US EPA ARCHIVE DOCUMENT

Data Evaluation Report on the Acute Oral Toxicity of IODOMETHANE to Avian Species Bobwhite Quail
PMRA Submission Number {.....} EPA MRID Number 45593716

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D280800
OECD Data Point	{.....}
EPA MRID	45593716
EPA Guideline	71-1

Test material: IODOMETHANE
Common name: IODOMETHANE
chemical name: IUPAC: (+)-(4S)-4-methyl-2-methylthio-4-phenyl-(1H)-1-phenylamino-2-imidazolin-5-one
CAS name: Not reported
CAS No.: 161326-34-7
Synonyms: Not reported

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature: Rebecca Bryan
Date: 3/28/02

QC Reviewer: Teri Myers, Ph.D.
Staff Scientist, Dynamac Corporation
Signature: Teri Myers
Date: 3/28/02

~~**Primary**~~ Secondary **Reviewer:** James Felkel
{EPA/OECD/PMRA}
Date: 9/17/02 J. Felkel

~~**Secondary**~~ Other **Reviewer(s):**
{EPA/OECD/PMRA}
Date:

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 000011

Date Evaluation Completed:

CITATION: Gallagher, S.P. and J.B. Beavers. 2001. IODOMETHANE: An Acute Oral Toxicity Study with the Northern Bobwhite Quail. Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory project 443-101. Study sponsored by Arvesta Corporation, San Francisco, California. Study initiated December 12, 2000 and completed February 2, 2001.

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EXECUTIVE SUMMARY:

The acute oral toxicity of IODOMETHANE to 19-week old bobwhite quail, *Colinus virginianus*, was assessed over 14 days. IODOMETHANE was administered to the birds by oral intubation at nominal concentrations of 0, 5, 10, 20, 40, 80, and 160 mg/kg. There was 100% mortality in the 80 and 160 mg/kg treatment levels. No mortality was observed at any other treatment level. Change in mean body weight was significantly reduced for males at the 40 mg/kg treatment level, but not for females. Food consumption was not significantly affected by IODOMETHANE during the study. Signs of toxicity were observed in the 20, 40, 80, and 160 mg/kg treatment levels. The 14-day acute oral LD_{50} was 57 mg/kg. According to the US EPA classification, IODOMETHANE is categorized as moderately toxic to bobwhite quail on an acute oral basis. The 14-day NOEC of IODOMETHANE was 10 mg/kg , based on signs of toxicity, including ruffled appearance and lethargy at the 20 mg/kg level.

This toxicity study is scientifically sound and is classified as Core. This study fulfills the US EPA requirements (Subdivision E, §71-1) for an acute avian oral toxicity study.

Results Synopsis

Test Organism Size/Age(mean Weight): 184 g; approx. 19 weeks (p. 8)
LD_{50}: 57 mg/kg 95% C.I.: 40 to 80 mg/kg
NOEC: 10 mg/kg (signs of toxicity) Probit Slope: N/A
Endpoint(s) Affected: mortality, male body weight change, and signs of toxicity

I. MATERIALS AND METHODS REPORTED

GUIDELINE FOLLOWED: Methods reported to be "based upon" USEPA Series 850 - Ecological Effects guidelines OPPTS Number 850.2100(1) and FIFRA, Subdivision E § 71-1.

Guideline deviations identified by reviewer(s) include:

The photoperiod (8:16) was less than required (10:14). This deviation was considered to be minor, so it did not affect the acceptability or the validity of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material IODOMETHANE

Description: Liquid

Lot No./Batch No. : Lot 007403-Batch 02

Purity: 99.7%

Stability of Compound Under Test Conditions: Not reported.
(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage Conditions of Test Chemicals: Ambient temperature.

2. Test organism:

Species (common and scientific names): Northern bobwhite quail, *Colinus virginianus*
Age at study initiation: 19 weeks old
Weight at study initiation: (mean and range): 184 g; range: 181-220 g
Source: Morris Quail Farm, Inc., Goulds, FL
(EPA recommends that either bobwhite quail or mallard duck be used. 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: N/A

b) Definitive Study

Table 1. Experimental Parameters

Parameter	Reported Details	Remarks *Criteria*
Acclimation period: conditions (same as test or not): feeding: health (any mortality observed):	17 days Same as test Birds were fed a game bird ration. Diet composition is listed on p. 24. Healthy birds were used.	*EPA recommends that birds be pre-conditioned to the test facilities for at least 15 days.* *OECD recommends that birds be pre-conditioned to the test facilities for at least 7 days.*
Pen size and construction materials	78 x 51cm floor space and 20-25 cm ceiling height; constructed of galvanized wire (side walls of galvanized sheeting).	*EPA requires: pens must conform to good husbandry practices and should not create crowding stress.* *OECD lists no criteria for pen construction other than stating that pens should be suitable for the captive rearing of that species.*
Test duration	14 days	*EPA requires a day for dosing and at least 14 days observation.*
Dose preparation [Indicate method of confirmation of dose]	The test substance was mixed with corn oil. The concentration of the test substance in the diluent was reported to be adjusted to provide a constant volume to body weight dosage for all treatment birds. A single preparation was made at six dose concentrations. Concentrations were not corrected for purity of the test substance.	
Mode of dose administration	Oral intubation using a stainless steel 14 gauge canula.	*Gavage or gelatin capsule.*
Dose levels nominal: measured:	0, 5, 10, 20, 40, 80, and 160 mg/kg Not reported.	*EPA requires a minimum of 5 treatment levels unless LD_{50} is demonstrated to be greater than 2000 mg ai/kg*
Solvent/vehicle, if used type: amount/bw:	Corn oil Final volume of the test substance and corn oil was 30mL in stock solutions. Dose volume was 4 ml/kg of body weight.	*EPA recommends that the test material be administered without a vehicle if possible. Maximum vehicle should not exceed 0.1 to 1.0% of body weight.*

Parameter	Reported Details	Remarks *Criteria*
Number of birds per groups/treatment for negative control: for solvent/vehicle control: for treated:	N/A 10 10 (5 males in one pen/5 females in second pen for control and treated)	*EPA recommends 10 birds per treatment group and 10 birds for each control and vehicle group.*
No. of feed withholding days before dosing	18 hours	*EPA recommends that food should be withheld for at least 15 hours prior to dosing.*
Test conditions Temperature: Relative humidity: Photoperiod:	22.2 ± 0.9°C 22 ± 17% 8 hours light/16 hours dark	The photoperiod was less than required. --- *EPA recommends that a 10 hr light/14 hr dark photo-period.*
Reference chemical, if used name: concentrations tested:	None used	

2. Observations:

Table 2: Observations

Criteria	Reported Details	Remarks *Criteria*
Parameters measured (mortality/individual body weight at test initiation and termination/ mean feed consumption/ others)	Mortality, body weight, and food consumption	*EPA recommends:* *Body weight measured at test initiation, on Day 14 and at end of the test if the test is extended beyond 14 days.* *Calculation of mortality. Mortality must NOT be more than 10% in controls.* *Feed consumption may be measured as average daily food consumption.*
Indicate if the test material was regurgitated	Regurgitation was not reported.	*Regurgitation is an indication that the does was rejected. The test may have to be repeated if the problem persists.*
Groups on which necropsies were performed	All test birds (see p. 18 re birds found dead).	*EPA recommends that gross necropsies be performed with inspections of the GI tract, liver, kidneys, heart, and spleen.*

Criteria	Reported Details	Remarks *Criteria*
Observation intervals	Mortality: daily. Body weight: days 0, 3, 7, and 14 Food consumption: the intervals of days 0 to 3, 4 to 7, and 8 to 14.	
Were raw data included?	Raw data for mortality and body weight were included. Mean food consumption data were provided.	

II. REPORTED RESULTS AND DISCUSSION:

A. MORTALITY:

By day 14, there was 100 % mortality in the 80 and 160 mg/kg treatment levels. No mortality was observed at any other treatment level.

Table 3: Effect of IODOMETHANE on mortality of bobwhite quail.

Nominal Treatment (mg/kg)		No. of birds	Cumulative mortality													
			d 1	d 2	d 3	d 4	d 5	d 6	d 7	d 8	d 9	d 10	d 11	d 12	d 13	d 14
Vehicle control		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80		10	9	10	10	10	10	10	10	10	10	10	10	10	10	10
160		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
NOEC		40 mg/kg														
LD_{50}		57 mg/kg														
Reference chemical	mortality	Not applicable.														
	LD_{50}															
	NOEC															

B. SUBLETHAL TOXICITY ENDPOINTS:

There were no treatment related effects on bodyweight in the 5 or 10 mg/kg treatment groups. Body weight losses were observed in the males of the 20 and 40 mg/kg treatment levels and the females in the 40 mg/kg treatment level from days 0-3.

There were no treatment related effects on food consumption in the 5, 10, or 20 mg/kg treatment levels. The feed consumption was reduced from days 0 to 3 in the 40 mg/kg treatment level.

The signs of toxicity, which appeared between 15 minutes and 3.5 hours after dosing, were ruffled appearance and lethargy in the 20 mg/kg level; ruffled appearance, lethargy, reduced reaction to external stimuli (sound and movement), and loss of coordination in the 40 mg/kg level; ruffled appearance, lethargy, reduced reaction to external stimuli (sound and movement), loss of coordination, lower limb weakness, prostrate posture, wing droop, minor muscle fasciculation, depression, convulsions, and comatose condition in the 80 mg/kg level; and lethargy, reduced reaction to external stimuli (sound and movement), loss of coordination, lower limb weakness, prostrate posture, wing droop, minor muscle fasciculation, depression, and convulsions in the 160 mg.kg level. These signs appeared dose responsive and treatment-related. There were no signs of toxicity in the control, 5, and 10 mg/kg treatment levels.

The gross necropsy revealed the presence of a gelatinus film or gelatinus material surrounding the crop. In several instances, the vessels of the crop wall were congested and the crop contained a clear fluid (presumably the dosing solution). Pale and/or small spleens and pale kidneys were noted in some birds. Details of the necropsy results are in Table 2, page 18.

Table 4: Sublethal effect of IODOMETHANE on bobwhite quail.

Treatment (mg/kg)		Observation							
		Mean body weight (g)				Mean food consumption			other endpoint
		day 0	day 3	day 7	day 14	days 0 to 3	days 4 to 7	days 8 to 14	
Vehicle control	M	198	204	207	208	23	21	14	None
	F	197	200	204	206	19	22	16	
5	M	198	202	206	209	16	18	13	
	F	198	200	205	207	23	24	16	
10	M	193	196	199	200	23	25	14	
	F	199	202	206	209	13	19	13	
20	M	202	199	205	209	20	23	16	
	F	194	199	201	201	19	21	15	
40	M	198	188	194	202	14	19	18	
	F	197	186	189	198	11	24	17	
80	M	204	-[1]	-	-	-	-	-	
	F	194	-	-	-	-	-	-	

Treatment (mg/kg)		Observation							
		Mean body weight (g)				Mean food consumption			other endpoint
		day 0	day 3	day 7	day 14	days 0 to 3	days 4 to 7	days 8 to 14	
160	M	200	-	-	-	-	-	-	
	F	194	-	-	-	-	-	-	
NOEC		10 mg/kg							
EC_{50}		Not reported							
Reference chemical	effect	Not applicable							
	NOEC								
	LD_{50}								

M = Male
F = Female
[1] No body weights, due to 100% mortality.

C. REPORTED STATISTICS: Mortality was analyzed using C.E. Stephan computer program. The LD_{50} value was determined using non-linear interpolation.

LD_{50}: 57 mg/kg 95% C.I.: 40 to 80 mg/kg
NOEC: 10 mg/kg Probit Slope: N/A
Endpoint(s) Affected: body weight, feed consumption, signs of toxicity

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The NOEC for mortality was determined visually. The LD_{50} was estimated using the bionomial method. Total change in body weight data for males and females was confirmed to be normally distributed and the variances were homogeneous. Data were subsequently analyzed using ANOVA, followed by Dunnett's test and William's test (for dose-dependent responses) to determine if treatment groups differed from the controls.

LD_{50}: 57 mg/kg 95% C.I.: 40 to 80 mg/kg
NOEC: 10 mg/kg (signs of toxicity) Probit Slope: N/A
Endpoint(s) Affected: mortality, male body weight change, and signs of toxicity

E. STUDY DEFICIENCIES:

The photoperiod was less than required (8:16 vs 10:14). This deviation was considered to be minor, so it did not affect the acceptability or the validity of the study.

F. REVIEWER'S COMMENTS:

With the exception of body weight change, the reviewer's conclusions were identical to the study authors. The 14-day

acute oral LD_{50} was 57 mg/kg, which categorizes IODOMETHANE as moderately toxic to bobwhite quail on an acute oral basis. The 14-day NOEC of IODOMETHANE was 10 mg/kg , based on signs of toxicity, including ruffled appearance and lethargy at the 20 mg/kg level. The reviewer analyzed total change in body weight (as opposed to body weight change for three separate intervals) and detected a significant reduction for males only at the 40 mg/kg treatment level.

G. CONCLUSIONS: This toxicity study is scientifically sound and is classified as Core. There was 100% mortality in the 80 and 160 mg/kg treatment groups. No mortalities were observed at any other treatment level. Total mean body weight losses were detected at the 40 mg/kg treatment level for males and feed consumption was unaffected by treatment. Signs of toxicity were observed in the 20, 40, 80, and 160 mg/kg treatment levels. The 14-day-acute oral LD_{50} was 57 mg/kg. According to the US EPA classification, IODOMETHANE is categorized as moderately toxic to bobwhite quail on an acute oral basis. The 14-day NOEC of IODOMETHANE was 10 mg/kg , based on signs of toxicity, such as ruffled appearance and lethargy. This study fulfills the US EPA requirements (Subdivision E, §71-1) for an acute avian oral toxicity study.

LD_{50}: 57 mg/kg 95% C.I.: 40 to 80 mg/kg
NOEC: 10 mg/kg (signs of toxicity) Probit Slope: N/A
Endpoint(s) Affected: body weight, feed consumption, signs of toxicity

III. REFERENCES:

U.S. Environmental Protection Agency, 1996. Series 850-Ecological Effects Test Guidelines (draft), OPPTS Number 850.2100: Avian Acute Oral Toxicity Test.

U.S. Environmental Protection Agency, 1982 (October). *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Subsection 71-1. Office of Pesticide Programs*. Washington, D.C. 86 pp.

National Research Council. 1996 *Guide for the Care and Use of Laboratory Animals*. Washington, D.C. National Academy Press. 125 pp.

Stephan, C.E. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal Communication.

Finney, D.J. 1971. *Statistical Methods in Biological Assay*, 2nd edition, Griffin Press, London.

Thompson, W.R. 1947. *Bacteriological Reviews*. Vol II, 2:115-145.

Stephan, C.E. 1977. Methods for Calculating an LC50. *Aquatic Toxicology and Hazard Evaluations*, Amer. Soc. Test Mat., Pub. No. STP 634:65-84.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

male body weight change
File: 3716bwcm Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	125.760	31.440	2.491
Within (Error)	20	252.400	12.620	
Total	24	378.160		

Critical F value = 2.87 (0.05,4,20)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

male body weight change
File: 3716bwcm Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	9.200	9.200		
2	5	10.800	10.800	-0.712	
3	10	7.200	7.200	0.890	
4	20	6.200	6.200	1.335	
5	40	4.400	4.400	2.136	

Dunnett table value = 2.30 (1 Tailed Value, P=0.05, df=20,4)

male body weight change
File: 3716bwcm Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	5			
2	5	5	5.168	56.2	-1.600
3	10	5	5.168	56.2	2.000
4	20	5	5.168	56.2	3.000
5	40	5	5.168	56.2	4.800

male body weight change
File: 3716bwcm Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	5	9.200	9.200	10.000
2	5	5	10.800	10.800	10.000

3	10	5	7.200	7.200	7.200
4	20	5	6.200	6.200	6.200
5	40	5	4.400	4.400	4.400

male body weight change
File: 3716bwcm Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	10.000				
5	10.000	0.356		1.72	k= 1, v=20
10	7.200	0.890		1.81	k= 2, v=20
20	**6.200**	**1.335**		**1.83**	**k= 3, v=20**
40	4.400	2.136	*	1.85	k= 4, v=20

s = 3.552
Note: df used for table values are approximate when v > 20.

female body weight change
File: 3716bwcf Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	292.160	73.040	1.457
Within (Error)	20	1002.400	50.120	
Total	24	1294.560		

Critical F value = 2.87 (0.05,4,20)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

female body weight change
File: 3716bwcf Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	9.400	9.400		
2	5	9.800	9.800	-0.089	
3	10	7.000	7.000	0.536	
4	20	0.600	0.600	1.965	
5	40	4.400	4.400	1.117	

Dunnett table value = 2.30 (1 Tailed Value, P=0.05, df=20,4)
fEmale body weight change
File: 3716bwcf Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	5			

2	5	5	10.298	109.6	-0.400
3	10	5	10.298	109.6	2.400
4	20	5	10.298	109.6	8.800
5	40	5	10.298	109.6	5.000

female body weight change
File: 3716bwcf Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	5	9.400	9.400	9.600
2	5	5	9.800	9.800	9.600
3	10	5	7.000	7.000	7.000
4	20	5	0.600	0.600	2.500
5	40	5	4.400	4.400	2.500

female body weight change
File: 3716bwcf Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	9.600				
5	9.600	0.045		1.72	k= 1, v=20
10	7.000	0.536		1.81	k= 2, v=20
20	2.500	1.541		1.83	k= 3, v=20
40	2.500	1.541		1.85	k= 4, v=20

s = 7.080
Note: df used for table values are approximate when v > 20.