Appendix 11. Evaluation of clinical sign data from avian acute oral toxicity studies

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An Evaluation of Clinical Sign Data from Avian Acute Oral Toxicity Studies,

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INTRODUCTION

The objective of this research is to provide information on sub-lethal clinical signs, clinical signs which occur before the reported LD50 value in order to further the development of alternate, more humane end points in avian acute oral toxicity testing. Such end points would reduce the amount of stress and pain to which birds are submitted during testing. A positive predictive value between the bird's sub-lethal symptoms and its LD50 value could prevent the killing of these birds by enabling an extrapolation of its LD50.

An evaluation of clinical signs was undertaken using 166 previously conducted acute avian oral toxicity tests from a broad list of pesticides. For all studies, the chemical dose of sub-lethal clinical sign onset as well as the LD50 dose were recorded. Using this data, an analysis was conducted to determine when sub-lethal signs occur in relation to the LD50 value. The analysis of sub-lethal signs was conducted across chemical classes and across different bird species.

In addition to evaluating sub-lethal clinical signs, an examination was made of the clinical signs first reported after the LD50 values. This allowed a comparison to be made between sub-lethal signs and signs first encountered after the LD50 in order to determine whether there is information lost when only sub-lethal signs are considered in the analysis.

MATERIALS AND METHODS

Selection of studies for the analysis of sub-lethal clinical signs

A positive predictive value between sub-lethal clinical signs and the LD50 is sought to enable a prediction of the LD50 value. Studies submitted by pesticide manufacturers to Canadian and U.S. authorities between 1962 and 1996, for registration purposes, were reviewed. The studies were selected based on the following criteria:

- study completed,
- reported mortalities,
- documented clinical signs at sub-lethal doses, and
- a precise LD50 reported. Note: in a few studies (3), the LD50 was not specified but the upper and lower 95% confidence intervals was given. In these three cases, the mean of the upper and lower values for the confidence interval was taken to arrive at a precise LD50 value.

Included in the sample were studies in which a precise LD50 was reported, but was above the typical 2000 mg/kg threshold (LD50 = 2000 mg/kg is the threshold above which a compound is no longer considered toxic). These studies were included in order not to bias results towards toxic products.

Examination of clinical signs first reported after the LD50 value

The initial sample of studies was reassessed for studies where clinical signs were encountered for the first time beyond the LD50 value. Each study, in addition to being complete,

- reported mortalities,
- reported a precise LD50 value, and
- documented clinical signs first reported after the LD50 value.

These studies were scanned for clinical signs not previously recorded at sub-lethal doses. This allowed a determination of the amount of information lost when clinical signs above the LD50 are not accounted for in the analysis. The following table shows the list of reviewed compounds.

TABLE 1
LIST OF CHEMICALS USED IN THE STUDIES
AND THEIR FREQUENCY OF OCCURRENCE

Chemicals (common name)					
Organophosphate and Carbamates	**N**	**All other Pesticides**	**N**	**All other Pesticides (con't)**	**N**
Aldicarb	8	Acifluorfen	1	Hydramethylnon	1
Aminocarb	1	Acticide 14	1	Hymexazol	1
Azamethiphos	3	Alachlor	1	Imazalil	1
Azinphos methyl	2	Amitraz	3	Imidacloprid	2
Bendiocarb	2	Azaconazole	1	Ioxynil	2
Bendiocarb	2	Bentazon	1	MCPA Acid	1
Carbofuran	1	Bifenthrin	1	MCPA Dimethylamine Salt	1
Chlorpyrifos	2	Bis Tributyltin Oxide	1	MCPA Sodium solution	1
Cloethocarb	1	Brodifacoum	2	Metalaxyl	1
Coumaphos	1	Bromethalin	2	Metaldehyde	1
Diazinon	2	Bromoxynil Butyrate	2	Methienamin	1
Disulfoton	2	Bromoxynil Heptanoate	1	Methylisothiocyanate	1
Disulfoton Sulfone	1	Bromoxynil Octanoate	1	Metobromuron	2
Disulfoton sulfoxide	1	Bromoxynil Phenol	1	Metribuzin	3
Fenamiphos	6	Calcium Polysulfide	1	Monolinuron	1
Fensulfothion	1	Chinomethionat	1	N,N-Diethyl-M-Toluamide	1
Fonofos/lambda-cyhalothrin	1	Chloflurenol-methyl	1	Oxadiazon	2
Fosetyl-al	1	Chlorothalonil	1	Oxycarboxin	1
Isazofos	2	Chromic acid	1	Penconazole	1
Isofenphos	1	Cladinafor-propargyl	1	Pentachlorophenol	1
Leptophos	4	Clopyralid	1	Phenmedipham	1
Methamidophos	2	Copper 8-Quinolinolate	1	Phostebupirim and Cyfluthrin	2
Methiocarb	2	Cycloheximide	1	Procloraz	2
Mexacarbate	1	Cyromazine	1	Propiconazole	1
Oxamyl	2	Dazomet	2	Tefluthrin	3
Oxydemeton-methyl	2	Deltamethrin	1	Triazine	1
Phorate and Fonofos	1	Dicamba	2	Triphenyltin Hydroxide	1
Pirimicarb	4	Dichlobenil	1		
Profenofos	1	Dicloran	1		
Propetamphos	2	Diflubenzuron	1		
Trichlorfon and Oxydemeton-methyl	2	Dimethipin	2	Yet to get common name:	
Trimethacarb	1	Dimethoxane	1	?Aquatol K?cas=2164-07-0	1
		Diniconazole	1	?Busan 11-M1?	1
		Endothall	1	?Compound 1339?	2
		Esfenvalerate	1	?CTAC Technical-cas=004080-31-3	1
		Fentin Hydroxide	1	?EXP 60655A?cas= 120068-37-3	1
		Fenvalerate	1	?Hydrothol 191?cas=66330-88-9	1
		Fipronil	4	?M&B 46513?	1
		Flucythrinate	1	?Omacide IPBC?-cas= 55406-53-6	1
		Glutaraldehyde (25%)	1	?Preventol CMK?-cas=59-50-7	1

	Glutaraldehyde (50%)	1	1,3-Dichloropropene and Methylisothiocyanate	1
	Hexazinone	1	2-(2,4 dichlorophenoxy) propionic acid dimethyl amine salt	1
			2,4-D dimethylamine salt	2
			2,4-Dichloropenoxy acetic acid	1
			4,4-Dimethyloxazolidine	1

Sorting of clinical signs

A total of 166 studies met our study criteria and were retained. From these 166 studies, a total of 128 clinical signs were listed by the laboratories that conducted the studies and these signs appear in Table 2. All clinical signs encountered in the selected studies were sorted, as a first step, based on synonyms for each clinical sign. Since no recording scheme exists for the documentation of symptomology in avian acute oral toxicity studies, a particular clinical sign can be reported with different names by different researchers. The synonymous symptoms which represented a single clinical sign were grouped together. We identified 31 distinct clinical signs. The grouping of synonymous clinical signs was done with standard toxicology tests, as well as expert opinion from wildlife veterinarians and veterinarians testing in the laboratories. Each clinical sign was then categorized into broad effect categories for purposes of convenience. The following table provides our sign classification.

TABLE 2
SYNONYMOUS SYMPTOMS FOR EACH CLINICAL SIGN, FROM SAMPLE OF 366 STUDIES, CATEGORIZED INTO BROAD EFFECT CATEGORIES

Clinical Sign	Synonyms	Sign Present in OECD Guideline for Mammalian Studies
	Respiration	
tachypnea	shallow rapid respiration - panting	tachypnea
dyspnea	labored breathing - gasping - heavy breathing - gaping	dyspnea
	Behavioral	
hyporeactivity	lethargy - reduced reaction to external stimuli - hypoactivity - decreased activity - quiet - sedated - subdued - withdrawal - depression – despondency - soporific symptoms - narcosis - lay with eyes closed - eyes closed – eyes shut - apathy – immobility – narcosis – less active – under active - inactive	immobile
piloerection	ruffled appearance - fluffed feathers - ruffled feathers – plumoerection – ungroomed	pilorection

	appearance -	
hyperexcitability	agitation - nervousness	excitable
	Loss of Muscle Control and/or Function	
loss of righting reflex	lateral position – lateral recumbency -	lateral position
prostration	prostrate posture– prone position – sternal recumbency – sprawled out with wings outstretched	ventral position
asthenia	lower limb weakness - inability to stand - unable to stand – sitting/resting on hocks – reluctance to stand – inability to walk – lying on pen floor	limp/lame
crouching	ventral, curved and hunched position	hunched position
ataxia	loss of balance - tumbling – stumbling - staggering gait - unsteady - loss of coordination – falling – difficulty walking – dropping – rolling motion	ataxia
wing drop	hanging wing	no
loss of flight	impaired flying - will not fly	no
paresis	lower limb rigidity – walking stiffly	paresis
paralysis	unable to move – inability to move	paralysis
coma	unconscious	coma
	Muscle Contraction	
opisthotonos	dorsal neck/head curl	opisthotonos
tremor	muscle fasciculation	tremor
convulsion	wing beat convulsion - tonic convulsion - muscle spasm - wings paddling – wing beating/flapping – head twitching - tetany	convulsion
walking on toes		walking on toes
	Bodily Fluids	
hypersalivation	excessive salivation - oral or nasal discharge - shaking of head	hypersalivation
lacrimation		lacrimation
conjunctivitis		conjunctivae swollen
	Feeding	
anorexia	decreased feed consumption	not eating
emesis	regurgitation	vomiting
diarrhea	loose faeces – chalky diarrhea – liquid excreta – chalky white diarrhea – yellow/green diarrhea – watery droppings	diarrhea
emaciation	decrease in body weight	emaciation
polydipsia		not drinking
constipation*	few faeces	constipation
	Blood	
hemorrhage	bleeding - blood in faeces – hemorrhage in area of wings – bruising – blood on mouth/nostrils	bleeding
	Other	
vocalization		vocalization
feather loss		no
	Unclassified clinical signs (too vague)	
	loss of muscle function, stress, weakness, sporadic movement	

Bird species used in studies

A variety of bird species were used in the selected studies. The species and their frequency are listed below:

TABLE 3
FREQUENCY OF TESTED SPECIES

Bird species	Proportion of studies where bird species was used (%)
Mallard Duck	25.4
Bobwhite Quail	55.5
Japanese Quail	7.29
Peking Duck	1.38

White Leghorn Hen	1.38
Pheasant	2.27
House Sparrow	5.38
Starling	0.92
Budgerigars	0.46
Pigeon	0.46
Red-winged Black Bird	0.46
Red-legged Partridge	0.90

Method of analysis

For each clinical sign, the dose of onset was calculated as a proportion of the LD50 value across all studies that administered at least two doses below the LD50. To ensure that clinical sign onset was not biased by a lack of dosage administration below the LD50, an additional analysis of clinical sign onset was conducted for studies that contained at least three doses below the LD50 and at least four doses below the LD50.

To test whether significant differences exist between the means of clinical sign onset to LD50 ratios, these means were transformed using an arcsin transformation and analyzed using the Tukey-HSD Multiple Range Test, at a significance level of 0.1, where all possible pair-wise combinations of the mean ratios were compared.

When the initial sample of studies was reassessed for studies where clinical signs were encountered for the first time beyond the LD50 value, only 24 studies reported clinical signs encountered for the first time beyond the LD50. These 24 are not included in the original sample of 166. Two new clinical sign descriptions were encountered: 'rolling on floor' and 'unconscious'. These two clinical signs are synonymous with sub-lethal clinical signs already seen in Table 2. 'Rolling on floor' is synonymous with ‘convulsion’ while 'unconscious' is synonymous with ‘paralysis’. In addition, 2 studies did not report any toxicity signs either before or after the LD50. These 26 studies were excluded from the frequency analysis reported below.

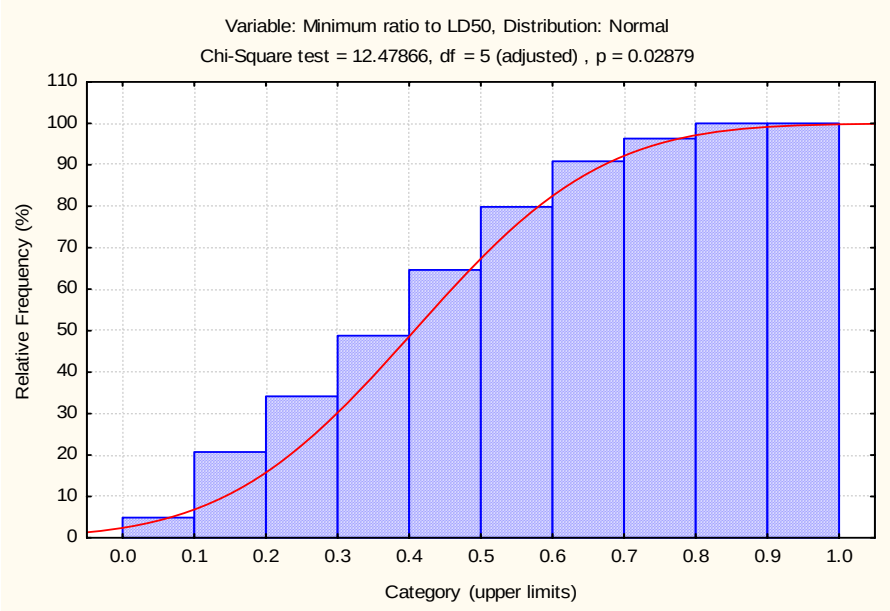
RESULTS

To determine whether clinical sign onset could occur sooner if lower doses were administered, the mean ratios of a clinical sign to the LD50 were calculated separately for studies that contained at least two, three and four doses below the LD50. Of the six most prominent clinical signs (‘hyporeactivity’, ‘asthenia’, ‘anorexia’, ‘ataxia’, ‘emaciation’, and ‘pilorection’)‘asthenia’, ‘ataxia’ and ‘pilorection’ showed a lower clinical sign onset when lower doses are administered. However, mean clinical sign onset was only decreased by approximately 10%. For the purpose of the following exercise, it was therefore decided to pool all studies.

Typically, mean ratios of a clinical sign to the LD50 were clustered together which indicates that clinical sign onset was similar for most clinical signs. All clinical sign onsets occurred between one and two doses below the LD50. Only three clinical signs did not fall into this interval: ‘conjunctivitis’ (n = 1), ‘hemorrhage’ (n = 2) and ‘walking on toes’ (n = 1). The number of observations for these three clinical signs are so small that their means are not significant. The six clinical signs previously mentioned, ‘hyporeactivity’, ‘pilorection’, ‘emaciation’, ‘asthenia’, ‘ataxia’, ‘anorexia’ all occurred at a similar fraction of the LD50.

We observed no clear progression of (subjectively assessed) symptom severity as dosage increased. For example, ‘hyporeactivity’, ‘prostration’ and ‘paresis’ all had mean ratios within 9% of one another. The failure to see a clear progression in clinical sign severity is most likely due to the biological onset of symptoms in avian acute oral toxicity tests; these symptoms all become apparent at the same time. Failure to see a progression in clinical sign severity could also be due, in some cases, to the large increases between administered doses. In order to compute a frequency distribution for

the purpose of this exercise, minimum ratios were therefore compiled across toxicity signs for each study. The distribution of those ratios for the 164 acute toxicity studies is shown in the figure below.



Based on this analysis, a cutoff value of 0.1 for the ratio of the first sign of toxicity to the LD50 would have been 'protective' in approximately 95% of the studies. A value of 0.1 of the LD50 is therefore proposed as the value to use as an estimate of the dose which may cause impairment and possibly endanger a reproductive effort in the field.

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