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PESTICIDE REREGISTRATION

REJECTION RATE ANALYSIS

ECOLOGICAL EFFECTS

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Introduction

This rejection rate analysis has been undertaken by the Special Review and Reregistration Division (SRRD) and the Environmental Fate and Effects Division (EFED) in the Office of Pesticide Programs (OPP) of the Environmental Protection Agency (EPA or "the Agency"). The purpose of this guideline-by-guideline analysis is to identify those factors that most frequently cause rejection of ecological effects studies required for reregistration. This information will enable OPP to (a) provide registrants with information on rejection factors to minimize their reoccurrence in future studies, (b) reassess the adequacy of its guidance, (c) determine the appropriate regulatory response to a future rejected study, and (d) make any internal changes in process, procedures, or criteria deemed appropriate.

The decision to analyze these factors was made after a FIFRA Reregistration recosting analysis, conducted in the spring of 1991, indicated that rejected studies posed the most significant potential for delays in the production of Reregistration Eligibility Documents (REDs). Reregistration eligibility decisions require that reasonable risk assessments be performed for all relevant human health and ecological end points for each chemical. Performing such risk assessments requires a substantially complete data base. A substantially complete data base requires that registrants submit studies of acceptable quality. Also, a significant reduction in rejection rates for most disciplines is required for OPP to be able to meet its production schedule for REDs.

Scope of analysis

The scope of this analysis is limited almost entirely to an examination of rejected studies. While a scientist's review could result in a finding of acceptable, upgradable, unacceptable, or supplemental, rejected (i.e., unacceptable) studies are the focus here because a rejected study will more than double the amount of time and resources required to satisfy that guideline. Upgrading usually does not require as much time to accomplish as repeating the study. A rating of supplemental by a scientist might require substantial new work and add additional time delays to the process.

The scope of this analysis is also limited primarily to List A studies, although for some guidelines rejected studies from List B were used to augment the sample size for the rejection factor identification process. The analysis was confined primarily to List A chemicals because List A chemicals: (1) represent those chemicals with the longest reregistration history—each chemical case had a Registration Standard published between 1980-1988; (2) are high-volume, food-use chemicals that could pose the greatest potential risk for human health and the environment, and therefore have the highest priority in reregistration; and (3) generate the most extensive data requirements.

To what extent are List A rejection factors representative of lists B, C, and D? Unfortunately, it is not possible at this time to make such a determination, since a random

sample of List A, B, C, and D studies was not chosen as the basis for this analysis. Such a sample was not feasible since List B chemicals completed Phase 4 (in fiscal year (FY) 91), List C chemicals completed Phase 4 in FY 92, and List D chemicals completed Phase 4 in FY 93. Consequently, at the time when the rejection rate project began in 1991, there was not an adequate pool of reviewed studies across lists for each guideline to support a randomly drawn data base. Furthermore, many List B and C study reviews conducted in Phase 4 were based on examination of the summaries only.

The rejection factors identified in this assessment of List A rejected studies could plausibly either *overstate* or *understate* the number of rejection factors likely to be found in any future assessment of List B, C, and D rejected studies. On the one hand, many List A studies were initiated in response to the Registration Standards prior to both the 1982 guidelines and development of acceptance criteria in Phase 3 (1989), and consequently may have been rejected by criteria that were not in place at the time the study was conducted. In this case, the corresponding rejection factors are not likely to be repeated in List B, C, and D studies, since the data call-ins have all been issued subsequent to OPP's publication of its guidelines and acceptance criteria. On the other hand, many of the studies judged to be acceptable now may be repeat studies. Consequently, the rejection factors identified here may omit factors that were responsible for previous submissions being rejected.

Process

The Agency first reviewed the data evaluation records (study reviews) on a guideline-by-guideline basis in order to:

(1) identify those factors that most frequently caused each guideline study to be rejected;

(2) determine the rejection rates and trends (where the sample size was adequate) for each guideline requirement;

(3) assess the adequacy of EPA's guidance documents with respect to each rejection factor; and

(4) determine if each rejection factor is avoidable.

Second, a draft was provided to an industry work group of scientists for review and comment, in order to (1) obtain from a user's perspective the adequacy of EPA's guidance documents corresponding to each rejection factor, and (2) better understand why the rejection factors occur. The industry work group included: Richard Balcomb, CIBA; Richard Brown, Zeneca; Peg Cherney, Rhone Poulenc; Joseph Dulka, DuPont; Reinhard Fischer, AgrEvo; Robert Graney, Miles; Richard Holt, DuPont; Catherine Holmes, BASF; Michael McKee, Monsanto; Ellen Mihaich, Rhone Poulenc; and John Schupner, AgrEvo. Industry and EPA scientists met on April 7 and 8, 1994, to discuss the problem areas in order to develop a

better understanding of them. The revised ecological effects chapter explicitly includes industry comments on each rejection factor, and EPA's response to them.

Description of the Discipline

Ecological effects data are used by the Agency to determine the toxicological hazards of pesticides to various terrestrial and aquatic nontarget organisms. These effects data are compared to environmental fate and exposure data when the Agency performs a risk characterization for a pesticide use.

The eco-effects tests include acute, subacute, chronic, and field studies, which are part of a testing scheme that moves from the basic acute laboratory studies to chronic and applied field studies and, essentially, from the least difficult studies to perform to the most difficult. The testing scheme is a tiered one; i.e., results from one tier are evaluated to determine potential toxicological hazards and if further testing is required in the higher tiers. In this system, surrogate organisms are used. Special testing (guidelines 70-1) may be required, but generally the tests move from the least expensive to the most expensive, and the adverse effects examined include the following: mortality, reduction in growth, reproductive impairment, changes in numbers of species, bioaccumulation of residues in nontarget organisms, and in the highest tier studies, structure and function changes in the ecosystem.

The Ecological Effects Branch (EEB), in conjunction with the Environmental Fate and Ground Water Branch (EFGWB), performs ecological risk characterizations based upon eco-effects and environmental fate data submitted to the Agency. For pesticide registration or reregistration, ecological risk characterizations generally consist of the following major activities:

(1) Review and evaluation of eco-toxicity data submitted to support the registration or reregistration of pesticides and their uses.

(2) Establishment of the endpoints of concern and the overall toxicity of the pesticide, and its use(s) to non-target, non-human organisms based upon the data submitted and evaluated.

(3) Calculation of risk quotients based upon the eco-toxicity data and the pesticide use data, fate and transport data, and estimates of exposure.

RISK QUOTIENT = EXPOSURE / TOXICITY

(4) Comparison of the risk quotients to established regulatory Levels of Concern (LOCs). An LOC is a measure of risk to nontarget organisms that may lead to regulatory action. Several ecological LOCs are used in OPP regulatory decision-making.

Specific levels of concern are defined in the table below. EEC is defined as the estimated environmental concentration.

Endpoint & Scenario	Risk Quotient	LOC - Non-Endangered	LOC - Endangered
Mammalian acute (granular)	LD_{50}/FT^2	0.5	0.1
Mammalian acute (spray)	EEC/LC_{50}	0.5	0.1
Mammalian chronic (granular)*	EEC/NOEL	1.0	1.0
Mammalian chronic (spray)	EEC/NOEL	1.0	1.0
Avian acute (granular)	LD_{50}/FT^2	0.5	0.1
Avian acute (spray)	EEC/LC_{50}	0.5	0.1
Avian chronic (granular)*	EEC/NOEL	1.0	1.0
Avian chronic (spray)	EEC/NOEL	1.0	1.0
Aquatic acute	EEC/LC_{50}	0.5	0.05
Aquatic chronic	EEC/NOEL**	1.0	1.0
Non-target insects	NOT QUANTIFIED		
Non-target plants	NOT QUANTIFIED		

* There are no standard procedures for estimating chronic exposure levels for granular pesticides.

** It is the goal of the Agency to regulate on the MATC where warranted. However, depending upon study results, the NOEL may be deemed appropriate for use in the risk assessment.

Description of Studies

The following is a description of the ecological effects guideline studies required by the Agency to support registration.

Wildlife and Aquatic Organisms

Avian Oral LD_{50} (guidelines 71-1): The avian oral LD_{50}, using either an upland game bird (e.g., bobwhite quail) or a waterfowl species (e.g., mallard duck), is an acute, single-dose laboratory study designed to determine the quantity of toxicant required to cause fifty percent mortality in a test population of birds. Technical grade active ingredient (TGAI) is administered by oral intubation to adult birds, and the results (expressed as LD_{50} milligrams (mg) active ingredient (a.i.)/kilogram (kg)) are used to make the following determinations:

Value of Information (questions answered):

(1) Determine the category of toxicity to birds (mg/kg):

<10	very highly toxic
10-50	highly toxic
51-500	moderately toxic
501-2000	slightly toxic
>2000	practically non-toxic

(2) Determine the need for precautionary label statements. (If LD_{50} < 100mg/kg, then "*This pesticide is toxic to birds*" is required.)

(3) Determine acute dose quotients (Q = quotient) (EEC (mg active ingredient available on the surface)/LD_{50}/bird = LD_{50}/ft^2).

(4) Establish the level of minimal acute risk to birds. (If Q < 0.2, then the pesticide has a minimum acute risk to birds, and no additional data are required.)

(5) Determine the need for Restricted Use classification. (If Q > 0.2, but < 0.5, then risk may be mitigated by Restricted Use classification.)

(6) Establish the level of acute risk to endangered birds (If Q > 0.1, then consultation with U.S. Fish and Wildlife Service is required.)

(7) Determine the need for further regulatory action (If Q > 0.5, then further regulatory action may be required, which could include mitigation, field test(s), screening study 71-5.)

(8) Establish the level of high acute risk to birds (If Q > 0.5, then the pesticide has a potential high acute risk to birds.)

Guideline Uses (Tier Progression):

(1) If Q < 0.2, then there are no additional data requirements.

(2) If Q > 0.5, further regulatory action may be required, which may include field test(s), screening study 71-5.

Regulatory Use(s):

(1) If Q < 0.2, then there is minimal acute risk to birds, and there are no additional data requirements; recommend registration/reregistration proceed.

(2) If Q > 0.2, but < 0.5, potential acute risk to birds may be mitigated by Restricted Use classification.

(3) If Q > 0.5, there is potential high acute risk to birds; further regulatory action may be required, which may include mitigation, field test(s), screening study 71-5.

(4) If Q > 0.5, and bird incident reports or field tests with bird kills are available, the recommend pesticide for special review or other risk-benefit balancing.

Avian Dietary LC_{50} (guidelines 71-2):

The avian dietary LC_{50}, using both an upland game bird (bobwhite quail) and a waterfowl species (mallard duck), is an acute, eight-day dietary laboratory study designed to determine the quantity of toxicant required to cause fifty percent mortality in a test population of birds. TGAI is administered by mixture into juvenile birds diets for five days (followed by three days "clean" diet), and the results (expressed as LC_{50} parts per million (ppm) active ingredient (a.i.)) are used to make the following determinations:

Value of Information (questions answered):

(1) Determine the category of toxicity to birds (ppm):

<50	very highly toxic
50-500	highly toxic
501-1000	moderately toxic
1001-5000	slightly toxic
>5000	practically non-toxic

(2) Determine the need for precautionary label statements. (If LC_{50} < 500ppm, then "*This pesticide is toxic to birds*" is required.)

(3) Determine acute dietary quotients (Q = quotient) for non-granular formulations (EEC (residue levels in avian food items from available field studies or Kenaga, 1973)/LC_{50}).

(4) Establish the level of minimal acute risk to birds. (If Q < 0.2, then the pesticide has a minimum acute risk to birds, and no additional data are required.)

(5) Determine the need for Restricted Use classification. (If Q > 0.2, but < 0.5, then acute risk may be mitigated by Restricted Use classification.)

(6) Establish the level of acute risk to endangered birds. (If Q > 0.1, then consultation with U.S. Fish and Wildlife Service is required.)

(7) Determine the need for further regulatory action. (If Q ≥ 0.5, then further regulatory action may be required which may include mitigation, field test(s), screening study 71-5.)

(8) Establish the level of high acute risk to birds. (If Q > 0.5, then the pesticide has a potential high acute risk to birds.)

Guideline Uses (Tier Progression):

(1) If Q < 0.2, then there are no additional data requirements.

(2) If Q > 0.5, further regulatory action may be required, which may include field test(s), screening study 71-5.

Regulatory Use(s):

(1) If Q < 0.2, then there is minimal acute risk to birds and no additional data requirements; recommend registration/reregistration proceed.

(2) If Q > 0.2, but < 0.5, potential acute risk to birds may be mitigated by Restricted Use classification.

(3) If Q > 0.5, then there is potential high acute risk to birds and further regulatory action may be required, which may include mitigation, field test(s), screening study 71-5.

(4) If Q > 0.5, and bird incident reports or field tests with bird kills are available, recommend pesticide for special review or other risk-benefit balancing.

Freshwater Fish LC_{50} (guidelines 72-1):

The freshwater fish LC_{50}, using both a cold water (rainbow trout) and a warm water (bluegill) species, is an acute, ninety-six-hour laboratory study designed to determine the concentration in water required to cause fifty percent mortality in a test population of fish. TGAI[1] is administered into water containing fish, providing exposure for ninety-six hours, and the results (expressed as ppm a.i.) are used to make the following determinations:

Endpoint:

Mortality: LC_{50} value in ppm with 95% confidence limits, lethal concentration in water likely to kill 50% of the cold water and warm water fish exposed

Value of Information (questions answered):

(1) Determine the category of toxicity to fish (ppm):

<0.1	very highly toxic
0.1-1	highly toxic
>1-10	moderately toxic
>10-100	slightly toxic
>100	practically non-toxic

(2) Determine the need for precautionary label statements. (If LC_{50} < 1 ppm, then "*This pesticide is toxic to fish*" is required.)

(3) Determine acute quotients (Q = quotient) for all formulations (EEC (residue levels in water from back-of-the-envelope calculations or fate models)/LC_{50})

(4) Establish the level of minimal acute risk to fish. (If Q < 0.1, then the pesticide has a minimum acute risk to fish and no additional data are required.)

(5) Determine the need for Restricted Use classification. (If Q > 0.1, but < 0.5, then acute risk may be mitigated by Restricted Use classification.)

(6) Establish the level of acute risk to endangered fish, (If Q > 0.05, then consultation with U.S. Fish and Wildlife Service is required.)

(7) Determine the need for chronic testing, fish early life-stage test 72-4. (If Q > 0.01, then fish early life-stage test 72-4 is triggered.)

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[1] Depending on certain criteria, testing with typical end-use product (TEP) may be done.

(8) Determine the need for further regulatory action. (If Q > 0.5, then further regulatory action may be required, which may include mitigation, field test(s), mesocosm, or actual aquatic field study 72-7.)

(9) Establish the level of high acute risk to fish. (If Q > 0.5, then the pesticide has a potential high acute risk to fish.)

Guideline Uses (Tier Progression):

(1) If Q < 0.1, then there are no additional data requirements.

(2) If Q > 0.5, further regulatory action may be required, which may include field test(s), mesocosm, or actual aquatic field study 72-7.

Regulatory Use(s):

(1) If Q < 0.1, then there is minimal risk and no additional data requirements; recommend registration/reregistration proceed.

(2) If Q > 0.1, but < 0.5, potential risk may be mitigated by Restricted Use classification.

(3) If Q > 0.5, there is potential high acute risk and further regulatory action may be required, which may include mitigation, field test(s), mesocosm, or actual aquatic field study 72-7.

(4) If Q > 0.5, and incident reports or field tests with fish kills are available, recommend pesticide for special review or other risk-benefit balancing.

Acute LC_{50} Freshwater Invertebrates (guidelines 72-2):

The freshwater invertebrate LC_{50}/EC_{50}, using a freshwater invertebrate (*Daphnia* sp.), is an acute, forty-eight-hour laboratory study designed to determine the concentration in water required to cause fifty percent mortality, or immobilization, in a test population of invertebrates. TGAI (see footnote 1) is administered into water containing invertebrates, providing exposure for forty-eight hours, and the results (expressed as ppm a.i.) are used to make the following determinations:

Endpoints:

Mortality: LC_{50} value in ppm with 95% confidence limits; lethal concentration in water likely to kill 50% of the aquatic invertebrates exposed; or,

Immobilization: EC_{50} in ppm with 95% confidence limits; concentration in water likely to immobilize 50% of the aquatic invertebrates exposed.

Value of Information (questions answered):

(1) Determine the category of toxicity to aquatic invertebrates (ppm):

<0.1	very highly toxic
0.1-1	highly toxic
>1-10	moderately toxic
>10-100	slightly toxic
>100	practically non-toxic

(2) Determine the need for precautionary label statements. (If LC_{50} < 1 ppm, then "*This pesticide is toxic to aquatic invertebrates*" is required.)

(3) Determine acute quotients (Q = quotient) for all formulations (EEC (residue levels in water from back-of-the-envelope calculations or fate models)/LC_{50}).

(4) Establish the level of minimal acute risk to aquatic invertebrates. (If Q < 0.1, then the pesticide has a minimum acute risk and no additional data are required.)

(5) Determine the need for Restricted Use classification. (If Q > 0.1, but < 0.5, then acute risk may be mitigated by Restricted Use classification.)

(6) Establish the level of acute risk to endangered aquatic invertebrates. (If Q > 0.05, then consultation with U.S. Fish and Wildlife Service is required.)

(7) Determine the need for chronic testing, invertebrate life cycle test 72-4. (If Q > 0.01, then invertebrate life cycle test 72-4 is triggered.)

(8) Determine the need for further regulatory action. (If Q > 0.5, then further regulatory action may be required, which may include mitigation, field test(s), mesocosm, or actual aquatic field study 72-7.)

(9) Establish the level of high acute risk to aquatic invertebrates. (If Q > 0.5, then the pesticide has a potential high acute risk to aquatic invertebrates.)

Guideline Uses (Tier Progression):

(1) If Q < 0.1, then there are no additional data requirements.

(2) If Q > 0.5, further regulatory action may be required, which may include field test(s), mesocosm, or actual aquatic field study 72-7.

Regulatory Use(s):

(1) If Q < 0.1, there is minimal risk and no additional data requirements; recommend that registration/reregistration proceed.

(2) If Q > 0.1, but < 0.5, potential risk may be mitigated by Restricted Use classification.

(3) If Q > 0.5, there is potential high acute risk and further regulatory action may be required, which may include mitigation, field test(s), mesocosm, or actual aquatic field study 72-7.

(4) If Q > 0.5, and incident reports or field tests with kills are available, recommend the pesticide for special review or other risk-benefit balancing.

Wild Mammal Toxicity (guidelines 71-3):

The wild mammal toxicity study, using a representative wild mammal, is typically a laboratory study designed to determine at least one of several eco-effects endpoints: an acute LD_{50}/LC_{50}, a no observable effect level (NOEL), or a maximum acceptable toxicant concentration (MATC). Typically, TGAI (see footnote 1) is administered either by oral intubation or via mixing in the diet, and the results, expressed either as mg a.i./kg or ppm a.i., are used to: provide additional support for precautionary labeling; establish eco-effects endpoints; compare with measured or estimated environmental concentrations (i.e., mg a.i./sq. ft. or ppm a.i.); and indicate if further wild mammal testing is required.

Avian Reproduction (guidelines 71-4):

The avian reproduction studies, using both an upland game bird (bobwhite quail) and a waterfowl species (mallard duck), are laboratory tests designed to determine the quantity of toxicant required to adversely affect the reproductive capabilities of a test population of birds. TGAI is administered by mixture into breeding birds diets throughout their breeding cycle, and the results (expressed as NOEL and various observable effect levels, such as Lowest Observed Effect Level (LOEL) in ppm a.i.) are used to assess effects on reproduction. Impaired reproduction is measured in terms of difference between treatment and controls for:

- # eggs laid per hen in eight weeks;
- eggs cracked of eggs laid (%);
- # viable embryos of eggs set;
- live three-week embryos of viable embryos (%);
- normal hatchlings of live three-week embryos (%);
- # 14-day old survivors of normal hatchlings; and
- # 14-day old survivors per hen.

Results of this test are used to make the following determinations:

Value of Information (questions answered):

(1) Determine chronic/reproductive quotients (Q = quotient) (EEC (residue levels in avian food items, from available field studies or Kenaga, 1973)/NOEL).

(2) Establish the level of minimal chronic risk to birds. (If Q(EEC/NOEL) < 1, then the pesticide has a minimum chronic risk to birds, and no additional data are required.)

(3) Establish the level of chronic risk to endangered birds. (If Q(EEC/NOEL) > 1, then consultation with U.S. Fish and Wildlife Service is required.)

(4) Determine the need for further regulatory action. (If Q(EEC/NOEL) > 1, then further regulatory action may be required, which may include mitigation, field test(s), screening study 71-5.)

(5) Establish the level of high chronic risk to birds. (If Q(EEC/NOEL) > 1, then the pesticide has a potential high chronic risk to birds.)

Guideline Uses (Tier Progression):

(1) If Q(EEC/NOEL) < 1, there are no additional data requirements.

(2) If Q(EEC/NOEL) > 1, further regulatory action may be required, which may include field test(s), screening study 71-5.

Regulatory Use(s):

(1) If Q(EEC/NOEL) < 1, there is minimal chronic risk to birds and no additional data requirements; recommend registration/reregistration proceed.

(2) If Q(EEC/NOEL) > 1, there is potential high chronic risk to birds and further regulatory action may be required, which may include mitigation, field test(s), screening study 71-5.

(3) If Q(EEC/NOEL) > 1, and bird incident reports or field tests with bird kills are available, recommend pesticide for special review or other risk-benefit balancing.

Acute LC_{50} Estuarine and Marine Organisms (guidelines 72-3):

The estuarine/marine organisms LC_{50}/EC_{50} studies, using marine/estuarine fish, shrimp, and mollusc species, are acute, forty-eight or ninety-six hour laboratory tests designed to determine the concentration in water required to cause fifty percent mortality, incomplete shell growth (oyster larvae), or reduced shell growth (oyster). TGAI (see footnote 1) is administered into water containing test organisms, providing exposure for the appropriate time period, and the results (expressed as ppm a.i.) are used to make the following determinations:

Endpoint:

Mortality: LC_{50} value in ppm with 95% confidence limits; lethal concentration in water likely to kill 50% of the organisms exposed. Applies to acute toxicity tests for estuarine and marine fish and shrimp.

Inhibition of Shell Growth: EC_{50} in ppm with 95% confidence limits. The concentration in water inhibiting shell deposition by 50%. Applies to oyster shell deposition study.

Reduction in Larval Development: EC_{50} value in ppm with 95% confidence limits. The concentration in water likely to result in a 50% reduction in successful development of free-swimming, fully-shelled veliger larvae from fertilized eggs. Applies to mollusc embryo larvae study.

Value of Information (questions answered):

(1) Determine the category of toxicity to fish and aquatic invertebrates (ppm):

<0.1	very highly toxic
0.1-1	highly toxic
>1-10	moderately toxic
>10-100	slightly toxic
>100	practically non-toxic

(2) Determine the need for precautionary label statements. (If LC_{50} < 1 ppm, then *"This pesticide is toxic to fish (and aquatic invertebrates)"* is required.)

(3) Determine the acute quotients (Q = quotient) for all formulations (EEC (residue levels in water from back-of-the-envelope calculations or fate models)/LC_{50}).

(4) Establish the level of minimal acute risk to fish and aquatic invertebrates. (If Q < 0.1, then the pesticide has a minimum acute risk and no additional data are required.)

(5) Determine the need for Restricted Use classification. (If Q > 0.1, but < 0.5, then acute risk may be mitigated by Restricted Use classification.

(6) Establish the level of acute risk to endangered estuarine fish and aquatic invertebrates. (If Q > 0.05, then consultation with U.S. Fish and Wildlife Service is required.)

(7) Determine the need for chronic testing, fish early life-stage and invertebrate life-cycle tests 72-4. (If Q > 0.01, then fish early life-stage and/*or* invertebrate life-cycle tests, 72-4 are triggered.) The determination to require either both the estuarine fish early life-stage study and the estuarine invertebrate life-cycle study or only one of the two studies will be made on a case-by-case basis to allow for flexibility. If the results from both freshwater chronic tests and other data raise concerns for chronic effects, then the Agency will ask for both estuarine chronic studies.

(8) Determine the need for further regulatory action. (If Q > 0.5, then further regulatory action may be required, which may include mitigation, field test(s), mesocosm, or actual aquatic field study 72-7.)

(9) Establish the level of high acute risk to estuarine fish and aquatic invertebrates. (If Q > 0.5, then the pesticide has a potential high acute risk to fish and aquatic invertebrates.)

Guideline Uses (Tier Progression):

(1) If Q < 0.1, then there are no additional data requirements.

(2) If Q > 0.5, then further regulatory action may be required, which may include field test(s), mesocosm, or actual aquatic field study 72-7.

Regulatory Use(s):

(1) If Q < 0.1, then there is minimal risk and no additional data requirements; recommend registration/reregistration proceed.

(2) If Q > 0.1, but < 0.5, potential risk may be mitigated by Restricted Use classification.

(3) If Q > 0.5, there is potential high acute risk and further regulatory action may be required, which may include mitigation, field test(s), mesocosm, or actual aquatic field study 72-7.

(4) If Q > 0.5, and incident reports or field tests with kills are available, recommend the pesticide for special review or other risk/benefit balancing.

Fish Early Life Stage and Aquatic Invertebrate Life-Cycle (guidelines 72-4):

The freshwater and estuarine/marine fish early life-stage and invertebrate life-cycle studies, using representative species, are laboratory tests designed to determine the quantity of toxicant required to adversely affect the reproductive capabilities of a test population of fish or invertebrates. TGAI is administered into water containing test organisms, providing exposure throughout a critical life-stage (fish) or a life-cycle (invertebrates), and the results (expressed as a MATC and a NOEL, in ppm a.i.) are used to make the following determinations:

Endpoint(s):

Impaired Reproduction and Development: Effects on reproduction and development are described as LOEL in ppm and NOEL in ppm in the aquatic environment of fish and aquatic invertebrates likely to be exposed. Identification of the lowest effect level is dependent upon study results and may be represented by either the NOEL or MATC. Impaired reproduction and development are measured in terms of difference between treatment and controls for:

Fish
- number of embryos hatched
- time to hatch
- mortality of embryos, larvae, and juveniles
- time to swim-up (if appropriate)
- growth-weight and length

Aquatic Invertebrates
- survival of first generation
- production of young by first generation
- length of first generation at end of test

NOEL and LOEL are determined for each endpoint.

Value of Information (questions answered):

(1) Determine the chronic/reproductive quotients for all formulations (EEC (residue levels in aquatic environment, from back-of-the-envelope calculations or fate models)/NOEL).[2]

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[2] It is the goal of the Agency to regulate on the MATC where warranted. However, depending upon study results, the NOEL may be deemed appropriate for use in the risk assessment.

(2) Establish the level of minimal chronic risk to fish and aquatic invertebrates. (If Q (EEC/NOEL) < 1 (see footnote 2), then the pesticide has a minimum chronic risk to fish and aquatic invertebrates, and no additional data are required.)

(3) Establish the level of chronic risk to endangered fish and aquatic invertebrates. (If Q(EEC/NOEL) > 1, then consultation with U.S. Fish and Wildlife Service is required.)

(4) Determine the need for additional chronic testing for fish, fish full life-cycle test 72-5. (If Q (EEC/NOEL) > 0.1 (see footnote 2), **and** there is information that other parts of the reproduction cycle (not tested in the fish early life-stage test) may be affected by the pesticide, then fish full life-cycle test 72-5 is triggered.)

(5) Determine the need for further regulatory action. (If Q > 1, then further regulatory action may be required, which may include mitigation, field test(s), mesocosm, or actual aquatic field study 72-7.)

(6) Establish level of high chronic risk to fish and aquatic invertebrates. (If Q > 1, then the pesticide has a potential high chronic risk to fish and aquatic invertebrates.)

Note: Item (2) above is a regulatory decision element. Item (4) above is a tier progression element. These two items are not contradictory.

Guideline Uses (Tier Progression):

(1) If Q (EEC/NOEL) < 0.1, then fish full life-cycle test 72-5 is not required.

(2) If Q (EEC/NOEL) > 0.1, and there is information that other parts of the reproduction cycle (not tested in the fish early life-stage test) may be affected by the pesticide, then fish full life-cycle test 72-5 is required.

Regulatory Use(s):

(1) If Q < 1, then there is minimal risk and no additional data requirements; recommend registration/reregistration proceed.

(2) If Q > 1, there is potential high chronic risk and further regulatory action may be required, which may include mitigation, field test(s), mesocosm, or actual aquatic field study 72-7.

(3) If Q > 1, and incident reports or field tests with kills are available, recommend the pesticide for special review or other risk-benefit balancing.

Fish Life-Cycle (guidelines 72-5):

The fish (freshwater and estuarine/marine) life-cycle study, using representative species, is a laboratory test designed to determine the quantity of toxicant required to adversely affect the reproductive capabilities and various life stages of a test population of fish. TGAI is administered into water containing test organisms, providing exposure throughout a full life-cycle (e.g., eggs from F1 adults through F2 eggs, embryos, larvae, and juveniles), and the results (expressed as a MATC and no observable effect concentration (NOEC) in ppm a.i.) are used to make the following determinations:

Endpoint:

Impaired Reproduction and Development: Effects on reproduction and development are described as Lowest Observed Effect Level (LOEL) in ppm and No Observed Effect Level (NOEL) in ppm in the aquatic environment of fish and aquatic invertebrates likely to be exposed. Identification of the lowest effect level is dependent upon study results and may be represented by either the NOEL or MATC. Impaired reproduction and development are measured in terms of difference between treatment and controls for:

(1) Effects on 1st and 2nd generation:

- number of days to complete hatching
- number of embryos hatched
- number of *surviving* larvae hatched

(2) Effects on 1st and 2nd generation juveniles:

- number of abnormal fish
- length of survivors
- weight of survivors

(3) Effects on 1st-generation juveniles and adults, and 2nd-generation juveniles:

- mean length
- mean weight
- number of survivors
- number of embryos

(4) NOEL and LOEL are determined for each endpoint.

Value of Information (questions answered):

(1) Determine chronic/reproductive quotients for all formulations (EEC (residue levels in aquatic environment, from fate models)/NOEL) (see footnote 2).

(2) Establish the level of minimal chronic risk to fish. (If Q (EEC/NOEL) < 1 (see footnote 2), then the pesticide has a minimum chronic risk to fish, and no additional data are required.)

(3) Establish the level of chronic risk to endangered fish. (If Q (EEC/NOEL) > 1 (see footnote 2), then consultation with U.S. Fish and Wildlife Service is required.)

(4) Determine the need for further regulatory action. (If Q > 1, then further regulatory action may be required, which may include mitigation, field test(s), mesocosm, or actual aquatic field study 72-7.)

(5) Establish the level of high chronic risk to fish. (If Q > 1, then the pesticide has a potential high chronic risk to fish.)

Guideline Uses (Tier Progression):

(1) If Q < 1, then there are no additional data requirements.

(2) If Q > 1, field test(s), mesocosm, or actual aquatic field study 72-7 is triggered.

Regulatory Use(s):

(1) If Q < 1, then there is minimal risk and no additional data requirements; recommend registration/reregistration proceed.

(2) If Q > 1, then there is potential high chronic risk and further regulatory action may be required, which may include mitigation, field test(s), mesocosm, or actual aquatic field study 72-7.

(3) If Q > 1, and incident reports or field tests with kills are available, recommend pesticide for special review or other risk-benefit balancing.

Simulated and Actual Field Testing - Mammals and Birds (guidelines 71-5(a) and (b)):

Simulated and actual field testing with mammals and birds consists of field tests designed: (1) to either refute the assumption (based on laboratory tests) that risks to wildlife will occur under conditions of actual pesticide use (Level One Study, 71-5a); or (2) to provide some quantification of what risks may occur during actual use (Level Two Study, 71-5b). Under most conditions TEP is applied: at maximum use rate(s), during actual use situations, under situations representing "worst case" risk, and in areas containing adequate numbers of wildlife. Results (expressed as the quantity of toxicant required to produce an adverse effect:

e.g., ppm a.i., mg a.i./kg, pounds (lbs) a.i./acre (A)) are used to: usually address field mortality of birds and mammals, but may also address sublethal toxic effects, altered behavior, reduced food resources, or lowered reproductive capabilities as well as effects to amphibians and reptiles; compare with measured or estimated environmental concentrations (i.e., ppm a.i., mg a.i./sf); possibly determine susceptibility differences between different species; provide support for additional precautionary labeling; indicate if further field or laboratory testing is required; and provide field data relative to the registrability of the pesticide use.

Specifically, the following determinations are made:

Level One Study, 71-5(a)

Endpoint:

Percent Mortality occurring less than a specified percent of the time (20% in most cases): Effects in the field are measured in terms of number of dead birds per test plot, acetylcholinesterase inhibition, abnormal behavior, density differences, diversity differences, residues in bird tissues, residues in bird food items.

Value of Information (questions answered):

(1) Determine if potential acute or chronic risk indicated by quotients (acute Q > 0.5; chronic Q > 1) are confirmed.

(2) Determine the need for precautionary label statements. (If field studies demonstrate that the use of the pesticide may result in fatality to birds, then the statement "*This pesticide is extremely toxic to wildlife*" is required.)

(3) Establish the level at which potential acute or chronic risk to birds is negated by results of field testing. (If no mortality is indicated in the screening study at not > 20%, no additional data are required.)

(4) Determine if further regulatory action is required. (If mortality is indicated in the screening study at > 20%, then further regulatory action may be required, which may include mitigation, field test(s), quantitative study 71-5(b).)

Guideline Uses (Tier Progression):

(1) If mortality or impaired reproduction at minimal levels, then there are no additional data requirements.

(2) If mortality is not shown to be < 20 %, further regulatory action may be required, which may include field test(s), quantitative study 71-5(b).

Regulatory Use(s):

(1) If mortality is not < 20 %, then the statement "*This pesticide is extremely toxic to wildlife*" is required.

(2) If mortality < 20 %, potential acute/chronic risk to birds is negated and there are no additional data requirements; recommend registration/reregistration proceed.

(3) If mortality is not shown to be < 20 %, then further regulatory action may be required, which may include mitigation, field test(s), quantitative study 71-5(b), or recommend pesticide for special review or other risk-benefit balancing.

Level Two Study, 71-5(b)

Endpoint:

Percent Mortality, percent Impaired Reproduction (as % of vulnerable local populations): Effects in the field may be measured in terms of mortality or survival rates, rates of reproductive impairment, survival of dependent young, acetylcholinesterase inhibition, abnormal behavior, age structure of the populations, residues in bird tissues, residues in bird food items.

Value of Information (questions answered):

(1) Determine if acute or chronic risk indicated by results from Tier 3 Testing (as % of birds that use the field) are confirmed and quantify impacts.

(2) Determine the need for precautionary label statements. (If field studies demonstrate that the use of the pesticide may result in fatality to birds, then the statement "*This pesticide is extremely toxic to wildlife*" is required.)

(3) Establish the level at which acute or chronic risk to birds is mitigated or limited in magnitude and/or duration. (If mortality or impaired reproduction on vulnerable local populations of birds are indicated in the quantitative study at minimal levels, indicating that no additional data are required.)

(4) Establish the level at which acute or chronic risk to birds is confirmed and quantified as to the magnitude and/or duration. (If mortality or impaired reproduction on

vulnerable local populations of birds is indicated in the quantitative study to be at levels potentially detrimental, further regulatory action may be required, which may include placing the pesticide in special review, or imposing other risk reduction measures.)

Guideline Uses (Tier Progression): If mortality or impaired reproduction is at minimal levels, then there are no additional data requirements.

Regulatory Use(s):

(1) If mortality or impaired reproduction is significant, then the statement "*This pesticide is extremely toxic to wildlife*" is required.

(2) If mortality or impaired reproduction is minimal, acute/chronic risk to birds is limited in magnitude and/or duration. There are no additional data requirements.

(3) If mortality or impaired reproduction is biologically significant (e.g., $\geq$ 20%), further regulatory action may be required, which may include mitigation, recommending the pesticide for special review or other risk-benefit balancing.

Simulated or Actual Field Testing - Aquatic Organisms (guidelines 72-7):

Simulated and actual field testing with aquatic organisms consists of field tests designed: (1) to either refute the assumption (based on laboratory tests) that risks to aquatic organisms will occur under conditions of actual pesticide use; and (2) to provide some quantification of what risks may be during actual use. Typically, the study design used is a mesocosm one, in which TEP is applied in a manner that simulates aquatic EECs likely to occur under actual pesticide use conditions. Results (expressed as the quantity of toxicant required to produce an adverse effect: e.g., ppm a.i.) are used to: establish what communities (e.g., benthic, zooplankton, phytoplankton, finfish) are adversely affected and the magnitude of such effects; compare with measured or estimated environmental concentrations (i.e., ppm a.i.); provide support for additional precautionary labeling; indicate if further field or laboratory testing is required; and provide field data relative to the registrability of the pesticide use.

Specifically, the following determinations are made:

Endpoint:

15-20% adverse effect (depending on measured parameter) in local populations of aquatic biota (e.g., plankton, macrophytes, macro-invertebrates, and finfish). Effects in the field are

measured in terms of adverse effects on survival, biomass, growth, reproduction, and population size in populations of aquatic organisms.

Value of Information (questions answered):

(1) Determine if potential acute or chronic risk indicated by quotients (acute Q > 0.5; chronic Q > 1) are confirmed.

(2) Determine the need for precautionary label statements. (If field studies demonstrate that the use of the pesticide may result in fatality to aquatic organisms, then the statement "This pesticide is *extremely* toxic to fish (and/or aquatic invertebrates)" is required.)

(3) Establish the levels at which acute or chronic risk to aquatic organism populations is negated, mitigated, or limited in magnitude or duration. (If adverse effects on exposed populations of aquatic organisms are indicated at < 15% (or 20%, depending on parameter measured), then no additional data are required.)

(4) Establish the level at which acute or chronic risk to aquatic organism populations is confirmed and quantified as to the magnitude and/or duration. (If adverse effects on exposed populations of aquatic organisms are indicated at > 15% (or 20%, depending on parameter measured), further regulatory action may be required, which may include mitigation, placing the pesticide in special review, or requiring other use restrictions.)

Guideline Uses (Tier Progression): If adverse effect is indicated at < 15% (or 20%, depending on parameter measured), then there are no additional data requirements.

Regulatory Use(s):

(1) If adverse effect is indicated at > 15% (or 20%, depending on parameter measured), then the statement, "This pesticide is *extremely* toxic to fish (and/or aquatic invertebrates)" is required.

(2) If adverse effect < 15% (or 20%), acute/chronic risk to aquatic organism populations is mitigated or limited in magnitude and/or duration, and no additional data are required; recommend registration/reregistration proceed.

(3) If adverse effect > 15% (or 20%), further regulatory action may be required, which may include mitigation, recommending pesticide for special review, or other risk-benefit balancing.

Special Tests (guidelines 70-1):

Special tests (typically using birds, mammals, or fish) are required on a case-by-case basis to address particular toxicological hazards of concern. Such tests are usually performed in the laboratory and may relate to metabolites, routes of exposure, or certain chemical properties. Examples of these tests include (but are not limited to):

1. fish, mammalian, or avian cholinesterase studies designed to address levels of cholinesterase inhibition;

2. avian, mammalian, or fish metabolism studies designed to address metabolites formed and their toxicity;

3. secondary toxicity studies with birds and mammals designed to address potential secondary, or tertiary, toxicity of pesticides (e.g., rodenticides and predacides); or

4. aquatic microcosm studies designed to address potential effects in aquatic communities may be accepted as supplemental data when appropriate.

Results from these studies are used to: provide support for additional precautionary labeling; establish toxicity or effect levels; compare, when possible, with measured or estimated environmental concentrations (i.e., ppm a.i., mg a.i./sf, lbs a.i./A); and indicate if further laboratory or higher tier testing is required.

Nontarget Plants

Tier I Nontarget Area Terrestrial Plant Phytotoxicity (guideline 122-1):

The terrestrial nontarget plant phytotoxicity tests are greenhouse or growth chamber tests that consist of three parts, a test for seed germination, a test for seedling emergence, and a test for vegetative vigor. In all three tests the test organisms are: the required three species corn, soybeans, and a root crop; plus 7 other species, usually tomato, cucumber, lettuce, cabbage, oat, ryegrass, and onion (6 species of at least 4 families of dicots and four species of at least 2 families of monocots). The soil or plant surface is treated with technical grade active ingredient (TGAI) at a concentration comparable to the maximum label application rate or at a concentration 3 times the estimated environmental concentration. Results are reported in grams or pounds a.i. per acre and are expressed as the percent of detrimental effect on germination or growth compared to the control after at least 5 days for the seed germination test and 14 days for the seedling emergence and vegetative vigor tests. Parameters measured include radicle length, % germination, % emergence, plant height, plant dry weight, and % phytotoxicity. The results are used to: establish acute toxicity levels, compare with measured

or estimated environmental concentrations, and to indicate if further testing at a higher tier is necessary.

Tier I Nontarget Area Aquatic Plant Phytotoxicity (guideline 122-2):

The aquatic nontarget plant phytotoxicity tests are laboratory tests that evaluate the acute toxicity of pesticides to the two aquatic species: *Selenastrum capricornutum,* (a freshwater green alga) and *Lemna gibba* (an aquatic macrophyte). A one dose concentration comparable to the maximum label application rate (or a concentration 3 times the estimated environmental concentration) is used in these static tests. The *Selenastrum* test is 5 days long and the *Lemna* test is 14 days long. Technical grade active ingredient is used, and the results are reported as the percent of detrimental effect on growth compared to the control. Parameters measured include number of cells or fronds and observed phytotoxicity. The results are used to: establish acute toxicity levels, compare with measured or estimated environmental concentrations, and to indicate if further testing at a higher tier is necessary.

Tier II Nontarget Terrestrial Plant Phytotoxicity (guideline 123-1):

The terrestrial nontarget plant phytotoxicity tests are greenhouse, growth chamber, or small field plot tests that consist of three parts: a seed germination test, a seedling emergence test, and a vegetative vigor test. These studies evaluate the effects of multiple dosage levels on plant growth, using less than the maximum label rate with dosages in a geometrical progression of no more than two-fold, and with subtoxic ($<EC_{50}$) and non-toxic (no observable effect level, NOEL) concentrations. The soil or plant surface is treated with technical grade active ingredient. Only those species with greater than 25% adverse growth effect in Tier I are tested in Tier II tests. Knowing that herbicides kill plants, many registrants start their herbicide testing at the Tier II level. Results are reported in grams or pounds a.i. per acre and are expressed as EC_{25}, EC_{50}, and NOEL values after at least 5 days for seed germination and 14 days for seedling emergence and vegetative vigor. Parameters measured include: radicle length, percent germination, percent emergence, plant height, plant dry weight, and % phytotoxicity. The results are used to establish acute toxicity levels, compare with measured or estimated environmental concentrations, and to indicate if further testing at a higher tier is necessary.

Tier II Nontarget Area Aquatic Plant Phytotoxicity (guideline 123-2):

The aquatic nontarget plant phytotoxicity tests are laboratory tests that evaluate the acute toxicity of pesticides to the five aquatic species: *Selenastrum capricornutum* (a freshwater green alga), *Lemna gibba* (an aquatic macrophyte), *Anabaena flos-aquae* (a blue-green alga), *Skeletonema costatum* (a marine diatom), and a freshwater diatom (unspecified, but usually *Navicula spp.*). These studies evaluate the effects of multiple dosage levels on plant growth,

using less than the maximum label rate with dosages in a geometric progression of no more than two-fold, and with subtoxic ($<EC_{50}$) and non-toxic (no observable effect level, NOEL) concentrations. Knowing that herbicides kill plants, many registrants start their herbicide testing at the Tier II level. The technical grade active ingredient is used, and the 5 day results are reported for *Selenastrum, Anabaena, Skeletonema,* and the unspecified diatom. Fourteen-day results are reported for *Lemna.* Results are reported as EC_{50} and NOEL values in mg a.i./liter. Parameters measured include number of cells or fronds, and observed phytotoxicity. The results are used to establish acute toxicity levels, compare with measured or estimated environmental concentrations, and to indicate if further testing at a higher tier is necessary.

Tier III Nontarget Plant Phytotoxicity Field Studies (guideline 124-1 (Terrestrial Plants), 124-2 (Aquatic Plants):

The Tier III terrestrial and aquatic nontarget plant phytotoxicity field studies are only required if greater than 25% adverse effects on plant growth for terrestrial plants and 50% adverse effects on plant growth for aquatic plants are expected to occur when the product is used as directed by the label (the estimated environmental concentration exceeds the EC_{25} terrestrial or EC_{50} aquatic value(s)). The Tier III tests are expected to provide information on detrimental effects to plants during critical stages of development. The typical end-use product (TEP) is used to assess effects on a broader range of nontarget plant species in a number of geographic areas. Due to the absence of test protocols, the registrants have not been required to perform Tier III tests to date.

Target Area Phytotoxicity Testing (guideline 121-1):

Data concerning the phytotoxic effects of a pesticide on desirable area plants are generally waived by 40 CFR Part 158. Under certain circumstances, the Agency may request these studies.

Nontarget Insects

Honey Bee Acute Contact LD_{50} (guidelines 141-1) :

The honey bee acute contact LD_{50}, using the honey bee, (*Apis mellifera*), is an acute, single-dose laboratory study designed to determine the quantity of toxicant required to cause fifty percent mortality in a test population of bees. Technical grade active ingredient (TGAI) is administered by one of two methods: whole body exposure to technical pesticide in a nontoxic dust diluent; or, topical exposure to technical pesticide via microapplicator. Results are expressed as LD_{50} in micrograms (μg) of a.i. per bee, and are used to make the following determinations:

(1) Determine Category of Toxicity to Bees (μg/bee):

< 2	highly toxic
2-10.99	moderately toxic
> 11	practically nontoxic

(2) Determine need for precautionary labeling

(3) Determine need for additional testing: If LD_{50} < 11 μg per bee, data are required from foliar residue testing.

Honey Bee - Toxicity of Residues on Foliage (guidelines 141-2):

The honey bee foliar residue study is a laboratory test designed to determine the length of time over which field-weathered foliar residues remain toxic to honey bees. The representative end-use product is applied to crop foliage, the foliage is harvested at predetermined intervals after application, and test bees are caged on the treated foliage. Results are expressed in terms of the length of time, following application, during which residues continue to cause significant mortality in honey bee test populations. Results are used to: provide support for precautionary labeling; establish extent of residual hazard of the pesticide to honey bees; and, indicate if additional testing is required.

Industry Comments on "Description of Discipline" Section

Avian Acute

(1) The use of estimated LC_{50} values calculated from LD_{50} values concerns NACA. Additional discussion of the validity and use of this approach is warranted prior to its use as a standard procedure.

EPA Response

The use of estimated LC_{50}[3] values calculated from LD_{50}[4] values is discussed in detail in Urban and Cook (1986), pp. 34-38. This Standard Evaluation Procedure has undergone peer review by the FIFRA Scientific Advisory Panel. This method is used primarily to convert rat LD_{50} values to estimated rat LC_{50} values. If actual rat LC_{50} values exist, these values would be used (i.e., McCann, *et al.*, 1981). A discussion of the variation in results using this method is also found in this reference. The Agency typically uses this method in

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[3] Median lethal concentration in the diet necessary to effect (kill) 50% of the test population.

[4] Median lethal dose necessary to effect (kill) 50% of the test population.

preliminary risk assessments. Refined risk assessments consider additional exposure and toxicity data.

(2) For the Restricted Use classification, Urban and Cook (1986) list the quotients as 0.2 to 1.0. In the Rejection Rate Analysis (RRA), the Agency has reduced the upper quotient limit from 1.0 to 0.5. Without specific justification and consideration of appropriate public comment, NACA believes the quotient should remain as outlined in Urban and Cook (1986).

EPA Response:

In Urban and Cook (1986), Table 1, it states that presumption of risk that may be mitigated by Restricted Use for birds occurs at 1/5 or 0.2 the $LC_{50} \leq EEC < LC_{50}$. Thus, the upper quotient[5] limit is equal to the LC_{50} or 1.0 for Restricted Use to birds. Beyond the quotient of 1.0 was presumption of unacceptable risk to birds. The reference for these limits dates to Agency regulations published in 1975[6]. Current regulations reaffirm the lower limit of 1/5 or 0.2 for birds[7], but do not specify a numerical upper limit[8]. Recent publicly released documents, the decisions from the Ecological, Fate, and Effects Task Force[9] and the Implementation Paper for the New Paradigm[10] establishes a numerical upper limit at 0.5 for birds. This 0.5 level of concern is consistent with that for aquatic organisms which was stated in the 1975 regulations. Support for the 0.5 limit for birds comes from the results of a preliminary retrospective analysis of 20 terrestrial field studies. The results of the analysis support the conclusion that bird kills in the field may occur at quotients at or near 0.5.

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[5] Quotient is the ratio of chemical exposure value(es) to toxicity value(es), e.g., 7 to 25 parts per million (ppm) estimated or measured in the diet of birds divided by LC_{50} values of 10 to 25 ppm yielding quotient values of 0.3 to 1.

[6] Part 162 - Regulations for the Enforcement of FIFRA [FR 40 (129): 28260-28265; 28281-28284; Thursday, July 3, 1975].

[7] 1992, 40 CFR Protection of the Environment. Part 152.170. Criteria for restriction to use by certified applicators, (c)(i)(A) and (B), criteria for hazard to non-target species.

[8] 1992, 40 CFR Protection of the Environment. Part 154.7 Criteria for Initiation of Special Review, (a)(3).

[9] U.S. Environmental Protection Agency. 1992. Memorandum from Linda J. Fisher, Assistant Administrator, to Douglas Campt, Director, Office of Pesticide Programs, dated October 29, 1992. Subject: Decisions on the Ecological, Fate and Effects Task Force.

[10] U. S. Environmental Protection Agency. 1993. Memorandum from Douglas. D. Campt, Office Director, OPP to Victor J. Kimm, Acting Assistant Administrator, OPPTS, dated August 25, 1993. Subject: Implementation Paper for the New Paradigm.

As previously noted, the Levels of Concern (LOCs)[11] for ecological effects have been established based on Agency regulations and guidance[12]. In 1975, a Rebuttable Presumption Against Registration (RPAR, the predecessor to Special Review), could be initiated when the estimated environmental exposure was equal to or greater than one-half the acute toxicity value for aquatic organisms. The "trigger" for birds at that time was equal to the acute avian toxicity value with no "safety factor." Internally, OPP always believed that some safety factor was needed, but the majority of pesticides for which avian field studies were required had estimated exposures much greater than the acute toxicity values for birds. Thus, OPP never established a number that captured the notion of "approaching" the bird toxicity value.

In the recent proposed revision to Part 158, OPP stated that the avian field study would be required when the estimated environmental exposure is equal to or greater than one-half the acute avian toxicity value. This is identical to the trigger for the aquatic mesocosm.

The establishment of regulatory LOCs depends on both science and policy, and they are designed to serve policy based decisions. EPA believes that the current LOCs have a strong regulatory and historical context and are supported by available data.

Industry Comment:

NACA continues to contend that there is minimal scientific justification for reducing the upper bound limit form 1.0 to 0.5. NACA requests that the Agency obtain external peer review of such a retrospective analysis before it is used as justification to change the guidelines.

(3) The concept of LD_{50}/ft^2 is included in the triggering process and regulatory uses. This trigger was not in the original guidelines and has not been thoroughly evaluated. In addition, the Agency states that this "trigger" can be used to conclude the "pesticide has a potential high acute risk to birds." NACA does not agree with this statement and requests that it be removed.

[Note: EEB believes that the following argument by NACA is outside the scope of this RRA, and that this section written by NACA enmeshes scientific issues leading to

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[11] The Level of Concern or LOC is a measure of risk to nontarget organisms or to groundwater which may lead to regulatory action.

[12] Examples include the regulations for Registration, Reregistration and Classification (40 FR 28242); the current regulations (40 CFR 154.7); the criteria for initiation of Special Review (40 CFR 154); Criteria for Restriction for Use by Certified Applicators [40 CFR 152.170(c)]; 1988 Guidance Document for Conducting Terrestrial Field Studies.

regulation (risk assessment) and the use of data in regulatory decision-making (risk management). During the April meeting, EEB stated that many of the issues discussed in this section are unrelated to the scope of the RRA. The RRA is focused on the science needed to make sound risk assessments, not on how the data are used to develop risk assessments. EEB believes that the issues addressed below should be addressed in a separate venue.]

NACA believes that the LD_{50}/sq-ft calculation, and the associated LOCs (e.g., significant hazard for values >0.5), have a number of weaknesses and in their current form should not be used for risk assessment. These weaknesses include:

1. The historical origins of the calculation are weak and not relevant to current chemistry.

   The original speculation that significant adverse effects on birds could be associated with applications equal to or greater than 1 LD_{50}/sq-ft are based on small-scale pen studies (DeWitt 1963) with persistent, bioaccumulative compounds (DDT, dieldrin, and heptachlor).

2. Correlation of LD_{50}/sq-ft calculations with the results of new field studies is incomplete and not publicly available.

   The agency has, at various times, made reference to a preliminary analysis of recent avian field studies which reportedly supports the use of the current LD_{50}/sq-ft model and associated LOCs. Until such an analysis is complete and available for public review it is inappropriate to reference this work.

3. The calculation has inherent flaws that lead to clearly untenable conclusions.

   Exploratory calculations for broadcast applications indicate that the LD_{50}/sq-ft model predicts that a chemical applied at 1 lb a.i./acre, having an LD_{50} of 400 mg/kg (moderately toxic rating), would exceed the LOC of highest concern (0.5) for a 50 gram songbird. Using the same example for a 20 gram songbird and a chemical with an LD_{50} of 1000 mg/kg (slightly toxic rating) produces a result also exceeding an LOC of 0.5. Using these same calculation procedures, it can be shown that a compound that is practically non-toxic ($LD_{50} > 2000$ mg/kg) would exceed the LOCs for restricted use (0.2) and for endangered species concern (0.1). NACA feels these examples demonstrate that the LD_{50}/sq-ft model is flawed and will not discriminate between safe and potentially toxic chemicals.

4. The calculation assumes no upper limit on granule consumption.

   Consumption of large numbers of granules is limited, among other things, by the repellent properties of most formulations, the relativity low attractiveness of most

commercial granules as grit and simply the dilution-effect of naturally occurring grit. Recent field studies (Fischer and Best 1994) with untreated granules indicate that consumption of greater than 20 granules would be unusual. With no upper limit, the current model results in products with relatively low toxicity triggering regulatory concern.

5. The LD_{50}/sq-ft model has not been adequately peer-reviewed.

   The model received cursory review by the SAP as part of the carbofuran Special Review. NACA strongly feels that this review can not be construed as a blanket endorsement for all granular compounds. Moreover, given the importance of this model as the primary assessment tool for this important class of formulations, it is essential that the model be the focus of an avian workshop or special scientific forum.

EPA Response:

The LD_{50}/ft^2 risk index concept is not a new one. It was first presented by Felthousen (1977) and has been used by OPP/EEB ever since. More recently it was presented in the Carbofuran Technical Support Document[13] for the carbofuran Position Document 2/3, which was issued for public comment through the Federal Register (54 FR 3744), January 25, 1989. As part of this process, the Agency presented the risk index to the FIFRA Scientific Advisory Panel for peer review[14]. The Panel supported the risk index as an acceptable initial approach to characterize avian risk and as a useful indication of potential risks of granular insecticides to birds.

In addition, in March 1992, the Agency released the "Comparative Analysis of Acute Avian Risk from Granular Pesticides"[15]. This document provided additional discussion, explanation, and analysis using LD_{50}/ft^2.

(4) Requirement for avian field studies conflicts with New Paradigm. Also need definition of "unusual circumstances."

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[13] U.S. Environmental Protection Agency. 1989 (January). CARBOFURAN: Special Review Technical Support Document. Office of Pesticides and Toxic Substances.

[14] FIFRA Scientific Advisory Panel. 1989. A set of scientific issues being considered by the Agency in connection with the Special Review of carbofuran. Report dated 2/23/89 in response to public review 2/15/89, Arlington, VA.

[15] U.S. Environmental Protection Agency. 1992 (March). Comparative Analysis of Acute Avian Risk from Granular Pesticides. Office of Pesticide Programs, Washington, D.C.

EPA Response:

Under the New Paradigm, requirements for avian field studies will not be based solely upon the criteria in the footnotes to Part 158, but will be the result of risk management decisions. The field study data requirement in Part 158 has not changed; rather, the criteria describing when these studies will be required has changed. Thus, the when-required criteria in the footnotes to this Part will be modified to reflect the policy changes.

The overriding emphasis of the policy is that risk managers should only ask for a study when the information from such a study will improve the Agency's ability to make decisions. Thus, if OPP has significant uncertainty about the risk of a chemical in the field which cannot be resolved without a field study, then a field study should be considered. However, before requiring such a study (or any other study) OPP should know how to use the results of the study.

The Task Force conclusion that the avian field study provides very limited new information to regulatory decision-making and thus would only be required under unusual circumstances was based to a large degree upon two factors. First, a consensus of Task Force members indicated that the results of the avian field studies reviewed by OPP always confirmed predictions of adverse effects, primarily bird mortality, based upon lower tiered studies. Second, a preliminary analysis of "in-house" avian field studies supported this conclusion. The Task Force recognized that the conclusion was based upon limited data on organophosphate and carbamate pesticides. Thus, the "unusual circumstances" where avian field studies may be required in the future could include new pesticides which exceed the LOCs for birds and which have chemistry and modes of action significantly different from organophosphate and carbamate pesticides. However, other cases may arise which also may warrant the requirement of a field study.

Avian Chronic

(1) Requirement for screening field study conflicts with New Paradigm.

EPA Response: See previous response concerning when field studies would be required.

(2) In the Rejection Rate Analysis (RRA), EPA proposes that a chronic risk quotient for evaluating and categorizing reproductive effects be calculated using the Kenaga EEC and the experimentally derived NOEL or LOEL. This conflicts with the New Paradigm, which states that the Kenaga data should only be used in the absence of "actual residue information."

Furthermore, the SEP for Ecological Risk Assessment clearly acknowledges the general inadequacy of the Kenaga data for chronic exposure estimation.

NACA believes that these recent documents (New Paradigm and RRA), in their simplistic approach to chronic exposure data, may promote a review process that will produce an unwarranted number of chronic toxicity triggers for pesticides.

EPA Response:

Recently, there has been considerable discussion concerning the conduct of the avian reproduction test, the interpretation of its results, and the use of the results in determining LOC's. At the request of OPP, the Office of Research and Development Environmental Research Laboratory at Corvallis, Oregon presented an overview of this test for evaluating effects of pesticides on reproduction in birds[16]. A number of changes in the conduct and design of the test were discussed. One important point of discussion in the report was that pesticide effects on avian reproduction are not simply a function of chronic exposure. Reproductive effects have been shown within days (e.g., 8 days or less) after treatment. Thus, short-term exposures should be considered in evaluating the potential for avian reproductive effects. In 1994, the Agency will be participating with OECD and SETAC in a review of this test and its use in ecological risk assessment. Until completion of that review, and based upon the ORD overview, the Agency will use short-term exposure values for determining avian chronic LOCs.

Residue data in Agency files usually lack residue values occurring immediately or shortly after application. As specified in the Decision on the Ecological, Fate and Effects Task Force, when actual residue data are lacking, the estimated residue values from Kenaga will be used in calculating avian chronic LOC's.

Simulated and Actual Field Testing

(1) The RRA uses 20% adverse effect as a benchmark for determining regulatory actions and requiring additional data. This appears to conflict with New Paradigm, which indicates that widespread and repeated avian mortality will not be tolerated in the face of low benefits (mortality of 20% could still be considered widespread).

EPA Response:

Quantification of effects as not less than 20% on exposed individuals is used as an important measurement endpoint for field studies. It is used to indicate that further regulatory action may be required. Whether this endpoint alone would satisfy the "widespread and repeated" risk/benefit standard for decision-making would be determined

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[16] Bennett, R. S. and L. M. Ganio. 1991 (July). Overview of methods for evaluating effects of pesticides on reproduction in birds. EPA 600/3-91/048. U.S. EPA, Environmental Research Laboratory, Corvallis, Oregon. pp.106.

after it was determined that further regulatory action is required. Changes were made in the document reflecting this response. As discussed earlier, under the New Paradigm policy, there is a reduced emphasis on using field studies to make a regulatory decision.

Aquatic Chronic

(1) The sections on value of information, guideline uses, and regulatory uses for the fish early life stage, fish full life cycle, and aquatic invertebrate (72-4) tests appear to contain a number of discrepancies. The risk criteria should compare the EEC directly to the NOEL or LOEL values, but instead they compare the EEC to a portion of the NOEL (0.10 or 0.5). The correct interpretation of the triggers is summarized by the Agency in the discussion of the flow charts presented in this document.

EPA Response:

We agree. The aquatic chronic LOC is the ratio of the EEC to the LEL, the lowest effect level. Identification of the LEL is dependent upon study results and may be represented by either the NOEL or MATC. It is the goal of the Agency to regulate on the MATC where warranted. However, depending upon study results, the NOEL may be deemed appropriate for use in the risk assessment. Additionally, the use of the data for either tier study progression decisions or regulatory decisions were clarified in the document. Appropriate changes were made in the document to reflect this.

(2) In the RRA, EPA states:

"A bioaccumulation study (guideline 165-4) is required if a significant concentration of the pesticide and/or its principal degradation product(s) are expected to occur in aquatic environments and may accumulate in aquatic organisms. If the potential for aquatic food chain effects is established, an aquatic organism accumulation study (guideline 72-6) is also required."

The requirement for also testing bioaccumulation according to guideline 72-6 is new. The new requirement is unnecessary and should be deleted.

EPA Response:

This requirement (guideline 72-6) is not new. It has been part of the data requirements since the establishment of Part 158 in 1984. This explanation was included to indicate the tiered requirements for potential aquatic food chain effects. The fish bioaccumulation test (guideline 165-4) in Subdivision N, is required first. Then if the results from this test indicate a potential for adverse effects on the aquatic food chain, the bioaccumulation test using other aquatic organisms (guideline 72-6) in Subdivision E is required.

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Felthousen, R. 1977. Memorandum to J. Akerman, dated September 9, 1977. Subject: Classification of granulated formulations.

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McCann, J.A., W. Teeters, D. J. Urban, and N. Cook. 1981. A Short-Term Dietary Toxicity Test on Small Mammals. Avian and Mammalian Wildlife Toxicology: Second Conference. ASTM STP 757. D.W. Lamb and E.E. Kenaga, Eds., American Society for Testing and Materials. pp.132-142.

Urban, D.J. and N. Cook. 1986. Hazard Evaluation Division Standard Evaluation Procedure - Ecological Risk Assessment. EPA-540/9-86-167. U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, D. C. 20460.

Rejection Rate Analysis - General Issues Raised by Industry

In the introductory material of the "NACA Response to EPA's Ecological Effects Rejection Rate Analysis," Industry provided a number of general comments. These comments addressed protocol and methodology problems, procedural and policy concerns, and a variety of other generic issues. Since these comments were general in nature, and did not relate to any specific rejection factor or specific study, the Agency's responses will be provided here in general terms.

Industry submitted several comments relating to the need for resolution of protocol and methodology problems. Concerns were raised for the following tests: fish full life-cycle, avian reproduction, nontarget plant studies, oyster studies, and *Daphnia* chronic studies. The issues raised are outside the scope of the RRA, and will be addressed by other venues (e.g., the guideline harmonization process and revision of SEPs).

One comment requested closure on analytical issues associated with aquatic tests. As noted in Industry's comment, these issues are discussed in the 1992 EPA/NACA guidance document, *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance.* This guidance document is considered closed and is an attachment to this Rejection Rate Analysis. (See Appendix A).

One comment questioned the need for NOELs in aquatic and avian acute studies. The Agency agrees that automatic rejection of acute studies which lack an NOEL is not justified. Currently, the NOEL is not required in acute studies.

A number of issues were raised relating to policy and procedure, the need for clarification of guidance, and the need for more flexibility on the part of the Agency. Two suggestions discussed in the April meeting are:

- Streamline the process for industry feedback to reviewers.
  For rapid feedback to questions from both sides, all contact should go through the Product Managers. NACA will help establish a procedure, similar to the SOP set up for rapid feedback from the EPA, for EPA scientists to use when a technical question arises in the review of a study.

- Integrate risk assessment into the study review process.
  If data is not an important component of the risk assessment, then repeating the study is not necessary. The problem is that the individual scientist will not have all of the necessary information available to him/her in order to make that determination. EEB suggested that a one page summary of all of the compound's uses, use rates, application methods, timing of use, maximum uses, etc., can be added as a cover letter to go with each study. This would help the scientist decide how important a study is to the risk assessment. It is important that EEB coordinate with BEAD and HED to evaluate comparable use rates, provided this is possible within program time constraints.

EPA Response:

The hazard assessment (ecotoxicology data) and the fate assessment (exposure data) are separate components of the risk process and must be adequately characterized independently prior to integration into the risk assessment. It is possible that a data requirement can be waived or a rejected study not be repeated. Coordination with BEAD and HED is only possible during special reviews and maybe RED's--not registration actions (sections 3, 5, 18, and 24(c)).

Note: EEB believes that the following issues raised by NACA are outside the scope of the RRA:

- provide clearer guidelines for the use of EEC values;
- consolidate all guidance into a single document;
- integrate the new paradigm into RRA;
- standardize criteria which define an acceptable study; and
- implement more flexible policies in areas of accepting test conditions outside recommended range, accepting chronic studies which lack NOEL's, reviewing non-standard studies, and reviewing studies conducted with very low toxicant concentrations.

Finally, although it was not presented in the introductory material, the need for a definition of "data" was expressed several times in the Industry's response document. The Agency intends this to mean the actual raw data sheets, or a summary of the raw data, with sufficient information to allow for independent statistical analysis of the data.

EPA Response/Comments:

NACA wants to include alternate test species not currently approved by the Agency. This issue is being addressed through internal and external (e.g., OECD) evaluation via the guideline harmonization process.

Under the Rejection Factors section of this document, the term "tabulated and unanalyzed data" is substituted for "raw data" to clarify what the Agency needs in order to evaluate the studies.

Current Rejection Rate

The following graphs demonstrate the current and historical rejection rates for each of the ecological effects guidelines. The historical rate includes all List A studies reviewed as of December 1, 1992, but does not include studies that were reviewed prior to the publication of the Registration Standards. Due to the limited number of cases, the results reported in this section have not been tested for statistical significance, and therefore caution should be exercised in their interpretation. The purpose here is not to develop an empirically defensible rejection rate value. Rather, the intent is to use rejection rates as the best indicator available of where additional Agency/registrant attention and efforts are warranted to improve the quality of the studies.

Figure 1 illustrates the overall rejection rate for ecological effects, which is now estimated at approximately 20%. This is down from the overall rate of 32% prior to 1986, and up slightly from the 1986-1988 rate of 17%. While there has been a significant reduction in rejection rates (38%) between the pre-1986 and the post-1988 time periods, the current (post-1988) rate still remains quite high.

Figures 2 through 4 illustrate what the Agency believes are the current rejection rates for each guideline. The avian acute toxicity studies rejection rates are low, but the avian chronic reproduction studies are very high (Figure 2). While five of the six fish acute toxicity studies have current rejection rates greater than 10%, they are significantly lower than the fish chronic reproduction studies (Figure 3). For both the avian and aquatic reproduction studies, the high rejection rates indicate that significant problems exist.

Figure 5 illustrates those guidelines whose rejection rates appear to have dropped significantly over time. All four of the guideline studies that have shown such improvement are acute toxicity studies.

Figure 6 illustrates those guidelines whose rejection rates have not demonstrated consistent and significant improvement over time.

Studies not included in this analysis due to insufficient data include 71-3 wild mammal toxicity, 71-5 avian field studies, 72-5 fish life cycle, 72-6 aquatic organism accumulation, and 72-7 mesocosm and pond field studies.

Summary

Key implications that might be drawn from these graphs include:

(1) overall rejection rates in ecological effects appear to have gone down significantly, but current rates remain high;

(2) for both avian and aquatic reproduction studies, the high rejection rates indicate significant problems;

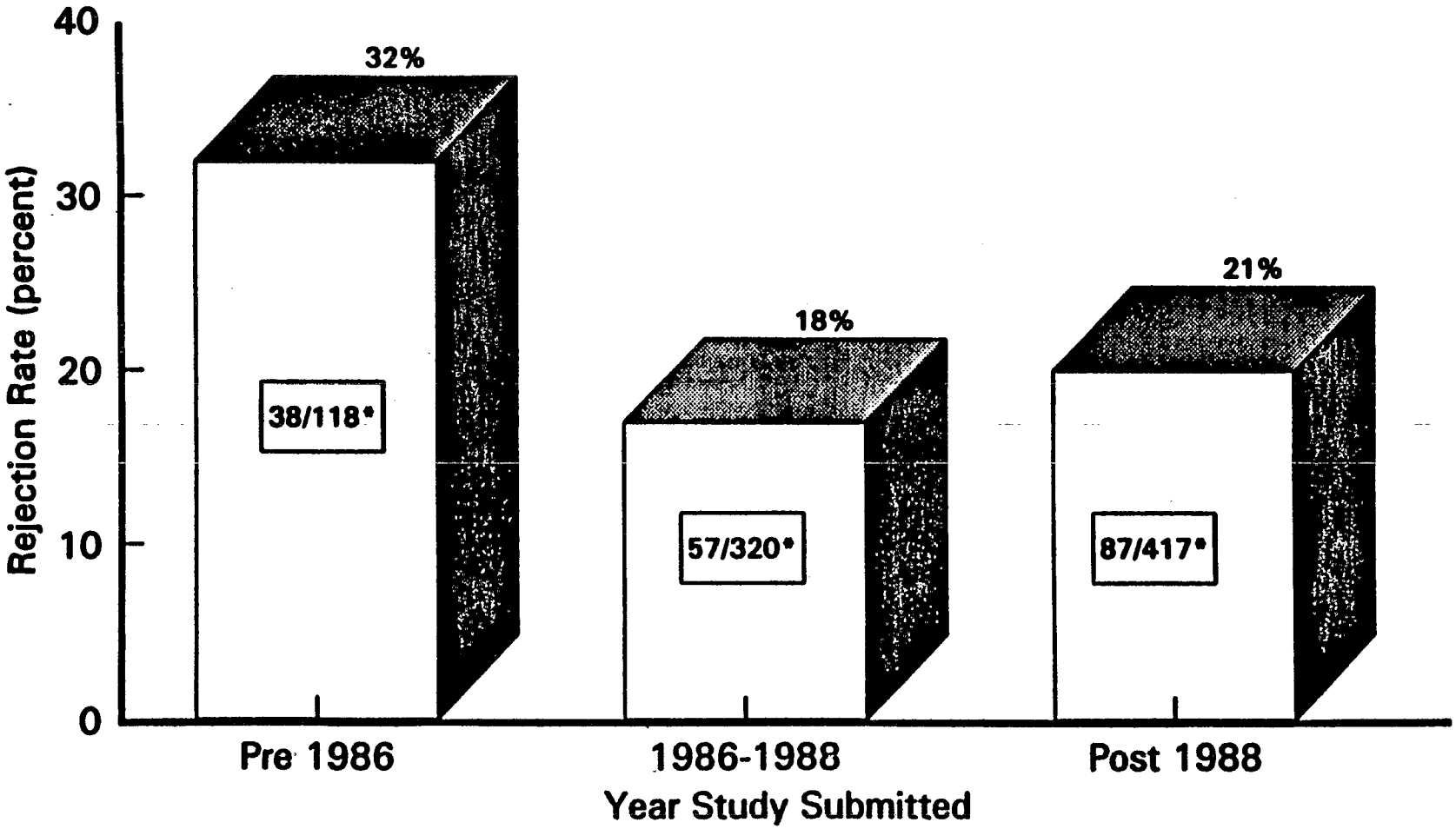
(3) five of the six fish acute toxicity studies have current (post-1988) rejection rates greater than 10%;

(4) four acute toxicity guidelines—71-1, acute avian oral; 72-1C, acute toxicity trout; 72-2, acute toxicity *Daphnia*; and 72-3B, acute toxicity mollusk—have shown encouraging and consistent reduction in their rejection rates over time; and

(5) none of the implications discussed above can be tested for statistical significance, and caution should therefore be exercised in interpreting them.

Figure 1

List A Rejection Rates for all Ecological Effects Guideline Requirements

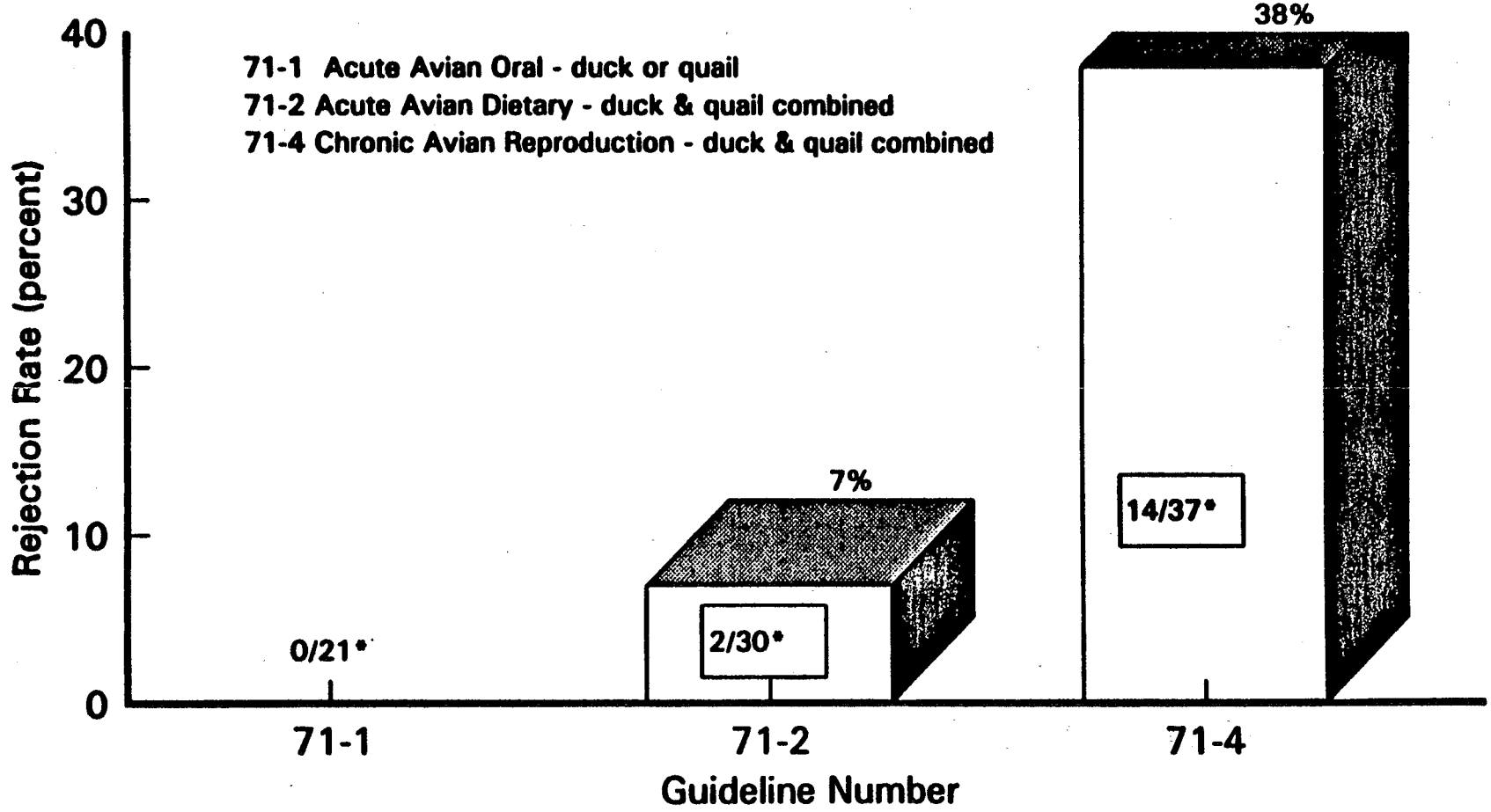


* # rejected studies/# studies reviewed

Note: Rejection rates include List A studies reviewed as of 12/1/92, but do not include studies submitted prior to Registration Standards.

Figure 2

List A - Current (Post 1988) Rejection Rates by Guideline - Avian Acute and Reproduction Toxicity

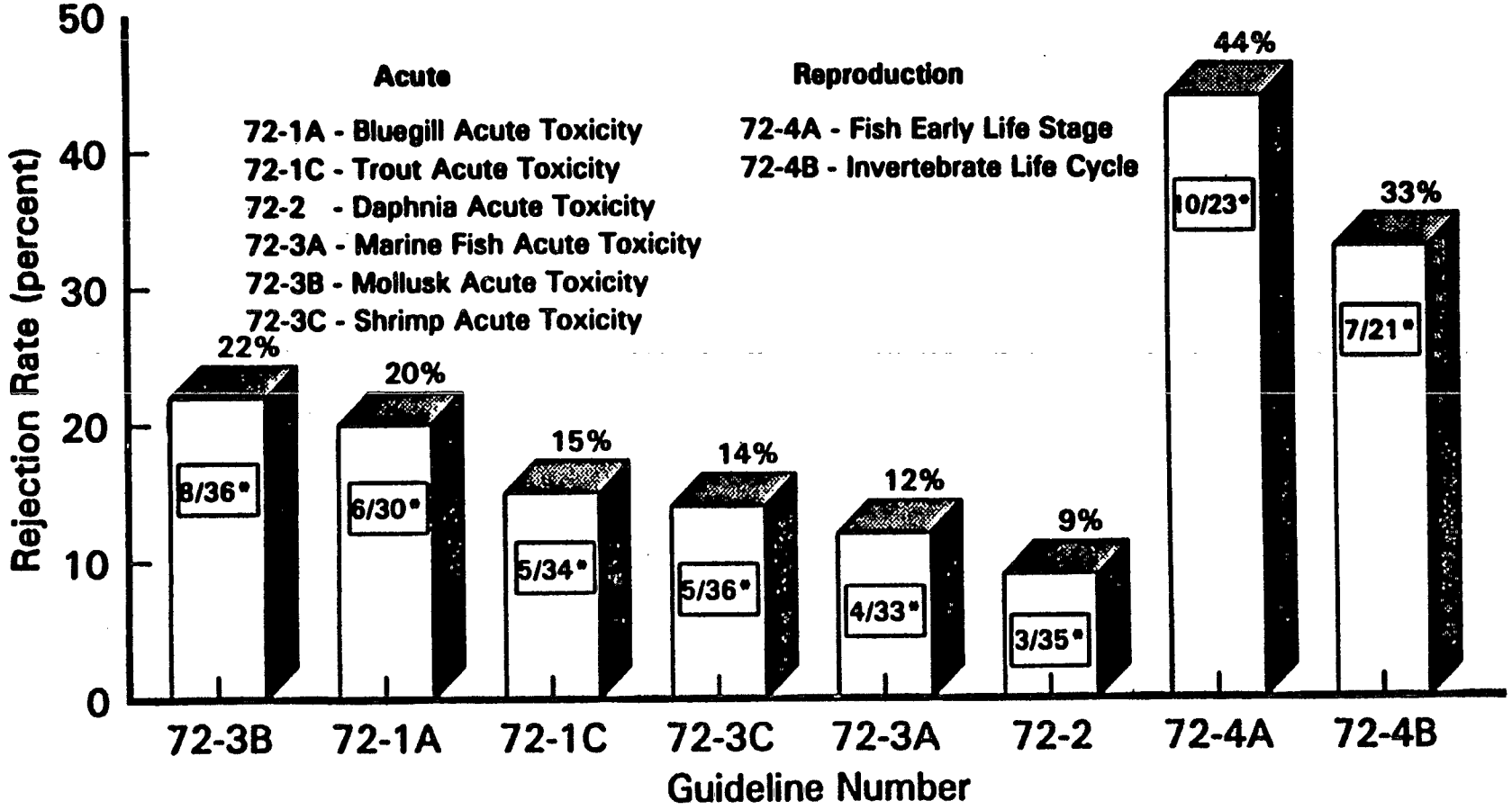


* # rejected studies/# studies reviewed

Note: Insufficient data to evaluate 71-3 and 71-5

Figure 3

List A - Current (Post 1988) Rejection Rates by Guideline - Fish Acute and Reproduction Toxicity

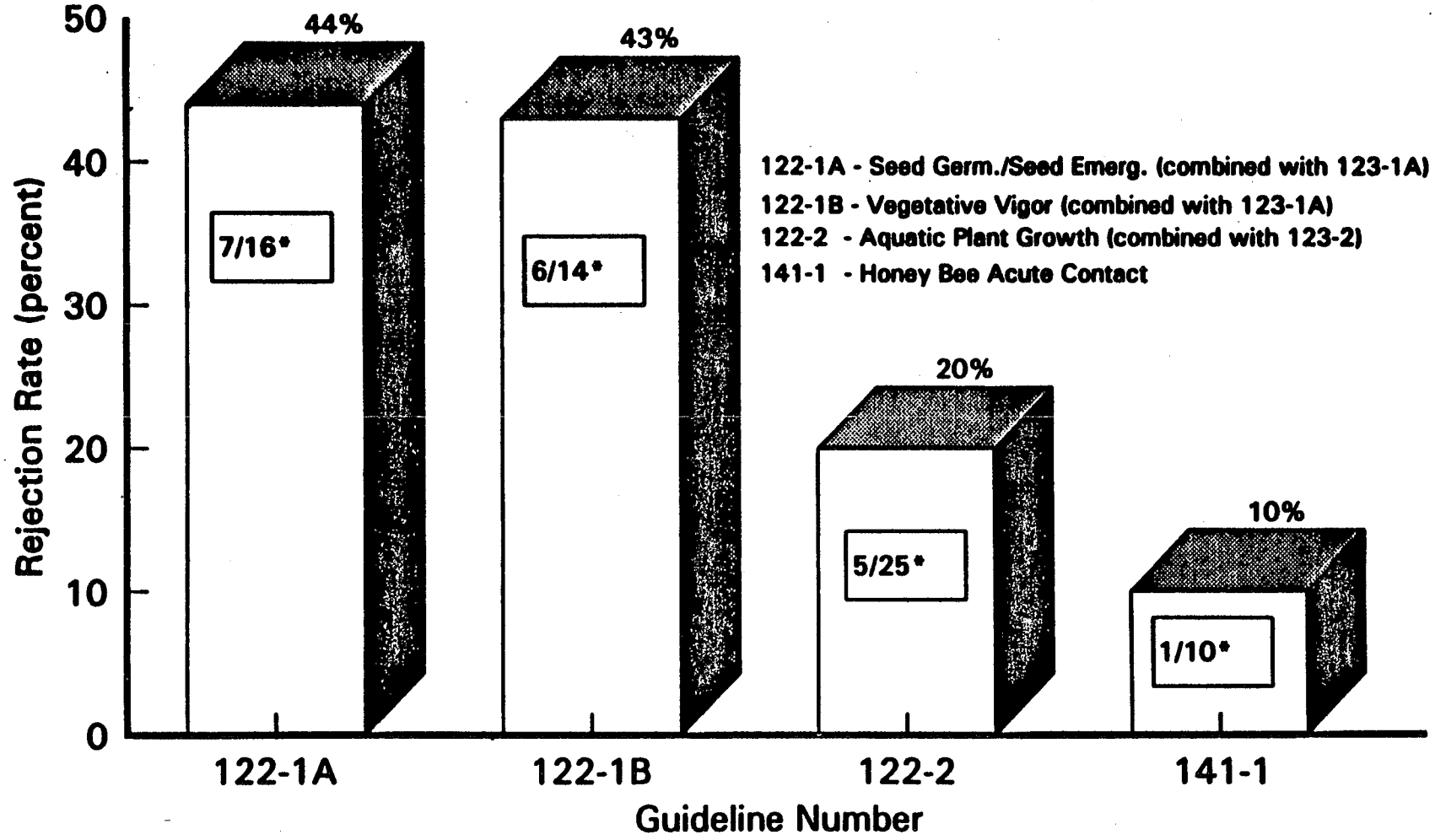


* # rejected studies/# studies reviewed

Note: Insufficient data to evaluate 72-5, 72-6, and 72-7

Figure 4

List A - Current (Post 1988) Rejection Rates by Guideline - Other



* # rejected studies/# studies reviewed

Note: Insufficient data to evaluate 124-1, 124-2, and 141-2

Figure 5

List A - Ecological Effects Guidelines with Lower Rejection Rates over Time

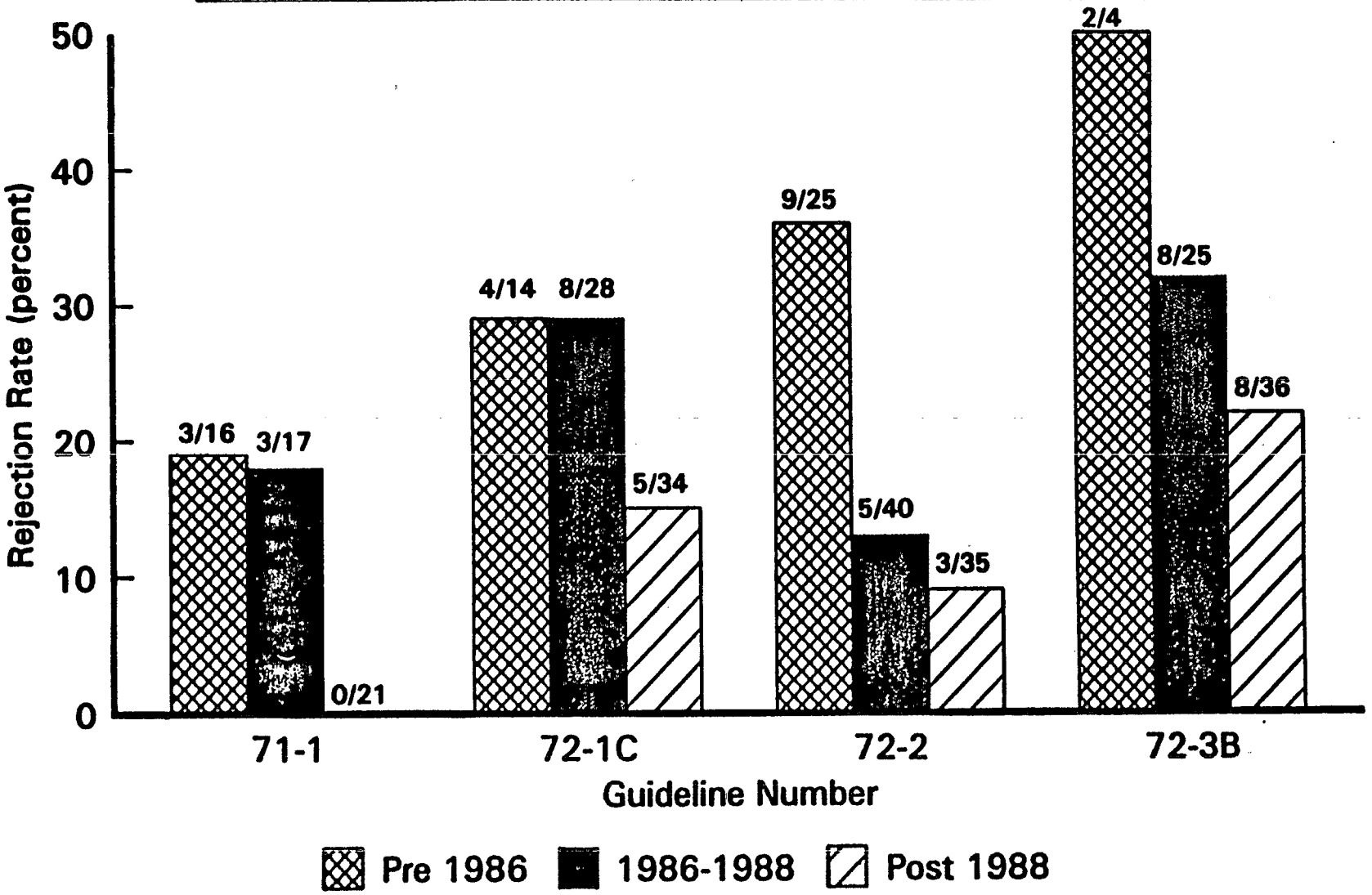
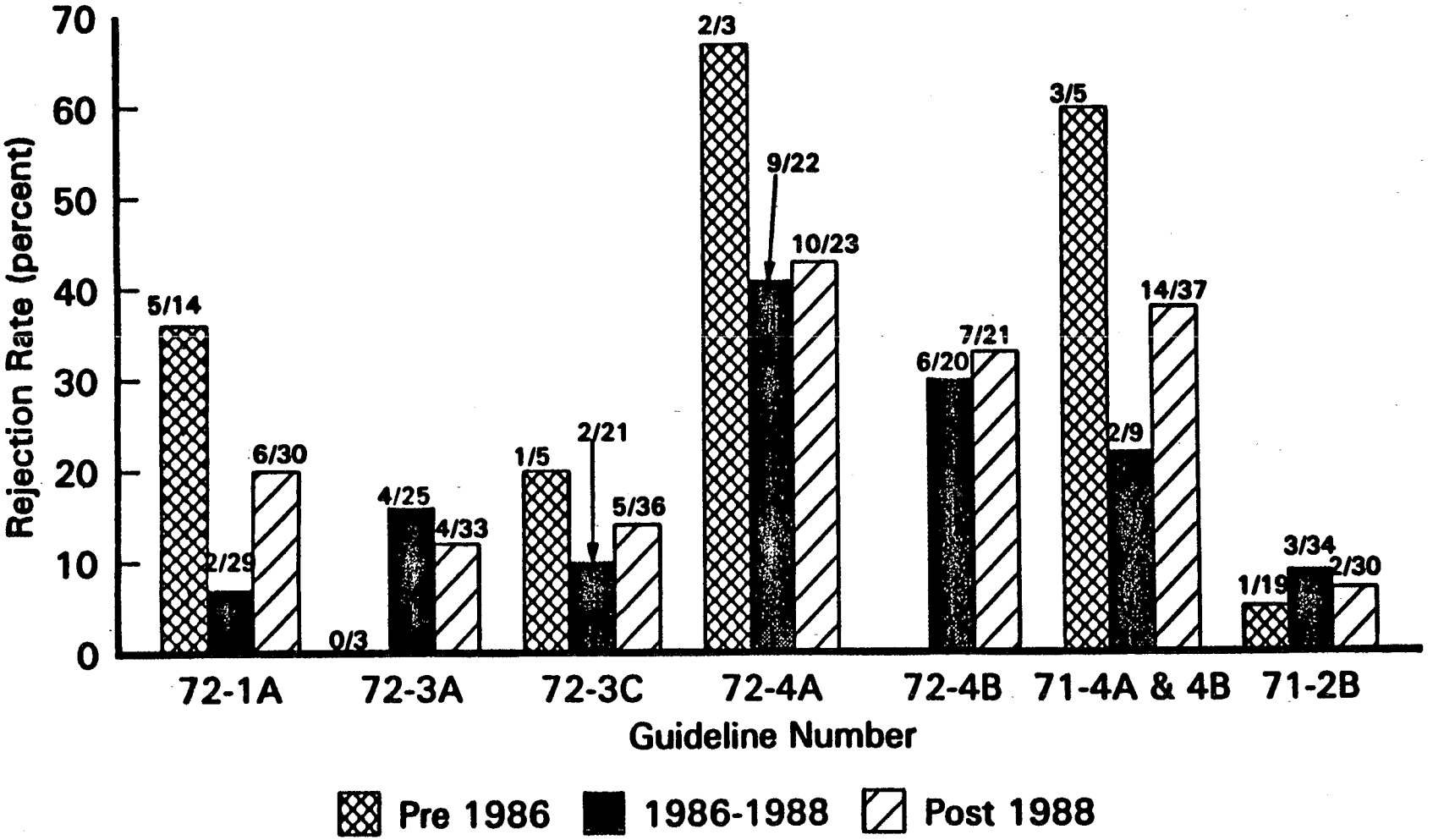


Figure 6

List A - Ecological Effects Guideline Rejection Rates Without Consistent Improvement Over Time



Rejection Factors

The following ecological effects guidelines were analyzed to determine the most common reasons that studies submitted to meet these guidelines were rejected. After each rejection factor, specific references to EPA guidance addressing that factor are given. The EPA guidance is analyzed and is referred to in this report to determine if the guidance documents available to registrants adequately cover the areas where problems have occurred. A list of all guidance documents available for ecological effects studies is provided in Appendix B.

After each rejection factor and the corresponding references to EPA guidance, an Industry Comment section has been provided with the industry scientists' assessment of the adequacy of the EPA guidance; an explanation of technical difficulties, if any, associated with the rejection factor; and recommendations. Following each industry comment is EPA's response to that comment.

After each guideline, the rejection factors are assessed in terms of the registrants' ability to avoid the factor in the future. This is presented in a list that represents what EPA would consider to be rejection factors that could or should be avoidable by the registrants. It is the intention of the Agency in the future to take the appropriate regulatory action should such avoidable factors cause a future study submission to be rejected. This standard will not be applied retrospectively to the studies analyzed in this assessment.

AVIAN ORAL LD_{50} (Guideline 71-1)

1. *Rejection Factor:* Study failed to establish a valid LD_{50} value with corresponding 95% confidence limits, or an LD_{50} greater than 2000 mg/kg.

Agency Guidance: Pesticide Assessment Guidelines - Subdivision E (EPA-540/9-82-024; October, 1982):[17] p. 33, no. 71- 1 (b)(4) and p. 36, 71-1 (d), *Dosage-mortality data* section. FIFRA Accelerated Reregistration - Phase 3 Technical Guidance (EPA-540/09-90-078; December 24, 1989),[18] p. C-14, 71-1, no. 9. and C-16, 71-1, no. 9., provide guidance on this subject.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

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[17] For the rest of this paper this document will be referred to as Subd-E.

[18] For the rest of this paper this document will be referred to as FIFRA-TG.

Industry Comment: As all of the 21 studies on list A chemicals since 1988 have been accepted, there is clearly not a rejection rate problem with this guideline.

EPA Response: Industry and the Agency agree.

2. *Rejection Factor:* A "split-dosing" procedure, which is inconsistent with other comparable acute toxicity testing methods, was used.[19]

Agency Guidance: Subd-E: p. 33, 71-1 Heading and no. (b) (4) and p. 34, 71-1, no. (d) *Acceptable protocol.*

Assessment of Guidance: Guidance on this subject is adequate.

Avoidable: Yes. Possibly not in some cases with Mallard Duck at the doses required. The guidance is not adequate if following the guidance with a recommended species at a recommended rate leads to a study rejection. Additional guidance should recommend the use of Bobwhites where regurgitation is a problem with Mallards.

Industry Comment: The quoted guideline does not actually specify that split dosing is not allowed, and split dosing should not be rejected out of hand if the doses are administered closely together. Regurgitation is a toxic response of Mallards to some chemicals and not necessarily related to the size of the dose, so split dosing does not always resolve the issue. Mallards also regurgitate if the volume exceeds 5% of the body weight. Bobwhite Quail are less prone to regurgitation and should be the species of choice.

EPA Response: The Agency agrees with Industry's comment that regurgitation may be an inevitable consequence of toxicity of some test compounds. Fortunately, this reaction has been relatively limited, and therefore should be addressed on a case-by-case basis. The result of the avian single-dose oral LD_{50} is an integral component of the Agency risk assessment. The Agency believes that, when the problem of regurgitation is encountered, a reasonable effort to resolve the problem

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[19] Another, possibly more important, rejection factor is that regurgitation occurred in all test levels. It appears that this may be why the study authors chose a "split-dosing" technique: to counteract the regurgitation.

should be made, so that satisfactory data are available from the study. As suggested in Industry comments, bobwhites are less prone to regurgitation and would be one way of avoiding the problem. However, the Agency believes that guidance on number of doses for the "Avian Single-dose Oral LD_{50}" is adequate.

3. *Rejection Factor:* The length of time birds were fasted prior to dosing was not specified.

Agency Guidance: Subd-E: p. 35, 71-1, no. (d) *Diet and fasting* and FIFRA-TG: p. C-13, 71-1, no. 4 and C-15, 71-1, no. 4.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: The length of time that the birds were fasted should have been reported.

EPA Response: Industry and the Agency agree.

AVIAN DIETARY LC_{50} (Guideline 71-2)

1. *Rejection Factor:* The reliability of the data was questionable because mortalities occurred that were attributed to interactions between animals (rather than to the test chemical), but no such interactions were observed in the controls.

Agency Guidance: The study author can draw his/her own conclusions concerning any mortalities that occur during a test, particularly when there is an inadequate dose-response pattern. P. 38 of Subd E 71-2(b)(4) indicates that satisfactory data should establish the 8-day dietary LC_{50}.

Assessment of Guidance: Not clear, in that it is subject to interpretation as to whether data are adequate to calculate an LC_{50}. Statistical means are available that will generate a "number for an LC_{50}," but the confidence in that number may be low if the dose response is poor.

Avoidable: No.

Industry Comment: Subd-E does not define "satisfactory data." The SEP says only that "Mortality that cannot be fully attributed to the effects of the test

material may be better understood if assessed in the light of behavioral observations." Other quail species, such as the Japanese Quail, are less aggressive than bobwhites, but they are not currently acceptable. The study should be allowed to be conducted with Japanese Quail, as is acceptable under European guidelines.

The SEP says only that "Mortality that cannot be fully attributed to the effects of the test material may be better understood if assessed in the light of behavioral observations." The SEP does mention the use of ten bobwhite quail to a pen (p 2. 2. Pen Facilities) and this species is known sometimes to express aggression under these circumstances. Other quail species, such as Japanese quail, are less aggressive than bobwhites.

NACA recommends the use of Japanese quail in cases where aggressive behavior cannot be avoided. EPA maintained that Japanese quail are unacceptable, although the use of Japanese quail is undergoing evaluation via internal and external (OECD) guideline harmonization. D. Barolo indicated that if one cannot eliminate aggressive behavior for a specific study, then results from Japanese quail studies will be considered as supplemental information and used in the "weight of evidence" evaluation of the need for additional data.

EPA Response: The Agency agrees with Industry's comment that chemical induced interaction between animals in initial tests is not avoidable. However, the Agency does not agree with the suggested solution to accept data on Japanese quail. The acceptability of this species is being addressed in the guideline harmonization process.

Resolution: NACA and EPA agreed that aggressive behavior can be avoided by caging the bobwhite quail individually, even though this is a protocol deviation.

2. *Rejection Factor:* The reliability of the test results were questionable because of high variability in the measured test concentrations in the test diet. The test material was known to be unstable at room temperature, so the treated test diet (bird food) was stored at 4 degrees C throughout the study. At the test termination, samples of feed were taken from the stored batch to verify concentrations through chemical analysis. These samples were then stored at -20 degrees C for about one-half year before chemical analysis. At that time, the chemical analysis results were highly variable.

Agency Guidance: Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species, Section 9.3 page 372.

Standard Evaluation Procedure; Avian Dietary LC_{50} Test, EPA-540/9-85-008, page 5, Section III D.

Subdivision E Guidelines for Hazard Evaluation for Wildlife and Aquatic Organisms, Section 70-3 (b)(1) page 26.

Assessment of Guidance: The guidance clearly requires that steps be taken to ensure that test concentrations are the same throughout the study. However, there is no specific guidance which specifies how soon after a sample is collected that chemical analysis must be performed. This was considered to be more a problem of scientific judgement than a disregarding of any specific guidance.

The Good Laboratory Practice Standards Subpart F of 40 CFR Section 160.105 (which is currently published, but was not in effect when these studies were performed) provide substantially more guidance on the need to verify concentrations, and to ensure that stored test material (and, implicitly, stored media that contains the test material) is tested to determine stability of test material under storage conditions. Even at that, currently there is nothing in the Standards that requires a specific maximum storage time such that this problem could be avoided in the future.

Avoidable: Unclear. On the one hand, it seems that a laboratory would not wait half a year to do chemical analysis. On the other hand, if the variability of the resulting measurements had been acceptably low, the procedure would have been accepted because it would have been confirmation that the test material was in the test diet at a given concentration. It is not clear that this type of problem would be avoidable, given the guidance provided.

Industry Comment: EPA has focused on the length of sample storage as the potential problem here, but Industry contends that there may be a number of other issues—such as over sampling, homogeneity, and recovery—that affect dietary analysis. It is well known that the accuracy of analytical measurement is affected by the size of the nominal concentration. Although concentrations within the range of hundreds of ppm can be measured very accurately, at the lower end of a dose-response range, if concentrations are in ppb or less, error will be proportionally greater due to a great excess of binding sites.

Although EPA does not directly refer to the magnitude of the problem, if it is within the 70 to 130% range, if the dilution and dosing procedures are good and well documented, and if there is a consistent dose-related response in the biological endpoints, the study should not be rejected purely on the basis of variation in the analysis of the diet. By setting a criterion of 70 to 130% for the measured doses compared with nominal, the requirements for avian dietary tests are then generally in line with those jointly established by EPA and NACA for aquatic laboratory testing (EPA/NACA (1992), *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance*, pp. 10-12).

EPA Response: The permissible variability of measured concentrations in the diet should be viewed in context of how it affects the ability to characterize toxicity. Generally, the greater the variability, the less certainty that can be placed in statistically calculated endpoints such as LC_{50}s, etc. Variability, in and of itself, should only be used as a study rejection factor if it is so great that it precludes distinction of test levels (i.e., the variability is so great that one test level is not significantly different than the next higher or next lower). The 70% and 130% may be a good rule of thumb for initial screening, but would not preclude under some conditions still requesting additional testing to better characterize risk, even if the variability was between 70% and 130%.

Resolution: EPA believes that the percent of nominal can be established to be used as a benchmark for acceptable analytical recoveries. A review of appropriate studies is needed to establish acceptable analytical limits. The limits of 70 to 130% established in the EPA/NACA guidance document apply to aquatic studies and may not be applicable to avian and mammalian studies.

Industry Comment: Designation of 70-130% as a "rule of thumb" means that a registrant will not know a priori if the study might be rejected on this parameter. Unless 70-130% is designated as an acceptable range, there is not agreement between EPA and Industry, and the issue remains unresolved.

3. *Rejection Factor:* Test material was not technical grade. The test material was 42.2% a.i., a formulation presumably, rather than the technical grade product.

Agency Guidance: Subdivision E Guidelines for Hazard Evaluation for Wildlife and Aquatic Organisms, Section 71-2 (b)(1) page 38.

Standard Evaluation Procedure; Avian Dietary LC_{50} Test, EPA-540/9-85-008, page 1, Section I C 1.

Assessment of Guidance: The guidance for this requirement is clearly stated and well recognized throughout the pesticide industry. Guidance states that avian subacute dietary testing must be done with the technical grade active ingredient unless the Agency specifically indicates a need for formulated product testing. No new guidance is needed to clarify this requirement.

Avoidable: Yes, this deficiency is clearly avoidable. Note that it would be possible for a test done with a formulation to provide useful supplemental information, but it does not fulfill requirements for registration unless specifically indicated by the Agency.

Industry Comment: From the information given, one cannot be certain whether the test material was a formulation or not. Some materials can, for good reasons, be described as being of low purity, or it may be difficult to define exactly what the active ingredient is.

EPA Response: The Agency agrees with Industry. In the rare event that the technical grade is relatively low in purity (e.g., 42%), the registrant should explain that fact in each study where the low purity technical grade test material is used. **Industry should be sure that the percent active ingredient is included in all reports.** This is because cover letters and other supporting documentation frequently get separated from tests, thus making the information found in them unavailable to the science reviewer. A study should not be rejected just because the technical grade is of relatively low purity.

4. *Rejection Factor:* The top test level was only 2110 ppm a.i., and no LC_{50} was established. The only condition where it is acceptable not to establish an LC_{50} is when testing is conducted up to 5000 ppm and it can be comfortably stated that the LC_{50} is likely to be greater than 5000 ppm.

Agency Guidance: Subdivision E Guidelines for Hazard Evaluation for Wildlife and Aquatic Organisms, Section 71-2 (b)(4) page 38.Standard Evaluation Procedure; Avian Dietary LC_{50} Test, EPA-540/9-85-008, page 3, Section II C 1.

Assessment of Guidance: The guidance for this requirement is clearly stated. Note that in terms of test material as a **formulated product**, the maximum test

concentrations were 5000, and the LC_{50} would be >5000 ppm for the 42.2% test material. No new guidance is needed to clarify this requirement.

Avoidable: Yes.

Industry Comment: This is clearly a problem associated with the study already discussed in Rejection Factor 3, and would inevitably follow from the original misunderstanding.

EPA Response: The Agency agrees with Industry. If the test material is a formulation of relatively low percentage a.i., it cannot fulfill the requirement for testing with the technical grade active ingredient. Tests using the formulation may, if there are no other deficiencies, provide useful supplemental information.

5. *Rejection Factor:* There was a 50% drop from nominal concentration by day 5. Inadequate justification and explanation were provided. Note that the primary problem was a lack of information explaining the graphs and results of the study to determine if binding caused the apparent decline in measured test concentrations. It appears that this study is capable of being upgraded if the problem is adequately addressed.

Agency Guidance: Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species, section 9.3, page 372.

Subdivision E Guidelines for Hazard Evaluation for Wildlife and Aquatic Organisms, Section 70-3 (b)(1), page 26.

Assessment of Guidance: The guidance primarily focuses on ensuring that test concentrations remain constant and that reliable comparisons can be made concerning relative toxicity and relative sensitivity. They do not specifically indicate the exact wording and nature of explanations required for variation in measured test concentrations compared to nominal test levels.

Avoidable: Yes.

Industry Comment: This rejection factor is avoidable, but should not necessarily undermine the study. The testing facility should understand and adapt to the

stability of the compound under test conditions and keep variability in the range of 70 - 130% of nominal.

EPA Response: The Agency agrees with Industry. Knowing the stability of the compound in avian test diet under test conditions is essential. A study rejected because of declining concentrations may be upgradeable if adequate explanation is provided and the decline curve is well characterized. Refer to the EPA Response and Resolution sections for Rejection Factor 2 above for comments regarding limitations of the 70 - 130% variability range.

WILD MAMMAL TOXICITY (Guideline 71-3)

1. *Rejection Factor:* The method used to prepare the treatment diets, first forming a stock feed and then mixing with clean feed, could lead to erroneous results because animals might choose to eat untreated rather than treated feed.

Agency Guidance: Subd-E: pp. 40 - 41, 71-2, (d)(5) *Test diets*[20] and FIFRA-TG: p. C-17, 71-2, no. 2, give guidance on diet preparation, and, initially, it appears to indicate this method of diet preparation is acceptable to use. However, diet mixing practices can still be open to question, and in this case the scientist who reviewed this study indicated that he questioned the homogeneity of the treatment diets and the mixing methods; e.g., how soon after the stock diet was prepared were the treatment diets prepared.

Assessment of Guidance: Agency guidance on this may need to be reexamined, since issues concerning diet preparation are recurring.

Avoidable: Probably not with current guidance.

Industry Comment: Guidance appears adequate, although the FIFRA-TG appears to be more relaxed than are the reviewers about acceptance criteria. The rejection factor quoted does not seem inconsistent with the guidelines. Industry welcomes the opportunity to establish exactly what problems EEB has with diet preparation. It is possible that contact with the registrant before issuing the DER could have resolved the issue.

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[20] This section is under 71-2, *Avian Dietary LC_{50}*, because 71-3, *Wild Mammal Toxicity*, refers to 71-2 for performing dietary tests.

EPA Response: While other issues concerning diet preparation, as previously suggested, may need to be reexamined, the Agency agrees with Industry's comments that guidance on mixing appears adequate. The question raised in this factor does not appear to be a generic issue, but a problem specific to this individual study. The issue relates to the description of preparation of the diets, which did not appear to meet the guidance given to ensure a uniform concentration in the feed.

Resolution: Use of a premix (i.e., prepare stock feed and mix with clean feed) for diet preparation is a commonly used, acceptable approach in toxicology and is not cause for rejection of a study. Methods must include adequate procedures to ensure that prepared feed concentration is uniform.

2. *Rejection Factor:* Extensive cannibalism occurred during the study, which may indicate that improper caging of animals was used.

Agency Guidance: Subd-E: p. 40, 71-2, (d)(1) *Pen facilities.*, nos. (1) and (4), and FIFRA-TG: p. C-18, 71-2, no. 6. provide guidance on pen facilities.

Assessment of Guidance: The guidance given is for pen facilities for birds. However, issues such as cannibalism are handled on a case-by-case basis. This is a chemical-specific decision that is important for the overall assessment of the hazards of the chemical.

Avoidable: No. It is not possible to prevent animals placed in cages from exhibiting cannibalism, but the registrant could have placed the animals in separate pens. Guidance is not specific in this case.

Industry Comment: Although the results are likely to be obscured by the cannibalism, they may still include sufficient information to conduct a risk assessment. On the grounds of animal welfare, repetition of the study should not be requested unless the results are completely unusable. The issue arises through lack of specific guidelines, though it appears that the animals may not have been cared for in the best possible way.

It should be routine that the protocol for this experiment be agreed upon by EPA and the registrant before the study is conducted. Expert advice should be sought where the behavior of the test species in captivity is not known by either party. The study should not have to be repeated unless the results of the current study add no value to the risk assessment.

This test is not normally required and a variety of species may be used. The behavior of the species in captivity may not be well known by either the registrant or the Agency. The SEP shows flexibility and Industry supports this. The rejection factor could have been avoided by a discussion between the Agency and the registrant, and by seeking specialist advice by both parties before initiation of the test.

EPA Response: The Agency agrees with Industry that extensive consultations with experts are needed before conducting these less-than-routine tests. The Agency disagrees with Industry that rejection, and subsequent repeating, only be imposed if the results are of *no* value. A study can be repeated if additional testing will increase the understanding of the risk to nontarget organisms. In some cases, the results may add some value, but still not provide sufficient information to allow for a mammal risk assessment in which high confidence can be placed. It is, however, Agency policy that the "value of information" should be taken into account before any study has to be repeated.

Resolution: EPA and NACA agree that these studies are seldom conducted and thus standard methods do not exist. Consultation with experts is recommended before conducting such studies, and inclusion of protocol review and consultation prior to test initiation is recommended. Problems such as cannibalism is certainly undesirable; however, they may be unavoidable given the lack of experience in conducting such tests.

EPA Additional Response: Unavoidable problems such as cannibalism may not result in automatic rejection of the study. However, if the extent of the problem is such that it precludes deriving useful toxicological information from the study that may be used to assess risk, then a repeat of the study may be requested. For example, if only one animal in ten, and in only one test level, succumbs to cannibalism, that cannibalism would probably not, by itself, result in study rejection. If, however, substantial cannibalism occurred at most of the test levels, the study would probably not provide sufficient information to characterize toxicity. The Agency would likely conclude that there would be high value of information for requesting that the study be conducted again.

It is recommended that when conducting tests which are less than routine, sufficient *preliminary* effort be expended to discover many of these problems and devise ways to avoid them. For example, groups of test animals similar in size and composition to those proposed in the study should be held for a period equivalent to the full duration of the

study. If cannibalism occurs, the test groups may have to be modified or reduced, or it is even possible that each individual would have to be housed separately.

AVIAN REPRODUCTION (BOBWHITE QUAIL) (Guideline 71-4)

1. *Rejection Factor:* The value of the control birds as a valid biological control was diminished because the percent of cracked eggs in the control group was higher than two of the treatment groups, and the highest incidence of mortality and excoriation was found in the control group.[21]

Agency Guidance: Subd-E: pp. 48 - 57, 71-4 (all sections) and FIFRA-TG: pp. C-27 - C-32, 71-4 (all sections) provide general guidance on conducting these types of studies. Specific guidance concerning acceptable levels for reproductive parameters is not available, since such levels are handled on a case-by-case basis.

Assessment of Guidance: Due to the complexities involved in performing these types of studies, these rejection issues are handled on a case-by-case basis.

Avoidable: No. Current testing guidance does not specify the maximum numbers or percent of cracked eggs in the control that are acceptable.

Industry Comment: Eggshell cracking may often be influenced by bird behavior, cage conditions, or handling procedures. Consequentially, rejection of studies based on eggshell cracking in controls is unlikely to improve assessment of pesticide uses that truly will affect bird reproduction. Before rejecting studies for eggshell cracking, the following questions should be considered: (1) is cracking in treatment groups significantly higher than in controls; (2) without reference to controls, does a dose-related trend in cracking exist; (3) is cracking elevated in other species tested; and (4) are other egg-related reproductive variables (fertility, viability, hatchability, shell thickness) affected in a dose-related manner?

EPA Response: The Agency does not agree with the Industry comments. The Agency has been consistent in rejecting studies in which there is more than 10% cracked eggs in the controls. A background rate of 10% as the limit of acceptability is reasonable as the Agency sees an average of

[21] There were four groups: a control, 20 ppm treatment level, 100 ppm, and 500 ppm.

5% from most research laboratories. The EPA-ERL at Corvallis was able to achieve background rates of <1% for mallard duck and <5% for bobwhite quail. A high rate of cracking leads to a loss of sensitivity of the study and a decrease in the statistical power of the test. The ability of the study to detect differences between control and treated birds for other avian reproduction parameters is diminished. The avian reproduction study does not achieve high statistical power for its parameters as is, and, therefore, further reduction in power is not desirable.

Industry Comment: 1. EPA states that the "EPA-ERL Corvallis was able to achieve background rates of < 1% for mallard duck and < 5% for bobwhite quail." It should be noted that the Corvallis lab does not conduct studies according to EPA or ASTM guidelines relative to light intensity. They have significantly reduced light intensity in order to reduce aggressive behavior and egg cracking. Given this, it is inappropriate to use this lab as a comparative example of what is attainable.

2. The Agency is incorrect in stating that a "high rate" of egg cracking leads to loss of statistical sensitivity or power. Statistical power depends upon the number of cages and not the number of eggs, per se. This concern has been rebutted by registrants in the past using actual statistical power analysis based on real data.

3. The Agency states that "the ability of the study to detect differences between control and treated birds for other avian reproduction parameters is diminished." NACA disagrees with this point.

Resolution: EPA asserts that the following rejection criterion shall be applied:

(1) < 10% cracked eggs in the control birds is acceptable (i.e., no rejection);

(2) ≥ 10% and < 13% will result in a review of eggshell cracking in relation to other study endpoints. However, this is not cause—by itself—for rejection; and

(3) ≥ 13% is grounds for rejection.

2. *Rejection Factor:* Data discrepancies diminish the integrity of the study; e.g., (1) the photoperiod used in the study appeared inappropriate, (2) postmortem examinations of chicks could not be performed because chicks were so

cadaverous, (3) it was not reported why medication was administered in the feed to adults and chicks, or (4) the statistical evaluation did not appear to include the total number of data points.

Agency Guidance: Subd-E: pp. 48 - 57, 71-4 (all sections) and FIFRA-TG: pp. C-27 - C-33, 71-4 (all sections) provide general guidance on conducting these types of studies. Specific guidance concerning how to handle data discrepancies or acceptable levels for reproductive parameters is not available, since such levels are handled on a case-by-case basis.

Assessment of Guidance: Due to the complexities involved in performing these types of studies, these rejection issues are handled on a case-by-case basis.

Avoidable: Possibly.

Industry Comment: The rejection factors are a collection of "data discrepancies." It is not stated whether each one of these issues is considered by itself to be a "fatal flaw," or whether it takes multiple issues of this type to result in study rejection (for other study types, EPA has generally listed such factors separately). Because three of the four issues are stated with qualifications ("appeared inappropriate"), it would be useful to know if the reviewers were essentially requesting additional information (and therefore deferring judgement on validity) or were classifying a study as scientifically unsound for these reasons.

Photoperiod: EPA guidelines provide recommendations on photoperiod that *should* be followed (not "must" be followed). Furthermore, the SEP states that "EEB does not endorse any one protocol . . . . This referenced protocol [Subd-E] provides flexible guidance to help researchers design scientific protocols . . ." (p. 9). Deviations from the recommendation should be considered grounds for rejection only if there is evidence that the photoperiod used did not stimulate the birds to reproductive activity. Under the current protocol, EPA recommends 16 to 17 hours of light during the stimulation period. This represents a significant daily photoperiod (equivalent to sunrise at 6:00 a.m. and sunset at 11:00 p.m.). Birds are stimulated to reproduction on shorter or longer daily photoperiod.

Postmortem examinations: EPA guidelines only require postmortem necropsy for adults (all must be necropsied). Necropsies are not routinely performed on offspring (0 to 14 days old) because their small size makes examination of internal organs difficult. Chicks are housed at high temperatures, which exacerbates the problem by promoting

rapid autolysis. If postmortem examination requirements were applied to progeny, the number of required analyses (> 2,000) would be excessive.

Medication use: Any medication given during the test must be documented and the reason for its use explained. However, EPA guidelines do not require an explanation for why such treatments are given. Future guideline revisions should clarify this point. In many cases, issues such as this can be most efficiently handled by telephone contacts with registrants, instead of going through the entire process of study rejection and registrant rebuttal.

Statistical evaluation: Any statistical analysis should include all the appropriate data points. Given the number of checks that such data currently receive prior to submission, this should not be a general problem. Before rejecting studies for such reasons, registrants could be contacted by telephone during the review process to resolve such matters.

Industry concurs that these are complex studies requiring case-by-case review. Given the high rejection rate reported for reproduction studies, it seems possible that studies are being rejected for data discrepancies that are not necessarily "fatal flaws." As a general rule, the more complex and lengthy a study, the greater the probability that some deviations from the ideal will exist in execution or biological performance. The need to repeat studies should be viewed in terms of the overall exposure and risk to birds, with clear documentation that an adequate safety margin can not be inferred from existing information. Finally, strong feelings exist within and outside EPA that the current avian reproduction test may be of limited usefulness for risk assessment for most currently registered chemicals. This factor should also be considered when the decision is made to request replacement studies. Industry encourages EPA to proceed expeditiously to review the existing test protocol.

EPA Response: The Agency agrees with Industry on most points:

photoperiod: [partially agree] The photoperiod should not be referenced as reason for rejecting study unless it is considered likely, scientifically, that the photoperiod adversely impacted the results, making them unreliable for risk assessment.

post mortem examination: [agree] The study should not be rejected just because the chicks were not necropsied.

medication: [agree tentatively] The issue is not so much whether the use of medication in the diet of test animals is acceptable; rather, it is whether it is explained, and whether it impacts the validity of the study. Medication of animals may be acceptable, but its use should be justified and explained in detail. The Agency must determine whether its use may have impacted the results. Further, the Agency must consider whether the fact that medication was needed suggests other problems with the study, such as unhealthy organisms or poor animal husbandry. These deficiencies are usually evidenced in other ways during the study, but may also be manifested by the need for medication. Use of unhealthy organisms or poor husbandry is likely to affect the acceptability of the study.

statistical analysis: [agree] Studies should never be rejected solely because of the statistical analysis the registrant did or did not do. It is the Agency's responsibility to conduct statistics and evaluate the results of the study using the data. The inclusion of data, and possibly the format of that data, may be an issue in acceptability of the "report" of the study. If a study report is deemed unacceptable because of missing data or data submitted in an inappropriate format, the study would be considered for upgrading when the needed information was provided in the proper format.

Resolution: Industry and the Agency were generally in agreement that:

(1) photoperiod should not be a rejection criteria unless it impacts on the scientific validity of the study,

(2) generally, the lack of a postmortem examination of chicks should not be a rejection criterion unless study-specific factors warrant it,

(3) an explanation of how and why animals were medicated should be included in the report, and

(4) all appropriate data should be provided in the report.

AVIAN REPRODUCTION (DUCK) (Guideline 71-4)

1. *Rejection Factor:* Data discrepancies diminish the integrity of the study; e.g., (1) the photoperiod used in the study appeared inappropriate, (2) egg collection procedures appeared inappropriate, (3) it was not reported why medication was administered in the feed to birds, or (4) the overall fertility of control birds appeared to be too low.

Agency Guidance: Subd-E: pp. 48 - 57, 71-4 (all sections) and FIFRA-TG: pp. C-27 -C-33, 71-4 (all sections) provide general guidance on conducting these types of studies. Specific guidance concerning how to handle data discrepancies or acceptable levels for reproductive parameters is not available, since such levels are handled on a case-by-case basis.

Assessment of Guidance: The guidance in Subd E for lighting period is specific (see page 53, 3rd and 4th para). The other issues are less clearly defined. Due to the complexities involved in performing these types of studies, these rejection issues are handled on a case-by-case basis. These types of decisions are important for the overall assessment of the hazards of the chemical.

Avoidable: The lighting deficiencies are avoidable, but it is not clear that the other deficiencies are specifically cautioned against in written guidance.

Industry Comment: The rejection factors are a collection of "data discrepancies." It is not stated whether each one of these issues is considered by itself to be a "fatal flaw," or whether it takes multiple issues of this type to result in study rejection (for other study types, the Agency has generally listed such factors separately). Because three of the four issues are stated with qualifications ("appeared inappropriate"), it would be useful to know if the reviewers were essentially requesting additional information (and therefore deferring judgement on validity) or were classifying a study as scientifically unsound for these reasons.

Photoperiod: EPA guidelines provide recommendations on photoperiod that *should* be followed (not "must" be followed). Furthermore, the SEP states that "EEB does not endorse any one protocol . . . . This referenced protocol [Subd-E] provides flexible guidance to help researchers design scientific protocols . . ." (p. 9). Deviations from the recommendation should be considered grounds for rejection only if there is evidence that the photoperiod used did not stimulate the birds to reproductive activity. Under the current protocol, EPA recommends 16 to 17 hours of light during the stimulation period. This represents a significant daily photoperiod (equivalent to sunrise at 6:00 a.m. and sunset at 11:00 p.m.). Birds are stimulated to reproduction on shorter or longer daily photoperiod.

Egg collection: This is unlikely to be a significant general factor in study rejection. Gross errors (e.g., no system existed to separate eggs collected from different treatment groups) should have been described in the EPA analysis.

Medication use: Any medication given during the test must be documented and the reason for its use explained. However, EPA guidelines do not require an explanation for why such treatments are given. Future guideline revisions should clarify this point. In many cases, issues such as this can be most efficiently handled by telephone contacts with registrants, instead of going through the entire process of study rejection and registrant rebuttal.

Egg Fertility: Critical parameters such as egg fertility should not be judged on so vague a basis as "appeared to be too low." Industry postulates two points in responding to this: (a) "normal" values have not been published by EPA nor do any consensus values exist in the scientific community at large; and (b) if the chemical is not exerting any deleterious effect, then any treatment group, including the controls, has an equal chance of being the lowest performing group. Given the cost and length of reproduction studies, EPA should allow use of laboratory historical data, where appropriate, as an additional indicator of reproductive performance. EPA should work with NACA and other groups to determine if normal or acceptable values can be established.

NACA concurs that these are complex studies requiring case-by-case review. Given the high rejection rate reported for reproduction studies, it seems possible that studies are possibly being rejected for data discrepancies that are not necessarily "fatal flaws." As a general rule, the more complex and lengthy a study, the greater the probability that some deviations from the ideal will exist in execution or biological performance. The need to repeat studies should be viewed in terms of the overall exposure and risk to birds, with clear documentation that an adequate safety margin can not be inferred from existing information. Finally, strong feelings exist within and outside EPA that the current avian reproduction test may be of limited usefulness for risk assessment for most currently registered chemicals. This factor should also be considered when the decision is made to request replacement studies. Industry encourages the Agency to proceed expeditiously to review the existing test protocol.

EPA Response: See Rejection Factor 2 above for comments regarding photoperiod and use of medication. It is unlikely that egg collection methods and/or frequency would be sole rejection criteria, unless they appeared to be rather haphazard and affected hatchability.

The Agency agrees with Industry's comment that critical parameters such as egg fertility should not be judged on so vague a basis as "appears to be too low," and this was not the case. This statement is a

summary of information discussed in the review of this study which addressed those points, such as historical data, which Industry indicated should be addressed. The range of egg fertility found in this type of study is stated and compared to the range reported for this study.

FRESHWATER FISH LC_{50} (BLUEGILL) (Guideline 72-1)

1. *Rejection Factor:* The concentration level selected was less than 100 mg/L and was not high enough to produce an LC_{50}.

Agency Guidance: Subd-E: p. 67, 72-1, no. (b)(3)(i)(A)(B) and (ii); pp. 67- 68, 72-1, no. (c)(1)(i)(A)(B). FIFRA-TG: p. C-38, 72-1, no. 8. and p. C-40, 72-1, no. 8. Stephan, Charles E. (EPA-660/3-75-009; April, 1975):[22] pp. 6 - 7 and pp. 45 - 49, nos. 12. and 13. plus Table 6 and Section F., no. 8.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes, unless the solubility of the test material is below the level at which any mortality occurs. In this case, the registrant is expected to submit a request for an exception to this requirement because of insolubility. The guidance for this type of request has been developed jointly by EPA and NACA and is contained in Appendix A, *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance (1992).*

Industry Comment: None of the guidance documents provides true guidance in the case of solubility problems. As the purpose of the study is to determine the acute toxicity expressed as an LC_{50} value, Industry basically agrees with EPA. Solubility problems frequently occur below 100 mg/L with pesticides, making it sometimes difficult to test up to this level. However, application of the theoretical rate of 10 lb/A directly into 0.5 ft. of water results in only 7.4 mg/L (Urban and Cook, 1986). Depending on the concentration achieved, the data may be used for a risk assessment. The expectation to request a waiver in these circumstances was not known to many registrants.

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue. However, the Agency

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[22] This reference provides examples of acceptable methods for conducting acute aquatic toxicity tests. For the rest of this paper this document will be referred to as Stephan.

stresses that resolution of solubility problems in aquatic studies is independent of risk assessment issues. Toxicity (hazard) must be characterized independently of an exposure estimate.

2. *Rejection Factor:* The test chambers were aerated.

Agency Guidance: FIFRA-TG: p. C-38, 72-1, no. 7 and Stephan: p. 16 and p. 31, no. 2.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes. Either the test chambers should not be aerated, or if aeration is necessary, chemical analysis of the test solution periodically throughout the test should be performed to verify exposure.

Industry Comment: Industry agrees with EPA's assessment. However, a rejection is only warranted if there is aeration but no analysis.

EPA Response: EPA and NACA agree.

3. *Rejection Factor:* The biological loading of test vessels was twice the recommended amount.

Agency Guidance: FIFRA-TG: p. C-37, 72-1, no. 7. and Stephan: pp. 33 - 34, no. 4.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry recognizes that the guidance documents clearly recommend levels for biological loading. However, if the dissolved oxygen and ammonia levels are within acceptable limits, if there is a distinct concentration-effect relationship, and if the concentrations are stable over time, the biological loading alone should not be reason for rejection. A flexible, case-by-case evaluation of an otherwise scientifically valid study should be considered.

EPA Response: The Agency agrees that this factor alone may not be sole reason for rejection of a study. However, a loading factor of twice the recommended amount is a significant deviation and can warrant

rejection of a study. Furthermore, in studies where loading exceeds recommended levels ammonia levels should be measured daily. This factor will be handled on a case-by-case basis.

4. *Rejection Factor:* Test substance purity was not identified.

Agency Guidance: Subd-E: p. 30, 70-4 (c)(2)(i). Stephan: p. 47, no. F.2.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry agrees that the guidance documents provide appropriate guidance, and that knowledge of the purity of the test substance is mandatory for a risk assessment. However, not all impurities may have to be known, unless there is reason to suspect the influence of impurities. In order to avoid unnecessary delays in time and effort for EPA and the registrant, industry recommends that the reviewer contact the registrant prior to finalizing and issuing the DER.

EPA Response: Both the Agency and Industry agree that this factor is avoidable. This information needs to be included with the study upon its first submission to the Agency.

5. *Rejection Factor:* Inappropriate test species were used and test species were not clearly identified.

Agency Guidance: Subd-E: p. 30, 70-4, (c)(3)(i) - (viii); p. 67, 72-1 (b)(2)(i) -(iii). FIFRA-TG: p. C-37, 72-1, no. 3. Stephan: pp. 20 - 22, no. D.1. and Table 3.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry concedes that a clear identification of the test species is essential. However, it is not quite clear which species are appropriate. While the FIFRA-TG and the SEP renders acceptable rainbow trout, brook trout, and Coho salmon as cold-water species, and bluegill, channel catfish, and fathead minnow as warm-water fish, Stephan and

ASTM also recommend the goldfish as a warm-water species. Subd-E requests one warm- and one cold-water fish, preferably bluegill and trout. Several other warm-water species are recommended by OECD. Although EPA has good reason for preferring certain species, use of other internationally accepted species in a scientifically valid test should not lead to automatic rejection, especially in view of international harmonization.

EPA Response: The preferred test species are rainbow trout and bluegill sunfish, which are listed in Subdivision E, FIFRA-TG and the SEP. EPA is in the process of harmonizing its testing guidelines with other groups, including OECD. A result of this process may be the acceptance of test species that are currently not on the preferred species list. However, accepting a study does not necessarily mean that an additional species that is more appropriate for the risk assessment will not be required. It is likely that EPA will always require trout and bluegill for comparative risk assessments.

Resolution: In the discussion, EEB indicated that it would be likely that EPA will harmonize with other groups and accept species that are not on the current preferred test species list. However, accepting a study does not necessarily mean that an additional species will not be required that is more appropriate for the risk assessment. It is likely that EPA will always require trout and bluegill for comparative risk assessments.

6. *Rejection Factor:* Fish were fed during the 96-hour exposure period of the study.

Agency Guidance: SEP Acute Toxicity Test for Freshwater Fish: p. 4, II.B.3. Stephan: p. 30, IV.D.7 p. 37, IV.E.7. ASTM E - 729: p. 388, 10.8.3 and p. 391, 11.6.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry agrees that the fish should not be fed during the 96-hour exposure period.

EPA Response: EPA and NACA are in agreement.

7. *Rejection Factor:* Minimum limit of detectability, or the minimum quantifiable limit, was not defined quantitatively.

Agency Guidance: Subd-E: p. 29, no. 70-4 (b)(1)(2), p. 32, no. 70-4 (c)(6)(ii)(F) and p. 68 no. 72-1 (c)(4). SEP Acute Toxicity Test for Freshwater Fish: p. 9, III.H. Stephan: p. 39 - 40, IV. E.10.b.1 and p. 49, IV.F.9. FIFRA-TG: 72-1, p. C-40, no. 8. ASTM E - 729: p. 394, 15.1.9.

Assessment of Guidance: Sufficient guidance did not exist until EPA/NACA produced *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance (1992).* The guidance provided in this document is adequate to address this issue.

Avoidable: No, due to lack of clarity in issues concerning analytical determinations. These issues are now resolved in the EPA/NACA document. Future submissions containing this factor will be rejected.

Industry Comment: Guidance to avoid this problem in the future is offered by EPA/NACA, *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance (1992).*

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

8. *Rejection Factor:* The variability limit for test concentrations was greater than 1.5.

Agency Guidance: ASTM E-729: p. 392, 11.9.3.4(2).

Assessment of Guidance: Sufficient guidance did not exist until EPA/NACA produced *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance (1992).* The guidance provided in this document is adequate to address this issue.

Avoidable: No, due to lack of clarity in issues concerning analytical determinations. These issues are now resolved in the EPA/NACA document. Future submissions containing this factor will be rejected.

Industry Comment: Variability of measurements may be encountered in spite of all efforts to limit the variation, especially if test concentrations are in the ppb or ppt range, and if the compound readily adsorbs to surfaces, has very limited solubility, or has limited stability. Coefficients of variation at

these concentrations are high, as noted in the literature, and variation is more likely to be related to analysis than to malfunction of a diluter (if used). In these or other circumstances where the analytical method is acceptable, as determined by spiked control samples, and serious efforts to produce less-variable data have not been successful, EPA should be flexible—a rejection does not seem warranted. On a case-by-case basis, EPA should accept studies with variable data, as long as an adequate defense has been made.

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

9. *Rejection Factor:* The dilution water contained higher levels than recommended of lead, iron, and aluminum.

Agency Guidance: Stephan: p. 15, IV. C. 2. ASTM E-729: 384, 8.2.2.1 and p. 385, 8.4.

Assessment of Guidance: Generally, the guidance given in this area is adequate.

Avoidable: Yes.

Industry Comment: While Stephan on page 15 recommends specific levels of lead, iron, and aluminum for *reconstituted* water, which is basically recommended, on page 14 acceptable water for acute toxicity tests is defined as "healthy test organisms will survive in it for the duration of acclimation and testing without showing signs of stress. . . . Because daphnids are more sensitive to many toxicants . . . a more realistic criterion for an acceptable dilution water is that first instar daphnids will survive in it for 48 hours without food." Similarly, ASTM gives as a minimal requirement for acceptable dilution water "that at least one test species will survive, grow, and reproduce satisfactorily in it." Taking criteria of biological responses to the test water into consideration, studies do not need to be rejected on this basis.

EPA Response: The Agency agrees with Industry that biological responses of organisms to the dilution water need to be considered. However, if these and other metals are present in the dilution water at concentrations which exceed acceptable criteria, the Agency should be notified prior to test initiation.

Resolution: The study will not be rejected solely on the basis that metals exceeded the criteria. The biological performance of the organisms will also be considered in evaluating the study.

10. *Rejection Factor* There was no solvent control.

Agency Guidance: Subd. E: p. 26, no. 70-3 (b)(1)(2)(3). ASTM E-729: p. 385 - 386, 9.2.5 - 9.2.7, p. 389, 11.1.1.1 and p. 393, 13.1.3. SEP Acute Toxicity Test for Freshwater Fish: p. 6, II.D.3. Stephan: p. 31, IV.E.1. FIFRA - TG: 72-1, p. C-36, no. 2 and p. C-39, no. 7.

Assessment of Guidance: The guidance documents which address this issue appear adequate.

Avoidable: Yes.

Industry Comment: Only Stephan and ASTM *require* solvent controls. The Subd-E guideline states that the solvent control "should be used." (ASTM defines "should" to mean that the specified condition is recommended and ought to be met, if possible. Although violation of one "should" is rarely a serious matter, violation of several will often render the results questionable.)

Industry agrees that the use of a solvent control is desirable and scientifically appropriate. However, if the solvent control would have displayed signs of intoxication, but was omitted, these signs of intoxication would be attributed to the test compound. Although this might not reflect the true toxicity, for risk assessment purposes it would reflect a "worse case" scenario, and safety for the assessment. It therefore may not be necessary to reject a study for this parameter alone.

EPA Response: The Agency guidance on this factor is adequate. Industry agrees with the Agency that using a solvent control is scientifically valid and correct.

Resolution: NACA suggested that the Agency indicate in guidance documents that a solvent control "must" (instead of "should") be included. The Agency agrees.

11. *Rejection Factor* The results for two of the test concentrations were obtained from a separate test conducted a few weeks after the definitive study.

Agency Guidance: Subd. E: p. 27, 70-3, (b)(4). Stephan: p. 31, IV.E.1. ASTM E-729: p. 380, 4.1.

Assessment of Guidance: The guidance documents which address this issue appear adequate.

Avoidable: Yes.

Industry Comment: Industry basically agrees with EPA's assessment. Adding concentrations at a later time may occur, however, if in cases of very flat concentration-effects relations, space in the diluter system or in the facility is insufficient. This may arise especially if the determination of a NOEL is required (studies of NACA members were rejected for this reason). Add-on studies must establish a continuation of the biological responses (e.g., by overlapping of tested concentrations). Study rejection therefore should be based on a case-by-case consideration.

EPA Response: Adding concentrations from a separate study and combining them with the ones used in the definitive study is inappropriate. The arguments provided by Industry are unpersuasive. If the definitive study does not generate an acceptable dose-response curve, a new study needs to be conducted. Note, however, that determination of a NOEL in acute toxicity studies is not required. It is the responsibility of the registrant to ensure that the design of the test system results in the study being conducted according to guideline requirements.

Resolution: NACA assumed that the results of the study were combined to produce a mortality/effect profile which would allow an LC_{50}/EC_{50} or a NOEL to be estimated. NACA agrees that combining results of tests conducted at different times is not desirable, especially to complete dose-response curves.

12. *Rejection Factor* Not all test solutions were measured at 96 hours as well as at zero hours. The concentration of the test material greatly decreased for at least one test level after 96 hours of exposure.

Agency Guidance: Subd. E: p. 29, 70-4 (b)(1)(2), p. 32, 70-4 (c)(6)(ii)(F). Stephan: p. 41 - 42, IV.E.10.B.(3). SEP Acute Toxicity Test for Freshwater Fish: p. 7, II.D.6 p. 9, III.H. and p. 11, IV.C.1. ASTM E - 729: p. 392, 11.9.3.3 and 11.9.3.4(1) and (3).

Assessment of Guidance: Sufficient guidance did not exist until EPA/NACA produced *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance (1992).* The guidance provided in this document is adequate to address this issue.

Avoidable: No, due to lack of clarity in issues concerning analytical determinations. These issues are now resolved in the EPA/NACA document. Future submissions containing this factor will be rejected.

Industry Comment: Industry agrees that rejections in this area were not avoidable due to lack of clarity. Studies were accepted in the past by EPA with and sometimes without analytical measurements.

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

13. *Rejection Factor* A control group of fish for the inert/carrier ingredients present in the formulation was lacking. (The test material was 42% a.i. and 58% inert/carrier ingredients.)

Agency Guidance: Stephan: p. 31, IV.E.1.

Assessment of Guidance: Normally, this guidance is adequate. However, in this case the scientist concluded that, without an inert/carrier control (i.e., blank formulation) it was not possible to discern whether the toxic effects were due to the a.i., the inerts/carrier, or to both. This is a chemical-specific decision that is important for the overall assessment of the hazards of the chemical.

Avoidable: No.

Industry Comment: While Stephan requires an additive control, it also states that "none of the ingredients of the mixture or formulation is considered an additive." Therefore, one cannot conclude that testing of blank formulations is needed. Since an a.i. will be applied to the environment only as a formulation, knowledge of the contribution of the formulation additives may not change the overall risk assessment, but may serve only scientific or comparative purposes. If the formulation is being tested for a reason other than low solubility of the a.i., then it is likely that data already exist for the TGAI. Comparison of the toxicities of the a.i. and the formulation should suffice to determine if the inert

ingredients cause greater toxicity. The inert/carrier control would therefore not be necessary.

EPA Response: The Agency does not routinely require testing of inert/carrier ingredients of pesticides. If this testing is necessary for the overall assessment of the hazards of a specific chemical, it will be stipulated in the Data Call-In Notice or in other correspondence. The Agency agrees with Industry that the guidance provided in the EPA/NACA document may be sufficiently adequate, on a chemical-specific basis, to address this issue without the need for further testing.

Resolution: NACA requested clarification as to what specifically was to be tested. The Agency indicated that the terms "inert/carrier control" and "blank formulation" are used interchangeably. If testing of the blank formulation is needed, this will be stipulated in the DCI or other correspondence.

14. *Rejection Factor* The acclimation period for the fish prior to initiation of the test was less than half the recommended length of time.

Agency Guidance: ASTM E - 729: p. 388, 10.7 - 8. SEP Acute Toxicity Test for Freshwater Fish: p. 4, II.B.3. Stephan: p. 28 - 29, IV.D.7.

Assessment of Guidance: The guidance documents which address this issue appear adequate.

Avoidable: Yes.

Industry Comment: The guidance documents appear contradictory:

ASTM requires maintaining the test organisms in the dilution water at the test temperature for at least 48 hours before they are placed in the test chambers;

SEP recommends $\geq$ 2-week acclimation to study conditions; and

Stephan says to acclimate to changing water/temperature for 2 days, and 2 days in that water/temperature.

Because this parameter may not be critical for the validity of a test, if the test organisms display normal behavior and reactions, rejection because of this parameter does not seem necessary.

EPA Response: There can be confusion regarding the terms "holding" and "acclimation." The SEP inadvertently used the wrong term. When the test organisms are brought into the laboratory, they are to held for at least 2 weeks in holding tanks under stable water quality and temperature conditions. After transfer to the acclimation tank from the holding tank, the water needs to be gradually changed from 100% holding water to 100% dilution water and test temperature over a period of 2 or more days. They must be kept in 100% dilution water and temperatures for at least 2 additional days before use in the toxicity study. The Agency guidance cited above, plus the procedures described in parts 801 and 810 of *Standard Methods for the Examination of Water and Wastewater,* provide adequate details. The Agency agrees that this factor alone may not be sole reason for rejection of a study. This factor will be handled on a case-by-case basis.

FRESHWATER FISH LC_{50} (RAINBOW) (Guideline 72-1)

1. *Rejection Factor:* The concentration level selected was less than 100 mg/L and was not high enough to produce an LC_{50}.

Agency Guidance Subd-E: p. 67, 72-1, (b)(3)(i)(A)(B) and (ii); pp. 67- 68, 72-1, (c)(1)(i)(A)(B). FIFRA-TG: p. C-38, 72-1, no. 8. and p. C-40, 72-1, no. 8. Stephan: pp. 6 - 7 and pp. 45 - 47, nos. 12. and 13. plus Table 6 and section F., no. 8.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes, unless the solubility of the test material is below the level at which any mortality occurs. In this case, the registrant is expected to submit a request for an exception to this requirement because of insolubility. The guidance for this type of request has been developed jointly by EPA and NACA and is contained in the guidance document found in Appendix A.

Industry Comment: Although the guidance documents cited clearly require the provision of an LC_{50}, none of the documents provides true guidance for solubility problems. Solubility problems frequently occur below 100 mg/L, making it sometimes difficult to test up to this level. However, application of the theoretical rate of 10 lb/A directly to 0.5 ft. of water results in only 7.4 mg/L (Urban and Cook, 1986). Depending on the concentration achieved, the data may be used for a risk assessment.

The expectation to request a waiver in these circumstances was not known to many registrants.

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

2. *Rejection Factor:* The test chambers were aerated.

Agency Guidance: FIFRA-TG: p. C-38, 72-1, no. 7 and Stephan: p. 16 and p. 31, no. 2.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes. Either the test chambers should not be aerated, or if aeration is necessary, chemical analysis of the test solution periodically throughout the test should be performed to verify exposure.

Industry Comment: The guidance documents do suggest that the water is not to be aerated unless the concentrations are measured; but, a rejection is only warranted if there is aeration but no analysis.

EPA Response: EPA and NACA agree.

3. *Rejection Factor:* The biological loading of test vessels was twice the recommended amount.

Agency Guidance: FIFRA-TG: p. C-37, 72-1, no. 7 and Stephan: pp. 33 - 34, no. 4.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry recognizes that the guidance documents clearly recommend levels for biological loadings. However, if the dissolved oxygen and ammonia levels are within acceptable limits, if there is a distinct concentration-effect relationship, and if the concentrations are stable over time, the biological loading alone should not be reason for rejection. A flexible, case-by-case evaluation of an otherwise scientifically valid study should be considered.

EPA Response: The Agency agrees that this factor alone may not be sole reason for rejection of a study. However, a loading factor of twice the recommended amount is a significant deviation and can warrant rejection of a study. Furthermore, in studies where loading exceeds recommended levels, ammonia levels should be measured daily. This factor will be handled on a case-by-case basis.

4. *Rejection Factor:* Test substance purity was not identified.

Agency Guidance: Subd-E: p. 30, 70-4, (c)(2)(i). Stephan: p. 47, no. F.2.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry agrees that the guidance documents provide appropriate guidance, and that knowledge of the purity of the test substance is mandatory for a risk assessment. However, not all impurities may have to be known, unless there is reason to suspect the influence of impurities. In order to avoid unnecessary delays in time and effort for EPA and the registrant, Industry recommends that the reviewer contact the registrant prior to finalizing and issuing the DER.

EPA Response: Both the Agency and Industry agree that this factor is avoidable. This information needs to be included with the study upon its first submission to the Agency.

5. *Rejection Factor:* Inappropriate test species were used and test species were not clearly identified.

Agency Guidance: Subd-E: p. 30, 70-4, (c)(3)(i) - (viii); p. 67, 72-1, (b)(2)(i) - (iii). FIFRA-TG: p. C-37, 72-1, no. 3. Stephan: pp. 20 - 22, no. D.1. and Table 3.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry concedes that a clear identification of the test species is essential. However, it is not quite clear which species are appropriate. While the FIFRA-TG and the SEP renders acceptable rainbow trout,

brook trout, and Coho salmon as cold-water species, and bluegill, channel catfish, and fathead minnow as warm-water fish, Stephan and ASTM also recommend the goldfish as a warm-water species. Subd-E requests one warm- and one cold-water fish, preferably bluegill and trout. Several other warm-water species are recommended by OECD. Although EPA has good reason for preferring certain species, use of other internationally accepted species in a scientifically valid test should not lead to automatic rejection, especially in view of international harmonization.

EPA Response: The preferred test species are rainbow trout and bluegill sunfish, which are listed in Subdivision E, FIFRA-TG and the SEP. EPA is in the process of harmonizing its testing guidelines with other groups, including OECD. A result of this process may be the acceptance of test species that are currently not on the preferred species list. However, accepting a study does not necessarily mean that an additional species that is more appropriate for the risk assessment will not be required. It is likely that EPA will always require trout and bluegill for comparative risk assessments.

Resolution: In the discussion, EEB indicated that it would be likely that EPA will harmonize with other groups and accept species that are not on the current preferred test species list. However, accepting a study does not necessarily mean that an additional species will not be required that is more appropriate for the risk assessment. It is likely that EPA will always require trout and bluegill for comparative risk assessments.

6. *Rejection Factor:* Fish acclimation records indicate the test fish were acclimated during the time the definitive study was conducted.

Agency Guidance: SEP Acute Toxicity Test for Freshwater Fish: p. 4, II.B.3. Stephan: pp. 28 - 29, IV.D.6 and 7. ASTM E - 729: p. 388, 10.8.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes. The contract laboratories are required to keep records regarding the acclimation conditions of the fish. Evidently, if the acclimation period overlapped the period for the definitive test, the fish were not properly acclimated to the test conditions and/or the laboratory was careless in conducting the study.

Industry Comment: The guidance documents appear contradictory:

- ASTM requires maintaining the test organisms in the dilution water at the test temperature for at least 48 hours before they are placed in the test chambers;

- SEP recommends ≥ 2-week acclimation to study conditions; and

- Stephan says to acclimate to changing water/temperature for 2 days, and 2 days in that water/temperature.

Industry agrees that fish should be properly acclimated prior to testing, and that appropriate records must document the acclimation period. But, the guidance documents should be clarified. In order to avoid unnecessary delays in time and effort for EPA and the registrant, Industry recommends that the reviewer contact the registrant prior to finalizing and issuing the DER.

EPA Response: This factor appears to be a Quality Assurance/Quality Control problem. The Agency is not stating that the fish were improperly acclimated to the test conditions prior to beginning the definitive study, but that the dates for the acclimation period and the testing period overlapped. The Agency's definition of holding and acclimation were described earlier. This factor is correctable by providing records that are accurately maintained and reported.

7. *Rejection Factor:* Fish were fed during the 96-hour exposure period of the study.

Agency Guidance: SEP Acute Toxicity Test for Freshwater Fish: p. 4, II.B.3. Stephan: p. 30, IV.D.7, p. 37, IV.E.7. ASTM E - 729: p. 388, 10.8.3 and p. 391, 11.6.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry agrees that the fish should not be fed during the 96-hour exposure period.

EPA Response: EPA and NACA are in agreement.

8. *Rejection Factor:* Minimum limit of detectability, or the minimum quantifiable limit, was not defined quantitatively.

Agency Guidance: Subd-E: p. 29, no. 70-4 (b)(1)(2), p. 32, no. 70-4 (c)(6)(ii)(F) and p. 68 no. 72-1 (c)(4). SEP Acute Toxicity Test for Freshwater Fish: p. 9, III.H. Stephan: p. 39 - 40, IV.E.10.b.1 and p. 49, IV.F.9. FIFRA-TG: 72-1, p. C-40, no. 8. ASTM E - 729: p. 394, 15.1.9.

Assessment of Guidance: Sufficient guidance did not exist until EPA/NACA produced *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance (1992).* The guidance provided in this document is adequate to address this issue.

Avoidable: No, due to lack of clarity in issues concerning analytical determinations. These issues are now resolved in the EPA/NACA document. Future submissions containing this factor will be rejected.

Industry Comment: Guidance to avoid this problem in the future is offered in EPA/NACA (1992), *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance.*

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

9. *Rejection Factor:* Chemical was recovered in the dilution water control at a level exceeding those in the two lowest test concentrations.

Agency Guidance: Subd. E: p. 26, no. 70-3 (b)(1)(2)(3). ASTM E - 729: p. 380, 4.1, p. 389, 11.1.1.1 and p. 393, 13.1.3. SEP Acute Toxicity Test for Freshwater Fish: p. 6, II.D.3. Stephan: p. 6, III.B and p. 45, IV.E.12.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes. Contamination of the controls with the test chemical is unacceptable.

Industry Comment: Industry agrees that the control water should not contain any test material. However, when working at very low concentrations (ppb and ppt), sporadic "findings" in the control water may occur, due to the fact that the method of chemical analysis is operated at its lowest level. False positive results may be as possible as false negative results. Depending on the level at which the test is performed and the nature of

the finding, the occurrence of positive findings should not be over-judged.

EPA Response: Contamination of the control water with the test material is unacceptable. If the chemical is being tested at very low concentrations that bracket the limit of quantification, and there is a possibility of obtaining false positive results, this concern must be clearly detailed being in the report.

10. *Rejection Factor* There was very low recovery of the test chemical from the stock solutions at zero and 96 hours. Measurements for the two lowest test concentrations were only given for 96 hours, as the chemical was not detected in the zero-hour samples.

Agency Guidance: Subd. E: p. 32, no. 70-4 (c)(6)(ii)(F). SEP Acute Toxicity Test for Freshwater Fish: p. 6, II.D.1, p. 7, II.D.6, p. 9, III.H. and p. 11, IV.C.1. Stephan: p. 45, IV.E.12. ASTM E -729: p. 392, 11.9.3.4(1) and (3).

Assessment of Guidance: Sufficient guidance did not exist until EPA/NACA produced *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance (1992).* The guidance provided in this document is adequate to address this issue.

Avoidable: No, due to lack of clarity in issues concerning analytical determinations. These issues are now resolved in the EPA/NACA document. Future submissions containing this factor will be rejected.

Industry Comment: Industry agrees that low recovery from stock solution and at 0 hours may cause the rejection of a study.

EPA Response: The Agency and Industry agree that this factor may affect the acceptance of a study. The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

11. *Rejection Factor* The variability limit for test concentrations was greater than 1.5.

Agency Guidance: ASTM E -729: p. 392, 11.9.3.4(2).

Assessment of Guidance: Sufficient guidance did not exist until EPA/NACA produced *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance (1992).* The guidance provided in this document is adequate to address this issue.

Avoidable: No, due to lack of clarity in issues concerning analytical determinations. These issues are now resolved in the EPA/NACA document. Future submissions containing this factor will be rejected.

Industry Comment: Variability of measurements may be encountered in spite of all efforts to limit the variation, especially if test concentrations are in the ppb or ppt range, and if the compound readily adsorbs to surfaces, has very limited solubility, or has limited stability. Coefficients of variation at these concentrations are high, as noted in the literature, and variation is more likely to be related to analysis than to malfunction of a diluter (if used). In these or other circumstances where the analytical method is acceptable, as determined by spiked control samples, and serious efforts to produce less-variable data have not been successful, EPA should be flexible—a rejection does not seem warranted. On a case-by-case basis, EPA should accept studies with variable data as long as an adequate defense has been made.

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

12. *Rejection Factor* The dilution water contained higher levels of lead, iron, and aluminum than recommended.

Agency Guidance: Stephan: p. 15, IV.C.2. ASTM E - 729: 384, 8.2.2.1 and p. 385, 8.4.

Assessment of Guidance: Generally, the guidance given in this area is adequate.

Avoidable: Yes.

Industry Comment: While Stephan on page 15 recommends specific levels of lead, iron, and aluminum for *reconstituted* water, which is basically recommended, on page 14 acceptable water for acute toxicity tests is defined as "healthy test organisms will survive in it for the duration of acclimation and testing without showing signs of stress. . . . Because daphnids are more sensitive to many toxicants . . . a more realistic criterion for an

acceptable dilution water is that first instar daphnids will survive in it for 48 hours without food." Similarly, ASTM gives as a minimal requirement for acceptable dilution water "that at least one test species will survive, grow, and reproduce satisfactorily in it." Taking criteria of biological responses to the test water into consideration, studies do not need to be rejected on this basis.

EPA Response: The Agency agrees with Industry that biological responses of organisms to the dilution water need to be considered. However, if these and other metals are present in the dilution water at concentrations which exceed acceptable criteria, the Agency should be notified prior to test initiation.

Resolution: The study will not be rejected solely on the basis that metals exceeded the criteria. The biological performance of the organisms will also be considered in evaluating the study.

13. *Rejection Factor* There was no solvent control.

Agency Guidance: Subd. E: p. 26, no. 70-3 (b)(1)(2)(3). ASTM E - 729: p. 385 - 386, 9.2.5 - 9.2.7, p. 389, 11.1.1.1 and p. 393, 13.1.3. SEP Acute Toxicity Test for Freshwater Fish: p. 6, II.D. 3. Stephan: p. 31, IV.E.1. FIFRA - TG: 72-1, p. C-36, no. 2 and p. C-39, no. 7.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Only Stephan and ASTM *require* solvent controls. The Subd-E guideline states that the solvent control "should be used." (ASTM defines "should" to mean that the specified condition is recommended and ought to be met, if possible. Although violation of one "should" is rarely a serious matter, violation of several will often render the results questionable.)

Industry agrees that the use of a solvent control is desirable and scientifically appropriate. However, if the solvent control would have displayed signs of intoxication, but was omitted, these signs of intoxication would be attributed to the test compound. Although this might not reflect the true toxicity, for risk assessment purposes it would reflect a "worst-case" scenario, and safety for the assessment. It

therefore may not be necessary to reject a study for this parameter alone.

EPA Response: The Agency guidance on this factor is adequate. Industry agrees with the Agency that using a solvent control is scientifically valid and correct.

Resolution: NACA suggested the Agency indicate in guidance documents that a solvent control "must" (instead of "should") be included. The Agency agrees.

14. *Rejection Factor* The results for several of the test concentrations were obtained from separate tests conducted a few weeks after the definitive study.

Agency Guidance: Subd. E: p. 27, 70-3, (b)(4). Stephan: p. 31, IV.E.1. ASTM E - 729: p. 380, 4.1.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry basically agrees with EPA's assessment. Adding concentrations at a later time may occur, however, if in cases of very flat concentration-effects relations, space in the diluter system or in the facility is insufficient. This may arise especially if the determination of a NOEL is required (studies of NACA members were rejected for this reason). Add-on studies must establish a continuation of the biological responses (e.g., by overlapping of tested concentrations). Study rejection therefore should be based on a case-by-case consideration.

EPA Response: Adding concentrations from a separate study and combining them with the ones used in the definitive study is inappropriate. The arguments provided by Industry are unpersuasive. If the definitive study does not generate an acceptable dose-response curve, a new study needs to be conducted. It is the responsibility of the registrant to ensure that the design of the test system can adequately conduct the study according to guideline requirements.

Resolution: NACA assumed that the results of the study were combined to produce a mortality/effect profile which would allow an LC_{50}/EC_{50} or a NOEL to be estimated. NACA agrees that combining results of tests conducted at different times is not desirable, especially to complete

dose-response curves. For clarification, NACA requested that the Agency reiterate in its response that NOELs are not required for acute studies.

15. *Rejection Factor* Not all test solutions were measured at 96 hours, as well as at zero hours.

Agency Guidance: Subd. E: p. 29, 70-4 (b)(1)(2), p. 32, 70-4 (c)(6)(ii)(F). Stephan: p. 41 - 42, IV.E.10.B.(3). SEP Acute Toxicity Test for Freshwater Fish: p. 7, II.D.6 p. 9, III.H and p. 11, IV.C.1. ASTM E - 729: p. 392, 11.9.3.3 and 11.9.3.4(1) and (3).

Assessment of Guidance: Sufficient guidance did not exist until EPA/NACA produced *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance (1992).* The guidance provided in this document is adequate to address this issue.

Avoidable: No, due to lack of clarity in issues concerning analytical determinations. These issues are now resolved in the EPA/NACA document. Future submissions containing this factor will be rejected.

Industry Comment: Industry agrees that rejections in this area were not avoidable due to lack of clarity. Studies were accepted in the past by EPA with and sometimes without analytical measurements.

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

16. *Rejection Factor* A control group of fish for the inert/carrier ingredients present in the formulation was lacking. (The test.material was 42% a.i. and 58% inert/carrier ingredients.)

Agency Guidance: Stephan: p. 31, IV.E.1.

Assessment of Guidance: Normally, this guidance is adequate. However, in this case the scientist concluded that, without an inert/carrier control (i.e., blank formulation) it was not possible to discern whether the toxic effects were due to the a.i., the inerts/carrier, or to both. This guidance may need strengthening.

Avoidable: No.

Industry Comment: While Stephan requires an additive control, it also states that "none of the ingredients of the mixture or formulation is considered an additive." Therefore, one cannot conclude that testing of blank formulations is needed. Since an a.i. will be applied to the environment only as a formulation, knowledge of the contribution of the formulation additives may not change the overall risk assessment, but may serve only scientific or comparative purposes. If the formulation is being tested for a reason other than low solubility of the a.i., then it is likely that data already exist for the TGAI. Comparison of the toxicities of the a.i. and the formulation should suffice to determine if the inert ingredients cause greater toxicity. The inert/carrier control would therefore not be necessary.

EPA Response: The Agency does not routinely require testing of inert/carrier ingredients of pesticides. If this testing is necessary for the overall assessment of the hazards of a specific chemical, it will be stipulated in the Data Call-In Notice or in other correspondence. The Agency agrees with Industry that the guidance provided in the EPA/NACA document may be sufficiently adequate, on a chemical-specific basis, to address this issue without the need for further testing.

Resolution: NACA requested clarification as to what specifically was to be tested. The Agency indicated that the terms "inert/carrier control" and "blank formulation" are used interchangeably. If testing of the blank formulation is needed, this will be stipulated in the DCI or other correspondence.

17. *Rejection Factor* The weights of the test fish exceeded the recommended range.

Agency Guidance: Subd. E: p. 28, 70-3 (d)(2) and p. 67, 72-1 (b)(2)(ii) and (iii). SEP Acute Toxicity Test for Freshwater Fish: p. 3, II.B.2. Stephan: p. 23 - 24, IV.D.3. ASTM E - 729: p. 386 - 387, 10.2. FIFRA -TG: 72-1, C-37, no. 3.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: While the guidance for this factor is appropriate, the size of the test fish may become a problem due to the availability of seasonally

spawning fish, especially if a test has to be performed to meet a specific deadline. Rejection may not be necessary, if the deviation is not excessive and the results of the study are comparable to others.

EPA Response: The range of preferred weights for test fish is rather wide, thereby giving the individual registrants flexibility in obtaining appropriate sized fish for the test. The Agency requires consistency in size of test fish to allow for comparison of test results among chemicals.

18. *Rejection Factor* The test temperature exceeded that recommended for rainbow trout.

Agency Guidance: FIFRA -TG: 72-1, C-37, no. 7. SEP Acute Toxicity Test for Freshwater Fish: p. 5, II.C.2 and p. 10, IV.C.1. ASTM E - 729: 386, Table 4. Stephan: p. 21, Table 3.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry agrees that a rejection due to this factor is avoidable. However, depending on the extent, it may not be necessary to reject a study, especially when considering harmonization issues. OECD allows testing at 13-17°C, Germany requires 15°C. Testing in this range, instead of 12 $\pm$ 1°C, may not lead to such different results as to justify a repetition of a test.

EPA Response: The issues of preferred test temperature for selected fish species as covered in the guidelines harmonization process is being addressed in other venues. No further comment on this factor is necessary.

19. *Rejection Factor* The biological loading of the system was greater than recommended.

Agency Guidance: SEP Acute Toxicity Test for Freshwater Fish: p. 5, II.C.5. FIFRA - TG: 72-1, C-37, no. 7. Stephan: p. 33 - 34, IV.E.4 and 5. ASTM E - 729: p. 390, 11.4.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry recognizes that the guidance documents clearly recommend the level for biological loading. However, if the dissolved oxygen level is within acceptable limits, and if there is a distinct concentration-effect relationship, the biological loading alone should not be reason for rejection. A flexible, case-by-case evaluation of otherwise scientifically valid studies should be considered.

EPA Response: The Agency agrees that this factor alone may not be sole reason for rejection of a study. However, a loading factor greater than the recommended amount is a significant deviation and can warrant rejection of a study. Furthermore, in studies where loading exceeds recommended levels, ammonia levels should be measured daily. This factor will be handled on a case-by-case basis.

20. *Rejection Factor* The acclimation period for the fish prior to initiation of the test was less than half the recommended length of time.

Agency Guidance: ASTM E - 729: p. 388, 10.7 - 8. SEP Acute Toxicity Test for Freshwater Fish: p. 4, II.B.3. Stephan: p. 28 - 29, IV.D.7.

Assessment of Guidance: Generally, the guidance in this area is adequate.

Avoidable: Yes.

Industry Comment: The guidance documents appear contradictory:

- ASTM requires maintaining the test organisms in the dilution water at the test temperature for at least 48 hours before they are placed in the test chambers;
- SEP recommends $\geq$ 2-week acclimation to study conditions; and
- Stephan says to acclimate to changing water/temperature for 2 days, and 2 days in that water/temperature.

This parameter may not be critical to the validity of the test, if the test organisms display normal behavior and reactions. In order to avoid unnecessary delays in time and effort for EPA and the registrant, industry recommends that the reviewer contact the registrant prior to finalizing and issuing the DER.

EPA Response: There can be confusion regarding the terms "holding" and "acclimation." The SEP inadvertently used the wrong term. When the test organisms are brought into the laboratory, they are to held for at least 2 weeks in holding tanks under stable water quality and temperature conditions. After transfer to the acclimation tank from the holding tank the water needs to be gradually changed from 100% holding water to 100% dilution water and test temperature over a period of 2 or more days. They must be kept in 100% dilution water and temperatures for at least 2 additional days before use in the toxicity study. The Agency guidance cited above, plus the procedures described in parts 801 and 810 of Standard Methods for the Examination of Water and Wastewater, provide adequate details. The Agency agrees that this factor alone may not be sole reason for rejection of a study. This factor will be handled on a case-by-case basis.

21. *Rejection Factor* Fish mortality during the acclimation period was higher than recommended.

Agency Guidance: ASTM E - 729: p. 388 - 389, 10.8 - 10.9. SEP Acute Toxicity Test for Freshwater Fish: p. 4, II.B.3. Stephan: p. 28 - 29, IV.D.7.

Assessment of Guidance: Generally, the guidance in this area is adequate.

Avoidable: Yes.

Industry Comment: Industry agrees that mortality above the levels specified in the guidance documents may indicate problems with the test organisms, and therefore may be reason to invalidate a study.

EPA Response: EPA and NACA are in agreement.

22. *Rejection Factor* The dissolved oxygen during the test was supersaturated (over 100% saturation).

Agency Guidance: SEP Acute Toxicity Test for Freshwater Fish: p. 7, II.D.5. Stephan: p. 31, IV.E.2. ASTM E - 729: p. 390, 11.4.2 and p. 393, 13.1.9.

Assessment of Guidance: Supersaturation of oxygen is not directly addressed in our guidance documents.

Avoidable: Not at this time.

Industry Comment: Oversaturation can occur after intensive aeration of test aquaria prior to testing at lower concentrations. Normally this should not be persistent over the entire testing period, but would be seen only at the beginning of the test. If the observation is only made then, if the control fish behave and look normal, and if chemical analysis verifies the concentration level, there should be no reason to reject the study. This factor may be solved on a case-by-case consideration, and not by a check-box approach.

EPA Response: The Agency agrees with Industry's comments. This factor will be handled on a case-by-case basis.

Resolution: NACA encouraged the Agency to indicate that if the animals in the control group perform normally, then the test is acceptable. The Agency indicated that it is unlikely that a study would be rejected for this factor alone.

ACUTE LC_{50} FRESHWATER INVERTEBRATES (Guideline 72-2)

1. *Rejection Factor:* Organisms were not randomly distributed to test vessels.

Agency Guidance: Subd-E: p. 28, 70-3, no. (d)(3). Stephan: p. 30, E.1 and p. 31, E.1. . . .cont.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: The guidance documents clearly state that organisms must be randomly distributed to test vessels and that this is a valid scientific precaution against skewing results. As long as some unbiased selection is used, however, it is doubtful that this factor would significantly impact the results of this study. In fact, it is not clear that a true random distribution is needed. Unless the dose-response curve shows erratic, highly variable data points, this should not be reason in itself to reject a study. Studies should not be rejected for this factor if the mortality data are not irregular.

EPA Response: The guidance is clear.

Resolution: EPA/NACA agreed to add a statement as follows: "Impartially distributing organisms among randomly placed test chambers is an acceptable procedure for randomizing organisms."

2. *Rejection Factor:* Temperature of the water was not monitored during the tests.

Agency Guidance: FIFRA-TG: p. C-41, 72-2, no. 2., and p. C-42, 72-2, no. 7., and p. C-44, 72-2, no. 7. Stephan: pp. 31 - 33, no. 3.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Unless temperature data can be supplied in some other way, this is a clear violation of the regulations. The study in all probability will have to be re-done, and this study will become supplemental data.

EPA Response: EPA and NACA are in agreement.

3. *Rejection Factor:* Chemical analyses (measurements of test concentration levels) were not performed on the test solutions.

Agency Guidance: Subd-E: p. 72, 72-2,(c)(4). Stephan: pp. 39 - 42, no. 10.a.b.(1)(2)(3).

Assessment of Guidance: The guidance documents which address this factor appear adequate. (In this particular situation, the registrant had submitted previous data which showed that measured concentrations were substantially lower than nominal concentrations. Based on this information, the Agency concluded that the present (i.e., "rejected") study should have utilized chemical analyses.)

Avoidable: Yes.

Industry Comment: There is an overall lack of clarity in the guidance documents concerning analytical determinations (e.g., how many samples to take, and when to take them).

Since previous tests showed that nominal concentrations were difficult to obtain/maintain, analysis should have been done (assuming the

variations between nominal and actual test concentrations in the previous tests were greater than 30%, and that physical data—i.e., stability and solubility—suggested that maintaining a constant exposure may be a problem). Test solutions should be maintained within 70% to 130% of nominal concentration, unless all efforts to do so fail and these efforts are carefully documented (EPA/NACA (1992), *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance*).

EPA Response: It is evident that in this test situation chemical analyses were necessary. More guidance on the need for measuring test concentrations is provided in the EPA/NACA document.

4. *Rejection Factor:* A control group of animals for the inert/carrier ingredients present in the formulation was lacking.[23]

Agency Guidance: Stephan: p. 31, E.1. . . . cont., 2nd paragraph.

Assessment of Guidance: Normally, this guidance is adequate. However, in this case the scientist found that, without an inert/carrier control (i.e., blank formulation) it was not possible to discern whether the toxic effects were due to the a.i., the inerts/carrier, or to both. This guidance may need strengthening.

Avoidable: Yes.

Industry Comment: While Stephan requires an additive control, it also states that "none of the ingredients of the mixture or formulation is considered an additive." Therefore, one cannot conclude that the testing of blank formulations is needed. And, as an a.i. will be applied to the environment as a formulation, knowledge of the contribution of the formulation additives may not change the overall risk assessment, but may serve only scientific or comparative purposes.

EPA Response: The Agency does not routinely require testing of inert/carrier ingredients (i.e., blank formulation) of pesticides. If this testing is necessary for the overall assessment of the hazards of a specific chemical, it will be stipulated in the Data Call-In Notice or in other correspondence. The Agency agrees with Industry that the guidance provided in the EPA/NACA document may be sufficiently adequate, on

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[23] The test material was 30% a.i. and 70% carrier/inert ingredients.

a chemical-specific basis, to address this issue without the need for further testing.

Resolution: NACA requested clarification as to what specifically was to be tested. The Agency indicated that the terms "inert/carrier control" and "blank formulation" are used interchangeably. If testing of the blank formulation is needed, this will be stipulated in the DCI or other correspondence.

5. *Rejection Factor:* Minimum limit of detectability, or the minimum quantifiable limit, was not defined quantitatively.

Agency Guidance: Subd-E: p. 29, no. 70-4 (b)(1)(2), p. 32, no. 70-4 (c)(6)(ii)(F) and p. 68 no. 72-1 (c)(4). SEP Acute Toxicity Test for Freshwater Invertebrates: p. 9, III.H. Stephan: p. 39 - 40, IV. E.10.b.1 and p. 49, IV.F.9. FIFRA-TG: 72-2, p. C-42, no. 11. ASTM E - 729: p. 394, 15.1.9.

Assessment of Guidance: Sufficient guidance did not exist until EPA/NACA produced *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance (1992).* The guidance provided in this document is adequate to address this issue.

Avoidable: No, due to lack of clarity in issues concerning analytical determinations. These issues are now resolved in the EPA/NACA document. Future submissions containing this factor will be rejected.

Industry Comment: Guidance to avoid this problem is offered in EPA/NACA (1992), *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance.*

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

6. *Rejection Factor:* The variability limit for test concentrations was greater than 1.5.

Agency Guidance: ASTM E -729: p. 392, 11.9.3.4(2).

Assessment of Guidance: Sufficient guidance did not exist until EPA/NACA produced *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance*

(1992). The guidance provided in this document is adequate to address this issue.

Avoidable: No, due to lack of clarity in issues concerning analytical determinations. These issues are now resolved in the EPA/NACA document. Future submissions containing this factor will be rejected.

Industry Comment: Variability of measurements may be encountered in spite of all efforts to limit the variation, especially if test concentrations are in the ppb or ppt range, and if the compound readily adsorbs to surfaces, has very limited solubility, or has limited stability. Coefficients of variation at these concentrations are high, as noted in the literature, and variation is more likely to be related to analysis than to malfunction of a diluter (if used). In these or other circumstances where the analytical method is acceptable, as determined by spiked control samples, and serious efforts to produce less-variable data have not been successful, EPA should be flexible—a rejection does not seem warranted. On a case-by-case basis, EPA should accept studies with variable data, as long as an adequate defense has been made.

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

7. *Rejection Factor:* Not all test concentrations were measured at both zero hours and 48 hours.

Agency Guidance: Subd. E: p. 29, 70-4 (b)(1)(2), p. 32, 70-4 (c)(6)(ii)(F). Stephan: p. 41 - 42, IV.E.10.B.(3). SEP Acute Toxicity Test for Freshwater Invertebrates: p. 7, II.D.6 p. 9, III.H. and p. 11, IV.C.1. ASTM E - 729: p. 392, 11.9.3.3 and 11.9.3.4(1) and (3).

Assessment of Guidance: Sufficient guidance did not exist until EPA/NACA produced *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance (1992).* The guidance provided in this document is adequate to address this issue.

Avoidable: No, due to lack of clarity in issues concerning analytical determinations. These issues are now resolved in the EPA/NACA document. Future submissions containing this factor will be rejected.

Industry Comment: EPA should not require that all test concentrations be analyzed, unless there is reason to suspect that there could be a problem with

maintaining exposure. If the data provided showed that the compound was stable over the 48-hour test period, and the assayed concentrations are within 70% to 130% of nominal, the study should be acceptable. The concern could be further mitigated by the physical characteristics of the compound.

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

Resolution: NACA agreed with the response, and EPA agreed that studies which analyzed low, medium, and high test concentrations would be "grandfathered" so they would not be rejected. Studies initiated after publication of the RRA need to follow the guidance document.

8. *Rejection Factor:* The dilution water contained higher levels of lead, iron, and aluminum than recommended.

Agency Guidance: Stephan: p. 15, IV. C. 2. ASTM E - 729: 384, 8.2.2.1 and p. 385, 8.4.

Assessment of Guidance: Generally, the guidance given in this area is adequate.

Avoidable: Yes.

Industry Comment: If EPA has data to show that the levels of these metals in the dilution water used in the test will have a detrimental effect on *Daphnia*, and if the controls show more than 10% mortality in a static system or 5% in a flow-through system, then the study should be rejected. Industry supports the Stephan concept. Taking criteria of biological responses to the test water into consideration, however, studies do not need to be rejected on this basis.

EPA Response: The Agency agrees with Industry that biological responses of organisms to the dilution water need to be considered. However, if these and other metals are present in the dilution water at concentrations which exceed acceptable criteria, the Agency should be notified prior to test initiation.

Resolution: The Agency responded that the study would not be rejected solely on the basis that metals exceeded the criteria. The biological performance of the organisms would also be considered in evaluating the study.

9. *Rejection Factor:* Percent a.i. of the tested formulation was not given. The test material was not identified by lot or batch numbers. There was no indication if the concentrations used in the study were based on the percent a.i. or total formulated product.

Agency Guidance: Subd. E: p. 30, 70-4 (c)(2)(i) and (ii). Stephan: p. 47, IV.F.2. SEP Acute Toxicity Test for Freshwater Invertebrates: p. 8, III.A. ASTM E-729: p. 394, 15.1.2.

Assessment of Guidance: The guidance documents which address this issue appear adequate.

Avoidable: Yes.

Industry Comment: EPA is reasonable in requiring a description of the test material. In order to avoid unnecessary delays in time and effort for EPA and the registrant, however, Industry recommends that the reviewer contact the registrant prior to finalizing and issuing the DER.

EPA Response: EPA and NACA are in agreement.

10. *Rejection Factor* The photoperiod was not 16-hour light/8-hour dark as recommended, but total darkness.

Agency Guidance: SEP Acute Toxicity Test for Freshwater Invertebrates: p. 5, II.C.4. Stephan: p. 10, IV.B.1. ASTM E-729: p. 381, 7.1.

Assessment of Guidance: A photoperiod of total darkness is not directly addressed in our guidance documents.

Avoidable: Not at this time.

Industry Comment: Unless there is data to show that *Daphnia* are adversely affected by changing the photoperiod over 48 hours, the requirement should only be a recommendation. In cases where a compound undergoes rapid aqueous photolysis, total darkness may be the photoperiod of choice. Furthermore, complete darkness is acceptable for the OECD 202 24-hour EC_{50} *Daphnia* acute mobilization test. In the interest of international harmonization of pesticide testing guidelines, this should not be a reason to reject a study if adequate explanations have been made.

EPA Response: A photoperiod is useful because it simulates natural conditions and *Daphnia* generally do not exist under total darkness.

It would be less stressful to the test organisms to experience standard periods of light and darkness, than to be shocked suddenly with light at observations times. The Agency agrees that photolytically sensitive chemicals should be tested in darkness or under flow-through conditions.

11. *Rejection Factor* No temperature data were provided to indicate if the chambers were monitored at least every 6 hours.

Agency Guidance: Subd. E: p. 29, 70-4 (b)(1), p. 31, 70-4 (c)(6)(ii)(B), p. 71, 72-2 (c)(3)(vii). Stephan: p. 49, IV.F.10. SEP Acute Toxicity Test for Freshwater Invertebrates: p. 7, II.D.5 and p. 9, III.G. FIFRA-TG: 72-2, p. C-44, no. 7. ASTM E-729: p. 392, 11.9.2.2 and p. 393, 13.1 and 13.1.10 to 13.1.12.

Assessment of Guidance: This guidance may need clarification. In general, the guidance states that the temperature range and average temperature should be reported. However, the study can be rejected if temperature is not measured enough or if the deviations are too great. Reporting only the range and mean will not provide sufficient data to determine the study's acceptability.

Avoidable: No.

Industry Comment: EPA needs to clarify what data should be submitted. Temperature data needs to be collected, but submission of tabled data should be acceptable. Studies that have been rejected due to the absence of raw data should be upgraded if the appropriate data is provided to EPA. If the reviewer or designee contacted the registrant for the needed information prior to finalizing and issuing the DER, significant time and rework could be avoided.

EPA Response: Submission of the daily range and daily average temperatures measured during the course of the study is sufficient. Any large deviations in temperature must be explained in the report for the study.

12. *Rejection Factor* Use of dechlorinated water as a portion of the dilution water is not recommended.

Agency Guidance: SEP Acute Toxicity Test for Freshwater Invertebrates: p. 4, II.C.1. ASTM E-729: p. 384, 8.2.3. Stephan: p. 15, IV.C.2.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: The guidance documents are somewhat contradictory. The SEP states (p. 4), "Dechlorinated water should not be used because removal of chlorine is rarely complete and residual chlorine can be quite toxic to aquatic organisms. The dilution water must be able to support the test animals without stress. Organisms should be able to survive, grow, and reproduce in acceptable diluent." If a laboratory establishes that reproduction is acceptable using in-house dechlorinated water, then the water is satisfactory and the test should be acceptable.

A key word in the above rejection factor is "recommended." Recommended guidance helps to establish "generally recognized as acceptable" boundaries. However, it may not be prudent to use them in all cases to reject a study. Taking criteria of biological responses to the test water into consideration, it may not have been appropriate to reject the study.

EPA Response: The Agency strongly recommends against the use of dechlorinated water for the reasons stated in the SEPs for freshwater organism testing. If the use of dechlorinated water cannot be avoided, the biological responses of the control organisms and chemical analyses must meet acceptable criteria.

ACUTE LC_{50} ESTUARINE AND MARINE ORGANISMS (FISH) (Guidelines 72-3A & D)

1. *Rejection Factor:* Unexplained variability between the 0-hour and 96-hour measured concentrations at the two highest test levels (i.e., mean measured concentrations substantially increased).

Agency Guidance: Subd-E: pp. 30 - 31, 70-4 (c)(4)(i) - (viii); p. 75, 72-3 (c)(3)(xi) and (c)(4). FIFRA-TG: pp. C-48, 72-3 no. 13. and C-50, 72-3, no. 9. Stephan: pp. 39 - 42, no. 10. a.b.(1)(2)(3).

Assessment of

Guidance: Generally, the guidance given in this area is adequate, and unexplained variations such as this are handled on a case-by-case basis.

Avoidable: Yes, if the variation was due to deficient chemical analysis techniques or inappropriate test conditions. However, if the variation was due primarily to chemical characteristics, it is unlikely that it could be avoided.

Industry Comment: Not avoidable. Measured concentrations may increase or decrease when testing close to the solubility of the compound in flow-through situations, if the materials is readily adsorbed to walls.

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

2. *Rejection Factor:* The concentration levels selected were less than 100 mg/L and because of solubility problems were not high enough to produce an LC_{50}.

Agency Guidance: LC_{50} Determinations: Subd-E: p. 73, 72-3 (b)(3)(i)(A)(B). FIFRA-TG: p. C-48, 72-3, no. 8. and 9. and p. C-50, 72-3, no. 8. Stephen: pp. 6 - 7 and pp. 45 - 49, nos. 12. and 13. plus Table 6 and section F., no. 8.

Solubility Issues: Stephen: p. 35, no. 5., 1st paragraph.

Assessment of Guidance: The guidance documents which address the LC_{50} or 100 mg/L factor appear adequate. The guidance addressing solubility issues may need improvement.

Avoidable: The problem may not be avoidable, but this eventuality should be accommodated by study evaluation policy. If solubility of the test material is below the level at which any mortality occurs, the registrant is expected to submit a request for an exception to this requirement because of insolubility. The guidance for this type of request is being developed and will be disseminated to the registrants.

Industry Comment: Although the guidance documents cited clearly require the provision of an LC_{50}, none of the documents provides true guidance for solubility problems. Solubility problems frequently occur below 100 mg/L, making it sometimes difficult to test up to this level. In these cases, the significance of the 100 mg/L criterion needs to be reconsidered. For example, assuming that a theoretical application rate of 10 lb/A is

applied directly to 0.5 ft. of water, the resulting concentration would be only 7.4 mg/L, well below the 100 mg/L criterion. Hence, depending on the concentration achieved, the data may be used for a risk assessment. Many registrants were not aware that they would need to request a waiver in these circumstances.

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

3. *Rejection Factor:* Dissolved oxygen levels fell below 60% saturation at 48 hours and below 40% saturation at 96 hours in two test levels.

Agency Guidance: SEP Estuarine Fish Acute Toxicity: P. 7, II.E.5. Stephan: p. 31, IV.E.2. ASTM E - 729: p. 390, 11.4.2 and p. 393, 13.1.9.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: While boundaries help to establish if a study is acceptable, it may not be necessary to use them in all cases to reject a study. Performing a test with solvent at higher temperatures and larger fish can lead to oxygen depletion. If the fish behave normally, results of the study may be acceptable. Also, fish stressed by lower oxygen levels may be a worse case than under normal circumstances. Therefore, flexible use of this rejection factor may be indicated.

EPA Response: The purpose of a toxicity test is to test organisms at optimal conditions to toxic agents. Lower D.O. generally results in altered metabolic rates, which alters chemical uptake. Therefore, it is possible to still get a good dose-response curve, but the slope of the curve could be greatly affected. A study is unacceptable if the dissolved oxygen levels fall below acceptable limits in the control and treatment chambers. Slight aeration is permissible to counter oxygen depletion below the acceptable limits provided chemical concentrations are adequately measured. Refer to ASTM E-729, 11.2.2 for guidance on how to properly aerate test chambers.

Resolution: The Agency agreed that rejection is not necessary if effective corrective action is taken immediately (i.e., the corrective action raises the D.O. to recommended levels by the next measurement time or within 24 hours, whichever is shorter, and there is no adverse effect noted in

control organisms). However, the study will be rejected if corrective measures fail to bring the D.O. to acceptable levels.

4. *Rejection Factor:* Although a static test system was used, the concentrations in the test vessels should have been measured, as other studies with the same test chemical showed that the concentrations varied as much as 30% during a 96-hour exposure period. Therefore, the nominal concentrations did not accurately reflect the true concentrations to which the fish were exposed.

Agency Guidance: SEP Estuarine Fish Acute Toxicity: p. 7 II.E.6 p. 10, III.C.1. ASTM E - 729: p. 392, 11.9.3.3.

Assessment of Guidance: Sufficient guidance did not exist until EPA/NACA produced *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance (1992).* The guidance provided in this document is adequate to address this issue. (In this particular situation, the registrant had submitted previous data showing that there was a substantial amount of variation between measured and nominal concentrations. Based on this information, the Agency concluded that the present (i.e., "rejected") study should have utilized chemical analyses.)

Avoidable: No, due to lack of clarity in issues concerning analytical determinations. These issues are now resolved in the EPA/NACA document. Future submissions containing this factor will be rejected.

Industry Comment: Industry agrees that, due to lack of clarity, rejections in this area were not avoidable. Studies were accepted in the past by EPA with and sometimes without analytical measurements. According to the new EPA/NACA guidance document, a variation of 30% is within acceptable ranges for demonstrating stability, and does not necessitate analytical verification of concentrations.

Variability of measurements may be encountered in spite of all efforts to limit the variation, especially if test concentrations are in the ppb or ppt range, and if the compound readily adsorbs to surfaces, has very limited solubility, or has limited stability. In these or other circumstances where the analytical method is acceptable, as determined by spiked control samples, and serious efforts to produce less-variable data have not been successful, EPA should be flexible and rejection does not seem warranted. On a case-by-case basis, EPA should accept

studies with variable data, as long as an adequate defense has been made.

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

ACUTE LC_{50} ESTUARINE AND MARINE ORGANISMS (MOLLUSKS) (Guidelines 72-3B and E)[24]

1. *Rejection Factor:* There was insufficient new shell growth in the control oysters to adequately determine the effect of the pesticide on shell deposition.

Agency Guidance: Subd-E: p. 26, 70-3 (b)(2) and (3). Stephan: pp. 30 - 31, no. E.1. and p. 39, no. 9., last paragraph.

Assessment of Guidance: Guidance in this area could be improved, since published guidance does not specifically address shell growth of oysters.

Avoidable: Possibly.

Industry Comment: It would not be possible to avoid rejection of the studies, given the current guidance, since there are no accepted EPA guidelines which specify minimum growth criteria for control oysters. Even if EPA adopted the current draft proposal of 2 mm of growth for control oysters, many of the studies would be rejected and would need to be repeated, resulting in delays, the wasting of resources, and the often needless use of additional test organisms.

It is difficult from the description of the rejection factor to determine how "insufficient" the growth of control oysters was in the rejected study. However, industry is aware of a draft document issued on 7 August 1990 by the EPA, in which a minimum growth criterion for control oysters of 2 mm in 96 hours is proposed. Industry will assume that the insufficient growth is based on the draft criteria of less than 2 mm.

Literature cited by EPA in justifying the 2 mm control shell growth (Epifanio et al. 1975; Epifanio and Mootz, 1976; Epifanio, 1979) are

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[24] Note that there are two types of mollusc studies: a 96-hour shell deposition test and a 48-hour embryo larvae study. The former measures shell growth; the latter measures mortality to embryos/larvae.

not supportive of the EPA position that it is normal to obtain up to 1.0 mm of peripheral shell growth per day. Under conditions similar to those used at many contract laboratories, Epifanio (1979) reported that over a 42-day test period employing 15 different diets, oysters grew between 1.22 mm and 8.09 mm. Ignoring the two lowest values as outliers, the remaining 13 diets produced a mean shell growth of 0.14 mm per day, or 0.56 mm in 96 hours. Epifanio and Mootz (1976) reported that the fastest growing oysters in their experiments in recirculating mariculture systems only deposited an average of 0.58 mm per week, or 0.33 mm in 96 hours. No reference to shell deposition rates up to 1.0 mm per day exist in the three Epifanio articles.

Examination of 16 groups of control oyster growth data obtained between August, 1990, and November, 1991, by Toxikon Environmental Sciences, showed a mean oyster growth rate range of 0.7 to 3.3 mm per group in 96-hour tests. Growth of individual oysters ranged from 0 to 5.6 mm. Toxikon also provided data from 7 random groups of control oysters tested in 1983 which showed that although new shell growth averaged 2.1 and 2.7 mm per group, 31% of these oysters produced less than 2 mm of growth over 96 hours. A summary of control oyster growth from Springborn Laboratories, Inc., consisting of 5 years of data and 68 studies (approximately 4,000 individuals tested) showed a mean oyster growth rate range of 0.9 to 3.8 mm in 96-hour studies.

A study was conducted by Springborn Laboratories, Inc., to determine the sensitivity of 2 different groups of oysters to a test material. The study compared the response of a group of *Crassostrea virginica* to a group of the "fast-growing" Wilde strain of *C. virginica*. This study was designed to determine if differences in rate of growth would affect the establishment of an EC_{50}. Using potassium chromate as the test substance, the EC_{50} for typical *C. virginica* was 5.6 mg/L and the EC_{50} for the Wilde strain of *C. virginica* was calculated to be 4.7 mg/L. The typical *C. virginica* deposited an average of 0.8 mm over the 96-hour test, and the Wilde strain deposited an average of 3.1 mm. The conclusion was that the 0.9 mg/L difference in EC_{50} values could easily be attributed to test biological variability in oyster response, and that the sensitivity to toxicants is not related to their inherent growth rate.

Discussions with oyster growers has identified important information concerning the growth endpoint. Growth rate in oysters varies greatly depending on season and strain. Even when a fast-growing strain such as the Wilde is used, the variability within a spawn can be large.

"Even under the best possible conditions healthy oysters may not on average grow the minimum 2 mm required during the 96-hour testing period" (M. Cummins, P. Cummins Oyster Company, personal communication, letter on file). Gary Wickfors of the National Marine Fisheries Service (Milford, CT) stated that 2 mm of growth in 4 days would occur only under the most optimal nutritional and temperature conditions (personal communication, letter on file). He also states that a 10-day acclimation period to a new food source or water temperature is insufficient to maximize optimal growth. Dr. Wickfors suggests that the seasonal variability in growth can be quite large and if the assay is needed in all seasons, then some changes in the protocol should be considered. He suggested that a modification of the acclimation schedule or relaxation of the 2 mm control growth requirement would be appropriate.

Oyster shell growth is variable, depending on size, age, source of the oyster, diet, or season of the year. Large variability in oyster growth is often exhibited in oysters from the same source and maintained under the same test conditions. The validity of a study should not be based on the highest growth rate obtained in reported studies or even on the average growth rate from many studies. Inherent biological variability in growth restricts the utility of a stringent growth performance standard. Since effects on oyster growth are calculated relative to the control growth for every study, the calculated EC_{50} and NOEL endpoints, as well as no-effect limit tests, would not be expected to be different if the shell growth was 0.8 mm (the lower end of the range of historical control data) or 2.0 mm over 96 hours. The range of the means, not the overall mean, is what should be considered in establishing a growth guideline. Reviewers must examine the data closely and not rely on a strict growth criteria that might invalidate reasonable and technically sound testing results.

EPA Response: In a memorandum from Paul Schuda, Deputy Director of EFED, to Daniel Barolo, Director of SRRD, and Lawrence Culleen, Acting Director of RD, dated Oct. 29, 1992, shell growth in mollusk studies was addressed. (See attachment 2). To summarize, the memo stated that EFED scientists, after consulting with experts in the field of estuarine organism testing (Roger Mann, Virginia Institute of Marine Science, and Jerry Zaroogian, USEPA - ERL, Narragnasett, RI), determined that 2 mm of new shell growth in clean water controls in a 96-hour period is easily obtainable and is the minimum growth to allow for valid comparisons.

EFED proposes to present this determination of minimum shell growth to the Science Advisory Panel (SAP) prior to formally amending the appropriate SEP. In the interim, if the sole deficiency in the study is insufficient shell growth (i.e., less than 2 mm in the controls) the study is supplemental (i.e., scientifically sound but does not fulfill guideline requirements). If the mollusks do not appear to be more sensitive than other tested organisms to that pesticide, the study does not need to be repeated. If mollusks do appear to be more sensitive, the potential for the pesticide to affect estuarine organisms will be considered before determining the need to repeat the study. Refer to attachment 2 for specific details. This memo provides the necessary guidance until the SEP is amended to address this issue.

The Epifanio studies were not cited by EPA (EEB) to justify the 2 mm control shell growth.

Resolution:

NACA consulted experts in the field (P. Cummins, Cummins Oyster Company and Gary Wickfors, National Marine Fisheries Service, Milford, CT) and found that 2 mm of growth in 96 hours was not "easily obtainable" and studies are often not submitted and are redone in order to obtain 2 mm of growth in controls, reflecting a much higher failure rate of studies than acknowledged by the rejection rate analysis. This was due to seasonal variation and differences between strains. NACA held that currently there was just only one supplier who could produce material to meet this criterion, but that they could not supply the whole industry and the source was not secure as it was based on a particular location in the Chesapeake prone to disturbance. EEB named Sammy Ray (Texas A&M) and Liam Anderson as experts advising that 2 mm of growth in Oysters was "easily obtainable" and added that at present they were not rejecting studies obtaining $<$ 2 mm but $>$ 1 mm growth in controls.

Listed is additional guidance regarding shell growth in control oysters:

Butler, P. A., and J. I. Lowe. 1978. Flowing sea water toxicity test using oysters (*Crassostrea virginica*). In: Bioassay Procedures for the Ocean Disposal Permit Program, EPA-600/9-78-010. Environmental Research Laboratory, U. S. Environmental Protection Agency, Gulf Breeze, FL. pp. 25-27.

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1985. Toxicity test procedures using Mollusks (Tentative). In: Standard Methods for

the Examination of Water and Wastewater. APHA, Washington, DC. pp. 796-797.

EEB plans to pursue a refinement of the minimum growth criteria. However, this will entail looking at historical data and round-robin testing before this criteria can be refined.

EPA and NACA were agreed that decisions should be made on a case-by-case basis as stated in the letter from Paul Schuda to Daniel Barolo and that due to apparent contradiction between experts, a resolution meeting of experts was proposed. Prior to any meeting, however, EEB would like to review the data from Toxikon Environmental Sciences and Springborn Laboratories, Inc, cited above, including the Springborn study comparing the sensitivity of two strains of *Crassostrea virginica*. The data should be presented in a tabulated, unanalyzed form to allow EEB to verify the conclusions stated above.

2. *Rejection Factor:* There were insufficient concentration levels to result in percent mortality to mollusc embryos/larvae of greater than 65%, thus resulting in a statistically less-reliable LC_{50}.

Agency Guidance: Stephan: pp. 43, 45, and 46, nos. 11. and 12.

Assessment of Guidance: Guidance in this area is generally adequate. However, as issues concerning concentration/dosage levels are recurring, the Agency handles these on a case-by-case basis.

Avoidable: Possibly. The registrant can choose to avoid submitting studies with results such as this, but it is possible that even with substantial preparation and care in study design, results such as this cannot be avoided.

Industry Comment: The criteria for needing greater than a 65% effect is not specified in the Subd-E, and as such should only be considered a recommendation, not a required criterion. Each study should be evaluated on the merit of the data produced, whether or not the dose-response is within some specified range. Solubility problems with the test compound may preclude testing at a high enough concentration to get the recommended response range. In addition, due to the typically flat dose-response curve in oyster studies, it is difficult to obtain both an EC_{50} and a NOEL. If there is a consistent slope for the effects data, then a valid

EC_{50} can be calculated even when a concentration does not produce more than 65% effect.

It is difficult to assess the above rejection factor, since it refers to 65% mortality and an LC_{50} level. The oyster embryo/larval study is an effect test, not a mortality test, and as such there is no requirement to obtain "greater than 65% mortality" or determine an "LC_{50} level." The SEP (1985) states that "the measured response in this test should be the percentage of larvae dying or failing to develop complete shells." Since the only required endpoints outlined in Subd-E are an EC_{50} and a NOEL, the above statement must refer to the identification of one endpoint that includes both dead and partially shelled organisms.

Another possible assumption regarding the above rejection criteria is that the reference to LC_{50} is a typographical error, and the issue is one of not obtaining an EC_{50}, due to solubility problems with the test compound. Current EPA policy allows chemicals that are poorly soluble to be tested up to the maximum water solubility obtainable for the given test conditions, provided that all measures to maximize water solubility are employed. While Industry agrees that the solubility of a compound should be maximized in order to determine an effect concentration, there are many instances where an LC_{50} or EC_{50} is not obtainable due to lack of solubility. Studies are often rejected due to the inability to obtain this 50% effect level, yet it is impossible to perform the study so that an effect level will be determined. EPA states in the "Avoidability" section that indeed it may not be possible to avoid this rejection factor.

If the first assumption of mortality versus effect is what caused the reviewed study to be rejected, then Industry recommends that clear guidance as to the actual assessment endpoint is needed. Since larvae that fail to develop a complete shell in 48 hours are considered ecologically non-viable (ASTM 1987), the only appropriate endpoint for this study combines both organisms that are dead and those that do not develop a full shell by the end of the test.

If the second assumption of solubility is what resulted in study rejection, Industry recommends that as long as the solubility was maximized in the test, the study should be accepted and the results reported with a complete description of measures taken to maximize solubility.

EPA Response: The Agency agrees with Industry that development is the valid endpoint in the mollusk embryo/larval study as calculated by an EC_{50} value.

This study is not a mortality study, although dead organisms are counted with those that do not fully develop a full shell. However, whether or not mortality or poor development were the endpoint, the Agency still requires that there must be at least one test concentration producing an effect of at least 65% to be statistically valid, provided there are no problems with chemical solubility.

Resolution: EPA could not accept the deletion of the study, though they classed it as "techniquey" but were unwilling to relax the criteria. As the NACA proposals were unacceptable to the EPA, this issue remains unresolved and so it is proposed that this test is re-assessed by a workgroup, possibly in conjunction with the shell deposition issue, following a review of historical control survival data.

3. *Rejection Factor:* Raw data on shell deposition were not provided.

Agency Guidance: Subd-E: p. 29, 70-4 (b)(1)(2). FIFRA-TG: p. C-57, 72-3 (c), no. 8.; p. C-58, 72-3 (c), no. 12.; p. C-59, 72-3 (c), no. 3.; p. C-60, 72-3 (c) nos. 8. and 9. Stephan: pp. 47 - 49, F.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry agrees that the raw data, defined as "enough data for the Agency to verify calculated statistical values . . ." (Subd-E: p. 29, § 70-4), should be included with the report in order for the reviewer to be able to verify the stated EC_{50} and NOEL based on shell deposition. Studies that have been rejected due to the absence of raw data should be upgraded, however, if the appropriate data is provided to EPA. If the reviewer or designee contacted the registrant for the needed information prior to finalizing and issuing the DER, significant time and rework could be avoided.

EPA Response: Growth measurements of individual oysters for each test concentration need to be provided so that the reported statistical values can be verified. Mean growth per test replicate or per test concentration is not sufficient.

4. *Rejection Factor:* The test was aerated without chemical analyses of test solutions.

Agency Guidance: FIFRA-TG: p. C-57, 72-3 (c), no. 7. and Stephan: p. 16 and p. 31, no. 2. and pp. 39 - 42, no. 10. a.b.(1)(2)(3).

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes. Either the test chambers should not be aerated, or if aeration is necessary, chemical analysis of the test solution periodically throughout the test should be performed to verify exposure.

Industry Comment: Industry disagrees that the referenced guidance is adequate. The FIFRA-TG (C-57, No. 7) only requires chemical analysis for embryo/larval studies when aeration is required. However, there is a requirement that all flow-through assays (FIFRA-TG C-58, No. 12) have concentration analysis. Stephan says that there should never be aeration, but that the dissolved oxygen should be between 60% and 100% for flow-through studies. No reference is made to chemical analysis if aeration is necessary in the Stephan document, although there is a requirement in this document to measure test concentrations during all studies.

Industry agrees that chemical analysis should be performed in cases where aeration of test solutions is necessary. Chemical analysis requirements have been proposed in EPA/NACA (1992), *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance*. Current guidelines recommend that test solutions be analyzed during flow-through studies; this would include the situation where aeration is needed. There does not appear to be a change needed in any guidance, and this should not be a problem in the future.

EPA Response: EPA and NACA are in agreement.

5. *Rejection Factor:* There were deviations from recommended test solution characteristics. For example, the dissolved oxygen concentration was below recommended values of 60% to 100% saturation.

Agency Guidance: Stephan: p. 31, no. 2.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes. Steps can be taken to maintain acceptable dissolved oxygen levels.

Industry Comment: A key word in the above rejection factor is "recommended." Recommended guidance helps to establish "generally recognized as acceptable" boundaries. However, it may not be prudent to use them in all cases to reject a study. Specific compound characteristics, solvents, and temperature can affect the dissolved oxygen concentration during a study. If the compound is volatile, then aeration may not be an option, and a study may have to be run at less than optimal dissolved oxygen concentrations.

Whether or not the rejection was avoidable depends on the extent of reduced dissolved oxygen, both in concentration and occurrence. It is unclear from the description in the rejection factor whether the dissolved oxygen levels were low in all concentrations, or just at one or a few test levels. Also, there is no clarification of what "below 60%" means. For example, there should be no reason to reject a study based on lower-than-recommended dissolved oxygen in the lowest dose of a test where the NOEL is the dose above the lowest.

If there is an adequate dose-response curve obtained at the end of the study, then it can be assumed that the deviation from recommended dissolved oxygen concentrations did not affect the outcome of the test. The study must be evaluated on the data produced, and on what effect the reduced dissolved oxygen might have had, not just on a recommended guideline.

EPA Response: The purpose of a toxicity test is to test organisms at optimal conditions to toxic agents. Lower D.O. generally results in altered metabolic rates, which alters chemical uptake. Therefore it is possible to still get a good dose-response curve, but the slope of the curve could be greatly affected. A study is unacceptable if the dissolved oxygen levels fall below acceptable limits in the control and treatment chambers. Slight aeration is permissible to counter oxygen depletion below the acceptable limits, provided chemical concentrations are adequately measured. Refer to ASTM E-729, 11.2.2 for guidance on how to properly aerate test chambers.

Resolution: The Agency agreed that no rejection is necessary if corrective measures were taken immediately. If corrective action increased the dissolved oxygen to recommended levels by the next measurement time period or within 24 hours, whichever is shorter, and no adverse effects on control organisms were observed, then the study should not be rejected solely on low dissolved oxygen. However, the study will be rejected if corrective measures fail to bring the D.O. to acceptable levels.

6. *Rejection Factor:* Dissolved oxygen levels were below 60% saturation in the last 48 hours of exposure in a flow-through test system.

Agency Guidance: SEP Estuarine Mollusc Acute Shell Deposition Study: p. 6, II.E.5. Stephan: 31, IV.E.2. ASTM E - 729: p. 390, 11.4.3 and p. 393, 13.1.9.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: This factor is not addressed in Subd-E. If the above listed references are required, rather than recommended, then that fact should be made clear.

Whether or not the rejection was avoidable depends on the extent of reduced dissolved oxygen, both in concentration and occurrence. It is unclear from the description in the rejection factor whether the dissolved oxygen levels were low in all concentrations, or just at one or a few test levels. Also, there is no clarification of what "below 60%" means.

If there is an adequate dose-response curve obtained at the end of the study, then it can be assumed that the deviation from recommended dissolved oxygen concentrations did not affect the outcome of the test. The study must be evaluated on the data produced, and on what effect the reduced dissolved oxygen might have had, not just on a recommended guideline.

EPA Response: The purpose of a toxicity test is to test organisms at optimal conditions to toxic agents. Lower D.O. generally results in altered metabolic rates, which alters chemical uptake. Therefore it is possible to still get a good dose-response curve, but the slope of the curve could be greatly affected.A study is unacceptable if the dissolved oxygen levels fall below acceptable limits in the control and treatment chambers. Slight aeration is permissible to counter oxygen depletion below the acceptable limits provided chemical concentrations are adequately measured. Refer to ASTM E-729, 11.2.2 for guidance on how to properly aerate test chambers.

Resolution: The Agency agreed that no rejection is necessary if corrective measures were taken immediately. If corrective action increased the dissolved oxygen to recommended levels by the next measurement time period or

within 24 hours, whichever is shorter, and no adverse effects on control organisms were observed, then the study should not be rejected solely on low dissolved oxygen. However, the study will be rejected if corrective measures fail to bring the D.O. to acceptable levels.

Other Industry Comments on Guidelines 72-3B and E:

Embryo/Larval percent control mortality by end of test:

According to the SEP for mollusc embryo/larval tests, the organisms should be placed into the exposure vessels within 1 hour of fertilization. This requirement has led to problems with test interpretation and acceptability, because only one cell division occurs during this one-hour period, making it very difficult to verify successful fertilization. Any non-viable embryos counted in the initial inoculum are therefore apparent mortalities at the end of the exposure. This increases the apparent mortality rate in the control vessels. Alternatively, the ASTM guidelines for this test require a count of viable embryos 3 hours after fertilization, after approximately 8 cell divisions. The number of organisms added to each test vessel can then be adjusted to reflect a more reliable count of viable embryos/ml. The SEP should be revised to account for the introduction of non-viable embryos into the test, which can result in biased survival data.

EPA Response: Industry makes a valid point. The Agency agrees that the additional 2-hour waiting period should not affect test results and would improve reliability of viable embryo counts.

Requirement of 70% control survival in the embryo/larval study:

The 70% survival/development performance criteria for control embryos has resulted in the failure of many embryo/larval studies, and the refusal by at least one contract laboratory to perform the study. Conversations with aquaculturists indicate that successful development of mollusc larvae 48 hours post-fertilization ranges from 10-90%, based on the inherent variability of the performance of the adult population.

One contract laboratory recommends reducing the requirement for survival/development in the control to 50% fully-shelled and an additional 10% underdeveloped. In order to have the minimum number of larvae to assess at the end of the test, the study could be started with 40,000 embryos/L. With 50% developing normally, the final number of embryos assessed would adhere to the minimum of 20,000/L indicated in the SEP. The whole issue of oyster growth and development studies should be reexamined. Amending the guidance may not prove beneficial if the study design is flawed.

EPA Response: The 70% survival requirement should be retained in order to assure that quality organisms and suitable culture conditions are maintained.

The conditions and specific methods used routinely by all aquaculturists surveyed should be analyzed closely. Consultations should be initiated with governmental (i.e., EPA-ERL at Gulf Breeze, Florida, or the Virginia Institute of Marine Science) and private laboratories to establish historical survival success. The Agency should do an internal analysis to determine what percentage of submitted studies have been identified as having survival less than 70 percent. From this data a statistically valid correction factor for control mortality will be developed, provided that control mortality does not exceed some predetermined criteria.

Resolution: Survival of <70% in the controls can be acceptable if the overall quality of the study (coefficients of variation (CV), etc.) are high; <70% survival alone is not a fatal flaw. EPA defines "high test quality" as including CV's under 20; CV's greater than 40 are unacceptable in most cases. A further evaluation of historical data and literature plus research at an EPA-ERL is required to determine the lower limit for acceptable control survival. EPA will address this issue on a case-by-case basis.

ACUTE LC_{50} ESTUARINE AND MARINE ORGANISMS (SHRIMP) (Guidelines 72-3C & F)

1. *Rejection Factor:* Test substance purity was not identified.

Agency Guidance: Subd-E: p. 30, 70-4 (c)(2)(i). Stephan: p. 47, no. F. 2.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: If the registrant cannot provide the test material characterization, the study should be rejected. In the case that the omission of purity information was an oversight, significant time and rework could be avoided if the reviewer contacts the registrant for the needed information prior to finalizing and issuing the DER.

EPA Response: Both the Agency and Industry agree that this factor is avoidable. This information needs to be included with the study upon its first submission to the Agency.

2. *Rejection Factor:* Chemical analyses (measurements of test concentration levels) were not performed on the test solutions.

Agency Guidance: Subd-E: p. 75, 72-3 (c)(4). Stephan: pp. 39 - 42, no. 10.a.b.(1)(2)(3).

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: The listed guidance is not adequate. Subd-E states that *if* chemical analysis is conducted, the methods must be described. Stephan does state that if the toxicant is known, the concentration must be measured; however, Stephan is only recommended—not required—guidance. If there is additional information on the stability of the test compound, there may be no need to measure test concentrations.

EPA Response: More guidance on the need for measuring test concentrations is provided in the EPA/NACA document.

3. *Rejection Factor:* The type of solvent used in the study, and the amount used in the solvent control and each treatment level, were not provided.

Agency Guidance: Subd-E: p. 28, 70-3 (c)(6)(i) - (iv) and p. 30, 70-4 (c)(4)(v). FIFRA-TG: p. C-51, 72-3 (b), no. 2.; p. C-52, 72-3 (b), no. 7.; and C-54, 72-3 (b), no. 2. Stephan: p. 31, 2nd paragraph and p. 35, no. 5., 1st paragraph.

Assessment of Guidance: The guidance documents which address this factor appear appropriate.

Avoidable: Yes.

Industry Comment: Industry agrees that the solvent used in a study should be identified, and that the concentration of solvent in the solvent control, in relation to the treatments, should be described.

EPA Response: EPA and NACA are in agreement.

4. *Rejection Factor:* Solubility needed to achieve LC_{50} was not obtained (and was questionable, since previously submitted data appeared to indicate that high levels of solubility could be achieved).

Agency Guidance: Stephan: p. 35, no. 5, 1st paragraph.

Assessment of Guidance: Issues of data discrepancies, relative to previously submitted data, are handled by the Agency on a case-by-case basis. However, guidance addressing solubility issues may need improvement.

Avoidable: Probably, but it may not have been avoidable even if higher solubility was achieved in other tests, since solubility can vary significantly under different test conditions. When the maximum solubility level is lower than the level which will allow calculation of an LC_{50}, the registrant must submit an explanation justifying an exception to that requirement. Guidance for this is being developed.

Industry Comment: Stephan does not address solubility issues and thus is inadequate guidance for this rejection factor. EPA should consider the guidance outlined in EPA/NACA (1992), *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance*. Also, it is unclear from the description of the rejection factor to what "high levels of solubility" refer. If that statement refers to the solubility reported under "perfect" conditions, then it is inappropriate to expect to achieve that level of solubility under actual test conditions. In addition, large differences in solubility have been noted between freshwater and saltwater systems. The solubility that should be used as a measure is that which is achievable given the test conditions.

EPA Response: The Agency believes that the guidance provided in the EPA/NACA document is adequate to address this issue.

Resolution: It is important to distinguish between "perfect" versus "real" solubility. The 1992 EPA/NACA (1992) document "Conducting Acceptable Aquatic Laboratory Studies : Proposed Guidance" outlines measures to take in order to maximize solubility. Solubility is defined as the amount of chemical retained in the supernatant of a conventionally centrifuged sample of test media. A measure is only considered to have increased solubility if the increase is two times or greater.

Other Industry Comments on Guideline 72-3(c):

Requirement that the age of mysids be less than 24 hours at the beginning of a study:

Neither Subd-E nor the SEP indicates an age requirement for the mysid shrimp acute study, though FIFRA-TG lists an age requirement of < 24 hours. The age requirement is based on the recommendation that exposure to the compound encompass an entire molt cycle in order to expose the organism during the most vulnerable time period. Industry agrees that a

uniform age is necessary to prevent cannibalism and to decrease the variability in response. However, it should be acceptable to begin a mysid study at 96 hours, in order to allow laboratories time to receive newly hatched mysids and acclimate them to the test conditions.

The concern surrounding the age requirement for mysids in acute toxicity tests appears to center around a possible increase in sensitivity to toxicants immediately post-molt. S. Lussier (EPA, Narragansett) has reported that mysids undergo 9 molts at 25°C in the first 12 days of life with a mean intermolt period of 12.3 hours (*Mysidopsis* sp.: Life History and Culture. Proceedings from a Workshop held in Gulf Breeze, Florida, 15-16 Oct 1986). This information suggests that a study performed from 96 hours to 192 hours (8 days) would include a full molt cycle.

Goodman et al. (1988) tested juvenile mysids from 3 age ranges (< 1, 5, and 10 days) to determine their sensitivity to 3 toxicants at the various ages. In these tests, the 96-hour LC_{50} values were within a factor of two for the 3 compounds tested in the 3 age groups. The authors concluded that age was not a significant factor in the acute toxicity of the test compound to *Mysidopsis bahia*.

In a comparative study by Nor-Am Chemical company, a compound was tested with < 24-hours-old and 4-day-old mysids. The LC_{50} and 95% confidence interval for the < 24-hours-old mysids, calculated by the moving average method, was 33.8 mg/L (26.4-46.4 mg/L). The LC_{50} and 95% confidence interval for the 4-day old mysids, again calculated by the moving average method, was 30.6 mg/L (23.9-40.9 mg/L). Again, age was not a factor in sensitivity to the toxicant.

Studies should not be rejected only on the basis of mysid shrimp being older than 24 hours, as long as the age of the mysids in the test is uniform within a specified range. As long as the mysids are juveniles during the study (up to approximately 9 days of age), data generated at the EPA Laboratory in Narragansett suggest that the mysids will be exposed to the test compound during a full molt cycle, and thus be exposed at potentially the most sensitive stage of their development.

EPA Response: Industry presents a valid point and the Agency suggests that all available data, including that of S. Lussier (EPA, Narragansett, and Nor-Am Chemical Company) cited above be analyzed to establish a reasonable age range (> 24 hours) adaptable to the use of uncultured test organisms.

Resolution: The EPA reconfirmed the <24 hour-old requirement until further data can be assessed to show that 24-, 48- or 96-hour-old shrimps are equally as susceptible. This data evaluation should be conducted as a peer review and must contain values for individual organisms. The data should be presented in a tabulated, unanalyzed form to allow EEB to verify the conclusions. This requirement is unchanged unless it is overturned by a subsequent review of data and further NACA/EPA discussions, according to joint priorities of EPA and NACA.

FISH EARLY LIFE STAGE (Guideline 72-4)

1. *Rejection Factor:* Significant mortality occurred at all test concentrations, making it impossible to determine an MATC.

Agency Guidance: ASTM[25]: p. 862, no. 9.3; p. 864, nos. 11.1.1.1 and 11.1.1.2.
FIFRA-TG: p. C-63, 72-4, no. 15. and C-65, 72-4, no. 10.

Assessment of Guidance: Generally, guidance on this factor is adequate because of the experience of the laboratories doing such studies. Issues such as this are handled on a case-by-case basis, but improvement in the guidance may be needed.

Avoidable: Yes.

Industry Comment: The FIFRA-TG document is inadequate. It does not address the issue of "significant" mortality at all tested concentrations. A footnote in the document indicates that a NOEL is "supplemental and may not be required for every study."

The ASTM guidance appears to be adequate in that it suggests that regression analysis may be used as an alternative to the hypothesis testing approach. It also discusses the issue of biological versus statistical significance. However, this document does not address what level of effect is of ecological significance.

Fish Early Life Stage studies are designed to evaluate the effects of a test substance on the survival and growth of embryos, larvae, and juvenile fish. Industry agrees that significant mortality at all test concentrations may prevent the determination of an MATC. A distinction must be made between statistical significance within the test and the normal or historical control variability (e.g., 20%). Based on the study design and the power of the statistical tests employed, a small difference in survival may be statistically significant but still within the acceptable range of mortality typical for control organisms. It is important to consider when and under what circumstances the mortality occurred. A careful review of the magnitude of the effect should be undertaken, considering the dose response and the other endpoints

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[25] Various versions are available. In this case, Vol. 11.04, 1991, which was readily available, was used and will be used for this study and the Aquatic Invertebrate Life-Cycle test.

evaluated in the study. The results need to be evaluated in relation to the risk assessment.

Studies such as this should be handled on a case-by-case basis. EPA should be flexible in their study reviews. Industry recommends that the magnitude of the effect on the fish in the lowest concentration group be reviewed carefully in respect to (1) when the mortality occurred, (2) statistical significance, (3) the overall concentration-effect relationship, and (4) relevance of the NOEL to the estimated environmental exposure level. Definition of a NOEL from regression analysis (e.g., LC_{20}) should be possible from studies with significant mortality in the lowest test concentration.

EPA Response: NOEL's are not required for acute toxicity studies, but are required in chronic toxicity studies for regulatory purposes. In order for a NOEL to be valid, there must be at least one concentration level with effects not significantly greater than the appropriate control, and a second concentration delineating an acceptable LOEL. The FIFRA-TG guidance states that an MATC value must be reported (C-62) and a NOEL determined (C-65). It was an inadvertent error that a footnote indicated that the NOEL is a supplemental data requirement.

Resolution: Studies will not automatically be rejected if a NOEL is not experimentally achieved. Registrants can submit a calculated NOEL for consideration. In this discussion, EPA also emphasized the general distinction between studies "not fully acceptable" versus "needing to be repeated." Studies that are not core (fully acceptable) may still provide enough information for a risk assessment and therefore need not be repeated. The need for a new study will be considered on a case-by-case basis.

2. *Rejection Factor:* Raw data were not submitted to allow the Agency to verify the MATC and NOEL.

Agency Guidance: Subd-E: p. 29, no. 70-4 (b)(1)(2) and p. 78, no. 72-4 (c)(1) - (11). FIFRA-TG: pp. C-61 - C-65, 72-4 (all sections). ASTM: pp. 867 - 868, no. 15.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry agrees that raw data, defined as "enough data for the Agency to verify calculated statistical values," should be included with the report in order for the reviewer to be able to verify the stated MATC and NOEL values. Studies that have been rejected due to the absence of raw data should be upgraded if the appropriate data are provided to EPA.

EPA Response: For the fish early life stage study, data in the following manner need to be submitted in the final report: number of embryos hatched, time to hatch, number of dead embryos and dead larvae are reported daily for each incubation cup; time to swim-up, number of dead juveniles are reported daily for each test chamber; and growth measurements are reported for each live individual fish for each test concentration and control at the end of the study. This will provide sufficient data for the reviewer to verify the reported MATC, NOEL, and LOEL values for each biological endpoint.

Resolution: Agreement was reached that data needed to calculate statistics must be submitted. EPA prefers to call this necessary data "tabulated and unanalyzed data."

3. *Rejection Factor:* The survival rate in the control group was substantially lower than recommended (70%).

Agency Guidance: Subd-E: p. 26, 70-3 (b)(2) and (3). (For background information: Stephan: pp. 30 - 31, no. E.1. and p. 39, no. 9., last paragraph). ASTM: p. 867, no. 13.1.8.

Assessment of Guidance: Generally, guidance on this factor is adequate because of the experience of the laboratories doing such studies. However, the guidance may need improvement.

Avoidable: Yes.

Industry Comment: Industry finds the guidance inadequate. Subd-E: p. 26, §70.3(b)(2) and (3) does not address control survival. The Stephen reference does not address fish early life stage tests. The ASTM document has variable criteria depending on species, and it differs from the SEP. The Agency should provide clear guidance as to how to calculate control survival.

Industry needs to know how many studies were rejected for poor control survival and what the survival was in each case. Industry

agrees that control survival should not be "substantially lower than recommended (70%)." The term "substantially lower" is open to interpretation. The calculation of control survival is handled differently among and within various documents. For example, ASTM recommends that control survival for fathead minnows be $\geq$ 70%, and be calculated for the period of 48 hours to 32 days. In the same document, survival must be $\geq$ 70% for rainbow trout, as calculated from the time of thinning to test termination. The SEP states that control survival at the end of the study must be 80% overall, and not less than 70% in any replicate.

If control mortality occurs later in a study (e.g., due to the malfunction of test equipment, outbreak of disease, etc.) resulting in overall control survival that is < 70%, the study should be acceptable. Repeating the study will not provide additional useful information, is wasteful of resources, and will impede the reregistration process.

Control survival below 60% should be considered to be "substantially lower" than recommended. Where survival is questionable, the issue should be handled on a case-by-case basis, with consideration of the time point within the study where survival dropped below the recommended criterion.

EPA Response: The Agency acknowledges differences among criteria as prescribed by the Agency SEP and various protocols. The Agency should consolidate appropriate criteria from the best guidance documents available into one SEP. Mortality within any replicate exceeding 30% would justify rejection of a study. The Agency agrees with Industry that the term "substantially lower" is vague. Stating that mortality was less than the criteria of 70% is adequate. The Agency will reconsider its study rejection levels to align them with the more conservative vis-a-vis more protective criteria prescribed by ASTM and Stephen (pp. 30-31, No. E.1 and p. 39, No. 9, last paragraph).

Resolution: EPA and NACA agreed that a flexible approach be used in the analysis of control survival. The difference between EPA recommendations (80% overall, 70% per replicate) and ASTM (70% overall) was noted.

4. *Rejection Factor:* Measured test concentrations showed highly erratic results throughout the test, raising questions about whether test equipment (i.e., diluter system) was functioning properly.

Agency Guidance: Subd-E: pp. 30 - 31, 70-4 (c)(4)(i) - (viii); p. 78, 72-4 (b)(4). FIFRA-TG: p. C-62, 72-4, no. 12.; p. C-63, 72-4, no. 15.; and C-65, 72-4, no. 10. ASTM: p. 867, nos. 13.1.14 and 13.1.15.

Assessment of Guidance: Generally, guidance on this factor is adequate because of the experience of the laboratories doing such studies. Further, unexplained variations such as these are handled on a case-by-case.

Avoidable: Yes.

Industry Comment: Industry finds the guidance inadequate. Subd-E: p. 30-31; §70.4(c)(4)(i) to (viii); and p. 78, §72-4(b)(4) does not address erratic analytical results. The pages and sections of the FIFRA-TG document do not give clear guidance on this issue. ASTM offers the clearest guidance, but it differs from EPA/NACA (1992) *Conducting Acceptable Aquatic Laboratory Studies: Proposed Guidance.*

Industry agrees that highly erratic results in measured test concentrations may indicate problems with the dosing apparatus. However, if there is no apparent problem with the dosing apparatus, and the test substance is highly unstable in aqueous solution, is poorly soluble in water, adsorbs to test apparatus, or the tested concentrations are near the detection limit of the analytical method, then the erratic results are probably unavoidable.

EPA Response: The Agency agrees that erratic results need to be handled on a case-by-case basis. The guidance provided in the EPA/NACA document should be adequate to address this issue provided the erratic results are caused by inherent properties of the test chemical. However, if inconsistent analytical results are due to malfunction of the test (diluter) system, the study will likely be rejected.

Resolution: It was agreed that long-term studies should not be automatically rejected for equipment failures. The registrant must be able to demonstrate the duration of the exposure fluctuation and that appropriate corrective actions were taken (e.g., repairs and sampling within 24 hours).

5. *Rejection Factor:* The raw data for the acclimation period and embryo hatchability, survival, and growth of the larvae were not provided. Therefore, the reported MATC and NOEL values could not be verified.

Agency Guidance: Subd-E: pp. 29 - 32, no. 70-4 (b) (1) and (2) and (c) (3), (5) - (7) and p. 78, no. 72-4 (c)(1) - (11). FIFRA-TG: 72-4, pp. C-61 - C-65 (all sections). ASTM E -1241: pp. 865 - 866, no. 11.8 and pp. 867 - 868, no. 15. SEP (Fish ELS): p. 6, II.A.7 and p. 9, II.D.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Although industry agrees that the guidance appears adequate, a clearer distinction should be made between the definition of raw data as used herein and that used in the Good Laboratory Practice Standards.

Raw data on acclimation is not pertinent to calculation of an MATC or NOEL. Industry agrees that raw data, defined as "enough data for the Agency to verify calculated statistical values," should be included with the report in order for the reviewer to be able to verify the stated MATC and NOEL values. Studies that have been rejected due to the absence of raw data should be upgraded if the appropriate data is provided to the Agency.

EPA Response: The Agency agrees that data for the acclimation period are not necessary for calculation of the MATC value. However, the data on the conditions during the acclimation period are needed for verification of maintenance of the test organisms. This data can take the form of ranges of data points confirming the condition and maintenance of the organisms. For the fish early life stage study, data in the following manner need to be submitted in the final report: number of embryos hatched, time to hatch, number of dead embryos and dead larvae are reported daily for each incubation cup; time to swim-up, number of dead juveniles are reported daily for each test chamber; and growth measurements are reported for each live individual fish for each test concentration and control. This will provide sufficient data for the reviewer to verify the reported MATC, NOEL, and LOEL values for each biological endpoint.

Other Industry Comments on Guideline 72-4

Individual Dry Weights Were Not Measured:

Dry weight is not a requirement in the guidance documents cited; measurement of wet weight is acceptable. In cases where the fish appear edematous, dry weight is more

appropriate. In the study design for the fish ELS test, the experimental unit is the replicate chamber. Industry believes that the use of individual fish as independent experimental units in the evaluation of growth parameters constitutes pseudo-replication and is not appropriate. Assuming the fish were not edematous, measurement of wet weight rather than dry weight should not be grounds for study rejection. Use of mean growth values of all fish within a replicate in the statistical analysis is appropriate if the replicate test chambers are the experimental units. Studies should not be rejected when the data is analyzed in this manner.

EPA Response: The Agency agrees that wet weight measurements are acceptable. In tests where the fish are edematous, dry weight measurements are valid. Individual measurements are important to determine "within" variances and therefore, are necessary. EEB would like to consult with statisticians on the use of individual versus mean replicate values for growth.

Only Two True Replicates Were Used for Embryo Exposure:

It is clear that Agency guidance documents recommend the use of four replicates in the fish ELS test. However, ASTM allows the use of two replicates. It is Industry's understanding that there has been some inconsistency on the part of EPA in respect to rejection of studies using two replicates. Some studies using two replicates have been accepted by EPA. The use of two replicates should not be the sole cause for rejection of a study.

EPA Response: The Agency requires four replicates for embryo exposure. However if Industry can provide scientifically valid reasons for using two rather than four replicates, the Agency will, on a case-by-case basis, judge whether a study should or should not be rejected.

Statistical Evaluation of Growth Data (Length and Weight) Used Mean Values Rather Than Individual Measurements:

In the study design for the fish ELS test, the experimental unit is the replicate chamber. Industry believes that the use of individual fish in the evaluation of growth parameters constitutes pseudo-replication and is not appropriate. Standardization of statistical procedures is required. Use of mean growth values of all fish within a replicate in the statistical analysis is appropriate if the replicate test chambers are the experimental units. Studies should not be rejected when the data is analyzed in this manner.

EPA Response: The Agency will accept mean growth data (Replicate = Experimental Unit) for statistical analysis with the following conditions: 1) individual length and weight data be provided to the Agency electronically and 2) time-weighted chemical averages be used for the analyses.

Resolution: Individual measurements are important to determine "within" variances and, therefore, are necessary. EEB would like to consult with statisticians on the use of individual versus mean replicate values for growth. Industry can do their calculations using mean data, but the Agency will continue to validate the results using the individual measurements until or unless the statistical consultation indicates otherwise.

Industry Comment: NACA requests the opportunity to be party to such consultation. NACA member companies have evaluated the issue statistically and have statistical expertise that would be useful to the discussion. The evaluation should result in a written document outlining the rationale and justification for the statistical procedures to be used.

Dissolved Oxygen Levels Were Below Recommended Levels:

Industry agrees that dissolved oxygen should not be substantially lower than recommended; however, the term "substantially lower" is open to interpretation, and recommended dissolved oxygen levels differ among the various guidance documents. ASTM recommends maintaining dissolved oxygen at $\geq$ 60% of saturation, based on a time-weighted average. ASTM also permits aeration of the test chambers. The SEP infers that dissolved oxygen be maintained at $\geq$ 75% of saturation and does not allow aeration of test chambers.

Studies such as this should be handled on a case-by-case basis. EPA should be flexible in their study reviews. Rejection should only occur if the dissolved oxygen concentrations are below a time weighted average of 60%. Industry recommends that low dissolved oxygen occurrences be reviewed carefully in respect to when and why they occurred, and the overall effect on the outcome of the study.

EPA Response: The purpose of a toxicity test is to test organisms at optimal conditions to toxic agents. A study is unacceptable if the dissolved oxygen levels fall below acceptable limits in the control and treatment chambers. Slight aeration is permissible to counter oxygen depletion below the acceptable limits, provided chemical concentrations are adequately measured. Refer to ASTM E-1241, 11.2 for guidance on how to properly aerate test chambers.

Resolution: The Agency agreed that rejection is not necessary if effective corrective action is taken immediately (i.e., the corrective action raises the D.O. to recommended levels by the next measurement time or within 24 hours, whichever is shorter, and there is no adverse effect noted in control organisms). However, the study will be rejected if corrective measures fail to bring the D.O. to acceptable levels.

Solvent Appeared to Affect Growth:

Use of a solvent is routine in aquatic toxicity tests of poorly soluble compounds. EPA's SEP and the ASTM guidelines contain recommendations for the solvents that can be used in

a test and the maximum concentration permitted. These solvents are generally regarded as non-toxic at the recommended concentrations. If there was an apparent effect on growth with the solvent, then the solvent control is used to compare with the other treatments. Studies where a solvent is used to solubilize the test compound should not be rejected if there appears to be an effect due to the solvent. In these cases, data evaluation should be based on comparisons with the solvent control.

EPA Response: The Agency will judge these issues on a case-by-case basis. If an acceptable solvent causes unacceptable control mortality or affects other biological endpoints, it should be determined whether solvent levels ($\leq 10\%$) are adequate or if a replacement solvent is needed. If the first two alternatives are not feasible, then the appropriateness of comparing the data to the solvent control will be evaluated.

Solvent Concentrations Not Equal Across Treatments:

There is no absolute requirement that solvent concentrations be equal across all treatments. EPA's SEP allows differing concentrations of solvent, as long as the solvent control contains at least as much solvent as in any of the other test chambers. Standard Mount and Brungs diluter systems that are used at many laboratories preclude the use of equivalent solvent concentrations. Studies performed using a cosolvent should not be rejected if solvent concentration is not equal across test chambers. As long as a solvent control is tested that contains solvent at a concentration equal to the highest level in any test chamber, the study should be acceptable.

EPA Response: The Agency agrees with Industry. The only additional comment is that the concentration of the solvent should not exceed 0.1 ml/L in any test concentration in a flow-through system.

Length Measurements Were Not Provided:

Neither EPA's SEP nor the ASTM guidance states that length must be measured. The FIFRA-TG document does list length as a growth parameter. Guidance documents are ambiguous on the issue of length measurements, and the need for measurement of length should be clarified.

EPA Response: Length measurements are a vital component of growth indices. Therefore, individual standard length measurements are required on the individual fish at the end of the study. The SEP, section II.D., states that length is one of the continuous measurements that needs to be statistically analyzed.

AQUATIC INVERTEBRATE LIFE CYCLE (Guideline 72-4, *Daphnia*)

1. *Rejection Factor:* Raw data were not submitted to allow the Agency to verify the MATC.

Agency Guidance: Subd-E: p. 29, 70-4 (b)(1)(2) and p. 78, no. 72-4 (c)(1) - (11). FIFRA-TG: p. C-67, no. 9. ASTM: p. 792, 13.1.9 and pp. 792 - 793, no. 15. SEP *Daphnia* Life Cycle: p.6, D.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry disagrees that guidance on this factor is adequate. Below is a list of the referenced EPA guidance with relevant text. Industry feels that guidance documents with asterisks clearly establish that some form of raw data is required; however, since "raw" data can be defined in a variety of ways, the guidance can cause confusion.

* • Subd-E: p. 29, §70-4(b)(1)(2). Both sections relevant, especially (2). Each test report should include ". . . enough data for the Agency to verify calculated statistical values."

• Subd-E: p. 78, §72-4(c)(1) to (11). All sections are not pertinent, only #4 ". . . include mortality data."

• ASTM: pp 792-793, No. 15. All sections are not pertinent, only 15.1.11 ". . . data in sufficient detail to allow independent statistical analyses . . ."

* • **SEP, *Daphnia* Life Cycle (not included in original analysis)**: p. 6, D. Requires that "results (of the study) must be accompanied by copies of the original (raw) data . . ."

A second source of confusion is the inconsistency with which EPA invokes various documents as guidance. For this rejection factor, for example, EPA has listed ASTM as guidance, but not EPA's own SEP.

In general, industry agrees with EPA that this was avoidable. However, clear delineation of appropriate guidance might help eliminate future problems. Requiring submission of raw data is not acceptable guidance, without a definition of "raw."

In addition, industry believes that:

1. Subd-E is the most appropriate guidance for this subject, since it establishes that only **enough** data needs to be submitted to allow independent verification of endpoints.

2. EPA should contact the registrant and request data needed, rather than rejecting the study because the data were not submitted. This would prevent needless rejection of studies.

EPA Response: Failure to initially include the SEP as guidance was an oversight. The citation is now included in the guidance for this rejection factor. For the *Daphnia* life-cycle study, data in the following manner need to be submitted in the final report: survival, mortality, and reproduction data for each replicate chamber for each day observed; lengths and or dry weights for each live individual daphnid for each test concentration and control at the end of the study. This will provide sufficient data for the reviewer to verify the reported MATC, NOEL, and LOEL values for each biological endpoint.

Resolution: Industry and EPA are in agreement with what needs to be submitted.

2. *Rejection Factor:* The study was unable to produce NOEL values for reproduction and growth; thus, correct MATC values cannot be established for reproduction and growth.

Agency Guidance: ASTM: p. 784, no. 4. p. 792, no. 13.1.9 and 15.1.11. FIFRA-TG: P. 67, 72-4(b), no. 9.; p. C-68, 72-4(b), no. 13. and p. C-70, 72-4(b), no. 9. SEP *Daphnia* Life Cycle: p.7, III.A. Subd-E: p. 29, no. 70-4 (b)(1) and (2) and p. 78 no. 72-4 (c)(1) to (11).

Assessment of Guidance: Generally, guidance on this factor is adequate because of the experience of the laboratories doing such studies. Issues such as this are handled on a case-by-case basis, but improvement in the guidance may be needed.

Avoidable: Yes.

Industry Comment: Industry agrees that guidance on this factor can be improved. Below is a list of the referenced EPA guidance with relevant text. Asterisks indicate guidance documents that are particularly clear in establishing the NOEL requirement. EPA's selection of guidance documents to

include in this report produced some confusion. For example, ASTM is listed, but not EPA's Subd-E Guidelines or SEP.

- ASTM, p. 784, no 4. Section does not require or mention MATC or NOEL (general description of study).

- ASTM, p. 792, No. 13.1.9. Section does not require or mention MATC or NOEL (discusses measurement endpoints for the study).

* • FIFRA-TG, §72-4(b), p. 67, No. 9. Requires ". . . MATC value reported in ppm."

* • FIFRA-TG, p. C-68, §72-4(b), No. 13. Requires ". . . no observed-effect level and reproductive effects defined."

* • FIFRA-TG, p. C-70, §72-4(b), No. 9. Requires MATC and NOEL.

* • **Subd-E:** p. 32, §70-4(c)(7) (not included in original analysis). General requirements for data analysis. §70-4(c)(7)(iii) specifically requires determination of NOEL. **Problems**: (1) These are general guidelines that apply to acute as well as chronic studies. Consequently, this implies that a NOEL is required for acute studies. (2) These requirements do not require an adverse effect level.

* • **Subd-E (not included in original analysis)**: p. 78, §72-4(c)(1) to (11). General requirements for reporting; (c)(3) requires no-effect level.

* • **SEP, *Daphnia* Life Cycle (not included in original analysis)**: p. 7, III.A. Requires verification of proper calculation of MATC.

In principle, Industry agrees that a NOEL and an adverse effect level are reasonable endpoints for this test. However, Industry feels that because of the complexity of these tests, it is important to remain flexible in interpreting results. The following three factors can influence NOEL determination:

First, selection of test concentrations that will yield a NOEL *and* a reproductive effect level (Required in FIFRA-TG, §72-4(b), p. C-68, No. 13) can be difficult. The selection problem is exacerbated by the routine spacing of test concentrations by 2X of the lower concentration. It would be helpful if EPA provided clear guidance that it is acceptable to increase the size of the interval.

Second, uncertainty exists regarding appropriate control groups to be used in statistical comparisons in determination of the NOEL. ASTM-1193, No. 9.2.4.3 suggests that if a solvent effect exists, then all comparisons should be made to the solvent control. In the absence of a solvent effect, both controls should be used in meeting requirements (Industry assumes this means pooling controls). It is important that guidance is clear on this issue, since these factors can influence NOEL identification.

Third, absence of a NOEL for a particular study should result in rejection only after considering the type of variable affected, the magnitude of the effect, the existence of a dose response relationship, the importance to EPA's risk assessment, and the possibility of estimating a theoretical NOEL.

Industry therefore suggests that EPA:

1. Cite only relevant guidance documents, as indicated with asterisks above.

2. Allow flexibility in establishing test concentrations for chronic tests. Do not *require* an adverse effect level unless it is necessary to complete the risk assessment. Allow test concentrations to be separated by levels greater than 2X the lower concentration.

3. Clearly indicate the type of statistical comparisons to be made (e.g., compare treatment levels to control, solvent control, or pooled control), since the NOEL is statistically derived by comparing to a control group, and since most aquatic studies have both a negative and a solvent control.

4. Not categorically reject studies if a NOEL is not identified. Weight of evidence based on such factors as magnitude of effect and importance to the risk assessment should be considered.

EPA Response: Failure to initially include the SEP and Subd-E as guidance was an oversight. The citations are now included in the guidance for this rejection factor. The Agency agrees to provide better guidance document citations, *advice* for test concentration selection, and test concentration intervals. The Agency can require data beyond the ASTM protocol. **See the Rapid Feedback SOP.**

When solvents are used, the solvent control should be used for data comparison. The negative control is designed to discern potential

effects of the solvent. In cases where there is no control mortality and no significant difference between the negative and positive controls, use of a pooled control may be permissible.

NOELs are not required for acute toxicity studies, but are required in chronic toxicity studies for regulatory purposes. In order for a NOEL to be valid, there must be at least one concentration level with effects not significantly greater than the appropriate control, and a second concentration delineating an acceptable LOEL.

Studies will not automatically be rejected if a NOEL is not experimentally achieved. Registrants can submit a calculated NOEL for consideration.

Resolution: There needs to be some flexibility in dose selection and spacing. The Agency needs to develop a scientifically sound method for spacing concentrations in addition to good general guidance for this chronic study. The Agency agreed to provide guidance (advice) through the rapid feedback process. The SOP developed for obtaining rapid feedback explains the documentation needed for inclusion in the final report for EPA review.

3. *Rejection Factor:* The survival rate in the control group (at one test level) was substantially lower than recommended.

Agency Guidance: Subd-E: p. 26, 70-3 (b)(2) and (3). (For background information: Stephan: pp. 30 - 31, no. E.1. and p. 39, no. 9., last paragraph). ASTM: p. 792, no. 13.1.10. SEP *Daphnia* Life Cycle: p.4, II.A.7. FIFRA-TG: 72-4(b), p. C-67, #12.

Assessment of Guidance: Generally, guidance on this factor is adequate because of the experience of the laboratories doing such studies. Issues such as this are handled on a case-by-case basis, but improvement in the guidance may be needed.

Avoidable: Yes.

Industry Comment: Industry agrees that improvement in guidance is needed. Below is a list of the referenced EPA guidance with relevant text. The guidance that Industry felt was particularly relevant (indicated below with asterisks) was not included as guidance in EPA's original rejection rate

analysis. These guidance documents were cited in other parts of this document, so it is unclear why they were not included here. Because of the inconsistencies in designating guidelines, it is not clear what the acceptability criteria is. For example, the ASTM guidance says > 20% mortality is unacceptable, whereas EPA's own guidance indicates > 30% mortality is unacceptable. None of the guidance indicates whether it is per replicate or the average of all replicates.

- Subd-E: p. 26, §70-3(b)(2) and (3). Passage addresses inclusion of solvent control (2) and maintenance of control groups similar to other test levels. Stephan: pp. 30-31, No. E.1 and p. 39, No. 9., last paragraph. This reference specifies 10% mortality in static and 5% in flow-through as being unacceptable.

- ASTM: p. 792, No. 13.1.10. Test rejected if controls have > 20% mortality, < 60 young per adult (13.1.11), or ephippia were produced (13.1.12).

* • **FIFRA-TG (not included in analysis)**: 72-4(b), p. C-67, #12. Success of controls—the study is rejected if ≥ 30% total mortality, ≤ 40 young were produced, or if ephippia are produced.

* • **SEP *Daphnia* (not included in analysis)**. Provides identical guidance as in FIFRA-TG

Also confusing is EPA's assessment that "guidance on this factor is adequate because of the experience of the laboratories doing such studies."

The rejection factor statement referred to "one test level in the control group." Industry assumed that this refers to the solvent and negative levels of the control groups.

Industry suggests that EPA:

1. Clarify why the study was rejected (e.g., What is one test level of the control group? What does "substantially" mean?).

2. Resolve conflicting acceptability criteria in guidance documents. Industry suggests following the 30% control mortality criteria established in guidance documents with asterisks. The acceptability criteria is based on the average of replicates where there are more than one animal per replicate.

3. Clarify the statement "this factor is adequate because of the experience of the laboratories doing such studies."

EPA Response: Failure to initially include the SEP and FIFRA-TG as guidance was an oversight. The citations are now included in the guidance for this rejection factor. The Agency acknowledges differences among criteria as prescribed by the Agency SEP and various protocols. The Agency also acknowledges that the wording, "one test level" is inappropriate. It should have read "one control group." Nevertheless, mortality within any replicate exceeding 30% would justify rejection of a study. The Agency will reconsider its study rejection levels to align them with the more conservative vis-a-vis more protective criteria prescribed by ASTM and Stephen (pp. 30-31, Nol E.1 and p. 39, No. 9, last paragraph).

Resolution: EPA and NACA agreed that a flexible approach be used in the analysis of control survival. The difference between EPA recommendations (80% overall, 70% per replicate) and ASTM (70% overall) was noted.

4. *Rejection Factor:* Growth parameters (length and weight) were not measured quantitatively on adults at the end of the study.

Agency Guidance: Subd-E: p. 29, no. 70-4 (b)(1)(2) and p. 78, no. 72-4 (c)(1) - (11). FIFRA-TG: 72-4(b) p. C-70, no. 9. ASTM E - 1193: p. 790 - 791, no. 11.10, pp. 792 - 793, no. 13, 14 and 15. SEP *Daphnia* Life Cycle: p. 5, II.A.8 and p. 6, II.D.

Assessment of Guidance: Guidance in this area needs to be improved. The EPA publications indicate that length measurements are adequate to analyze effects on growth. However, in 1987 ASTM determined that weight is a better growth indicator than length. An amendment to the SEP was drafted in September 1990 and indicated that dry weight and length measurements are needed. However, these changes need to be incorporated formally into EPA documents, particularly the SEP.

Avoidable: No.

Industry Comment: We agree with EPA that guidance in this area needs to be improved. Below is a list of the referenced EPA guidance with relevant text. We feel that the guidance indicated with the asterisks are most appropriate. Industry could not check the accuracy of the statement that "ASTM, in 1987, determined that (dry) weight is a better growth indicator than

length," since no reference was included in the ASTM document. Industry does not believe that EPA should reject studies solely on the basis that dry weight measurements were not made, until it is clearly mandated in the guidance documents.

- Subd-E: p. 29, §70-4(b)(1) and (2); p. 78, §72-4(c)(1) to (11). General data requirements; no reference to *Daphnia* or growth.

* - FIFRA-TG: 72-4(b) p. C-70, No. 9. Requires length of first generation organisms.

- ASTM E-1193: p. 790-791, No. 11.10. Requires dry weight determination of individual *Daphnia* **or** length of individual *Daphnia*, if it has been determined length and dry weight are correlated.

- ASTM E-1193: pp. 792-793, Nos. 13, 14, and 15. No. 13.1.9 requires data on growth. No. 14.1 requires dry weight or length. No. 15.1.11 requires data sufficient to allow recalculation of growth MATC.

* - SEP, *Daphnia* Life Cycle: p. 5, II.A.8 requires length of first generation. P. 6, II.D requires statistical analysis of length.

- Amendment to Ecological Effects SEP (not included in original analysis): Section (8). Changes SEP to say "include data on . . . the dry weight of first generation daphnids at the end of the test."

The issue relative to this rejection factor is principally related to the additional requirement of measuring dry weight, as well as length, as an estimate of growth. Industry does not feel that addition of this endpoint is warranted, for the following reasons:

First, Industry does not agree with EPA's statement on assessment of guidance that ASTM determined that dry weight was a better indicator of growth rate. ASTM guidance states that "dry weight of each individual first-generation daphnid . . . must be determined to the nearest microgram, *except* that length may be determined in place of dry weight if a correlation has been shown between length and dry weight." Kooijman and Metz (1984) noted that length of daphnids is proportional (correlated) to the third root of wet weight. Gurney, et al. (1990), point out that even though length and weight are correlated, carapace length will tend to fluctuate less than total body mass. This is because carapace length remains constant during the molting cycle but weight can fluctuate. Accordingly, body mass would tend to be more

variable in a changing environment compared to length. A less variable parameter such as length could be a "better" indicator of growth.

Second, growth and reproduction of individual daphnids is closely related (Kooijman and Metz, 1984). Since the principal endpoint of this test is reproduction, it is important that balance be maintained in the power of statistical comparisons for growth and reproduction. Industry agrees that a measure of growth is necessary, but maintains that reproduction should be the focus of the study and that length serves as an adequate estimate of growth.

Gurney, W.S.C., E. McCauley, R.M. Nisbet, and W.W. Murdoch (1990) The physiological ecology of *Daphnia*: A dynamic model of growth and reproduction. Ecology 71:716-732.

Kooijman, S.A.L.M. and J.A.J. Metz (1984) On the dynamics of chemically stressed populations: The deduction of population consequences from effects on individuals. Ecotox. Environ. Safety 8:254-274.

Industry suggests that EPA:

1. Clearly indicate whether dry weight or length were not measured.

2. Do a rigorous statistical power analysis on any new endpoints added to this test. Since this is principally a test for determining reproductive effects, it is important that the statistical power for growth measurements do not deviate greatly from the power inherent to the reproduction test. Moreover, Industry believes that one measure of growth should be acceptable.

3. Compile new guidance specific for this test system, since many *Daphnia* Life-Cycle studies are flow-through systems. Specific attention should be focused on the types of endpoints that should be included and statistical power.

EPA Response: The Agency recommends that both length and dry weight be determined, however no study reporting length measurements only will be rejected. The Agency concurs that appropriate guidance should be provided for the flow-through test design, and specific guidance provided on preferred end-points.

Resolution: Industry and EPA agree that, while both length and weight are preferable, only length is required until the Agency provides specific guidance indicating otherwise.

There was agreement that guidance is needed for the flow-through *Daphnia* Life-Cycle study. EEB is working toward developing a protocol and SEP for this study. ASTM has a draft protocol for the flow-through design which is still in the process of comment and revision. It cannot be recommended as a general guidance document by the Agency until it is finalized.

5. *Rejection Factor:* Failure to obtain a NOEL. Thus, correct MATC values for reproduction and growth cannot be established.

Agency Guidance: ASTM: p. 784, no. 4. p. 792, no. 13.1.9 and 15.1.11. FIFRA-TG: P. 67, 72-4(b), no. 9.; p. C-68, 72-4(b), no. 13. and p. C-70, 72-4(b), no. 9. SEP Daphnia Life Cycle: p.7, III.A. Subd-E: p. 29, no. 70-4 (b)(1) and (2) and p. 78 no. 72-4 (c)(1) to (11).

Assessment of Guidance: Generally, the guidance in this area is adequate.

Avoidable: Yes.

Industry Comment: Industry suggests that guidance on this factor can be improved. Below is a list of the referenced EPA guidance with relevant text. Guidance documents indicated with asterisks are particularly clear in establishing the NOEL requirement. EPA's selection of guidance documents to include for this factor caused some confusion. For example, the guidance documents listed for this factor are not the same as those listed for the very similar factor no. 2 above.

- Subd-E: p. 29, §70-4(b)(1) and (2). General data requirements; no mention of NOEL or MATC.

* • Subd-E: p. 78, §72-4(c)(1) to (11). General requirements for reporting; (c)(3) requires no effect level.

* • FIFRA-TG: p. C-67, 72-4(b), No. 9. Requires MATC value to be reported in ppm.

* • *Daphnia* Life Cycle: p. 7, III.A. Requires verification of proper calculation of MATC.

In principle, Industry agrees that a NOEL and an adverse effect level are reasonable endpoints for this test. However, Industry feels that because of the complexity of these tests, it is important to remain flexible in interpreting results. Three of the factors that can affect NOEL determination are described below:

First, selection of test concentrations that will yield a NOEL *and* a reproductive effect level (Required in FIFRA-TG, §72-4(b), p. C-68, No. 13) can be difficult. The selection problem is exacerbated by the routine spacing of test concentrations by 2X of the lower concentration. It would be helpful if the Agency provided clear guidance that it is acceptable to increase the size of the interval.

Second, uncertainty exists regarding appropriate control groups to be used in statistical comparisons in determination of the NOEL. ASTM-1193, No. 9.2.4.3 suggests that if a solvent effect exists, then all comparisons should be made to the solvent control. In the absence of a solvent effect, both controls should be used in meeting requirements (industry assumes this means pooling controls). It is important that guidance is clear on this issue, since these factors can influence NOEL identification.

Third, absence of a NOEL for a particular study should result in rejection only after considering the type of variable affected, the magnitude of the effect, the existence of a dose response relationship, the importance to EPA's risk assessment, and the possibility of estimating a theoretical NOEL.

EPA Response:

The guidance for this rejection factor and factor No. 2 for this guideline study have been corrected and are now identical. The Agency agrees to provide better guidance document citations, guidance for test concentration selection, and test concentration intervals. The Agency can require data beyond the ASTM protocol.

When solvents are used, the solvent control should be used for data comparison. The negative control is designed to discern potential effects of the solvent. In cases where there is no control mortality and no significant difference between the negative and positive controls, use of a pooled control may be permissible.

NOELs are not required for acute toxicity studies, but are required in chronic toxicity studies for regulatory purposes. In order for a NOEL to be valid, there must be at least one concentration level with effects

not significantly greater than the appropriate control, and a second concentration delineating an acceptable LOEL.

Studies will not automatically be rejected if a NOEL is not experimentally achieved. Registrants can submit a calculated NOEL for consideration.

Resolution: There needs to be some flexibility in dose selection and spacing. The Agency needs to develop a scientifically sound method for spacing concentrations in addition to good general guidance for this chronic study. The Agency agreed to provide guidance (advice) through the rapid feedback process. The SOP developed for obtaining rapid feedback explains the documentation needed for inclusion in the final report for EPA review.

6. *Rejection Factor:* The raw data for production of offspring, adult survival, weight, and length were not provided. Therefore, the reported MATC and NOEL values could not be verified.

Agency Guidance: Subd-E: p. 29, no. 70-4 (b)(1) and (2) and p. 78, no. 72-4 (c)(1) - (11). ASTM E - 1193: p. 792, 13.1.9 and p. 792, 15.1.11. FIFRA-TG: 72-4(b), p. C-67, no. 9.

Assessment of Guidance: Generally, the guidance in this area is adequate.

Avoidable: Yes.

Industry Comment: Industry disagrees that guidance on this factor is adequate. Below is a list of the referenced EPA guidance with relevant text. Industry feels that guidance documents with asterisks above clearly establish that some form of "raw" data is required. However, since raw data can be defined in a variety of ways, the guidance can cause confusion.

* • Subd-E: p. 29, §70-4(b)(1) and (2). Both sections are relevant, especially (2). Each test report should include ". . . enough data for the Agency to verify calculated statistical values."

• Subd-E: p. 78, §72-4(c)(1) to (11). All sections are not pertinent, only #4 ". . . include mortality data."

• ASTM E-1193: p. 792, 13.1.9. Requires data on survival, growth, and reproduction.

- ASTM E-1193: p. 792, 15.1.11. Requires data sufficient to allow recalculation of growth MATC.

- FIFRA-TG: p. C-67, 72-4(b), No. 9. MATC value reported in ppm. Raw data provided.

* • SEP *Daphnia* Life Cycle: p. 6, D. Requires that "results (of the study) must be accompanied by copies of the original (raw) data . . ."

A second source of confusion is the inconsistency with which EPA invokes various documents as guidance. For example, the guidance documents listed for this factor are not the same as those listed for the very similar rejection factor no. 1 above.

In general, Industry agrees that this is avoidable. However, clear delineation of appropriate guidance might help eliminate future problems. Raw data must be clearly defined.

In addition, Industry believes that:

1. Subd-E provides the best guidance for this subject, since it establishes that only **enough** data needs to be submitted to allow independent verification of endpoints.

2. EPA should contact registrant and request data needed prior to finalizing and issuing the DER, rather than rejecting the study because the data were not submitted. This would prevent needless rejection of studies.

EPA Response: For the *Daphnia* life-cycle study, data in the following manner need to be submitted in the final report: survival, mortality, and reproduction data for each replicate chamber for each day observed; lengths and or dry weights for each individual live daphnid for each test concentration and control at the end of the study. This will provide sufficient data for the reviewer to verify the reported MATC, NOEL, and LOEL values for each biological endpoint.

Resolution: Industry and EPA are in agreement with what needs to be submitted.

Other Industry Comments on Guideline 72-4

A *Daphnia* chronic study was initially rejected by EEB because of three factors: 1) variable test concentrations, 2) no raw data, and 3) experimental design did not follow

SEP (static renewal protocol with 7 beakers containing individual adults and 3 beakers containing 5 individuals per beaker). All factors were ultimately resolved and the study was accepted as core.

Industry included this case study to illustrate the lack of sufficient guidance in identifying an appropriate protocol for the *Daphnia* chronic study. EPA's SEP is written for a static renewal system, whereas many *Daphnia* chronic studies are performed under flow-through conditions. Consequently, the experimental design in the SEP may be difficult to implement under conditions imposed by splitter devices or beaker turnover rates.

EPA Response: The Agency suspects that the study was rejected primarily on the basis of the absence of tabulated and unanalyzed data coupled with erratic test concentrations. It has been established historically through numerous written and verbal communications that data (all unsummarized and unanalyzed data) is needed by the Agency to facilitate its evaluation. The Agency concurs that the SEP in particular, and the study design generally, need complete revisions to reflect flow-through conditions.

INVERTEBRATE LIFE CYCLE (ESTUARINE SPECIES (Mysid)) (Guideline 72-4)

1. *Rejection Factor:* Reproduction rate of the mysids in the controls and in the test concentrations was very low and could not be statistically analyzed.

Agency Guidance: ASTM E - 1191: p. 764, 13.1.10. FIFRA-TG: 72-4(c), p. C-72, no. 9 C-75, no. 9.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Some confusion may arise if laboratories performing the study do not have explicit instructions to follow the ASTM protocol. This should be clearly identified in official guidance. Subd-E Guidelines reference the protocol by Nimmo et al. (1978) as the only example of an acceptable protocol for the mysid chronic reproduction test. This reference was not listed as guidance in the present analysis. Perhaps it will be necessary to update the Guidelines to acknowledge this change, so that laboratories will know which protocol to follow. EPA should provide an official list of protocols that will yield acceptable studies if followed.

EPA Response: The Agency concurs that acceptable protocols should be listed by the Agency. Two suggested protocols are listed below.

American Society for Testing Materials. 1990. Standard Guide for Conducting Life-Cycle Toxicity Tests With Saltwater Mysids. E 1191-90. Published by ASTM Committee E-47 on Biological Effects and Environmental Fate, Philadelphia, PA, 19103.

Nimmo, D.E., L. H. Bahner, R.A. Rigby, J.M. Sheppard, and A.J. Wilson, Jr. 1977. *Mysidopsis bahia*: An estuarine species suitable for life cycle bioassays in determining sublethal effects of a pollutant. In Aquatic Toxicology and Hazard Evaluation (F.L. Mayer and J. L. Hamelink, eds.), American Society For Testing Materials, Philadelphia, PA, STP 634, pp. 109-116.

Nimmo, D.E., T.L. Hamaker, and C.A. Sommers. 1978. Entire life-cycle toxicity test using mysids (*Mysidopsis bahia*) in flowing water. Pp. 64-68 in Bioassay Procedures for the Ocean Disposal Permit Program. U.S. Environmental Protection Agency, Office of Research and Development. EPA-600/9-78-010.

2. *Rejection Factor:* Adult body lengths were not measured at the end of study.

Agency Guidance: ASTM E - 1191: p. 762, 11.9.4 and p. 764, 13.1.8. FIFRA-TG: 72-4(c), p. C-72, no. 9 and C-75, no. 9.

Assessment of Guidance: The guidance documents which address this factor appear adequate.

Avoidable: Yes.

Industry Comment: Industry questions the need to have two estimates of mysid growth in a study principally aimed at determining reproductive effects in chemicals. EPA should (1) provide an official list of protocols that will yield acceptable studies if followed, and (2) require only one estimation of growth. Industry recommends length as the principal measure of growth.

EPA Response: The Agency concurs that acceptable protocols should be listed by the Agency. Suggested protocols are listed below.

American Society for Testing Materials. 1990. Standard Guide for Conducting Life-Cycle Toxicity Tests With Saltwater Mysids. E 1191-90. Published by ASTM Committee E-47 on Biological Effects and Environmental Fate, Philadelphia, PA, 19103.

Nimmo, D.E., L. H. Bahner, R.A. Rigby, J.M. Sheppard, and A.J. Wilson, Jr. 1977. *Mysidopsis bahia*: An estuarine species suitable for life cycle bioassays in determining sublethal effects of a pollutant. In Aquatic Toxicology and Hazard Evaluation (F.L. Mayer and J. L. Hamelink, eds.), American Society For Testing Materials, Philadelphia, PA, STP 634, pp. 109-116.

Nimmo, D.E., T.L. Hamaker, and C.A. Sommers. 1978. Entire life-cycle toxicity test using mysids *(Mysidopsis Bahia)* in flowing water. Pp. 64-68 in Bioassay Procedures for the Ocean Disposal Permit Program. U.S. Environmental Protection Agency, Office of Research and Development. EPA-600/9-78-010.

The Agency concurs that this test method was designed primarily for determining reproductive effects. However, a complete environmental assessment includes data on growth, survival, and reproduction. Consequently, the Agency believes that growth data is important for the overall risk assessment. The Agency believes that growth is two-dimensional, longential as well as transversal. Therefore, the Agency would prefer to see both length and weight measurements for mysids.

Resolution: NACA raised the question of whether one measure of growth would be sufficient for the mysid, since EPA has agreed that only one measure of growth (length) is necessary in the *Daphnia* Life-Cycle test.

EPA did not agree during the meeting that only one measure of growth is acceptable. EPA wants to work with the ERL at Gulf Breeze to resolve this issue. As data becomes available comparing the two measures of growth, NACA may like to follow-up with EPA on this point.

3. *Rejection Factor:* The feeding rate of the mysids was below the recommended daily ration.

Agency Guidance: ASTM E - 1191: p. 760, 10.5 and p. 761-762, 11.5.

Assessment of Guidance: Generally, the guidance given in this area is adequate.

Avoidable: Yes.

Industry Comment: Industry recognizes that ASTM provides guidance on the appropriate feeding rate for mysid shrimp in the chronic study. However, Industry

feels that deviations from recommended feeding rates is not sufficient cause for rejection of the study. Rejection of the study would not be appropriate if the control group was meeting all acceptability criteria. Also, Industry feels that some confusion may arise if laboratories performing the study do not have explicit instructions to follow the ASTM protocol. This should be clearly identified in guidelines or the SEP. EPA should provide an official list of protocols that will yield acceptable studies if followed.

EPA Response: Guidance is adequate for *recommended* mysid feeding rates. While study guidance recommends acceptable feeding rates, a study will not be rejected solely because feeding deviates from recommended levels. Biological response (i.e., survival, growth, reproduction, lack of cannibalistic behavior) will also be evaluated in determining if optimal feeding was maintained during a study.

Resolution: NACA does not believe that feeding rate should be a reason for rejection if the controls are growing and surviving. Mysids are cannibalistic if there is insufficient food. The biological response is what needs to be optimal, not the feeding rate. EEB disagreed and said that the feeding should be optimal.

NACA pointed out that the feeding rate was only mentioned in one recommended protocol and is probably not the only feeding rate which is acceptable. EEB agreed that biological response is important, and agreed that there would be more flexibility within reason. EEB continues to emphasize that in order for toxicity studies to be truly comparative, absolute standardized, optimal conditions must be used.

4. *Rejection Factor:* The raw data for adult mortality and growth, production, and survival of offspring were not provided. Therefore, the reported MATC and NOEL values could not be verified.

Agency Guidance: Subd-E: p. 29, no. 70-4 (b)(1) and (2) and p. 32, no. 70-4 (c)(7) and p. 78, no. 72-4 (c)(1) - (11). ASTM E - 1191: p. 764, 13.1.8 and p. 764-765, 15.1.11. FIFRA-TG: 72-4(c), p. C-72, no. 9 and 10.

Assessment of Guidance: Generally, the guidance given in this area is adequate.

Avoidable: Yes.

Industry Comment: Industry disagrees that guidance on this factor is adequate. Below is a list of the referenced EPA guidance with relevant text. Industry feels that guidance documents with asterisks clearly establish that some form of raw data is required; however, since raw data can be defined in a variety of ways, the guidance can cause confusion.

A second source of confusion is the inconsistency with which EPA invokes various documents as guidance. Guidance documents cited for this rejection are conflicting. For example, one document requires sufficient data to calculate statistical endpoints, whereas another requires submission of raw data.

* • Subd-E: p. 29, §70-4(b)(1) and (2). Both sections relevant, especially (2). Each test report should include ". . . enough data for the Agency to verify calculated statistical values."

• Subd-E: p. 32, §70-4(c)(7). Not relevant; specifies types of data analysis.

• Subd-E: p. 78, §72-4(c)(1) to (11). All sections are not pertinent, only #4 ". . . include mortality data."

• ASTM E-1192: p. 764, 13.1.8. Requires data on survival, growth and reproduction.

• ASTM E-1192: p. 764-765, 15.1.11. Provide a table of data on survival, growth, and reproduction of mysids ". . . in sufficient detail to allow independent statistical analysis."

* • FIFRA-TG: p. C-72, 72-4(c), No. 9 and 10. No. 10 specifically requires raw data.

In general, Industry agrees that this was avoidable. However, clear delineation of appropriate guidance might help eliminate future problems. Requiring submission of raw data is not acceptable guidance, without an definition of "raw."

In addition, Industry believes that:

1. Guidance provided by Subd-E is most adequate for this subject, since it establishes that only **enough** data needs to be submitted to allow independent verification of endpoints.

2. EPA should contact registrant and request data needed, rather than rejecting the study because the data were not submitted. This would prevent needless rejection of studies.

EPA Response: For the mysid life cycle study, data in the following manner need to be submitted in the final report: daily survival, mortality, and reproduction data for each replicate chamber; lengths and dry weights for each individual live mysid for each test concentration and control alive at the end of the study. This will provide sufficient data for the reviewer to verify the reported MATC, NOEL, and LOEL values for each biological endpoint.

Other Industry Comments on Guideline 72-4

Mysid spawn can be affected by individual or group matings. Guidance is not clear, but EPA rejected this study because spawns were individual rather than groups. Principally, this is a problem of inadequate guidance. Official guidance should be published that addresses appropriate experimental designs for mysid chronic studies.

EPA Response: The Agency agrees with Industry that it needs to provide better guidance for all aspects of this study.

Resolution: EPA agreed that the guidance is not clear. ASTM standard guide E-1191 requires that mature mysids be paired prior for reproduction, whereas the Nimmo protocols require group spawning. Until the Agency determines that one method is preferable to the other, it will be flexible in reviewing the studies, provided that the study clearly states which protocol it is following.

Additional Industry Comment:

Concerns Regarding the Study:

- Rejection rate is high.
- Studies are expensive.
- The utility of the data is unclear. Of the 20 or more Life-cycle studies performed, it is difficult to determine how they were used in the risk assessment.
- The restart rate for the studies is high, more of a problem since variability is often seen late in a study.
- Difficult to conduct the study.

There was a NACA/EPA Work Group which began to address the issues in 1992. NACA submitted a protocol to the EPA for review. Due to EPA resource constraints, the

review of the protocol was suspended. Concerning the use of the data, EPA said there is a difference in how the data is used for regulatory purposes and how OPP uses the data to understand the compound. It is still unclear how useful the study actually is and since it is so problematic, it should be reviewed.

NACA Recommendation: A small, focused work group consisting of 2-3 NACA representatives and 2-3 EPA personnel should be formed, with an established completion date (6-12 months) to evaluate the Fish Life-cycle study.

[EPA priorities must weigh strongly in determining when this can be accomplished.]

Additional EPA Comment:

The mysid test is relevant. This study determines whether a pesticide present in an estuarine environment will affect reproduction in exposed estuarine invertebrates. This study is the true counterpart to the freshwater *Daphnia* invertebrate toxicity test.

Resolution:

1) NACA stated that due to difficulties in study design/conduct, the sensitivity of the study to identify reproductive effects is not optimal. EPA does not agree that the sensitivity of the study is not optimal. EPA wants to consult the literature and the ERL at Gulf Breeze in addition to its own data records prior to determining if it agrees with this statement.

2) The reproductive NOELs from the Early Life-Stage are often similar to the NOEL from the Life-cycle study.

3) Time extensions will be granted (assuming adequate justification is provided) when requested as long as it is not the last study controlling the finalization of the RED.

4) NACA agreed to resubmit the protocol developed in the original work group by the end of April and EPA agreed to review.

5) Consideration should be given to the development of a test that addresses reproductive endpoints more effectively.

6) Rejection rate analysis for the submitted Fish Life-Cycle studies would be useful, although resource constraints must be considered.

SIMULATED AND ACTUAL FIELD TESTING - MAMMALS AND BIRDS (SIMULATED STUDY) (Guideline 71-5A) and (ACTUAL STUDY) (Guideline 71-5B)[26]

A discussion of the rejection factors, Agency guidance, or assessment of guidance is not presented because, essentially, this experimental design (i.e., simulated (pen) study) is no longer acceptable for addressing potential terrestrial hazards. With publication of the *Guidance Document for Conducting Terrestrial Field Studies* (EPA-540/09-88-109)[27] in September 1988, simulated (or pen) studies are no longer done or considered appropriate by the Agency.

In addition, with the publication of the New Paradigm, terrestrial field studies, under most conditions, are no longer required. Therefore, there are no comments for Guideline 71-5B.

Additional Industry Comment:

Industry commented on the following guidelines:

GUIDELINE §§122-1(a) and 123-1(a)—SEED GERMINATION

EPA did not include non-target plant testing in the rejection rate analysis of ecological effects studies. Industry has encountered the following issues in meeting EPA acceptance criteria for non-target plant-testing requirements:

1. *Industry Concern:* **Seedling germination test not included in registration package.**

EPA Guidance:

- Subd-J: §122-2
- SEP: Non-Target Plants, Growth and Reproduction of Aquatic Plants - Tier 1, 30 July 1985
- SEP: Growth and Reproduction of Aquatic Plants - Tier 2, Draft
- FIFRA-TG: §122-2

Industry Assessment of Guidance: Industry questions the need for a separate set of tests to study germination, as germination is part of the seedling emergence test. If a shoot does not emerge above the soil line, it is clear that the plant has been affected. Whether the product is embryocidal or impacts emergence of the shoot is immaterial.

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[26] This section concerns studies done using the GDTFS.

[27] For the rest of this paper this document will be called GDTFS.

Industry Assessment of Avoidability: Avoidable. The germination test can continue to be part of the registration submittal. However, Industry questions whether this study substantially adds to the risk assessment process, as no company has observed a test substance passing the emergence test but failing the germination test.

Industry Recommendations: Industry requests that EPA evaluate the need for this test. Industry believes that elimination of these germination tests would not severely impede EPA's ability to assess the potential impact of products to non-target terrestrial plants, while it would relieve the number and cost of studies to the registrant, and reduce EPA cost and manpower requirements to review these studies. EPA personnel could focus on review of the seedling emergence and vegetative vigor/shoot length test results.

EPA Response: The Agency will consider a waiver of seed germination studies (122-1, 123-1).

Resolution: NACA requested that the Agency consider a waiver of seed germination studies. The testing scheme proposed by the Agency suggests that all seed germination studies (as currently required under Subdivision J of the guidelines, 122-1 and 123-1) be waived. Percent germination standards are discussed in the new proposed testing scheme.

2. *Industry Concern:* **Insufficient germination in the control group.**

EPA Guidance:

- Subd-J: §122-2
- SEP: Non-Target Plants, Growth and Reproduction of Aquatic Plants - Tier 1, 30 July 1985
- SEP: Growth and Reproduction of Aquatic Plants - Tier 2, Draft
- FIFRA-TG: §122-2

Industry Assessment of Guidance: Rejection has been noted if the percent germination is below 70%. There is no set number given in any of the guidance documents stating or suggesting that seeds must demonstrate 70% germination. Rather, the guidelines do state that the seed batch should be "healthy and germinable." Not all plant species will provide 70% germination. Additionally, the test conditions recommended by EPA are not optimal for all species. Few seed companies use this type of test for determining seed germination rates. Industry questions the need for this test, as germination is part of the seedling emergence test (see comments in next section).

Industry Assessment of Avoidability: Avoidable. Rather than basing acceptance on a set percent germination number, focus should be given to whether the study answers/refutes the question of whether the test material produces a 25% effect.

Industry Recommendations: Some companies have designed this test so that there is sufficient replication based on percent germination for each specific species. The number of seeds per replicate is set to statistically refute a 25% effect, even though the germination may be less than 70%. The approach advances any species to Tier II if a presumption of 25% inhibition cannot be refuted. EPA has also stated that the germination rate for each seed lot should be reported. We believe that controls and historic control data produced from germination trials are more appropriate and should serve this purpose.

EPA Response: The Agency has never invalidated a plant study solely because the seed germination fell below 70%. We would, however, consider a study invalid if a sufficient number of control plants died or did not germinate so that a statistical analysis was not possible. Minimum percent germination is not currently addressed by Subdivision J Guidelines, however, in the interests of Good Laboratory Practices, the minimum acceptable percent seed germination must be determined for each seed lot tested if the study is to have statistical validity. The Agency suggests that Industry supply percent germination information from the seed packages for Agronomic crops. For vegetable crops, the Agency suggests that the Association of Official Seed Analysts (AOSA) and Federal Seed Act test standards be used. Minimum acceptable seed germination standards are set by the USDA under Federal Seed Act Regulations (7 CFR, Section 201.31) The values for the agronomic crops are based on the experience of the agronomists in EEB and will be used for study rejection purposes. The Agency does not believe that historic control data from previous tests are more appropriate or serve this purpose because certified germination will vary from year to year and from packet to packet.

VEGETABLE	MIN. % GERM	AGRON. CROPS	MIN % GERM
artichoke	60	alfalfa	70
asparagus	70	small grain[28]	80
asparagus bean	75	bean, field	70
bean, garden	70	beet, field	65
bean, lima	70	sugar beet	55
bean, runner	75	bentgrass	65
beet	65	bluegrass	65
broadbean	75	bermudagrass	75
broccoli	75	bluestem	50
brussels sprout	70	brome	65
burdock, great	60	buckwheat	60
cabbage	75	buffalograss	50
cabbage, tronchuda	75	canarygrass	60
cantaloupe	75	corn, field	85

[28]Small grains include sorghum, barley, wheat, oats, rye, millet, and rice.

cardoon	60
carrot	55
cauliflower	75
celeriac	55
celery	55
chard, Swiss	65
chicory	65
chinese cabbage	75
chives	50
citron	65
collards	80
corn, sweet	75
cornsalad	70
cowpea	75
cress, garden	75
cress, upland	60
cress, water	40
cucumber	80
dandelion	60
eggplant	60
endive	70
kale, all types	75
kolhrabi	75
leek	60
lettuce	80
mustard, all types	75
okra	50
onion	70
pak-choi	75
parsley	60
parsnip	60
pea	80
pepper	55
pumpkin	75
radish	75
rhubarb	60
rutabaga	75
salisfy	75
soybean	75
spinach	60
spinach, New Zealand	40
squash	75
corn, pop	75
vetch	75
fescue, tall	75
fescue, other	60
grama	50
lespedeza	60
lupine	70
panicgrass	50
orchardgrass	70
rape, all types	75
redtop	55
ryegrass	75
safflower	75
soybean	75
sudangrass	70
tobacco	55
wheatgrass	50
clover	70

tomato	75
tomato, husk	50
turnip	80
watermelon	70

The susceptibility or tolerance of plant species to pesticides can vary significantly. Data on plant sensitivity generated in pre-registration efficacy screening trials should be considered when choosing species that are most appropriate for Subdivision J testing. The separation of cold hearty and warm season plants for optimum seed germination, seedling emergence, and vegetative vigor under different temperatures is suggested.

3. *Industry Concern:* **Presence of a seed treatment.**

EPA Guidance:

- Subd-J: §122-2
- SEP: Non-Target Plants, Growth and Reproduction of Aquatic Plants - Tier 1, 30 July 1985
- SEP: Growth and Reproduction of Aquatic Plants - Tier 2, Draft

Industry Assessment of Guidance: Species selection is generally based on species that are known to be affected by the product. Laboratories select a suite of species that will produce a spectrum of response for the compound. Therefore, for crop protection products that are known to be selective (grass control versus broadleaf control), the same suite of species may not be used for every compound tested. This is not always an issue for non-selective compounds. For crop selective products it is also known, *a priori*, that a grass-control agent is likely to be more selective toward monocotyledons and therefore little-to-no effect might be observed to dicotyledon species, and *visa versa*. Where possible, laboratories conducting these tests have used treatment-free seeds. However, this is not always possible for all species. To select species on the basis of availability of treatment-free seed would be inappropriate. There are also problems arising from fungal contamination with some species, making testing extremely difficult. The seed treatment, when compared to appropriate controls, should not interfere with developing adequate response data. Additionally, EPA has given verbal approval to the use of treated seed if no source of untreated seed was available.

Industry Assessment of Avoidability: Avoidable. Laboratories conducting these tests could limit species selection to only those available from suppliers as seed-treatment-free. However, this would limit testing and might not allow the spectrum of compound sensitivities to be established for risk assessment purposes.

Industry Recommendations: Industry believes that seed treatments do not impact results on the control group, and therefore EPA should accept studies performed with treated seeds.

EPA Response: The Subdivision J Guidelines currently specify that seeds may be steam sterilized; however, no other seed treatments are allowed. Verbal approval to use treated seeds has been given by Agency staff as long as the registrant proves that there are no synergistic or antagonistic interactions with the test chemical, or direct phytotoxicity to each of the tested plants (all 10). This would, of course, require the submission of phytotoxicity data for each seed treatment and combinations of them if used, as compared to a negative control. Industry may provide research data that supports their position that the use of other chemicals in the test will not adversely impact study results. This data must be presented in a form that will allow Agency reviewers to apply it to all reviews. The Agency suggests a published, peer-reviewed document.

4. *Industry Concern:* **Lack of negative controls.**

EPA Guidance: None

Industry Assessment of Guidance: EPA has rejected some studies that do not include a solvent-free control when a solvent is used to apply the test substance and only solvent controls are used. This includes tests where the test substance has been applied in a solvent, the solvent is allowed to evaporate, and water is added for germination. Such studies have been rejected due to concerns that residual acetone may have resulted in reduced germination in the controls. Additionally, when differences exist between solvent controls and solvent-free controls (e.g., aquatic non-target plant testing), the guidelines state that the EC_{50} must be calculated based on the solvent control. Industry questions the need for a solvent-free control, as it is not used in the data analysis.

Industry Assessment of Avoidability: Avoidable. Tests conducted to evaluate the effects of the solvent should be performed to verify that the solvent does not reduce germination.

Industry Recommendations: If it can be shown from a control study that the prescribed solvent used in a test on a specific species does not result in effects, routine inclusion of a negative control (solvent free control) should not be necessary. If a difference is apparent, then the percent inhibition data should be developed for the solvent control and the solvent control study should be referenced whenever it is used in subsequent studies. The percent inhibition due to the test substance, however, should still be determined from the solvent control data.

EPA Response: The Subdivision J Guidelines do specify that a negative control is required at all times, even when a solvent control is used. Without the negative control, there is no way to determine if a reduction in growth in the solvent control (if and when it occurs) is a significant reduction if no comparative data are available. Once again, the Agency has been flexible with Industry regarding this point. The Agency encourages that Industry provide research data to support the position that the solvents routinely used in the plant studies are not directly phytotoxic to plants, and that they are not interactive with the test compound. If

a significant adverse effect to plant growth occurs in the solvent control, then the study should be repeated using a less toxic solvent. If there is no significant difference between the two sets of control data as determined by the Williams test, then the data may be pooled for use in the statistical analysis.

GUIDELINE §§122-1 and 123-1—SEEDLING EMERGENCE and VEGETATIVE VIGOR

1. *Industry Concern:* **Presence of a seed treatment.**

EPA Guidance:

- Subd-J: §122-2
- SEP: Non-Target Plants, Growth and Reproduction of Aquatic Plants - Tier 1, 30 July 1985
- SEP: Growth and Reproduction of Aquatic Plants - Tier 2, Draft

Industry Assessment of Guidance: All species where seed treatments have been used have been rejected.

Industry Assessment of Avoidability: Avoidable.

Industry Recommendations: See Industry Concern above under "Germination."

EPA Response: Refer to Agency response to NACA Concern 3, above.

Resolution: Untreated seeds must be used wherever possible for test and control plants. Studies with treated seeds will be categorized as supplemental (that is unless other rejection factors render the study invalid) until or unless industry shows with testing that the seed treatment did not influence the toxicity of the test material or adversely affect seed germination and emergence. These supplemental studies will not have to be repeated unless it is deemed necessary, by the Agency, for risk assessment or risk reduction evaluation.

2. *Industry Concern:* **Lack of negative controls.**

EPA Guidance: None

Industry Assessment of Guidance: For low-solubility compounds, the test substance is sometimes applied with a solvent in the carrier liquid. Some tests conducted where only a control that has been sprayed with a water and solvent have been rejected or deemed supplemental. EPA insists that a water-only control for comparison to evaluate solvent effects is necessary. As described above, tests comparing a solvent control against a water-

only control for both emergence and vigor testing could be used to determine whether the solvent concentration produces an effect. However, if it does, the solvent control—not the water control—is appropriate for use in calculation of the EC_{25}.

Industry Assessment of Avoidability: Avoidable.

Industry Recommendations: See Industry Concern above under "Germination."

EPA Response: Refer to Agency response to NACA Concern 4, above.

3. *Industry Concern:* **Test duration of 14 days is not adequate.**

EPA Guidance:

- Subd-J: §122-2
- SEP: Non-Target Plants, Growth and Reproduction of Aquatic Plants - Tier 1, 30 July 1985
- SEP: Growth and Reproduction of Aquatic Plants - Tier 2, Draft
- FIFRA-TG: §122-2

Industry Assessment of Guidance: Guidelines indicate that the duration of the germination and seedling growth test is 14 days. It can be extended for a longer period to allow for vigor measurements at the registrants discretion.

Industry Assessment of Avoidability: Not avoidable. This is not avoidable as 14 days is given as the study duration in the guidelines.

Industry Recommendations: Industry believes that 14 days is generally sufficient for test duration, as stated in the guidelines.

EPA Response: The Agency finds the 14-day rating period unacceptable for many slow acting, foliar-applied herbicides (translocate slowly to the active growing points in the plant and result in a slow phytotoxic effect). Some recent labels clearly inform growers of a delay before phytotoxic symptoms appear. These statements are placed on labels to inform growers that the herbicide is slower acting than previously used herbicides. The pesticide registrants often know the mode-of-action, weed-control spectrum, and longevity of efficacy of their product before Subdivision J tests are conducted. The Agency recommends a minimum test duration of 4 weeks for seedling emergence and vegetative vigor studies.

Resolution: Generally, the test period should remain 14 days. However, if the compound is a slow-acting foliar herbicide, the test period should be extended from 14 days to 4 weeks. Registrants should make best effort up front to meet this requirement.

4. *Industry Concern:* **Use of pest control agents, fertilizer, or other materials to maintain healthy plants is unacceptable.**

EPA Guidance:

- Subd-J: §122-2
- SEP: Non-Target Plants, Growth and Reproduction of Aquatic Plants - Tier 1, 30 July 1985
- SEP: Growth and Reproduction of Aquatic Plants - Tier 2, Draft
- FIFRA-TG: §122-2

Industry Assessment of Guidance: This issue is not addressed in the guidelines. While it is preferable to not have pests and diseases in the greenhouse during these tests, it is not uncommon that plants reared in greenhouses may sometimes be subjected to outbreaks of such pests as aphids, white flies, mites, and fungal pathogens. While some greenhouses incorporate the use of natural predators to control some of these pests, this approach is not always effective for every pest. To prevent the plants from becoming impacted by these pests, appropriate pest control products may be used occasionally to prevent the loss of a test. It is Industry's belief that as long as the products used and reasons for use are clearly documented, this should not be a reason for rejection. This is consistent with animal testing, where animal health treatments are permitted under well-documented circumstances. Fertilization is necessary to establish healthy control plants, as soils—especially those used in these tests—may lack sufficient nutrients to grow plants successfully.

Industry Assessment of Avoidability: Not avoidable. Treatment of greenhouses with pest control agents is critical for greenhouse culturing of plants. For the longer-term studies (emergence and vigor studies), an outbreak may occur which will impact the validity of the study if not controlled. These treatments will not impact the outcome of the study. Conversely, the studies could be discarded and restarted. However, it is unlikely that this effort will result in more reliable results.

Industry Recommendations: Even under the best controlled conditions, and particularly during certain times of the year, pest outbreaks will occur under greenhouse conditions and pest control must be exercised to maintain control in the greenhouse and for the study. Under these circumstances, the curative products used, the period during the test when these conditions existed, and "historical" phytotoxicity data on the compounds used as curative treatments, should be presented in the report. For example, if a company is required to use a particular compound during a test to control whiteflies and has generated data to indicate that this compound is not phytotoxic, then referencing such data should be adequate, and additional "controls" for such treatments should not be required. This is critical, because it is not possible logistically or practically to have extra controls available to test any product that might be needed to control diseases or infestations during conduct of a test, nor would any such controls be a representative control for the test. NACA requests that the following statement be added to the "Resolution" section: "In lieu of treating additional controls during

the test with any curative compounds, potential phytotoxicity of curative compounds can be established in separate trials, and those results could be referenced to support use of such compounds during the test."

EPA Response: The logic for not using pesticide treated seed was previously presented. This same logic holds for other pesticide treatments that may be applied during the course of the study. The introduction of additional pesticides during the study adds variability to an already highly variable study. The Agency is aware of no plant studies that were rejected because fertilizer was used in the study. Excessive use or lack of fertilizer are factors that are considered during the study review.

Resolution: If the use of pest controls is absolutely necessary, Industry should apply compound to all treatments and additional controls. One completely untreated control must be maintained for the duration of the study. Industry should provide information on the antagonistic/synergistic effects of the pest control agent and the test substance. It was agreed that a Workshop/Working Group should be established to further evaluate this issue, as the Agency and NACA have not reached a resolution.

5. *Industry Concern:* **Guidelines state that the test substance must be the technical grade of the active ingredient. The test substance was a formulated product rather than the technical grade active ingredient.**

EPA Guidance:

- Subd-J: §122-2
- SEP: Non-Target Plants, Growth and Reproduction of Aquatic Plants - Tier 1, 30 July 1985
- SEP: Growth and Reproduction of Aquatic Plants - Tier 2, Draft
- FIFRA-TG: §122-2

Industry Assessment of Guidance: Currently, the guidelines require testing to be done on TGAI. However, to apply TGAI to plants it is necessary to use a solvent and/or make a simple formulation. Though it is not considered necessary to test all formulations or to remove the option of testing TGAI, it is proposed that the guideline should be modified to permit the testing of either TGAI or a typical end-use single formulation.

Industry Assessment of Avoidability: Industry is unaware of studies being rejected on the basis of testing appropriate formulations, and so believes the guidelines should reflect this practice.

Industry Recommendations: Industry believes that the guidelines should be modified to state that the testing of a formulation and/or the end-use product should be allowable.

EPA Response: The Agency agrees with this statement, and encourages the use of TEP in future plant testing. If the pesticide registrant has many different formulations registered, the one with the highest percentage of a.i. and /or the one most widely used should be tested. Additional tests might be required upon the introduction of new formulations.

Resolution: It was agreed that either TGAI or TEP would be acceptable until the time new direction is given by the Agency via 40 CFR part 158 or the guidelines, Subdivision J.

6. *Industry Concern:* **Watering of pots in the greenhouse from the top for emergence testing.**

EPA Guidance: None

Industry Assessment of Guidance: Some studies have been rejected because the plants have been watered from the top. This is a usual testing practice, even in efficacy testing and is not excluded in the current guideline. Therefore, it should not be a reason for rejection if the registrant can provide sufficient evidence that the test chemical was retained within the test system and would be in contact with the seeds, roots, etc., during the study. Bottom watering could be done, but can lead to a higher incidence of plant failure through root disease.

Industry Assessment of Avoidability: Avoidable. However, because of the points mentioned above, Industry does not believe that watering of the plant from the top should be cause for rejection.

Industry Recommendations: The most important point is that the chemical is contained within the system. This cannot be done easily with bottom watering. With top watering, water will quickly penetrate the dry soil and the net movement of water will be upwards due to evapotranspiration. Therefore, watering from the top should be an acceptable practice.

EPA Response: One particular study was rejected because it was believed that a highly soluble pesticide may have been washed through the soil because of excessive watering events. In this case, bottom watering was recommended so that the chemical would move upward through the soil with evaporation, thus coming in contact with the germinating seeds. There are pluses and minuses to both methods of watering. The Subdivision J guidelines are not specific with regard to method of watering or frequency of watering, for good reason. Each greenhouse or growth chamber may have different environmental conditions that would require a different watering frequency and/or methodology. The Agency does **not** routinely reject plant studies solely on the basis of watering method or frequency. The Agency requests that Industry utilize data regarding the solubility, volatility, and Kd value of the test chemical to determine its affinity to the growth media prior to conducting the study so that the best method and frequency of plant watering is used. The Agency would prefer that

seedling emergence studies utilize bottom watering. For vegetative vigor studies, top watering under the foliage or bottom watering can be used.

The Agency recommends that non-porous plant containers be used. Peat and clay containers may adsorb the test chemical. With regard to propagation media, soil mixes containing >2% organic matter should be avoided. At the other extreme, 100% acid washed sand, glass beads, or rockwool are not recommended either.

Resolution: Watering from the bottom is preferred. However, when watering from the top, care should be taken not to wash the compound off of the plant, and chemical physical properties of the test substance should be considered (solubility, K_{ow}, K_{od} etc.)

7. *Industry Concern:* **Placing more than one plant of the same species in a pot per replicate.**

EPA Guidance: None

Industry Assessment of Guidance: Rejection of a study due to the presence of more than one plant per pot is inappropriate. It is usual greenhouse practice to grow more than one plant in a pot when appropriate for that species. Pots for each plant species are selected to minimize interplant competition and allow treatment of all plants by the application. Not to place more than one plant per pot for this test would be impractical.

Industry Assessment of Avoidability: Not avoidable. It is impractical to grow plants in individual containers for all species.

Industry Recommendations: Industry recommends that as long as the density of plants does not impact the treatment of the plants, plants may be grown with more than one plant to a pot. This practice should not be a reason for rejection.

EPA Response: The Subdivision J guidelines do not currently specify the number of individual plants that are allowed per pot. Different types and sizes of pots are used in different greenhouses in different parts of the country. Occasionally, we review studies in which the plants are obviously crowded for the size and type of container. If we consider this a significant detriment to plant growth, the study will be rejected on this basis. In the absence of specific guidance in Subdivision J, the Agency suggests the following: 15 cm diameter X 15 cm deep pot, use a maximum of 1 plant per pot for corn, soybean, tomato, and cucumber; use a maximum of 3 plants per pot for sugarbeet, rape, and pea; use a maximum of 6 plants per pot for onion, wheat, and sorghum. All others, use a maximum of 1 plant per pot if uncertain.

Resolution: The Agency agrees with the NACA proposal. However, if care is not taken to prevent overcrowding, the study may be rejected on that basis.

GUIDELINE §§123-1 and 123-2—PLANT PROTECTION TIER II

1. *Industry Concern:* **Selection of rates for Tier II.**

EPA Guidance:

- Subd-J: §122-2
- SEP: Non-Target Plants, Growth and Reproduction of Aquatic Plants - Tier 1, 30 July 1985
- SEP: Growth and Reproduction of Aquatic Plants - Tier 2, Draft
- FIFRA-TG: §122-2

Industry Assessment of Guidance: The guidelines suggest five rates in geometric progression in steps of 2X. At times, test rates in increments of 4X or grouping of test rates in appropriate increments to better define the EC_{25} are appropriate, depending on the slope of the dose-response curve. If steps of 2X were used, it would require a much greater number of rates to be tested, and would require much greater greenhouse space and resources to complete the test. These additional rates are not required, provided that the rates selected and the magnitude of the steps between rates adequately define the response curve. Industry believes that both EC_{50} and EC_{25} can be routinely determined.

Industry Assessment of Avoidability: Avoidable. Studies conducted in only 2X geometric increments can be conducted, but it is unlikely that they will improve the quality of data if the appropriate end-points can be calculated in a reputable fashion.

Industry Recommendations: Industry suggests that studies be accepted or rejected on the reliability of the study end-points. If concentrations other than 2X geometric increments appropriately define the study end-points, then the study should be deemed acceptable.

EPA Response: The Subdivision J guidelines clearly state that dosages in a 2X geometric progression be used. The Agency agrees with Industry with regard to this request. A number of waivers of the 2X requirement have already been given, however, the Agency must have a sufficient number of test dosages at 2X, 3X, or 4X to perform a proper statistical analysis. A preliminary range finding study is suggested prior to final rate selection. Studies will be rejected if there is not at least one dose greater than the EC50 value and one dose lower than the EC25 value.

2. *Industry Concern:* **Development of appropriate dose-response curves for each parameter tested.**

EPA Guidance:

- Subd-J: §122-2

- SEP: Non-Target Plants, Growth and Reproduction of Aquatic Plants - Tier 1, 30 July 1985
- SEP: Growth and Reproduction of Aquatic Plants - Tier 2, Draft
- FIFRA-TG: §122-2

Industry Assessment of Guidance: Study end-points are based on the most sensitive end-point measures. These are generally selected by observations made in a preliminary range-finding test. Quantitatively, the effect on shoot weight has typically been observed as the most sensitive measure, and provides the most useful and accurate dose-response curve. Due to variability introduced with root washing, this measure is believed to be less reliable. Shoot height and visual injury symptoms can be used, but these tend to be less quantitative. It is impossible to provide a dose-response curve on root inhibition that covers the EC_{50} and EC_{25} in most instances, since rates of compound producing these effects would have killed the plant through shoot effects. If a valid response curve is provided for the most sensitive of the parameters (e.g., shoot weight), it should not be necessary to do additional work to establish a complete response curve for all other possible measures.

Industry Assessment of Avoidability: Avoidable. As stated in the SEP, it is not always possible, nor is it useful, to evaluate an EC_{25}, EC_{50}, and a NOEL for all growth measures. A valid dose-response curve for the most sensitive measure should suffice to perform a risk assessment analysis.

Industry Recommendations: Industry recommends that the study end-points be based on the most sensitive end-point measure (shoot weight, if appropriate, based on observations in the Tier 1 tests) and if possible provide end-points for other plant growth measures when available. However, if other less sensitive end-point growth measures cannot be measured reliably, this should be stated in the report.

EPA Response: The Agency is in general agreement with the Industry recommendation. Study end-points were briefly discussed in the 1990 Corvallis Non-target Plant Testing Workshop. Another workshop was recommended by the Corvallis workgroup so that further discussion on this and other concerns could occur. The Agency recommends that a second workshop be conducted.

GUIDELINE §§122-2 and 123-2—AQUATIC PLANT GROWTH

1. *Industry concern:* **Registration and reregistration of several products, principally herbicides and fungicides, have been hampered when a complete battery of aquatic non-target plant studies [*Selenastrum capricornutum, Lemna gibba, Anabaena flos-aqua,* a freshwater diatom (e.g., *Naviculla pelliculosa*) and a marine diatom (e.g., *Skeletonema costatum*)] have not been submitted as part of the registration/reregistration package.**

EPA Guidance: Subd-J: §122-2

Industry Assessment of Guidance: More-specific guidance is needed to establish when non-target aquatic plant studies are required, and which species and tests should be used. Subd-J states: "Data on the toxic effects of a pesticide on growth and reproduction of aquatic plants are required by 40 CFR Part 158 on a case-by-case basis to support the registration of each end-use product intended for outdoor pesticide application." However, these tests now seem to be required routinely for all pesticides or at least for *all* herbicides and fungicides.

Industry Assessment of Avoidability: Avoidable

Industry Comments: Aquatic non-target plant testing should initially be limited to *S. capricornutum*, as suggested in the guidelines, or to another comparable internationally acceptable green algae (e.g., *Scenedesmus subspicatus*, *Chlorella vulgaris*). If the EC_{50} for *S. capricornutum* is greater than the EEC, then further testing on other species should not be required. Additionally, data generated by F.L. Mayer, EPA (Presentation to Aquatic Effects Dialogue Group, 1991) support the concept that testing *S. capricornutum*, along with other the aquatic toxicity tests (*Daphnia* and fish acute toxicity), is a reasonable approach to screen products for potential adverse effects to the aquatic environment.

Industry Recommendations: A tiered approach developed jointly by EPA and NACA should be used when requiring aquatic non-target plant testing, to determine if and when an entire suite of aquatic non-target plant tests is necessary.

EPA Response: Because of the potential for phytotoxicants to reach aquatic habitats, an analysis of the potential for adverse effects to nontarget and endangered aquatic plants is conducted. In order to perform such a risk assessment, the 5 above-referenced aquatic plant dose-response studies are required.

A number of issues are driving the need to improve our plant toxicity data base, and to clarify when and which studies are required for Section 3 and reregistration.

Required plant studies for new chemicals (Section 3 actions) should be submitted along with the required basic 6 ecotoxicology studies. The Agency data base is generally devoid of phytotoxicity data for insecticides, miticides, nematicides, lampricides, molluscicides, or piscicides. In the future, plant toxicity ecological risk assessments will be conducted for all pesticide registration and reregistration actions.

2. *Industry Concern:* **Study is rejected because it was performed according to OECD, TSCA, or Canadian guidelines, rather than EPA guidelines.**

Rejections are most commonly due to differences in cell inoculum levels (1×10^4 rather than 3×10^3 cells/ml), light intensity, study duration (96 hours rather than 120 hours for algae, and 120 hours rather than 14 days for *Lemna*), and/or growth medium.

EPA Guidance:

- Subd-J: §122-2
- SEP: Non-Target Plants, Growth and Reproduction of Aquatic Plants - Tier 1, 30 July 1985
- SEP: Growth and Reproduction of Aquatic Plants - Tier 2, Draft

Industry Assessment of Guidance: Studies conducted according to Canadian, OECD, TSCA, or FIFRA Subd-J guidelines all reach the same end-point: a 50% reduction in algal growth or *Lemna* frond development within a prescribed period of time. Differences in cell inoculum, light intensity, medium, and test duration were selected to reach a predetermined growth level within a specified time, and should have little effect on the end-point relative to the growth observed for the control group.

From a policy perspective, EPA has signed agreements with the European Community (EC) to mutually accept studies conducted according to mutually accepted protocols. This agreement should be acknowledged in order for FIFRA- conducted studies to be mutually accepted by the EC.

Industry Assessment of Avoidability: Avoidable

Industry Recommendations: To maximize the use of available data, reduce costs, and reduce duplication of effort, Industry proposes that studies performed under EC, OECD, Canadian, FIFRA, and other scientifically acceptable testing procedures be mutually accepted by EPA and other regulatory agencies around the world. Furthermore, if a study has already been accepted by one country, review time in another country might be reduced or eliminated.

EPA Response: The harmonization of Agency guidelines with Canadian and OECD guidelines is currently in progress at the Agency. In the meantime, the Agency will continue to evaluate each study on its individual scientific merits. With regard to inoculum levels, the Agency is currently allowing up to 10,000 cells at the beginning of any of our algae studies. The Data Evaluation Record (DER) for a given study contains a section called "Test Procedure," in which deviations from test procedures in Subdivision J guidelines or the SEPs are listed. The study is rarely rejected based on improper lighting, temperature, humidity, and other environmental factors alone, unless the control organisms are significantly affected.

Subdivision J guidelines clearly specify that algae studies must be of 120-hour duration. At the time that the initial guidelines were written, Industry provided comments on the draft guidelines. Industry, academia, and the Agency agreed to the 120-hour test interval for algae studies. Generally, it was believed at that time that the additional day was necessary to demonstrate recovery from initial toxic effects (if recovery was to occur). A 96-hour test is currently required by TSCA. The Agency would welcome a scientific analysis of studies in which 96- and 120-hour test results are compared for a number of chemical classes.

The American Society for Testing and Materials (ASTM) has published a protocol for a 7-day *Lemna gibba* study. Before adopting this 7-day standard, the Agency would again welcome a scientific analysis of studies that compare 7- versus 14-day test results for a number of chemical classes. Data in OPP files suggests that the 7- and 14-day results are not similar for most studies.

Additional scientific input is requested with regard to 96- versus 120-hour test intervals for algae and 7 days versus 14 days for *Lemna gibba*. These issues are topics for workshop discussion.

Resolution: Some effort is being made to harmonize with other guidelines. It was agreed that while the 120-hour test is preferred, if a valid 4 day study is submitted it will not lead to an automatic rejection. If the study does not serve the purpose for risk assessment, the Agency can ask for more information. NACA indicated it has no objection to the 14 day *Lemna* test.

3. *Industry Concern:* **Studies have been rejected or considered supplemental if a NOEL is not obtained.**

EPA Guidance:

- Subd-J: §122-2
- SEP: Non-Target Plants, Growth and Reproduction of Aquatic Plants - Tier 1, 30 July 1985
- SEP: Growth and Reproduction of Aquatic Plants - Tier 2, Draft
- FIFRA-TG §122-2

Industry Assessment of Guidance: Subd-J and the SEPs do not require that a NOEL be determined in the Tier 1 and 2 non-target plant toxicity studies. However, FIFRA-TG requires that a NOEL be reported in Tier 2 studies.

Industry Assessment of Avoidability: Not avoidable

Industry Comments: A NOEL cannot be routinely calculated from an EC_{50} study. The main purpose of an EC_{50} study is to determine a mean effect concentration for comparison to a control group. Typically, five treatment concentrations are used that will bracket the EC_{50}. It is often not possible to run a study where sufficient effects are observed to determine an EC_{50}, and also have a low enough concentration to determine a NOEL.

Industry Recommendations: The dose-response relationship generated in an EC_{50} study is generally adequate for determining an EC_{25}, as discussed in the guidelines, and this can be used for Ecological Risk Assessment and for protection of endangered species.

EPA Response: Subdivision J does require that the NOEL be reported for Tier II studies; however, in many cases the NOEL estimate is higher than the EC25 value. Because plant studies are subject to a high degree of biological variation due to differences in soil type, watering regimes, nutrients used, lighting, temperatures, greenhouses and growth chambers, seed sources, seed germination, and varietal or hybrid differences, the number of replicates must be increased from 3 to 5 to reduce this inherent variability. Further, all studies should have at least one dosage above the EC50 value and at least one dosage below the EC25 value. A no-effect concentration will become increasingly important in our risk assessments and in our "may affect" determinations for endangered/threatened species. When the expected down-wind concentration is matched with the plant no-effect values, the Agency should be able to derive a reasonable buffer distance for drift. The Agency sees merit in striving for the no-effect values, especially for low-dose herbicides, but will not invalidate studies when it cannot be obtained. Instead, we will now require that the EC50 value be determined for endangered species purposes. In the future, we expect to set bounds regarding acceptable and unacceptable confidence intervals.

The Agency is currently evaluating a recent publication of a statistical method for continuous biological data: Bruce, R.D. and D.J. Versteeg. 1992. A Statistical Procedure For Modeling Continuous Toxicity Data. Environmental Toxicology and Chemistry. Vol. 11, pp. 1485-1494. This statistical method is considered to be a more appropriate method than the currently used probit method.

4. *Industry Concern:* **Degradation of test substance during test period due to the presence of the algae or *Lemna* sp.**

EPA Guidance:

- Subd-J: §122-2
- SEP: Non-Target Plants, Growth and Reproduction of Aquatic Plants - Tier 1, 30 July 1985
- SEP: Growth and Reproduction of Aquatic Plants - Tier 2, Draft
- FIFRA-TG: §122-2

Industry Assessment of Avoidability: Not avoidable. Industry continues to develop compounds that complete their designated function and then degrade. Rejection of a study because the test substance is not persistent seems to contradict the goal of both EPA and Industry—that of protection of non-target organisms.

Industry Recommendations: Industry and EPA discussed this problem under NACA's ERAWG, and agreed that the test solutions should be analyzed both before and after completion of a study, and that the EC_{50} should be determined based on the measured initial concentrations, not the concentration after the study. Industry strongly supports this previous conclusion and requests that EPA incorporate this into its guidance.

EPA Response: The Agency will abide by the mutually agreed-upon conclusion of the NACA/EPA guidance document.

Additional Discussion and Resolution:

There were several discussions concerning the draft Non-Target Plant testing scheme proposed by the Agency under 40 CFR Part 158. Following is a summary of the resolutions of those discussions. It was suggested that several of these issues be further addressed by a work group.

1. **Discussion and Resolution:** Minimum USDA standards for seed germination will be used to determine seed viability. Separate testing of viability rates is not necessary. It was agreed that the final document will contain the standard USDA numbers for all ten selected terrestrial species. NACA will check to see if USDA standards can be met with non-treated seeds.

2. **Discussion and Resolution:** In the discussion of testing with TEP plus adjuvants, it was decided that only a single representative adjuvant (designed to increase efficacy) recommended on the label requires testing. No testing of possible interaction between the adjuvant and the TEP is required. Testing requirements when more than one class of adjuvant is recommended (anionic, ionic, non-ionic, etc.) were not resolved and should be further discussed in a work group setting.

3. **Discussion and Resolution:** It was clarified that this document is NOT requiring native species testing be initiated. This issue should be referred to a work group/workshop.

4. **Discussion and Resolution:** During the discussion of phytotoxicants and non-target plants, it was decided that all phytotoxicants (not just herbicides) should be subjected to Tier II terrestrial testing.

5. **Discussion and Resolution:** It was clarified that all aquatic non-target plant testing could be completed with TGAI.

6. **Discussion and Resolution:** The possible use of efficacy data to determine terrestrial EC_{50} phytotoxicity data for compounds non-phytotoxic substances was discussed. It was decided that because this data is generally not collected under GLP, efficacy data would be evaluated as support information but would be considered supplemental for risk assessment purposes. This issue should be further discussed in a work group setting.

Summary Table of Rejection Factors

GUIDELINE	REJECTION FACTORS
Avian Oral LD_{50} 71-1	-Failure to establish a valid LD_{50} value with corresponding 95 % confidence limits or an LD_{50} greater than 2000 mg/kg. -Use of a "split-dosing" procedure. -Fasting period prior to dosing not specified.
Avian Dietary LC_{50} 71-2	-Mortalities attributed to interactions between animals (rather than to test chemical), when such interactions were not observed in the controls. -High variability in the measured test concentrations in the test diet. -Test material was not technical grade. -LC_{50} not established when testing at dose levels < 5000 ppm a.i. -Variation in test concentration and/or failure to adequately justify the variations.
Freshwater Fish LC_{50} (Bluegill) 72-1	-Concentration level < 100 mg/l, not high enough to produce an LC_{50}. -Aeration of test chambers. -Biological loading of test vessels twice the recommended amount. -Test substance purity not identified. -Inappropriate test species and/or test species not clearly identified. -Fish fed during the exposure period. -Minimum limit of detectability, or the minimum quantifiable limit, not defined quantitatively. -Test concentrations variability limit > 1.5. -Levels of lead, iron and aluminum present in dilution water higher than recommended. -No solvent control. -Results for some test concentrations obtained from tests conducted after the definite study. -Not all test solutions measured at 96 and 0 hours. -No control group for the inert/carrier ingredient component of the formulation. -Acclimation period half that recommended.

GUIDELINE	REJECTION FACTORS
Freshwater Fish LC_{50} (Rainbow) 72-1	-All of the rejection factors listed above for guideline 72-1, Bluegill. -Fish acclimation period overlapping with the definite study period. -Contamination of the controls with test chemical. -Low recovery of test chemical from the stock solutions. -Fish weights exceeding the recommended range. -Test temperature exceeding the recommended for the test species. -Biological loading of the system greater than recommended. -Fish mortality during the acclimation period higher than recommended. -Supersaturation of oxygen.
Acute LC_{50} Freshwater Invertebrates 72-2	-Organisms not randomly distributed to test vessels. -Water temperature not monitored. -Chemical analyses (concentration levels) not performed on test solutions. -No control group for the inert/carrier ingredient component of the formulation. -Minimum limit of detectability, or the minimum quantifiable limit, not defined quantitatively. -Test concentrations variability limit > 1.5. -Not all test concentrations measured at 0 and 48 hours. -Levels of lead, iron and aluminum present in dilution water higher than recommended. -Percent of a.i. of the test formulation not identified. -Photoperiod not as recommended. -Raw temperature data not provided. -Use of dechlorinated water as a portion of the dilution water.
Wild Mammal Toxicity 71-3	-Diet preparation method not adequate. -Improper animal caging, as indicated by extensive cannibalism.
Avian Reproduction Quail 71-4	-Percent of cracked eggs in the control higher than in treatment groups. -Data discrepancies: =inappropriate photoperiod; =reasons for administration of medication not provided; =total number of data points not included in statistical evaluation.
Avian Reproduction Duck 71-4	-Data discrepancies: =inappropriate photoperiod; =inappropriate egg collection procedures; =low overall fertility of control birds.
Acute LC_{50} Estuarine and Marine Organisms Fish 72-3D	-Unexplained variations in concentrations. -Concentration level < 100 mg/l, not high enough to produce an LC_{50}. -Dissolved oxygen levels lower than recommended. -Analytical determination of the concentration in the test vessels not provided.

GUIDELINE	REJECTION FACTORS
Acute LC_{50} Estuarine and Marine Organisms Mollusc 72-3B and E	-Insufficient new shell growth in control oysters. -Insufficient dosage levels to produce a reliable LC_{50}. -Raw data on shell deposition not provided. -Aeration of test chambers without chemical analyses of test solutions. -Dissolved oxygen levels lower than recommended.
Acute LC_{50} Estuarine and Marine Organisms Shrimp 72-3C	-Test substance purity not identified. -Chemical analysis of test solutions concentration not performed. -Type and quantity of solvent used not provided. -Solubility needed to achieve LC_{50} not obtained.
Fish Early Life Stage 72-4	-Mortality too high at all concentrations. -Raw data not submitted. -Survival rate in the control group lower than recommended. -Erratic results in measured test concentrations.
Aquatic Invertebrate Life Cycle 72-4	-Raw data not submitted. -NOEL values for reproduction and growth cannot be established from study results. -Survival rate in the control group lower than recommended. -Adults' growth (length and weight) not measured quantitatively.
Invertebrate Life Cycle Estuarine Species 72-4	-Reproduction rates too low to be statistically analyzed. -Adult body lengths not measured at the end of the study. -Feeding rate below the recommended daily ration. -Raw data not provided.

Unresolved Issues
In Order of NACA's Priority for Resolution

Study	Issue	Action/Resolution
1. Terrestrial and Aquatic Non-Target Plants	General testing methodology and risk assessment procedures	Some issues resolved, others remain open; work group may be necessary to resolve remaining issues.
2. Avian Risk Assessment	Exposure assessment issues	NACA proposes work group formation to address avian issues.
3. Fish Full Life Cycle	Methodology for study conduct	NACA proposes work group to address issue.
4. Oyster deposition	Shell deposition of 2 mm	Temporary solution in place. Follow-up needed for review of historical growth information.
5. Oyster Larvae	Acceptable Control Mortality	Evaluation of historical survival data.
6. Shrimp Acute	Age of test organisms	Evaluation of data.

Conclusions

The overall rejection rate for ecological effects has shown improvement, having declined from a pre-1986 rate of 36 percent to the current 20 percent. Four acute toxicity guidelines have shown consistent improvement: avian oral (71-1), trout (72-1C), *Daphnia* (72-2), and mollusk (72-3B). Despite this encouraging individual progress, however, the aggregate rejection rate for ecological effects remains unacceptably high.

Avian and aquatic reproduction studies have current rejection rates near or above 40 percent—a clear indication that serious problems exist. Two other studies (both of which are combined with 123-1A, Tier II nontarget terrestrial plant phytotoxicity) with rejection rates over 40 percent are seed germination/seed emergence (122-1A) and vegetative vigor (122-1B). Plant toxicity testing requirements are relatively new compared to animal testing requirements. The number of rejected studies is expected to sharply diminish as testing laboratories become more familiar with OPP data requirements and guidelines. Further, a number of laboratories have only recently (within the past two years) begun to conduct plant studies.The following studies have rejection rates of substantially less than 40 percent, but over time their rejection rates have not improved significantly: bluegill acute toxicity (72-1A), marine fish acute toxicity (72-3A), shrimp acute toxicity (72-3C), invertebrate life cycle (72-4B), and avian dietary (71-2B).

Progress on rejection rates for Tier I avian and aquatic studies is therefore mixed. The avian Tier I studies have lower rejection rates than their aquatic counterparts, but the avian dietary guideline has not shown significant improvement. Several aquatic Tier I guidelines have shown improvement, but for the most part their rejection rates remain high.

With rejection rates greater than 30 percent, the Tier II reproduction guidelines for both aquatic and avian species have higher rejection rates than the Tier I guidelines. Rejection factors common to the aquatic and avian reproduction guidelines include data discrepancies and lack of tabulated and unanalyzed data submission.

In 1982, the Environmental Fate and Effects Division (then called the Hazard Evaluation Division) issued three Pesticide Assessment Guidelines on ecological effects. The Division also issued six Standard Evaluation Procedures during the period 1985-1986. Appendix B gives a complete list of the ecological effects guidance documents.

The Agency's dedication to reducing ecological effects rejection rates goes beyond improving its guidance. When future studies are rejected for factors where the Agency believes its guidance to be adequate, and where it deems the factor avoidable, regulatory action may be appropriate.

EPA is confident that this assessment will accelerate the downward trend in the ecological effects rejection rate. This cooperative effort between EPA and Industry scientists furthers OPP's goal of meeting its production schedule for REDs.

Recommendations

As a result of the rejection rate analysis and the ensuing discussions with Industry, both EPA and Industry realize the need for more in-depth follow-up on various ecological effects data requirements. In order of priority for resolution are:

(1) Terrestrial and Aquatic Non-target Plants
(2) Fish Full Life Cycle
(3) Oyster Deposition
(4) Oyster Larvae
(5) Shrimp Acute Toxicity

Industry/EPA work groups have been proposed to resolve these critical issues, and EPA intends to address them in priority order as resources permit.

Finally, SRRD intends to continue tracking rejection rates for ecological effects guideline studies. If significant reductions in the rejection rates for these studies are not realized, further regulatory action may be required.

Appendix A: Additional Supporting Documents

CONDUCTING ACCEPTABLE AQUATIC LAB STUDIES:

PROPOSED GUIDANCE

INTRODUCTION

The primary goal of this proposed guidance is to provide registrants with more detailed information on how to design and conduct aquatic laboratory studies for pesticide risk assessment. It should be noted that this document is a "working" proposed guidance which may be subject to further scientific review within the Agency, and that no new policy is being introduced at this time. This guidance emphasizes the importance of adequate characterization of the test material and proper understanding of how the material reacts under test conditions. This document also attempts to:

1. Provide guidance to help pesticide registrants develop the best toxicity information possible, taking into consideration any constraints in the experimental design and test material: information that is scientifically defensible and protective of human health and the environment.

2. State EFED's position on specific areas of concern. In this guidance, provisions are made for exceptions, while at the same time maintaining a high level of scientific integrity.

3. State EFED's position and reasonable approach for enforcing testing criteria, limits and standards. Standards are set with the recognition that certain problems will arise and provisions must be made to accommodate unavoidable problems.

4. Interpret those areas that need to be defined and set limits for designing and conducting laboratory studies.

DEFINITIONS AND SITUATIONS

Analysis, 0-Hour

In static or static renewal tests, when 0-hour measurement is indicated, it is assumed that the method of mixing the test material in the test solution allows adequate time for complete dissolution equilibrium such that these initial measurements accurately reflect exposure throughout the test container. The registrant may need to justify an exception from the 30-minute requirement for adding test organisms if the characteristics of the test material and test system require a longer equilibrium time. If preliminary trials have been done, this delay should be predictable.

In flow-through tests, the study should be conducted with knowledge of the time it will take for the test material to reach equilibrium or steady-state in the test container. Initiation of the test and scheduling the sampling times must be based on this information. In some cases, a flow-through system may have to be run for an extended-time "pre-test" to try to achieve equilibrium or steady-state. If equilibrium or steady-state cannot be achieved, and/or it appears that the measured concentrations will be substantially below (<70%) nominal, the study report should reflect that the laboratory was aware of this problem. The study report should clearly identify the problem, indicate the steps taken to mitigate it and justify the study design and dosing levels. However, if sufficient analytical methods are available and acceptable toxicity data are produced, additional testing and evaluation with the sole objective of obtaining initial measured concentrations greater than 70% of nominal, will not be required.

ASTM Recommendations

In many cases, ASTM guidance makes recommendations based on "when needed" or "when necessary." When referring to testing performed to support pesticide risk assessment, EFED is responsible for defining "when needed" or "when necessary".

Chemistry Method

When a chemistry method is used in preliminary trials, in range-finding tests, in establishing percent purity of batches of test material, or in measuring concentrations in test containers, it must be submitted with the study or referenced by MRID #. The documentation must include a complete description of the method so that a bench chemist can determine the necessary equipment and perform the analysis. It must also include the raw data, standards and chromatogram from a representative analysis using the method. This representative analysis must be conducted with the specific media for which it will be used during the test; i.e., under test conditions. The actual minimum detection level and level of quantification must be identified.

GLP Standards

According to GLP regulations, tests not conducted according to those regulations should not be used to support permits such as the registration of pesticides. Among the specific GLP requirements are that the test material's solubility and stability be known and that chemical analysis of the batch test material be performed. Determining the solubility and stability of the test material in the mixture or test solution is an important part of these studies.

Nominal Concentration

For aquatic tests, the nominal test level is the concentration that would occur if all test material added to the test solution were completely dissolved and did not dissipate in any way.

EFED Recommended

Recommended means that the procedure or test is preferred in order to avoid problems, but it is not required. If the recommended procedure or test is not performed, the study will not necessarily be rejected.

Renewal Cycle

Static renewal is one method to ensure relatively continuous concentrations when the test material is not stable under test conditions. At a minimum, the renewal cycle[1] should be based on the stability of the test material under test conditions. The time to renewal (renewal cycle) should be shorter than the time it took for the concentration of the test material to decline to <70%.

Replicates and Concentration Measurement

When replicate test containers and measurement of test concentration are required, each replicate in each test concentration must be analyzed separately. This is because the responses in each replicate are viewed as independent and it is necessary to know what the concentrations were so variation can be determined. Exceptions to this occur when:

1. Treatment replicate containers under static tests or static renewal conditions are filled from a bulk preparation. In this case, only samples from the bulk supply for each test level must be analyzed; or

2. A "splitter" is used in a flow-through test to feed more than one replicate. In this case, only samples from one replicate per treatment level require analysis. It is recommended that samples be collected from all replicates and be stored, in case anomalous concentrations are measured in the one that is analyzed. Analyzing the other replicates may shed light on the cause and extent of the anomalous measurements.

[1] The renewal cycle may be shorter than required by stability characteristics of the test material because of other factors, such as dissolved oxygen, feeding, etc.

Replicates receiving flow from a splitter should be sampled and analyzed alternately. In other words, if there are two replicates (A and B), replicate A should be analyzed in the first week and replicate B in the second week, etc.

Average concentrations of replicates are used in regression analysis.

Stability

A test material is considered to be stable under test conditions if, under those conditions, it does not degrade, volatilize, dissipate, precipitate, sorb to test container walls, or otherwise decline to concentrations less than 70% of the day-0 measured concentration during the study period. If it is expected to decline to less than 70% of the day-0 measured concentration during the study period, either static renewal or flow-through design is needed to try to ensure that the test concentration is maintained at levels greater than or equal to 70%. The only exception is testing with algae and diatoms, which cannot be tested in static renewal or flow-through systems (see discussion on testing with algae and diatoms).

Sample Storage

If samples of growth medium, stock solutions, or test solutions collected for chemical analysis cannot be analyzed immediately, they should be handled and stored appropriately to minimize loss of the test material. Loss could be caused by such processes as microbial degradation, hydrolysis, oxidation, photolysis, reduction, sorption, volatilization, etc. Stability determination under storage conditions, whether it refers to storing the test material before testing or storing samples awaiting analysis, is required by GLP regulation.

Under Test Conditions

The behavior of a test material should not be based on experiments which are conducted under conditions different from those occurring during the test. When there is a problem with chemical solubility and/or stability, the behavior of a test material should be determined under test conditions or those conditions that are likely to affect the behavior of the test material. These include but are not limited to:

- test solution characteristics (salt or freshwater)
- temperature, pH, conductivity, lighting
- with test organisms in place
- use of the same test containers
- use of the same flow-through systems where appropriate

Variability in Measured Concentrations

The goal for limiting variability of measurements between replicates of the same concentration, and over time in the same concentration, is maintaining the ratio of the highest concentration to the lowest concentration at 1.5 or less. A test may be rejected if variability exceeds this amount.

An important factor in considering the limits of variability is the avoidance of overlapping mean test concentrations between test levels. High variability puts into question the reliability of the environmental chemistry method and/or the concentrations on which to base statistical analysis and toxicological conclusions. If variability beyond the 1.5 ratio occurs, an exception to it should be justified.

This justification should clearly state the problem, explain why it occurred, provide scientific justification, and identify all measures taken to mitigate the problem. The justification also should include the fully developed chemistry method, including the documentation necessary for a bench chemist to review and evaluate it.

For cases in which variability problems are suspected, registrants wishing to avoid possible rejection of a study are strongly advised to conduct extensive preliminary trials. If it becomes clear that high variability cannot be avoided, an exception should be justified.

It is recommended that any justification be provided in advance. EFED scientists will decide on the validity of the rationale for the exception, and possibly recommend other methods to reduce potential variability.

PRELIMINARY TRIALS

EFED recommends that more effort be expended in designing acceptable studies before they are conducted, especially for problem chemicals. Subpart F (S 160.105 of 40 CFR), Good Laboratory Practice Standards, requires that certain information be known before beginning toxicity tests used to support permits by EPA. The information indicated as necessary in the GLP guidance should be developed under test conditions. This information can be gained while doing the currently required range-finding studies. Below is a list of recommended preliminary tests:

A. Stability trials should be conducted under test conditions. These trials must be documented and submitted to EFED for review with the study to which they apply.

B. Solubility trials should be conducted under test conditions. These trials must be documented and submitted with the study to EFED for review.

C. If solubility is a problem (<100 ppm), trials should be conducted under test conditions using various solvents that are most likely to be effective and that are widely recognized as being nontoxic and other means to ensure that the appropriate methods are used during the laboratory tests to enhance solubility. Once a solvent is chosen based upon more simplistic, comparative evaluations, the decision should be confirmed in the range-finder preliminary trials with only that solvent.

D. Chemical Analysis Methods used during laboratory studies must be provided (or referenced by MRID #) in the laboratory study. The submitted documentation should specify the LOD and LOQ, and the precision, based on trials conducted using the Method with samples representing test conditions. The description of the chemical method must be in sufficient detail that a chemist could evaluate the method.

E. Stability of the test material in the samples to be collected for chemical analyses should be determined during the laboratory studies. This includes determining whether and how samples can be stored for future analysis.

Laboratory studies must be designed taking into account this preliminary information. This means the above trials are to be conducted before the definitive laboratory studies are initiated.

Publications, such as ASTM, rarely specify exactly how a study should be designed in the detail necessary to be used as "cookbook protocols." Suggestions are provided for study designs with qualifying phrases such as "as needed" or "if necessary." This implies that the behavior of the test material under test conditions is known and assumes that the best methods and procedures will be chosen by the laboratory doing the study.

CONFIRMING EXPOSURE WITH ANALYSIS

Acute Testing

Acute Static Tests: Except for acute aquatic algae and diatom studies (122-2 and 123-2, which can only be conducted as static tests), acute static tests may be conducted only if, among other things, the test material has been shown to be stable under the

test conditions, as defined above.[2] In an acute static test with a test material that is stable and readily soluble at the treatment levels, measurements of each test concentration are not absolutely required. However:

1. ASTM guidance indicates that for static tests, if possible, the concentration of toxicant should be measured at the beginning and end of the test in all test chambers.[3] Further, measurement of the toxicant's degradation products is desirable, but not required.

2. The study may be rejected if the following occurs: 1) the test material was not stable under test conditions, 2) precipitates formed, or 3) solubility was likely to have been a problem at the levels tested. However, if the recommended chemical measurements were made to verify exposure levels, then the study may not be rejected. Whether the study design was modified in a scientifically defensible attempt to accommodate these chemical characteristics will also be considered.

If variability is expected to be a problem, it is recommended that measurements of test concentrations be made at each test level at 0-hour, 48 hours and, for tests longer than 48 hours, at test termination. Replicate test containers should be measured separately, except as explained above.

Acute Static Renewal: See the general discussion of replicates above.

If a static renewal test is conducted, each test chamber must be sampled for chemical analysis at the 0-hour, at the end of the first (or longest) cycle, and at test termination. It is recommended that measurements be made at the end of each renewal cycle.

[2] Other factors not addressed in this guidance may preclude conducting a static test even if the test material is stable under test conditions conditions. These include, but are not limited to, problems in maintaining dissolved oxygen levels, feeding requirements, and concern for bacterial/microbial contaminants.

[3] EFED certainly considers it "possible" to measure concentrations of toxicants. Increased expense does not make it impossible.

Acute Flow-Through: See the general discussion of replicates above.

If a flow-through test is conducted[4], each test concentration must be measured at the 0-hour and at test termination. It is recommended that for 96-hour tests, an intermediate measurement be made at 48 hours to verify mid-test exposure if variability is expected to be a problem.

Chronic Testing

Chronic Static Renewal: See the general discussion of replicates above.

Concentrations must be measured at each test level at 0-hour, at the end of the last renewal cycle (at test termination), and at the beginning and end of an intervening cycle at least once per week. The longest cycle in a sequence should be used if variable-cycle periods are employed.

Chronic Flow-Through: See the general discussion of replicates above.

In each concentration, measure at 0-hour, every 7 days, and at test termination. At the beginning of a study, the exact flow of the system and water output at each splitter must be documented. In addition, system flow must be metered and monitored visually or mechanically on a daily basis (every 24 hours), and it is recommended that the system flow be metered and monitored twice a day (approximately every 12 hours). Measurement of test concentration is required each time metering fluctuation or malfunction is detected or observed. A record of the regular inspections must be maintained and provided with the study report.

[4] All acute aquatic algae and diatom tests must be conducted as static. Flow-through and static renewal systems are not recommended for these test, since they are conducted with microscopic organisms that cannot be protected from loss when renewing or draining water from the test containers. Static tests for Lemna gibba can be conducted, regardless of stability.

TOXICITY TESTS WITH POORLY SOLUBLE MATERIALS

Existing OPP guidelines for aquatic toxicity tests require that chemicals be tested up to a maximum dissolved concentration of 100 ppm (mg/l) in an effort to obtain an LC_{50} or EC_{50}. Current policy allows chemicals that are poorly soluble (solubility < 100 ppm) to be tested up to the maximum water solubility obtainable for the given test conditions employed, provided that certain prerequisites apply:

1) The technique used to maximize chemical dissolution in the test media under standard conditions for the test is justified. Consideration of the optimum technique should include use of non-toxic solvents, saturation (solubility) columns, sonication, minor adjustments to environmental conditions (i.e., temperature, pH, etc.), etc., as appropriate. Minor adjustments should not extend outside the recommended range of conditions for the specific test organism.

2) Testing with a more soluble formulation (e.g., emulsifiable concentrate), if one exists, is provided in addition to testing with the technical-grade material. Testing with a more soluble formulation will not be required if it does not increase the solubility by 2X.

3) Measured concentrations of test media at appropriate intervals and from appropriate test chambers of all test levels are determined from centrifuged supernatant.

Studies that involve radical changes in environmental test conditions outside the recommended range of values for temperature, salinity, pH, etc. will be considered on a case-by-case basis.

Solubility is defined as the amount of chemical retained in the supernatant of a conventionally centrifuged sample of test media. This amount of test material is considered to represent a conservative measure of the most bioavailable fraction which may include some colloidal material not removed by centrifugation in addition to the truly dissolved fraction. A condition or mechanism is only considered to have increased solubility if the increase is two times or greater.

OTHER ISSUES OF SPECIAL CONCERN

Position on Solubility Enhancement

Saturator columns: The use of saturation columns as an aid in the dissolution of test material and in confirming maximum solubility is recommended but not required for nonvolatile test chemicals with test media solubilities of 10 ppm or less. Methods for using these columns in aquatic toxicity tests can be adapted from the methods established for their use in determining water solubility under OECD's Column Elution Method (Ref. No. 105). Saturator columns will be considered to generate test solutions for static studies and not for flow-through studies. Furthermore, saturator columns for static studies need only be considered if conventional techniques for dosing the water do not result in water concentrations within 2X of the stated solubility of the compound.

Emulsifiers and Formulation Testing: Testing with a more soluble formulation, if one exists and which may contain emulsifiers, dispersants, solubilizing agents, etc., is required for all active ingredients subject to aquatic organism testing and having a water solubility less than 100 ppm and less than an EC/LC_{50}. A defined EC/LC_{50} provides a greatly improved basis for risk assessment.

Effect of Temperature: Solubility is a function of temperature and is especially sensitive at the limits of solubility. It is recognized that increases of as much as 10 degrees may affect the solubility no more than a factor of two. However if test solutions are close to saturation, small changes in temperature may result in supersaturated solutions. In addition, control of temperature is important because of its well-known effects on the actual toxicity of the compound.

Centrifugation: Conventional centrifugation is required for all test media where undissolved test material, precipitate, flocculant, colloidal suspension, etc., is/are observed in the test chambers or where the solubility and hence bioavailability are in question. Filtration may be used instead of centrifugation if the analytical method is validated over a range of acceptable concentrations. For aquatic toxicity tests, EFED defines solubility as the extractable chemical in the centrifuged or filtered supernatant.

Position on Nominal Versus Measured Concentrations

When a laboratory test design has been specifically modified to accomodate the instability of test material or other factors likely to cause variability in test concentrations, and the design is judged adequate based on sufficient preliminary

information[5], then the study will not be rejected solely on the grounds that measured concentrations varied by more than 30% of the nominal concentration. An increase in measured test concentration of more than 30% from the nominal concentration during the test will generally not result in rejection, provided that the following conditions are met:

1. a reasonable and scientific explanation is given, and the variability of results produced by the chemical analysis method is adequately characterized.

2. all test containers exhibit a similar (but not necessarily identical) shift[6],

3. the variability of the measured concentrations is acceptable,

4. a statistically valid endpoint can be derived from the measured concentrations[7], and

5. the preliminary stability information is provided with complete documentation and description of methods used to derive such information.

When High Variability Cannot Be Avoided

In some cases, high variability cannot be avoided because the test concentrations are approaching the limit of detection or because of unavoidable binding of the test material to the chemical analysis apparatus. When the ratio of the highest

[5] This assumes that the preliminary stability tests were conducted under test conditions essentially identical to the actual test conditions.

[6] If concentrations in some containers go up substantially (>30%) and test concentrations in other containers go down substantially (>30%), they will not be considered to have exhibited a similar shift. The most important criterion is that test levels must not experience a shift in "order." That is, the highest test level should remain highest, the next should remain second, etc. If orders are shifted, the test may be rejected, since regression analysis would not yield statistically sound median lethal concentrations and confidence limits.

[7] Either an LC_{50}, EC_{50}, or that the LC_{50} or EC_{50} is greater than 100 ppm.

concentration to the lowest measured concentration is expected to vary by more than 1.5, the registrant is strongly advised to justify an exception to this requirement in advance of conducting the aquatic laboratory studies. This exception justification should consist of:

1. documentation of the preliminary trials indicating this problem;

2. the specific steps that will be taken to reduce the variation;

3. the fully developed chemical analysis method; and

4. the raw data, standards and chromatogram from a representative analysis using the method. For each chemistry method, the actual minimum detection level and level of quantification must be identified.

EFED will decide on each exception justification on a case-by-case basis. However, if a series of aquatic tests are to be conducted with one chemical and it is anticipated that these limits will be exceeded, one exception justification may cover more than one study. EFED will then exercise judgment in evaluating studies with test materials that are difficult to measure.

Test Material Decline in Algae and Diatom Tests

Conducting flow-through or static renewal tests with aquatic algae and diatoms is not feasible with the current state of the practice. Therefore, the following is recommended for a test material that, based on preliminary stability testing, is expected to degrade to less than 70% of the nominal concentration. The study should be conducted normally, with concentrations measured at 0-hour and at test termination. Although it is undesirable to allow the concentrations to decline throughout the study, the problem may be unavoidable. In this case, the LC_{50} regression analysis is based on 0-hour test levels.

For purposes of consistency, the aquatic test with a vascular plant (Lemna gibba) need not be done using a flow-through or static renewal system with the sole purpose of maintaining test concentrations. There may be other reasons for conducting a static renewal study.

Appendix

Why Require Measured Concentrations Instead of Nominal Concentrations?

Where measured concentrations are indicated, they are considered necessary because:

1. There are concerns that the actual concentrations to which the test organisms are exposed may be different than "nominal." This variation may be due to chemical characteristics, test conditions or mechanical apparatus.

2. Measured concentrations confirm that the test system was designed appropriately and is operating acceptably. Characteristics that make testing difficult (low solubility, short half-life, high binding potential, etc.) must be accounted for in the exposure estimates (see below). They are not a reason for developing misleading toxicity values from laboratory tests.

Measurement of test concentrations is not done <u>just</u> to determine if the technician knows how to mix the test solution once. Among other things, it also ensures that the test solution was mixed correctly each time: It corroborates the precision of the technician or mechanics of the test system.

If test levels are not measured, the nominal values are used to calculate the LC_{50}, EC_{50}, NOEL and LOEL. If the test material has degraded or has become unavailable because of insolubility or sorption, the pesticide may be characterized as less toxic than it really is. For example, if based on nominal test levels, the LC_{50} is 5 ppm, the pesticide would be considered moderately toxic. No higher tier testing would be required and that value (5 ppm) would be the basis for developing concern levels with which to compare EECs. But if, in reality, the concentrations to which the organism was actually exposed were only between 0.1 and 1 ppm, the LC_{50} may well be closer 0.5 ppm. This would result in labeling, and could trigger higher tier tests. More importantly, it would yield substantially lower concern levels with which to compare exposure levels.

Why Use Measured Concentrations When They Are Available?

Measured concentrations are used when they are available because they indicate what the exposure was in the test chambers.

Why Be Concerned with Pesticides That Are Difficult to Test?

Presumably, a safer pesticide is one that that may degrade rapidly, has low solubility, and is used at low rates. While these characteristics may result in lower exposure levels in the field, the risk they represent can only be determined if the

actual toxicity of the pesticide is known[8]. When potentially low, realistic exposure levels are calculated and used for risk assessments, it is imperative that the actual toxicity of the pesticide at those levels be determined. If the test were conducted using nominal concentrations, the results could reflect a higher apparent effect concentration (e.g. LC_{50}, EC_{50} or NOEL, as discussed above). As a result, potential risk may be missed because the comparison would be between a low "realistic" exposure and a high nominal test level that was not the true toxicity level. A risk assessment based on such a comparison and data would be faulty and could not be scientifically defended.

Pesticides that are used at very low levels tend to have high biological activity. For this reason, it is imperative that the toxicity data developed for these pesticides be accurate and scientifically defensible.

Why Analyze at Test Termination?

Where indicated, measurement at test termination is considered necessary to determine if the test organisms were exposed to the test material throughout the entire study and at what levels. A significant change in test concentration during the last part of the study may substantially alter the results. For example, if the test concentration dropped dramatically during the last few days of a study, the effects that may have been caused by such exposure may not occur. The EC_{50} or LC_{50} developed from that study would be midleading if it is called a "96-hour LC_{50}".

Test endpoints are used as if the organisms were exposed to the test material at the statistically developed value (LC_{50} or EC_{50}) for the entire test duration. Granted, one aspect of the risk assessment is to compare concern levels based on LC_{50}s to initial immediate concentrations. However, it is assumed that field conditions exist in which concentrations that may be of acute concern may last longer or occur frequently enough to be comparable to the 48-hour, 96-hour or 120-hour test duration. Even though a pesticide may degrade rapidly under one condition (in water, for example), the possibility of repeated exposure needs to be considered. Repeated loadings from reservoirs of the active ingredient, occurring in compartments where persistence is greater, may occur. Without taking into account these eventualities, EFED would not be able to generate risk assessments that adequately protect the aquatic ecosystem.

[8] Or at least know with high certainty the level below which the pesticide is not likely to result in 50% mortality (i.e., an "LC_{50} > X concentration" situation.

Costs to Achieve Current Test Standards

The costs of developing chemical analysis methods, performing chemical analysis in most toxicity tests, conducting range-finding tests and preliminary trials, and measuring the active ingredient in batch supplies of the test material should not be considered an extra cost. These activities are part of the overall test procedure, and have been included in our cost estimates.

The GLP requires that batch mixes be analyzed and that solubility and stability be known. Both require methods development. Therefore, the cost of methods development should not be factored into the additional chemical analysis that may be recommended to do scientifically sound and acceptable studies for pesticide risk assessment.

Cost estimates provided by NACA did not include cases in which the pesticide active ingredient does not cause mortality at maximum practical solubility (see solubility discussion). In these cases, only one treatment level is required with 30 test organisms. If it can be shown that no mortality occurred throughout the test period, the LC_{50} is assumed to be greater than that concentration and no more testing would be required. The fact that this may occur should be known from range-finding tests. In such cases, the cost for chemical analysis would be reduced dramatically because there would be fewer samples to analyze. Adequate preliminary trials to determine the behavior of the test material under test conditions could avoid unnecessary sampling and analyses.

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

OCT 29 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Reevaluation of Previously Rejected
Mollusk Shell Deposition Studies 72-3[b]

FROM: Paul F. Schuda, Deputy Director
Environmental Fate and Effects Division H7507C

TO: Daniel Barolo, Director
Special Review and Reregistration Division H7508W

Lawrence E. Culleen, Acting Director
Registration Division H7505C

SUMMARY

Mollusk shell deposition studies (72-3[b]) that were rejected because of inadequate amount of shell growth in control organisms will be re-examined to determine if the study has to be repeated. The need for a certain minimum amount of shell growth in the control mollusks is a relatively new requirement, of which the registrants have not been officially notified. This memorandum presents the criteria for when the EFED will, or will not recommend that the rejected mollusk studies be repeated. It also explains what the new criteria are and how they will be promulgated.

BACKGROUND

One of the 3 studies used by EFED to characterize potential toxicity of a chemical to estuarine and marine species is the 96-hour mollusk shell deposition study. This study is required for a pesticide when significant exposure in estuaries or marine habitats is expected. This study depends on a reduction in the shell-growth of mollusks in response to exposure to various concentrations of a pesticide compared to the shell-growth of control mollusks in clean water. In the 72-3 guidelines and in the Standard Evaluation Procedure (SEP) for this study, no minimum amount of shell growth was established for the control mollusks (those exposed to clean water). It was stated that 1 mm per day could be expected from healthy mollusks, but that was not established as a criteria for rejection.

Since the SEP's were published, the EFED scientists have received information from experts in the field of estuarine organism testing indicating that there should be a minimum amount of shell-growth that occurs in the control to make the study acceptable. With inadequate shell-growth of the control organisms, these experts believe there is a likelihood that reductions in the shell-growth of the mollusks exposed to the pesticide may not be detectable and the resulting EC50 developed from such a study may not reliably indicate toxicity. In other words, the poor performance of the control mollusks could mask affects on shell growth caused by the pesticide. The consensus of the experts is that 2 mm of shell growth in a 96-hour period is easily obtainable and is the minimum they would allow for a valid comparison with responses from mollusks exposed to pesticides.

PROPOSAL

The EFED is proposing to implement the requirement that, for a mollusk shell deposition study to be acceptable, the organisms in the clean water control must produce a minimum average of 2 mm of shell growth during the 96 hours. The EFED will present this proposal to the Science Advisory Panel (SAP). With the comments from SAP, the EFED will formally amend the SEP and notify the registrants of this change.

IMPLEMENTATION

The EEB has begun incorporating this change into its evaluation criteria and has, in the past few years, rejected several studies solely because the average shell growth in the clean water control was less than 2 mm during the 96-hour test. However, in light of the fact that this change was not formally established and communicated to RD and SRRD, and the registrants, the EEB will reconsider the category of these rejected studies and evaluate them under the following interim procedures.

Old studies, and future studies begun before the proposed formal notification takes effect, will be evaluated in the following way. If they were rejected because shell growth in the clean water control is less than 2 mm, and that is the primary problem with the study (i.e. no other deficiencies were noted that, by themselves, would cause the study to be unacceptable[1]), the study will be called supplemental (i.e. not fulfilling guideline requirements). Then a value of data assessment will be made. The results of the mollusk study will be considered with other available freshwater and estuarine organism test data to see if mollusks seem more or less sensitive to that pesticide. If mollusks do not appear to be more sensitive, EFED will not recommend that the study be repeated. The toxicity values for

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[1] If another deficiency caused the study to be unacceptable this interim procedure will not apply.

the more sensitive species will be used for characterizing risk to freshwater or estuarine organisms.

If, for a given pesticide, it is determined that the mollusk may be more sensitive than any other freshwater or aquatic organism, the EFED will then appraise the hazard represented by the pesticide and determine the level of concern it represents. The study may still not have to be repeated for a pesticide that has a low potential for adverse effects, even if the mollusk was the most sensitive.

If the mollusk is the most sensitive, and if there appear to be risks to aquatic or estuarine organisms, the EFED will maintain the position that the mollusk study should be repeated. This is because of the possibility that risk would be based on test results that do not adequately characterize hazard.

Mollusk shell deposition studies initiated after the modification takes effect and is distributed to the registrants will be evaluated based on the new criteria. At that time, the EFED will recommend those not meeting the criteria be repeated.

BENEFIT OF THIS INTERIM SOLUTION

This proposed interim procedure will likely permit OPP to reduce substantially the number of mollusk studies that need to be repeated. In a review of a random group of pesticides where data from mollusks and other estuarine and freshwater organisms were available, the mollusk was usually not the most sensitive.

IDENTIFYING REJECTED MOLLUSK STUDIES

The EFED is requesting that the product managers or chemical review managers identify mollusk studies that have been rejected solely because of problems with shell growth. The EEB scientists will determine which of those studies do not need to be repeated. An alternative is to submit this proposal to the registrants that have done mollusk studies and allow them to identify the studies they feel were rejected based on this criterion.

If you have questions, please contact Douglas Urban, Acting Chief, EEB or Daniel Rieder (305-5314).

cc: Stephanie Irene
Peter Caulkins

Rapid Feedback SOP

Appendix B: List of EPA Guidance Documents

The following is a list of guidance documents that outline procedures for conducting ecological effects studies. Specific references to these materials are made under each of the rejection factors listed.

Hazard Evaluation Division, Standard Evaluation Procedures:

Acute Toxicity Test for Estuarine/Marine Organisms (1985).

Acute Toxicity Test for Freshwater Fish (1985).

Acute Toxicity Test for Freshwater Invertebrates (1985).

Avian Dietary LC_{50} Test.

Daphnia Life Cycle Chronic Toxicity Test (1986).

Ecological Risk Assessment (1986).

Pesticide Assessment Guidelines:

Subdivision E: Hazard Evaluation, Wildlife and Aquatic Organisms (1982).

Subdivision J: Hazard Evaluation, Nontarget Plants (1982).

Subdivision L: Hazard Evaluation, Nontarget Insects (1982).

Guidance Document for Conducting Terrestrial Field Studies (1988).

FIFRA Accelerated Reregistration Phase 3 Technical Guidance (1989).

Stephan, Charles E., "Committee on Methods for Toxicity Tests with Aquatic Organisms." 1975. *Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians*. US EPA, Ecol. Res. Series.

ASTM Standard E, American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA.

Standard Operating Procedure for Obtaining Rapid Feedback from EPA on Technical Issues Associated with Ecological and Environmental Fate Studies

Background:

Rejection rates for Ecological Effects and Environmental Fate studies may be reduced if feedback from the Agency on specific technical issues was obtained prior to initiating the studies. Given the strict time-lines associated with reregistration, it can be difficult to obtain such definitive feedback in a short time period. This limitation was identified by both the Agency and Registrants and it was determined that a specific mechanism should be established for communication.

Procedure:

When registrants have specific questions concerning the technical conduct of studies, the following procedure should be used to obtain guidance from the Agency:

1. For minor problems requiring rapid response from EPA, the registrant should call the Branch Chief or Deputy Branch Chief of EEB or the Branch Chief of EFGWB as appropriate for the problem.

2. The Branch Chief will determine if the problem is minor and urgent, and if so, will assign it to the appropriate Section Head or form an ad hoc team. Minor problems will be defined as those which can be solved within one week, with less than approximately one staff hour of work.

3. To facilitate discussion, it may be best to send data or information to the Branch Chief or appropriate Section Head by FAX or mail before calling. When appropriate, it is assumed that the registrants will perform adequate preliminary research for the compound and test system of concern and, if necessary, have this data available to support any position they are defending.

The current Section Heads (SH) and Branch Chiefs (BC) phone numbers are:

Ecological Effects	Phone No. 703-305-	Environmental Fate	Phone No. 703-305-
N. Cook (SH)	5322	P. Mastradone (SH)	5335
H. Craven (SH)	5320	M. Shamim (SH)	5025
D Rieder (SH)	5314	A. Abramovitch (SH)	5975
A. Stavola (SH)	5354	H. Behl (SH)	6128
L. Touart (SH)	6134	H. Nelson (SH)	7356
D. Urban (Deputy BC)	5746	H. Jacoby (BC)	5734
T. Maciorowski (BC)	7347		

4. EPA's decision on the problem raised by the registrant will be communicated back to the registrant by telephone.

5. A memorandum to the Branch chemical file will be prepared and the registrant will immediately (within one week) follow up the telephone communication with a submission through the appropriate registration division (RD or SRRD) documenting the request and decision. A written response will be made to that submission.

6. When the final study report is submitted to the EPA, it should reiterate any agreed-upon decisions and document when they were requested and granted.

If the request is not considered minor and cannot be decided within one week with less than approximately one staff hour of work, the registrant will be informed as soon as practical (within one week). If the registrants wish to pursue the matter, they must submit their request with necessary supporting studies and explanation through the appropriate division (RD or SRRD). They may, if necessary, submit a request for a time extension.

Relative to field or monitoring studies, every effort will be made by the Agency to accommodate questions and concerns of the registrants.

When contacting EPA for advice, questions should only relate to technical issues. Questions regarding the status of reviews, etc., should be addressed through the product managers in RD or chemical review managers in SRRD. Contacting reviewers directly is discouraged.

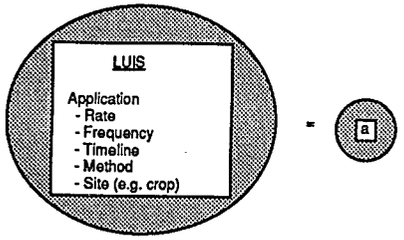
(1)

1	2
3	4
5	6
7	8

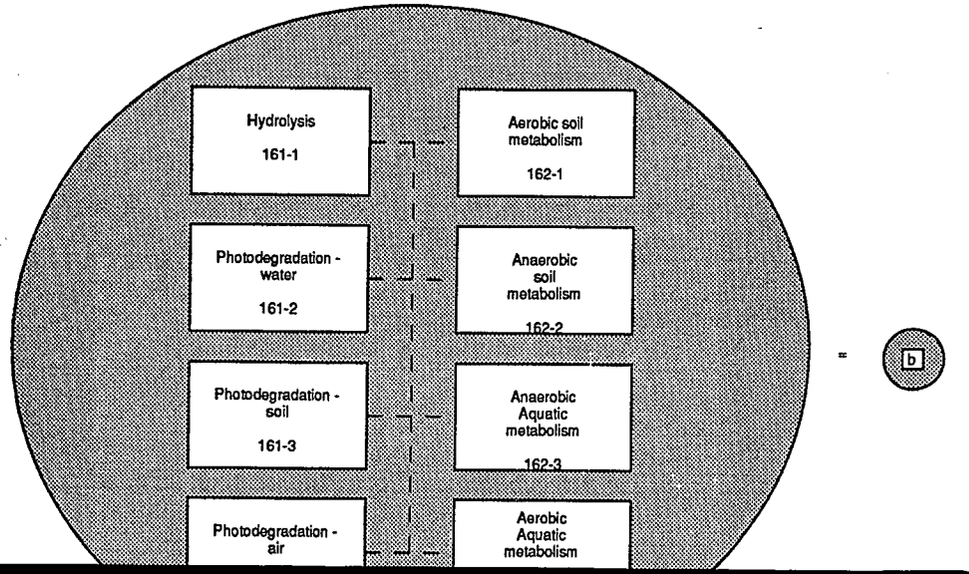
GUIDE FOR CHART ASSEMBLY

CHART
ECOLOGICAL EF FE
AQUATIC - FISH AND AQU

Label Information



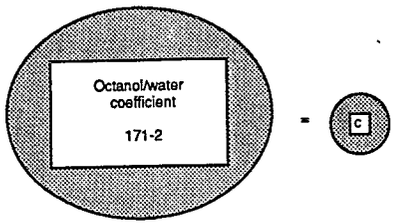
Environmental Fate Information



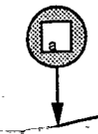
(2)

1
ECTS BRANCH
ATIC INVERTEBRATES

Physical/Chemical Properties



Marine / Estuarine



Fresh Water

161-4 162-4

Tier 1
Acute

Acute toxicity (96 hr) warm water fish (bluegill) 72-1(a) R

1 YR

Data generated:
1. LC50 (ppm) at 95% confidence level
2. NOEL

Acute toxicity (96 hr) coldwater fish (trout) 72-1(c) R

1 YR

Acute toxicity (48 hr) invertebrates (daphnia) 72-2 R

1 YR

Data generated:
1. LC50 or EC50 (ppm) at 95% confidence level
2. NOEL

Acute toxicity marine fish 72-3(a) CR

1 YR

1

2

a b c

1. Applied directly to water, or
2. Contiinuously present in water, or
3. LC50 or EC50 < 1ppm, or
4. EEC >= .01(LC50,or EC50) or
5. EEC < .01(LC50 or EC50) but
   - 1/2 life in water > 4 days
   - Potential cummulative effects (e.g., KOW >1000; 171-2)

Yes

a b

EEC < 0.1 LC 50

Ye

No

a b

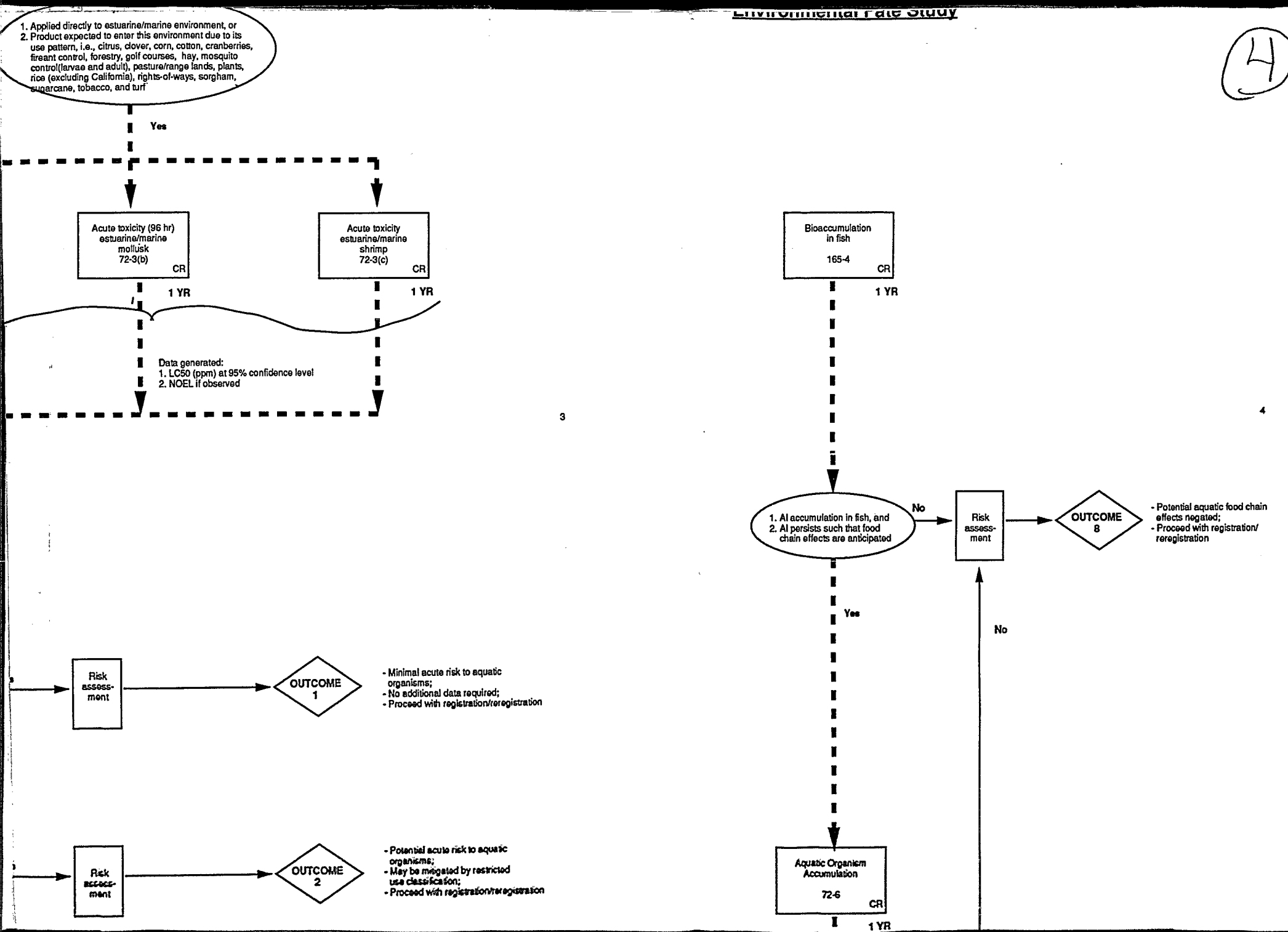
0.5 LC 50 > EEC >= 0.1 LC 50

Ye

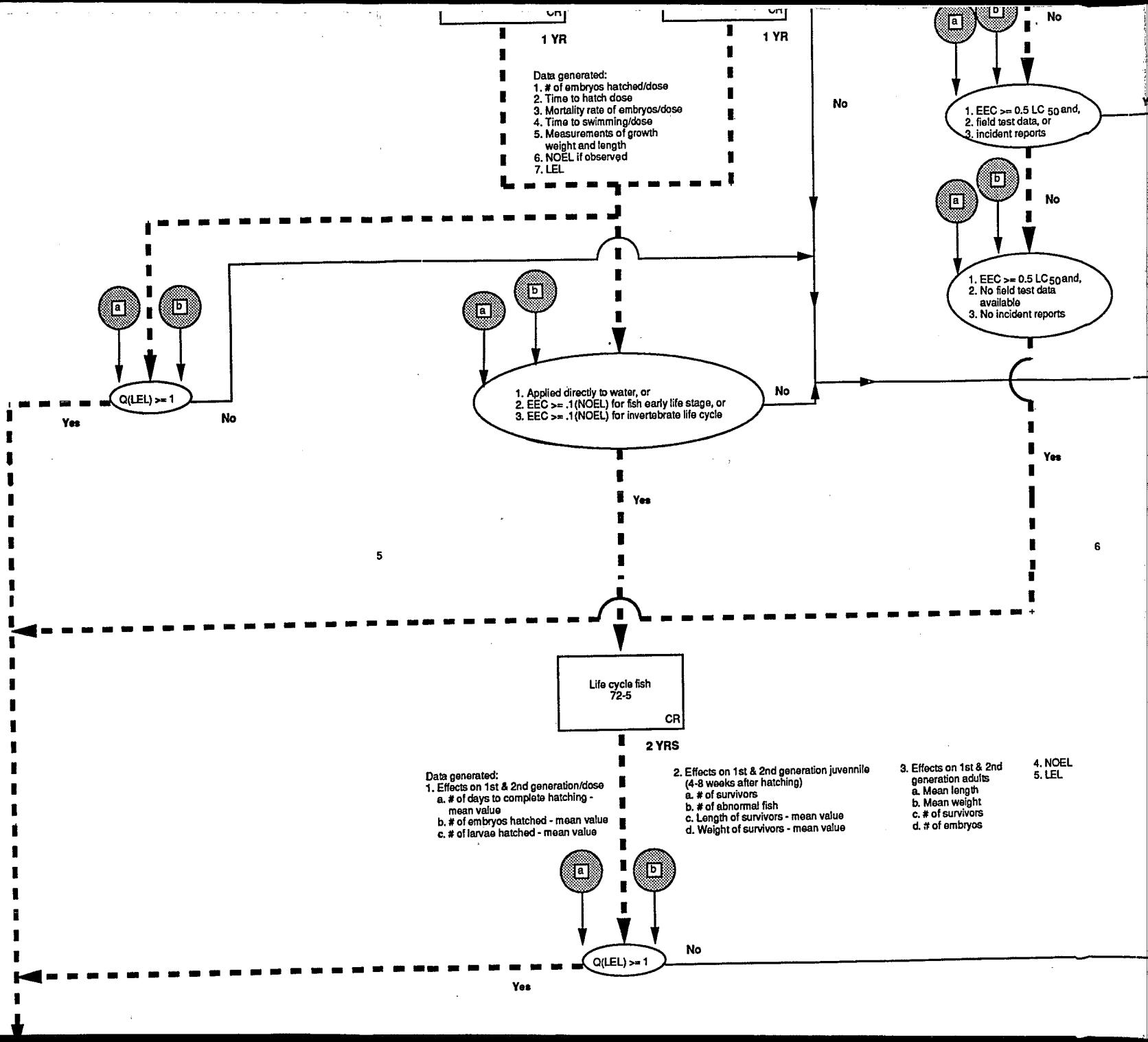
Tier 2
Chronic Aquatic
Invertebrate And Fish

Fish early life stage 72-4(a) CR

Invertebrate life cycle 72-4(b) CR

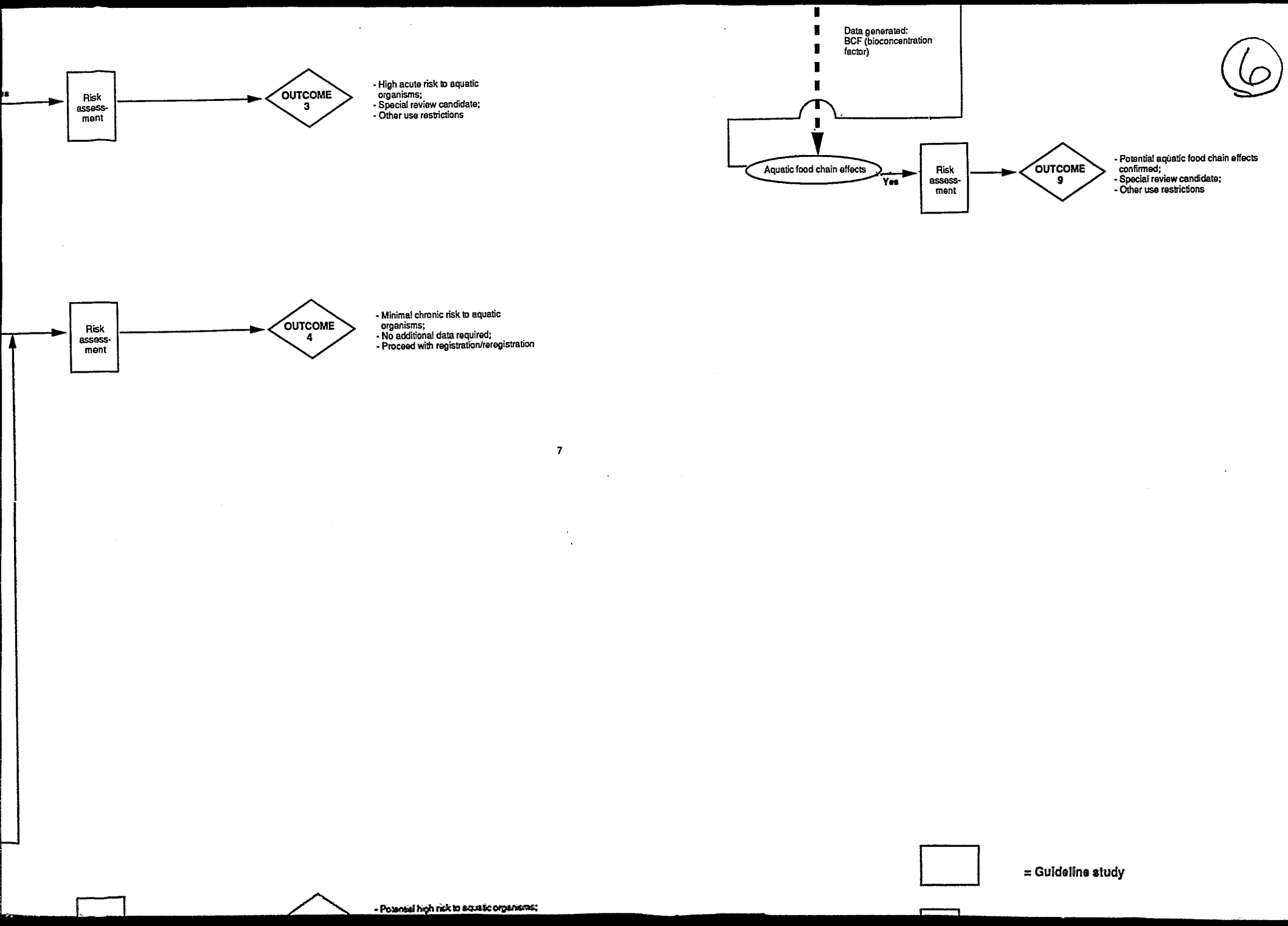

1. Applied directly to estuarine/marine environment, or
2. Product expected to enter this environment due to its use pattern, i.e., citrus, clover, corn, cotton, cranberries, fireant control, forestry, golf courses, hay, mosquito control(larvae and adult), pasture/range lands, plants, rice (excluding California), rights-of-ways, sorgham, sugarcane, tobacco, and turf
Yes
Acute toxicity (96 hr) estuarine/marine mollusk 72-3(b) CR
1 YR
Acute toxicity estuarine/marine shrimp 72-3(c) CR
1 YR
Data generated:
1. LC50 (ppm) at 95% confidence level
2. NOEL if observed
3
Bioaccumulation in fish 165-4 CR
1 YR
4
1. AI accumulation in fish, and
2. AI persists such that food chain effects are anticipated
No
Risk assess-ment
OUTCOME 8
- Potential aquatic food chain effects negated;
- Proceed with registration/ reregistration
Yes
No
Risk assess-ment
OUTCOME 1
- Minimal acute risk to aquatic organisms;
- No additional data required;
- Proceed with registration/reregistration
Risk assess-ment
OUTCOME 2
- Potential acute risk to aquatic organisms;
- May be mitigated by restricted use classifcation;
- Proceed with registration/reregistration
Aquatic Organism Accumulation 72-6 CR
1 YR

5



Tier 3

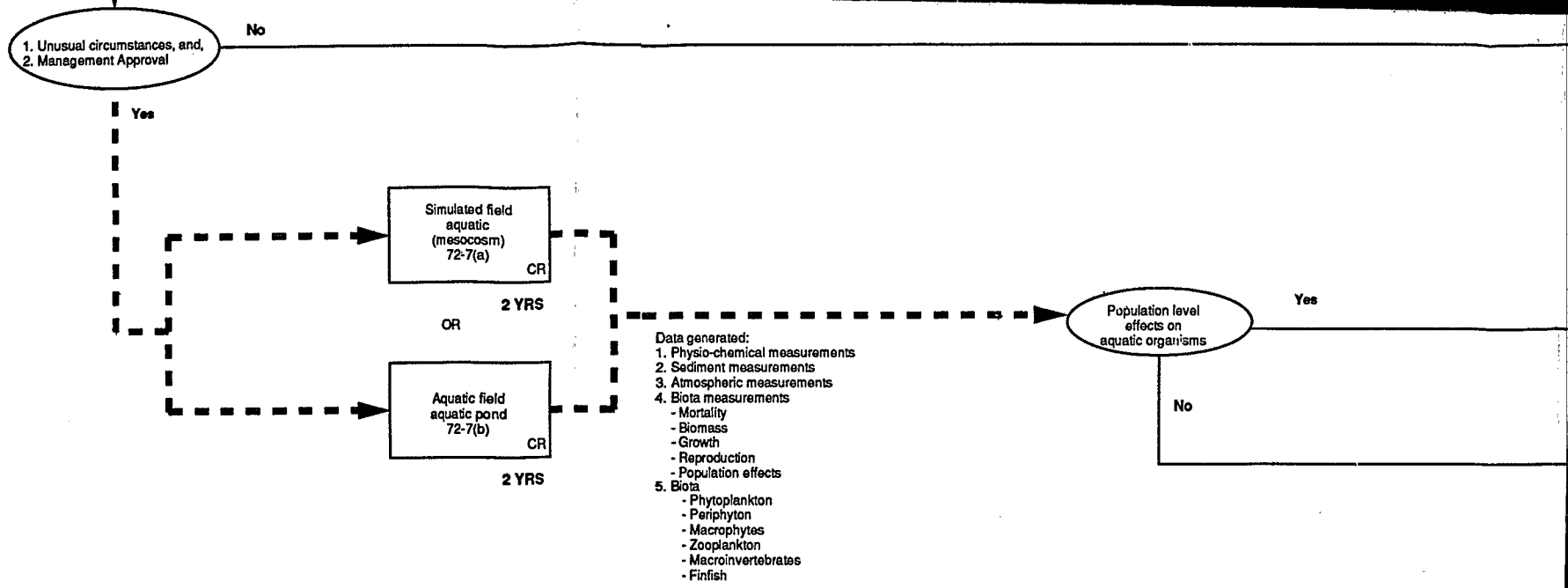
Life Cycle Fish

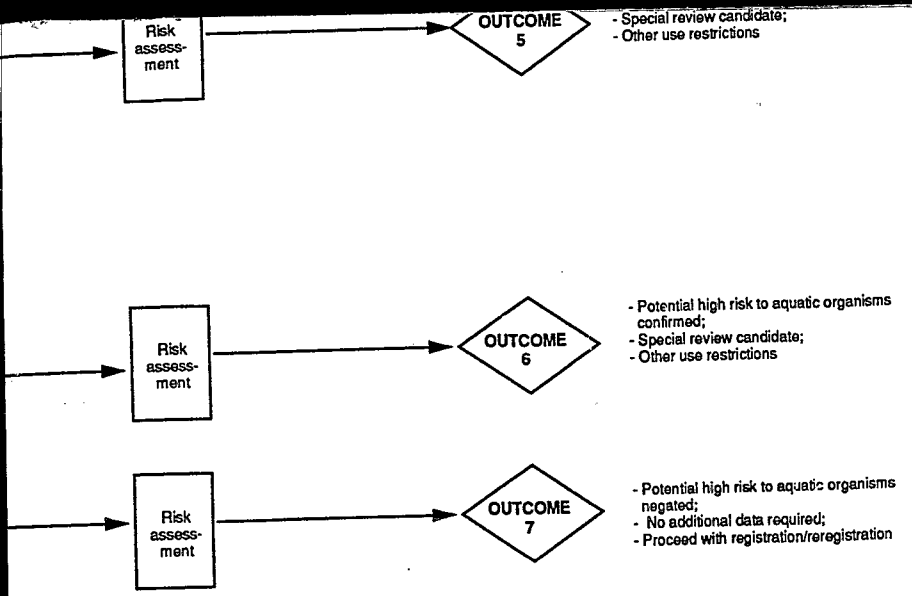

Risk assess-ment
OUTCOME 3
- High acute risk to aquatic organisms;
- Special review candidate;
- Other use restrictions
Data generated: BCF (bioconcentration factor)
Aquatic food chain effects
Yes
Risk assess-ment
OUTCOME 9
- Potential aquatic food chain effects confirmed;
- Special review candidate;
- Other use restrictions
Risk assess-ment
OUTCOME 4
- Minimal chronic risk to aquatic organisms;
- No additional data required;
- Proceed with registration/reregistration
= Guideline study
- Potential high risk to aquatic organisms;

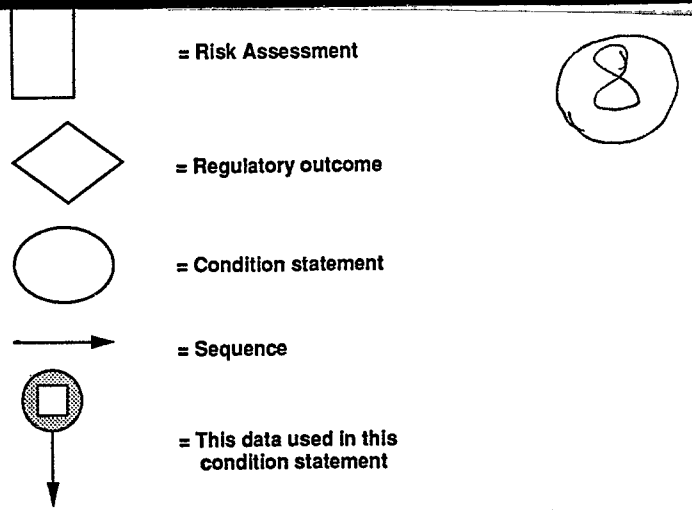
7

Tier 4

Field







EEC = Estimated Environmanetal Concentration

Q = Quotient

= Trigger

06/17/92