Appendix B. Ecological Effects Characterization

This appendix presents available submitted and open literature studies available on imazapyr. Studies that are submitted to the Agency in support of pesticide registration or re-registration are categorized as either acceptable, supplemental, or invalid. Acceptable means that all essential information was reported, the data are scientifically valid, and the study was performed according to recommended protocols. Studies in the "acceptable" category fulfill the corresponding data requirement in 40 CFR Part 158 and are appropriate for use in risk assessment. Supplemental studies are also scientifically valid; however, they were either performed under conditions that deviate from recommended guideline protocols or certain data necessary for complete verification are missing. Supplemental studies may be used quantitatively in the risk assessment and can, at the Agency's discretion, fulfill the corresponding data requirement in 40 CFR Part 158. Invalid studies are not scientifically valid, or deviate substantially from recommended protocols such that they are not useful for risk assessment. Invalid studies do not fulfill the corresponding data requirement in 40 CFR Part 158.

With respect to the open literature, studies may be classified as either qualitative, quantitative, or invalid. The degree to which open literature data are quantitatively or qualitatively characterized is dependent on whether or not the information is relevant to the assessment endpoints (i.e., maintenance of the survival, reproduction, and growth of the California red legged frog and PCEs of its designated critical habitat) identified in the problem formulation. Open literature studies classified as qualitative are not appropriate for quantitative use but are of good quality, address issues of concern to the risk assessement, and, when appropriate, are discussed qualitatively in the risk characterization discussion. Those open literature studies that are classified as

quantitative are appropriate for quantitative use in the risk assessment including calculation of RQs. It should be noted that this appendix includes all relevant data taken from the 2005 RED imazapyr ecological effects appendix. For the 2005 RED, open literature data were obtained from the data provided to EFED by ORD on 06/21/2005. In addition, updated ECOTOX information was obtained on February 9, 2007. The February, 2007 ECOTOX search included all open literature data for imazapyr (i.e., pre- and post-RED). Data that pass the ECOTOX screen described in Section 4.1 of the assessement are evaluated along with the registrant-submitted data, and may be incorporated qualitatively or quantitatively into this endangered species assessment. In general, effects data in the open literature that are more conservative than the registrant-submitted data are considered for quantitative use.

All open literature that was not considered as part of this assessment because it was either rejected by the ECOTOX screen or accepted by ECOTOX but not used (e.g., the endpoint is less sensitive and/or not appropriate for use in this assessment) is included in Appendix G. Appendix G also includes a rationale for rejection of those studies that did not pass the ECOTOX screen and those that were not evaluated as part of this endangered species assessment. Further detail on the ECOTOX exclusion categories is provided in the Agency's *Guidance of the Evaluation Criteria for Ecological Toxicity Data in the Open Literature* (U.S. EPA, 2004).

In order to be included in the ECOTOX database, papers must meet the following minimum criteria:

- the toxic effects are related to a single chemical exposure
- the toxic effects are on an aquatic or terrestrial plant or animal species
- there is a biological effect on live, whole organisms
- a concurrent environmental chemical concentration/dose or application rate is reported and
- there is an explicit duration of exposure.

Data that passes the ECOTOX screen is evaluated along with the registrant-submitted data, and may be incorporated qualitatively or quantitatively into the risk assessment. In general, effects data in the open literature that are less than or more conservative than the registrant-submitted data are considered. The degree to which open literature data is quantitatively or qualitatively characterized is dependent on whether the information is relevant to the assessment endpoints (i.e., maintenance of survival, reproduction, and growth) identified in the problem formulation. For example, endpoints such as behavior modifications are likely to be qualitatively evaluated, because it is unclear whether such modifications cause a reduction in species survival, reproduction, and/or growth. Specific open literature data that are considered include the following:

- the endpoint is more sensitive than those identified in the registrant data
- the data is for under represented taxa (i.e., amphibians) and
- the data includes endpoints not normally evaluated in registrant studies, but

ecologically relevant

An examination of the studies found in the open literature (ECOTOX) did not provide any lower endpoints than the studies submitted by the Registrant, however, an aquatic in situ microcosm study was available to observe the effects to benthic marcoinvertebrates.

Ecological effects studies have been submitted for both imazapyr (technical product imazapyr acid) and the product, ARSENAL® (EPA Reg. No. 241-346), which is formulated as a concentrated aqueous solution of the isopropyl amine salt of imazapyr. The amine salt comprises 28.7% of the weight of ARSENAL®, which corresponds to an imazapyr acid equivalent (a.e.) composition of 22.6% by weight or 2.0 lbs of imazapyr a.e. per gallon of product. In the ecological effects characterization section and in this Appendix, all exposure concentrations and toxicity values are expressed on the basis of either percent active ingredient (a.i.) or acid equivalent (a.e.) imazapyr. Both terms are comparable to each other in this case.

Studies are with imazapyr acid, unless otherwise noted.

B.1 Toxicity to Terrestrial Animals

Given limited ecotoxicity data for terrestrial phase amphibians, avian acute oral, subacute dietary, and chronic reproduction data are used as a surrogate for the terrestrial phase of the California red legged frog. No amphibian or reptile toxicity studies were either submitted or located in the open literature. Ecotoxicity data for birds and terrestrial phase amphibians are discussed in Sections B.1.1 through B.1.6.

B.1.1 Birds/Terrestrial Phase Amphibians: Acute Oral Studies

An oral toxicity study using the technical grade of the active ingredient (TGAI) is required to establish the acute toxicity of imazapyr to birds. The preferred guideline test species is either mallard duck (a waterfowl) or bobwhite quail (an upland gamebird). The submitted acute data indicate that imazapyr is practically non-toxic to waterfowl and upland gamebirds with acute oral LD_{50} values >2,150 mg a.i./kg. There were no mortalities or clinical signs of toxicity in either the bobwhite quail or the mallard ducks (MRID 00131633, MRID 00131634). The available acute oral study on mallard ducks for the salt indicates that it is no more toxic than the acid. Results of the studies on the acid are summarized below in Table B-1.1.

Table B-1.1 Avian Acute Oral Toxicity for Imazapyr Acid.					
Species	**% ae**	**LD_{50} (mg ae/kg-bw)**	**Toxicity Category**	**MRID No. Author, Year**	**Study Classification**
Northern bobwhite quail *(Colinus virginianus)*	93	>2,150	Practically non-toxic	00131633 Bio-Life Assoc., 1983	Acceptable
Mallard duck *(Anas platyrhynchos)*	93	>2,150	Practically non-toxic	00131634 Bio-Life Assoc., 1983	Acceptable

Imazapyr isopropylamine salt (22.6% formulated product)

Mallard ducks. MRID 00153774. The acute oral toxicity of Arsenal® Herbicide (Salt of Imazapyr) to 27-30-week old Mallard ducks (*Anas platyrhynchos*) was assessed over 21 days. Test material was administered to the birds by oral gavage at nominal concentrations of 0 (tap water control), 1470, and 2150 mg Arsenal/kg bw. No mortality occurred in any control or treatment group during the 21-day study. The acute LD_{50} was >2150 mg Arsenal/kg bw, the highest level tested, which categorizes Arsenal® Herbicide (Salt of Imazapyr) as practically non-toxic to Mallard duck on an acute oral basis. No sublethal effects were observed in the control or treatment groups. No treatment-related effects on body weight or feed consumption were observed. The NOAEL for sublethal effects, body weight gain, and feed consumption was 2150 mg Arsenal/kg bw. This toxicity study is scientifically sound and is classified as Acceptable.

B.1.2 Birds/Terrestrial Phase Amphibians: Subacute Dietary Studies

Two dietary studies using the TGAI are required to establish the subacute toxicity of imazapyr to birds. The preferred test species are mallard duck and bobwhite quail. The data that were submitted show that the 8-day acute dietary LC_{50} for both species was >5,000 ppm; therefore, imazapyr is categorized as practically non-toxic to avian species on a subacute dietary basis. In the bobwhite quail study, there was one mortality at one of the lower concentration levels but none at the higher concentration levels. There were no clinical signs of toxicity in either. The available subacute dietary study on bobwhite quail for the salt indicates that it is no more toxic than the acid. Results of the studies on the acid are summarized in Table B-1.2.

Table B-1.2 Avian Subacute Dietary Studies for Imazapyr Acid.					
Species	**% ae**	**8-Day LC_{50} (mg ae/kg-diet)**	**Toxicity Category**	**MRID No. Author, Year**	**Study Classification**
Northern bobwhite quail *(Colinus virginianus)*	93	>5,000	Practically non-toxic	00131635 Bio-Life Assoc., 1983	Acceptable

Table B-1.2 Avian Subacute Dietary Studies for Imazapyr Acid.					
Species	**% ae**	**8-Day LC_{50} (mg ae/kg-diet)**	**Toxicity Category**	**MRID No. Author, Year**	**Study Classification**
Mallard duck *(Anas platyrhynchos)*	93	>5,000	Practically non-toxic	00131636 Bio-Life Assoc., 1983	Acceptable

Imazapyr acid

Bobwhite Quail. MRID 00131635 (Acceptable). In an 8-day dietary study, imazapyr acid was determined to be practically non-toxic to upland game birds (bobwhite quail) with an LC_{50} >5000 ppm. The study is scientifically sound and generally followed guideline protocols; however, there was some unexplainable low weight gains and mortality at the 625 ppm test concentration.

Mallard. MRID 00131636 (Acceptable). In an 8-day dietary study, imazapyr acid was determined to be practically non-toxic to mallard ducklings with an LC_{50} >5000 ppm.. The study is scientifically sound and generally followed guideline protocols.

Imazapyr isopropylamine salt (22.6%)

Bobwhite Quail. MRID 00147115 (Acceptable). In an 8-day dietary study, the isopropylamine salt of imazapyr was determined to be practically non-toxic to upland game birds (bobwhite quail) with an LC_{50} >5000 ppm. The study is scientifically sound and generally followed guideline protocols. This study was conducted with the formulated product to ensure that isopropylamine did not affect the toxicity of the active ingredient.

Mallard Ducks. MRID 00153776 (Supplemental). The acute dietary toxicity of Arsenal® Herbicide (Salt of Imazapyr) to 5-day-old mallard ducklings (*Anas platyrhynchos*) was assessed over 8 days. The test material was administered to the birds in the diet at nominal concentrations of 0 (negative control), 312, 625, 1250, 2500, and 5000 mg Arsenal/kg-diet. **The nominal dietary concentrations were not corrected for the purity of the active ingredient**. It was reported that samples of the diet were analyzed for concentrations of the test material, however results were not reported. All toxicity values were reported based on the nominal dietary concentrations. No mortality was observed during the study. No clinical signs of toxicity or treatment-related effects on body weight or food consumption were observed. The results of the analytical verification of the test material in the diets were not reported, so the actual concentrations that test organisms were exposed to are unknown. Toxicity values and categorization derived using nominal test concentrations may not be indicative of exposure to the test substance under these study conditions. This study is scientifically sound; however, the test concentrations in the diets were not analytically verified. Toxicity values are based

on the nominal concentrations. This study is classified as SUPPLEMENTAL for a formulated product.

B.1.3 Birds/Terrestrial Phase Amphibians: Chronic Toxicity Studies

Avian reproduction studies using the TGAI are usually required for pesticide registration because birds may be subject to repeated or continuous exposure to the pesticide, especially preceding or during the breeding season. The preferred test species are mallard duck and bobwhite quail. The submitted data indicate no evidence of adverse reproductive effects to bobwhite quail at concentrations up to 1,670 ppm (MRID 45119714) and 2000 ppm (MRID 43831401), and to mallard ducks at concentrations up to 1890 ppm (MRID 43831402). Results of these studies are summarized in Table B-1.3.

Table B-1.3 Avian Reproduction for Imazapyr Acid

Species	% ae	NOAEC/LOAEC (mg ae/kg-diet)	LOAEC Endpoints	MRID No. Author, Year	Study Classification
Northern bobwhite quail *(Colinus virginianus)*	100	1,670/>1,670	No treatment-related toxicity	45119714 Ahmed *et al.*, 1999	Acceptable
Northern bobwhite quail *(Colinus virginianus)*	Technical - % not stated	2000/>2000	No treatment-related toxicity	438314011987	Acceptable
Mallard duck *(Anas platyrhynchos)*	Technical - % not stated	1890/>1890 (2000 nominal)	No treatment-related toxicity	438314021987	Acceptable

Bobwhite Quail. MRID 45119714a (Acceptable). In a one-generation reproductive toxicity study, imazapyr acid produced no evidence of treatment-related adverse effects on adult or reproductive parameters with an NOAEC of 1670 ppm. The study is scientifically sound and generally followed guideline protocols.

Mallard. MRID 45119714b (Invalid). In a one-generation reproductive toxicity study, imazapyr acid resulted in a significant reduction in the ratio of viable embryos/eggs at the 1,670 ppm treatment level. However, the study was determined to be invalid due to bacterial contamination and high embryonic mortality in the controls. EFED recommended that another study be conducted to determine the reproductive toxicity of imazapyr to waterfowl.

Bobwhite Quail. MRID 43831401 (Originally Supplemental; Reclassified Core). In a one-generation reproductive toxicity study, imazapyr acid resulted in reduced hatchlings/live embryo at 2000 ppm (LOEC; NOEC = 1000 ppm); however, the study was originally determined to be supplemental due to guideline deficiencies (primarily,

EECs would be higher than highest dose tested and control egg shell cracking was 13%). EFED reevaluated the studies and determined that the dosing did reflect the maximum EEC and that the handling and measurement deficiencies did not reflect a dose-response relationship; consequently, the study was reclassified as core and the NOEC was changed to 2000 ppm.

Mallard. MRID 43831402 (Originally Supplemental; Reclassified Core). In a one-generation reproductive toxicity study, imazapyr acid produced no evidence of treatment-related adverse effects on adult or reproductive parameters with an NOAEC of 1890 ppm (measured concentration; 2000 ppm nominal concentration). However, the study was originally determined to be supplemental due to guideline deficiencies (primarily, EECs would be higher than highest dose tested, inaccurate measurement of egg shell thickness, and insufficient pre-egg laying period. EFED reevaluated the studies and determined that the dosing did reflect the maximum EEC and that the measurement deficiencies did not reflect a dose-response relationship; consequently, the study was reclassified as core and the NOAEC was established at 2000 ppm.

B.1.4 Birds/Terrestrial Phase Amphibians: Open Literature Data

No bird or terrestrial amphibian toxicity studies were submitted or located in the open literature.

B.1.5 Mammals: Acute Oral Studies

Wild mammal testing is required on a case-by-case basis, depending on the results of lower tier laboratory mammalian studies, intended use pattern and pertinent environmental fate characteristics. In most cases, rat or mouse toxicity values obtained from the Agency's Registration Division (RD) and Health Effects Division (HED) substitute for wild mammal testing. These toxicity values are reported below.

The results indicate that imazapyr acid is categorized as practically non-toxic to small mammals on an acute oral basis (LD_{50} value >5,000 mg/kg bw, both sexes). The available acute oral studies with rats for the salt indicate that it is no more toxic than the acid. Results of the study on the acid are summarized in Table B-1.5.

Table B-1.5 Mammalian Acute Toxicity for Imazapyr Acid.

Species	% ae	Toxicity	Affected Endpoints	MRID No. Author, Year	Study Classification
Rat (Sprague-Dawley)	93	LD_{50} >5,000 mg ae/kg bw (males/females)	Mortality	00132030 American Cyanamid Co., 1983	Acceptable

Imazapyr acid

Rat. MRID 132030 (Acceptable). In an acute oral study, imazapyr acid was determined to have a low toxicity (Toxicity Category III) to rats with an LD_{50} >5000 mg/kg. The study is scientifically sound and meets guideline protocols.

Imazapyr isopropylamine salt

Rat. MRID 00147049 (Acceptable). In an acute oral study, imazapyr isopropylamine salt was determined to exhibit no toxicity (Toxicity Category IV) to rats with an LD_{50} >5000 mg/kg (>4090 mg ae/kg). The study is scientifically sound and meets guideline protocols.

Rat. MRID 44735301 (Acceptable). In an acute oral study, imazapyr isopropylamine salt was determined to exhibit no toxicity (Toxicity Category IV) to rats with an LD_{50} of >5000 mg/kg (>4090 mg ae/kg). The study is scientifically sound and meets guideline protocols.

B.1.6 Mammals: Chronic Toxicity Studies

In a 2-generation reproduction study with rats exposed to imazapyr acid, no treatment-related effects were observed. Consequently, the NOAEL for parental systemic, reproductive, and offspring was 738 mg/kg bw/day for males and 933.3 mg/kg bw/day for males. The NOAEC is 10000 ppm. The NOAEC/NOAEL from this study will be used in assessment of risk.

In developmental toxicity studies, administration of imazapyr acid by gavage resulted in no treatment-related effects in developmental parameters at doses up to and including 1000 and 400 mg/kg bw/day in the rat and rabbit, respectively. In the rat study, the only maternal toxicity observed at 300 mg/kg bw/day was salivation during gestation days 8 - 15. This effect is not likely to affect reproduction, growth or survival. Therefore, it is not be used quantitatively in assessment of risk. The salivation is likely due to the route of administration (gavage) with a potentially irritating substance (an acid). In the rabbit study, no maternal toxicity was observed at 400 mg/kg bw/day, the highest dose tested. Mortality was observed in the does at 250 mg/kg/day and above in the pilot study (MRID 00131614). Microscopic examination of the does that died showed gastric ulcers and lesions in the gastrointestinal tract. These effects are not considered to be effects that would occur following chronic exposure. They are considered to be acute effects and are more likely a result of the route of administration (gavage with imazapyr acid, a probable irritating substance). Results of these studies are summarized in Table B-1.6.

Table B-1.6 Mammalian Developmental/Reproductive Toxicity for Imazapyr Acid

Species	% Purity	Test Type	Toxicity	Affected Endpoints	MRID No. Study author Classification
Rat (Sprague Dawley)	93	Developmental	NOAEL/LOAEL = 300/1000 mg/kg bw/day NOAEL = 1000 mg/kg bw/day	Maternal tox[1] Developmental	00131611 Salamon & Mayhew, 1983 Acceptable
Rabbit (New Zealand White)	93	Developmental	NOAEL = 400 mg/kg bw/day NOAEL = 400 mg/kg bw/day	No effects	00131613 Mayhew & Salamon, 1983 Acceptable
Rat (Sprague Dawley)	99.5	Reproduction	NOAEL = 738 mg/kg bw/day - Males NOAEL = 933.3 mg/kg bw/day - Females or 10000 ppm for both.	No effects	41039505 Robinson, 1987 Acceptable

[1]Maternal toxicity - Gravid dams exhibited salivation during gestation days 8 - 15 (likely related to gavage route of administration).
Developmental toxicity - No treatment-related effects in developmental parameters; no treatment-related malformations.

83-3 Mammalian Developmental

Rat. MRID 00131611 (Acceptable). In a developmental toxicity study, imazapyr acid produced maternal toxicity in Sprague Dawley rats at 1000 mg ai/kg/day (LOAEL), based on salivation in the gravid dams between gestation days 8-15. The findings were determined to be treatment-related. The NOAEL was 300 mg /kg bw/day. No treatment-related effects were reported for developmental parameters. The study is scientifically sound and meets guideline protocols.

Rabbit. MRID 00131613 (Acceptable). In a 2-generation teratology study, imazapyr acid produced no treatment-related effects for maternal or developmental parameters; consequently, the NOAEL for both endpoints was ≥400 mg/kg bw/day in New Zealand white rabbits. The study is scientifically sound and meets guideline protocols.

83-4 Mammalian Reproduction

Rat. MRID 41039505 (Acceptable). In a 2-generation reproduction study, imazapyr acid produced no treatment-related effects for maternal or developmental parameters; consequently, the parental systemic, reproductive, and offspring NOAEL was ≥738 mg/kg bw/day in males and 933.3 mg/kg bw/day in females. The study is scientifically sound and meets guideline protocols.

B.1.7 Terrestrial Invertebrates: Acute Toxicity Studies

A honey bee acute contact study using the TGAI is required for imazapyr because its foliar application treatment use will result in honey bee exposure. The acute contact LD_{50}, using the honey bee, Apis mellifera, is an acute contact, single-dose laboratory study designed to estimate the quantity of toxicant required to cause 50% mortality in a test population of bees. The acute contact LD_{50} for imazapyr is > 100 µg/bee and it is, therefore, classified as practically non-toxic to bees on a contact exposure basis. Table B-1.7 summarizes the study.

Table B-1.7 Non-target Insects - Acute Contact (Imazapyr Acid).

Species	% ae	LD_{50} (µg ae/bee)	Toxicity Category	MRID No. Author/Year	Study Classification
Honey Bee (Apis mellifera)	Tech	>100	Practically non-toxic	00131637 Atkins, 1983	Acceptable

Honey Bee. MRID 00131637 (Acceptable). In a 48-hour acute contact study with the honey bee, imazapyr acid was determined to be practically non-toxic to honey bees and the LD_{50} was >100 µg/bee. The study is scientifically sound and meets guideline protocols.

B.2 Toxicity to Freshwater Animals

B.2.1 Freshwater Fish and Aquatic Amphibia, Acute Toxicity Studies

Fish toxicity studies for two freshwater species using the TGAI are required to establish the acute toxicity of imazapyr acid to fish. The preferred test species are rainbow trout (a coldwater fish) and bluegill sunfish (a warmwater fish). Acute studies that were submitted for three freshwater fish species (rainbow trout, bluegill sunfish, channel catfish) showed that imazapyr is practically non-toxic with 96-hr LC50 values of >100 mg/L (NOAEC = 100 ppm) for all three species. No mortalities and no clinical signs of toxicity were observed in any of the studies. EFED will use this value for evaluating acute toxic exposure to freshwater fishes and aqueous phase amphibians. The available fish toxicity data for the salt are no more toxic than the acid. Results of the studies on the acid are summarized in Table B-2.1.

Table B-2.1 Freshwater Fish Acute Toxicity for Imazapyr Acid.

Species	% ae	96-hour LC50 (mg/L)	Toxicity Category	MRID No. Author/Year	Study Classification

Table B-2.1 Freshwater Fish Acute Toxicity for Imazapyr Acid.					
Species	**% ae**	**96-hour LC50 (mg/L)**	**Toxicity Category**	**MRID No. Author/Year**	**Study Classification**
Bluegill sunfish (Lepomis macrochirus)	93	>100	Practically non-toxic	00131630 ABC Laboratories, 1983	Acceptable
Rainbow trout (Oncorhynchus mykiss)	93	>100	Practically non-toxic	00131629 ABC Laboratories, 1983	Acceptable
Channel catfish (Ictalurus punctatus)	93	>100	Practically non-toxic	00131631 ABC Laboratories, 1983	Acceptable

Imazapyr acid

Rainbow Trout. MRID 00131629 (Acceptable). In a 96-hour acute test, imazapyr acid was determined to be practically non-toxic to rainbow trout with an LC_{50} of >100 mg/L. The NOEC was determined to be 100 mg/L. The study is scientifically sound and meets guideline protocols.

Bluegill Sunfish. MRID 00131630 (Acceptable). In a 96-hour acute test, imazapyr acid was determined to be practically non-toxic to bluegill sunfish with an LC_{50} of >100 mg/L. The NOEC was determined to be 100 mg/L. The study is scientifically sound and meets guideline protocols.

Channel Catfish. MRID 00131631(Acceptable). In a 96-hour acute test, imazapyr acid was determined to be practically non-toxic to channel catfish with an LC_{50} of >100 mg/L. The NOEC was determined to be 100 mg/L. The study is scientifically sound and meets guideline protocols.

Imazapyr isopropylamine salt (22.6%)

Rainbow Trout. MRID 45119713 (Acceptable). In a 96-hour flow-through test, imazapyr isopropylamine salt was determined to be practically non-toxic to rainbow trout with an LC_{50} of >110 mg ae/L (mean measured concentration; nominal concentration 120 mg ae/L). The NOEC was determined to be 110 mg ae/L. The study is scientifically sound and meets guideline protocols.

Bluegill Sunfish. MRID 00147116 (Acceptable). In a 96-hour test, imazapyr isopropylamine salt was determined to be practically non-toxic to bluegill sunfish with an LC_{50} of >818 mg ae/L (1000 mg ai/L). The study is scientifically sound and meets guideline protocols.

Rainbow Trout MRID 00153778 (Supplemental). In a 96-hour acute toxicity study,

Rainbow Trout (*Salmo gairdneri*) were exposed to Arsenal (Salt of Imazapyr) at nominal treatment concentrations of 0 (negative control), 32, 56, 100, 180, and 320 mg Arsenal/L under static conditions. Analytical verification of the test material in the test solution was not conducted at any point during the definitive test. By 96-hours, mortality was 0% in the negative control and nominal 56 mg Arsenal/L treatment level and 10, 40, 90 and 90% in the nominal 32, 100, 180 and 320 mg Arsenal/L (6.0, 10.4, 18.9, 33.4 and 59.3 mg ae/L) treatment levels, respectively. Sub-lethal effects included surfacing, loss of equilibrium, dark discoloration, fish on bottom and quiescence in the nominal 100, 180, and 320 mg Arsenal/L treatment groups by 96 hours. The test solutions were not analytically verified in this study, so the actual concentrations that test organisms were exposed to are unknown. Toxicity values and categorization derived using nominal test concentrations may not be indicative of exposure to the test substance under these study conditions. The 96-hour LC_{50} is 112 mg Arsenal/L (20.8 mg ae/L) with a NOAEC of 10.4 mg ae/L and a LOAEC of 18.9 mg ae/L. This study is scientifically sound; however, the test fish weight (0.402-1.074 g) ranged lower than EPA recommendations (0.5-5.0 g) and the test concentrations were not analytically verified. Toxicity values are based on the nominal concentrations. This study is classified as SUPPLEMENTAL for a formulated product.

B.2.2 Freshwater Fish and Aquatic Amphibia, Chronic Toxicity Studies

A freshwater fish early life-stage test using the TGAI is normally required for pesticide registration because the end-use product may be transported to water from the intended use site, and the following conditions are met: (1) imazapyr is intended for use such that its presence in water is likely to be continuous or recurrent regardless of toxicity, and (2) imazapyr is persistent in water (e.g., photolytic half-life of 300 - 700 days). A chronic early life stage study conducted on rainbow trout showed a decrease in larval survival at a mean measured concentration of 92.4 mg/L (MRID 413158040). The NOAEC was 43.1 mg ae/L. The study was originally classified as invalid because survival of control embryos following thinning was below 70%. However, it was upgraded to supplemental because the SEP was met and the data were still considered useful for the purpose of risk assessment. The results from this study will be used for risk assessment purposes. A chronic early life stage study conducted on the fathead minnow showed no treatment-related effects at 118 mg ae/L (highest concentration tested). A full life cycle study was also submitted for fathead minnow which showed no treatment-related effects at 120 mg ai/L. This study was classified as supplemental because the F1 generation was maintained for 4 weeks instead of 8 weeks. Results of these studies are summarized in Table B-2.2.

Table B-2.2 Freshwater Fish Chronic Toxicity for Imazapyr Acid

Species	% ae	NOAEC/LOAEC (mg ae/L)	Endpoints Affected	MRID No. Author/Year	Study Classification
Early Life-Stage Study under Flow-through Conditions					

Table B-2.2 Freshwater Fish Chronic Toxicity for Imazapyr Acid					
Species	**% ae**	**NOAEC/LOAEC (mg ae/L)**	**Endpoints Affected**	**MRID No. Author/Year**	**Study Classification**
Rainbow Trout (Oncorhyncus mykiss)	99.5	43.1/92.4	Larval survival	41315804 Ward, 1988	Supplemental
Fathead Minnow (Pimephales promelas)	99.6	118/>118	No treatment-related effects	45119711 Drottar et al., 1998	Acceptable
Full Life cycle Study under Flow-through Conditions					
Fathead Minnow (Pimephales promelas)	100	120/>120	No treatment-related effects	45119712 Drottar et al., 1999	Supplemental

72-4a Freshwater Fish Early Life Stage

Fathead Minnow. MRID 45119711 (Acceptable). In an early life-stage flow-through test, imazapyr acid produced no treatment-related effects on embryonic survival, time to hatch, alevin survival, terminal length, or wet and dry weight. The NOEC was determined to be 118 mg ai/L (mean measured concentration; nominal concentration 120 mg ai/L). The study is scientifically sound and meets guideline protocols.

Rainbow Trout. MRID 41315804 (Supplemental). In an early life-stage flow-through test, imazapyr acid resulted in significantly reduced percent hatch and an observed reduction on survival at 92.4 mg/L (mean measured concentration; nominal concentration 100 mg/L). No abnormalities in embryonic or juvenile development were observed. The MATC was >43.1 and <92.4 mg/L; thus the geometric mean MATC was 63.1 mg/L. The study did not meet all guideline requirements (feeding limited the growth of replicates with higher fish densities).

72-5 Freshwater Fish Life Cycle

Fathead Minnow. MRID 45119712 (Supplemental). In a full life cycle flow-through test, imazapyr acid produced no treatment-related effects on growth, embryo survival, time to hatch, or larval and juvenile survival of the F0 and F1 generations. No treatment-related effects were observed on percent spawning frequency, mean number of eggs produced per female or mean percent fertilization success. The NOEC was reported at the nominal concentration of 120 mg ai/L (mean measured concentration 118 mg ai/L). The study is scientifically valid but did not meet all guideline requirements (F1 generation was maintained for 4 weeks instead of 8 weeks).

B.2.3 Freshwater Fish/Aquatic Amphibians, Open Literature Data

No freshwater fish or aquatic phase amphibian toxicity studies were submitted or located in the open literature.

B.2.4 Freshwater Invertebrates, Acute Toxicity Studies

A freshwater aquatic invertebrate toxicity test using the TGAI is required to establish the toxicity of imazapyr to aquatic invertebrates. The preferred test species is Daphnia magna. Submitted data indicate that imazapyr is practically non-toxic to Daphnia magna with an acute 48-hour EC_{50} value of >100 mg/L. There were no mortalities and no clinical signs of toxicity in this study. EFED will use this value for evaluating acute toxic exposure to freshwater invertebrates. The available freshwater invertebrate toxicity data for the salt are no more toxic than the acid. Results of the study on the acid are summarized in Table B-2.4.

Table B-2.4 Freshwater Invertebrate Acute Toxicity for Imazapyr Acid.

Species	% ae	48-hour EC_{50} (mg/L)	Toxicity category	MRID No. Author/Year	Study Classification
Waterflea (Daphnia magna)	93	>100	Practically non-toxic	00131632 ABC Laboratories, 1983	Acceptable

Imazapyr acid

Daphnia. MRID 00131632 (Core). In a 48-hour acute test, imazapyr acid was determined to be practically non-toxic to daphnids with an EC_{50} of >100 mg/L. The study is scientifically sound and meets guideline protocols.

Imazapyr isopropylamine salt

Daphnia. MRID 00147117 (Core). In a 48-hour static test, imazapyr isopropylamine salt was determined to be practically non-toxic to daphnids with an EC_{50} of 614 mg ae/L (750 mg ai/L). The study is scientifically sound and meets guideline protocols.

Daphnia. MRID 00153779 (Supplemental). The 48-hour acute toxicity of Arsenal® Herbicide (Salt of Imazapyr) to the water flea, *Daphnia magna*, was studied under static conditions. Daphnids were exposed to the test material at nominal concentrations of 0 (negative control), 32, 56, 100, 180, 320, 560, and 1000 mg Arsenal/L (0, 5.9, 10.4, 18.5, 59.3, 103.8, 185.3 mg ae/L). Analytical verification of the test material in the test solution was not conducted at any point during the definitive test. After 48-hours of exposure, immobility was 0% in the control and nominal 32-180 mg Arsenal/L treatment levels and 45, 90 and 100% in the nominal 320, 560 and 1000 mg Arsenal/L treatment level, respectively. After 48-hours of exposure, daphnids were observed on the bottom in

the 320 (4 daphnids total) and 560 (1 surviving daphnid) mg Arsenal/L treatment levels. All surviving daphnids were reported to be normal in the negative control and nominal 32-180 mg Arsenal/L treatment levels. The test solutions were not analytically verified in this study, so the actual concentrations that test organisms were exposed to are unknown. Toxicity values and categorization derived using nominal test concentrations may not be indicative of exposure to the test substance under these study conditions. The acute EC_{50} is 64.9 mg ae/L and a NOAEC/LOAEC of 59.3/103.8 mg ae/L. This study is scientifically sound; however, the test concentrations were not analytically verified. Toxicity values are based on the nominal concentrations. This study is classified as SUPPLEMENTAL for a formulated product.

B.2.5 Freshwater Invertebrates, Chronic Toxicity Studies

A freshwater aquatic invertebrate life-cycle test using the TGAI is normally required for pesticide registration because the end-use product may be transported to water from the intended use site, and the following conditions are met: (1) the presence of imazapyr in water is likely to be continuous or recurrent and (2) fate properties indicate that imazapyr is persistent in the aquatic environment (e.g., photolytic half-life of 300- 700 days). The preferred test is a 21-day life cycle on Daphnia magna. The data that were submitted show that imazapyr concentrations up to 97.1 mg/L did not significantly affect survival, reproductive success, or growth of first generation daphnids. The NOAEC of 97.1 mg/L will be used in assessing risk. Results of the study on the acid are summarized in Table B-2.5.

Table B-2.5 Freshwater Aquatic Invertebrate Chronic Toxicity for Imazapyr Acid

Species/ Flow-through	% ae	21-day NOAEC (mg/L)	Endpoints Affected	MRID No. Author/Year	Study Classification
Waterflea (Daphnia magna)	99.5	97.1	No effects on growth or reproduction	41315805 Manning, 1988	Acceptable

Daphnia. MRID 41315805 (Acceptable). In a life cycle flow-through test, imazapyr acid produced no treatment-related effects on survival, growth and reproduction of first generation daphnids. No physical or behavioral abnormalities were observed. The MATC and NOEL were determined to be ≥97.1 mg/L. The study is scientifically sound and meets guideline protocols.

B.2.6 Freshwater Macroinvertebrates Microcosm Study – Open Literature

An *in situ* microcosm study, published in the open literature and accessed via ECOTOX was conducted to assess the effects of a single application of imazapyr (stirred into the water column) at the following mean concentrations: 0.19, 2.1 or 19.8 mg/L (equivalent to 1, 10 or 100 times the expected environmental concentration from a normal application

rate) on the macroinvertebrate community of a logged pond cypress dome. *In situ* microcosms were set up in schedule-40 polyvinyl chloride water pipes (diameter 7.62 cm; height 45.7 cm; area 45.6 cm^2) driven approximately 12 cm into the substrate and leaving a mean water column depth of 32.1 cm. The microcosms were immediately dosed with the selected treatments of imazapyr and left undisturbed for two weeks. Forty eight microcosms were set up (3 blocks of 16, each block consisting of 4 replicates of 3 treatment levels and a control). In addition, 12 cypress dome cores, divided equally among the 3 blocks were sampled at the end of the study. These allowed for testing for microcosm influences on the measured parameters. Macroinvertebrates were hand picked from each sample and prepared for identification. Organisms other than chironomids were identified at the family level or to the lowest practical taxonomic level. Chironomids were identified to the genus level. Effects on aquatic plants were not examined. Changes in the macroinvertebrate composition, chironomid biomass and chironomid head-capsule deformities were assessed. A total of 2,904 individuals representing 44 taxa were collected. The following taxa were represented: *Caecidotea, Crangonyx,* Dipteran, Chironomid, *Polypedilum, Chironomus, Ablabesmyia,* and *Procladius*. There were three rain events following treatment. The half-life of imazapyr was calculated to be 3.2, 3.2 and 3.4 days for the 0.19, 2.1 and 19.8 mg/L concentrations, respectively. Imazapyr did not appear to affect any of these parameters at the concentrations tested (ECOTOX Ref. 68204). However, these results are of limited value because potential effects at the species level were not examined. Individual species could have been affected and the results may not have picked it up because the analysis was conducted at higher taxonomic levels. In addition, effects on aquatic plants were not examined.

B.3 Toxicity to Plants

B.3.1 Terrestrial Plants

Terrestrial Tier II studies are required for all low dose pesticides (those with the maximum use rate of 0.5 lbs ai/acre or less) and for any pesticide showing a negative response equal to or greater than 25% in Tier I studies. Tier II terrestrial plant toxicity studies were conducted to establish the toxicity of imazapyr and the isopropylamine salt of imazapyr to non-target terrestrial plants. The recommendations for seedling emergence and vegetative vigor studies are for testing of (1) six species of at least four dicotyledonous families, one species of which is soybean (Glycine max), and the second of which is a root crop, and (2) four species of at least two monocotyledonous families, one of which is corn (Zea mays). Due to problems of overcrowding and 'fresh weight' endpoints with the seedling emergence and vegetative vigor studies with imazapyr acid, only results that were classified by EFED as supplemental will be used to assess risk to imazapyr acid (seedling emergence for 3 monocots and 2 dicots; vegetative vigor for 3 monocots and 4 dicots). Tier II vegetative vigor studies were performed with the isopropylamine salt of imazapyr for one monocot (onion) and two dicots (soybean and sugar beet). This data will be used to assess risk to the isopropylamine salt of imazapyr.

Results of Tier II toxicity studies with monocots and dicots indicate that seedling emergence and vegetative vigor are severely impacted by exposure to imazapyr acid and to the isopropylamine salt of imazapyr. Seedling emergence, based on weight, was adversely impacted in monocots (wheat) at an EC_{25} of 0.0046 lb ae/are and in dicots (sugar beet) with an EC_{25} of 0.0024 lb ae/acre. In the wheat, severe stunting, interveinal chlorosis, and cessation of growth occurred at doses >0.0078 lb ae/acre. After 28 days, imazapyr acid resulted in >60% crop injury in sugar beets at all doses >0.031 lb ae/acre. Vegetative vigor in monocots, based on weight, was adversely impacted by both imazapyr acid and the isopropylamine salt of imazapyr at an EC_{25} of 0.012 lb ae/acre in wheat and 0.012 lb ae/acre in onion, respectively. In vegetative vigor studies with dicots, imazapyr acid was more toxic than the isopropylamine salt of imazapyr with an acid EC_{25} of 0.0009 lb ae/acre (cucumber) versus salt EC_{25} of 0.002 lb ae/acre (sugar beet), respectively. The observed effects to monocots and dicots included stunting, chlorosis, and plant death were observed for isopropylamine salt (MRID 40003711). The results of these studies are summarized in Table B-3.1

Table B-3.1 Tier II Terrestrial Non-target Plant Toxicity[C]*								
Species	**Seedling Emergence**		**Endpoint Affected**	**Vegetative Vigor**		**Endpoint Affected**	**MRID No. Author/Year**	**Study Classification**
	EC_{25} (lb ae/acre)	**NOAEC/[EC_{05}]****		**EC_{25} (lb ae/acre)**	**NOAEC/[EC_{05}]**			
Isopropylamine Salt of Imazapyr*								
Monocots								
Onion	--[A]	--	--	0.012	[0.005]	Dry weight	43889101 Feutz & Canez, 1995	Acceptable
Dicots								
Soybean	--	--	--	0.034	0.008	Shoot length		
Sugar beet	--	--	--	0.002	0.001	Dry weight		
Imazapyr Acid[B]								
Monocots								
Corn	--	--	--	>0.0156	0.0078	Weight	40811801 Banks, 1988	Supplemental
Oat	0.054	0.0156	Height	0.013	0.0039	Height		Supplemental
Onion	0.034	[0.01]	Weight	--	--	--		Supplemental
Wheat	0.0046	[0.00099]	Weight	0.012	0.0039	Weight		Supplemental
Dicots								
Sugar beet	0.0024	[0.00017]	Weight	0.00097	[0.00039]	Weight		Supplemental
Sunflower	--	--	--	0.0054	0.0039	Weight		Supplemental

Table B-3.1 Tier II Terrestrial Non-target Plant Toxicity[C]*								
Species	**Seedling Emergence**		**Endpoint Affected**	**Vegetative Vigor**		**Endpoint Affected**	**MRID No. Author/Year**	**Study Classification**
	EC_{25} (lb ae/acre)	**NOAEC/[EC_{05}]****		**EC_{25} (lb ae/acre)**	**NOAEC/[EC_{05}]**			
Cucumber	--	--		0.0009	[0.000064]	Height		Supplemental
Tomato	0.008	0.0003	Weight	>0.0156	0.00097	Weight		Supplemental

*The EC_{50}/NOAEC values from the toxicity tests with the isopropylamine salt of imazapyr are expressed in acid equivalents (a.e.).
**If the NOAEC value is above the EC_{25}, equal to the EC_{25}, or below the lowest concentration, an EC_{05} value is used instead.
[A] –- = no data [B] No data for pea and soybeans tested with acid, and the study was invalid. [C] Bold values are used in risk assessment.

123-1(a) Seedling Emergence - Tier II

Imazapyr Acid

Monocots (4 species) and Dicots (4 species). MRID 40811801 (Supplemental). In a Tier II seedling emergence study, the most sensitive monocot tested was wheat (EC_{25} 0.0046 lb ae/acre, EC_{05} 0.00099 lb ae/acre; shoot weight). The most sensitive dicot tested was sugarbeet (EC_{25} 0.0024 lb ae/acre, EC_{05} 0.00017 lb ae/acre; shoot weight). Acceptable data endpoints were used in the risk assessment..

123-1(b) Vegetative Vigor - Tier II

Imazapyr acid

Monocots (4 species) and Dicots (4 species). MRID 40811801 (Supplemental). In a Tier II vegetative vigor study, the most sensitive monocot tested was wheat (EC_{25} 0.012 lb ae/acre, NOEC 0.0039 lb ae/acre; shoot weight). The most sensitive dicot tested was cucumber (EC_{25} 0.0009 lb ae/acre, EC_{05} 0.000064 lb ae/acre; shoot height). Acceptable data endpoints were used in the risk assessment.

Imazapyr isopropylamine salt

Monocots (3 species) and Dicots (2 species). MRID 43889101 (Core). In a Tier II vegetative vigor study, chlorosis, stunting, and plant death. The most sensitive monocot tested was onion (EC_{25} 0.010 lb ae/acre, NOEC 0.004 lb ae/acre; shoot weight). The most sensitive dicot tested was sugar beet (EC_{25} 0.0016 lb ae/acre, NOEC 0.0008 lb ae/acre; shoot weight). The study is scientifically sound and meets guideline protocols.

Monocots (4 species) and Dicots (4 species). MRID 40003711 (Supplemental). This study was a modified Tier II vegetative vigor study that did not meet guideline requirements. Only descriptive summary data was presented; consequently effect levels were not determined. Observed effects included chlorosis, stunting, leaf tip burning, necrosis, and plant death.

B.3.2 Aquatic Plants

Several aquatic plant toxicity studies using the TGAI are required to establish the toxicity of imazapyr to non-target aquatic plants. The recommendation is for testing of five species: freshwater green alga (Selenastrum capricornutum), duckweed (Lemna gibba), marine diatom (Skeletonema costatum), blue-green algae (Anabaena flos-aquae), and a freshwater diatom. The 14-day EC_{50} for the freshwater vascular plant (duckweed) is 0.024 mg/L (NOAEC = 0.01 mg/L), based on inhibition of population growth and reduced frond production; and the lowest 7-day EC_{50} for the freshwater non-vascular plant (blue-green algae) is 12.2 mg/L (NOAEC = 9.6 mg/L), based on reduced cell

counts. In the non-vascular plant studies, the study authors concluded that imazapyr acid was not expected to exert detrimental effects at the maximum application rate up to 1.5 lbs ai/acre. The toxicity of the isopropylamine salt of imazapyr to duckweed was similar to the acid, with a 14-day EC_{50} of 0.018 mg ae/L (NOAEC = 0.011 mg ae/L). The isopropylamine salt of imazapyr was more toxic to the green algae than imazapyr acid and more closely resembled the toxic response of blue-green algae (see Table B-3.2 below). Since the toxicity of the isopropylamine salt is more toxic to aquatic vasculars and non-vasculars (based on duckweed and green algae) than imazapyr acid, it will be used in the risk assessment. The results of these studies are summarized in Table B-3.2.

Table B-3.2 Non-target Aquatic Plant Toxicity for Imazapyr Acid and Isopropylamine Salt of Imazapyr.

Species [Tier II]	% ae	EC_{50}/NOAEC (mg/L)	Endpoints Affected	MRID No. Author, Year	Study Classification
Isopropylamine Salt of Imazapyr*					
Duckweed (Lemna gibba)	23.3	0.018/0.011 (mg ae/L)	Frond production	43889102 Hughes et al., 1995	Acceptable
Green Algae (Selenastrum capricornutum)	23.3	11.5/7.16 (mg ae/L)	Slight change in cell shape	43889102 Hughes et al., 1995	Acceptable
Imazapyr Acid					
Duckweed (Lemna gibba)	99.5	0.024/0.01	Population growth Frond production	40811802 Hughes, 1987	Acceptable
Green Algae (Selenastrum capricornutum)	99.5	71/50.9	Cell density	40811802 Hughes, 1987	Acceptable
Blue-green Algae (Anabaena flos-aquae)	99.5	12.2/9.6	Cell density	40811802 Hughes, 1987	Acceptable
Diatom (Navicula pelliculosa)	99.5	>41/41	Cell density	40811802 Hughes, 1987	Acceptable
Diatom (Skeletonema costatum)	99.5	92/15.9	Cell density	40811802 Hughes, 1987	Acceptable

*The EC_{50}/NOAEC values from the toxicity tests with the isopropylamine salt of imazapyr are expressed in acid equivalents (a.e.)

122-2 Aquatic Plant Nonvascular

Imazapyr acid

Green algae. MRID 40811802 (Acceptable). In a Tier II toxicity test with Selenastrum capricornutum, the 7 day EC_{50} for cell density was 71 mg ai/L (NOEC = 50.9 mg ai/L).

The study is scientifically sound and meets the guideline protocols.

Blue-green algae. MRID 40811802 (Acceptable). In a Tier II toxicity test with Anabaena flos-aquae, the7-day EC_{50} for cell density was 12.2 mg ai/L (NOEC = 9.6 mg ai/L). The study is scientifically sound and meets the guideline protocols.

Marine diatom. MRID 40811802 (Acceptable). In a Tier II toxicity test with Skeletonema costatum, the7-day EC_{50} for cell density was 92 mg ai/L (NOEC = 15.9 mg ai/L). The study is scientifically sound and meets the guideline protocols.

Diatom. MRID 40811802 (Acceptable). In a Tier II toxicity test with Navicula pelliculosa, the 7-day EC_{50} for cell density was >41 mg ai/L (NOEC = 41 mg ai/L). The study is scientifically sound and meets the guideline protocols.

Imazapyr isopropylamine salt

Green algae. MRID 43889102 (Acceptable). In a Tier II toxicity test with green algae, the 7-day EC_{50}, based on slight changes in cell shape was 11.5 mg ae/L (NOEC = 7.16 mg ae/L). The study is scientifically sound and meets the guideline protocols.

123-2 Aquatic Plant Vascular

Imazapyr acid

Duckweed. MRID 40811802 (Acceptable). In a 14-day toxicity test with duckweed, the EC_{50} for frond production was 0.024 mg ai/L and the NOEC was 0.01 mg ai/L. Imazapyr is considered highly toxic and expected to exert a detrimental effect on vascular aquatic plants. The study is scientifically sound and meets guideline protocols.

Imazapyr isopropylamine salt

Duckweed. MRID 43889102 (Acceptable). In a 14-day toxicity test with duckweed, the EC_{50} for frond production was 0.018 mg ai/L and the NOEC was 0.011 mg ai/L. The study is scientifically sound and meets guideline protocols.