1485.00	167.00	502.00	29.4450	17.0	
bromophos-ethyl	3	350.00	200.00	300.00	26.2340	11.4	
naled	6	135.00	36.90	74.55	10.5630	7.1	
acephate	6	852.00	106.00	143.00	10.0310	14.3	
oxydemeton-methyl	9	120.00	14.50	53.90	7.2291	7.5	
trichlorfon	10	249.00	22.40	53.15	6.5096	8.2	
temephos	12	240.00	18.90	53.15	6.2249	8.5	
dimethoate	8	84.00	17.80	29.70	4.4244	6.7	
chlorpyrifos-ethyl	16	75.60	8.41	33.40	4.1951	8.0	1.2405
fonofos	10	43.10	10.00	17.35	3.8734	4.5	
dichlorvos(DDVP)	9	26.60	7.78	15.60	3.6663	4.3	3.9057
carbaryl	6	2290.00	56.20	1830.25	3.6616	499.8	
methamidophos	3	10.10	8.00	8.48	3.2842	2.6	
methomyl	12	168.00	10.00	23.60	3.1169	7.6	1.2092
pirimicarb	6	32.80	8.20	19.75	2.8045	7.0	
ethoprop	9	13.30	4.21	7.50	1.5947	4.7	1.3894
propoxur	21	120.00	3.55	10.60	1.1770	9.0	1.0699
fenitrothion	10	1190.00	11.00	70.30	1.0404	67.6	
mexacarbate	16	27.70	2.64	5.86	1.0359	5.7	0.5990
sulprofos	3	72.00	28.10	47.00	0.9860	47.7	
methyl-parathion	8	23.70	3.08	7.89	0.9532	8.3	
methiocarb	32	270.00	1.33	7.50	0.9053	8.3	0.3701
phoxim	7	75.00	5.62	23.70	0.8886	26.7	
bufencarb	8	88.00	4.22	32.95	0.8716	37.8	0.8716
azinphos-methyl	6	136.00	8.25	79.55	0.8533	93.2	
chlorfenvinphos	12	178.00	3.20	20.75	0.8510	24.4	0.3489
phosphamidon	14	21.70	2.25	3.71	0.7791	4.8	1.3906
fenthion	21	25.90	1.33	5.86	0.7307	8.0	0.7009
demeton	13	15.10	1.33	8.48	0.6951	12.2	0.9328
dichlofenthion	5	316.00	15.90	75.00	0.5960	125.8	
dicrotophos	15	9.63	1.30	2.83	0.5699	5.0	0.4922
thionazin	7	7.50	1.68	3.16	0.5554	5.7	
carbophenothion	8	269.00	5.62	45.80	0.4355	105.2	
aldicarb	12	6.70	0.75	3.28	0.3516	9.3	0.1712
trichloronat	8	85.30	2.91	12.65	0.3431	36.9	
EPN	14	274.00	3.08	6.70	0.3218	20.8	0.3012
aminocarb	4	212.00	22.50	46.20	0.3104	148.8	
coumaphos	12	32.00	1.00	4.66	0.2629	17.7	0.4469
mevinphos	11	23.70	1.10	3.80	0.2223	17.1	0.1808
disulfoton	6	27.50	2.37	9.22	0.2060	44.8	
monocrotophos	20	16.20	0.19	2.20	0.2006	11.0	0.4245
parathion	18	24.00	0.19	5.62	0.2005	28.0	0.4067
diazinon	12	213.00	2.70	5.25	0.1709	30.7	0.0624
fenamiphos	5	1.83	0.70	0.80	0.1653	4.8	
methidathion	6	225.00	8.40	34.10	0.1649	206.8	
phosmet	5	1830.00	17.80	435.80	0.1370	3181.0	
formetanate	3	42.00	12.00	22.00	0.1360	161.8	
fensulfothion	12	1.78	0.24	0.66	0.0968	6.8	0.0676
carbofuran	14	8.00	0.24	1.33	0.0774	17.2	0.0573
TEPP	3	10.10	3.56	4.22	0.0561	75.2	
oxamyl	3	9.40	3.16	4.18	0.0497	84.1	
fenchlorphos	3	611.00	77.50	364.00	0.0412	8835.0	
bendiocarb	4	45.00	3.10	15.75	0.0294	535.7	
ethion	4	1297.00	36.00	89.65	0.0190	4718.4	
famphur	3	9.87	1.78	4.22	0.0039	1082.1	
isophenphos	4	972.00	8.80	22.50	0.0003	75000.0	
isazophos	3	26.50	1.50	11.10	0.0000	236170.2	

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[1] TLD$_5$ calculated with LD$_{50}$ values of all species

[2] TLD$_5$ calculated with with LD$_{50}$ values of 8 selected species

Table 6: Safety factors for 11 bird species derived from TLD_5 calculated with LD_{50} values of all species and of 8 selected species

	Bird species										
	Pheasant	Mallard	Bobwhite	Japanese quail	Red-winged blackbird	Starling	House sparrow	Common grackle	Rock dove	Red partridge	Grey partridge
TLD_5 (all spp)											
Safety factor											
geometric mean:	16.80	10.88	15.24	17.07	5.87	19.77	10.69	9.26	13.09	21.62	10.26
max:	298.24	113.35	141.01	173.91	18.72	1246.34	43.89	48.42	55.23	87.78	79.80
min:	2.00	2.12	2.40	3.10	2.28	3.76	2.13	2.13	3.51	10.49	3.58
n:	22	22	16	22	22	22	22	22	22	7	7
Protection reliability (%)[1]:	41	36	38	50	41	41	45	50	50	43	29
TLD_5 (8 spp)											
Safety factor											
geometric mean:	20.76	13.45	20.89	21.09	7.26	24.42	13.21	11.45	16.17	25.14	11.93
max:	729.53	245.06	343.94	424.19	51.28	3413.46	120.19	120.19	138.93	43.28	39.34
min:	2.89	1.99	1.88	2.59	1.78	4.04	2.27	1.91	2.08	10.84	4.24
n:	22	22	16	22	22	22	22	22	22	7	7
Protection reliability (%):	41	27	37	50	45	36	45	50	50	57	57

[1] Percentage of chemicals for which mean safety factor insufficient to obtain 95% level of protection

Table 7. Validation of mean safety factors with cholinesterase inhibiting insecticides used to derive the safety factors. For each insecticid the TLD'5 was calculated using the LD50 and mean safety factor for each of the five hypothetical test species. The number of insecticides where the LD50 of at least one species was less than the TLD'5 is determined.

TEST SPECIES (Safety factor)	Range of the number of toxicity values available per insecticide	No. of insecticides where LD50 < TLD'5 for at least one species/ Total No. of insecticides (%)	Average % of species unprotected across all insecticides	Average % of species unprotected for insecticides where LD50<TLD'5 for at least one species
Mallard Duck (13.4)	8-32	3/22 (13.6)	1.0	7.2
Bobwhite Quail (20.9)	9-32	3/16 (18.7)	1.3	7.0
Japanese Quail (21.1)	8-32	3/22 (13.6)	1.0	7.6
House Sparrow (13.2)	8-32	3/22 (13.6)	0.8	6.0
Rock Dove (16.2)	8-32	3/22 (13.6)	0.9	6.4

Table 8. Validation of mean safety factors with cholinesterase inhibiting insecticides not used to derive the safety factors. For each insecticide the TLD'5 was calculated using the LD50 and mean safety factor for each of the five hypothetical test species. The number of insecticides where the LD50 of at least one species was less than the TLD'5 is determined.

TEST SPECIES (Safety factor)	Range of the number of toxicity values available per insecticide	No. of insecticides where LD50 < TLD'5 for at least one species/ Total No. of insecticides (%)	Average % of species unprotected across all insecticides	Average % of species unprotected for insecticides where LD50<TLD'5 for at least one species
Mallard Duck (13.4)	2-14	5/35 (14.3)	4.9	34.4
Bobwhite Quail (20.9)	2-12	0/18 (0)	0.0	--
Japanese Quail (21.1)	2-12	2/26 (7.7)	2.2	33.3
House Sparrow (13.2)	5-12	1/10 (10.0)	3.0	30.0
Rock Dove (16.2)	2-12	1/17 (5.6)	1.0	16.7

Table 9. Validation of mean safety factors with other pesticides. The mean safety factor used was derived from TLD5s calculated from toxicity data on 8 species. For each substance the TLD'5 was calculated using the LD50 and mean safety factor for each of the five hypothetical test species. The number of substances where the LD50 of at least one species was less than the TLD'5 is determined.

TEST SPECIES (Safety factor)	Range of the number of toxicity values available per substance	No. of substances where LD50 < TLD'5 for at least one species/ Total No. of substances (%)	Average % of species unprotected across all substances	Average % of species unprotected for substances where LD50<TLD'5 for at least one species
Mallard Duck (13.4)	2-57	12/64 (18.7)	7.3	39.4
Bobwhite Quail (20.9)	2-31	0/34 (0)	0.0	--
Japanese Quail (21.1)	2-57	2/23 (8.7)	1.7	17.6
House Sparrow (13.2)	3-57	5/21 (23.8)	7.1	29.8
Rock Dove (16.2)	4-57	2/23 (8.7)	0.6	7.1

Table 10. Validation of mean safety factors with other pesticides. The mean safety factor used was derived from TLD5s calculated from toxicity data on all available species. For each substance the TLD'5 was calculated using the LD50 and mean safety factor for each of the five hypothetical test species. The number of substances where the LD50 of at least one species was less than the TLD'5 is determined.

TEST SPECIES (Safety factor)	Range of the number of toxicity values available per substance	No. of substances where LD50 < TLD'5 for at least one species/ Total No. of substances (%)	Average % of species unprotected across all substances	Average % of species unprotected for substances where LD50<TLD'5 for at least one species
Mallard Duck (10.9)	2-57	18/64 (28.1)	11.1	39.3
Bobwhite Quail (15.2)	2-31	0/34 (0)	0.3	11.1
Japanese Quail (17.1)	2-57	3/23 (13.0)	1.8	13.6
House Sparrow (10.7)	3-57	6/21 (28.6)	9.0	31.4
Rock Dove (13.1)	4-57	3/23 (13.0)	1.0	8.0