Data Evaluation Report on the Acute Oral Toxicity of Cycocel to Japanese Quail

PMRA Submission Number {.....} EPA MRID Number 467152-10

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	D325185
	OECD Data Point	{.....}
	EPA MRID	467152-10
	EPA Guideline	850.2100

Test material: Cycocel (chlorcholine chloride) **Purity:** 37.5% a.i.
Cycocel (chlorcholine chloride + choline chloride) 41.2% a.i. + 28.8% choline chloride
Common name: Chlormequat chloride
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: BASF Cycocel; CCC

Primary Reviewer: Brian Kiernan **Date:** 04/20/06
EPA/OPP/EFED/ERBIV

10/17/2006

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	018101	

Date Evaluation Completed:

CITATION: Ross, D.B., and N.L. Roberts. 1974. The acute toxicity (LD_{50}) of two formulation of Cycocel to the Japanese quail. Unpublished study performed by Huntingdon Research Centre, Huntingdon, Cambridge, England. Report No. BSF 55/74727. BASF Registration Document No. 1974/10039. Study submitted by BASF Corporation, Research Triangle Park, NC. Study initiated June 1974, and submitted October 21, 1974.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute oral toxicity of a pesticide to avian species. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

The acute oral toxicity of two formulations of Cycocel to young adult (not further specified) Japanese quail (scientific name not reported) was assessed over 14 days. Each compound was administered to the birds by oral gavage with corn oil as the carrier.

The first formulation, containing chlorcholine chloride (37.5% ai), was administered at 0 (vehicle control), 233, 466, 932, 1398, 1864, and 2796 mg product/kg bw. The 14-day LD_{50} (with 95% C.I.) was 1482 (985-2124) mg product/kg bw, equivalent to 556 (369-797) mg ai/kg bw. The 14-day NOAEL of chlorcholine chloride based on mortality was 1864 mg product/kg bw (699 mg ai/kg bw). According to the US EPA classification, this formulation of Cycocel (containing 37.5% chlorcholine chloride) would be classified as slightly toxic to Japanese quail on an acute oral basis.

All mortality occurred within 24 hours of dosing and totaled 0, 0, 0, 1, 2, 3, and 5 at the 0, 233, 466, 932, 1398, 1864, and 2796 mg product/kg bw test levels, respectively. While only group means were provided, it appeared that body weights decreased in a dose-dependent manner, particularly by day 14. Based on this effect, the reviewer determined the NOAEC to be 466 mg product/kg bw (175 mg ai/kg bw). There was no clear effect on food consumption. Necropsy examinations were not performed.

The second formulation, containing chlorcholine chloride (41.2% ai) plus choline chloride (28.8%), was administered at 0 (vehicle control), 241, 483, 966, 1449, 1931, and 2897 mg product/kg bw. The 14-day LD_{50} (with 95% C.I.) was 2205 (1323-31999) mg product/kg bw, equivalent to 908 (545-13184) mg ai/kg bw. The 14-day NOAEL of chlorcholine chloride plus choline chloride was 1931 mg product/kg bw (796 mg ai/kg bw). According to the US EPA classification, this formulation of Cycocel (containing 41.2% chlorcholine chloride and 28.8% choline chloride) would be classified as practically non-toxic to Japanese quail on an acute oral basis.

Aside from a single death on day 11 at the 1449 mg product/kg bw level, all mortality occurred within 24 hours of dosing and totaled 0, 0, 1, 0, 1, 2, and 4 at the 0, 241, 483, 966, 1449, 1931, and 2897 mg product/kg bw test levels, respectively. No apparent treatment-related effects on body weight or food consumption were observed. Necropsy examinations were not performed.

This toxicity study is scientifically sound, however, as numerous deviations from OPPTS 850.2100 were observed. This study does not fulfill the guideline requirement as is classified as SUPPLEMENTAL.

Results Synopsis

Test Organism Size/Age(Mean Weight): Young adult (not otherwise specified); group mean weights 109-120 g

1) Chlorocholine chloride (37.5% ai):
LD_{50}: 1482 mg product/kg bw 95% C.I.: 985-2124 mg product/kg bw
Probit slope: 5.52 95% C.I.: 1.55-9.49
NOAEL: 466 mg product/kg bw (body weight)

Based on a.i.
LD_{50}: 556 mg ai/kg bw 95% C.I.: 369-797 mg ai/kg bw
NOAEL: 175 mg ai/kg bw

Endpoint(s) Affected: mortality and body weight

2) Chlorocholine chloride (41.2% ai) + choline chloride:
LD_{50}: 2205 mg product/kg bw 95% C.I.: 1323-31999 mg product/kg bw
Probit slope: 2.50 95% C.I.: 0.361-4.64
NOAEL: 1931 mg product/kg bw

Based on a.i.
LD_{50}: 908 mg ai/kg bw 95% C.I.: 545-13184 mg ai/kg bw
NOAEL: 796 mg ai/kg bw

Endpoint(s) Affected: mortality

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was submitted to fulfill the OPPTS 850.2100 guideline requirement. Deviations from OPPTS 850.2100 included:

1. The batch number, physical description, and storage conditions of the test compounds were not reported.
2. The initial age, scientific name, and source of the test birds was not provided. Furthermore, northern bobwhite quail are the recommended species for upland game bird testing.
3. Acclimation of the test birds was not described.
4. The pen size allotted approximately 43 in^2 per bird, whereas the recommended floor size is 75 in^2 per bird.
5. The dosing volume was approximately 26 ml/kg bw, based on the 3 ml administered to control birds and an initial mean group weight of 0.115 kg. This dosing volume was more than three times the maximum allowable volume of 8 ml/kg bw.
6. Only five birds per test level was tested, whereas a minimum of 10 per level are required.
7. The birds were apparently not fasted prior to treatment.
8. The photoperiod was not reported.
9. Gross pathology examinations should have been conducted on at least two or three birds dying at each dosage level.
10. Group mean body weight data for the vehicle control group for the chlorocholine chloride experiment were apparently not provided.
11. The method used to calculate the 14-day LD_{50} and associated 95% C.I. for each test compound was not reported. In addition, NOAEL values were not provided for any endpoint.

These deficiencies do not affect the validity of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study preceded and therefore was not conducted according to 40 CF Part 160 GLP requirements.

A. MATERIALS:

1. Test Material 1) Chlorocholine chloride (ai)
2) Chlorocholine chloride (ai) + choline chloride

Description: Not reported

Lot No./Batch No. : Not reported

Purity: 1) 37.5%
2) 41.2% + 28.8%, respectively

Stability of compound under test conditions: Not reported [typically not necessary for acute oral (gavage) experiments]

Storage conditions of test chemicals: Not reported

Physicochemical properties of chlorocholine chloride.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test Organism

Species (common and scientific names): Japanese quail (not further specified)

Age at study initiation: Young adult (not further specified)

Weight at study initiation (mean and range): Group means of 109-120 g

Source: Not reported

(EPA recommends using either bobwhite quail or mallard duck. Birds should be at least 16 weeks old at test initiation and should be uniform in size and weight as well as phenotypically indistinguishable from wild birds).

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: None reported.

b. Definitive study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	Not reported	During the study, birds were offered a commercial chick diet *ad libitum*. Water was available at all times. *The recommended acclimation period is a minimum of 15 days. OECD recommends a minimum of 7 days.*
Pen size and construction materials	Metal cages measured 18 x 12 x 8"	The pen size allotted approximately 43 in^2 per bird. *Pen size and construction should conform to good husbandry practices and should not create crowding stress.* *OECD recommends that pens be suitable for the captive rearing of that species.*
Test duration	14 days	*Recommended test duration is one day for dosing and at least 14 days observation.*
Dose preparation [Indicate method of confirmation of dose]	Not specified	
Mode of dose administration	Oral gavage	*Gavage or gelatin capsule is recommended*
Dose levels nominal: measured:	Nominal 1) 0 (vehicle control), 233, 466, 932, 1398, 1864, and 2796 mg product/kg bw 2) 0 (vehicle control), 241, 483, 966, 1449, 1931, and 2897 mg product/kg bw Measured: Not reported	*Dose levels should be a minimum of 5 treatment levels unless LD_{50} is demonstrated to be greater than 2000 mg ai/kg*

Parameter	Details	Remarks *Criteria*
Solvent/vehicle, if used type: amount/bw:	 Corn oil Approx. 26 ml/kg bw (2.6% of bw)	Reviewer-calculated: 3 ml dosing volume for control birds ÷ 0.115 kg bw = 26 ml/kg bw. The maximum allowable volume is 8 ml/kg bw. *The test material should be administered without a vehicle if possible. Maximum vehicle should not exceed 0.1 to 1.0% of body weight.*
Number of birds per groups/treatment for negative control: for solvent/vehicle control: for treated:	 N/A 5 5/level	*Recommended number of birds in a treatment group is 10 and 10 birds for each control and vehicle group.*
No. of feed withholding days before dosing	Not reported	*Food should be withheld for at least 15 hours prior to dosing.*
Test conditions Temperature: Relative humidity: Photoperiod:	 60°F Not reported Not reported	*The recommended photoperiod is 10 hours of light and 14 hours of dark.*
Reference chemical, if used name: concentrations tested:	 N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured (mortality/individual body weight at test initiation and termination/ mean feed consumption/ others)	- Mortality - Clinical signs of toxicity - Body weight - Food consumption	Control mortality was 0% for both experimental groups. *Body weight should be measured at test initiation, on day 14 and at the end of the test if the test is extended beyond 14 days. Mortality should not be more than 10% in controls.* *Feed consumption should be measured as average daily food consumption.*

Criteria	Details	Remarks *Criteria*
Indicate if the test material was regurgitated	None reported.	*Regurgitation is an indication that the dose was rejected. If this problem persists, the test should be repeated.*
Groups on which necropsies were performed	None performed.	*Gross necropsies should be performed with inspections of the GI tract, liver, kidneys, heart, and spleen.*
Observation intervals	Birds were examined for mortality and clinical signs of toxicity several times following dosing, and once daily thereafter. Body weights were determined at 0, 7, and 14 days, and food consumption was determined at 7-day intervals.	
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

1) Chlorocholine chloride: All mortality occurred within 24 hours of dosing and totaled 0, 0, 0, 1, 2, 3, and 5 at the 0, 233, 466, 932, 1398, 1864, and 2796 mg product/kg bw test levels, respectively. The 14-day LD_{50} (with 95% C.I.) was 1479 (973-2237) mg product/kg bw, equivalent to 555 (365-839) mg ai/kg bw.

Table 3: Effect of Chlorocholine Chloride on Mortality of Japanese Quail.

Treatment (mg product/kg bw)		No. of Birds	Cumulative Mortality				
			day 1	day 2	day 3	day 7	day 14
Vehicle control		5	0	0	0	0	0
233		5	0	0	0	0	0
466		5	0	0	0	0	0
932		5	1	1	1	1	1
1398		5	2	2	2	2	2
1864		5	3	3	3	3	3
2796		5	5	5	5	5	5
NOAEL		Not reported					
LD_{50}		1479 (973-2237) mg product/kg bw [555 (365-839) mg ai/kg bw]					
Reference chemical	mortality	N/A					
	LD_{50}	N/A					
	NOAEL	N/A					

2) Chlorocholine chloride + choline chloride: Aside from a single death on day 11 at the 1449 mg product/kg bw level, all mortality occurred within 24 hours of dosing and totaled 0, 0, 1, 0, 1, 2, and 4 at the 0, 241, 483, 966, 1449, 1931, and 2897 mg product/kg bw test levels, respectively. The 14-day LD_{50} (with 95% C.I.) was 2348 (790-7301) mg product/kg bw, equivalent to 967 (325-3008) mg ai/kg bw.

Table 4: Effect of Chlorocholine Chloride + Choline Chloride on Mortality of Japanese Quail.

Treatment (mg product/kg bw)		No. of Birds	Cumulative Mortality				
			day 1	day 2	day 3	day 7	day 14
Vehicle control		5	0	0	0	0	0
241		5	0	0	0	0	0
483		5	1	1	1	1	1
966		5	0	0	0	0	0
1449		5	0	0	0	0	1
1931		5	2	2	2	2	2
2897		5	4	4	4	4	4
NOAEL		Not reported					
LD_{50}		2348 (790-7301) mg product/kg bw [967 (325-3008) mg ai/kg bw]					
Reference chemical	mortality	N/A					
	LD_{50}	N/A					
	NOAEL	N/A					

B. SUBLETHAL TOXICITY ENDPOINTS:

Birds became lethargic prior to death and there was a loss of muscle function. Surviving birds recovered rapidly and no other toxic signs were observed. The bird from the chlorcholine chloride + choline chloride study that died on day 11 was in very poor condition. No other clinical signs of toxicity were described.

No treatment-related effects on body weight changes or food consumption were reported. Statistical comparisons were not conducted for either endpoint. Reductions in body weight were observed in birds dosed at ≥932 mg product/kg bw, whereas slight increases were observed at the lower levels. The study author, however, noted that it is difficult to draw conclusions as the number of surviving birds in each group varies considerably.

Table 5: Sublethal Effect of Chlorocholine Chloride on Japanese Quail.

Treatment (mg product/kg bw)		Observation					
		body weight, g			food consumption, g/bird/day		other endpoint
		day 0	day 7	day 14	days 0-7	days 8-14	% affected
Vehicle control		NR[a]	NR	NR	15	15	N/A
233		115	119	122	12	13	N/A
466		117	118	118	14	12	N/A
932		115	109	111	9	14	N/A
1398		111	111	108	13	12	N/A
1864		109	106	107	14	10	N/A
2796		117	---[b]	---	---	---	N/A
NOAEL		Not reported					
EC_{50}		Not reported					
Reference chemical	effect	N/A					
	NOAEL	N/A					
	LD_{50}	N/A					

[a] Control bird body weights were not reported (NR).
[b] 100% mortality observed.

Table 6: Sublethal Effect of Chlorocholine Chloride + Choline Chloride on Japanese Quail.

Treatment (mg product/kg bw)		Observation					
		body weight, g			food consumption, g/bird/day		other endpoint
		day 0	day 7	day 14	days 0-7	days 8-14	% affected
Vehicle control		115	116	116	12	14	N/A
241		155	119	118	15	16	N/A
483		119	121	123	12	13	N/A
966		120	117	115	9	12	N/A
1449		114	114	114	10	10	N/A
1931		113	109	107	12	13	N/A
2897		116	114	115	14	9	N/A
NOAEL		Not reported					
EC_{50}		Not reported					
Reference chemical	effect	N/A					
	NOAEL	N/A					
	LD_{50}	N/A					

C. REPORTED STATISTICS:

The method used for calculating the 14-day LD_{50} and associated 95% C.I. for each test compound was not reported. No statistical analyses were applied for the endpoints of food consumption and body weight. NOAEL values were not provided for any endpoint.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer subject mortality data to the Probit slope model to determine the LD_{50} values via Toxanal statistical software. The NOAEL values for mortality were determined using Fisher's Exact test via Toxstat statistical software. Replicate data were not provided for body weight and food consumption, but the reviewer visually determined the NOAEL for the study based on apparent reductions in body weight at concentrations of 932 mg product/kg bw and higher (for the chlorcholine chloride formulation).

1) Chlorocholine chloride (37.5% ai):
LD_{50}: 1482 mg product/kg bw 95% C.I.: 985-2124 mg product/kg bw
Probit slope: 5.52 95% C.I.: 1.55-9.49
NOAEL: 466 mg product/kg bw (body weight)

Based on a.i.
LD_{50}: 556 mg ai/kg bw 95% C.I.: 369-797 mg ai/kg bw
NOAEL: 175 mg ai/kg bw

Endpoint(s) Affected: mortality and body weight

2) Chlorocholine chloride (41.2% ai) + choline chloride:
LD_{50}: 2205 mg product/kg bw 95% C.I.: 1323-31999 mg product/kg bw
Probit slope: 2.50 95% C.I.: 0.361-4.64
NOAEL: 1931 mg product/kg bw

Based on a.i.
LD_{50}: 908 mg ai/kg bw 95% C.I.: 545-13184 mg ai/kg bw
NOAEL: 796 mg ai/kg bw

Endpoint(s) Affected: mortality

E. STUDY DEFICIENCIES:

There were no deficiencies that affected the scientific soundness of this study. However, numerous deficiencies from OPPTS 850.2100 guideline were observed. The most notable deviations included: the Japanese quail is not the required species for acute oral toxicity testing of an upland game bird; detailed descriptions of the methods were not provided (e.g., batch number and storage conditions of test materials, initial age and acclimation of birds, fasting prior to treatment, photoperiod, method used for calculation of LD_{50} values); the dosing volume exceeded the maximum allowable by >3X; an insufficient number of birds were tested; and gross pathological examinations were not performed on decedent birds. Although these deficiencies do not affect the scientific integrity of the study, this study does not fulfill guideline requirements.

F. REVIEWER'S COMMENTS:

The reviewer's LD_{50} values were similar to the study authors'. The reviewer additionally determined NOAEL values for mortality and body weight. The reviewer's results are presented in the Executive Summary and Conclusions sections.

This study was conducted during June and July 1974 using two formulations of Cycocel.

G. CONCLUSIONS:

This study is scientifically sound, however, as numerous deviations from OPPTS 850.2100 were observed, this study does not fulfill the guideline requirement as is classified as SUPPLEMENTAL.

1) Chlorocholine chloride (37.5% ai):
LD_{50}: 1482 mg product/kg bw 95% C.I.: 985-2124 mg product/kg bw
Probit slope: 5.52 95% C.I.: 1.55-9.49
NOAEL: 466 mg product/kg bw (body weight)

Based on a.i.
LD_{50}: 556 mg ai/kg bw 95% C.I.: 369-797 mg ai/kg bw
NOAEL: 175 mg ai/kg bw

Endpoint(s) Affected: mortality and body weight

2) Chlorocholine chloride (41.2% ai) + choline chloride:
LD_{50}: 2205 mg product/kg bw 95% C.I.: 1323-31999 mg product/kg bw
Probit slope: 2.50 95% C.I.: 0.361-4.64
NOAEL: 1931 mg product/kg bw

Based on a.i.
LD_{50}: 908 mg ai/kg bw 95% C.I.: 545-13184 mg ai/kg bw
NOAEL: 796 mg ai/kg bw

Endpoint(s) Affected: mortality

III. REFERENCES:

A reference list was not provided.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

CCC

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1614.271

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.4514306	1456.144	1026.225	2212.074

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.5174307	1	.9280158

SLOPE = 5.517826
95 PERCENT CONFIDENCE LIMITS = 1.548707 AND 9.486944

LC50 = 1482.551
95 PERCENT CONFIDENCE LIMITS = 984.8499 AND 2124.387

LC10 = 872.6628
95 PERCENT CONFIDENCE LIMITS = 194.3784 AND 1196.873

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	5	0	
1	233	5	0	
2	466	5	0	
3	932	5	1	
4	1398	5	2	
5	**1864**	**5**	**3**	
6	2796	5	5	*

CCC + CC

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 2129.412

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	1.221141	2101.575	0	+INFINITY

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.7321221	1	.3226828

SLOPE = 2.498888

95 PERCENT CONFIDENCE LIMITS = .3607361 AND 4.637039

LC50 = 2204.71
95 PERCENT CONFIDENCE LIMITS = 1322.791 AND 31999.43

LC10 = 684.1094
95 PERCENT CONFIDENCE LIMITS = 5.788461 AND 1173.845

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	5	0	
1	241	5	0	
2	483	5	1	
3	966	5	0	
4	1449	5	1	
5	**1931**	**5**	**2**	
6	2897	5	4	*