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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND
TOXIC SUBSTANCES

June 28, 2002

MEMORANDUM

Product Name: Advantix™ 10 for Dogs
EPA File Symbol: 11556-RGE
DP Barcode: D280825
Case No: 071346
Submission: S608992
Chemicals: 109701 Permethrin, mixed cis, trans
129099 Imidacloprid

From: Byron T. Backus, Ph.D., Toxicologist
Technical Review Branch
Registration Division (7505C)

To: William Sproat/Arnold Layne, PM 03
Insecticide Branch
Registration Division (7505C)

Registrant: Bayer Corp.

ACTION REQUESTED: "Please review the attached studies (MRID 45563010, 45563009, 45563007, 45563008, 45563006, 45563005, 45563004, 45563003, 45563002), label data matrix and CSF for EPA Reg. No. 11556-RGE. This product is identical to 11556-RGL, RGU and RGG..."

BACKGROUND: According to the label received by TRB, this product [Advantix™ 10 Topical Solution] has the following ingredient declaration:

Active Ingredients:
Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine.....8.8%
*Permethrin.....44.0%
Inert Ingredients:.....47.2%
*cis/trans ratio: Max 55% (±) cis and min 45% (±) trans

1/53
(1)

As part of this package, TRB has received the following acute toxicity studies: acute oral LD50 in rats (MRID 45563004); acute dermal LD50 in rats (MRID 45563005); acute inhalation LC50 in rats (MRID 45563006); primary eye irritation in rabbits (MRID 45563007); primary dermal irritation in rabbits (MRID 45563008), and dermal sensitization in guinea pigs (MRID 45563009) with a validation (positive control study) for the dermal sensitization assay (MRID 45563010). In addition, there are two companion animal safety studies, one in adult dogs (MRID 45563002) and one in puppies (MRID 45563003).

COMMENTS AND RECOMMENDATIONS:

1. The study titled: "General Safety of Imidacloprid with Permethrin Topical Solution in the Dog" (MRID 45563002) generally followed the pertinent guidelines for a companion animal safety study (OPPTS 870.7200), and is classified as Acceptable in demonstrating an adequate (approximately 5X in terms of overt symptoms) margin of safety in adult dogs. While administration of the vehicle or 5X the label dosage of test material on days 7 and 14 was followed by a decrease in food consumption on days 8 and 15, food consumption quickly recovered in a day or so. It is noted that not only were these dogs treated at a 5X single-dose application rate, but that they were also given four 5X treatments in a 21-day period (as the label specifies once-a-month treatment at the 1X label, these dogs then received a cumulative of 20X the monthly dose specified on the label). Refer to the attached DER for MRID 45563002 for a complete executive summary.

2. The study titled: "Evaluation of the General Safety of 8.8% Imidacloprid with 44.0% Permethrin Formulation in the Target Species, 7-Week Old Puppies" (MRID 45563003) generally followed the pertinent guidelines for a companion animal safety study (OPPTS 870.7200), and is classified as Acceptable in demonstrating an adequate (approximately 5X in terms of overt symptoms) margin of safety in puppies. While treated puppies did not eat as much as their controls following on days 1 and 8 following applications of the test material on days 0 and 7, their food consumption recovered in a day or so, and there was little or no evidence of any reduction in food consumption following the third and fourth applications of the test material. It is also noted that not only were these puppies treated at a 5X single-dose application rate, but that they were also given four 5X treatments in a 21-day period (as the label specifies once-a-month treatment at the 1X label, these puppies then received a cumulative of 20X the monthly dose specified on the label). Refer to the attached DER for MRID 45563003 for a complete executive summary.

3. The six submitted acute toxicity studies (MRIDs 45563004 through 45563009) have also been reviewed and have been classified as acceptable. It is noted that the dermal sensitization study (in MRID 45563009) was not validated by the positive control study in MRID 45563010, as that was dated approximately 21 months prior to the study in MRID 45563009. However, the study in MRID 45563009 was validated by a study (Bayer AG Report No. 32137, dated June 27, 2002, with experimental dates January 15, 2002 to February 14, 2002) which was made available by Bayer.

4. Based on the results of the reviewed acute toxicity studies, the following is the acute toxicity profile for EPA File Symbol 11556-RGE, Advantix™ 10 Topical Solution:

Acute Oral LD50	Acceptable	Tox. Cat. III (MRID 45563004)
Acute Dermal LD50	Acceptable	Tox. Cat. III (MRID 45563005)
Acute Inhalation LC50	Acceptable	Tox. Cat. IV (MRID 45563006)
Primary Eye Irritation	Acceptable	Tox. Cat. II (MRID 45563007)
Primary Dermal Irritation	Acceptable	Tox. Cat. IV (MRID 45563008)
Dermal Sensitization	Acceptable	Negative (MRID 45563009)

5. Based on the acute toxicity profile given above and based on the proposed label use directions, the following is the appropriate precautionary labeling for this product, as obtained from the Label Review System:

PRODUCT ID #: 011556-00132

PRODUCT NAME: ADVANTIX™ 10 FOR DOGS

PRECAUTIONARY STATEMENTS

SIGNAL WORD: WARNING

Hazards to Humans and Domestic Animals:

Causes substantial but temporary eye injury. Harmful if absorbed through skin. Harmful if swallowed. Do not get in eyes. Avoid contact with skin or clothing.. Wash thoroughly with soap and water after handling. wash contaminated clothing before reuse.

First Aid:

If in eyes:
-Hold eye open and rinse slowly and gently with water for 15-20 minutes.
-Remove contact lenses, if present, after the first 5 minutes, then continue rinsing.
-Call a poison control center or doctor for treatment advice.

If on skin:
-Take off contaminated clothing.
-Rinse skin immediately with plenty of water for 15-20 minutes.
-Call a poison control center or doctor for treatment advice.

If swallowed:
-Call a poison control center or doctor immediately for treatment advice.
-Have person sip a glass of water if able to swallow.
-Do not induce vomiting unless told to by a poison control center or doctor.
-Do not give anything to an unconscious person.

NOTE TO PHYSICIAN: Note to PM/CRM/Registrant: The proposed label should contain a "Note to Physician". The following statements are suggested types of information that may be included, if applicable: - technical information on symptomatology; - use of supportive treatments to maintain life functions; - medicine that will counteract the specific physiological effects of the pesticide; - company telephone number to specific medical personnel who can provide specialized medical advice.

Have the product container or label with you when calling a poison control center or doctor or going for treatment. You may also contact 1-800-xxx-xxxx for emergency medical treatment information.

Reviewer:Byron T. Backus, Ph.D. _____ _____

Secondary Reviewer: _____ _____

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety/Dog [OPPTS 870.7200]

EPA I.D. NUMBERS: DP BARCODE: D280825; MRID NUMBER: 45563002

TEST MATERIAL: Imidacloprid with Permethrin Topical Solution; Imidacloprid 8.75% & Permethrin 43.66%; Lot No. A-01-061-G804-01-02-78

STUDY NUMBER 01-S26-HB

TESTING FACILITY: Bayer Corporation
Agriculture Division
Toxicology
17745 South Metcalf
Stilwell, KS 66085

SPONSOR: Bayer Corporation, Agriculture Division, Animal Health, 9009 W. 67th St., Merriam, KS 66202

TITLE OF REPORT: General Safety of Imidacloprid with Permethrin Topical Solution in the Dog

AUTHORS: R.E. Mueller

STUDY COMPLETION DATE: December 6, 2001

EXECUTIVE SUMMARY: ***In a companion animal safety study (MRID 45563002) Imidacloprid (8.8% w/w) and Permethrin (44.0% w/w) Topical Solution (Lot No. A-01-061-G804-01-02-78), containing 99.6 mg of imidacloprid and 496.8 mg permethrin per mL, was administered via syringe to the dorsal midline of each dog in a group of 6 male and 6 female young adult (10-11 months old) beagles at 5X the proposed use dosage rate on days 0, 7, 14 and 21. (The proposed 1X rate is 0.4 mL for dogs weighing ≤ 10 lbs; 1.0 mL for dogs weighing 11-20 lbs; 2.5 mL for dogs weighing 21-55 lbs, and 4.0 mL for dogs weighing ≥ 55 lbs.) A second (control) group of 6 male and 6 female dogs of the same age received a weekly 5X application of the test substance less the active ingredients on days 0, 7, 14 and 21. All test material males and 4/6 females received 12.5 mL/week/dog; 2/6 females consistently received 5.0 mL/week/dog. All placebo group males and 3/6 females received 12.5 mL/week/dog; 3/6 females consistently received 5.0 mL/week/dog. For test group males exposure to the actives ranged from 85.9 to 132.4 mg Imidacloprid/kg body weight and from 428.3 to 660.7 mg permethrin/kg body weight. For females, ranges were from 55.6 to 122 mg Imidacloprid/kg and from 279.2 to 608.9 permethrin/kg.***

Clinical observations were conducted hourly for 4 hr following each treatment; on other days each dog was observed at least twice a day. Blood was taken by jugular venipuncture (following

TABLE 3. Selected clinical observations in dogs treated		
Animal # & Treatment Group	**Sex**	**Observation**
HB0001 (placebo)	M	Normal throughout observation period
HB0002 (placebo)	M	Vomiting & mucoid feces (day 1); a few pinpoint-sized red areas at base of hair shafts at dose site (day 7); dry scabs (day 5).
HB0003 (placebo)	M	Mucoid feces (days 1 & 31); a few pinpoint-sized red areas at base of hair shafts at dose site (day 21); eye discharge (days 0-3, 5, 8-11, 32)
HB0004 (placebo)	M	Mucoid feces (day 1), vomiting (days 22 & 41)
HB0005 (placebo)	M	Vomiting (days 3, 10, 17, 26 & 39); mucoid feces (days 1 & 22)
HB0006 (placebo)	M	A few pinpoint-sized red areas at base of hair shafts at dose site (day 21)
HB0101 (placebo)	F	Vomiting (days 24 & 28)
HB0102 (placebo)	F	Vomiting (days 12 & 39); a few pinpoint-sized red areas at base of hair shafts at dose site (days 14 & 21)
HB0103 (placebo)	F	Vomiting (day 1); a few pinpoint-sized red areas at base of hair shafts at dose site (day 14)
HB0104 (placebo)	F	Vomiting (days 17, 24 & 27)
HB0105 (placebo)	F	Vomiting (day 1)
HB0106 (placebo)	F	Vomiting (day 8); eye discharge (days 0, 4 & 31)
HB1001 (5X)	M	Vomiting (days 20 & 35); mucoid feces (day 37)
HB1002 (5X)	M	Vomiting (days 11, 29 & 42); eye discharge (days 0 & 7); Loose or soft feces (days 12 & 13)
HB1003 (5X)	M	Loose or soft feces (days 12 & 27)
HB1004 (5X)	M	Mucoid feces (day 1)
HB1005 (5X)	M	Vomiting (days 25, 26, 27 & 40)
HB1006 (5X)	M	Normal throughout observation period
HB1101(5X)	F	Vomiting (days 13, 20, 26 & 32); Multiple pinpoint-sized red areas at base of hair shafts at dose site (days 14, 15, 16 & 17)
HB1102(5X)	F	Vomiting (days 27, 36 & 37)
HB1103(5X)	F	Normal throughout observation period
HB1104(5X)	F	Vomiting (day 21); Loose feces (day 20)
HB1105(5X)	F	Vomiting (days 13, 27, 28, 29, 30, 33, 40 & 41); Soft feces (day 20)
HB1106(5X)	F	Eye discharge (days 9, 10 & 11)

Data extracted from p. 235-246 of MRID 45563002

Mucoid feces and/or vomiting were observed in 7 dogs (6 controls, 1 5X) on day 1.

D. Bodyweight and weight gain

From information on pages 248-251 of MRID 45563002, no significant differences in mean bodyweight were observed among test groups during the study period. The 5X males showed a mean weight gain of 333.2 g in the period from day 0 to day 42; whereas their controls showed a mean weight loss of 224.6 g for the same period. The 5X females showed a mean

weight gain of 178.2 g from day 0 to day 42 and their controls showed a mean weight gain of 82.8 g for this period.

E. Food consumption

There were no significant differences between groups during the test period. However, there were noticeable drops in mean food consumption in both groups on days following application treatments. This was particularly evident on days 1 and 22, but it also occurred to a lesser extent on days 8 and 15:

Table 4 - Mean amounts of food consumed/dog on application days and the 2 days following (food consumption for the following day is in bold and underlined):

Group/ Sex	Day 0	Day 1	Day 2	Day 7	Day 8	Day 9
Vehicle/Male	365.50	**133.50**	262.67	308.67	**231.83**	391.33
5X/ Male	340.17	**175.17**	318.83	317.50	**281.17**	326.67
Vehicle/ Female	346.17	**83.50**	271.33	345.67	**294.17**	315.67
5X/ Female	282.0	**96.83**	297.00	271.33	**230.67**	291.00

Group/ Sex	Day 14	Day 15	Day 16	Day 21	Day 22	Day 23
Vehicle/Male	360.83	**212.17**	364.50	388.17	**90.33**	377.50
5X/ Male	374.83	**202.50**	298.83	396.33	**100.50**	384.67
Vehicle/ Female	239.00	**203.17**	271.33	337.67	**59.83**	379.50
5X/ Female	300.83	**205.00**	327.33	316.50	**85.83**	356.83

Data extracted from p. 256-268 of MRID 45563002

In addition, some dogs consumed no (or little) food on the days following administration of the control material or test material with actives. The following is a listing of dogs/group consuming less than 100 grams of food on the same days as those indicated above:

Table 5 - Number of dogs/group consuming less than 100 grams of food on:

Group/ Sex	Day 0	Day 1	Day 2	Day 7	Day 8	Day 9
Vehicle/Male	0	3	1	0	0	0
5X/ Male	0	0	1	0	0	0
Vehicle/ Female	0	2	0	0	0	0
5X/ Female	0	3	1	1	1	0

Group/ Sex	Day 14	Day 15	Day 16	Day 21	Day 22	Day 23
Vehicle/Male	0	2	0	0	2	1
5X/ Male	0	1	0	0	2	0
Vehicle/ Female	0	1	0	0	4	0
5X/ Female	0	1	0	1	3	0

Data extracted from p. 256-268 of MRID 45563002

For the 7 dogs (6 vehicle, one 5X) which exhibited vomiting and/or mucoid feces on day 1, the following were individual food consumptions on days 0, 1 and 2:

Table 6. Individual food consumptions on days 0, 1 and 2 of dogs which exhibited vomiting and/or mucoid feces on day 1.

Group/ Sex	Animal #	Day 0	Day 1	Day 2
Vehicle/Male	HB0002	326	173	182
Vehicle/Male	HB0003	417	31	303
Vehicle/Male	HB0004	400	283	317
Vehicle/Male	HB0005	399	0	377
Vehicle/Female	HB0103	223	159	302
Vehicle/Female	HB0105	443	145	364
5X/Male	HB1004	459	198	432

Data extracted from p. 256-260 of MRID 45563002

However, there were a considerable number of dogs with reduced food consumption on day 1 (male control: HB0006: 98 g; 5X males: HB1001: 143 g; HB1005: 105 g; female controls: HB0101: 80 g; HB0102: 3 g; HB0104: 114 g; HB0106: 0 g; 5X females: HB1101: 9 g; HB1102: 46 g; HB1104: 106 g; HB1105: 46 g) which did not show vomiting and/or mucoid feces.

A closer examination of the food consumption data indicates similar food consumption decreases on days -12, -5, and 39. As these were the days blood was taken (and blood was taken as well on days 1 and 22), it is concluded that the decreases in food consumption on days 1 and 22 involved blood collection procedures (and the fact - refer to p. 18 of MRID 45563002 - **that dogs were fasted overnight?**) on these days. However, the possibility of an additional contributing factor from exposure to the test material and/or vehicle (such as that observed on days 8 and 15) appears to be present.

Table 7 - Mean amounts of food consumed/dog on days -5 and day 39, as well as on the days preceding and following:

Group/ Sex	Day -6	Day -5	Day -4	Day 38	Day 39	Day 40
Vehicle/Male	242.00	**178.17**	383.00	334.50	**110.17**	445.50
5X/ Male	285.33	**177.33**	492.83	395.17	**172.50**	456.33
Vehicle/ Female	171.33	**133.83**	320.83	285.50	**73.33**	361.67
5X/ Female	218.67	**154.59**	334.67	323.50	**126.00**	371.50

F. Hematology

From information on p. 32 of MRID 45563002: "The day 1 monocytes...in 5X males was increased compared to the same sex concurrent control. This change was considered to not be adverse because values [4.4% and 2.9% respectively] were within the range of normal for healthy dogs measured in this lab. Day 1 WBC in 5X females was decreased ($p \leq 0.05$ vs concurrent control by ANOVA + Dunnett's Test) compared to the same sex concurrent control. The day 22 MONO [monocytes] in 5X females was increased ($p \leq 0.05$ vs concurrent control by ANOVA + Dunnett's Test). These changes were considered not adverse since they were not biologically meaningful and were well within our historical control range."

Although not stated in the text, the day 1 mean MONO for 5X females was also significantly increased relative to their controls (4.4% vs 3.4%); the day 22 values were 4.3% (for the 5X females) and 3.2% (for the female controls). These values are well within normal range and the differences are not biologically significant.

G. Clinical chemistry

LD (lactate dehydrogenase, expressed as U/L) values were noticeably - but apparently not significantly - elevated on day 22 in both groups:

Table 8 - Mean Lactic Dehydrogenase activities (with Standard Deviations) in U/L during the study:

Group/ Sex	Day -12	Day -5	Day 1	Day 22	Day 39
Vehicle/Male	100 (38)	83 (25)	108 (39)	125 (47)	104 (28)
5X/ Male	97 (44)	90 (29)	110 (36)	191 (83)	87 (31)
Vehicle/ Female	118 (37)	155 (44)	187 (54)	236 (181)	150 (56)
5X/ Female	115 (62)	110 (40)	167 (67)	215 (99)	124 (58)

Data extracted from p. 150-169 of MRID 45563002

Although the slight elevation in lactate dehydrogenase on day 22 (after 4 treatment applications) is suggestive, the data provided (see pages 214-217 of MRID 45563002) indicate these mean lactate dehydrogenase activities were well within historical ranges (males: 35-390; females: 41-356).

H. Necropsy findings

As there were no mortalities, no necropsies were performed.

IV. DISCUSSION

In a companion animal safety study (MRID 45563002) Imidacloprid (8.8% w/w) and Permethrin (44.0% w/w) Topical Solution (Lot No. A-01-061-G804-01-02-78), containing 99.6 mg of imidacloprid and 496.8 mg permethrin per mL, was administered via syringe to the dorsal midline of each dog in a group of 6 male and 6 female young adult (10-11 months old) beagles at 5X the proposed use dosage rate on days 0, 7, 14 and 21. (The proposed 1X rate is 0.4 mL for dogs weighing ≤ 10 lbs; 1.0 mL for dogs weighing 11-20 lbs; 2.5 mL for dogs weighing 21-55 lbs, and 4.0 mL for dogs weighing ≥ 55 lbs.) A second (control) group of 6 male and 6 female dogs of the same age received a weekly 5X application of the test substance less the active ingredients on days 0, 7, 14 and 21. All test material males and 4/6 females received 12.5 mL/week/dog; 2/6 females consistently received 5.0 mL/week/dog. All placebo group males and 3/6 females received 12.5 mL/week/dog; 3/6 females consistently received 5.0 mL/week/dog. For test group males exposure to the actives ranged from 85.9 to 132.4 mg Imidacloprid/kg body weight and from 428.3 to 660.7 mg permethrin/kg body weight. For females, ranges were from 55.6 to 122 mg Imidacloprid/kg and from 279.2 to 608.9 permethrin/kg.

Clinical observations were conducted hourly for 4 hr following each treatment; on other days each dog was observed at least twice a day. Blood was taken by jugular venipuncture (following overnight fasting) for hematology and clinical chemistry measurements on pre-treatment days -12, -5, and then during the treatment/observation period on days 1, 22 and 39. Physical examinations were conducted on days -3 and 42 [p. 16 of MRID 45563002 indicates the physical examination was on day 39]. Body weights were measured on days -13, -7, -1, 0, 7, 13, 14, 21, 28, 35 and 42. Food consumption was measured on a daily basis from days -13 to 42. The first application treatment was on August 27, 2001, which would indicate day 42 was October 8, 2001.

No mortality occurred. The report states that there was no evidence of adverse or irreversible clinical signs demonstrated by the animals on the days of dosing or at other times during the study. However, there were noticeable reductions in mean food consumption for both the placebo and 5X groups on days -12, -5, 1, 22, 39, and, to a lesser extent, for days 8 and 15 (the vehicle or test material was administered on days 0, 7, 14 and 21). The reduced food consumptions on days -12, -5, 1, 22 and 39 were presumably largely due to blood taking and/or overnight fasting, which (for days 1 and 22) would have masked any effects from exposure to the test material. However the reductions for days 8 and 15 (Day 8 relative to day 7: vehicle males: -24.9%; 5X males: -11.4%; vehicle females: -14.9%; 5X females: -15.0%; Day 15 relative to day 14: vehicle males: -41.2%; 5X males: -46.0%; vehicle females: -15.0%; 5X females: -31.9%) were presumably associated with administration of the vehicle and test material (the vehicle is not toxicologically inert). Mean food consumption values recovered in all groups for days 9 and 15.

There were sporadic occurrences of vomiting, mucoid feces, with a "clustering" of these around day 1. These may have resulted from stress on the dogs from the combination of fasting, blood taking and exposure to the vehicle or test material.

A number of dogs in both the vehicle and 5X groups showed a "few" to "multiple" pinpoint-sized red areas at the base of hair shafts at the dose site on days 7, 14 and/or 21. In one case, these red areas persisted for several days.

A few statistically significant increases in day 1 and/or day 22 monocytes in 5X males and/or 5X females relative to their vehicle controls were not considered as adverse effects because all resulting values were within the normal range for healthy dogs at this laboratory facility.

Mean lactate dehydrogenase activities were noticeably - but apparently not significantly - elevated on day 22 in both vehicle control and 5X dogs relative to earlier and subsequent mean values. However, they were still within the normal range.

All animals survived to the end of the study, and there were no terminal sacrifices (not required for this type of study).

It is noted that not only were these test material treated dogs received a 5X single-dose application rate, but that they were also given four 5X treatments in a 21-day period (as the label specifies once-a-month treatment at the 1X label, these dogs then received a cumulative of 20X the monthly dose specified on the label).

This study generally followed the pertinent guidelines for a companion animal safety study (OPPTS 870.7200), and is classified as Acceptable in demonstrating an adequate (approximately 5X in terms of overt symptoms) margin of safety. While administration of the vehicle or test material on days 7 and 14 was followed by a decrease in food consumption on days 8 and 15, food consumption recovered in a day or so

.Reviewer:Byron T. Backus, Ph.D. _____ _____

Secondary Reviewer: _____ _____

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety/Puppy [OPPTS 870.7200]

EPA I.D. NUMBERS: DP BARCODE: D280825; MRID NUMBER: 45563003

TEST MATERIAL: Imidacloprid with Permethrin Topical Solution; Imidacloprid 8.84% & Permethrin 43.73%; Lot No. A-01-048-G804-01-02-11

STUDY NUMBER 01-S26-DM

TESTING FACILITY: Bayer Corporation
Agriculture Division
Toxicology
17745 South Metcalf
Stilwell, KS 66085

SPONSOR: Bayer Corporation, Agriculture Division, Animal Health, 9009 W. 67th St., Merriam, KS 66202

TITLE OF REPORT: Evaluation of the General Safety of 8.8% Imidacloprid with 44.0% Permethrin Formulation in the Target Species, 7-Week Old Puppies

AUTHOR: R.D. Jones

STUDY COMPLETION DATE: December 4, 2001

EXECUTIVE SUMMARY: ***In a companion animal safety study (MRID 45563003) Imidacloprid (8.84% w/w) and Permethrin (43.73% w/w) Topical Solution (Lot No. A-01-048-G804-01-02-11), containing 100.8 mg of imidacloprid and 498.5 mg permethrin per mL, was dermally applied as a single 2 mL (5X the proposed use dosage rate of 0.4 mL) dose to the dorsal cervical area of each puppy in a group of 6 male and 6 female young (≤ 7 weeks old at initiation of dosage) on days 0, 7, 14 and 21. (The proposed 1X rate is 0.4 mL for dogs and puppies weighing ≤ 10 lbs; 1.0 mL for dogs weighing 11-20 lbs; 2.5 mL for dogs weighing 21-55 lbs, and 4.0 mL for dogs weighing ≥ 55 lbs.) A second (control) group of 6 male and 6 female dogs of the same age received an application of 1.0 mL vehicle (formulation without active ingredients) on days 0, 7, 14 and 21.***

For test group males on day 0 individual doses ranged from 931.4 mg test substance (82.3 mg Imidacloprid and 407.3 mg Permethrin)/kg to 1614 mg test substance (142.7 mg Imidacloprid and 795.6 mg Permethrin)/kg. In test group females individual doses on day 0 ranged from 1220.6 mg test substance (107.9 mg Imidacloprid and 533.7 mg Permethrin)/kg to 1581.1 mg test substance (139.8 mg Imidacloprid and 691.4 mg Permethrin)/kg. On day 28 in the treated group males individual doses ranged from 636.0 mg test substance (56.2 mg Imidacloprid and 278.1 mg

Permethrin)/kg to 1108.4 mg test substance (98.0 mg Imidacloprid and 484.7 mg Permethrin)/kg. In the treated group females individual doses on day 28 ranged from 807.7 mg test substance (71.4 mg Imidacloprid and 353.2 mg Permethrin)/kg to 1301.4 mg test substance (115.0 mg Imidacloprid and 569.1 mg Permethrin)/kg.

Clinical observations were conducted hourly for 4 hr following each treatment; on other days each puppy was observed at least twice a day.

Blood was collected by jugular venipuncture using a small gauge needle and vacutainer tubes on study days 0 (preexposure), 1, 22 and 35 for hematology and clinical chemistry parameters. Puppies were not fasted prior to blood collection. Physical examinations were conducted on days 1 and 35. Body weights were taken on days 0 (pretreatment), 13, 28 and 35 (or days 0, 12, 27 and 34). The first application treatment was on March 2, 2001, and the experimental completion date was the week of April 6, 2001.

One female puppy (DM1102) in the 5X dose group died on day 25. This puppy was necropsied and found to have had a congenital digestive/cardiac condition. However, it had been evident for some time that there was a problem (this puppy had weighed 1522 g on day 0, and had dropped to 1117 g on day 12; this puppy had also been noted as thin on day 9 and had subsequently been given occasional supplemental nutrition. It had also been single-housed starting on day 9, while the others were usually co-housed).

The report states that there was no evidence of adverse or irreversible clinical signs demonstrated by the puppies on the days of dosing or at other times during the study, and that the test substance and vehicle were well retained within the haircoat. However, while the report states (p. 26 of MRID 45563003) that: "Food consumption was not considered to have been influenced by compound administration (females or males) during the course of the study" mean food consumption for the 5X puppies on day 1 was considerably lower than that for their controls (food consumption data for both 5X and controls are for combined males and females); 323.75 g vs. 478.25 g. On day 8 mean food consumption for the 5X puppies was 580.25 g vs. the control value of 686.25 g. The food consumption differences on days 1 and 8 (day 1: 5X animals consumed 32.3% less food per pup than their controls; day 8: 5X puppies consumed 28.4% less food per pup than their controls) were the largest daily differences observed during this study. There was also a somewhat lower food consumption in the period from day 14 to day 16, although this was not as pronounced as those following the first two application treatments. There is no evidence that there was lower food consumption in the 5X puppies following the fourth (day 21) application treatment. It is also noted that 5X puppies recovered their appetites by the third day after each treatment.

There was a clustering of soft or mucoid red feces on day 20. In addition, red mucoid feces were noted in some of the puppies during the first four days of the study which were confirmed by fecal flotation as a mild, subclinical coccidiosis, and all puppies were treated with Tribrissen, 60 mg, sid for three days starting on day 4. Another puppy received a dose of kaopectate on day 3.

There was no evidence of any other adverse effect in these puppies. Sporadically statistically significant differences between groups with respect to hematology and clinical chemistry parameters were not biologically significant (values were still within the normal range, or mean values were skewed by the value from puppy DM1102).

It is noted that not only were these puppies treated at a 5X single-dose application rate, but that they were also given four 5X treatments in a 21-day period (as the label specifies once-a-month treatment at the 1X label, these puppies then received a cumulative of 20X the monthly dose specified on the label).

This study generally followed the pertinent guidelines for a companion animal safety study (OPPTS 870.7200).

The study is classified as Acceptable in demonstrating an adequate (approximately 5X in terms of overt symptoms) margin of safety. While treated puppies did not eat as much as their controls following on days 1 and 8 following applications of the test material on days 0 and 7, their food consumption recovered in a day or so, and there was little or no evidence of any reduction in food consumption following the third and fourth applications of the test material.

COMPLIANCE: Signed and dated Quality Assurance (p. 8-10), Data Confidentiality (p. 2), and Good Laboratory Practice Compliance (p. 3) Statements are included.

I. MATERIALS:

A. Test material: Imidacloprid (8.8% w/w) and Permethrin (44.0% w/w) Formulation, containing 100.8 mg of imidacloprid and 498.5 mg permethrin per mL. [refer to p. 23 of MRID 45563003]
Description: A liquid with a specific gravity of 1.14.
Lot/Batch No.: A-01-048-G804-01-02-11
Active Ingredients: Imidacloprid: 8.8%; Permethrin: 44.0%.
Storage Conditions: Room temperature, as supplied in a capped, amber glass bottle

B. Placebo: Topical Solution placebo (the test material less the active ingredients Imidacloprid and Permethrin)
Description: A liquid
Lot/Batch No.: A-01-049-G804-01-02-08
Active Ingredients: None
Storage Conditions: Room temperature, as supplied in a capped, amber glass bottle

C. Administration:

The puppies were treated on days 0, 7, 14 and 21 with a single 2 mL dermal dose of test substance or equivalent volume (1 mL) of vehicle applied to the dorsal cervical area. From p. 20 of MRID 45563003: "The hair coat of the animals was able to accommodate the volume applied."

D. Test animals

Species: Dog *(Canis familiaris)*
Breed: Beagle
Age and weight at study initiation: 7 weeks of age or less (from information on p. 34 of MRID 45563003 birthdates ranged from 01/12/01 to 01/22/01, so the puppies ranged in age from 39 to 49 days at the time of first dosing). From p. 15 of MRID 45563003: "The puppies

could not be shipped at an age younger than what was used in this study, and remain in good health for the study." Day 0 weights for puppies treated with the proposed product: Males: 1.413 - 2.448 kg; Females: 1.442 - 1.75; for puppies treated with the placebo: Males: 1.448 - 2.381 kg; Females: 1.488 - 1.906 kg.

Source: White Eagle Laboratories, Doylestown, PA

Housing: cohoused, two or three in stainless steel cages, except on days of dosage when they were housed individually (for observational purposes). One puppy with a congenital digestive/cardiac anomaly was housed individually from day 9 through day 25.

Diet: Puppy feeding formula (first 3 days), then a combination of puppy feeding formula and dry feed for 2 weeks, then dried feed, generally *ad libitum*. The one puppy with a congenital digestive/cardiac anomaly "was noted as being thin and was given occasional supplemental nutrition (NutriCal, vitamin B-complex, chicken baby food; subcutaneous lactated ringers) in response to progressive dehydration and emaciation."

Purina® Laboratory Canine, *ad libitum*, except for overnight fasts prior to blood collection.

Water: tap water, *ad libitum*

Environmental conditions:

Temperature: 64-84°F

Humidity: 30-70%

Photoperiod: 12 hr dark/light

Acclimation period: There was no acclimation period. From p. 33 of MRID 45563003: "Due to age requirements for the start of the test and availability of animals of that age, acclimation period was dispensed with."

Medications, therapies and immunizations: From p. 20 of MRID 45563003: "There were no meaningful concomitant medications administered during the treatment phase of the study that could be considered to have confounded the study. During the study several control and treated animals were noted to have red, mucoid feces that was confirmed by fecal flotation as a mild, subclinical coccidiosis. Therefore on day 4 each dog was administered Tribrissen, 60 mg, *sid* for three days. This is common veterinary practice in a kennel environment and was not considered to be a confounding variable. Vaccinations were administered to all puppies on day 5 for Parvovirus, and day 32 for Canine Distemper-Adenovirus Type 2-Parainfluenza-Parvovirus." From information on page 20 of MRID 45563003 one puppy was given a dose of Kaopectate on day 3.

Animal removal: From p. 20 of MRID 45563003: "After the animals were randomized into dose groups, no animal was removed from the study. However, puppy DM1102 [a female in the 5X Imidacloprid-Permethrin formulation exposure group] died of a congenital digestive/cardiac condition on day 25." This animal was not replaced.

II. STUDY DESIGN

A. In life dates

Start: March 2, 2001 (first application of the test material); end: the week of April 6, 2001 (April 6, 2001 would be 35 days after the first application of the test material).

B. Animal assignment/ Dosage and Administration

From p. 15 of MRID 45563003: "Each dog was uniquely identified with an ear tattoo." According to information on p. 36 cage cards were also used. From information received by this reviewer from Bayer on 5 June 2002 all puppies were assigned a study identification number (Study ID) and these numbers are cross-referenced in the following table:

Table 1 - animal assignment:

Group/ Sex	Animal	ID	Age at First Dose	Group/ Sex	Animal	ID	Birth Date
A/Male	DM0001	1AAF4	49 days	A/Female	DM0101	1AAJ6	40 days
A/Male	DM0002	1AAG4	49 days	A/Female	DM0102	1AAL6	39 days
A/Male	DM0003	1AAI2	42 days	A/Female	DM0103	1AAH4	43 days
A/Male	DM0004	1AAJ8	40 days	A/Female	DM0104	1AAH3	43 days
A/Male	DM0005	1AAH2	43 days	A/Female	DM0105	1AAK5	39 days
A/Male	DM0006	1AAG5	49 days	A/Female	DM0106	1AAI6	42 days
B/Male	DM1001	1AAF1	49 days	B/Female	DM1101	1AAI4	42 days
B/Male	DM1002	1AAG3	49 days	B/Female	DM1102	1AAI5	42 days
B/Male	DM1003	1AAF2	49 days	B/Female	DM1103	1AAJ7	40 days
B/Male	DM1004	1AAI1	42 days	B/Female	DM1104	1AAG6	49 days
B/Male	DM1005	1AAF3	49 days	B/Female	DM1105	1AAI3	42 days
B/Male	DM1006	1AAG2	49 days	B/Female	DM1106	1AAK5	39 days

Table taken from information on p. 34 of MRID 45563003, as well as material received via fax by this reviewer from Bayer on 5 June 2002.
Group A = placebo (vehicle at 1 mL/puppy/treatment); Group B = treated with proposed formulation (at 2 mL/puppy/treatment).

From p. 16 of MRID 45563003: "Twenty-four dogs were purchased and were randomly allocated (6/sex/dose) by body weight to study groups using a computerized randomization procedure..."

The test article was used as supplied. The proposed label use directions specify the application of the contents of one 0.4 mL tube on a once-a-month basis to dogs and puppies weighing less than 10 pounds (with 1X application rates of 1.0 mL for 11 to 20 lbs, 2.5 mL for 21 to 55 lbs, and 4.0 mL for >55 lbs). From p. 24 of MRID 45563003: "The six males and six females in group B weighed between 1.4 and 2.3 kg, and therefore they were dermally dosed with 2 mL of test substance for a 5X use rate volume exposure. The six males and six females in group A were treated with a 1 mL equivalent of vehicle (negative control) once a week for four consecutive weeks."

overnight fasting) for hematology and clinical chemistry measurements on pre-treatment days -12, -5, and then during the treatment/observation period on days 1, 22 and 39. Physical examinations were conducted on days -3 and 42 [p. 16 of MRID 45563002 indicates the physical examination was on day 39]. Body weights were measured on days -13, -7, -1, 0, 7, 13, 14, 21, 28, 35 and 42. Food consumption was measured on a daily basis from days -13 to 42. The first application treatment was on August 27, 2001, which would indicate day 42 was October 8, 2001.

No mortality occurred. The report states that there was no evidence of adverse or irreversible clinical signs demonstrated by the animals on the days of dosing or at other times during the study. However, there were noticeable reductions in mean food consumption for both the placebo and 5X groups on days -12, -5, 1, 22, 39, and, to a lesser extent, for days 8 and 15 (the vehicle or test material was administered on days 0, 7, 14 and 21). The reduced food consumptions on days -12, -5, 1, 22 and 39 were presumably largely due to blood taking and/or overnight fasting, which (for days 1 and 22) would have masked any effects from exposure to the test material. However the reductions for days 8 and 15 (Day 8 relative to day 7: vehicle males: -24.9%; 5X males: -11.4%; vehicle females: -14.9%; 5X females: -15.0%; Day 15 relative to day 14: vehicle males: -41.2%; 5X males: -46.0%; vehicle females: -15.0%; 5X females: -31.9%) were presumably associated with administration of the vehicle and test material (the vehicle is not toxicologically inert). Mean food consumption values recovered in all groups for days 9 and 15.

There were sporadic occurrences of vomiting, mucoid feces, with a "clustering" of these around day 1. These may have resulted from stress on the dogs from the combination of fasting, blood taking and exposure to the vehicle or test material.

A number of dogs in both the vehicle and 5X groups showed a "few" to "multiple" pinpoint-sized red areas at the base of hair shafts at the dose site on days 7, 14 and/or 21. In one case, these red areas persisted for several days.

A few statistically significant increases in day 1 and/or day 22 monocytes in 5X males and/or 5X females relative to their vehicle controls were not considered as adverse effects because all resulting values were within the normal range for healthy dogs at this laboratory facility.

Mean lactate dehydrogenase activities were noticeably - but apparently not significantly - elevated on day 22 in both vehicle control and 5X dogs relative to earlier and subsequent mean values. However, they were still within the normal range.

All animals survived to the end of the study, and there were no terminal sacrifices (not required for this type of study).

It is noted that not only were these test material treated dogs received a 5X single-dose application rate, but that they were also given four 5X treatments in a 21-day period (as the label specifies once-a-month treatment at the 1X label, these dogs then received a cumulative of 20X the monthly dose specified on the label).

This study generally followed the pertinent guidelines for a companion animal safety study (OPPTS 870.7200), and is classified as Acceptable in demonstrating an adequate (approximately 5X in terms of overt symptoms) margin of safety. While administration of the vehicle or test

material on days 7 and 14 was followed by a decrease in food consumption on days 8 and 15, food consumption quickly recovered in a day or so.

COMPLIANCE: Signed and dated Quality Assurance (p. 4), [No] Data Confidentiality (p. 2), and Good Laboratory Practice Compliance (p. 3) Statements are included.

I. MATERIALS:

A. Test material: Imidacloprid (8.8% w/w) and Permethrin (44.0% w/w) Topical Solution, containing 99.6 mg of imidacloprid and 496.8 mg permethrin per mL.
Description: A liquid with a specific gravity of 1.138 at 20°C.
Lot/Batch No.: A-01-061-G804-01-02-78
Active Ingredients: Imidacloprid: 8.8%; Permethrin: 44.0%.
Storage Conditions: Room temperature, as supplied in a capped, amber glass bottle

B. Placebo: Topical Solution placebo (the test material less the active ingredients Imidacloprid and Permethrin)
Description: A liquid
Lot/Batch No.: A-01-074-G804-01-02-77
Active Ingredients: None
Storage Conditions: Room temperature, as supplied in a capped, amber glass bottle

C. Administration:
The dogs were treated on days 0, 7, 14 and 21 using a syringe without a needle for application. The test material or placebo was administered at the 5X dose level from the dorsal mid-back to the cervical area, against the grain of the hair on the back in order for the applied material to come into contact with the skin.

D. Test animals

Species: Dog *(Canis familiaris)*
Breed: Beagle
Age and weight at study initiation: 10-11 months (birthdates ranged from 09/30/00 to 11/03/00). Weights for dogs treated with the proposed product: Males: 9.4 - 14.5 kg; Females: 8.4 - 13.3 kg; weights for dogs treated with the placebo: Males: 11.0 - 15.0 kg; Females: 8.3 - 11.3 kg.
Source: White Eagle Laboratories, Doylestown, PA
Housing: Individual, in stainless steel cages
Diet: Purina® Laboratory Canine, *ad libitum*, except for overnight fasts prior to blood collection.
Water: tap water, *ad libitum*
Environmental conditions:
Temperature: 18-29°C
Humidity: 30-70%
Photoperiod: 12 hr dark/light

Acclimation period: 27 days (July 31, 2001 to August 27, 2001), except for one dog which was received as a replacement on August 16, 2001, and released for study use on August 27, 2001 (11 days).

Medications, therapies and immunizations: From p. 19 of MRID 45563002: "No concomitant medication was administered during the treatment phase of the study. Upon receipt of each shipment, each dog was administered Tribrissen, 120 mg *sid* from August 1, 2001, through August 10, 2001, to prevent coccidiosis..." The replacement dog was treated with Tribrissen from August 16, 2001, through August 20, 2001. "This is common veterinary practice in a kennel environment and was not considered to be a confounding variable in this study. Dogs were vaccinated for Parvovirus on August 3, 2001 and Canine Distemper-Hepatitis-Parainfluenza-Parvovirus on August 15, 2001..." The replacement dog and most dogs received Canine Distemper-Hepatitis-Leptospirosis-Parainfluenza-Parvovirus on August 24, 2001."

Animal removal and replacement: One female in the test material group was removed from the study after assignment but prior to initiation of treatment "for fractious and aggressive behavior." This female was replaced with another female (selected as an age-matched replacement) received August 16, 2001.

II. STUDY DESIGN

A. In life dates

Start: July 31, 2001 (start of acclimation), August 27, 2001 (first application of test material); end: October 8, 2001 (day 42).

B. Animal assignment/ Dosage and Administration

"Each dog was uniquely identified with an ear tattoo. All dogs were assigned a study identification number (Study ID) and these numbers are cross-referenced in the following table:

Table 1 - animal assignment:

Group/ Sex	Animal	ID	Birth Date	Group/ Sex	Animal	ID	Birth Date
A/Male	HB0001	0AGO3	10/14/00	A/Female	HB0101	0AGW3	10/21/00
A/Male	HB0002	0AGV2	10/20/00	A/Female	HB0102	0AGW5	10/21/00
A/Male	HB0003	0AGL2	10/03/00	A/Female	HB0103	0AGR5	1016/00
A/Male	HB0004	0AGL3	10/03/00	A/Female	HB0104	0AGM5	10/06/00
A/Male	HB0005	0AGF1	09/30/00	A/Female	HB0105	0AGF7	09/30/00
A/Male	HB0006	0AGM1	10/06/00	A/Female	HB0106	0AGP3	10/15/00
B/Male	HB1001	0AGE2	11/03/00	B/Female	HB1101	0AGJ8	10/03/00
B/Male	HB1002	0AGN2	10/13/00	B/Female	HB1102	0AGN3*	10/13/00
B/Male	HB1003	0AGR1	10/16/00	B/Female	HB1103	0AGW6	10/21/00
B/Male	HB1004	0AGV3	10/20/00	B/Female	HB1104	0AGX6	10/24/00
B/Male	HB1005	0AGX1	10/24/00	B/Female	HB1105	0AGY6	10/28/00
B/Male	HB1006	0AGV1	10/20/00	B/Female	HB1106	0AGV5	10/20/00
-	-	-	-	B/Female	HB1102	0AGY7*	10/28/00

Table taken from information on p. 14 of MRID 45563002.
*0AGY7 female was excluded due to fractious and aggressive behavior after randomization. 0AGN3 female was the replacement for 0AGY7, and therefore was not randomized.
Group A = placebo (vehicle at 5X); Group B = treated with proposed formulation at 5X

"Twenty-four dogs...were randomly allocated (six/sex/dose) by body weight to study groups using DATATOX...a validated computerized randomization procedure... One dog excluded from this study for fractious and aggressive behavior (0AGY7-female), which was replaced by dog (0AGN3-female) 11 days prior to the first dose. Although dog 0AGN3 was not randomized, it was age matched to dog 0AGY7 and was acclimated to the facility for 11 days prior to the day of the first dose. The exclusion and replacement action had no measurable impact on the outcome of the study..."

The test article was used as supplied. "The one X (1X) Intended Labeled Use is: **0.4 mL**/≤10 lbs [4.5 kg], **1.0 mL**/11 to 20 lbs [5.0 - 9.1 kg], **2.5 mL**/21 to 55 lbs [9.2 - 25 kg], and **4.0 mL**/>55 lbs [>25 kg] for each of the four body weight ranges... Six male and six female dogs

received 5X the monthly use volume (**2.0 mL**/≤ 10 lbs [≤ 4.5 kg], **5.0 mL**/11 to 20 lbs [5.0 - 9.1 kg], **12.5 mL**/21 to 55 lbs [9.2 - 25 kg], and **20 mL**/>55 lbs [>25 kg] body weight)."

TABLE 2. Experimental design

Group	No. of animals		Dose Level	Number of treatments[a]
	Male	Female		
A	6	6	5X (vehicle)[b]	4
B	6	6	5X[c]	4

Data taken from information on p. 19, MRID 45563002.
[a]Treatments were on days 0, 7, 14 and 21.
[b]6/6 males and 3/6 females in group A were consistently dosed at 12.5 mL placebo/application; 3/6 females were consistently dosed at 5.0 mL placebo/application.
[c]6/6 males and 4/6 females in group B were consistently dosed at 12.5 mL formulation/application; 2/6 females were consistently dosed at 5.0 mL formulation/application.

"Group A consisted of six male and six female dogs that received weekly a dermal application (dorsal mid-line) of topical solution...minus the active ingredients for four weeks...at 5X the monthly dose. Group B consisted of six male and six female dogs that received weekly a dermal application (dorsal mid-line) of topical solution...plus the active ingredients...at 5X the monthly dose...for four weeks."

C. Study objective and dose rationale

From p. 11 of MRID 45563002: "This controlled laboratory study was designed to provide assurance that an adequate margin of safety exists for the use of Imidacloprid/ Permethrin Topical Solution (imidacloprid 8.8%/permethrin 44.0%) when administered dermally to dogs at five times (5X) the recommended dermal dose weekly for four consecutive weeks. Six, 10 - 11 month old dogs/sex/dose were administered a five (5X) times the recommended dermal unit dosage, or vehicle minus the active ingredients, weekly for four consecutive weeks..."

D. Clinical observations

From p. 20 of MRID 45563002: "the dogs were observed twice daily during acclimation and twice daily for the remainder of the study (except on the days of dosing), and no clinical signs dictated the need for more frequent observations. On dosing days, dogs were observed prior to dosing, hourly (±0.5 hr) for four hours post-dosing, with a four-hour observation which was used as the required P.M. observation. Observations included those specifically made for neurological signs, including depression, ataxia, mydriasis, abnormal salivation and abnormal muscle fasciculations..."

E. Pathological parameters

From p. 18 of MRID 45563002: "Blood was drawn by jugular venipuncture using small gauge needles and vacutainer tubes. On study days -12, -5, 1, 22, and 39, blood was drawn for

hematology and clinical chemistry parameters. All animals were fasted overnight prior to sampling..." The CHECKED (X) parameters were measured.

a. Hematology

X		X	
	Hematocrit (HCT)*		Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count		Reticulocyte count
	Blood clotting measurements		
X	(Partial Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)*		
	(Activated partial thromboplastin time)*		
	Erythrocyte morphology		

*Recommended in OPPTS 870.7200 Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*		Total Cholesterol
X	Potassium*	X	Globulin*
X	Sodium*	X	Glucose*
		X	Total and direct bilirubin*
	ENZYMES	X	Total serum protein*
X	Serum alkaline phosphatase(SAP)*		(TP)
X	Lactic acid dehydrogenase(LDH)		Triglycerides
X	Serum alanine aminotransferase (also SGPT)*		Serum protein electrophoresis
X	Serum aspartate aminotransferase(also SGOT)*		Albumin/globulin (A/G) ratio (calculated)
	Gamma glutamyl transferase(GGT)		
X	Amylase		
	Glutamate dehydrogenase		

*Recommended in OPPTS 870.7200 Guidelines.

F. Statistics

From p. 21 of MRID 45563002: "Continuous data were examined statistically during the study for evidence of abnormalities following the pre-treatment blood collection. A second evaluation was done for evidence of acute effects due to treatment after day 1. A third comprehensive examination was done for subacute toxicological effects due to treatment on day 22. A final comprehensive evaluation was done for reversibility on day 39. Dependent variables were analyzed initially by an analysis of variance (ANOVA) and Dunnett's test to determine if statistical differences exist among groups tested vs. concurrent control... If significance was suggested, each treatment group was compared to the control using Kruskal-Wallis ANOVA or Mann/Whitney U-test.

"A probability value of $p \leq 0.05$ was accepted as significant, which is standard practice for hypothesis testing in toxicology and pharmacology studies... DATATOX...is a closed system that uses a minimum $p \leq 0.05$ level or greater to assess differences due to treatment.

"In evaluating the data for potential treatment-related effects in this study, weight-of-evidence approach was used based on clinical experience with canines, accepted principles of veterinary toxicology, statistical analysis and historical (reference) data from this laboratory."

G. Disposition of animals

From p. 20 of MRID 45563002: "Upon completion of the study, the animals were added to a reserve pool for potential use in future investigations."

H. Compliance

Signed and dated Quality Assurance (p. 4), [No] Data Confidentiality (p. 2), and Good Laboratory Practice Compliance (p. 6-9) Statements are included within the report.

III. RESULTS

A. Exposure levels

In the treated group males individual doses ranged from 981 mg test substance (containing 85.9 mg Imidacloprid and 428.3 mg Permethrin)/kg to 1513.3 mg test substance (containing 132.4 mg Imidacloprid and 660.7 mg Permethrin/kg. In the treated group females individual doses ranged from 639.3 mg test substance (containing 55.6 mg Imidacloprid and 279.2 mg Permethrin)/kg to 1394.6 mg test substance (containing 122.0 mg Imidacloprid and 608.9 mg Permethrin)/kg. [Note: there are errors on p. 30 of MRID 45563002 for females HB1103 and HB1105 in the doses of test substance as given in mg/kg, although the values given for the active ingredients Imidacloprid and Permethrin are correct].

B. Mortality

No dogs died during the study.

C. Clinical signs

Selected clinical observations are presented in Table 3.

TABLE 2. Experimental design				
Group	No. of puppies		Dose Level	Number of treatments[a]
	Male	Female		
A	6	6	5X (vehicle)[b]	4
B	6	6	5X[c]	4

Data taken from information on p. 17, MRID 45563003.
[a]Treatments were on days 0, 7, 14 and 21.
[b]6/6 males and 6/6 females in group A were consistently dosed with 1.0 mL placebo/application.
[c]6/6 males and 6/6 females in group B were consistently dosed with 2.0 mL proposed product.

From p. 18 of MRID 45563003: "This study had two groups (A and B); each group had six male and six female puppies (7 weeks of age or less on study day 0). The puppies in group A received a single weekly dermal dose (dorsal cervical area) of vehicle equivalent (1 mL) in volume to the 5X use rate volume of the test substance. The puppies in group B received a single daily dermal (dorsal cervical - back of neck to shoulder area) dose of 5X [5 x 0.4 mL = 2.0 mL] based on the intended label volume of test substance."

C. Study objective and dose rationale

From p. 12 of MRID 45563003: "The objective of this controlled laboratory study was to provide pivotal information regarding the safety of 8.8% Imidacloprid with 44.0% Permethrin (W/W) formulation on puppies to support an EPA registration of this formulation for the treatment of external pests. The study was designed to evaluate the safety of 8.8% Imidacloprid with 44.0% Permethrin (w/w) formulation on puppies applied at 5 times the use volume rate at one week intervals for a total of 4 weeks. The treatment groups included a vehicle control group (equal to 5X in volume) and a 5X-treatment group. Each group included six animals/sex, for a total of 24 puppies that were 7 weeks of age or less on the first day of treatment."

D. Clinical observations

From p. 21 of MRID 45563003: "The dogs were observed twice daily during the study (except on the day of dosing), and no clinical signs dictated the need for more frequent observations. On the day of dosing, dogs were observed prior to dosing, immediately post-dosing, and at approximately 1, 2, 3, and 4 hours post-dosing. During treatment and conduct of physical exams, observations were performed by (blinded) personnel that did not participate in dose administration. Routine morbidity checks were not performed blind at other times during the study. The application site(s) was observed at least once daily for signs of irritation..."

E. Body Weights

From information on p. 39-40 of MRID 45563003 the puppies were weighed on days 0 (pretreatment), 12, 27 and 34.

F. Physiological parameters

From p. 19 of MRID 45563003: "Blood was collected by jugular venipuncture using a small gauge needle and vacutainer tubes. On study days 0 (hematology/chemistry/ coagulation) as experimental control, 1, 22 and 35, blood was drawn for hematology and clinical chemistry parameters: for the hematology profile, and for a clinical chemistry profile." There is no indication within the text of the report or within the protocol (pages 19 and 230 of MRID 45563003) that the puppies were fasted prior to blood collection, and this reviewer spoke with a sponsor representative on June 26, 2002 and it was confirmed that puppies were not fasted. The CHECKED (X) parameters were measured:

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)*
X	Platelet count		Reticulocyte count
	Blood clotting measurements	X	RDW (The RDW is the coefficient of variation of the RBC Volume Histogram)
	(Partial Thromboplastin time)		
	(Clotting time)	X	HDW (The HDW is the standard deviation of the RBC Hemoglobin Concentration histogram)
	(Prothrombin time)*		
	(Activated partial thromboplastin time)*		
	Erythrocyte morphology		

*Recommended in OPPTS 870.7200 Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*		Total Cholesterol
X	Potassium*	X	Globulin*
X	Sodium*	X	Glucose*
		X	Total and direct bilirubin*
	ENZYMES	X	Total protein*
X	Alkaline phosphatase(SAP)*		Triglycerides
	Lactic acid dehydrogenase(LDH)		Serum protein electrophoresis
X	Serum alanine aminotransferase (also SGPT)*		Albumin/globulin (A/G) ratio (calculated)
X	Serum aspartate aminotransferase(also SGOT)*		
	Gamma glutamyl transferase(GGT)		
	Amylase		
	Glutamate dehydrogenase		

*Recommended in OPPTS 870.7200 Guidelines.

G. Statistics

From p. 21 of MRID 45563003: "Continuous data were examined statistically during the study for evidence of abnormalities following the pre-treatment blood collection. A second evaluation was done for evidence of acute undesirable (adverse) effects due to treatment after day 1. A third comprehensive examination was done for subacute toxicological effects due to treatment on day 17. Dependent variables were analyzed initially by an analysis of variance (ANOVA) to determine if statistical differences exist among the groups tested vs concurrent negative control using SAS within DATATOX. If significance was suggested, each treatment group was compared to the control using a Student's t-test, Kruskal-Wallis Anova or Mann/Whitney U-test. If needed, a Student's t-test was used to compare group Day 1 values to pre-treatment (experimental control) values to interpret potential acute effects, including pooling sex to rigorously assess the probability of an adverse effect due to treatment. In addition, a repeated measures ANOVA using the pre-treatment values as the experimental control followed by a Student's t-test was done."

"A probability value of $p \leq 0.05$ was accepted as significant, which is standard practice for hypothesis testing in toxicology and pharmacology studies... In addition per Sponsor request analysis at $P<0.10$ (10%) was conducted. DATATOX tables were word-processed, to footnote the label for statistical hypothesis testing at the $P<0.10$ (10%) level."

"In evaluating the data for potential treatment-related effects in this study, weight-of-evidence approach was used based on clinical experience with the canine species..., accepted principles of veterinary toxicology, statistical analysis and reference data from this laboratory."

H. Disposition of animals

From p. 20 of MRID 45563003: "...puppy DM1102 died of a congenital digestive/ cardiac condition on day 25... Puppy DM1102 was necropsied and found to have had a congenital digestive/cardiac condition... After the study was completed, the other animals were added to a reserve pool to be used in any future studies."

I. Compliance

Signed and dated Quality Assurance (p. 8-10), Data Confidentiality (p. 2), and Good Laboratory Practice Compliance (p. 3) Statements are included.

III. RESULTS

A. Exposure levels

From information on p. 23-24 of MRID 45563003 on Day 0 in the treated group males individual doses ranged from 931.4 mg test substance (82.3 mg Imidacloprid and 407.3 mg Permethrin)/kg to 1614 mg test substance (142.7 mg Imidacloprid and 795.6 mg Permethrin)/kg. In the treated group females individual doses on day 0 ranged from 1220.6 mg test substance (107.9 mg Imidacloprid and 533.7 mg Permethrin)/kg to 1581.1 mg test substance (139.8 mg Imidacloprid and 691.4 mg Permethrin)/kg. On day 28 in the treated group males individual doses ranged from 636.0 mg test substance (56.2 mg Imidacloprid and 278.1 mg Permethrin)/kg to 1108.4 mg test substance (98.0 mg Imidacloprid and 484.7 mg

Permethrin)/kg. In the treated group females individual doses on day 28 ranged from 807.7 mg test substance (71.4 mg Imidacloprid and 353.2 mg Permethrin)/kg to 1301.4 mg test substance (115.0 mg Imidacloprid and 569.1 mg Permethrin)/kg.

B. Mortality

Female puppy DM1102 (5X test material group) died on day 25. From p. 20 of MRID 45563003: "The puppy was normal on initial physical examination, with normal clinical chemistry values on days 0 and 1. On day 21 the Study Director suspected a congenital digestive condition based on low total protein, albumin and globulin without evidence of other clinical chemistry alterations or clinical signs. On day 25 puppy 1102 was found dead, and necropsy confirmed a congenital pyloric sphincter constriction likely impaired the ability to digest solid food... In addition, the heart also appeared enlarged with thrombi in both atria. It is noteworthy that despite this congenital condition (inherent in the canine population) the puppy exhibited no clinical chemistry alterations or clinical signs suggestive of an adverse effect of four dermal treatments with the test substance." It is noted that this puppy (ID 1AAI5) had, from information on p. 54 of MRID 45563003, been single-housed since day 9 (the remaining puppies had been co-housed, except on days of treatment).

C. Clinical signs

Selected clinical observations are presented in Table 3.

TABLE 3. Selected clinical observations in dogs treated

Animal # & Treatment Group	Sex	Observation
DM0001 (vehicle)	M	
DM0002 (vehicle)	M	
DM0003 (vehicle)	M	
DM0004 (vehicle)	M	
DM0005 (vehicle)	M	Soft feces (day 20)
DM0006 (vehicle)	M	
DM0101 (vehicle)	F	Red mucoid feces (day 20)
DM0102 (vehicle)	F	Red mucoid feces (day 20)
DM0103 (vehicle)	F	Red mucoid feces (day 20)
DM0104 (vehicle)	F	
DM0105 (vehicle)	F	
DM0106 (vehicle)	F	Red mucoid feces (day 20)
DM1001 (5X)	M	
DM1002 (5X)	M	Enlarged nictitating membrane left eye (days 13 & 15)
DM1003 (5X)	M	Red mucoid feces (day 21)
DM1004 (5X)	M	
DM1005 (5X)	M	
DM1006 (5X)	M	
DM1101(5X)	F	
DM1102(5X)	F	Red mucoid feces (day 20); found dead (day 25)
DM1103(5X)	F	Red mucoid feces (day 20)
DM1104(5X)	F	Yellow vomit (day 20)
DM1105(5X)	F	Red mucoid feces (day 20)
DM1106(5X)	F	

Data extracted from p. 45-52 of MRID 45563003

A number of puppies had red mucoid feces on day 20. From information on p. 54 of MRID 45563003 there had been a change in diet (from puppy feeding formula + dry feed to just dry feed) on day 18, but it is uncertain as to whether this was a contributing factor (the symptom is also consistent with coccidiosis, but there is no indication that the puppies were subsequently treated for this).

The symptoms reported on pages 45-52 of MRID 45563003 do not include an observation (as indicated on p. 20 of MRID 45563003) of red mucoid feces in the period of days 0-4. This reviewer made an inquiry to Dan Van Goethem of Bayer regarding this on June 26, 2002 and the faxed response included the following: "...at the time the study technicians observed the

puppies on study day 4, there were no clinical signs observed. However, at a subsequent time on study day 4, the study director observed slight mucus, blood tainted stool in the cage of puppies. Based on this, he suspected subclinical coccidiosis and directed prophylactic treatment with 60 mg Tribrissen pos sid for 3 days. The study director's observations were made in the Log File database, but were inadvertently not transferred to the Clinical Observation database. Thus, while his observation and the treatment are discussed in the first paragraph in Section 13.4 on p. 19 [an additional page was subsequently added to the report to make this p. 20], the clinical signs and observations table in the report does not reflect this study day 4-observation."

D. Bodyweight and weight gain

It is stated (p. 23 of MRID 45563003) that: "There was no meaningful or statistical difference ($p \leq 0.05$ or $p < 0.10$) that suggested a biological effect on body weights after dosing at the conclusion of the study." An examination of the individual and group mean weights for males (p. 39) shows no substantive difference between the 5X puppies and their controls on the days (0, 12, 27 and 34) when body weights were taken. For females (p. 40) the initial (day 0) group means for 5X and controls were 1645 and 1684 g respectively; on day 12 the means were 1799.5 and 2077.8 g respectively; in part this was due to the low mean body weight of puppy DM1102 on day 12 (it dropped from 1522 g on day 0 to 1117 g on day 12), but even without the weight of that puppy included the mean of the remaining members of the 5X group was 1936 g. As puppy DM1102 died on day 25 it was not included in the mean weights for the 5X females on days 27 and 34. Even so, the mean weights of the remaining members were slightly lower than that of their controls on these days, due largely to one female puppy (DM1105) that weighed only 1974 g on day 34 (all other females on that day weighed over 2300 g).

E. Food consumption

It is stated (p. 26 of MRID 45563003) that: "Food consumption was not considered to have been influenced by compound administration (females or males) during the course of the study." However, mean food consumption for the 5X puppies on day 1 was considerably lower than that for their controls (food consumption data for both 5X and controls are for combined males and females); 323.75 g vs. 478.25 g. On day 8 mean food consumption for the 5X puppies was 580.25 g vs. the control value of 686.25 g.

Table 4 - Mean amounts of food consumed/dog on a daily basis.

Day	Control	5X	5X as % of control	% difference
1	478.25	323.75	67.7	-32.3
2	567.25	510.5	90.0	-10.0
3	597.75	536.0	89.7	-10.3
4	645.5	618.75	95.9	-4.1
5	628.75	517.5	82.3	-17.7
6	665.5	631.75	94.9	-5.1
7	686.25	580.25	84.6	-15.4
8	624.0	446.5	71.6	-28.4
9	683.0	596.25	87.3	-12.7
10	740.75	703.25	94.9	-5.1
11	737.25	665.0	90.2	-9.8
12	731.25	660.5	90.3	-9.7
13	720.75	691.5	95.9	-4.1
14	726.25	632.5	87.1	-12.9
15	696.25	614.5	88.3	-11.7
16	733.25	651.5	88.9	-11.1
17	569.0	526.25	92.5	-7.5
18	446.5	411.25	92.1	-7.9
19	447.0	417.5	93.4	-6.6
20	446.25	415.0	93.0	-7.0
21	418.5	381.25	91.1	-8.9
22	447.25	412.5	92.2	-7.8
23	448.0	471.0	105.1	+5.1
24	446.0	452.0	101.3	+1.3
25	442.25	451.5	102.1	+2.1
26	442.25	407.25	92.1	-7.9
27	444.5	408.5	91.9	-8.1
28	443.75	407.25	91.8	-8.2
29	441.75	409.25	92.6	-7.4

Data extracted from p. 53 of MRID 45563003

From Table 4, above, it appears that on days 1 and 8 mean food consumption in the 5X group of puppies was noticeably depressed (although this may not have been statistically significant) relative to that of their controls. There was also a somewhat lower food consumption in the period from day 14 to day 16, although this was not as pronounced as those following the first two application treatments. There is no evidence of lower food consumption in the 5X puppies following the fourth (day 21) application treatment.

F. Hematology

From information on p. 25 and 26 of MRID 45563003 there were sporadic findings (day 0 decreased % RDW in females and increased WBC in males, day 1 decreased %RDW and VAR in females, day 1 decreased MCV in males, the day 21 increased Aniso in males, and the day 34 increased Eosin% in females), which, while statistically significantly different from controls, were not biologically relevant as they were all well within this laboratory's reference range.

G. Clinical chemistry

From information on p. 25 and 26 of MRID 45563003 there were sporadic findings (day 0 increased Cl and increased total bilirubin in females, day 1 decreased phosphorus, decreased glucose, decreased ALP and increased total bilirubin in females, day 21 increased Cl and decreased AST in males, day 34 increased albumin in males and decreased total bilirubin in females) which, while statistically significant, were not biologically relevant as they were within this laboratory's reference range for these parameters. Some factors (decreased total protein and globulin, decreased albumin and calcium) in females on day 21 were influenced by the condition of DM1102, which subsequently died on day 25.

H. Necropsy findings

Animal DM1102 (a female in the 5X dose group) died on day 21. From information on p. 20 of MRID 45563003 it "was necropsied and found to have had a congenital digestive/cardiac condition. There were no clinical chemistry, hematology or clinical signs other than progressively thin body condition and low serum proteins. This was not considered to be compound related. Histopathology was not done since the gross necropsy was definitive and there was no evidence of undesirable (adverse) effects suggestive of toxicity due to treatment with the test substance."

IV. DISCUSSION

In a companion animal safety study (MRID 45563003) Imidacloprid (8.8% w/w) and Permethrin (44.0% w/w) Topical Solution (Lot No. A-01-048-G804-01-02-11), containing 100.8 mg of imidacloprid and 498.5 mg permethrin per mL, was dermally applied as a single 2 mL (5X the proposed use dosage rate of 0.4 mL) dose to the dorsal cervical area of each puppy in a group of 6 male and 6 female young (≤ 7 weeks old at initiation of dosage) on days 0, 7, 14 and 21. (The proposed 1X rate is 0.4 mL for dogs and puppies weighing ≤ 10 lbs; 1.0 mL for dogs weighing 11-20 lbs; 2.5 mL for dogs weighing 21-55 lbs, and 4.0 mL for dogs

weighing ≥ 55 lbs.) A second (control) group of 6 male and 6 female dogs of the same age received an application of 1.0 mL vehicle (formulation without active ingredients) on days 0, 7, 14 and 21.

For test group males on day 0 individual doses ranged from 931.4 mg test substance (82.3 mg Imidacloprid and 407.3 mg Permethrin)/kg to 1614 mg test substance (142.7 mg Imidacloprid and 795.6 mg Permethrin)/kg. In test group females individual doses on day 0 ranged from 1220.6 mg test substance (107.9 mg Imidacloprid and 533.7 mg Permethrin)/kg to 1581.1 mg test substance (139.8 mg Imidacloprid and 691.4 mg Permethrin)/kg. On day 28 in the treated group males individual doses ranged from 636.0 mg test substance (56.2 mg Imidacloprid and 278.1 mg Permethrin)/kg to 1108.4 mg test substance (98.0 mg Imidacloprid and 484.7 mg Permethrin)/kg. In the treated group females individual doses on day 28 ranged from 807.7 mg test substance (71.4 mg Imidacloprid and 353.2 mg Permethrin)/kg to 1301.4 mg test substance (115.0 mg Imidacloprid and 569.1 mg Permethrin)/kg.

Clinical observations were conducted hourly for 4 hr following each treatment; on other days each puppy was observed at least twice a day.

Blood was collected by jugular venipuncture using a small gauge needle and vacutainer tubes on study days 0 (preexposure), 1, 22 and 35 for hematology and clinical chemistry parameters. Puppies were not fasted prior to blood collection. Physical examinations were conducted on days 1 and 35. Body weights were taken on days 0 (pretreatment), 13, 28 and 35 (or days 0, 12, 27 and 34). The first application treatment was on March 2, 2001, and the experimental completion date was the week of April 6, 2001.

One female puppy (DM1102) in the 5X dose group died on day 25. This puppy was necropsied and found to have had a congenital digestive/cardiac condition. However, it had been evident for some time that there was a problem (this puppy had weighed 1522 g on day 0, and had dropped to 1117 g on day 12; this puppy had also been noted as thin on day 9 and had subsequently been given occasional supplemental nutrition. It had also been single-housed starting on day 9, while the others were usually co-housed).

While the report states (p. 26 of MRID 45563003) that: "Food consumption was not considered to have been influenced by compound administration (females or males) during the course of the study" mean food consumption for the 5X puppies on day 1 was considerably lower than that for their controls (food consumption data for both 5X and controls are for combined males and females); 323.75 g vs. 478.25 g. On day 8 mean food consumption for the 5X puppies was 580.25 g vs. the control value of 686.25 g. The food consumption differences on days 1 and 8 (day 1: 5X animals consumed 32.3% less food per pup than their controls; day 8: 5X puppies consumed 28.4% less food per pup than their controls) were the largest daily differences observed during this study. There was also a somewhat lower food consumption in the period from day 14 to day 16, although this was not as pronounced as those following the first two application treatments. There is no evidence that there was lower food consumption in the 5X puppies following the fourth (day 21) application treatment. It is also noted that 5X puppies recovered their appetites by the third day after each treatment.

There was a clustering of soft or mucoid red feces on day 20. In addition, red mucoid feces were noted in some of the puppies during the first four days of the study which were confirmed by fecal flotation as a mild, subclinical coccidiosis, and all puppies were treated with Tribrissen, 60 mg, sid for three days starting on day 4. Another puppy received a dose of kaopectate on day 3.

There was no evidence of any other adverse effect in these puppies. Sporadically statistically significant differences between groups with respect to hematology and clinical chemistry parameters were not biologically significant (values were still within the normal range, or mean values were skewed by the value from puppy DM1102).

It is noted that not only were these puppies treated at a 5X single-dose application rate, but that they were also given four 5X treatments in a 21-day period (as the label specifies once-a-month treatment at the 1X label, these puppies then received a cumulative of 20X the monthly dose specified on the label).

This study generally followed the pertinent guidelines for a companion animal safety study (OPPTS 870.7200).

The study is classified as **Acceptable** in demonstrating an adequate (approximately 5X in terms of overt symptoms) margin of safety. While treated puppies did not eat as much as their controls following on days 1 and 8 following applications of the test material on days 0 and 7, their food consumption recovered in a day or so, and there was little or no evidence of any reduction in food consumption following the third and fourth applications of the test material.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (870.1100, formerly §81-1)

Product Manager: 03 **Reviewer:** Byron T. Backus, Ph.D.
MRID No.: 45563004

CITATION: Johnson, K.L. G804S16 Insecticide (Imidacloprid 8.8%/Permethrin 44.0%) An Acute Oral LD_{50} Study in the Rat. Toxicology Study ID No. 01-A12-HF. Unpublished study prepared by Bayer Corporation Agriculture Division Toxicology 17745 South Metcalf Ave, Stilwell, KS 66085-9104. Study Completion Date: November 26, 2001. MRID 45563004.

SUBMITTER: Bayer Corporation, Agricultural Division, Animal Health

TEST MATERIAL: G804S16 Insecticide (Imidacloprid 8.8%/Permethrin 44.0%), Lot No.: A-01-061-G804-01-02-78; a clear, colorless to slight amber liquid. According to an analysis dated 08/16/01 the test material assayed 8.75% Imidacloprid and 43.66% Permethrin; according to a subsequent analysis on 10/16/01 it assayed 8.60% Imidacloprid and 43.58% Permethrin.

SPECIES: Rat, Wistar Hanover (Crl: WI[Glx/BRL/HAN]GS BR)
AGE(at dosing): approximately 8-12 weeks of age
WEIGHT (fasted): Males: 232-266 g; Females: 153-167 g
SOURCE: Charles River Laboratories Inc., Raleigh, NC

EXECUTIVE SUMMARY: ***In an acute oral toxicity study (MRID 45563004), fasted (overnight) young adult (8-12 weeks old) Wistar Hanover (Crl: WI[Glx/BRL/HAN]GS BR) rats (6 males and 6 females/dose level) were orally gavaged with 500, 1000 or 2000 mg/kg G804S16 Insecticide (active ingredients: imidacloprid 8.8%/permethrin 44.0%) administered at a constant dose volume of 10 mL/kg in vehicle (0.5% aqueous carboxymethylcellulose). A control group of 6 males and 6 females was dosed with vehicle only.***

There was one mortality (occurring the day following dosing) involving a female dosed at 1000 mg/kg. There were no deaths at any of the other doses (0, 500 or 2000 mg/kg). The vehicle (0.5% aqueous carboxymethylcellulose) controls showed no symptoms. Symptoms (persisting to day 5 in 2000 mg/kg rats) in other groups included whole body tremors, nasal discharge and perigenital staining. All surviving rats showed body weight gains, although the mean weight gain in 2000 mg/kg males (65 g) was somewhat less (although apparently not significantly so) than that observed in other groups (controls: 79 g; 500 mg/kg: 77 g; 1000 mg/kg: 86 g).

Gross pathological findings in the rat which died included wet ventrum and nasal discharge; one 2000 mg/kg female had a single pinpoint crusty zone on the nose. No significant findings were observed in the other rats which survived to termination.

Oral LD50 Male > 2000 mg/kg,
Oral LD50 Females > 2000 mg/kg

G804S16 Insecticide (active ingredients: imidacloprid 8.8%/permethrin 44.0%) is in toxicity category III in terms of oral toxicity.

Study Classification: Acceptable

COMPLIANCE: Signed and dated GLP Compliance (p. 3), Quality Assurance (p. 6-7), and Data Confidentiality (p. 2) statements were provided.

Procedure (including deviations from 870.1100): The test material was administered at a constant dose volume of 10 mL/kg using 0.5% aqueous carboxymethylcellulose as vehicle. Groups of 6 male and 6 female rats received a single dose of 0 (vehicle only), 500, 1000 or 2000 mg/kg of the test material, with subsequent 14-day observation.

Results:

Dosage (mg/kg)	Number of Deaths/Number Tested		
	Males	Females	Total
0	0/6	0/6	0/12
500	0/6	0/6	0/12
1000	0/6	1/6	1/12
2000	0/6	0/6	0/12

Observations: One female in the 1000 mg/kg dose group was found dead on day 1. Some rats at all dose levels showed whole body tremors, which persisted up to day 5 in the 2000 mg/kg group. Other symptoms (particularly in the 2000 mg/kg rats) included stains in the perigenital area and nasal discharge. All surviving rats showed body weight gains, although the mean weight gain in 2000 mg/kg males (65 g) was somewhat less (although apparently not significantly so) than that observed in other groups (controls: 79 g; 500 mg/kg: 77 g; 1000 mg/kg: 86 g).

Gross Necropsy: According to information on p. 14 of MRID 45563004: "The 1000 mg/kg female that was found dead, had wet ventrum and nasal discharge." According to the gross observations summary on p. 25 of MRID 45563004 there were no gross observations in any of the females dosed at 1000 mg/kg (although one of them was found dead), and the wet/stained ventrum and nasal discharge were observed in a female or females dosed at 2000 mg/kg. One of the females at 2000 mg/kg had a nasal red crusty zone, and this is consistent with what is stated on p. 8 for the 14-day survivors ("Gross observational findings consisted of a red pinpoint crusty zone on the nose. All other surviving animals were normal.").

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (870.1200, formerly §81-2)

Product Manager: 03 **Reviewer:** Byron T. Backus, Ph.D.
MRID No.: 45563005

CITATION: Johnson, K.L. G804S16 Insecticide (Imidacloprid 8.8%/Permethrin 44.0%) An Acute Dermal LD_{50} Study in the Rat. Toxicology Study ID No. 01-A22-HJ. Unpublished study prepared by Bayer Corporation Agriculture Division Toxicology 17745 South Metcalf Ave, Stilwell, KS 66085-9104. Study Completion Date: November 26, 2001. MRID 45563005.

SUBMITTER: Bayer Corporation, Agricultural Division, Animal Health

TEST MATERIAL: G804S16 Insecticide (Imidacloprid 8.8%/Permethrin 44.0%), Lot No.: A-01-061-G804-01-02-78; a clear, colorless to slight amber liquid. According to an analysis dated 08/16/01 the test material assayed 8.75% Imidacloprid and 43.66% Permethrin; according to a subsequent analysis on 10/16/01 it assayed 8.60% Imidacloprid and 43.58% Permethrin.

SPECIES: Rat, Wistar Hanover (Crl: WI[Glx/BRL/HAN]GS BR)
AGE(at dosing): approximately 8-12 weeks of age
WEIGHT: Males: 245-293 g; Females: 194-213 g
SOURCE: Charles River Laboratories Inc., Raleigh, NC

EXECUTIVE SUMMARY: ***In an acute dermal toxicity study (MRID 45563005), a group of 6M & 6F young adult (approximately 8-12 weeks old) Wistar Hanover (Crl: WI[Glx/BRL/HAN]GS BR) rats were dermally exposed for 24 hrs (occluded exposure) to 2000 mg/kg G804S16 Insecticide Lot No. A-01-061-G804-01-02-78 (active ingredients: imidacloprid 8.8% & permethrin 44.0%). A control group was exposed to a placebo (Lot No. A-01-074-G804-01-02-77), which consisted of the test formulation excluding the active ingredients. However, the report does not indicate the amount of placebo material that was administered to the control group.***

There was no mortality. One male and two females in the placebo (control) group had lesions/sores within or close to the dose site, as did one male in the 2000 mg/kg test material dose group. Lacrimal and nasal staining from red discharge, as well as perigenital staining, occurred with similar incidences in both the control and test material-exposed groups. These were attributed to the use of Elizabethan collars.

At necropsy 3/6 control females and 5/6 2000 mg/kg females had a wet/stained ventrum

Dermal LD50 Males > 2000 mg/kg (0/6 died at this dose level)
Dermal LD50 Females > 2000 mg/kg (0/6 died at this dose level)
Combined > 2000 mg/kg (0/12 rats died at this dose level)

G804S16 Insecticide Lot No. A-01-061-G804-01-02-78 (active ingredients: imidacloprid 8.8% & permethrin 44.0%) is in toxicity category III in terms of dermal toxicity.

Study Classification: Acceptable

COMPLIANCE: Signed and dated GLP Compliance (p. 3), Quality Assurance (p. 6-7), and Data Confidentiality (p. 2) statements were provided.

Procedure (including deviations from 870.1200): "Animals were treated with the test material (G804S16 Insecticide; Imidacloprid 8.8%/Permethrin 44.0%) as received (neat) by pipetting the

appropriate quantity directly onto the back of the animal. A two-ply gauze pad with plastic backing was applied over the treatment site... Control and treated rats were treated in an identical manner with the exception that controls were treated with placebo. All dosing preparations (test substance or placebo) were administered as a single dermal dose... Dose groups consisted of 6 animals/sex/group. The hair from the scapulae (shoulders) to the wing of the ileum (hipbone) and half-way down the flank on each side of the animal was removed using...electric clippers... Animals were clipped approximately 24 hours before the first dose was administered... The test substance was administered directly to the back of the animal as a single dose for a duration of 24 hours... After dosing, the gauze with plastic backing was applied and the site was secured with hypoallergenic tape. Next, the gauze and plastic backing was secured with Vetrap ®...bandage, which was also secured with tape. All animals wore Elizabethan collars...for the duration of the exposure with the exception that one animal was found out of its collar shortly after dosing... After approximately 24 hours (minimum), the bandages and gauze were removed and the dose site was wiped using paper towels dampened with tap water to remove as much of the residual test substance as feasible without damaging the skin..."

Results:

Dosage (mg/kg)	Number of Deaths/Number Tested		
	Males	Females	Combined
0	0/6	0/6	0/12
2000	0/6	0/6	0/12

Observations: There was no mortality. "One male and two females in the control (placebo) group, and one male in the treated group displayed lesions/sores within, or close to, the dose site. However, all lesions/sores (with subsequent scabbing) and incidences of perigenital staining, were considered incidental and unrelated due to comparable incidence between control and treated animals. Lacrimal and nasal staining, in the form of red discharge, in both control and treated animals in all dose groups, were attributed to the animals wearing Elizabethan collars, which interfere with normal rodent preening, and were not considered to be compound-related... All animals showed body weight gain during the study and were not affected by treatment..."

Gross Necropsy: From information on p. 27 of MRID 45563005 3/6 control females and 5/6 females treated with 2000 mg G804S16 Insecticide/kg had wet/stained ventrum.

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (870.1300, formerly §81-3)

Product Manager: 03
MRID No.: 45563006

Reviewer: Byron T. Backus, Ph.D.

CITATION: Pauluhn, J. G804 Insecticide (c.n.: Imidacloprid & Permethrin) Study on Acute Inhalation Toxicity in Rats according to OECD No. 403. Bayer AG Study No. T9070589; Bayer AG Report No. PH 31214. Unpublished study prepared by Bayer AG Department of Toxicology, Friedrich-Ebert-Strasse 217-333, D-42096 Wuppertal, Germany. Study Completion Date: July 10, 2001. MRID 45563006.

SUBMITTER: Bayer Corporation, Agricultural Division, Animal Health

TEST MATERIAL: G804 Insecticide (Imidacloprid 8.8%/Permethrin 44%), Lot No.: A-01-048-G804-01-02-11; a translucent, yellowish-brownish colorless liquid. According to an analysis dated 01/26/01 the test material assayed 8.84% Imidacloprid and 43.73% Permethrin.

SPECIES: Rat, Wistar, Hsd Cpb:WU (SPF)
AGE(at exposure): young adult, approximately 2 months old
WEIGHT (at exposure): Males: 167-198 g; Females: 168-175 g;
SOURCE: Harlan-Winkelmann GmbH, Borchen, Germany

EXECUTIVE SUMMARY: ***In an acute inhalation toxicity study (MRID 45563006), a group of 5 male and 5 female young adult (approximately 2 months old) Wistar rats received a 4-hour nose-only exposure to a mean concentration of 3.565 mg/L of the test material (G804 Insecticide: Lot No. A-01-048-G804-01-02-11, containing 8.84% Imidacloprid and 43.73% Permethrin). The mean MMAD was 1.92 µm and the mean GSD was 2.34. A control group of 5M and 5F was similarly exposed to air only.***

There were no mortalities. Symptoms observed (mostly on the day of exposure) included piloerection, ungroomed haircoat, bradypnea, reduced motility, limpness, tremor, high-legged gait, blepharospasm, serous nasal discharge, nostrils/muzzle reddened and with red encrustations, and salivation. The most pronounced symptoms were those indicative of respiratory tract irritation. Symptoms were gone by day 3. The rats were hypothermic (rectal temperatures significantly depressed) at 30 minutes following exposure (control males: 37.7°C; exposed males: 29.2°C; control females: 37.9°C; exposed females: 31.9°C). For G804-exposed rats, 1/5 males and 3/5 females lost weight in the period from day 0 to day 3, but all had normal weight gains in the period from day 0 to day 14.

A battery of reflex measurements was made on day 1. One male rat exposed to the test substance displayed decreased tonus. The other rats which had been exposed gave normal responses.

There were no test article-related gross findings on necropsy following sacrifice on day 14.

Inhalation LC50 Males > 3.565 mg/L (0/5 died)
Inhalation LC50 Females > 3.565 mg/L (0/5 died)
Combined LC50 > 3.565 mg/L (0/10 rats died)

G804 Insecticide: Lot No. A-01-048-G804-01-02-11, containing 8.84% Imidacloprid and 43.73% Permethrin) is in toxicity category IV in terms of acute inhalation toxicity, based on the LC50 > 3.565 mg/L.

Study Classification: Acceptable

COMPLIANCE: Signed and dated GLP (p. 3), Quality Assurance (p. 6-7) and Data Confidentiality (p. 2) statements are provided.

Procedure (including deviations from 870.1300): Exposure was nose-only for four hours. "Animals were exposed to the aerosolized test item in Plexiglas exposure tubes applying a *directed-flow* nose-only exposure principle. Tubes were chosen that accommodated the animal's size. These tubes were designed so that the rat's tail remained outside the tube, thus restrained-induced hypothermia can be avoided...The test substance was nebulized using a binary nozzle with conditioned compressed air (15L/min; dispersion pressure approximately 500 kPa...)."

Mean Exposure Concentration mg/L (Gravimetrically Determined)	Number of Deaths/Number Tested		
	Males	Females	Combined
0	0/5	0/5	0/10
3.565[a]	0/5	0/5	0/10

[a]Four samples were collected during exposure; values were 1.945, 1.905, 1.960, and 1.890 mg/L. As the test material contained 46% volatile substances, the resulting value of 1.925 mg/L was multiplied by 100/54 (=1. 85185) to obtain the value of 3.565 mg/L. From p. 18 of MRID 45563006: "The test-substance concentration was determined by gravimetric analysis (filter: Glass-Fibre-Filter...). After sampling, filters were dried for 120-min (@ 70°C). The mass collected by the filter was converted to the test substance taking into account the concentration of constituents contained in it that are prone to evaporate subsequent to nebulization. The relative proportion of constituents prone to evaporate is determined as follows: aliquots of the test substance were added onto glass fiber filters and filters were allowed to dry under the conditions described as above... Stability was attained after 120-min and the mass-fraction evaporated was equal to 46.1%. Accordingly, the mass collected by filter analyses was corrected using a factor of 100/54."

The nominal concentration was 17.333 mg/L [from p. 27 of MRID 45563006: "Specific density assumed to be 1 g/mL (no data provided by sponsor)."]

Clinical Observations: There were no mortalities. Symptoms observed (mostly on the day of exposure) included piloerection, ungroomed haircoat, bradypnea, reduced motility, limpness, tremor, high-legged gait, blepharospasm, serous nasal discharge, nostrils/muzzle reddened and with red encrustations, and salivation. Symptoms were gone by day 3. The rats were hypothermic (rectal temperatures significantly depressed) at 30 minutes following exposure (control males: 37.7°C; exposed males: 29.2°C; control females: 37.9°C; exposed females: 31.9°C). For G804-exposed rats, 1/5 males and 3/5 females lost weight in the period from day 0 to day 3, but all had normal weight gains in the period from day 0 to day 14.

A battery of reflex measurements (visual placing response, vertical grip strength, horizontal grip strength, tonus, cornea reflex, light reflex, pinna reflex, startle reflex/sound, startle reflex/touch, tail-pinch reflex, open-field righting reflex, drop-method righting reflex) was made on day 1. One male rat exposed to the test substance displayed decreased tonus. All other responses of this rat were normal. The other rats which had been exposed gave normal responses.

Gross Necropsy: There were no test article-related gross findings on necropsy following sacrifice on day 14.

Chamber Atmosphere		
Grav. Conc. (mg/L)	**Mean MMAD (µm)**	**Mean GSD**
3.565	1.92	2.34

Particle Size Distribution: Approximately 40% of the particles by weight had an effective cut-off diameter of 2.0 µm or less, and > 76% had an effective cut-off diameter of 4.0 µm or less.

Chamber Environment	
Internal Chamber Volume	3.8 L
Mean Airflow (inlet)	15 LPM
Mean Temperature	22°C
Mean Relative Humidity	< 5%

DATA REVIEW FOR PRIMARY EYE IRRITATION TESTING (870.2400, formerly §81-4)

Product Manager: 03 **Reviewer:** Byron T. Backus, Ph.D.
MRID No.: 45563007

CITATION: Kuhn, J.O. Acute Eye Irritation Study in Rabbits: G804 Insecticide, Formula No. A-01-061, Lot No. A-01-061-G804-01-02-58. STILLMEADOW Inc. Study No. 6422-01; Sponsor Study No. 01C-I35-FO. Unpublished study prepared by STILLMEADOW, Inc. 12852 Park One Drive, Sugar Land, TX 77478. Study Completion Date: November 9, 2001. MRID 45563007.

SUBMITTER: Bayer Corporation, Agricultural Division, Animal Health

TEST MATERIAL: G804 Insecticide (Imidacloprid 8.8%/Permethrin 44.0%), Formula No. A-01-061, Lot No.: A-01-061-G804-01-02-58; described as a pale yellow liquid. According to an analysis dated 05/25/01 the test material assayed 8.79% Imidacloprid and 43.75% Permethrin. The pH was 5.12.

SPECIES: Rabbit, New Zealand White (1 male and 2 females used)
AGE: adult, 99 days old when treated
WEIGHT: 2.05 - 2.6 kg
SOURCE: Ray Nichols Rabbitry, Lumberton, TX

EXECUTIVE SUMMARY: ***In a primary eye irritation study (MRID 45563007), 0.1 mL of undiluted G804 Insecticide, Formula No. A-01-061, Lot No. A-01-061-G804-01-02-58, a pale yellow liquid with a pH of 5.12 (containing 8.79% Imidacloprid and 43.75% Permethrin as the active ingredients) was instilled into one eye of each of 3 adult (99-day old) New Zealand white rabbits.***

At 1 hour 2/3 eyes scored positive for corneal irritation and all eyes were positive (scores of 2) for conjunctival redness and chemosis. At 24 hours 2/3 eyes were positive for corneal irritation and all 3 eyes were positive (score of "2" or more) for redness and 1/3 was positive for chemosis. On day 7 1/3 eyes was still positive for corneal opacity and the same eye was also positive (score of 2) for redness. This eye was still positive for corneal opacity on day 10, but had completely cleared (all scores zero) by day 14.

The test material, G804 Insecticide, Formula No. A-01-061, Lot No. A-01-061-G804-01-02-58, a pale yellow liquid with a pH of 5.12 (containing 8.79% Imidacloprid and 43.75% Permethrin as the active ingredients) is in toxicity category II in terms of primary eye irritation potential, based on the presence of corneal opacity in 1/3 eyes on day 7 which had cleared by day 14.

Study Classification: Acceptable

COMPLIANCE: Signed and dated GLP (p. 3), Quality Assurance (p. 5) and Data Confidentiality (p. 2) statements are provided.

Procedure (including deviations from 870.2400): "On day 0, a dose of 0.1 mL of the undiluted test substance was placed into the conjunctival sac of the right eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance was dropped. The lids were gently held together for one second to prevent loss of material. The untreated left eyes served as comparative controls."

Results:

Observations	Number scoring positive/total number						
	1 hr	24 hrs	48 hrs	72 hrs	7 days	10 days	14 days
Corneal Opacity	2/3	2/3	1/3	1/3	1/3	1/3	0/3
Iritis	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Conjunctivae:							
Redness[a]	3/3	3/3	3/3	1/3	1/3	0/3	0/3
Chemosis[a]	3/3	1/3	3/3	1/3	0/3	0/3	0/3
Discharge[a]	3/3	2/3	3/3	1/3	1/3	0/3	0/3

[a]Score of 2 or more considered positive.

"The maximum average irritation score of 25.3, obtained at 1 hour after treatment, was used to rate G804 Insecticide, Formula No. A-01-061, Lot No. A-01-061-G804-01-02-58 moderately irritating. Since all "positive" effects had cleared by Day 14 after dosing, the test substance is assigned to Toxicity Category II."

DATA REVIEW FOR PRIMARY DERMAL IRRITATION TESTING (870.2500, formerly §81-5)

Product Manager: 03
MRID No.: 45563008

Reviewer: Byron T. Backus, Ph.D.

CITATION: Kuhn, J.O. Acute Dermal Irritation Study in Rabbits: G804S16 Insecticide, Formula No. A-01-061, Lot No. A-01-061-G804-01-02-58. STILLMEADOW Inc. Study No. 6423-01; Sponsor Study No. 01C-I25-FP. Unpublished study prepared by STILLMEADOW, Inc. 12852 Park One Drive, Sugar Land, TX 77478. Study Completion Date: November 9, 2001. MRID 45563008.

SUBMITTER: Bayer Corporation, Agricultural Division, Animal Health

TEST MATERIAL: G804S16 Insecticide (Imidacloprid 8.8%/Permethrin 44.0%), Formula No. A-01-061, Lot No.: A-01-061-G804-01-02-58; described as a pale yellow liquid. According to an analysis dated 05/25/01 the test material assayed 8.79% Imidacloprid and 43.75% Permethrin. The pH was 5.12.

SPECIES: Rabbit, New Zealand White (1 male and 2 females used)
AGE: adult, 94 days old when treated
WEIGHT: 2.6 - 3.15 kg
SOURCE: Ray Nichols Rabbitry, Lumberton, TX

EXECUTIVE SUMMARY: ***In a dermal irritation study (MRID 45563008), 0.5 mL of G804S16 Insecticide, Formula No. A-01-061, Lot No. A-01-061-G804-01-02-58 (a pale yellow liquid with a pH of 5.12 containing 8.79% Imidacloprid and 43.75% Permethrin as active ingredients) was applied to an intact skin site on the back of each of 3 New Zealand white rabbits, with 4-hr semi-occluded exposure.***

Very slight (grade 1) erythema was present at 2/3 application sites at 1 hour. All subsequent (24, 48 and 72 hour) scores for erythema were zero. All scores (1, 24, 48 and 72 hours) for edema were zero. The Primary Irritation Index (average of 1, 24, 48 and 72 hour scores) = 0.17. The test material, G804S16 Insecticide, Formula No. A-01-061, Lot No. A-01-061-G804-01-02-58 (containing 8.79% Imidacloprid and 43.75% Permethrin as active ingredients) is in toxicity category IV in terms of primary skin irritation potential, based on the relatively low level of dermal irritation observed in this study.

Study Classification: Acceptable

COMPLIANCE: Signed and dated GLP (p. 3), Quality Assurance (p. 5) and Data Confidentiality (p. 2) statements were provided.

Procedure (including deviations from 870.2500): "Each animal was prepared on the day prior to treatment by clipping the dorsal area of the trunk free of hair to expose an area at least 8 x 8 cm... A single intact exposure site was selected as the test site... On Day 0, 0.5 mL of the undiluted test substance was applied to each test site and covered with a surgical gauze patch measuring 2.5 x 2.5 cm and four single layers thick. Each patch was secured in place with a strip of non-irritating adhesive tape. The entire trunk of each animal was loosely wrapped with a semi-permeable dressing (orthopedic stockinette) which was secured on both edges with strips of tape to retard evaporation of volatile substances and to prevent possible ingestion of the test substance... After four hours, the patches and wrappings were removed. The test sites were gently washed with room temperature tap water and a clean cloth to remove as much residual test substance as possible."

Results: Very slight (grade 1) erythema was present at 2/3 application sites at 1 hour. All subsequent (24, 48 and 72 hour) scores for erythema were zero. All scores (1, 24, 48 and 72 hours) for edema were zero. The Primary Irritation Index (average of 1, 24, 48 and 72 hour scores) = 0.17.

DATA REVIEW FOR DERMAL SENSITIZATION TESTING (870.2600, formerly §81-6)

Product Manager: 03
MRID No.: 45563009

Reviewer: Byron T. Backus, Ph.D.

CITATION: Vohr, H.-W. G804 Insecticide Study for the Skin Sensitization Effect in Guinea Pigs (Buehler Patch Test). Bayer AG Study No. T 2071176; Bayer AG Report No. PH 31522. Unpublished study prepared by Bayer AG Department of Toxicology, Friedrich-Ebert-Strasse 217-333, D-42096 Wuppertal, Germany. Study Completion Date: November 22, 2001. MRID 45563009.

SUBMITTER: Bayer Corporation, Agricultural Division, Animal Health

TEST MATERIAL: G804S16 Insecticide (Imidacloprid 8.75%/Permethrin 43.66%), Batch No.: A-01-061-G804-01-02-78; Formula No. A-01-61. Described as a clear to yellowish/amber liquid.

SPECIES: Guinea Pig, strain Hsd Poc:DH; SPF-bred (females only)
AGE(at initiation of induction): Females: 3-5 weeks
WEIGHT(at initiation of induction): Females: 298-386 g
SOURCE: Harlan Winkelmann GmbH Laboratory Animal Breeders in 33176 Borchen

EXECUTIVE SUMMARY: ***In a dermal sensitization study (MRID 45563009) using a Buehler patch test, 20 female Hsd POC:DH guinea pigs each received a total of three six-hour occluded induction exposures, at one week intervals (days 0, 7 & 14) to 0.5 mL of undiluted G804S16 Insecticide, Batch No. A-01-061-G804-01-02-78 (containing 8.75% Imidacloprid and 43.66% Permethrin as active ingredients).***

Two weeks after the last induction dose, previously induced guinea pigs were challenged (at a previously unexposed dermal site), along with a previously unexposed control group of 10 female guinea pigs, to a six-hour exposure to 0.5 mL of undiluted G804S16 Insecticide, Batch No. A-01-061-G804-01-02-78 (containing 8.75% Imidacloprid and 43.66% Permethrin as active ingredients).

During induction, the maximum score for irritation at 30 hrs following each treatment was "1," and this occurred in one animal (#27) following the third induction treatment. All other induction scores were zero. Following challenge, all guinea pigs (the 20 previously exposed, as well as the 10 controls) scored zero at both 30 and 54 hours after the start of exposure. Therefore there was no indication of a dermal sensitization reaction.

The report cited a positive control study which used alpha-Hexylcinnamaldehyde or HCA as the test material, and this was submitted (MRID 45563010) separately. However, the completion date for the positive control study is February 9, 2000, or more than 21 months before the completion date for the study with G804S16 insecticide.

In response to this finding, Bayer made available a second positive control study (Bayer AG Report No. 32137; Bayer AG Study No. T 4071402; study completion date: June 27, 2002) conducted at Bayer AG Department of Toxicology using "ALPHA-HEXYLZIMTALDEHYD" (alpha-Hexylcinnamaldehyde, or HCA). The results were appropriate. According to information in the positive control study report the experimental starting date was January 15, 2002 and the completion date was February 14, 2002; from information in MRID 45563009 the starting date was October 16, 2001 and the completion date was November 15, 2001. It is concluded that this second positive control study (Bayer AG Report No. 32137; Bayer AG Study No. T 4071402) does validate the findings of the study in MRID 45563009.

Study Classification: Acceptable (using the positive control study in Bayer AG Report No. 32137 as validation). There were no indications that the test material, G804S16 Insecticide, Batch No. A-01-061-G804-01-02-78 (containing 8.75% Imidacloprid and 43.66% Permethrin as active ingredients), is a dermal sensitizer.

COMPLIANCE: Signed and dated GLP (p. 3), Quality Assurance (p. 6-7) and Data Confidentiality (p. 2) statements were provided.

Procedure: For induction: "The animals were dermally treated with the test item three times at intervals of seven days. The suitable areas of the body were shaved one day (24 hours) before each treatment... The 1st to 3rd induction were performed with the 100% test item concentration. The animals in the test item group were treated with a hypoallergenic patch loaded with the test item applied to the left flank and held in place on the skin with "ORABAND"® adhesive plaster... In the case of the control group animals, a hypoallergenic dry patch was applied to the left flank and fixed with a strip of "ORABAND"® in each of the first to third inductions. The patches were removed after an exposure period of six hours and any remaining test item was washed off the skin with physiological saline solution... The volume [of test material] applied per animal was 0.5 mL... The treatment areas were visually assessed 30 hours after initiation of exposure. For this, the treatment areas were not shorn or chemically depilated..."

For challenge:"The challenge was performed four weeks after the first (two weeks after the last) dermal induction... For the challenge the backs and right flanks of the animals were shorn one day prior to the challenge. During the challenge a hypoallergenic patch loaded with the 100% test item was applied and fixed to the right flank of each animal in the control and test item group. As a control a dry patch was applied and fixed also to the right flank, cranial to the test item patch. The patches were held securely in place on the skin with a ORABAND self-adhesive tape for 6 hours... The volume applied per animal was 0.5 mL... At the end of the six-hours exposure period, the patches were removed and the remaining test item was rinsed away with physiological saline solution. Twenty-one hours later the skin of the animals was shorn in the region of the treatment sites... The skin reactions were assessed 30 hours after initiation of the induction exposures, and 30 and 54 hours after the beginning of the challenge with the following pattern... 0 = no reaction 1 = slight localized redness [2 = moderate confluent redness, 3 = severe redness and swelling].

Results: During induction, the maximum score for irritation at 24 hrs following each treatment was "1," and this occurred in only one animal following the third induction treatment. All scores following challenge (at both 24 and 48 hours after patches were removed) were zero in both the previously induced as well as the naive guinea pigs. Therefore there was no indication of a dermal sensitization reaction.

The report cited a positive control study which used alpha-Hexylcinnamaldehyde or HCA as the test material, and this was submitted (MRID 45563010) separately. However, the completion date for the positive control study in MRID 45563010 was February 9, 2000, or more than 21 months before the completion date for the study with G804S16 insecticide. This study could not be used to validate the study in MRID 45563009.

In response to this finding, Bayer made available a second positive control study (Bayer AG Report No. 32137; Bayer AG Study No. T 4071402; study completion date: June 27, 2002) conducted at Bayer AG Department of Toxicology using "ALPHA-HEXYLZIMTALDEHYD" (alpha-Hexylcinnamaldehyde, or HCA). The results were appropriate. According to information in the positive control study report the experimental starting date was January 15, 2002 and the completion date was February 14, 2002; from information in MRID 45563009 the starting date was October 16, 2001 and the completion date was November 15, 2001. It is concluded that this second positive

control study (Bayer AG Report No. 32137; Bayer AG Study No. T 4071402) does validate the findings of the study in MRID 45563009.

As there were no indications that the test material, G804S16 Insecticide, Batch No. A-01-061-G804-01-02-78 (containing 8.75% Imidacloprid and 43.66% Permethrin as active ingredients), is a dermal sensitizer, and an acceptable positive control study was conducted within six months, the study is classified as **Acceptable.**

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D280825
2. **PC CODE:** 109701 Permethrin, mixed cis, trans; 129099 Imidacloprid
3. **CURRENT DATE:** June 28 2002
4. **TEST MATERIAL:** [EPA File Symbol: 11556-RGE]; Advantix™ 10 for Dogs; G804S16 Insecticide (Imidacloprid 8.8%/Permethrin 44.0%), Lot No.: A-01-061-G804-01-02-78 [used in acute oral LD50, dermal LD50 and dermal sensitization studies] a clear, colorless to slight amber liquid. According to an analysis dated 08/16/01 the test material assayed 8.75% Imidacloprid and 43.66% Permethrin; according to a subsequent analysis on 10/16/01 it assayed 8.60% Imidacloprid and 43.58% Permethrin. The lot number used in the inhalation study was A-01-048-G804-01-02-11, containing 8.84% Imidacloprid and 43.75% Permethrin. The lot number used in the eye irritation and dermal irritation studies was A-01-061-G804-01-02-58, assaying 8.79% Imidacloprid and 43.75% Permethrin, with a pH of 5.12.

Study/Species/Lab Study #/Date	MRID	Results	Tox. Cat.	Core Grade
Acute oral toxicity/rat/ Bayer Corporation Agriculture Division Toxicology/ID No. 01-A12-HF/NOV-26-2001	45563004	LD_{50}(M) > 2000 mg/kg (0/6 died at this dose level); LD_{50}(F) > 2000 mg/kg (0/5 died at this dose level, but 1/6 died at 1000 mg/kg). Symptoms included whole body tremors, nasal discharge and perigenital staining. All surviving rats showed body weight gains, though the mean wt gain in 2000 mg/kg males was somewhat less than that observed in other groups.	III	A
Acute dermal toxicity/rat/ Bayer Corporation Agriculture Toxicology/ID No. 01-A22-HJ/NOV-26-2001	45563005	LD_{50} > 2000 mg/kg (males, females, combined; no deaths at this exposure level). Symptoms (lacrimal & nasal staining from red discharge, perigenital staining) attributed to use of Elizabethan collars.	III	A

Acute inhalation toxicity/rat/ Bayer AG Dept. of Toxicology Germany/Bayer AG Study No. T9070589/JUL-10-2001	45563006	LC_{50} > 3.565 mg/L (0/5 F, 0/5 M died at this exposure level). Nose-only exposure. MMAD = 1.92 µm & mean GSD = 2.34. Symptoms observed (mostly on the day of exposure) included piloerection, reduced motility, limpness, tremor, high-legged gait, blepharospasm, serous nasal discharge, nostrils/ muzzle reddened and with red encrustations, and salivation. The most pronounced symptoms were those indicative of repiratory tract irritation. Symptoms were gone by day 3. Rats were hypothermic (rectal temperatures significantly depressed) at 30 minutes following exposure. 1/5 males & 3/5 females lost weight between day 0 and day 3, but all had normal weight gains in the period from day 0 to 14.	IV	A
Primary eye irritation/rabbit/ STILLMEADOW Inc./Study No. 6422-01/NOV-9-2001	45563007	3 NZ white rabbit eyes exposed. 2/3 were positive for corneal opacity and all were positive for conjunctival redness and chemosis at 1 hr. On day 10 1/3 eyes was still positive for corneal opacity, but this eye had completely cleared by day 14.	II	A
Primary dermal irritation/ rabbit/ STILLMEADOW Inc./ Study No. 6423-01/NOV-9-2001	45563008	PII (av. of 1, 24, 48 & 72 hr scores) = 0.17. Very slight (score 1) erythema at 2/3 sites at 1 hr; all subsequent scores for erythema (24, 48 & 72 hrs) were zero. All scores for edema were zero.	IV	A
Dermal sensitization/guinea pig/Bayer AG Dept. of Toxicology Germany/Bayer AG Study No. T2071176/ NOV-22-2001	45563009	Buehler patch test. Induction used 0.5 mL aliquots of undiluted test material, with occluded exposures at 1 week intervals; challenge also used 0.5 mL aliquots of undiluted test material to both previously induced (20F guinea pigs), and control (10Fguinea pigs). No indication of a dermal sensitization reaction; positive control study which validates this study is Bayer AG Report No. 32137, with a study completion date of June 27, 2002.	Not a sensitizer	A

Core Grade Key: **A =Acceptable, S = Supplementary, U = Unacceptable, V = Self Validated**

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D280825
2. **PC CODE:** 109701 Permethrin, mixed cis, trans; 129099 Imidacloprid
3. **CURRENT DATE:** June 28 2002
4. **TEST MATERIAL:** [EPA File Symbol: 11556-RGE]; Advantix™ 10 for Dogs; G804S16 Insecticide (Nominal Imidacloprid 8.8%/Permethrin 44.0%), Lot No.: A-01-061-G804-01-02-78; containing Imidacloprid 8.75% and Permethrin 43.66%.

Study/Species/Lab Study #/Date	MRID	Results	Core Grade
Companion Animal Safety/dog [beagle]/ Bayer Corporation Agriculture Division Toxicology/01-S26-HB/DEC-6-2001	45563002	Test material was administered to a 6M & 6F young adult beagle dogs at 5X the single-application dose rate on days 0, 7, 14 & 21. 6M & 6F beagles received placebo (formulation less the active ingredients). Noticeable reductions in mean food consumption in both placebo and 5X groups on days 8 & 15; reduced food consumptions on days -12, -5, 1, 22 & 39 were presumably due to blood taking and overnight fasting and could have masked effects due to test material or placebo applications on days 1 and 22. A number of dogs in both groups showed a few to multiple pinpoint-sized red areas at the base of hair shafts at the application site on days 7, 14 and/or 21. In one case these persisted for several days. Statistically significant differences in hematology and clinical chemistry were sporadic, and not biologically relevant as they were all within normal ranges. Noted that test material treated dogs received four 5X single-dose applications in a month, or a cumulative of 20X the monthly label-dose rate. As food consumption reductions were transient, an adequate safety margin (approximately 5X in terms of overt symptoms) was demonstrated.	A

Core Grade Key: **A =Acceptable, S = Supplementary, U = Unacceptable, V = Self Validated**

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D280825
2. **PC CODE:** 109701 Permethrin, mixed cis, trans; 129099 Imidacloprid
3. **CURRENT DATE:** June 28 2002
4. **TEST MATERIAL:** [EPA File Symbol: 11556-RGE]; Advantix™ 10 for Dogs; G804S16 Insecticide (Nominal Imidacloprid 8.8%/Permethrin 44.0%), Imidacloprid with Permethrin Topical Solution; Imidacloprid 8.84% & Permethrin 43.73%; Lot No. A-01-048-G804-01-02-11

Study/Species/Lab Study #/Date	MRID	Results	Core Grade
Companion Animal Safety puppy [7-wk old beagle at start of study]/Bayer Corporation Agriculture Division Toxicology/01-S26-DM/DEC-4-2001	45563003	Test material was dermally applied to a group of 6M & 6F beagle dog puppies (7 weeks old at start of study) at 2.0 mL/puppy or 5X the single-application dose rate specified on the label on days 0, 7, 14 & 21. 6M & 6F beagles received placebo (1.0 mL application of formulation less the active ingredients). One female puppy died on day 25 from a congenital digestive/cardiac condition. There were noticeable food consumption differences between the 5X group and their controls on days 1 and 8 (day 1: 323.75 g vs. 478.25 g or -32.3%; day 8: 580.25 vs. 686.25 g or -28.4%). There was also a somewhat lower food consumption for 5X puppies in the period from day 14 to day 16, although this was not as pronounced. There was no evidence for lower food consumption following the fourth (day 21) treatment. There were no other indications of any adverse effects in the puppies as statistically significant differences in hematology and clinical chemistry were sporadic, and not biologically relevant as they were all within normal ranges. It is noted that test material treated puppies received four 5X single-dose applications in a month, or a cumulative of 20X the monthly label-dose rate. As food consumption reductions were transient, an adequate safety margin (approximately 5X in terms of overt symptoms) was demonstrated.	A

Core Grade Key: **A =Acceptable, S = Supplementary, U = Unacceptable, V = Self Validated**