

US EPA ARCHIVE DOCUMENT




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

MAR 8 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: SIMAZINE Second Round Review (SRR)

FROM: 
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TO: Christine Dively
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Attached to this cover memorandum are the Topical Summaries, Disciplinary Review and Generic Data Requirements for the Ecological Effects Branch Chapter of the Simazine SRR.

cc: Carol Stangel, SPSMS (memorandum only)
Esther Saito, SIPS coordinator

SIMAZINE 080807

Ecological Effects Chapter

INTRODUCTION

The Ecological Effects Chapter for the initial Simazine Science Chapter identified two outstanding data requirements, an avian reproduction test with an upland gamebird and a coldwater fish acute toxicity test with a typical end-use product. Studies to fulfill these requirements have been accepted. Subsequent to issuance of the Simazine Registration Standard (March 1984) the regulations for data requirements of pesticides were issued (40 CFR Part 158, November 1984). In accordance with these regulations, additional data are required for support of the simazine registration. Also, certain tests accepted in the earlier standard review have been found to be deficient in this review and will have to be upgraded. Tests requiring replacement include an avian reproduction study with waterfowl, an estuarine mollusc acute test and an estuarine fish acute test. Data newly required by this Second Round Review as a result of an assessment of available information and promulgation of the regulations for data requirements include an aquatic field study and Tier II phytotoxicity tests for non-target plants.

TOPICAL SUMMARIESEffects on Birds

Eleven studies in seven citations have been evaluated under this topic. These studies were used in performing a hazard assessment.

<u>Author</u>	<u>MRID#</u>
Beavers & Breslin	163134
Beliles <u>et al.</u>	0043671
Beliles <u>et al.</u>	0043672
Fink	0043672
Fink	0072798
Gough & Shellenberger	0070001 00139393
Hill <u>et al.</u>	0034769 00022923

In order to establish the toxicity of simazine to birds, the minimum data required on the technical material are:

- An avian single-dose LD50 test with either one species of waterfowl, preferably the mallard, or one species of upland gamebird, preferably bobwhite (section 71-1); and
- Two avian dietary LC50 tests, one with a species of waterfowl, preferably the mallard, and one with a species of upland gamebird, preferably the bobwhite (section 71-2).

Avian Acute Oral Toxicity - Technical

An acceptable acute oral toxicity studies on simazine is listed below.

<u>Species</u>	<u>Test Material</u>	<u>Results</u>	<u>Author</u>	<u>Date</u>	<u>MRID</u>	<u>Fulfills Req.</u>
Mallard	Tech.	LD50=4640 mg/kg	Fink	1976	0072798	yes

Avian Dietary Toxicity - Technical

The acceptable avian dietary toxicity studies on technical simazine are listed below.

<u>Species</u>	<u>Test Material</u>	<u>Results</u>	<u>Author</u>	<u>Date</u>	<u>MRID</u>	<u>Fulfills Req.</u>
Bobwhite	98.9% LC50>20,000 ppm		Gough & Shellenberger	1972	00139393 0070001	yes
Bobwhite	98.9% LC50>2000 ppm (11 week)		Gough & Shellenberger	1972	00139393 0070001	no ^{1/}
Bobwhite	99.1% LC50>5000 ppm		Hill <u>et al.</u>	1975	00022923 0034796	yes
Japanese quail	99.1% LC50>3720 ppm		Hill <u>et al.</u>	1975	00022923 0034796	no ^{2/}
Ring-necked pheasant	99.1% LC50>5000 ppm		Hill <u>et al.</u>	1975	00022923 0034796	yes
Mallard	99.1% LC50>5000 ppm		Hill <u>et al.</u>	1975	00022923 0034796	yes

new numbers
JA
1/23/90

1/ Extended exposure period and a maximum test concentration below accepted minimum of 5000 ppm.

2/ Not a recommended test species.

The guideline requirements for acute avian toxicity testing have been fulfilled. These test results show that simazine is practically non-toxic to birds.

Avian Dietary Toxicity - Formulated Product

The acceptable avian dietary toxicity studies on formulated simazine are listed below.

<u>Species</u>	<u>Test Material</u>	<u>Results</u>	<u>Author</u>	<u>Date</u>	<u>MRID</u>	<u>Fulfills Req.</u>
Bobwhite	80% WP LC50=11,000 ppm		Beliles <u>et al.</u>	1965	0043671	no ^{3/}
Mallard	80% WP LC50>32,000 ppm		Beliles <u>et al.</u>	1965	0043671	no ^{4/}

3/ Birds were older than recommended age.

4/ Birds were younger than the recommended age.

Avian Reproduction Studies - Technical Simazine

Avian reproduction studies may be required (section 71-4) for pesticides which are stable in the environment to the extent that potentially toxic amounts may persist in avian feed. Simazine may persist in the environment (up to 40 days in soil, up to 700 days in water) and therefore subject birds to repeated and/or continuous exposure.

The acceptable avian reproduction toxicity studies on technical simazine are listed below.

<u>Species</u>	<u>Test Material</u>	<u>Results</u>	<u>Author</u>	<u>Date</u>	<u>MRID</u>	<u>Fulfills Req.</u>
Mallard	99.1%	NOEL>20 ppm	Fink	1974	0043678	no ^{5/}
Bobwhite	97.0%	NOEL=100 ppm LOEL=500 ppm	Beavers & Breslin	1986	163134	yes

5/ The maximum test concentration is below concentrations reasonably anticipated on waterfowl foodstuffs.

The guideline requirements for chronic avian toxicity testing have been fulfilled only for upland gamebirds, the requirement for waterfowl has not been satisfied. The NOEL is based on no observation of effect to body weight, food consumption, eggs laid, eggs cracked, viable embryos, live three-week embryos, normal hatchlings, 14-day old survivors and eggshell thickness. The LOEL for bobwhite is based on an observed reduction in number of eggs laid.

Field Testing

Avian field testing may be required depending on the results of the avian toxicity studies and available fate and exposure information. Also, though unusual and rare, field testing may be required if toxicity information on other organisms and related fate and exposure information indicate that indirect effects to birds may be expected. No requirements for avian field testing are imposed at this time.

Precautionary Labeling

Based on the available information, no toxicity labeling for birds is needed.

Effects on Freshwater Fish

Twelve studies in ten citations have been evaluated under this topic. These studies were used in performing a hazard assessment.

<u>Author</u>	<u>MRID#</u>
Beliles <u>et al.</u>	00025438
Bowman	40245701
Gilderhus	00025433
Kuc	00043666
Kuc	163136
Mayer & Sanders	00043676
Sleight	00033309
Swabey & Schenk	00034214
Thompson & Forbis	163135
Zak <u>et al.</u>	00043668

✓ Fish Acute Toxicity Tests - Technical

The minimum data required for establishing the acute toxicity of simazine to fish are the results from two 96-hour studies with the technical product. One with coldwater species, preferably rainbow trout, the other with a warm water species, preferably bluegill sunfish (section 72-1). The acceptable fish studies are listed below.

<u>Species</u>	<u>Test Material</u>	<u>Results</u>	<u>Author</u>	<u>Date</u>	<u>MRID</u>	<u>Fulfills Req.</u>
Rainbow trout	Tech.	LC50=70.5 ppm	Kuc	1976	0043666	yes
Bluegill sunfish	99.1%	LC50=16 ppm	Beliles	1965	0025438	yes
Fathead minnow	Tech.	LC50=6.4 ppm	Sleight	1971	0033309	yes
Rainbow trout	97.6%	LC50>10 ppm	Thompson & Forbis	1983	163135	no ^{1/}

^{1/} The study did not expose animals to a maximum recommended test concentration of 100 ppm or demonstrate that a maximum solubility/dispersion concentration was achieved.

These studies fulfill the guideline requirement for fish acute toxicity tests for simazine with technical material. They show that technical simazine is moderately toxic to finfish in acute exposures.

✓ Fish Acute Toxicity Tests - Formulated Product

Data on the acute toxicity of formulated products of simazine to fish are required for uses with direct application to water. Two studies are minimally required for fulfillment of formulated product testing, one with coldwater species, preferably rainbow trout, the other with a warm water species, preferably bluegill sunfish (section 72-1). The acceptable fish studies are listed below.

<u>Species</u>	<u>Test Material</u>	<u>Results</u>	<u>Author</u>	<u>Date</u>	<u>MRID</u>	<u>Fulfills Req.</u>
Rainbow trout	80% WP	LC50>82 ppm	Bowman	1987	40245701	yes
Rainbow trout	80% WP	LC50=60 ppm (7° C) LC50=40.5 ppm (12° C) LC50=44.6 ppm (17° C)	Kuc	1976	163136	no ^{2/}
Emerald shiner	50%	LC50>18ppm	Swabey & Schenk	1963	0034214	no ^{3/}

2/ Test material precipitated in test solutions with no verification of exposure concentrations.

3/ Not a recommended test species and maximum test concentration below accepted level.

These studies fulfill the guideline requirement for fish acute toxicity tests for simazine with formulated product. They show that formulated simazine is moderately toxic to finfish in acute exposures.

✓ Fish Early Life Stage/Reproductive Tests

Fish chronic toxicity studies may be required (section 72.5). Simazine is expected to persist in water with a 1/2-life from 50 to 700 days, therefore, a fish chronic toxicity study is required. The acceptable fish chronic studies are listed below.

<u>Species</u>	<u>Test Material</u>	<u>Results</u>	<u>Author</u>	<u>Date</u>	<u>MRID</u>	<u>Fulfills Req.</u>
Rainbow trout	Tech.	28-day LC50 > 2.5 ppm	Zak	1973	00043668	no ^{4/}
Fathead minnow	80% WP	NOEL=1.2 ppm LOEL=2.5 ppm	Mayer & Sanders	1975	00043676	no ^{5/}

4/ Test unable to identify a No Observable Effect Concentration.

5/ Use of formulated material and failure to report measured concentration values.

The guideline requirements for finfish chronic toxicity testing have been fulfilled by the combination of the above tests. The NOEL for fathead minnow is based on no observation of effect to spawning, hatching, larval/fry growth and survival. The LOEL for fathead minnow is based on an observed reduction in fry growth.

Field Studies

Field studies may also be required to determine exposure or effects to finfish (section 72-7). Aquatic field testing may be required depending on the results of laboratory toxicity studies and available fate and exposure information. Also, though unusual and rare, field testing may be required if toxicity information on other organisms and related fate and exposure information indicate that indirect effects to finfish may be expected.

A simulated field study by Gilderhus (1967, 00025433) shows that at < 5 ppm simazine is effective for control of Elodea canadensis. The study was conducted in nine 0.01-acre concrete ponds filled with Mississippi River water. Each pond had 4 inches of loam on the bottom and was initially stocked with Elodea. A total of 150 bluegill and 150 goldfish were placed in each pond.

Three ponds were untreated and served as controls, three were treated once with 1, 2.5 and 5 ppm, respectively, and the remaining three were treated with 1, 2.5 and 5 ppm, respectively, every 4 weeks for a total of 5 treatments each. The 5 ppm concentration did not reduce survival of goldfish during the study, but it did reduce survival of bluegill. Fish survival during the study was not reduced at 2.5 ppm for either species. Some physiological stress was observed. Goldfish and bluegills from pools treated monthly were subjected to histopathological examination at 5, 10 and 20 weeks after treatment. Goldfish at all concentrations with repeated treatments had lifted lamellar epithelium and edematous lamellae in the gills. Those exposed to repeated treatments of 2.5 and 5 ppm showed ulceration of the gastric mucosa and after 20 weeks those exposed to 5 ppm developed liver damage. This study is not considered adequate to fulfill the guideline requirement for aquatic field testing, if imposed.

No requirements for aquatic field testing with finfish are imposed at this time.

Precautionary Labeling

Based on the available information, no toxicity labeling for fish is needed.

Effects on Aquatic Invertebrates

Nine studies in four citations have been reviewed and used to perform a risk assessment on aquatic invertebrates.

<u>Author</u>	<u>MRID#</u>
Gilderhus	00025433
Mayer & Sanders	00043676
Mayer & Ellersieck	40098001
Walker	RIOSIM01

✓ Aquatic Invertebrate Acute Toxicity Tests - Technical

The minimum data requirement for establishing the acute toxicity of simazine to aquatic invertebrates is the result from one 48-hour (or 96-hour) acute toxicity test with the technical product (section 72-2). Acceptable tests are listed below.

<u>Species</u>	<u>Test Material</u>	<u>Results</u>	<u>Author</u>	<u>Date</u>	<u>MRID</u>	<u>Fulfills Req.</u>
<u>Daphnia magna</u>	98.1%	EC50>10 ppm 48hr	Mayer & Ellersieck	1986	40098001	no ^{1/}
<u>Cypridopsis vidua</u>	98.1%	LC50=3.7 ppm 48hr	"		"	yes
<u>Gammarus fasciatus</u>	98.1%	LC50=130 ppm 96hr	"		"	yes
<u>Palaemonetes kadiakensis</u>	98.1%	LC50>5.6 ppm 24hr	"		"	yes
<u>Pteronarcys californica</u>	98.1%	LC50=1.9 ppm 96hr	"		"	yes

1/ Test concentration below maximum accepted test concentration.

These studies fulfill the requirements for an acute toxicity testing with aquatic invertebrates and shows that technical simazine is moderately toxic to this group in acute exposures.

✓ Aquatic Invertebrate Acute Toxicity Tests - Formulated Product

Data on the acute toxicity of formulated products of simazine to aquatic invertebrates are required for uses with direct application to water. The acceptable aquatic invertebrate studies are listed below.

<u>Species</u>	<u>Test Material</u>	<u>Results</u>	<u>Author</u>	<u>Date</u>	<u>MRID</u>	<u>Fulfills Req.</u>
<u>Daphnia magna</u>	80% WP 48hr	EC50>10 ppm	Mayer & Ellersieck	1986	40098001	no ^{2/}

2/ Maximum test concentration below maximum accepted test concentration.

This study fulfills the guideline requirement for aquatic invertebrate acute toxicity testing for formulated simazine when combined with the chronic daphnid test performed with formulated product (Mayer and Sanders, 1975). Acceptable aquatic invertebrate This study shows that formulated simazine is moderately toxic to aquatic invertebrates in acute exposures.

✓ Reproductive Aquatic Invertebrate Testing

Reproductive testing with aquatic invertebrates may be required (section 72-4). Since simazine is registered for major outdoor uses (forests, corn, alfalfa, ponds, etc.) and persistent in water (1/2-life from 50 to 700 days) aquatic invertebrate reproductive testing is required. Acceptable aquatic invertebrate toxicity tests are listed below.

<u>Species</u>	<u>Test Material</u>	<u>Results</u>	<u>Author</u>	<u>Date</u>	<u>MRID</u>	<u>Fulfills Req.</u>
<u>Daphnia magna</u>	80% WP	NOEL=2.5 ppm	Mayer & Sanders	1975	0043676	no ^{3/}
<u>Benthic organisms</u>	Tech.	NOEL>10 ppm < 20 ppm	Walker	1964	RIOSIM01	no ^{4/}

3/ Test material was formulated product.

4/ Test methods (scope) insufficient to fulfill a full-scale aquatic field test.

In Walker (1964, RIOSIM01), waterbugs, mayfly nymphs, horsefly larvae, common midges, mosquitoes, phantom midges, biting midges, caddisfly larvae, dragonfly nymphs, damsel fly nymphs, water beetles, aquatic worms, leeches, clams and snails found in natural mud samples were submerged in buttermilks. Concentrations of 10 ppm resulted in little more mortality than the controls. This does not meet any guideline requirement because it is not a standard protocol.

Field Studies

Field studies may also be required to determine exposure or effects to aquatic invertebrates (section 72-7). Aquatic field testing may be required depending on the results of laboratory toxicity studies and available fate and exposure information. Also, though unusual and rare, field testing may be required if toxicity information on other organisms and related fate and exposure information indicate that indirect effects to aquatic invertebrates may be expected.

A simulated field study by Gilderhus (1967, 00025433) shows that at < 5 ppm simazine is effective for control of Elodea canadensis. The study was conducted in nine 0.01-acre concrete ponds filled with Mississippi River water. Each pond had 4 inches of loam on the bottom and was initially stocked with Elodea. A total of 150 bluegill and 150 goldfish were placed in each pond.

Three ponds were untreated and served as controls, three were treated once with 1, 2.5 and 5 ppm, respectively, and the remaining three were treated with 1, 2.5 and 5 ppm, respectively, every 4 weeks for a total of 5 treatments each.

The total production of zooplankton over the 5-month test was similar in all ponds. The bottom fauna populations in the controls, 1 ppm yearly and 1 ppm monthly, were similar. Their mean number of organisms per square foot and the peak populations were nearly identical, revealing no effect of simazine. The 2.5 and 5 ppm yearly and monthly treatments appeared to suppress bottom fauna for 2 or 3 weeks following treatments but then those ponds developed populations which were far in excess of those in controls or the 1 ppm concentrations. The researcher concluded that the temporary reductions were due to oxygen depletion due to destruction of the vegetation. Whether this conclusion is correct or not, the study does show that concentrations of simazine which control vegetation do not totally eliminate all benthic fauna. However, no information was provided on species diversity, so the study does not unequivocally show that simazine did not affect aquatic invertebrates. This study alone would not fulfill the requirement for a aquatic field test.

Such testing is reserved pending receipt of outstanding aquatic organism testing and environmental fate information.

Precautionary Labeling

Based on the available information, no toxicity labeling for aquatic invertebrates is needed.

✓ Effects on Estuarine and Marine Organisms

Four studies in three citations were reviewed and used to perform the hazard assessment on marine and estuarine organisms.

<u>Author</u>	<u>MRID#</u>
Cook and Smith	00043667
Sleight and Macek	00023331
Wright and Beliles	00043677

✓ Acute Toxicity Tests - Technical

Toxicity testing with estuarine and marine organisms may be requested (section 72-3). The requirements under this category include a 96-hour LC50 for an estuarine fish, a 96-hour LC50 for a crustacean and either a 48-hour embryo-larvae study or a 96-hour shell deposition study with oysters. The following studies are acceptable.

<u>Species</u>	<u>Test Material</u>	<u>Results</u>	<u>Author</u>	<u>Date</u>	<u>MRID</u>	<u>Fulfills Req.</u>
Eastern oyster	99.1%	96hrEC50>1 ppm	Wright & Beliles	1966	00043677	no ^{1/}
Pink shrimp	98.9%	96hrLC50=113ppm	Sleight & Macek	1973	00023331	yes
Mud crab	"	96-hr LC50>1000ppm	"	"	"	no ^{2/}

1/ Maximum test concentration below maximum accepted test concentration.

2/ Test organism not a recommended species.

These data fulfill the requirements for estuarine/marine crustacean testing. They demonstrate that simazine is moderately toxic to crustaceans. They tentatively demonstrate that simazine is moderately toxic to molluscs. These data do not satisfy the requirements for estuarine/marine testing with molluscs and fish.

✓ Acute Toxicity Tests - Formulated Product

Data on the acute toxicity of formulated products of simazine to estuarine/marine organisms may be required for uses with direct application to estuarine water, certain uses of simazine (e.g., rights-of way) could be construed to meet this requirement, but

such direct exposure occurrences would be expected to be minimal and no requirement is imposed for formulated testing. Nonetheless, an acceptable fish study is listed below.

<u>Species</u>	<u>Test Material</u>	<u>Results</u>	<u>Author</u>	<u>Date</u>	<u>MRID</u>	<u>Fulfills Req.</u>
Striped bass	80% WP	96hrNOEL=3 ppm	Cook & Smith	1967	00043677	no ^{1/}

^{1/} Maximum test concentration below maximum accepted test concentration.

This study is not considered adequate to fulfill a formulated product test requirement if one were imposed.

Precautionary Labeling

Based on the available information, no toxicity labeling for estuarine and marine organisms is needed.

Effects on Non-target Plants

No studies were received under this topic. To determine toxicity of simazine to non-target plants, the following studies are required:

- 123-1: Seed germination/seedling emergence and Vegetative Vigor;
- 123-2: Aquatic plant growth.

These tests are required for terrestrial and aquatic non- food uses including forests, rights-of-way, ditchbanks, etc.

Higher tier testing may be required depending on the results of the lower tier tests.

Effects on Beneficial Insects

The following study was received and reviewed under this topic.

<u>Author</u>	<u>MRID#</u>
Atkins <u>et al.</u>	00036935

<u>Species</u>	<u>Test Material</u>	<u>Results</u>	<u>Author</u>	<u>Date</u>	<u>MRID</u>	<u>Fulfills Req.</u>
Honey bee	Tech.	At 96.69 ug/bee mortality was 6.52% (relatively nontoxic)	Atkins <u>et al.</u>	1975	00036935	yes

There is sufficient information to characterize simazine as relatively nontoxic to honey bees.

DISCIPLINARY REVIEW

I Ecological Effects Profile

A Technical Simazine

1. Avian Studies

Three studies show that simazine is practically nontoxic to birds in acute or subchronic exposures. An acute oral study (Fink, 1976, 00072798) resulted in an LD50 > 4640 mg/kg for mallard duck. Avian dietary studies (Hill *et al.*, 1975, 00034796) demonstrate an LC50 of >5000 ppm for both mallards and bobwhite quail.

The chronic toxicity of simazine to upland gamebirds can be characterized by an avian reproduction test (Beavers and Breslin, 1986, 163134) which demonstrates a reduction in eggs laid to bobwhite quail exposed to 500 ppm. No effect to body weight, food consumption, eggs laid, eggs cracked, viable embryos, live three-week embryos, normal hatchlings, 14-day old survivors and eggshell thickness. A study with mallards (Fink, 1974, 0043678) tested simazine only up to 20 ppm, but demonstrated no observed effects at this concentration.

2. Aquatic Studies

Simazine has been demonstrated to be moderately toxic to finfish with a 96-hr LC50 of 6.4 ppm to the fathead minnow, 16 ppm to the bluegill, and 70.5 ppm to the rainbow trout (Sleight, 1971, 00033309; Beliles, 1965, 00025438; and Kuc, 1976, 00043666, respectively). A 28-day test with rainbow trout (Zak, 1973, 00043668) reveals an LC50 of > 2.5 ppm.

Mayer and Ellersieck (1986) reports a 48hr EC50 of greater than 10 ppm to Daphnia magna and a 96hr LC50 of 1.9 ppm to Pteronarcys californica which characterizes simazine as moderately toxic to aquatic invertebrates in acute exposures.

3. Estuarine Studies

Sleight and Macek (1973, 0002331) report a 96-hr LC50 of 113 ppm to pink shrimp and Wright and Beliles (1966, 00043677) report a 96-hr EC50 for shell deposition of greater than 1 ppm which characterizes simazine as moderately toxic to estuarine molluscs and practically nontoxic to estuarine crustaceans.

B Formulated Product Testing

Simazine toxicity of formulated products to non-target fish and wildlife has been assessed by a variety of tests with the 80% active ingredient wettable powder.

1. Avian Studies

Two studies show that formulated simazine is practically nontoxic to birds in acute dietary exposures. Beliles *et al.* (1965, 0043671) report an LC50 of >32,000 ppm and =11,000 for mallards and bobwhite quail, respectively.

2. Aquatic Studies

Formulated simazine has been demonstrated to be slightly toxic to finfish with a 96-hr LC50 of 40.5 ppm to the rainbow trout (Kuc, 1976, 163136). A fathead minnow partial chronic test (Mayer and Sanders, 1975, 00043676) revealed a reduction in fry growth at 2.5 ppm but no observed effects to spawning, hatching, larval/fry growth and survival at 1.2 ppm.

A simulated field study (Gilderhus, 1967, 00025433) conducted in 0.01-acre concrete ponds demonstrated mortalities to bluegill at 5 ppm, and sublethal effects (behavioral and histopathological) occurred at single treatments of 2.5 ppm and above and at repeated treatments of 1 ppm (lowest rate tested) and above. The 2.5 ppm and 5 ppm treatments temporarily reduced benthic invertebrates after initial treatment.

II Uses

Simazine is a selective or non-selective, triazine herbicide used to control grasses, broadleaf, woody plants and algae. Simazine is formulated as a granular, soluble granular, emulsifiable concentrate, and wettable powder.

A Use Sites

Terrestrial, food uses include: citrus, corn, apples, blueberries, grapes, pastures, and walnuts.

Terrestrial, non-food uses include: turf, ornamentals, non-crop areas, rangeland, rights-of-way, Christmas tree plantations, forest nurseries, and forest plantings.

Aquatic, non-food use: ponds, swimming pools and cooling water.

Uses cancelled since the initial registration standard include alfalfa, drainage ditch banks, hay, grass grown for seed, tree plantations grown for timber and tree seedlings.

B Discussion of Uses

According to the Preliminary Quantitative Use Assessment, October, 1987, the primary usage of simazine is for corn (29%), aquatic (27%), alfalfa (10%), citrus (9%), grapes (4%) and apples (3%).

III Environmental Fate Information

Information presented in the original standard and presented here indicate that available data are insufficient to fully assess the environmental fate of simazine. Simazine is relatively persistent in soil (1/2-life reported from 10 to 40 days) with microbial action the primary degradation pathway. In water, simazine is very persistent (1/2-life reported up to 700 days in pond water). Simazine is stable to photolysis and is not considered to be bioaccumulative.

Simazine is absorbed by plant roots and is translocated to the stem and leaves. Within the plant cell, it inhibits photosynthesis. The greater the amount of vegetation, the faster simazine dissipates.

IV Hazard Assessment

A Discussion

The available information indicates that simazine is practically non-toxic to terrestrial wildlife and moderately toxic to aquatic organisms in acute exposures. Technical simazine is a moderate eye and dermal irritant (Toxicity Category III) and has low oral and dermal toxicities (Toxicity Category IV) in mammals. The chronic toxicity of simazine to terrestrial organisms is generally low based on the observations that reproduction is not impaired at 20 ppm or 100 ppm in mallards or bowwhite quail, respectively. Slight impairment observed at 500 ppm in bobwhite quail provides evidence that a potential chronic concern is justified only at relatively high residues (> 100 ppm). For concentrations above 100 ppm, additional information on mallard reproduction is needed for an adequate assessment. With a fish chronic NOEL of 1.2 ppm and adverse effects to aquatic invertebrates observed at 1 ppm in a simulated field test, aquatic organisms are potentially at risk to chronic exposure of concentrations above 1 ppm.

Simazine is registered for numerous outdoor uses, including agricultural crops such as corn, and non-agricultural uses such as rights-of-way and ponds. Exposure to non-target organisms can result from residues of direct applications, spray drift from treated areas and runoff from treated areas. Such exposures would be both acute and chronic. Due to the absence of a complete set of environmental fate and non-target organism toxicity data, a full Ecological Effects Hazard Assessment cannot be completed at this time.

Agricultural uses have application rates typically up to 4 lbs. a.i./acre, although a few uses (e.g., citrus) allow rates up to 9.6 lbs. a.i./acre, and non-crop uses up to 40 lbs. a.i./acre.

TABLE 1 - Expected Residues (ppm)

Vegetation/Surface Type	Application Rate (a.i.)			
	1 lb.	4 lbs. maximum (typical)	9.6 lbs.	40 lbs.
Short grass	240(125)	960(500)	2304(1200)	9600(5000)
Long grass	110(92)	440(368)	1056(883)	4400(3680)
Leafy crops	125(35)	500(140)	1200(336)	5000(1400)
Forage/Small Insects	58(33)	232(133)	557(317)	2320(1320)
Seeds/Pods/Large Insects	12(3)	48(12)	115(29)	480(120)
Fruit	7(1.5)	28(6)	67(14)	280(60)
Soil (0.5 inches)	4.4		42	176
Water (0.5 feet)	0.7		7	29

TABLE 2 - Measured Simazine Residues on Various Crops

Crop	Rate lbs. a.i. per acre	Days after application	Residue (ppm)
Bermuda grass	2.4	30	≤ 13.00
	2.56	28	≤ 34.00
	4.0	60	≤ 0.23
	5.0	50	≤ 0.91
	16.0	90	≤ 0.70
Red Fescue & Perrennial ryegrass	3.0	29	1.19
	3.0	36	0.45
Alfalfa	1.6	23	11.00
	1.6	52	0.65
	2.0	14	17.00
	4.0	19	30.00

Measured residues indicate a cumulative half-life on vegetation of between 5 and 10 days. This rate includes dissipation and degradation rates. Initial residues will generally dissipate very rapidly moving off plant surfaces into the soil to be later translocated into the plant. It can be reasonably concluded from the above tables that at typical agricultural rates up to 4 lbs. a.i./acre initial rates on vegetation will be well below acutely toxic concentrations (LC50 > 5000 ppm) and will be below laboratory derived NOEC for bobwhite reproduction within 1 week on typical avian forage. The highest concentration tested for mallards was 20 ppm which showed no adverse effects, but additional data at higher test concentrations are needed to allow assessment of use rates greater than 1 lb. a.i./acre which will allow residues on waterfowl forage to exceed 20 ppm for greater than one week. Although rates above 4 lbs. a.i./acre are not expected to pose any acute risks to terrestrial organisms, concentrations which exceed the laboratory derived NOEC for bobwhite reproduction can be expected for several weeks after treatment. In considering chronic risks to birds, it must be understood that the laboratory derived LOEC of 500 ppm for bobwhite quail identified only a minor, albeit significant, reduction in eggs laid. Also, non-crop uses which allow high rates of application generally involve treatment of areas with little or no vegetation as a pre-emergent control and vegetation treated at such high rates would also be destroyed. Therefore, forage at such sites would be very limited except in margin areas between treated and untreated sites.

With a half-life in pond water of up to 700 days, residues of simazine which reach aquatic environments will likely pose serious chronic risks at concentrations above 1 ppm. Agricultural uses, even at maximum rates of 9.6 lbs. a.i./acre, are not expected to allow contamination of aquatic environments to concentrations reaching 1 ppm. Specifically for 9.6 lbs. a.i./acre use,

Drift (5% from aerial) = 0.48 lbs. a.i./acre loading
 in 6 inches of water = 352 ppb
 in 6 feet of water = 29.4 ppb

Runoff (1% in 10-to-1 basin) = 0.96 lbs. a.i./acre loading ✓
 in 6 inches of water = 705 ppb
 in 6 feet of water = 58.6 ppb.

These concentrations are below laboratory observed effect concentrations and are not expected to pose any direct acute or chronic risks to aquatic organisms. This conclusion is further supported by STORET data. Of 5849 surface water samples analyzed for simazine since 1966, 941 samples had detectable residues. One sample, taken 10-16-86 in California, was 1.3 ppm. Twenty samples revealed concentrations from 100 to 700 ppb. All other samples with detectable residues had concentrations generally below 30 ppb. This information, though not specifically targeting areas where simazine use or exposure potential is high, suggests that concentrations of simazine which could threaten aquatic fauna are predominately not exceeded and despite being persistent does not accumulate in the environment.

Non-crop uses (rights-of-way, forestry, etc.) with rates up to 40 lbs. a.i./acre will marginally exceed the 1 ppm critical level identified for aquatic organisms. In worst case scenarios, 20% of the aerially applied or even ground applied (primarily ditchbank uses only) simazine could reach aquatic environments. At maximum rates, this translates into water concentrations of 5.9 ppm in six inches of water and 0.49 ppm in six feet of water. Small streams and shallow ponds would be at risk. For most of these uses, ground cover would be at a minimum as recommended by the label. Without groundcover, areas treated with simazine would have a greater susceptibility for runoff loss than vegetated or cultivated areas. With this consideration, 5% runoff loss of simazine is not unrealistic in non-crop areas such as rights-of-way adjacent to aquatic habitat. A scenario of 5 treated acres for every acre-foot of adjacent water would yield water concentrations of 3.7 ppm. Uses which allow direct application to water (ponds, etc.) have rates of up to 3.4 lbs. a.i./acre-foot in ponds without drainage and 12 lbs. a.i./acre-foot in ponds with drainage. This would allow for concentrations of simazine in pond water to be 2.5 ppm in ponds without drainage and 8.8 ppm in ponds ✓

with drainage. Both are likely to pose substantial risk to fish and invertebrates within the treated ponds, also for ponds with drainage receiving streams for such drainage could be at risk. Additional data (aquatic field testing) are needed to fully evaluate the potential direct and indirect effects to aquatic systems.

B Summary of Hazard

No adverse effects to non-target fish and wildlife are indicated for acute exposure to simazine at any application rate. Chronic risks to aquatic organisms and wildlife may occur at non-crop use rates. Aquatic organisms would also be at high risk from maximum aquatic use rates. Additional information is needed to assess potential risks from these chronic exposures and non-target plant exposures.

V Endangered Species

Because of its expected toxicity to non-target plant species (based on its label claims as a herbicide) and its intended use pattern, simazine has been identified by the Office of Endangered Species (OES), U. S. Fish and Wildlife Service (FWS), as being likely to jeopardize endangered species when used on forests and/or rangeland. Based on this determination, OES specified reasonable and prudent alternatives to avoid jeopardizing the continued existence of the identified species by these uses. EPA is working with the FWS and other Federal and State agencies to implement the alternatives in a technically sound manner.

In May 1987, EPA issued PR Notices 87-4 and 87-5 in response to OES findings that certain pesticides, including simazine, jeopardized the continued existence of endangered species. Those PR Notices directed registrants to add labeling to their products which referred users to additional information that, in turn, explained limitations on use of the pesticide within the range of jeopardized endangered species. Subsequent to issuance of these PR Notices, EPA identified a number of significant technical errors and inconsistencies in the information to which users would have been referred. Therefore, on January 26, 1988 the Agency issued PR Notice 88-1 which withdrew 87-4 and 87-5 pending development of a more focused program to protect endangered species.

EPA is working to correct these errors prior to requiring labeling to protect endangered species. When that program is fully developed, notice of any labeling necessary to protect endangered species will be issued.

VI Precautionary Labeling

A. Manufacturing Use

"Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or public waters unless this product is specifically identified and addressed in an NPDES permit. Do not discharge effluent containing this product to sewer systems without previously notifying the sewage treatment plant authority. For guidance, contact your State Water Board or Regional Office of the EPA."

B. End-Use Products

1. Non-aquatic use sites:

"Do not apply directly to water or wetlands (swamps, bogs, marshes, and potholes). Do not contaminate water when disposing of equipment washwaters."

2. Aquatic use sites:

"Do not contaminate or allow discharge into domestic, livestock, or irrigation water supplies, or lakes or streams. Do not contaminate water when disposing of equipment washwaters."

VII CLASSIFICATION

The available information warrants that simazine be classified for restricted use for all aquatic uses and all non-crop uses with application rates > 10 lbs. a.i./acre.

VIII DATA REQUIREMENTS

The required data are provided in Table A, attached.

TABLE A
Generic Data Requirements for SIMAZINE

Data Requirement	Composition ^{1/}	Use Pattern ^{2/}	Does EPA have data to satisfy this requirement? (Yes, No or Partially)	Bibliographic Citation	Must additional data be submitted under FIFRA Section 3(c)(2)(B)?
\$158.145 Wildlife and Aquatic Organisms					
Avian and Mammalian Testing					
71-1 Avian Oral LD50	TGAI	A,B,D,G	Yes	72798	No
71-2 Avian Dietary LC50 a-waterfowl b-upland game	TGAI	A,B,D,G	Yes	00022923 34796	No
	TGAI	A,B,D,G	Yes	00022923 34796; 70004 00139393	No
71-3 Wild Mammal Toxicity	TGAI	A,B,D,G	No		No
71-4 Avian Reproduction	TGAI	A,B,D,G	Partially	43678, 163134	Yes ^{3/}
71-5 Simulated and Actual Field Testing - Mammals	TGAI	A,B,D,G	No		No ^{4/}
Aquatic Organism Testing					
72-1 Freshwater Fish LC50					
a-warmwater	TGAI	A,B,D,G	Yes	25438, 33309	No
	TEP	D,G	Yes	40098001	No
b-coldwater	TGAI	A,B,D,G	Yes	✓ 43666	No
	TEP	D,G	Yes	✓ 40245701	No
72-2 Freshwater Invertebrate Acute EC50					
	TGAI	A,B,D,G	Yes	40098001	No
	TEP	D,G	Yes	✓ 40098001, 43676	No

TABLE A (Continued)
Generic Data Requirements for SIMAZINE

Data Requirement	Composition ^{1/}	Use Pattern ^{2/}	Does EPA have data to satisfy this requirement? (Yes, No or Partially)	Bibliographic Citation	Must additional data be submitted under FIFRA Section 3(c)(2)(B)?
72-3 Estuarine/Marine Organism Acute EC50	a-finfish	TGAI	No		Yes ^{5/}
	b-crustacean	TGAI	Yes	23331	No
	c-mollusc	TGAI	No		Yes ^{5/}
72-4 ^{1a} Fish Early-Life Stage and Aquatic Invertebrate Life-Cycle	TGAI	A,B,D,G	Yes	43668, 43676	No
72-5 Aquatic Organism Accumulation	TGAI	D,G	Partially	43668, 43670	No ^{6/}
72-6 Life-Cycle Tests with Fish	TGAI	A,B,D,G	Yes	43676	No
72-7 Simulated or Actual Field Testing - Aquatic	TEP	A,B,D,G	Partially	25433	Yes ^{7/}

TABLE A (Continued)
Generic Data Requirements for SIMAZINE

Data Requirement	Composition ^{1/}	Use Pattern ^{2/}	Does EPA have data to satisfy this requirement? (Yes, No or Partially)	Bibliographic Citation	Must additional data be submitted under FIRRA Section 3(c)(2)(B)?
§158.150 Plant Protection Testing					
Tier I:					
122-1 Seed Germination/Seedling Seedling Emergence	TGAI	B,D,G	No		No ^{8/}
Vegetative Vigor	TGAI	B,D,G	No		No ^{8/}
122-2 Aquatic Plant Growth	TGAI	B,D,G	No		No ^{8/}
Tier II:					
123-1 Seed Germination/Seedling Seedling Emergence	TGAI	B,D,G	No		Yes ^{9/}
Vegetative Vigor	TGAI	B,D,G	No		Yes ^{9/}
123-2 Aquatic Plant Growth	TGAI	B,D,G	No		Yes ^{9/}
Tier III:					
124-1 Terrestrial Field	TGAI	B,D,G	No		No ^{10/}
124-2 Aquatic Field	TGAI	B,D,G	No		No ^{10/}

- 1/ Composition: TGA1 = Technical grade of the active ingredient; PA1 = Pure active ingredient; TEP = Typical end-use product.
- 2/ The use patterns are coded as follows: A=Terrestrial, Food Crop; B=Terrestrial Non-Food Crop; C=Aquatic, Food Crop; D=Aquatic, Non-Food Crop; E=Greenhouse, Non-Food Crop; G=Forestry; H=Domestic Outdoor; I=Indoor.
- 3/ Avian reproduction testing with waterfowl, e.g., mallard duck, at test concentrations of 100, 500 and 2500 is needed to evaluate high expected exposures from simazine uses > 10 lbs. a.i. per acre.
- 4/ Reserved pending receipt of waterfowl reproduction requirements.
- 5/ Required for citrus, corn, rights-of-way, and turf.
- 6/ Available data insufficient to fulfill data requirement but are considered adequate to indicate a very low potential to bioaccumulate (BCF < 6x) and additional data requirements are waived.
- 7/ Hazard assessment for aquatic uses and high-rate terrestrial uses (e.g., >10 lbs. a.i./acre) indicated a high risk to aquatic organisms. A comprehensive aquatic field study to quantify direct and indirect effects on aquatic organisms if the hazard indicated by available laboratory and field evidence is ecologically significant. The EEB recommends that a protocol for conducting this study be submitted prior to initiation of the field work. A guidance document outlining acceptable methods for conducting an aquatic mesocosm test (Touart, 1988, NITS) can be consulted in designing an appropriate comprehensive test. A mesocosm test is recommended because it is believed to be extrapolatable to the variety of simazine uses.
- 8/ These test are not required as simazine is registered for use as a herbicide.
- 9/ Required for all terrestrial non-food and aquatic uses.
- 10/ Higher tier testing is reserved pending receipt of the lower tier tests.

- 1/ Composition: TGA1 = Technical grade of the active ingredient; PA1 = Pure active ingredient; TEP = Typical end-use product.
- 2/ The use patterns are coded as follows: A=Terrestrial, Food Crop; B=Terrestrial Non-Food Crop; C=Aquatic, Food Crop; D=Aquatic, Non-Food Crop; E=Greenhouse, Non-Food Crop; G=Forestry; H=Domestic Outdoor; I=Indoor.
- 3/ Avian reproduction testing with waterfowl, e.g., mallard duck, at test concentrations of 100, 500 and 2500 is needed to evaluate high expected exposures from simazine uses > 10 lbs. a.i. per acre.
- 4/ Reserved pending receipt of waterfowl reproduction requirements.
- 5/ Required for citrus, corn, rights-of-way, and turf.
- 6/ Available data insufficient to fulfill data requirement but are considered adequate to indicate a very low potential to bioaccumulate (BCF < 6x) and additional data requirements are waived.
- 7/ Hazard assessment for aquatic uses and high-rate terrestrial uses (e.g., >10 lbs. a.i./acre) indicated a high risk to aquatic organisms. A comprehensive aquatic field study to quantify direct and indirect effects on aquatic organisms if the hazard indicated by available laboratory and field evidence is ecologically significant. The EEB recommends that a protocol for conducting this study be submitted prior to initiation of the field work. A guidance document outlining acceptable methods for conducting an aquatic mesocosm test (Touart, 1988, NTIS) can be consulted in designing an appropriate comprehensive test. A mesocosm test is recommended because it is believed to be extrapolatable to the variety of simazine uses.
- 8/ These test are not required as simazine is registered for use as a herbicide.
- 9/ Required for all terrestrial non-food and aquatic uses.
- 10/ Higher tier testing is reserved pending receipt of the lower tier tests.

DATA EVALUATION RECORD

PAGE 1 OF

CASE: GS0070

SIMAZINE FRSTR

CONT-CAT: 01

GUIDELINES: 72-1

MRID: 163136

Kuc, W. (1976) Acute Toxicity of Aquazine 80W ... to the Rainbow Trout, *Salmo gairdneri* Richardson at 7.0 [Degree] C: AES Proj. #7637-500. Unpublished study prepared by Aquatic Environmental Sciences. 10 p.

REVIEW RESULTS:

VALID

INVALID

INCOMPLETE

GUIDELINE:

SATISFIED

PARTIALLY SATISFIED

NOT SATISFIED

DIRECT RVW TIME =

START DATE:

END DATE:

REVIEWED BY:

Les Touart

TITLE:

FISHERIES BIOLOGIST

ORG:

CEB/CFED

LOC/TEL:

557-2438

SIGNATURE:

[Signature]

DATE:

3-6-89

APPROVED BY:

TITLE:

ORG:

LOC/TEL:

SIGNATURE:

DATE:

DATA EVALUATION RECORD

1. Chemical: Simazine
2-chloro-4,6-bis(ethylamino)-s-triazine
2. Test Material: Simazine 80W
3. Study Type: Freshwater Fish 96-Hour LC₅₀
Species Tested: Salmo gairdneri
4. Study ID: Kuc, W.J. (1976) Acute Toxicity of Aquazine 80W Batch No. FL-760951 to the Rainbow Trout, Salmo gairdneri. Prepared by: Aquatic Environmental Sciences, Union Carbide Corporation, Tarrytown, NY. Submitted by Ciba-Geigy Corporation. AES Project No. 7636-500. EPA Accession No. 265011.
5. Reviewed By: Thomas M. Armitage
Fisheries Biologist
EEB/HED
Signature: *Thomas M. Armitage*
Date: 10-30-86
6. Approved By: Raymond W. Matheny
Supervisory Biologist
EEB/HED
Signature: *Raymond W. Matheny*
Date:
7. Conclusions:

The study documents a 96-hour LC₅₀ = 40.5 mg/L (95% c.i. = 34.0 to 48.4 mg/L). It does not, however, fulfill the Guidelines requirement for a freshwater fish LC₅₀ determination. This is because a precipitate appeared in the upper concentration 48 hours after the start of the test. An additional deficiency in the study is that the amount of solvent used was not reported.
8. Recommendations:

The study should be repeated using different solvents to eliminate precipitation of the test material. A flow-through system with analytically measured concentrations of toxicant must be used if the chemical cannot be dissolved in a static system.
9. Background:

The study, an acute toxicity determination for a coldwater fish species with Simazine 80W, was submitted to fulfill a data requirement identified in the reregistration guidance package.
10. Discussion of Individual Test: N/A.

11. Materials and Methods: (Definitive Test)

- a. Test Animals: Were rainbow trout cultured from eggs obtained from a commercial hatchery in Washington. At the time of the test the fish had a mean length of 41 mm and a mean weight of 0.61 g.

Test System: 5-gallon glass jars containing 15 liters of water. Loading was 0.41 g/L. Water temperature was 12.0 °C. Initial pH of the test high concentration water was 7.74, final pH was 7.23. Test was 96 hours in duration.

- b. Dose: Static bioassay using nominal concentrations. DMF was used as a solvent.
- c. Design: Ten fish per level. Five dose levels plus solvent and freshwater controls (6.0, 10.0, 17.0, 29.0, and 50.0 ppm).
- d. Statistics: LC₅₀'s were determined using the Spearman-Kärber method (Finney 1971).

12. Reported Results:

The 96-hour LC₅₀ with 95 percent confidence intervals for Aquazine 80W at 12 °C to rainbow trout is 40.5 (34.0 to 48.4) mg/L.

13. Study Author's Conclusions/QA Measures:

96-hour LC₅₀ = 40.5 ppm (95% c.i. = 34.0 to 48.4 ppm).

96-hour no-effect level < 6.0 mg/L.

No QA measures indicated.

14. Reviewer's Discussion and Interpretation of the Study:

- a. Test Procedures: The following deficiencies were noted: A precipitate formed in the highest concentration 48 hours after initiation of the study. Also, the amount of solvent used was not reported.
- b. Statistical Analysis: EEB statistical analysis (results attached) confirms the author's reported result.
- c. Discussion/Results: The study documents a 96-hour LC₅₀ = 40.5 ppm (95% c.i. = 34.0 to 48.4). It does not, however, fulfill the Guidelines requirement. This is because a precipitate was observed in the highest test concentration 48 hours after the start of the test.

Also, the amount of solvent used to dissolve the test material was not reported.

d. Adequacy of Study:

- (1) Classification: Supplemental.
- (2) Rationale: Precipitate in test chambers, amount of solvent used was not reported.
- (3) Repairability: No repair possible. Test must be repeated as described in section 8 above.

15. Completion of One-Liner for Study:

One-liner form completed October 21, 1986.

16. CBI Appendix: Data attached.

TABLE 1
 PERCENT MORTALITIES AND LC50 VALUES - CIBA-GEIGY CORPORATION
 Aquazine 80W - Rainbow Trout

Aquazine 80W Nominal Conc. mg/l	Percent Mortality						
	Control	6.0	10.0	17.0	29.0	50.0	Solvent Control
24 Hour	0%	0%	0%	0%	0%	10%	0%
48 Hour	0%	0%	0%	0%	0%	80%	0%
96 Hour	0%	10%	0%	0%	0%	80%	0%

LC50 Values

LC50 mg/l	95% Confidence Interval		
	24 Hour	48 Hour	96 Hour
62.0	42.7	40.5	40.5
55.7	37.1	34.0	34.0
68.9	49.2	48.4	48.4

The 96 hour no effect level is <6.0 mg/l

ARMITAGE SIMAZINE RAINBOW TROUT 96 HR LC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
50	10	8	80	5.46875
29	10	0	0	.0976563
17	10	0	0	.0976563
10	10	0	0	.0976563
6	10	1	10	1.07422

THE BINOMIAL TEST SHOWS THAT 29 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 42.122

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	9.45182	7.8678	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.78677
 95 PERCENT CONFIDENCE LIMITS = -5.78082 AND 11.3544

LC50 = 48.2018
 95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 16.8787
 95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

DATA EVALUATION RECORD

1. Chemical: Simazine
2-chloro-4,6-bis(ethylamino)-S-triazine
2. Test Material: Simazine 80W
3. Study Type: Freshwater Fish 96-Hour LC₅₀

Species Tested: Salmo gairdneri

4. Study ID: Kuc, W.J. (1976) Acute Toxicity of Aquazine 80W Batch No. FL-760951 to the Rainbow Trout, Salmo gairdneri at 7.0 °C. Prepared by: Aquatic Environmental Sciences, Union Carbide Corporation, Tarrytown, NY. Submitted by Ciba-Geigy Corporation. AES Project No. 7637-500. EPA Accession No. 265011.

5. Reviewed By: Thomas M. Armitage
Fisheries Biologist
EEB/HED

Signature: *Thomas M. Armitage*
Date: 10-30-86

6. Approved By: Raymond W. Matheny
Supervisory Biologist
EEB/HED

Signature: *Raymond W. Matheny*
Date: 10-31-86

7. Conclusions:

The study documents a 96-hour LC₅₀ = 60.0 mg/L (95% c.i. = 49.8 to 72.3 mg/L). It does not fulfill the Guidelines requirement for a freshwater fish LC₅₀ determination. This is because the study was conducted using water at 7.0 °C. The recommended test temperature for rainbow trout is 12.0 °C. The study is also unacceptable because a precipitate formed in the three highest concentrations approximately 24 hours after introduction of the toxicant. An additional deficiency is that the amount of solvent used is not reported.

8. Recommendations:

The study should be repeated using a water temperature of 12.0 °C. Alternative solvents should be used to eliminate precipitation. A flow-through system with analytically measured concentrations of toxicant must be used if the chemical cannot be dissolved in a static system.

9. Background:

The study, an acute toxicity determination for a coldwater fish species with Simazine 80W was submitted to fulfill a data requirement identified in the reregistration guidance package.

10. Discussion of Individual Test: N/A.

11. Materials and Methods: (Definitive Test)

- a. Test Animals: Were rainbow trout cultured from eggs obtained from a commercial hatchery in Washington. At the time of the test the fish had a mean length of 45 mm and mean weight of 0.94 g.

Test System: 5-gallon glass jars containing 15 liters of water. Loading was 0.63 g/L. Water temperature was 7.0 °C. Initial pH was 7.58. Test was 96 hours in duration.

- b. Dose: Static bioassay using nominal concentrations. DMF was used as a solvent.

- c. Design: Ten fish per level. Five dose levels plus solvent and freshwater controls (0, 11.2, 19.6, 34.3, 60.0, and 105.0 ppm).

- d. Statistics: LC₅₀'s were determined using the Spearman-Kärber method (Finney 1971).

12. Reported Results:

The 96-hour LC₅₀ with 95 percent confidence intervals for Aquazine 80W at 7.0 °C is 60.0 (49.8 to 72.3) mg/L.

13. Study Author's Conclusions/QA Measures:

96-hour LC₅₀ = 60.0 mg/L (95% c.i. = 49.8 to 72.3).

96-hour no-effect level = 34.3 mg/L.

No QA measures indicated.

14. Reviewer's Discussion and Interpretation of the Study:

- a. Test Procedures: The following deficiencies were noted: The test was conducted at 7.0 °C rather than the recommended 12.0 °C. A precipitate formed in the top three concentrations 24 hours after the initiation of the study. Other test procedures appeared to conform to accepted protocol. However, the amount of solvent used was not reported.
- b. Statistical Analysis: EEB statistical analysis (results attached) confirms the author's reported result.
- c. Discussion/Results: The study documents a 96-hour LC₅₀ = 60.0 mg/L (95% c.i. = 49.8 to 72.3 mg/L). It does not fulfill the Guidelines requirement for a freshwater fish LC₅₀ study. This is because the study was conducted

using test water at 7.0 °C. The recommended temperature for a rainbow trout study is 12.0 °C. The study is also unacceptable because a precipitate formed in the three highest concentrations approximately 24 hours after introduction of the toxicant.

d. Adequacy of Study:

- (1) Classification: Supplemental.
- (2) Rationale: Incorrect test temperature, precipitate in test chambers.
- (3) Repairability: No repair possible; test must be repeated using a different solvent or a flow-through apparatus. Solvent concentration must be reported.

15. Completion of One-Liner for Study:

One-liner form completed October 21, 1986.

16. CBI Appendix: Data attached.

PERCENT MORTALITIES AND LC50 VALUES - CUMULATIVE
Aquazine 80W Rainbow Trout (7.0°C)

Nominal Conc., mg/l	Percent Mortality		Solvent Control				
	Control	Control	11.2	19.6	34.3	60.0	105.0
24 Hour	0%	0%	0%	0%	0%	10%	100%
48 Hour	0%	0%	0%	0%	0%	30%	100%
96 Hour	0%	0%	0%	0%	0%	50%	100%

LC50 Values

95% Confidence Interval	LC50 mg/l			
	Low	High	24 Hour	96 Hour
	67.1	84.0	75.1	60.0
	56.6	79.7	67.1	49.8

The 96 hour no effect level was observed to be 34.3 mg/l.

ARMITAGE SIMAZINE RAINBOW TROUT 96 HOUR LC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
105	10	10	100	.0976563
60	10	5	50	62.3047
34.3	10	0	0	.0976563
19.6	10	0	0	.0976563
11.2	10	0	0	.0976563

THE BINOMIAL TEST SHOWS THAT 34.3 AND 105 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 60

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

DATA EVALUATION RECORD

1. Chemical: Simazine
2-chloro-4,6-bis(ethylamino)-s-triazine

2. Test Material: Simazine 80W

3. Study Type: Freshwater Fish 96-Hour LC₅₀

Species Tested: Salmo gairdneri

4. Study ID: Kuc, W.J. (1976) Acute Toxicity of Aquazine 80W Batch No. FL-760951 to the Rainbow Trout, Salmo gairdneri at 17 °C. Prepared by: Aquatic Environmental Sciences, Union Carbide Corporation, Tarrytown, NY. Submitted by Ciba-Geigy Corporation. AES Project No. 7638-500. EPA Accession No. 265011.

5. Reviewed By: Thomas M. Armitage
Fisheries Biologist
EEB/HED

Signature: *Thomas M. Armitage*

Date: 10-30-86

6. Approved By: Raymond W. Matheny
Supervisory Biologist
EEB/HED

Signature: *Raymond W. Matheny*

Date: 10-31-86

7. Conclusions:

The study documents a 96-hour LC₅₀ = 44.6 mg/L (95% c.i. = 37.6 to 52.9). It does not, however, fulfill the Guidelines requirement for a freshwater fish LC₅₀ determination. This is because the study was conducted at a temperature of 17 °C. The recommended test temperature for rainbow trout is 12.0 °C. The study is also unacceptable because a precipitate formed in the three highest test concentrations approximately 24 hours after the introduction of the toxicant. An additional deficiency is that the amount of solvent used is not reported.

8. Recommendations:

The study should be repeated using a water temperature of 12.0 °C. Alternative solvents should be used to eliminate the precipitate. A flow-through system with analytically measured concentrations of toxicant must be used if the chemical cannot be dissolved in a static system.

9. Background:

The study, an acute toxicity determination for a coldwater fish species with Simazine 80W, was submitted to

fulfill a data requirement identified in the reregistration guidance package.

10. Discussion of Individual Test: N/A.

11. Materials and Methods: (Definitive Test)

- a. Test Animals: Were rainbow trout cultured from eggs obtained from a commercial hatchery in Washington. At the time of the test the fish had a mean length of 40 mm and mean weight of 0.64 g.

Test System: 5-gallon glass jars containing 15 liters of water. Loading was 0.43 g/L. Water temperature was 17.0 °C. Initial pH of the dilution water was 7.65. Test was 96 hours in duration.

- b. Dose: Static bioassay using nominal concentrations. DMF was used as a solvent.
- c. Design: Ten fish per level. Five dose levels plus solvent and freshwater controls (0, 9.3, 16.3, 28.6, 50.0, and 87.5 ppm).
- d. Statistics: LC₅₀'s were determined using the Spearman-Kärber method (Finney 1971).

12. Reported Results:

The 96-hour LC₅₀ with 95 percent confidence intervals for Aquazine 80W at 17 °C is 44.6 mg/L (37.6 to 52.9) mg/L.

13. Study Author's Conclusions/QA Measures:

96-hour LC₅₀ = 44.6 (37.6 to 52.9) mg/L.

No QA measures indicated.

14. Reviewer's Discussion and Interpretation of the Study:

- a. Test Procedures: The following deficiencies were noted: The test was conducted at 17 °C rather than the recommended 12.0 °C. A precipitate formed in the three highest test concentrations 24 hours after introduction of the toxicant.
- b. Statistical Analysis: EEB statistical analysis (results attached) confirms the author's reported result.
- c. Discussion/Results: The study documents a 96-hour LC₅₀ = 44.6 mg/L (95% c.i. = 37.6 to 52.9). It does not, however, fulfill the Guidelines requirement. This is because a precipitate was observed in the three highest test concentrations 24 hours after the start of the test. Also, the amount of solvent used to dissolve the test material was not reported.

d. Adequacy of Study:

- (1) Classification: Supplemental.
- (2) Rationale: Precipitate in test chambers, amount of solvent used was not reported.
- (3) Repairability: No repair possible. Test must be repeated as described in section 8 above.

15. Completion of One-Liner for Study:

One-liner form completed October 22, 1986.

16. CBI Appendix: Data attached.

PERCENT MORTALITIES AND IC50 VALUES - CIBA-GEIGY CORPORATION
Aquazine 80W Rainbow Trout (17.0°C)

Percent Mortality

Aquazine 80W Nominal Conc. mg/l	Control	Solvent Control				
		9.3	16.3	28.6	50.0	87.5
24 Hour	0%	0%	0%	0%	40%	100%
48 Hour	0%	0%	0%	0%	70%	100%
96 Hour	0%	0%	0%	0%	70%	100%

IC50 Values

IC50 mg/l	95% Confidence Interval		
	Low	High	IC50 Value
24 Hour	43.9	63.3	52.7
	37.6	52.9	44.6
48 Hour	37.6	52.9	44.6
	37.6	52.9	44.6

The 96 hour no effect level was observed to be 28.6 mg/l.

ARMITAGE SIMAZINE RAINBOW TROUT 96 HOUR LC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
87.5	10	10	100	.0976563
50	10	7	70	17.1875
28.6	10	0	0	.0976563
16.3	10	0	0	.0976563
9.3	10	0	0	.0976563

THE BINOMIAL TEST SHOWS THAT 28.6 AND 87.5 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 44.0138

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

DATA EVALUATION RECORD

PAGE 1 OF

CASE: GS0070

SIMAZINE FRSTR

CONT-CAT: 01

GUIDELINES: 72-1

MRID: 163135

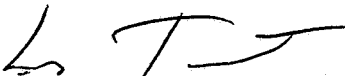
Thompson, C.; Forbis, A. (1983) Acute Toxicity of Aquazine (Simazine) to Rainbow Trout (*Salmo gairdneri*): Static Bioassay Rept. #30452. Unpublished study prepared by Analytical Bio-Chemistry Laboratories, Inc. 14 p.

REVIEW RESULTS:

VALID INVALID _____ INCOMPLETE _____

GUIDELINE: SATISFIED _____ PARTIALLY SATISFIED _____ NOT SATISFIED

DIRECT RVW TIME = START DATE: END DATE:

REVIEWED BY: LES TOWART
TITLE: FISHERIES BIOLOGIST
ORG: EEB / EFED H7507C
LOC/TEL: 557-2438
SIGNATURE: 

DATE: 3-C-89

APPROVED BY:
TITLE:
ORG:
LOC/TEL:
SIGNATURE:

DATE:

DATA EVALUATION RECORD

1. Chemical: Simazine
2-chloro-4,6-bis(ethylamino)-S-triazine
2. Test Material: Simazine Technical 97.6% Active Ingredient
3. Study Type: Freshwater Fish 96-Hour LC₅₀

Species Tested: Salmo gairdneri

4. Study ID: Thompson, C.M.; Forbis, A.D. (1983) Acute Toxicity of Aquazine (Simazine) to Rainbow Trout (Salmo gairdneri). Prepared by: Analytical Bio-Chemistry Laboratories, Inc., Columbia, MD. Submitted by: Ciba-Geigy Corporation, Greensboro, North Carolina. ABC Static Bioassay Report No. 30452. EPA Accession No. 265011.

5. Reviewed By: Thomas M. Armitage
Fisheries Biologist
EEB/HED

Signature: *Thomas M. Armitage*
Date: 10-31-86

6. Approved By: Raymond W. Matheny
Supervisory Biologist
EEB/HED

Signature: *Raymond W. Matheny*
Date: 10-31-86

7. Conclusions:

The study documents a 96-hour LC₅₀ > 10 mg/L for rainbow trout exposed to technical simazine. The study does not, however, fulfill the Guidelines requirement for a freshwater fish LC₅₀ determination. This is because a statistically calculated LC₅₀ with 95 percent confidence limits is not reported. The slope of the dose-response line should also be calculated and reported. At the highest dose level tested, 10 mg/L, no effect was observed. A statistically calculated LC₅₀ need not be reported only if it is demonstrated that a chemical will have an LC₅₀ greater than 100 mg/L. This conclusion must be reached by testing at least 30 animals at a concentration of 100 mg/L or greater. Clearly this study design does not fulfill this requirement.

8. Recommendations:

The study must be repeated using dose levels high enough to report a definitive LC₅₀ and 95 percent confidence limits. Alternatively, it can be demonstrated that the 96-hour LC₅₀ is greater than 100 mg/L. At least 30 test animals must be tested at exposure levels of 100 mg/L or greater.

9. Background:

The study, an acute toxicity determination for coldwater fish species with technical simazine, was submitted to fulfill a data requirement identified in the reregistration guidance package.

10. Discussion of Individual Test: N/A.

11. Materials and Methods: (Definitive Test)

- a. Test Animals: Were rainbow trout obtained from Spring Creek Trout Hatchery in Lewistown, Montana. The fish had a mean weight of 1.0 (\pm 0.25) g and a mean standard length of 40 (\pm 3.4) mm.

Test System: 5-gallon glass vessels, 15 liters of test solution. Static exposure for 96 hours. Initial pH of 7.2 to 7.6. Day 0 dissolved O₂ of 10.0 mg/L. Test vessels were kept in a water bath at 12.0 °C (\pm 1.0 °C).

- b. Dose: Static bioassay using nominal concentrations; acetone was used as a solvent at 0.5 mL/mL of test water.
- c. Design: Ten fish per level. Five dose levels plus solvent and freshwater controls (1.0, 1.8, 3.2, 5.6, and 10.0 mg/L).
- d. Statistics: No mortality was observed at the highest dose level. No statistical analysis was required.

12. Reported Results:

The 24-, 48-, and 96-hour LC₅₀ values for Aquazine were > 10 mg/L. The highest concentration tested (10 mg/L) produced no mortality or observed adverse effects.

13. Study Authors' Conclusions/QA Measures:

96-hr LC₅₀ > 10 mg/L.

"In accordance with ABC Laboratories intent that all studies conducted at our facilities are designed and function in conformance with good laboratory practice regulations and the protocols for individual laboratory studies, an inspection of the final report for Aquazine was conducted and found to be in an acceptable form by a member of our Quality Assurance Unit. An inspection of the daily mortality rate of the test organisms prior to the initiation of the study indicated they were in good health and should not bias the observed mortality in the study. A procedure audit was conducted on protocol #7601 (rainbow trout) on May 9, 1983. A final inspection of all data and records on May 27, 1983 indicated that the report submitted to you is an accurate reflection of the study as it was conducted by ABC Laboratories."

14. Reviewer's Discussion and Interpretation of the Study:

- a. Test Procedures: With the following exception, the procedures followed were in accordance with protocols recommended by the Guidelines. A definitive LC₅₀ with 95 percent confidence limits was not reported. Unless it can be demonstrated that the LC₅₀ is greater than 100 ppm, the LC₅₀ and 95 percent confidence limits must be reported. The highest dose level tested, 10 mg/L, is not high enough to demonstrate an LC₅₀ > 100 mg/L.
- b. Statistical Analysis: No mortality was observed. Therefore, no statistical analysis was required.
- c. Discussion/Results: The study documents a 96-hour LC₅₀ > 10 mg/L. The study does not fulfill the requirement for a freshwater fish acute LC₅₀ determination because, unless it can be demonstrated by testing 30 or more animals at dose levels of 100 mg/L or higher that the LC₅₀ is > 100 mg/L, a definitive LC₅₀ and 95 percent confidence limits must be reported.
- d. Adequacy of Study:
 - (1) Classification: Supplemental.
 - (2) Rationale: See section 7 above.
 - (3) Reparability: No repair possible. Study must be repeated using higher dose levels.

15. Completion of One-Liner for Study:

One-liner form completed October 21, 1986.

16. CBI Appendix: Data attached.

TABLE 3
Mortality Rates and Water Quality Measurements During the Acute Toxicity Test
of Aquazine^a to Rainbow Trout (*Salmo gairdneri*)

Concentration mg/l	Percent Mortality Hours			Water Quality						
	24	48	96	0 hours		48 hours		96 hours		
				Temp. °C	D.O. ^a mg/l	Temp. °C	D.O. mg/l	Temp. °C	D.O. mg/l	pH
Control	0	0	0	12	10.0	12	8.5	12	8.4	7.1
Solvent Control	0	0	0							
1.0	0	0	0	12	10.2	12	8.5	12	8.2	7.2
1.8	0	0	0							
3.2	0	0	0	12	10.2	12	7.6			
5.6	0	0	0							
10	0	0	0	12	10.3	12	8.6	12	8.1	7.2

^aDissolved oxygen concentrations - Dissolved Oxygen Probe (YSI Model 54).

^bpH - pH Probe (Corning Model 476182) used with a Corning Model 125 pH and mV meter.

NOTE: Dissolved oxygen saturation at the test temperature of 12°C is 10.8 mg/l.

DATA EVALUATION RECORD

PAGE 1 OF

CASE: GS0070

SIMAZINE FRSTR

CONT-CAT: 01

GUIDELINES: 71-4

MRID: 163134

Beavers, J. (1986) Simazine Technical: A One-generation Reproduction Study with the Bobwhite (Colinus virginianus): Final Rept: Project No.: 108-245. Unpublished study prepared by Wildlife International Ltd. 124 p.

REVIEW RESULTS:

VALID INVALID INCOMPLETE

GUIDELINE: SATISFIED PARTIALLY SATISFIED NOT SATISFIED

DIRECT RVW TIME = START DATE: END DATE:

REVIEWED BY:

LES TOUANT

TITLE:

FISHERIES BIOLOGIST

ORG:

EEB/EFED (H7507-C)

LOC/TEL:

557-2438

SIGNATURE:

[Signature]

DATE: 3-6-89

APPROVED BY:

TITLE:

ORG:

LOC/TEL:

SIGNATURE:

DATE:

DATA EVALUATION RECORD

1. Chemical: Simazine
2-chloro-4,6-bis(ethylamino)-s-triazine
2. Test Material: Simazine Technical 97.0% Active Ingredient
3. Study Type: One-Generation Reproduction Study
Species Tested: Colinus virginianus
4. Study ID: Beavers, J.B.; Breslin, J.C. (1986) Simazine Technical: A One-Generation Reproduction Study with the Bobwhite (Colinus virginianus). Prepared by Wildlife International Ltd., Solitude Creek Farm, St. Michaels, Maryland. Submitted by: Ciba-Geigy Corporation, Greensboro, North Carolina. Wildlife International Project No. 108-245. EPA Accession No. 265011.

5. Reviewed By: Thomas M. Armitage
Fisheries Biologist
EEB/HED

Signature: *Thomas M. Armitage*
Date: 10-30-86

6. Approved By: Raymond W. Matheny
Supervisory Biologist
EEB/HED

Signature: *Raymond W. Matheny*
Date: 10-31-86

7. Conclusions:

The study is scientifically sound. It indicated that exposure to dietary concentrations of 20 and 100 ppm did not affect reproductive parameters. At a dose level of 500 ppm, there was an observed reduction in the number of eggs laid, and there appeared to be a possible reduction in the number of eggs set. The no-observed-effect concentration was 100 ppm based upon an effect on reproductive performance at 500 ppm. The study fulfills the Guidelines requirement for an avian reproductive study.

8. Recommendations: N/A.

9. Background:

The study, an avian reproduction study using bobwhite quail, was identified by EEB as a data gap in the reregistration guidance package (Registration Standard) for simazine.

10. Discussion of Individual Test: N/A.

11. Materials and Methods: (Definitive Test)

- a. Test Animals: Were 25-week-old quail obtained from Fritt's Quail Farm, RD #3, Box 362, Phillipsburg, New Jersey.
- b. Dose: Nominal dietary concentrations of 0, 20, 100, and 500 ppm active ingredient. Control diet, and test diets were prepared weekly. Dietary concentrations were adjusted for purity of test material. Diets were formulated by Wildlife International by Agway. They contained 28 percent protein, 2.5 percent fat, 5 percent fiber. Neither adults nor offspring received any form of medication. Samples of the control diet and each of the test diets were taken for analysis immediately after mixing.
- c. Design: [Excerpted from Submission]

Study Phases

The primary phases of the study and their durations were:

1. Acclimation - 5 weeks.
2. Pre-photostimulation - 8 weeks.
3. Pre-egg laying (with photostimulation) - Approximately 3 weeks.
4. Egg laying - Approximately 8 weeks.
5. Post-adult sacrifice (final incubation, hatching, and 14-day offspring rearing period) - 5 weeks.

Treatment Groups

Treatment levels were based upon known toxicity data. One hundred and twenty-eight (128) bobwhite (64 cocks and 64 hens) were randomly distributed into four groups. Sex of the birds was determined by a visual examination of the feather coat.

Simazine Technical PPM Active Ingredient

<u>Nominal Concentration</u>	<u>Mean Measured Concentration</u>	<u>Number Of Pens</u>	<u>Birds Per Pen</u>	
			<u>Cocks</u>	<u>Hens</u>
1 - 0	--	16	1	1
2 - 20	19	16	1	1
3 - 100	107	16	1	1
4 - 500	551	16	1	1

Procedure

Each group contained sixteen pairs of birds one male and one female per pen. Three of the groups were fed a diet containing 20, 100 or 500 mg of simazine technical as active ingredient per kg of diet. The fourth group was fed control diet. Each of the four groups of adult birds were fed the appropriate diet from the initiation of the test until terminal sacrifice.

The test birds were acclimated to the facilities for 5 weeks prior to initiation of the test. During acclimation and upon initiation of the study, the birds were maintained under a photoperiod of eight hours of light per day. During Week 9 the photoperiod was increased to seventeen hours of light per day to induce egg laying. The first eggs were set for incubation during Week 13. The birds were held under a photoperiod of seventeen hours of light per day until terminal sacrifice.

All adult birds were observed at least once daily throughout the study for signs of toxicity or abnormal behavior. A record was maintained of all mortalities and observations. All birds that died during the study were necropsied. In addition, at the conclusion of the adult exposure period all birds were sacrificed by cervical dislocation and necropsied.

Adult body weights were measured at study initiation, on Weeks 2, 4, 6, 8, and at terminal sacrifice. Body weights were not measured during egg laying because of the possible adverse effects handling may have on egg production. Feed consumption was measured for each pen for a seven day period every week throughout the study.

Eggs were collected daily from all pens and marked according to the pen of origin. The eggs were then stored in a cold room until incubated. At weekly intervals all eggs were removed from the cold room and candled with a Speed King (Model 32) egg candling lamp to detect egg shell cracks. Cracked eggs were discarded. All eggs that were not cracked or used for egg shell thickness measurements were then fumigated to prevent pathogen contamination and placed in a Petersime Incubator (Model No. S20). Eggs were candled again on Day 11 of incubation to determine embryo viability; and on Day 21 to determine embryo survival. On Day 21 of incubation, the eggs were placed in a Petersime Hatcher (Model No. S6-H) and allowed to hatch. Pedigree baskets constructed of galvanized steel wire mesh were used to keep hatchlings separated by pen.

All hatchlings, unhatched eggs and egg shells were removed from the hatcher on Day 25 or 26 of incubation. The average body weight of the hatchlings by pen was then determined. Hatchlings were banded or toe clipped for identification by pen of origin and then housed according to the appropriate parental concentration grouping in brooding pens until 14 days of age. The hatchlings were fed untreated diets. At 14 days of age the average body weight by parental pen of all surviving chicks was determined. The chicks were sacrificed with chloroform and disposed of by incineration.

Housing and Environmental Conditions

The adult birds were housed indoors in pens manufactured by G.Q.F. Manufacturing (Model 206) measuring approximately 30 X 51 cm. The pens had sloping floors, which resulted in ceiling heights ranging from 21 to 26 cm. External walls and ceilings were constructed of galvanized wire grid, while the common walls were of galvanized sheeting. Floors were constructed of galvanized wire mesh.

Each pen was equipped with a feeder. Each week, sufficient feed for seven days was placed in clean feeders for each pen and presented to the birds. Waterers were changed and water added as necessary to provide potable water (generally every 1 or 2 days).

The birds were maintained in a separate study room which helped avoid excessive disturbances. The average temperature in the adult bobwhite study room during the course of the study was $20^{\circ}\text{C} \pm 3^{\circ}\text{C}$ (SD) ($68^{\circ}\text{C} \pm 5^{\circ}\text{F}$), with an average relative humidity of 50%. The air was heated or cooled as needed to maintain the desired temperature. The system was designed to vent approximately five to eight room air volumes every hour and replace it with fresh air.

The photoperiod in the adult bobwhite study room was maintained by a time clock. The photoperiod for the first 8 weeks of the study was eight hours of light per day. It was then increased to seventeen hours of light per day until sacrifice of adult birds. The birds received approximately 129 lux (12 footcandles) of illumination throughout the study, provided by Chroma 50 fluorescent lights which closely approximate noon-day sunlight (noon-day - 4870° Kelvin, Chroma 50 - 5000° Kelvin).

Hatchlings were placed in batteries of brooding pens manufactured by Beacon Manufacturing (Model B735Q). Each pen measured 72 X 90 X 23 cm high. The external walls and ceilings of each pen were constructed of galvanized wire mesh and galvanized sheeting. Floors were of galvanized wire mesh. Thermostats in the brooding compartment of each pen were set to maintain a temperature of approximately 38°C (100°F) from the time of hatching until the birds were 14 days of age. The photoperiod for the hatchlings was maintained by a time clock at 17 hours of light per day.

Incubation and Hatching

Eggs were collected daily and stored in a cold room at 12.5°C \pm 1.0°C (SD) (55°F \pm 2°F) and approximately 70% relative humidity. All eggs to be incubated were fumigated to reduce the possibility of pathogen contamination. The eggs were fumigated by placing them in an airtight cabinet equipped with a circulating fan. Formaldehyde gas was generated by adding thirty-eight grams of potassium permanganate and thirty-seven ml of 40% commercial grade formalin to a porcelain bowl in the base of the cabinet. The gas was then allowed to circulate for approximately two hours.

Eggs were set for incubation on a weekly basis. The eggs were placed in the incubator where the temperature was maintained at 37.375°C \pm 0.125°C (SD) (99.2°F \pm 0.2°F) with a wet bulb temperature of 29.5°C \pm 0.5°C (SD) (85°F \pm 1°F) or a relative humidity of 56%. The incubator was equipped with a pulsator fan and blades that produced a mild breathing air movement that was designed to eliminate intracabinet temperature and humidity variation during incubation. In order to prevent adhesion of the embryo to the shell membrane, the incubator was also equipped with an automatic egg rotation device, designed to rotate the eggs from 50° off of vertical in one direction to 50° off of vertical in the opposite direction (total arc of rotation is 100°) each hour through Day 21 of incubation. The eggs were transferred to the hatcher on Day 21. Eggs were not rotated in the hatching compartment. The temperature in the hatching compartment was 37.250°C \pm 0.05°C (SD) (99.0°F \pm 0.1°F), and the wet bulb temperature was raised to 33.5°C \pm 0.5°C (SD) (92°F \pm 1°F) or a relative humidity of 76%.

- d. Statistics: Upon completion of the study, Dunnett's method was used to determine statistically significant differences between the control group and each of the treatment groups. Sample units were the individual pens within each experimental group. Pens in which a mortality occurred were not used in statistical comparisons of the reproductive data. Each of the following parameters was analyzed statistically:
1. Adult Body Weight - Individual body weight was measured at initiation, Weeks 2, 4, 6, 8 and at termination of the study. Statistical comparisons were made between the control group and each treatment group at each weighing interval by sex.
 2. Adult Feed Consumption - Feed consumption expressed as grams of feed per bird per day was examined by pen for each 7 day period during the study. Statistical comparisons were made between the control and each treatment group.
 3. Eggs Laid of Maximum Laid - The number of eggs laid per hen was divided by 53, the largest number of eggs laid by any one hen. This transformation was used to convert the number of eggs laid to a percentile value less than or equal to 100.
 4. Eggs Cracked of Eggs Laid - The number of eggs determined to be cracked by candling divided by the number of eggs laid, per pen.
 5. Viable Embryos of Eggs Set - The number of viable embryos at the Day 11 candling was divided by the number of eggs set, per pen.
 6. Live 3-Week Embryos of Viable Embryos - The number of live embryos at the Day 21 candling was divided by the number of viable embryos, per pen.
 7. Hatchlings of 3-Week Embryos - The number of hatchlings removed from the hatcher was divided by the number of live 3-week embryos, per pen.
 8. 14-Day Old Survivors of Hatchlings - The number of 14-day old survivors was divided by the number of hatchlings per week, by pen.
 9. 14-Day Old Survivors of Eggs Set - The number of 14-day old survivors was divided by the number of eggs set per week, by pen.

10. Hatchlings of Maximum Set - The number of hatchlings per hen was divided by 48, the largest number of eggs set from any one hen. This transformation was used to convert the number of hatchlings to a percentile value equal to or less than 100.
11. 14-Day Old Survivors of Maximum Set - The number of 14-day olds per pen was divided by 48, the largest number of eggs set.
12. Offspring's Body Weight - The group body weights of hatchlings and 14-day old survivors was measured by parental pen group.
13. Egg Shell Thickness - The average egg shell thickness of randomly selected eggs per hen, was measured.

12. Reported Results:

Mature bobwhite received simazine technical at nominal dietary concentrations of 20 ppm, 100 ppm and 500 ppm active ingredient for 20 weeks. A control group was maintained concurrently with the treatment groups.

Results of Diet Analysis

Samples of test diet were submitted to EN-CAS Analytical Laboratories for analysis of simazine technical. Results of the analysis ranged from 80% to 128% of nominal.

Mortalities

Two adult mortalities occurred during the study. One female from the 20 ppm group was found dead during Week 14 with extensive head lesions. The bird also exhibited loss of body muscle mass and a regressing ovary. The male from this pen was sacrificed by cervical dislocation. Necropsy findings were not remarkable.

A female from the 100 ppm group was found dead during Week 20. Necropsy revealed extensive feather loss on the head, neck and body, extensive lesions of toe picking of both feet and hocks and bruising along the keel. Additionally, hemorrhagic enteritis in the upper small intestine and a regressing ovary were observed.

Both mortalities were considered to be due to aggression by the pen mate. No mortalities occurred in the 0 ppm or 500 ppm treatment groups.

Clinical Observations

No overt signs of toxicity were observed during the course of the study. Only those lesions or abnormal behavior normally associated with pen wear and tear or aggression were observed during the study.

Gross Necropsy

All birds found dead during the study as well as birds sacrificed at the termination of the study from the control and treatment groups were subjected to gross necropsy. At the 500 ppm concentration one male was observed to have slight regressing testes and three males were observed with regressing testes. All other overt lesions observed in the treatment groups were considered to be incidental to treatment.

Adult Body Weight and Feed Consumption

There were no treatment related effects upon body weight at any of the concentrations tested. When compared to the controls there were no statistically significant differences ($p < .05$) in the body weights of males or females at 20, 100 or 500 ppm.

There was no treatment related effect upon feed consumption at 20, 100 or 500 ppm. In all instances the differences in feed consumption were slight, and did not appear to be treatment related.

Reproductive Results

There were no apparent treatment related effects on reproductive parameters at the 20 or 100 ppm concentrations. When the treated groups were compared to the controls, there were no statistically significant differences ($p < .05$) in any reproductive parameters at the 20 ppm concentration. At both the 100 ppm and 500 ppm concentrations there was a statistically significant ($p < .01$) decrease in the number of cracked eggs as a percent of eggs laid. The differences observed appeared to be the result of a high percent of cracked eggs in two control pens, and did not appear to be treatment related. There was no effect on any other reproductive parameter at 100 ppm.

In the 500 ppm concentration, while not statistically significant, there was an apparent effect on the number of eggs laid, and a possible effect on the number of viable

embryos of eggs set. These effects also were reflected in the number of 14-day old survivors as a percent of eggs set and hatchlings and 14-day old survivors as a percent of maximum set.

Egg Shell Thickness

There were no treatment related reductions in egg shell thickness in the 20, 100, and 500 ppm treatment groups. When treated groups were compared with the control, there were no statistically significant differences ($p < .05$) in egg shell thickness at any concentration tested.

Offspring Body Weights

There were no treatment related differences in the body weight of hatchlings or in the body weight of the 14-day old survivors at any concentration tested. When compared with the controls, there was a statistically significant increase ($p < .05$) in hatchling body weight at 20 ppm. The difference was very slight, and did not appear to be meaningful. There were no statistically significant differences ($p < .05$) in hatchlings body weight at 100 ppm or 500 ppm, or body weight of 14-day old survivors at any concentration tested.

[End of excerpt from submission]

13. Study Authors' Conclusions/QA Measures:

Dietary concentrations of simazine technical of up to 500 ppm did not result in mortality, overt signs of toxicity or effects upon adult body weight or feed consumption during the 20 week exposure period. There were no apparent treatment related effects upon reproductive parameters at 20 ppm or 100 ppm. While not statistically significant, at 500 ppm there was an effect on the number of eggs laid and there appeared to be a reduction in the number of viable embryos of eggs set. The no-observed-effect concentration for simazine technical in this study was 100 ppm, based upon effects on reproductive parameters at 500 ppm.

"This study was conducted so as to conform with good laboratory practices as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs (FEDERAL REGISTER, Volume 48, No. 230, November 29, 1983, pages 53946-53969."

14. Reviewer's Discussion and Interpretation of the Study:

- a. Test Procedures: The procedures followed were in accordance with protocols recommended by the Guidelines. There were no problems in this regard.
- b. Statistical Analysis: EEB statistical analysis (results attached) confirms the authors' reported result.
- c. Discussion/Results: Based upon observed reproductive parameters, reproduction in bobwhite quail does not appear to be affected at dose exposures of 100 ppm and 50 ppm.
- d. Adequacy of Study:
 - (1) Classification: Core.
 - (2) Rationale: Study was conducted according to accepted protocol.
 - (3) Reparability: N/A.

15. Completion of One-Liner for Study:

One-liner form completed October 20, 1986.

16. CBI Appendix: Data attached.

EGGS LAYED

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = c

TOTAL OBSERVATIONS: 16

RESPONSE

N OF CASES	16
MEAN	35.125
STANDARD DEV	11.621

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = x

TOTAL OBSERVATIONS: 15

RESPONSE

N OF CASES	15
MEAN	39.867
STANDARD DEV	11.643

No significant difference

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = y

TOTAL OBSERVATIONS: 15

RESPONSE

N OF CASES	15
MEAN	36.133
STANDARD DEV	10.623

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = z

TOTAL OBSERVATIONS: 16

RESPONSE

N OF CASES	16
MEAN	28.188
STANDARD DEV	10.821

SUMMARY STATISTICS FOR RESPONSE

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES = .195
APPROXIMATE F = .063 DF = 3, 6039 PROBABILITY = .974

65

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	1112.685	3	370.895	2.963	.039
WITHIN GROUPS	7259.654	58	125.166		

DUNCAN MULTIPLE RANGE TESTS
 ORDERED MEANS DIFFER AT ALPHA = .050 IF THEY EXCEED FOLLOWING GAPS

GAP ORDER	DIFFERENCE
2	8.048
3	8.464
4	8.739

THIS TEST ASSUMES THE COUNTS PER GROUP ARE EQUAL

SYSTAT PROCESSING FINISHED

INPUT STATEMENTS FOR THIS JOB:

USE ANOVAD
 BY TRT\$
 OUTPUT @
 STATISTICS/DUNCAN=.05

THE FOLLOWING RESULTS ARE FOR:
 TRT\$ = c

EGGS CRACKED

TOTAL OBSERVATIONS: 17

RESPONSE

N OF CASES	17
MEAN	2.824
STANDARD DEV	2.430

No significant difference

THE FOLLOWING RESULTS ARE FOR:
 TRT\$ = x

TOTAL OBSERVATIONS: 15

RESPONSE

N OF CASES	15
MEAN	2.867
STANDARD DEV	3.378

THE FOLLOWING RESULTS ARE FOR:
 TRT\$ = y

TOTAL OBSERVATIONS: 16

RESPONSE

N OF CASES 16
 MEAN 1.375
 STANDARD DEV 1.258

THE FOLLOWING RESULTS ARE FOR:
 TRT\$ = z

TOTAL OBSERVATIONS: 16

RESPONSE

N OF CASES 16
 MEAN 0.750
 STANDARD DEV 0.931

SUMMARY STATISTICS FOR RESPONSE

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES = 27.489
 APPROXIMATE F = 8.948 DF = 3, 6449 PROBABILITY = .000

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	53.905	3	17.968	3.705	.016
WITHIN GROUPS	290.954	60	4.849		

DUNCAN MULTIPLE RANGE TESTS
 ORDERED MEANS DIFFER AT ALPHA = .050 IF THEY EXCEED FOLLOWING GAPS

GAP ORDER	DIFFERENCE
2	1.558
3	1.639
4	1.692

THIS TEST ASSUMES THE COUNTS PER GROUP ARE EQUAL

SYSTAT PROCESSING FINISHED

INPUT STATEMENTS FOR THIS JOB:

USE ANOVAD
 BY TRT\$
 OUTPUT @
 STATISTICS/DUNCAN=.05
 A:

THE FOLLOWING RESULTS ARE FOR:
 TRT\$ = c

TOTAL OBSERVATIONS: 16

EGGS SET

RESPONSE

N OF CASES 16
 MEAN 28.875
 STANDARD DEV 10.899

THE FOLLOWING RESULTS ARE FOR:
 TRT\$ = x

TOTAL OBSERVATIONS: 15

RESPONSE

N OF CASES 15
 MEAN 33.067
 STANDARD DEV 10.892

THE FOLLOWING RESULTS ARE FOR:
 TRT\$ = y

TOTAL OBSERVATIONS: 15

RESPONSE

N OF CASES 15
 MEAN 31.333
 STANDARD DEV 10.055

THE FOLLOWING RESULTS ARE FOR:
 TRT\$ = z

TOTAL OBSERVATIONS: 16

RESPONSE

N OF CASES 16
 MEAN 24.062
 STANDARD DEV 10.240

SUMMARY STATISTICS FOR RESPONSE

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES = .150
 APPROXIMATE F = .049 DF = 3, 6039 PROBABILITY = .980

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	716.417	3	238.806	2.154	.103
WITHIN GROUPS	6430.954	58	110.879		

No significant differences

DUNCAN MULTIPLE RANGE TESTS
ORDERED MEANS DIFFER AT ALPHA = .050 IF THEY EXCEED FOLLOWING GAPS

GAP ORDER	DIFFERENCE
-----------	------------

2	7.575
---	-------

3	7.966
---	-------

4	8.225
---	-------

THIS TEST ASSUMES THE COUNTS PER GROUP ARE EQUAL

SYSTAT PROCESSING FINISHED

INPUT STATEMENTS FOR THIS JOB:

USE ANOVAD
BY TRT\$
OUTPUT @
STATISTICS/DUNCAN=.05

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = c

TOTAL OBSERVATIONS: 16

Viable Embryos

RESPONSE

N OF CASES	16
MEAN	26.687
STANDARD DEV	11.086

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = x

TOTAL OBSERVATIONS: 15

RESPONSE

N OF CASES 15
MEAN 27.800
STANDARD DEV 9.065

*This shows a difference (significant)
between the highest treatment
group and the control*

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = y

TOTAL OBSERVATIONS: 15

RESPONSE

N OF CASES 15
MEAN 27.867
STANDARD DEV 11.765

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = z

TOTAL OBSERVATIONS: 16

RESPONSE

N OF CASES 16
MEAN 19.125
STANDARD DEV 9.316

SUMMARY STATISTICS FOR RESPONSE

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES = 1.411
APPROXIMATE F = .457 DF = 3, 6039 PROBABILITY = .716

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	833.453	3	277.818	2.585	.062
WITHIN GROUPS	6233.321	58	107.471		

DUNCAN MULTIPLE RANGE TESTS

ORDERED MEANS DIFFER AT ALPHA = .050 IF THEY EXCEED FOLLOWING GAPS

GAP ORDER	DIFFERENCE
2	7.457
3	7.843
4	8.098

THIS TEST ASSUMES THE COUNTS PER GROUP ARE EQUAL

THIS TEST ASSUMES THE COUNTS PER GROUP ARE EQUAL

SYSTAT PROCESSING FINISHED

INPUT STATEMENTS FOR THIS JOB:

USE ANOVAD
BY TRT\$
OUTPUT @
STATISTICS/DUNCAN=.05

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = c

3 week Embryos

TOTAL OBSERVATIONS: 16

RESPONSE

N OF CASES	16
MEAN	26.437
STANDARD DEV	11.111

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = x

TOTAL OBSERVATIONS: 15

RESPONSE

N OF CASES	15
MEAN	27.667
STANDARD DEV	9.053

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = y

TOTAL OBSERVATIONS: 15

RESPONSE

N OF CASES	15
MEAN	27.800
STANDARD DEV	11.736

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = z

TOTAL OBSERVATIONS: 17

RESPONSE

This shows a significant difference between the highest treatment group and the controls.

N OF CASES 17
 MEAN 17.588
 STANDARD DEV 9.274

SUMMARY STATISTICS FOR RESPONSE

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES = 1.459
 APPROXIMATE F = .473 DF = 3, 6224 PROBABILITY = .705

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	1184.212	3	394.737	3.695	.017
WITHIN GROUPS	6303.788	59	106.844		

DUNCAN MULTIPLE RANGE TESTS
 ORDERED MEANS DIFFER AT ALPHA = .050 IF THEY EXCEED FOLLOWING GAPS

GAP ORDER	DIFFERENCE
2	7.374
3	7.755
4	8.007

THIS TEST ASSUMES THE COUNTS PER GROUP ARE EQUAL

SYSTAT PROCESSING FINISHED

INPUT STATEMENTS FOR THIS JOB:

USE ANOVAD
 BY TRT\$
 OUTPUT @
 STATISTICS/DUNCAN=.05

Number Hatched

THE FOLLOWING RESULTS ARE FOR:
 TRT\$ = c

TOTAL OBSERVATIONS: 16

RESPONSE

N OF CASES 16
 MEAN 24.312
 STANDARD DEV 10.916

THE FOLLOWING RESULTS ARE FOR:
 TRT\$ = x

TOTAL OBSERVATIONS: 15

RESPONSE

RESPONSE

N OF CASES 15
 MEAN 25.533
 STANDARD DEV 8.070

THE FOLLOWING RESULTS ARE FOR:
 TRT\$ = y

TOTAL OBSERVATIONS: 15

RESPONSE

N OF CASES 15
 MEAN 26.600
 STANDARD DEV 11.388

*This shows a significant
 difference between highest
 treatment group and controls*

THE FOLLOWING RESULTS ARE FOR:
 TRT\$ = z

TOTAL OBSERVATIONS: 16

RESPONSE

N OF CASES 16
 MEAN 16.250
 STANDARD DEV 9.406

SUMMARY STATISTICS FOR RESPONSE

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES = 1.990
 APPROXIMATE F = .645 DF = 3, 6039 PROBABILITY = .590

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	1046.826	3	348.942	3.464	.022
WITHIN GROUPS	5841.771	58	100.720		

DUNCAN MULTIPLE RANGE TESTS

ORDERED MEANS DIFFER AT ALPHA = .050 IF THEY EXCEED FOLLOWING GAPS

GAP ORDER	DIFFERENCE
2	7.219
3	7.593
4	7.840

THIS TEST ASSUMES THE COUNTS PER GROUP ARE EQUAL

SYSTAT PROCESSING FINISHED

INPUT STATEMENTS FOR THIS JOB:

USE ANOVAD
BY TRT\$
OUTPUT @
STATISTICS/DUNCAN=.05

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = c

TOTAL OBSERVATIONS: 16

RESPONSE

N OF CASES	16
MEAN	23.000
STANDARD DEV	10.551

Number Hatched

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = x

TOTAL OBSERVATIONS: 15

RESPONSE

N OF CASES	15
MEAN	22.933
STANDARD DEV	6.892

This shows a significant
difference between highest
treatment group and control.

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = y

TOTAL OBSERVATIONS: 15

RESPONSE

N OF CASES	15
MEAN	24.600
STANDARD DEV	10.822

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = z

TOTAL OBSERVATIONS: 17

RESPONSE

N OF CASES	17
MEAN	15.647
STANDARD DEV	8.595

SUMMARY STATISTICS FOR RESPONSE

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES = 3.500
APPROXIMATE F = 1.135 DF = 3, 6224 PROBABILITY = .334

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	792.441	3	264.147	3.022	.037
WITHIN GROUPS	5156.416	59	87.397		

DUNCAN MULTIPLE RANGE TESTS

ORDERED MEANS DIFFER AT ALPHA = .050 IF THEY EXCEED FOLLOWING GAPS

GAP ORDER	DIFFERENCE
2	6.669
3	7.014
4	7.242

THIS TEST ASSUMES THE COUNTS PER GROUP ARE EQUAL

SYSTAT PROCESSING FINISHED

INPUT STATEMENTS FOR THIS JOB:

USE ANOVAD
BY TRT\$
OUTPUT @
STATISTICS/DUNCAN=.05

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = c

TOTAL OBSERVATIONS: 16

RESPONSE

N OF CASES	16
MEAN	0.240
STANDARD DEV	0.012

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = x

TOTAL OBSERVATIONS: 15

RESPONSE

N OF CASES	15
MEAN	0.239
STANDARD DEV	0.014

THE FOLLOWING RESULTS ARE FOR-

Eggshell Thickness

No significant differences

TRT\$ = y

TOTAL OBSERVATIONS: 15

RESPONSE

N OF CASES	15
MEAN	0.242
STANDARD DEV	0.010

THE FOLLOWING RESULTS ARE FOR:
TRT\$ = z

TOTAL OBSERVATIONS: 16

RESPONSE

N OF CASES	16
MEAN	0.242
STANDARD DEV	0.011

SUMMARY STATISTICS FOR RESPONSE

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES = 1.674
 APPROXIMATE F = .542 DF = 3, 6039 PROBABILITY = .658

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	0.000	3	0.000	.265	.851
WITHIN GROUPS	0.008	58	0.000		

DUNCAN MULTIPLE RANGE TESTS
 ORDERED MEANS DIFFER AT ALPHA = .050 IF THEY EXCEED FOLLOWING GAPS

GAP ORDER	DIFFERENCE
2	0.009
3	0.009
4	0.009

THIS TEST ASSUMES THE COUNTS PER GROUP ARE EQUAL

SYSTAT PROCESSING FINISHED

INPUT STATEMENTS FOR THIS JOB:

USE ANOVAD
 BY TRT\$
 OUTPUT @
 STATISTICS/DUNCAN=.05

SIMAZINE 090807

RIN 1646-93

Page ___ is not included in this copy.

Pages 78 through 80 are not included.

The material not included contains the following type of information:

- Identity of product inert ingredients.
 - Identity of product impurities.
 - Description of the product manufacturing process.
 - Description of quality control procedures.
 - Identity of the source of product ingredients.
 - Sales or other commercial/financial information.
 - A draft product label.
 - The product confidential statement of formula.
 - Information about a pending registration action.
 - FIFRA registration data.
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DATA EVALUATION RECORD

PAGE 1 OF

CASE: GS0070

SIMAZINE FRSTR

CONT-CAT: 01

GUIDELINES: 72-1

MRID: 40245701

Bowman, J. (1987) Acute Toxicity of Aquazine 80W to Rainbow Trout
(Salmo gairdneri) in a Static Renewal System: Final Report: Lab
Study No. 35578. Unpublished study prepared by Analytical Bio-
Chemistry Labs, Inc. 27 p.

REVIEW RESULTS:

VALID

INVALID _____

INCOMPLETE _____

GUIDELINE:

SATISFIED

PARTIALLY SATISFIED _____

NOT SATISFIED _____

DIRECT RVW TIME =

START DATE:

END DATE:

REVIEWED BY:

LES Touant

TITLE:

FISHERIES BIOLOGIST

ORG:

EEB/EFED (H7507C)

LOC/TEL:

557-2438

SIGNATURE:

LES Touant

DATE:

3-6-89

APPROVED BY:

TITLE:

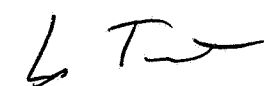

ORG:

LOC/TEL:

SIGNATURE:

DATE:

DATA EVALUATION RECORD

1. CHEMICAL: Simazine
2. TEST MATERIAL: 88.6% a.i. formulated product
3. TEST TYPE: Coldwater fish acute toxicity test
4. STUDY IDENTIFICATION: Bowman, J. (1987) Acute toxicity of Aquazine 80W to rainbow trout (Salmo gairdneri) in a static renewal system. Unpublished study prepared by Analytical Bio-Chemistry Labs for Ciba-Geigy Corp. [MRID: 40245701]
5. REVIEWED BY:
Les Touart
Fisheries Biologist
Ecological Effects Branch
Signature: 
Date: 3-6-87
6. APPROVED BY:
Raymond Matheny
Supervisory Biologist
Ecological Effects Branch
Signature: 
Date: 3-9-87
7. CONCLUSIONS:

The study is acceptable and it fulfills the Guidelines requirement for a coldwater fish acute toxicity test. With a 96-hr LC50 of > 82 ppm (simazine a.i.), Aquazine 80W can be characterized as practically non-toxic to coldwater fishes.
8. RECOMMENDATIONS: N/A
9. BACKGROUND:
10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. METHODS AND MATERIALS:

A. Test Organisms: rainbow trout, Salmo gairdneri.

Age/Size at test initiation: 0.87 g mean weight, 39 mm mean length

Source: Mt. Lassen Trout Farm, Red Bluff, CA.

B. Dosage Form:

Solvents/Vehicles: dimethylformamide

C. Referenced Protocol:

Test Levels: 0.38, 0.72, 1.4, 2.8, 5.4, 11, 22, 47 and 82 ppm
mean measured with appropriate controls.

Number per level: 10 fish/concentration

Temperature: 12 - 13 degrees C

Dissolved Oxygen: 8.0 - 9.5 ppm

pH: 7.1 - 7.6

Source of Dilution Water: soft reconstituted water

Test Vessels/Test System: 5-gallon glass vessels, static
renewal (24 hr)

Aeration: none

Photoperiod: 16 hrs. light

Observation period: 96 hours

Statistical Methods: Stephan's program.

12. REPORTED RESULTS:

Refer to the attached table.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

"The results of the four day static fish toxicity study using Aquazine 80W are ..." 96-hr LC50 > 82 mg/l. " Also, the results indicated a 96-hour, no-observed effect concentration could be estimated at 22 mg/l, which was based on the lack of mortality and abnormal effects. Abnormal effects of surfacing and/or fish on the bottom of the test chamber were observed during the 96-hour exposure period in the 47 and 82 mg/l test concentrations.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF THE STUDY:

A. Test Procedures: The methods used were generally consistent with recommended procedures.

B. Statistical Analysis: N/A

C. Discussion/Results: The data support the conclusions drawn.

D. Adequacy of Test:

1. Validation Category: Core (for formulated product testing).

2. Rationale: N/A

3. Repairability: N/A

15. COMPLETION OF ONE-LINER FOR TEST

SIMAZINE 090907

RIN 1646-93

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 - Identity of product impurities.
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