A CRITIQUE OF THE AVIAN 5-DAY DIETARY TEST (LC_{50}) AS THE BASIS OF AVIAN RISK ASSESSMENT

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Technical Report Series No. 215
Headquarters 1994
Canadian Wildlife Service

This publication may be cited as:

Mineau, P., B. Jobin and A. Baril. A Critique of the Avian 5-Day Dietary Test (LC_{50}) as the Basis of Avian Risk Assessment. Technical Report No. 215, Canadian Wildlife Service, Headquarters

Issued under the authority of the
Minister of the Environment
Canadian Wildlife Service

Catalogue No. CW69-5/215E
ISBN 0-662-22733-6

Copies may be obtained from:

Canadian Wildlife Service
National Wildlife Research Centre
100 Gamelin Blvd.
Hull, PQ
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Résumé: Ce document critique la façon dont on calcule le risque que représentent les pesticides et autres substances chimiques pour les oiseaux. Le risque est présentement calculé à l'aide d' un test mesurant la concentration léthale moyenne du produit offert pendant 5 jours dans la nourriture de jeunes oiseaux. Notre analyse démontre que ce test est souvent peu fiable, le résultat étant très fortement influencé par les conditions exactes du test. L'incertitude quant à la quantité de pesticide ingéré est en partie responsable du problème. Cependant, même les résultats du test ayant trait a d'autres aspects du produit testé, comme par exemple les effets répulsifs de certains produits observés en laboratoire, se sont avérés peu indicatifs des conditions réelles observées sur le terrain.

1. INTRODUCTION

Avian dietary toxicity tests determine the median lethal concentration (LC_{50}) of a chemical defined as the quantity of toxicant in the diet calculated to kill fifty percent of the test population. The U.S. EPA (1985) protocol is considered the 'industry standard'. The test consists of feeding young birds with a contaminated diet for 5 days followed by a 3-day recovery period during which birds are fed a clean diet. The test product is mixed with the food in various concentrations (minimum is 4) and is given to groups of ten birds per concentration. Concurrent control and vehicle control groups are required for each LC_{50} test. Typically, up to 5 control groups are included in the test with ten birds in each. Individual body weights are measured at the beginning and the end of the study and presented as pen means. Food consumption is recorded at the beginning and the end of the treatment period and at the end of the 3-day recovery period. Mallards (*Anas platyrhynchos*) of 5-10 days of age and Bobwhite quail (*Colinus virginianus*) of 10-14 days are the species and ages specified by the EPA. Japanese quail (*Coturnix japonica*) have also been tested and are currently being proposed as surrogates for Bobwhites (Romijn *et al.* undated).

The LC_{50} test currently provides the endpoints which drive the risk assessment process in a number of countries including the U.S. (Urban and Cook 1986). This is because the test is thought to be most representative of exposure conditions in the wild. The concentration of a contaminant which causes mortality when given in the feed can be compared directly to the level of the same contaminant present on treated crops, vegetation, sprayed insects, etc...

We intend to argue that our reliance on the LC_{50} test as a meaningful endpoint in the risk assessment process is misguided and that the test does not offer useful information, at least for several large classes of insecticides most likely to result in wildlife poisoning incidents. Our first line of argument is that the test is inherently unreliable in that the exact conditions of the test have an inordinate impact on the test endpoints. Secondly, we intend to demonstrate that the LC_{50} values which are obtained are not meaningful *per se* and appear to be at odds with the available field evidence. Finally, we explore whether the LC_{50} test offers any insight which would make the test worthwhile.

2. METHODS

We restricted our attention to organophosphorus and carbamate insecticides that were the subject of the companion discussion paper on LD_{50} values (Baril *et al.* 1994). Data for the Mallard and the Bobwhite were often obtained from proprietary industry submissions so that endpoints cannot be attributed to specific compounds for reasons of confidentiality, except where those data are in the public domain having been the subject of administrative or judicial public hearings. We also used the extensive work of Hill and Camardese (1986) on *Coturnix*. A list of studies which were used in this discussion paper is given in table 1. Throughout this document, products are coded, either as organophosphate (OP) or carbamate (CAB) insecticides. The coding is consistent throughout -- the same codes correspond to the same products throughout the document.

Most of the studies were carried out according to the current (1985) EPA protocol. However, the age of the birds tested proved to be quite variable and, for the Mallard especially, exceeded the limits specified by the EPA. Also, LC_{50} tests on Bobwhites sometimes lasted 7 days followed by a 3-day recovery period rather than the accepted 5 days on the treatment diet followed by a 3-day recovery period. These tests were conducted in the mid 60's, prior to the acceptance of the current protocol. Unfortunately, this discrepancy means that the LC_{50} values are not comparable to those obtained following the standard 5-day feeding period and they were therefore omitted from the current analysis.

3. RESULTS AND DISCUSSION

3.1 Is the LC_{50} a meaningful value?

Current risk assessment procedures which are based on the LC_{50} assume that the value of the LC_{50} *per se* is meaningful. For an LC_{50} to be considered an unbiased measure of the inherent dietary toxicity of a compound, the consumption of food at each concentration would have to be the same. In other words, the consumption of the toxicant should predictably increase with the dose level mixed into the feed. Yet, many researchers have shown that the consumption of treated feed often decreases as the concentration of the toxicant in the feed increases with the net result that the intake of toxicant is often similar accross dose groups. This feature of dietary toxicity tests was recently reviewed by Luttik (1993). Luttik also reviewed how mortality occurs in dietary tests conducted with cholinesterase inhibiting insecticides. Typically, birds either die of acute poisoning, usually on the first day of the test (Type A mortality) or, they die towards the end of the test (Type B mortality). In Type B mortality, birds demonstrate pronounced weight loss of a magnitude associated with starvation and this can be linked directly to a decrease in food consumption. The only resonable conclusion one can draw from these observations is that, in many cases, an LC_{50} test is not so much a measure of a product's inherent toxicity as much as a measure of a bird's ability to survive a period of drastic food reduction after the presence of the test compound has been detected. The importance of food consumption to the interpretation of the LC_{50} was deduced over a decade ago -- e.g. " *However, normal reductions of food consumption (and toxic exposure) in proportion to body weight coupled with increased fat for endogenous energy were probably the predominant factors*

Table 1. LC50 studies which were used in the various analyses reported.

PRODUCT	TYPE	MALLARD	BOBWHITE	COTURNIX
aldicarb	carbamate		X	X
azinphos-methyl	organophosphorus	X	X	
bendiocarb	carbamate	X	X	
carbofuran	carbamate	X	X	X
carbophenothion	organophosphorus		X	X
chlorpyrifos-ethyl	organophosphorus	X	X	X
coumaphos	organophosphorus	X	X	
diazinon	organophosphorus	X	X	X
disulfoton	organophosphorus	X	X	
EPN	organophosphorus	X	X	
fenamiphos	organophosphorus	X	X	X
fenitrothion	organophosphorus	X	X	
fensulfothion	organophosphorus	X	X	X
fenthion	organophosphorus	X	X	
fonofos	organophosphorus		X	X
formetanate	carbamate	X	X	X
isazophos	organophosphorus	X	X	
isophenphos	organophosphorus	X	X	X
methidathion	organophosphorus	X	X	X
methomyl	carbamate	X	X	
methyl-parathion	organophosphorus	X	X	
mexacarbate	carbamate	X	X	X
oxamyl	carbamate	X	X	
parathion	organophosphorus	X	X	
phorate	organophosphorus	X	X	
pirimicarb	carbamate	X		
pirimiphos-methyl	organophosphorus	X	X	
propetamphos	organophosphorus	X	X	
sulprofos	organophosphorus	X	X	
terbufos	organophosphorus	X	X	X

responsible for general increases of LC_{50} during early maturation for all the compounds and may have masked certain changes of response that could influence hazard assessment." (Hill and Camardese 1982).

It follows that any factor which influences the ability of young birds to withstand starvation will have a direct effect on the determination of most LC_{50} values. Only a few of these factors are currently under experimental control in the current protocols. This presents us with serious problems in the interpretation of LC_{50} values.

3.1.2 How test conditions influence the outcome of the LC_{50} test

3.1.2.1 Age of test birds

The age of the test birds is one factor which can be controlled although current protocols allow for a certain latitude. According to current specifications, Mallards should be 5-10 days old and Bobwhites 10-14 at the beginning of the test. Several of the early Mallard tests were performed when the birds were 14 days old. Hill and Camardese (1982) have clearly demonstrated that LC_{50} values for *Coturnix* increase as birds grow older. LC_{50} values of adult birds are higher than those of young birds in large part because the older chicks can better withstand food deprivation brought about by their refusal to eat the treated diet. Young birds, being physiologically more constrained, are either forced to continue feeding even though the food is contaminated hence causing more mortality amongst them, or they stop feeding and die of starvation. Because birds gain weight very rapidly when they are young, a difference of a few days between the age of test birds will result in large differences in LC_{50} values. For example, Hill and Camardese (1982) showed that LC_{50} values increased more than threefold for carbamates (aldicarb, carbofuran) and organophosphorus insecticides (ethoprop, thionazin) for *Coturnix* birds aged between 1 and 21 days. For similar reasons, LC_{50} values cannot be reliably obtained with adult birds. For example, the toxicity of technical diazinon (86.6% purity) in the Mallard increased from < 47 ppm to 510 ppm to > 1500 ppm when the birds increased from 10 to 31 to 87 days of age (Grimes and Jaber 1987a) reflecting the ability of older birds to better withstand starvation. In contrast to this, extremes in the susceptibility of several pesticides to Mallards ranging from 1.5 days to 6 months of age were generally less than 3-fold as measured by a single acute dose (LD_{50}) (Hudson *et al.* 1972).

3.1.2.2 The condition of test birds

The weight, size and general condition of the test birds is not under any experimental control in the current protocols. Yet, it is reasonable to predict that increased fat levels and body reserves should allow birds to better withstand food deprivation and/or allow them to reduce their intake of contaminated feed. A comparison of the intra- and inter-test differences in these parameters indicate the extent to which the determination of an LC_{50} is likely to be dependant on test conditions.

Mallards

Analysis of control groups of 6 products (table 2) where birds were of the same age (14 days) at the beginning of the treatment period revealed a high variability in bird weight

Table 2. Mean body weight at the beginning of the treatment period, mean food consumption during the 5-day treatment period and weight gain after 8 days for control groups of 14-day old Mallard ducklings.

Product	Control group[1]	Body weight (day 0) (g)	Food consumption (g/bird/day)	Food consumption (g/kg/day)	Weight gain (% of initial mean group weight)
CAB 2	1	192	70.0	364.6	101.6
	2	215	67.0	311.6	81.4
	3	207	73.5	355.1	95.7
	4	190	71.0	373.7	103.7
	5	197	78.5	398.5	108.1
CAB 7	1	230	65.0	282.6	82.6
	2	218	67.0	307.3	75.2
	3	220	65.5	297.7	82.7
	4	217	66.0	304.1	86.6
	5	227	63.0	277.5	82.8
OP 16	1	291	63.6	218.6	69.8
	2	291	68.5	235.3	61.5
	3	294	64.3	218.6	54.8
	4	294	69.4	236.1	68.0
	5	292	67.8	232.1	70.9
CAB 6	1	198	63.5	320.7	91.9
	2	190	65.5	344.7	93.7
	3	182	67.5	370.9	84.1
	4	198	68.5	346.0	84.3
	5	195	69.5	356.4	95.9
OP 11	1	172	66.5	386.6	115.1
	2	192	74.0	385.4	100.5
	3	192	60.5	315.1	97.9
	4	173	64.5	372.8	116.8
	5	175	62.0	354.3	111.4
OP 19	1	167	93.0	556.9	85.6
	2	160	88.5	553.1	109.4
	3	170	90.0	529.4	94.1
	4	155	91.5	590.3	111.0
	5	172	95.0	552.3	98.8

[1] Each control group consists of a pen with 10 birds .

reflecting different genetic stocks of birds and/or different body conditions[1] : mean group weight (N = 10 birds/group) varied from a low of 155 g to 294 g . Potentially significant differences were also observed between control groups within a single experiment, possibly reflecting a high inter-bird variance and/or a non-random assignation of birds to pens. In three of the 6 studies examined, there was a greater than 10% difference between the lowest and highest initial mean among-control-pen weights.

Mean food consumption among control groups during the 5-day treatment period varied from 61 g/bird/day to 95 g/bird/day, the higher food consumption being found in the experiment with the lowest reported initial body weights. Within experiments, considerable differences were also seen in the food consumption data among control pens: e.g. 17% and 19% differences between extremes for CAB 2 and OP 19 respectively. Accurate food consumption can be difficult to measure because of the problems encountered with spillage and variability in this measurement alone might not be cause for concern. However, the variability in weight gain (an easy parameter to measure) is also large and this is more worrisome. Among experiments and pens, average weight gain after 8 days in the control pens varied from a low of 55% to 117% of the initial mean group weight. Within experiments, the among-pen extremes in measured weight gain were 29% or greater for half of the studies.

Finally, mean food consumption per experiment corrected for mean pen weight (expressed as g food/kg of bird/day) ranged from a low of 219 to a high of 590 or a difference of 2.7 fold.

Bobwhite

As was the case for the Mallard , we compared bird condition for 6 studies where chicks were 14-days of age at the start of the experiment (table 3). Once again the comparison revealed high inter-test and inter-pen variance. Overall mean weight ranged from 17 g to 35 g. Food consumption during the 5-day treatment period also varied greatly ranging from 5.5 g/bird/day to 12.6 g/bird/day while weight gain after 8 days was between 6% and 120% of the initial mean group weight. We also detected large differences between control groups within a single experiment. For example, mean food consumption between control groups during the toxicity test for CAB 4 varied by more than twofold, from 5.5 to 12.0 g/bird/day. Large differences were also observed with weight gain data (for example with CAB 4 and CAB 5). Mean food consumption per product corrected for weight (g/kg/day) ranged between 243 and 601.

[1] It is assumed here that test procedures were as described and that results were accurately reported in all submitted studies.

Table 3: Mean body weight at the beginning of the treatment period, mean food consumption during the 5-day treatment period and weight gain after 8 days for control groups of 14-day old Bobwhite quail .

Product	Control group[1]	Body weight (day 0) (g)	Food consumption (g/bird/day)	Food consumption (g/kg/day)	Weight gain (% of initial mean group weight)
CAB 4	1	20	8.5	426.0	120.0
	2	20	12.0	601.1	85.0
	3	20	6.5	326.0	70.0
	4	21	7.5	358.1	81.0
	5	21	5.5	261.9	61.9
CAB 2	1	22	8.5	386.4	54.5
	2	23	10.5	456.5	47.8
	3	20	11.5	575.0	60.0
	4	22	11.4	518.2	59.1
	5	22	10.0	454.5	68.2
CAB 7	1	35	10.5	300.0	37.1
	2	35	9.0	257.1	51.4
	3	35	10.0	285.7	57.1
	4	35	8.5	242.9	42.9
	5	35	9.5	271.4	34.3
CAB 5	1	19	6.9	363.2	6.3
	2	17	6.9	405.9	69.9
	3	19	8.0	421.1	65.8
CAB 6	1	32	8.2	255.6	68.8
	2	28	7.6	272.9	75.0
	3	31	8.9	287.7	67.7
	4	32	8.5	264.4	56.3
	5	30	9.0	300.0	73.3
OP 19	1	30	9.0	300.0	56.7
	2	30	10.5	350.0	56.7
	3	30	12.6	420.0	50.0
	4	30	11.0	366.7	50.0
	5	30	9.9	330.0	60.0

[1] Each control group consists of a pen with 10 birds .

Japanese quail (*Coturnix*)
The only data available to us were those of Hill and Camardese (1982, 1986). All tests were performed in the same laboratory and the inter-test variance in the parameters described earlier is expected to be lower. Of interest here, Hill and Camardese (1982) explored the inter-test variability in LC_{50} determinations. They found the level of variance to be acceptable, at least for compounds with an irreversible mode of action. For carbofuran however, a reversible cholinesterase inhibitor, they obtained very erratic results with LC_{50} values changing by as much as two-fold between subsequent repeat trials. These authors hypothesized that perhaps even slight differences between tests, such as the exact length of time (measured in minutes) between removal of clean feed pre-test and presentation of treated feed might be responsible for these discrepancies. Again this emphasizes the point that the determination of the LC_{50} is extremely sensitive to test conditions.

3.1.2.3 The quality of the feed

The caloric and nutritional quality of the feed used in dietary tests is seldom measured, and even when it is, there is no way in which the results of the analysis can currently be used. Yet, we can predict that there should be a direct relationship between the quality of the feed given to the birds on test and the resulting LC_{50}. A feed that is twice as nutritious as another will allow birds to feed half as much thus reducing their intake of toxicant by half. Currently, protocols only specify that standard commercial diets should be used without regard to their caloric value or nutritional character.

This problem was recognised early on in avian hazard assessment ...

e.g. "*It is recognized that a two- or three-fold difference in the daily rate of food consumption could occur in the diet of a bird, depending on the moisture content and the caloric and nutritive values of its food. .... Birds eating a high percentage of food with a high moisture content may consume greater quantities of pesticide than birds with diets containing a low moisture content because of the increased bulk needed for equivalent nutritive value.*" (Kenaga 1973) ... but unfortunately was never systematically or adequately dealt with by regulatory bodies.

3.1.3 Comparison of LC_{50} values among the different species tested

It is only possible to test a very limited number of bird species. Yet, inferences of risk need to be generally applicable to birds at large. A companion analysis of avian LD_{50} values for a large group of cholinesterase-inhibiting chemicals (Baril *et al.* 1994) has shown that, despite the occasional exception, there is acceptable among-species consistency in the toxicity of these products. In other words, the very toxic products tend to be very toxic to most species and likewise for products at the other extreme. Indeed, such consistency among species is a *sine qua non* prerequisite of any hazard assessment procedure. How does the LC_{50} test perform in this regard?

To carry out this comparison, we identified a group of cholinesterase-inhibiting insecticides, for which we had both an LD_{50} and LC_{50} for each of the two usual test species: Northern Bobwhite and Mallard. Bobwhites were consistently tested at ages 10-14 days. Most

Bobwhite and Mallard. Bobwhites were consistently tested at ages 10-14 days. Most Mallard tests were conducted at 10 days of age with a few tests conducted at 5 days of age and some at 11-14 days. Rank correlations for LC_{50} and LD_{50} values (the latter on adult birds) were performed separately for compounds where the Mallards were either 5-10 days (as per current guidelines) or 10-14 days. Compounds where the age of the Mallards was 10 days at testing are common to both analyses. In four cases however, two LC_{50} values were available - one at 5 days and the other at 10. In those cases, the 10 day test results were used in the 10-14 day analysis only. Results of these correlations are given in the following tables.

Table 4. Spearman rank comparison of 5 day LC_{50} values between the Mallard (either 5-10 or 10-14 days) and Bobwhite (10-14 days) for groups of cholinesterase-inhibiting insecticides.

Age of Mallards	Mallard LC_{50} (ppm)		Bobwhite LC_{50} (ppm)		Spearman Rank Correlation
	Lowest value	Highest value	Lowest value	Highest value	
5-10 days (N = 16)	76	4908	30	1100	0.29 NS
10-14 days (N = 19)	43	5025	30	1100	0.16 NS

NS = Non statistically significant

These results suggest that we cannot rely on the LC_{50} value of one species to "predict" the relative LC_{50} value of the other. If we see no relation between the sensitivity of the 2 species (Mallard and Bobwhite) currently used in dietary studies, we believe that the extrapolation of results of LC_{50} studies to other avian species is unreliable and it follows that the LC_{50} should not form the basis of any risk assessment intended to protect birds at large. On the other hand, the significant correlation between the LD_{50} values in the Mallard and Bobwhite despite the limited range of values, at least in the Bobwhite, suggests that interspecies extrapolation on the basis of LD_{50} values is possible (table 5).

Table 5. Spearman rank comparison of LD_{50} values between adult Mallards and Bobwhite for the same groups of cholinesterase-inhibiting insecticides featured in table 4 .

Compounds for which the age of Mallards in the LC_{50} test was:	Mallard LD_{50} (mg/kg)		Bobwhite LD_{50} (mg/kg)		Spearman Rank Correlation
	Lowest value	Highest value	Lowest value	Highest value	
5-10 days (N = 16)	0.62	1190	1.2	32	0.45 *
10-14 days (N = 19)	0.40	1190	1.2	47	0.47 *

* Statistically significant at $P < 0.05$

3.1.4 Comparison of lab-derived LC_{50} values with field incidents

As mentioned earlier, the attraction of the LC_{50} as a basis of risk assessment procedures is that the units (ppm residue in feed) can be compared directly to expected residue levels in wild foods (EEC - Expected Environmental Concentration; or PEC - Predicted Environmental Concentration, depending on which side of the Atlantic one is from). This facilitates the current 'quotient' approach to wildlife risk assessment (Urban and Cook 1986) which simply entails deriving a prediction of likely hazard by taking a ratio of the PEC over the LC_{50}. One problem with this approach is that an LC_{50}-based risk assessment makes it very difficult to integrate routes of non-oral exposure such as dermal or inhalation uptake. Dermal exposure especially was shown to be a very important route of exposure for at least two organophosphorous insecticides - fenitrothion (Mineau *et al.* 1990) and methyl parathion (Driver *et al.* 1991). A case of bird mortality was attributed to dermal exposure to the pesticide diazinon when a dead Downy woodpecker (*Picoides pubescens*) was recovered with 46 700 ppm of diazinon on its feathers (Stone and Gradoni 1985). A pen study reported extensive mortality of American robins (*Turdus migratorius*) exposed to turf treated with chlorpyrifos but given clean food and water (Brunet and Cyr 1990). However, this may currently be considered to be a minor problem because only rarely are there data which allow for the consideration of routes of exposure other than the oral one.

More importantly, there is growing evidence that the lab-derived LC_{50} value *per se* is a poor predictor of hazard. Two examples are given here:

Diazinon

In the course of the judicial hearings on the use of diazinon on turf, a great deal of laboratory, semi-field and field data were generated. To date, these data provide one of the best examples available for lab to field extrapolation in birds. The accepted Mallard LC_{50} value used for all the original diazinon risk assessments was that obtained by Hill *et al.* (1975) for 10-day old ducklings. That value was 191 ppm with an extrapolated LC_{10} of 86 ppm and LC_{90} of 424 ppm. A large number of incidents have been reported following the use of diazinon on turf, both in the U.S. (Stone 1987) and in Canada (Frank *et al.* 1991). Grazing waterfowl (ducks or geese) are repeatedly killed when they consume diazinon-treated grass. A review of the residue levels measured in the grass blades recovered from the dead birds showed that even in cases where there had been very extensive mortality and where grass was very quickly recovered from the upper digestive tracts of birds, residue values were substantially lower than the accepted waterfowl LC_{50}. More precisely, the highest value ever recovered from any species was 79 ppm from a pooled grass sample retrieved from a few of the 700 Brant geese (*Branta bernicla*) killed on a Long Island golf course (Stone and Gradoni 1985). All the birds that died in that incident were adults. Efforts to determine an LC_{50} value on adult or even slightly older waterfowl gave a value of approximately 3000 ppm for adult Canada Geese (Grimes and Jaber 1987b) and of greater than 1500 ppm in the 87 day old Mallard (Grimes and Jaber 1987a). However, in a repeat study on 10 day old birds, all birds died at a feed concentration of 47 ppm casting some doubts as to the 'proper' endpoint - 191 ppm or <47 ppm. The inter-test variation exhibited by the Mallard as well as the lack of relationship between actual lethal residues and at least some of the laboratory dietary toxicity values both underscore our inability to predict risk on the basis of laboratory dietary tests. In fact, even small pen tests where birds were held on treated grass (Wildlife International 1986) were unable to predict the type of mortality seen in the wild. The most parsimonious explanation for these discrepancies was that the rate of food intake of wild birds was the critical parameter not adequately modelled, either in the laboratory or in small pens.

Carbofuran

Carbofuran is another cholinesterase-inhibiting insecticide with an extensive bird kill record. The five day LC_{50} values in the 10 and 14 day Mallard duckling were determined to be 190 ppm and 79 ppm respectively (Hill *et al.* 1975, Wildlife International 1976). As discussed earlier, carbofuran was one of the products which gave a high inter-test variance in Hill's laboratory. The Mallard is one of the most sensitive of the approximately 15 species tested with this chemical. Yet, extensive mortality of California gulls (*Larus californicus*) was recorded when these fed on grasshoppers containing between 4.2 and 7.2 ppm of carbofuran (Leighton and Wobeser 1987; Leighton 1988). Large flocks of Canada geese (*Branta canadensis*) grazing in alfalfa have been killed by concentrations possibly as low as 3.6 ppm of carbofuran (reviewed in Mineau 1993). Some Mallard ducklings walking through a sprayed pasture with grass residue levels measured at between 9.1 and 11.5 ppm showed marked signs of intoxication such as spasms and convulsions (Martin *et al.* 1991). None of the ducklings died but dietary intake was thought to be minimal. Again, these observations reflect poorly on the adequacy of LC_{50} values to predict risk to birds.

3.2. The use of the LC_{50} study to infer whether birds will avoid feeding on treated food

It has been suggested (Luttik 1993) that the LC_{50} test could be used to derive a 'detection' or 'repellency' threshold. Luttik showed that the food consumption data from LC_{50} studies provided detection thresholds very similar to those obtained with more sophisticated choice tests. All the products which formed the basis of Luttik's review were cholinesterase inhibitors. These products are not thought to be repellent in the sense of presenting the test animal with noxious sensory cues such as bad taste or smell. Rather, animals are only 'repelled' once they undergo toxicosis, either because they are physiologically unable to continue eating (pesticide-induced anorexia *sensu* Grue 1982) or because they form a conditioned response to the product following the toxicosis (conditioned aversion response *sensu* Avery 1984). The term 'food avoidance' will be used here to refer to any reduction in feeding regardless of the mechanism. We prefer that the term 'repellant' be restricted to sensory repellents. It has been argued that a demonstrated avoidance of contaminated feed may mitigate the risk posed by a toxic pesticide by reducing the likelyhood of exposure and some avoidance tests are currently in use in some jurisdictions (BBA 1993). Therefore, even if the LC_{50} value itself is unreliable and somewhat meaningless as shown above, is the test worth preserving for any insight it might provide on the real-world likelyhood of exposure?

Our contention is that lab-derived food avoidance data obtained through an LC_{50} test (or even possibly through more specialised choice test) may be unreliable and largely artefactual for much the same reasons that the LC_{50} value is unreliable and largely dependant on test conditions. We again offer two lines of evidence: 1) That the inter-species variability in avoidance thresholds makes the use of any one value suspect; and 2) More importantly, that the laboratory-derived avoidance data appears to be at odds with available field information.

3.2.1 Inter-species comparisons

Food consumption of test birds was examined for several insecticides to determine the chemical concentration at which birds start reducing their food intake. This was achieved in two ways. First, when 3 to 5 control groups were present, we calculated the 95% confidence interval for daily food consumption. The lowest concentration at which daily food consumption fell below the confidence interval was deemed to be the level at which avoidance was manifest. An avoidance threshold would therefore be lower than this particular concentration. Second, when 1 or 2 control groups only were present, we looked at the raw data of daily food consumption (g/bird/day) of birds in the different treatment groups and determined subjectively the concentration at which food consumption appeared to decrease. The second method was utilized only 3 and 6 times for Mallard and Bobwhite respectively but for all chemicals in the trials with *Coturnix*. Food consumption data for *Coturnix* and presented in Hill and Camardese (1986) must be interpreted with caution because only two concentrations (second lowest and second highest) of the chemicals are available.

The concentrations at which birds detected the chemical in the diet are presented in table 6. For several products, we cannot either prove or disprove differential sensitivity for

Table 6: Concentration at which birds detected the presence of the chemical in the diet in LC50 studies

	Mallard			Bobwhite			Coturnix[1]		
Product	Age (days)	Concentration (ppm)	LC50	Age (days)	Concentration (ppm)	LC50	Age (days)	Concentration (ppm)	LC50
CAB 4				14	56.2 < x < 100	71	14	< 356	387
CAB 2	14	< 464	1466	14	< 215	749			
CAB 7	14	46 < x < 100	79	14	< 100	714	14	526 < x < 911	746
OP 2				56	< 100	320	14	1768 < x < 5000	4434
OP 6	5-7	10 < x < 30	361	10-12	100 < x < 300	449			293
OP 4	6	< 50	90	42-56	< 20	68	14	< 85	167
OP 16	14	< 46.4	316	13	15 < x < 22	38	14	< 33	59
OP 14	10	< 15	47	13	10 < x < 15	22	14		85
OP 1				49-63	100 < x < 180	400	14	< 249	90
CAB 5	9	100 < x < 500	2086	14	500 < x < 1000	3963	14	< 600	993
OP 22	10	30 < x < 60	163.2	10	60 < x < 120	131			
OP 17	10-15	125 < x < 250	> 1000	13	< 32	145	14	< 200	299
OP 21	6	< 128	328	42-56	< 128	240	14	< 921	980
CAB 8	5	39 < x < 78	150	15	< 312	730	14	< 526	605
CAB 6	14	< 215	5025	14	215 < x < 464	225			
CAB 3	12	< 250	741						
OP 11	14	< 215	633	11	< 163	304			
OP 19	14	< 464	4752	14	215 < x < 464	568			
OP 18	10-15	68.1 < x < 147	185	13	178 < x < 316	157	14	< 197	284

[1] Data presented in Hill and Camardese 1986 only for two experimental groups, second lowest and second highest concentrations

Mallard, Bobwhite and *Coturnix* because threshold levels were not determined exactly and the range of concentration at which contaminated food was avoided by birds overlapped. However, there were several cases where the range of product avoidance did not overlap between different species of similar ages. For example, the concentration at which birds were deterred from feeding on food treated with CAB 7 was obviously higher for *Coturnix* than for the Bobwhite despite very similar LC_{50} values. CAB 5, OP 22 and OP 18 demonstrated a similar situation (differing avoidance levels despite similar dietary toxicity values) between the Mallard and the Bobwhite. In the case of OP 17, the detection thresholds in the Mallard and Bobwhite mirrored their relative LC_{50}s whereas with CAB 6, this trend was reversed: the species with the highest LC_{50} had the lowest avoidance thresholds. It is noteworthy in the case of this chemical that the susceptibility of the two species as determined by the LD_{50} is reversed from what it is with the LC_{50}.

The difficulty in extrapolating an avoidance level from one species to another is a clear problem from a risk analysis point of view. To be fair, data on food avoidance seem to be much more 'robust' (*i.e.* more consistent) between ages and species than the LC_{50} values. However, there is good experimental evidence to show that the exact test conditions under which birds are placed (e.g. the number of treated and untreated food bowls) determine whether avoidance will be manifest in the laboratory, at least for some cholinesterase-inhibiting compounds (Bennett 1989). One can only assume that the exact conditions under which the LC_{50} test is performed will likewise influence the determination of avoidance levels.

3.2.2 The lack of evidence for avoidance of toxic feed in the field

Inherent in Luttik's logic and central to the value of a laboratory test of avoidance is the belief that food avoidance seen in the laboratory will result in a reduced exposure in the field. Hart (pers. comm.) has even proposed a modification of the LC_{50} test that would have birds taken off contaminated feed as soon as they exhibit weight loss and their eventual survival would be assessed on clean feed in order to mimic a switch to a clean food source in the field. We believe that, for a few well documented cholinesterase inhibitors at least, the evidence is that birds do not avoid contaminated feed in the wild despite a well demonstrated avoidance in the laboratory.

Diazinon

Again, it is useful to turn to this well-studied pesticide. The laboratory data readily demonstrate that this is one chemical for which several species readily show avoidance in the laboratory. For example, the avoidance level was determined to be below 47-50 ppm for Mallards of either 10, 31, or 87 days of age. This was an extremely consistent response given that the concomitently-determined LC_{50} values for those birds ranged from less than 47 ppm to more than 1500 ppm (Grimes and Jaber 1987a). Food avoidance developed very rapidly in the laboratory and was seen typically on the first day of the test. Stromborg (1982) similarly reported food avoidance in breeding female Bobwhite at levels of 50 ppm and up. Had these observations been the only data available, the clearly demonstrated (and highly consistent) avoidance at very low levels of feed contamination would have led to the prediction that, in the wild, birds (and Mallards in particular) would avoid foods treated at this level or above. This assessment would have been seriously in

error and would have grossly underestimated the hazard of this chemical to Mallards and other bird species.

Residue values recovered from dead birds are extremely variable and many are indeed lower than 47-50 ppm (Stone 1987). Diazinon is labile and therefore loss is expected depending on the time between death and proper preservation of the samples. Also, by the time the samples are analysed, especially if these are from the lower digestive tract, much of the insecticide has already been absorbed by the body. Nevertheless, we have listed a number of well documented waterfowl incidents (table 7) which indicate that the avoidance level of < 47-50 ppm seen in the laboratory appears to be meaningless. Birds in these incidents were not deterred by residue concentrations much higher than the laboratory-derived avoidance threshold. Cases where liquid diazinon was utilized are more convincing than those with granular diazinon because the ingestion of a few granules adhering to grass blades can result in extremely high crop contents residue concentrations in dead birds. If grazing species cannot be deterred, it is even more unlikely that species feeding on seeds, insects or other discrete food items of potentially high residue concentration can ever be deterred. Many extreme examples can be found in the literature that cast further doubt on food avoidance being important in real life -- two will suffice here: Blue Jays *Cyanocitta cristata*), Common grackles (*Quiscalus quiscula*) and House sparrows (*Passer domesticus*) feeding on bread purposely contaminated with 14,300 ppm of diazinon; Rock doves (*Columba livia*) feeding on treated wheat seeds coated with 3,500 ppm of diazinon (Stone and Gradoni 1985).

Fensulfothion

Data for other chemicals are more difficult to obtain, these products not having been reviewed as extensively as diazinon. Stone (1979) reported the death of 25 Canada geese in 1977 on a golf course in New York State previously sprayed with fensulfothion (Dasanit). Crop and gizzard contents of two birds revealed residue concentrations of 25 and 4 ppm; the residue concentration in a sample of sod was 13 ppm. A case of fensulfothion-related mortality involving Herring (*Larus argentatus*) and California gulls happened in Prince Albert, Saskatchewan in 1986 (Leighton and Wobeser 1987). Sixty birds were found dead near a municipal landfill site and the esophagal contents of one of the birds revealed a fensulfothion residue concentration of 26.1 ppm . Residues in the crop contents of a Red-tailed hawk (*Buteo jamaicensis*) which was secondarily poisoned after feeding on contaminated ducks were measured at 30 ppm (Elliott *et al.* in prep.). In the available LC_{50} tests, both Mallards and Bobwhite showed feed avoidance of fensulfothion at levels which are less than half of the peak residue values reported in these incidents (proprietary).

Carbofuran

Several cases of large scale avian mortality following carbofuran application have been reported in the past but residue level of carbofuran in ingesta of dead birds was never higher than the concentration at which Mallards detects its presence in the laboratory. These examples of carbofuran poisoning involved both granular and flowable carbofuran and caused mortality to different types of birds, from small passerines to large raptors (Mineau 1993). Carbofuran residues in alfalfa fields can be as high as 150 ppm (Mineau 1993) which, based on the LC_{50} data should easily lead to detection and food avoidance

Table 7: Field evidence of waterfowl kills caused by ingestion of diazinon contaminated food showing higher concentration than laboratory repellency level (< 47-50 ppm).

Location, date	Mortality	Application site	Residue measured	Formulation	Reference
Sudden Valley, Washington; 1986	85 widgeons	golf course	4 fairway grass samples ranged from 100 ppm to 332 ppm	liquid	Institute of Wildlife Toxicology, 1987
Nassau County, New York; 1984	700 brants	golf course	grass in esophagus: 79 ppm	liquid	Stone, 1987 case # 18
Suffolk County, New York; 1980	81 Canada geese	condominium lawn	grass from gizzard: 64.4 ppm grass blades: 236 and 38.8 ppm	liquid	Stone, 1987 case # 31
Oakland County, Michigan; 1979	25 Canada geese	lawn	grass from esophagus: 55 ppm	liquid	Stone, 1987 case # 34
Greenwich, Connecticut; 1973	31 Canada geese	estate lawn	grass from esophagus: 61 ppm	liquid	Stone, 1987 case # 50
Markham, Ontario; 1987	10 Canada geese	turf	turf sample: 390 ppm	liquid	Frank *et al*., 1991
Monroe County, New York; 1986	7 domestic ducks	home lawn	upper alimeantary canal contents: 13 - 63 ppm	granular	Stone, 1987 case # 6
Indianapolis, Indiana; 1985	40 mallards	condominium lawn	grass ans soil: 141 ppm	granular	Stone, 1987 case # 8
King County, Washington; 1986	3 mallards	lawn	gizzard and stomach contents: 60.9 ppm cranefly larvae from lawn: 98.8 ppm	granular	Stone, 1987 case # 25
Fairfield, Connecticut; 1978	24 mallards	golf course	stomach contents: 72 ppm	granular	Stone, 1987 case # 36
Monroe County, New York;1986	3 mallard ducklings	home lawn	gizzard and crop contents: 227 and 62 ppm	fertilizer 22-5-9 with diazinon	Stone, 1987 case # 3
Ventura County, California; 1987	2 mallards	office building lawn	gizzard contents: 50 and 70 ppm	not specified	Stone, 1987 case # 1
King County, Washington; 1985	50 widgeons and gadwalls	lawn	grass: 50 ppm	not specified	Stone, 1987 case # 12
Saratoga County, New York; 1986	2 domestic ducks	not specified	crop contents: 421 ppm	not specified	Stone and Gradoni, 1987
Ontario County, New York; 1986	12 mallards, 1 black duck	not specified	esophagus contents: 57.7 ppm	not specified	Stone, pers. comm.

(proprietary). Yet, kills of grazing waterfowl commonly take place in freshly-sprayed fields. A more extreme case is when a flock of 800 European starlings (*Sturna vulgaris*) and House sparrows died after feeding on potato waste treated with liquid carbofuran. Levels as high as 3,500 ppm were recorded in one bird's ingesta (CWS unpublished). Stone (1979) reported a number of species as diverse as Red-winged blackbirds (*Agelaius phoeniceus*) and Herring gulls being poisoned by wheat seed treated with 2000 ppm carbofuran. This is more than ten-fold the level which should have resulted in food avoidance according to the laboratory information.

Organophosphorus seed dressings

At least two organophosphorus seed dressings have repeatedly given rise to bird mortality in Britain: carbophenothion and fonofos. A number of bird species have been involved including several gallinaceous species in the case of fonofos. Based on label rates, the concentration of pesticide on seed is about 1200 ppm and 980 ppm for carbophenothion and fonofos respectively. Again, for both of these products, the level at which clear food avoidance is seen in the Bobwhite LC_{50} study is at least ten-fold lower (proprietary).

The limited field information currently at our disposal suggests that the food avoidance behaviour seen in the LC_{50} test does not have much field applicability. If, as suggested by Luttik (1993), avoidance thresholds obtained with more specialised laboratory tests are in accordance with those obtained with the LC_{50}, there is reason to doubt the validity of those other tests as well. The situation may be different for chemicals which show true sensory repellency but, judging from the difficulty researchers have had in designing effective bird repellents, we believe these products are rare or at least not typical of the bulk of pesticides likely to give rise to poisoning incidents. Avoidance may be more pronounced in the lab than in the field because of a slower food intake and more time for a conditioned aversion response to form; alternatively, the laboratory situation where the food is given in food hoppers and where air movement is more limited may provide the birds with sensory cues (e.g. olfaction, occular irritation etc...) not as readily available in the field. It has been shown that the presence of cues can enhance the formation of a conditioned aversive response (Avery 1984).

3.3 Use of the LC_{50} as a measure of 'chronicity'

Kenaga (1973) suggested that, comparing a prolonged dietary or repeat-dosing exposure to a single acute dose could provide an indication of a pesticide's potential to cause chronic or cumulative toxicity. He compared the relative performance of an acute oral, a repeated (30 day) oral and a dietary exposure test with dimethoate (an organophosphate), mexacarbate (a carbamate) and DDT (an organochlorine insecticide). Not surprisingly, he concluded that an acute oral exposure made DDT look safe while the carbamate was 'maligned' even though the latter was undoubtedly preferable because, although acutely toxic, it did not bioaccumulate nor did it cause delayed effects. For this and other reasons (e.g. the 'naturalness' of a dietary exposure) Kenaga recommended the dietary test as a basis of avian risk assessment.

Although we believe that, for the many reasons discussed earlier, reliance on a dietary test is misguided, it is still relevant to head Kenaga's warnings about the hazard of DDT-like

compounds. The above discussion has focussed on cholinesterase-inhibiting insecticides which, although responsible for the majority of wildlife poisonings, generally demonstrate a very low level of cumulative toxicity on account of a fairly high clearance rate and/or reversibility of toxic symptoms (for carbamates especially). A risk assessment based on acute toxicity may well underestimate the hazard of compounds with a cumulative action and it would be advisable to perform a test of subchronic exposure with compounds shown to have bioaccumulation potential or that result in cumulative injury. It remains to be seen whether such a subchronic exposure should be achieved through a dietary route of exposure or repeat dosing.

For a group of carbamates and organophosphates, we transformed the dietary dose at the LC_{50} into actual pesticide uptake expressed as mg of pesticide/kg of bird/day (table 8). In order to do so, food intake was estimated by interpolating, on a linear scale, the food intake of the two dose groups which bracketed the LC_{50}. This effectively gives a measure of the number of LD_{50} equivalents consumed by the birds at the LC_{50} which is a measure of the chronicity of the product. The higher the number of lethal doses consumed, the lower the chronicity or, in other words, the faster the clearance of the product and/or the recovery from intoxication.

Table 8. Chronicity index expressed as the number of LD_{50} equivalent doses consumed per day by young Mallard or Bobwhite in the 5 day dietary LC_{50} test at the determined LC_{50}.

Pesticide	Mallard (age in days)	Bobwhite (age in days)
CAB 7	40.0 (14)	11.5 (14)
CAB 8	12.8 (5)	4.25 (15)
CAB 2	19.0 (14)	8.95 (14)
CAB 6	64.9 (14)	5.43 (14)
CAB 5	10.4 (9)	ND
CAB 3	4.36 (12)	ND
CAB 4	ND	9.50 (14)
OP 4	2.40 (6)	3.52 (42-56)
OP 21	5.36 (6)	ND

OP 14	1.47 (10)	2.30 (13)
OP 16	2.00 (14)	6.30 (13)
OP 17	ND	1.38 (13)
OP 19	3.35 (14)	ND
OP 22	ND	4.68 (10)
OP 1	ND	1.50 (49-63)
OP 18	ND	3.80 (13)
OP 6	0.49 (5-7)	2.06 (10-12)

ND = Not determined. Necessary data unavailable.

The first observation is that, not unexpectedly, there was a tendency for birds to ingest more LD_{50} equivalents per day in the case of carbamates. Some of the high values may be confounded by spillage which is indistinguishable from feed consumption in the index. The propensity of Mallards to spill their food and mix it with water may account for the generally higher index numbers seen in that species. There is therefore a limit to the possible interpretation of these data but they do offer some insight into the various products. For example, a product like OP 14 would be considered amongst the most inherently hazardous to birds on account of a very low LD_{50} (not shown here) as well as a very low index value in table 8. This is borne out by field evidence. On the other hand, a high index value may be insufficient to prevent poisoning through dietary exposure given a product of very high acute toxicity. This appears to be the case with CAB 7 where wildlife poisonings are frequent despite a relatively high capacity for clearance of the product and/or recovery from toxicosis. It might be possible to improve on this chronicity index by considering the dietary dose at which the first mortalities are recorded (rather than the LC_{50}) or by restricting the analysis to Type A mortality - those deaths which result from intoxication rather than food deprivation.

4. CONCLUSIONS

There are serious problems associated with the determination of the LC_{50}. Unfortunately, results of this test are only partially dependent on the nature of the products themselves and highly susceptible to test conditions. The end result is that the endpoint of the test is highly variable and, we argue, largely artefactual.

However, in deciding whether the LC_{50} test has any predictive value, it may not be relevant that the test endpoint *per se* be a meaningful value. Could a calculated risk index,

such as a ratio of PEC to $LC_{50,}$ be an adequate relative predictor of risk even if the LC_{50} value itself is not a suitable predictor of field toxicity? For any calculated risk index to be a good predictor of relative impact, it is critical that the between-chemical ratios be consistent between species. This was shown in our analysis not to be the case.

It is therefore clear to us that the LC_{50} value should not be used as a trigger for higher tiered studies (such as field studies) or be the basis of any risk assessment procedure which tries to predict risk for birds at large.

Does the LC_{50} study provide us with any useful data? On the basis of the limited field evidence available, we believe that the food avoidance levels obtained from LC_{50} studies have little or no predictive field value. This criticism may extend to a number of the more sophisticated laboratory food avoidance tests. If our insights about diazinon are correct, the rate of food intake may be of such an overwhelming importance as to make field exposure difficult to simulate in the laboratory. If there is an area where the LC_{50} test offers some new insight, it might be in the area of chronicity. A test of subchronic exposure, whether a dietary test corrected for food intake or a test with repeat dosing, does highlight differences in the metabolism and/or recovery from intoxication. As such, some form of subchronic test is probably worthwhile; it remains to be seen whether this test should be the LC_{50} with all the problems inherent to its interpretation.

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