Aminopyralid

Human Health and Ecological Risk Assessment – FINAL REPORT

Prepared for:
USDA/Forest Service
and
National Park Service

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
AChE	acetylcholinesterase
AEL	adverse-effect level
a.e.	acid equivalents
a.i.	active ingredient
ATSDR	Agency for Toxic Substances and Disease Registry
ATV	all terrain vehicle
BCF	bioconcentration factor
bw	body weight
CBI	confidential business information
CEQ	Council on Environmental Quality
ChE	cholinesterase
CI	confidence interval
cm	centimeter
CNS	central nervous system
DAA	days after application
DAT	days after treatment
DER	data evaluation record
d.f.	degrees of freedom
DMF	dimethylformamide
DMSO	dimethylsulfoxide
EC_x	concentration causing X% inhibition of a process
EC_{25}	concentration causing 25% inhibition of a process
EC_{50}	concentration causing 50% inhibition of a process
EHE	2-ethylhexyl ester
EFED	Environmental Fate and Effects Division (U.S. EPA/OPP)
ExToxNet	Extension Toxicology Network
F	female
FH	Forest Health
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FOB	Functional Observation Battery
FQPA	Food Quality Protection Act
FS	Forest Service
g	gram
GLP	Good Laboratory Practices
ha	hectare
HED	Health Effects Division (U.S. EPA/OPP)
HQ	hazard quotient
IARC	International Agency for Research on Cancer
IRED	Interim Reregistration Eligibility Decision
IRIS	Integrated Risk Information System

ACRONYMS, ABBREVIATIONS, and SYSMBOLS *(continued)*

k_a	absorption coefficient
k_e	elimination coefficient
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
$K_{o/w}$	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound
LC_{50}	lethal concentration, 50% kill
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
LOC	level of concern
m	meter
M	male
MEI	most exposed individual
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
MLE	maximum likelihood estimate
mM	millimole
mPa	millipascal, (0.001 Pa)
MOS	margin of safety
MRID	Master Record Identification Number
MSDS	material safety data sheet
MSMA	monosodium methanearsonate
MW	molecular weight
NAWQA	USGS National Water Quality Assessment
NCI	National Cancer Institute
NCOD	National Drinking Water Contaminant Occurrence Database
NEPA	National Environmental Policy Act
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOS	not otherwise specified
NRC	National Research Council
NPS	National Park Service
NTP	National Toxicology Program
OECD	Organization for Economic Co-operation and Development (Europe)
OM	organic matter
OPP	Office of Pesticide Programs
OPPTS	Office of Pesticide Planning and Toxic Substances
OSHA	Occupational Safety and Health Administration
Pa	Pascal

ACRONYMS, ABBREVIATIONS, and SYSMBOLS *(continued)*

PBPK	physiologically-based kinetic
PHED	Pesticide Handlers Exposure Database
ppm	parts per million
RBC	red blood cells
RED	re-registration eligibility decision
RfD	reference dose
SERA	Syracuse Environmental Research Associates
TEP	typical end-use product
T.G.I.A.	Technical grade active ingredient
TIPA	Triisopropanolamine
TRED	Tolerance Reassessment Eligibility Decision
UF	uncertainty factor
U.S.	United States
USDA	U.S. Department of Agriculture
U.S. EPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WHO	World Health Organization

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

To convert ...	Into ...	Multiply by ...
acres	hectares (ha)	0.4047
acres	square meters (m^2)	4,047
atmospheres	millimeters of mercury	760
centigrade	Fahrenheit	1.8℃+32
centimeters	inches	0.3937
cubic meters (m^3)	liters (L)	1,000
Fahrenheit	centigrade	0.556℉-17.8
feet per second (ft/sec)	miles/hour (mi/hr)	0.6818
gallons (gal)	liters (L)	3.785
gallons per acre (gal/acre)	liters per hectare (L/ha)	9.34
grams (g)	ounces, (oz)	0.03527
grams (g)	pounds, (oz)	0.002205
hectares (ha)	acres	2.471
inches (in)	centimeters (cm)	2.540
kilograms (kg)	ounces, (oz)	35.274
kilograms (kg)	pounds, (lb)	2.2046
kilograms per hectare (hg/ha)	pounds per acre (lb/acre)	0.892
kilometers (km)	miles (mi)	0.6214
liters (L)	cubic centimeters (cm^3)	1,000
liters (L)	gallons (gal)	0.2642
liters (L)	ounces, fluid (oz)	33.814
miles (mi)	kilometers (km)	1.609
miles per hour (mi/hr)	cm/sec	44.70
milligrams (mg)	ounces (oz)	0.000035
meters (m)	feet	3.281
ounces (oz)	grams (g)	28.3495
ounces per acre (oz/acre)	grams per hectare (g/ha)	70.1
ounces per acre (oz/acre)	kilograms per hectare (kg/ha)	0.0701
ounces fluid	cubic centimeters (cm^3)	29.5735
pounds (lb)	grams (g)	453.6
pounds (lb)	kilograms (kg)	0.4536
pounds per acre (lb/acre)	kilograms per hectare (kg/ha)	1.121
pounds per acre (lb/acre)	mg/square meter (mg/m^2)	112.1
pounds per acre (lb/acre)	µg/square centimeter ($\mu g/cm^2$)	11.21
pounds per gallon (lb/gal)	grams per liter (g/L)	119.8
square centimeters (cm^2)	square inches (in^2)	0.155
square centimeters (cm^2)	square meters (m^2)	0.0001
square meters (m^2)	square centimeters (cm^2)	10,000
yards	meters	0.9144

Note: All references to pounds and ounces refer to avoirdupois weights unless otherwise specified.

CONVERSION OF SCIENTIFIC NOTATION

Scientific Notation	Decimal Equivalent	Verbal Expression
$1 \cdot 10^{-10}$	0.0000000001	One in ten billion
$1 \cdot 10^{-9}$	0.000000001	One in one billion
$1 \cdot 10^{-8}$	0.00000001	One in one hundred million
$1 \cdot 10^{-7}$	0.0000001	One in ten million
$1 \cdot 10^{-6}$	0.000001	One in one million
$1 \cdot 10^{-5}$	0.00001	One in one hundred thousand
$1 \cdot 10^{-4}$	0.0001	One in ten thousand
$1 \cdot 10^{-3}$	0.001	One in one thousand
$1 \cdot 10^{-2}$	0.01	One in one hundred
$1 \cdot 10^{-1}$	0.1	One in ten
$1 \cdot 10^{0}$	1	One
$1 \cdot 10^{1}$	10	Ten
$1 \cdot 10^{2}$	100	One hundred
$1 \cdot 10^{3}$	1,000	One thousand
$1 \cdot 10^{4}$	10,000	Ten thousand
$1 \cdot 10^{5}$	100,000	One hundred thousand
$1 \cdot 10^{6}$	1,000,000	One million
$1 \cdot 10^{7}$	10,000,000	Ten million
$1 \cdot 10^{8}$	100,000,000	One hundred million
$1 \cdot 10^{9}$	1,000,000,000	One billion
$1 \cdot 10^{10}$	10,000,000,000	Ten billion

EXECUTIVE SUMMARY

OVERVIEW

Aminopyralid is a new herbicide that has been registered by the U.S. EPA for the control of invasive weeds. The control of invasive weeds is a major component in programs conducted by both the USDA/Forest Service and the National Park Service (NPS). Both of these organizations have begun using aminopyralid in weed management programs and both organizations are considering expanding the use of aminopyralid in other weed management programs.

The U.S. EPA has judged that aminopyralid appears to be a *reduced risk* herbicide. This judgment by the U.S. EPA is supported by the current risk assessment. Aminopyralid is an effective herbicide. As with any effective herbicide applied to terrestrial weeds, adverse effects in nontarget terrestrial plants are plausible. There is no indication, however, that adverse effects on workers, members of the general public or other nontarget animal species are likely.

This assessment of aminopyralid is tempered by the lack of information on aminopyralid in the open literature. All of the information on the toxicity of aminopyralid comes from studies that have been submitted to the U.S. EPA in support of aminopyralid registration. While these studies have been reviewed and the bulk of these studies appear to have been appropriately designed, conducted and reported, the available information on aminopyralid is much less diverse than the information that is available on herbicides that have been used for many years and for which the open literature is rich and varied. This situation will exist for any new herbicide or other new pesticide.

PROGRAM DESCRIPTION

Two formulations of aminopyralid are specifically considered in this risk assessment: Milestone and Milestone VM. Both of these formulations contain the triisopropanolamine (TIPA) salt of aminopyralid (40.6 % w a.i./v, equivalent to 21.1% a.e. or 2 lbs a.e./gal). These formulations contain no inert ingredients other than water and triisopropanolamine.

The most likely uses of aminopyralid will involve applications to forest and rangelands, rights-of-way, and developed recreational areas such as campgrounds, picnic areas and trails. Application methods have and will likely continue to include backpack (selective foliar), hydraulic spray, and aerial applications. The labeled application rates for aminopyralid are 0.03 to 0.11 lb a.e./acre. The upper bound of this range is likely to be used for rhizomatous weeds. For non-rhizomatous weeds, the application rate will generally be about 0.078 lb a.e./acre. Again, specific application rates will vary with site-specific considerations. Consequently, the current risk assessment considers the full range of labeled application rates for broadleaf weeds as well as all labeled application methods.

Dow AgroSciences, the registrant for aminopyralid, has suggested that this herbicide may be used as an alternative to herbicides such as picloram, clopyralid, 2,4-D, dicamba, monosodium methanearsonate, and metsulfuron methyl. While the decision to use any particular herbicide is based on a number of site-specific considerations, the Forest Service and NPS have begun to use

aminopyralid at some sites rather than herbicides such as picloram, clopyralid, glyphosate, and dicamba.

HUMAN HEALTH RISK ASSESSMENT

Hazard Identification – Because aminopyralid is a new herbicide, no information is available in the published literature on the toxicity of aminopyralid to humans or other mammalian species. The only information on aminopyralid that is available for assessing potential hazards in humans is a series of toxicity studies that have been submitted to and evaluated by the U.S. EPA's Office of Pesticides in support of the registration for aminopyralid.

Although the mechanism of action of aminopyralid and other pyridine carboxylic acid herbicides is fairly well characterized in plants, the mechanism of action of aminopyralid in mammals is not well characterized. The weight-of-evidence suggests that aminopyralid may not have any remarkable systemic toxic effects. The effects that are most commonly seen involve effects on the gastrointestinal tract after oral exposure and these may be viewed as portal of entry effects rather than systemic toxic effects. The location of these effects within the gastrointestinal tract appears to vary among species with the ceca being the most common site of action in rats and the stomach being the most common site of action in dogs and rabbits. Mice do not seem to display any remarkable gastrointestinal effects after oral doses of aminopyralid. The reason for these differences among species is not clear but may simply reflect differences in methods of exposure (gavage versus dietary) and/or differences in anatomy.

In one acute oral toxicity study in rats using the aminopyralid TIPA formulation, lacrimation and cloudy eyes were noted in all test animals on the first day of the study but not on subsequent days. Clouding of the eyes is an unusual effect that has not been noted in other studies on aminopyralid, either the acid or the TIPA salt. The significance of this observation, if any, is unclear.

Aminopyralid is rapidly absorbed and excreted and is not substantially metabolized in mammals. As a consequence of rapid absorption and excretion, gavage and dietary exposures probably lead to very different patterns in the time-course of distribution in mammals. The oral LD_{50} of aminopyralid has not been determined because aminopyralid does not cause any mortality at the dose limits set by the U.S. EPA for acute oral toxicity studies – i.e., up to 5,000 mg/kg bw. Similarly, subchronic and chronic toxicity studies have failed to demonstrate any clear signs of systemic toxic effects. Developmental studies involving gavage administration, however, have noted signs of incoordination in adult female rabbits. The incoordination was rapidly reversible and did not persist past the day of dosing. Two chronic oral bioassays have been conducted, one in mice and the other in rats, and a 1-year feeding study is available in dogs. Based on the results of the chronic bioassays as well as the lack of mutagenic activity in several mutagenicity screening assays, there is no basis for asserting that aminopyralid is a carcinogen. Similarly, based on the chronic bioassays and several additional subchronic bioassays in mice, rats, dogs, and rabbits, there is no basis for asserting that aminopyralid will cause adverse effects on the immune system or endocrine function. The potential for effects on the nervous system is less clear. Aminopyralid has also been subject to several bioassays for developmental toxicity and

one multi-generation study for reproductive performance. No adverse effects on offspring have been noted in these studies other than decreased body weight in offspring that is associated with decreased food consumption and decreased body weight in adult females.

Exposure Assessment – For workers applying aminopyralid, three types of application methods are modeled: directed ground spray, broadcast ground spray, and aerial spray. In non-accidental scenarios involving the normal application of aminopyralid, central estimates of exposure for workers are approximately 0.001 mg/kg/day for aerial and backpack workers and about 0.002 mg/kg/day for broadcast ground spray workers. Upper ranges of exposures are approximately 0.012 mg/kg/day for broadcast ground spray workers and 0.006 mg/kg/day for backpack and aerial workers. All of the accidental exposure scenarios for workers involve dermal exposures. Except for the scenario involving a spill on the lower-legs for 1 hour (an upper bound dose of 0.003 mg/kg/event), the accidental exposures lead to dose estimates that are substantially lower than the general exposure levels estimated for workers. This is not uncommon and it reflects the fact that the general exposure estimates are based on field studies of workers in which accidental and/or incidental events such as spills probably occurred and in some cases were specifically noted to occur.

For the general public, acute levels of exposures range from minuscule (e.g., $1x10^{-8}$ mg/kg/day) to about 0.4 mg/kg bw at the typical application rate of 0.078 lb a.e./acre. The upper bound of exposure, 0.4 mg/kg bw, is associated with the consumption of contaminated water by a child shortly after an accidental spill. This exposure scenario is highly arbitrary. The upper bound of the dose associated with the consumption of contaminated vegetation, a more plausible but still extreme exposure scenario, is about 0.1 mg/kg bw. The other acute exposure scenarios lead to much lower dose estimates – i.e., ranging from near zero to about 0.042 mg/kg for the accidental direct spray of a child. The lowest acute exposures are associated with swimming in or drinking contaminated water.

The modeled chronic or longer-term exposures are much lower than the corresponding estimates of acute exposures. The highest longer-term exposures are associated with the consumption of contaminated vegetation and the upper bound for this scenario is about 0.027 mg/kg/day. This is followed by the scenario for the longer-term consumption of contaminated fruit with an upper bound of 0.003 mg/kg/day. As with the acute exposures, the lowest longer-term exposures are associated with the consumption of surface water.

Dose-Response Assessment – The Office of Pesticide Programs of the U.S. EPA has derived a chronic RfD of 0.5 mg/kg/day for aminopyralid. This RfD is based on a chronic rat NOAEL of 50 mg/kg/day and an uncertainty factor of 100. The Office of Pesticide Programs has also derived an acute RfD of 1 mg/kg bw/day based on a NOAEL from a reproduction study of about 100 mg/kg/day. In deriving both of these RfD values, the U.S. EPA used an uncertainty factor of 100, a factor of 10 for extrapolating from animals to humans and a factor of 10 for extrapolating to sensitive individuals within the human population. Both of these RfD values are based on NOAELs for the most sensitive endpoint in the most sensitive species and studies in which LOAEL values were identified. In addition, both of the NOAEL values are supported by other

studies. Thus, the RfD values recommended by the U.S. EPA are adopted directly in the current risk assessment.

Risk Characterization – The risk characterization for both workers and members of the general public is reasonably simple and unambiguous: based on a generally conservative and protective set of assumptions regarding both the toxicity of aminopyralid and potential exposures to aminopyralid, there is no basis for suggesting that adverse effects are likely in either workers or members of the general public even at the maximum application rate that might be used in Forest Service or NPS programs.

For workers, no exposure scenarios, acute or chronic, exceeds the RfD at the upper bound of the estimated dose associated with the highest application rate of 0.11 lb a.e./acre. The hazard quotients for directed ground spray, broadcast ground spray, and aerial applications are below the level of concern by factors of 33 to 200 over the range of application rates considered in this risk assessment.

For members of the general public, upper bounds of hazard quotients at the highest application rate are below a level of concern by factors of 100 to 125,000 for longer term exposures. For one accidental exposure scenario, the consumption of contaminated water by a child immediately after an accidental spill of aminopyralid into a small pond, the hazard quotient is 0.6, approaching the level of concern (1.0). This is an intentionally extreme exposure scenario that typically leads to the highest hazard quotient in pesticide risk assessments similar to the current assessment on aminopyralid. The upper bounds of acute exposure scenarios for contaminated vegetation or fruit are below the level of concern by factors of 10 to 50. Acute non-accidental exposure scenarios for members of the general public that involve contaminated water are below the level of concern by factors of about 140 to 14,000.

The risk characterization given in this risk assessment is qualitatively similar to that given by the U.S. EPA: no risks to workers or members of the general public are anticipated. The current risk assessment derives somewhat higher hazard quotients than those in the U.S. EPA human health risk assessment because the current risk assessment uses a number of extreme exposure scenarios that are not used by the U.S. EPA.

ECOLOGICAL RISK ASSESSMENT

Hazard Identification – The mammalian toxicity of aminopyralid is relatively well-characterized in experimental mammals in a series of toxicity studies that are required for pesticide registration. In standard experimental toxicity studies in rats, mice, rabbits, and dogs, aminopyralid has low acute and chronic oral toxicity. It seems reasonable to assume the most sensitive effects in wildlife mammalian species will be the same as those in experimental mammals (e.g., changes in the gastrointestinal tract, weight loss, and incoordination).

Results of acute exposure studies in birds indicate that avian species appear no more sensitive than experimental mammals to aminopyralid in terms of acute lethality. In terms of non-lethal effects, however, birds may be somewhat more sensitive than mammals to aminopyralid after

gavage exposures. In developmental studies involving gavage administration, NOAEL values for mammals are in the range of 200 mg a.e./kg bw/day. In birds, the single dose gavage NOAEL is 14 mg a.e/kg bw. Birds are much less sensitive to dietary exposures compared to gavage exposures with NOAEL values for 5-day dietary exposures of over 1000 mg a.e./kg bw/day. While chronic studies (i.e., those approach the lifespan of the animal) are not available in birds, two standard reproduction studies have been conducted in bobwhite quail and one reproduction study has been conducted in mallard ducks. One of the reproduction studies in bobwhite quail appears to be a failed study but the second study in bobwhites, although not yet reviewed by the U.S. EPA, appears to acceptable. The study in mallards, which has been reviewed and accepted by the U.S. EPA, yielded the lowest NOAEL, 184 mg a.e./kg bw/day, comparable to the reproductive NOAEL values in mammals.

A standard set of toxicity studies are also available on terrestrial plants. Dicots (i.e., broadleaf plants) are substantially more sensitive to aminopyralid than monocots (e.g., grasses). This is consistent with the proposed uses of aminopyralid and the quantitative aspects of this difference in sensitivity are discussed further in the dose-response assessment for terrestrial plants. Relatively little information is available on the toxicity of aminopyralid to terrestrial invertebrates or terrestrial microorganisms. Relatively little information is available on the toxicity of aminopyralid to terrestrial invertebrates or terrestrial microorganisms. Based on bioassays in honeybees, earthworms, and soil microorganisms, aminopyralid does not appear to be very toxic to terrestrial invertebrates or soil microorganisms.

There is no indication that aminopyralid is likely to be toxic to aquatic animals based on standard acute and chronic bioassays in fish and invertebrates as well as one acute toxicity study in a species of frog. As would be expected from a herbicide, some aquatic plants are more sensitive than aquatic animals to the effects of aminopyralid. Duckweed, the one macrophyte on which a bioassay of aminopyralid has been conducted, does not appear to be sensitive to aminopyralid.

Exposure Assessment – In acute exposure scenarios, the highest exposure for terrestrial vertebrates involves the consumption of contaminated insects by a small bird, which could reach up to about 3 mg/kg. There is a wide range of exposures anticipated from the consumption of contaminated vegetation by terrestrial animals: central estimates range from 0.1 mg/kg for a small mammal consuming fruit to 2.1 mg/kg for a large bird with upper bound estimates of about 0.2 mg/kg for a small mammal consuming fruit and 6 mg/kg for a large bird consuming grasses. The consumption of contaminated water will generally lead to much lower levels of acute exposure – i.e., in the range of about 0.00002 to 0.007 mg/kg. A similar pattern is seen for chronic exposures. The central estimate for daily doses for a small mammal from the longer term consumption of contaminated vegetation at the application site is about 0.002 mg/kg/day, with an upper estimate of about 0.01 mg/kg/day. Dose estimates associated with the consumption of contaminated water are in the range of 0.00001 mg/kg bw/day to 0.003 mg/kg bw/day for a small mammal. Based on general relationships of body size to body volume, larger vertebrates will be exposed to proportionately lower doses than small vertebrates under comparable exposure conditions. Because of the apparently low toxicity of aminopyralid to animals, the rather substantial variations in the different exposure assessments have little impact

on the assessment of risk to terrestrial animals.

For terrestrial plants, five exposure scenarios are considered quantitatively: direct spray, spray drift, runoff, wind erosion and the use of contaminated irrigation water. Unintended direct spray is expressed simply as the application rate – i.e., 0.078 lb a.e./acre for the typical application rate. For directed foliar applications, this scenario should be regarded as an extreme/accidental form of exposure that is not likely to occur in most applications. For broadcast applications, the direct spray scenario is much more plausible. Spray drift is based on estimates from AGDRIFT. The proportion of the applied amount transported off-site from runoff is based on standard GLEAMS modeling of clay, loam, and sand. The amount of aminopyralid that might be transported off-site from wind erosion is based on estimates of annual soil loss associated with wind erosion and the assumption that the herbicide is incorporated into the top 1 cm of soil. Exposure from the use of contaminated irrigation water is based on the same data used to estimate human exposure from the consumption of contaminated ambient water. All of these exposure scenarios are dominated by situational variability because the levels of exposure are highly dependent on site-specific conditions. Thus, the exposure estimates are intended to represent conservative but plausible ranges that could occur but these ranges may over-estimate or under-estimate actual exposures in some cases.

Exposures of aquatic plants and animals to aminopyralid are based on essentially the same information used to assess the exposure to terrestrial species from contaminated water. The peak estimated rate of contamination of ambient water associated with the application of aminopyralid is 0.1 (0.002 to 0.6) mg a.e./L at a normalized application rate of 1 lb a.e./acre. For longer-term exposures, estimated rate of contamination of ambient water is 0.04 (0.001 to 0.26) mg a.e./L at a normalized application rate of 1 lb a.e./acre. For the assessment of potential hazards to aquatic species, these water contamination rates are adjusted based on the application rates considered in this risk assessment.

Dose-Response Assessment – The available toxicity data support separate dose-response assessments in eight classes of organisms: terrestrial mammals, birds, terrestrial invertebrates, terrestrial plants, fish, aquatic invertebrates, aquatic algae, and aquatic macrophytes. Different units of exposure are used for different groups of organisms depending on how exposures are likely to occur and how the available toxicity data are expressed. When possible, a range of toxicity values based on the most sensitive and most tolerant species within a given group of organisms are given.

For terrestrial mammals, the dose-response assessment for aminopyralid is based on the same data as the human health risk assessment (i.e., an acute gavage NOAEL of 104 mg/kg bw and a chronic dietary NOAEL of 50 mg/kg/day). In terms of acute toxicity, birds appear to be more sensitive than mammals to aminopyralid with an acute NOAEL of 14 mg a.e./kg/day from a gavage study. In terms of longer-term toxicity, however, the toxicity value for birds is 184 mg a.e./kg bw/day, somewhat higher than the corresponding value in mammals. It should be noted that the acute NOAEL for birds is lower than the chronic NOAEL for birds. This is an atypical situation. Birds appear to be much more sensitive to aminopyralid after gavage administration

than after dietary administration. This difference in sensitivity results in the lower acute NOAEL (gavage) relative to the chronic NOAEL (dietary). Basing the acute NOAEL for birds on a gavage study is a conservative, and perhaps grossly conservative, approach. This is discussed further in the risk characterization.

For terrestrial invertebrates, no mortality would be expected following acute exposure to doses up to 1075 mg/kg based on direct spray studies in honey bees. Based on a single bioassay in earthworms, soil invertebrates do not appear to be sensitive to aminopyralid with a NOEC value of 5000 mg a.e./kg soil. Similarly, a single bioassay on soil microorganisms does not suggest that adverse effects would be expected at concentrations of up to about 8 mg a.e./kg soil.

The toxicity of aminopyralid to terrestrial plants is relatively well-characterized. Aminopyralid is more toxic to dicots than monocots. The most sensitive species have a NOEC value of 0.00048 lbs a.e./acre based on seeding emergence studies (soil exposures) and a NOEC value of 0.0002 lb a.e./acre based on foliar exposure. Tolerant species have NOEC values of 0.11 lb a.e./acre for both soil and foliar exposures.

Aminopyralid has a low order of acute toxicity to aquatic animals, with acute NOEC values falling within a narrow range: 50 mg a.e./L for sensitive fish to 100 mg a.e./L for tolerant fish. Acute toxicity values for amphibians and aquatic invertebrates fall within this range. Algae and aquatic macrophytes are only somewhat more sensitive with NOEC values for algae in the range of 6 mg a.e./L to 23 mg a.e./L and a single NOEC of 44 mg a.e./L for an aquatic macrophyte. The lowest aquatic toxicity value is 1.36 mg a.e./L from an egg-and-fry study in fathead minnow. Aquatic invertebrates are much less sensitive to longer-term exposures to aminopyralid with NOEC values in the range of 102 mg a.e./L to 130 mg a.e./L.

Risk Characterization – Aminopyralid is an effective herbicide that is designed to damage certain types of terrestrial plants, particularly broadleaf weeds. Consequently, nontarget plants that are similar to target species in sensitivity to aminopyralid may also be adversely affected by aminopyralid applications. Aminopyralid is selective to the extent that dicots (broadleaf plants) are much more sensitive to aminopyralid than monocots (e.g. grasses). Consequently, some nontarget dicots that are directly sprayed with aminopyralid at or near effective application rates are likely to be adversely affected. Direct spray scenarios for sensitive species of plants result in risk quotients in the range of 150 to 550 over application rates from 0.03 lb a.e./acre to 0.11 a.e./acre. For all forms of broadcast applications, the direct spray scenario seems plausible and relevant. The direct spray of nontarget species could be much less likely in directed foliar applications (e.g., backpack). Of the indirect exposure scenarios (i.e., drift, runoff, and wind erosion), drift appears to present the highest potential risks to sensitive species of plants. At distances from about 25 feet to about 300 feet downwind, hazard quotients for sensitive plant species are in the range of about 2 to 10 for ground applications and 2 to about 80 for aerial applications. Except in areas that are highly susceptible to runoff such as hard packed and predominantly clay soils, offsite losses associated with runoff do not appear to pose a substantial risk. Similarly, risks associated with transport of the herbicide by wind erosion appear to be insubstantial. All of the individual exposure scenarios for nontarget vegetation could be highly

variable depending on a large number of site-specific considerations.

There is no indication that other groups of organisms will by adversely affected by aminopyralid. These groups include tolerant species of terrestrials plants (such a grasses), aquatic plants (algae or macrophytes), mammals, birds, aquatic or terrestrial invertebrates, terrestrial microorganisms, fish, and amphibians.

As with all ecological risk assessments, the current risk assessment is based on tests in only a limited number of species and under conditions that may not well-represent populations of free-ranging nontarget species. For some groups of organisms including soil microorganisms and amphibians, this limitation is severe in that the available information is sparse and not well-suited to quantitative risk assessment. In other groups of organisms, there are uncertainties in the application of the different types of information that are available for the characterization of risk. These uncertainties are particularly evident in the assessment of potential risks to birds in which the current risk assessment takes an extremely conservative approach in the application of gavage toxicity data to the assessment of risks from dietary exposures.

1. INTRODUCTION

Aminopyralid is a new herbicide that has been registered by the U.S. EPA (U.S. EPA/OPPEPTS 2005). The control of undesirable vegetation is a major component in programs conducted by both the USDA/Forest Service and the National Park Service (NPS). Both of these organizations have begun using aminopyralid in weed management programs (e.g., USDA/FS 2006) and both organizations are considering expanding the use of aminopyralid in other weed management programs. The present document provides a risk assessment for human health effects and ecological effects to support an assessment of the environmental consequences of using aminopyralid in both Forest Service and NPS programs.

When discussing target organisms in the Forest Service and NPS programs, the terms *weed*, *invasive weeds*, *invasive plants*, and *target vegetation* are used interchangeably throughout this document. Program focus for the use of aminopyralid by both the Forest Service and NPS is the control or elimination of invasive plants (weeds) potentially affecting the management of ecosystems to generate a predefined desired future condition in the area being treated.

This document contains four chapters, including the introduction, program description, risk assessment for human health effects, and risk assessment for ecological effects or effects on wildlife species. Each of the two risk assessment chapters has four major sections, including an identification of the hazards associated with aminopyralid and its commercial formulation, an assessment of potential exposure to the product, an assessment of the dose-response relationships, and a characterization of the risks associated with plausible levels of exposure.

Although this is a technical support document and addresses some specialized technical areas, an effort was made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical concepts, methods, and terms common to all parts of the risk assessment are described in plain language in a separate document (SERA 2007a).

Most pesticide risk assessments – e.g., triclopyr (SERA 2003a), picloram (SERA 2003b), and clopyralid (SERA 2004) – are based on two general types of information: studies published in the open literature and studies submitted to the U.S. EPA's Office of Pesticides in support of the registration of the pesticide under review. Because aminopyralid is a new pesticide, very little information on aminopyralid is available in the open literature. As of the date of the current document, no published studies on the toxicity of aminopyralid were identified in searches of TOXLINE (http://toxnet. nlm.nih.gov/) or the AGRICOLA (http://agricola.nal.usda.gov/). The only published article is an efficacy study by Ferrell et al. (2006) on the use of aminopyralid for the control of tropical soda apple.

Because of the lack of information on aminopyralid in the open literature, this risk assessment is and must be based almost exclusively on studies submitted to the U.S. EPA in support of the registration of aminopyralid. These studies are typically classified as

Confidential Business Information (CBI) and are not typically released or available to individuals outside of the U.S. EPA Office of Pesticides.

In the preparation of this risk assessment, however, full copies of these studies were requested from Dow AgroSciences, the registrant for aminopyralid. With the exception of some proprietary information (discussed further below), Dow AgroSciences provided copies of all studies that were submitted to the U.S. EPA to support the registration of aminopyralid. Dow AgroSciences has provided full copies of 110 studies, as listed in Section 5 (References). Initially, 107 studies were provided to SERA, Inc. by John Jachetta, the product manager for aminopyralid at Dow AgroSciences. Dow AgroSciences declined to release two submissions: Ghaoui (2004)/MRID 46235701 and Jensen 2004a/MRID 46235702. These studies both involve information on product identity, including manufacturing methods. In addition, some of the studies submitted to the U.S. EPA contain confidential attachments. These types of studies and attachments are regarded as proprietary and are exempt from FOIA. The studies on product chemistry as well as confidential attachments were not provided to SERA by Dow AgroSciences. As discussed further in Section 2, however, Dow AgroSciences has released some important information on impurities and inerts. Two of the studies initially provided by Dow AgroSciences were flawed: a study on reproduction in bobwhite quail (Mach 2003b) and an algal bioassay (Hoberg 2002c). Dow AgroSciences has repeated both studies and full copies of the new studies in quail (Temple et al. 2007) and algae (Hancock et al. 2007) have been provided to SERA by Dow AgroSciences. Additional studies provided by Dow AgroSciences include a gavage developmental toxicity study in rats (Carney and Tornesi 2004c), a response concerning aminopyralid to the FAO/WHO Joint Meetings on Pesticide Residues (Dow AgroSciences 2006a), a metabolism study on aminopyralid in rabbits (Hansen et al. 2005), and a study on triisopropanolamine (McCollister et al. 1981).

Under the Freedom of Information Act (FOIA), SERA also requested full copies of the studies on aminopyralid from the U.S. EPA. In addition, SERA requested copies of the two risk assessments on aminopyralid that have been conducted by the U.S. EPA: the human health risk assessment conducted by Health Effects Division (HED) of the Office of Pesticides (U.S. EPA/OPP-HED 2004) and the ecological risk assessment conducted by the Environmental Fate and Effects Division of the Office of Pesticides (U.S. EPA/OPP-EFED 2004). Lastly, the FOIA requested all data evaluation records on aminopyralid. The original FOIA was submitted to the U.S. EPA on May 15, 2006. The U.S. EPA has provided copies of the requested risk assessments as well as most of the DERs. The specific DERs that have been provided are identified by MRID number in the appendices to this risk assessment. The utility of the DERs and the role that these play in data quality evaluation is discussed further below. SERA did not received full copies of the requested studies from the U.S. EPA. This does not impact this risk assessment because all studies that could have been released by the U.S. EPA under FOIA as well as some additional studies not submitted to the U.S. EPA were provided by Dow AgroSciences.

Some additional information on aminopyralid has been identified from the Internet. Citations to this information are listed in Section 5 (References). Some of citations found on the Internet include evaluations of aminopyralid by other organizations – e.g., California

EPA 2006; Gajanayake 2006 (Australian Pesticides and Veterinary Medicines Authority). While these reviews have been consulted in the preparation of this risk assessment, they do not contain any important information that is not included in the studies submitted to the U.S. EPA. Other citations include various reports and reviews by the U.S. EPA as well as abstract of presentations on the use of aminopyralid in specific applications. Again, this information is included for completeness but has little impact on the current risk assessment.

The Forest Service and NPS are aware of and are sensitive to concerns with risk assessments that are based chiefly on studies submitted to the U.S. EPA in support of product registration. The general concern can be expressed as follows:

> *If the study is paid for and/or conducted by the registrant, the study may be designed and/or conducted and/or reported in a manner that will obscure any adverse effects that the compound may have.*

This type of concern is largely without foundation. While any study (published or unpublished) can be falsified, concerns with the design, conduct and reporting of studies that are submitted to the U.S. EPA for pesticide registration are minor. The design of studies that are submitted for pesticide registration is based on strict guidelines for both the conduct and reporting of studies. These guidelines are developed by the U.S. EPA and not by the registrants. Full copies of the guidelines for these studies are available at http://www.epa.gov/opptsfrs/home/guidelin.htm. All studies are conducted under Good Laboratory Practices (GLPs). GLPs are an elaborate set of procedures that involve documentation and independent quality control and quality assurance that substantially exceed the levels typically seen in open literature publications. Lastly, each study that is submitted to the U.S. EPA is reviewed by the U.S. EPA for adherence to the relevant study guidelines. These reviews most often take the form of Data Evaluation Records (DERs). While the nature and complexity of DERs will vary with the nature and complexity of the differing studies, each DER involves an independent assessment of the study to ensure that the EPA Guidelines are followed. In addition, each DER undergoes internal review (and sometimes several layers of review).

There are real and legitimate concerns with risk assessments that based solely on registrant submitted studies but data quality and data integrity are not substantial concerns. The major limitation of risk assessments that are based solely on registrant submitted studies involve the nature and diversity of the available studies. The studies required by the U.S. EPA are based on a relatively narrow set of studies in a relatively small subset of species. For some pesticides (e.g., picloram, clopyralid, and triclopyr), a very large base of published studies are available, many of which are generated by academics who have a fundamental interest in understanding both the toxicology of a compound as well as underlying biological principles (e.g., physiology, biochemistry, ecology, etc.). Such studies tend to be non-standard but highly creative and can substantially contribute to or even form the basis of a risk assessment. Because aminopyralid is a new pesticide, this type of information from the open literature is not available. This is a limitation that is acknowledged.

As the open literature on aminopyralid develops, it is likely that this risk assessment will be updated at some point. The Forest Service and NPS welcome input from all interested parties on the selection of studies included in the risk assessment. This input is helpful, however, only if recommendations for including additional studies specify why and/or how the new or not previously included information would be likely to alter the conclusions reached in the risk assessments.

Almost no risk estimates presented in this document are given as single numbers. Usually, risk is expressed as a central estimate and a range, which is sometimes very large. Because of the need to encompass many different types of exposure as well as the need to express the uncertainties in the assessment, this risk assessment involves numerous calculations. Most of the calculations are relatively simple and the very simple calculations are included in the body of the document. Some of the calculations, however, are cumbersome. For those calculations, an EXCEL workbook, consisting of a set of worksheets, is included as an attachment to the risk assessment. The worksheets provide the details for the estimates cited in the body of this document. The worksheets are divided into the following sections: general data and assumptions, chemical specific data and assumptions, exposure assessments for workers, exposure assessments for the general public, and exposure assessments for effects on nontarget organisms. SERA (2005) contains documentation for the use of the EXCEL workbooks.

2. PROGRAM DESCRIPTION

2.1. OVERVIEW

Aminopyralid is a new herbicide that has been registered provisionally as a *reduced risk* pesticide for the control of broadleaf weeds. Dow AgroSciences, the registrant for aminopyralid, has suggested that this herbicide may be used as an alternative to herbicides such as picloram, 2,4-D, dicamba, monosodium methanearsonate (MSMA), and metsulfuron methyl. While the decision to use any particular herbicide is based on a number of site-specific considerations, the Forest Service and NPS have begun to use aminopyralid at some sites rather than herbicides such as picloram, clopyralid, glyphosate, and dicamba.

Two formulations of aminopyralid are specifically considered in this risk assessment: Milestone and Milestone VM. Both of these formulations contain the triisopropanolamine (TIPA) salt of aminopyralid (40.6 % w a.i./v, equivalent to 21.1% a.e. or 2 lbs a.e./gal). These formulations contain no inert ingredients other than water and triisopropanolamine.

The most likely uses of aminopyralid will involve applications to forest and rangelands, rights-of-way, and developed recreational areas such as campgrounds, picnic areas and trails. Application methods have and will likely continue to include backpack (selective foliar), hydraulic spray, and aerial applications. The labeled application rates for aminopyralid are 0.03 to 0.11 lb a.e./acre. The upper bound of this range is likely to be used for rhizomatous weeds. For non-rhizomatous weeds, the application rate will generally be about 0.078 lb a.e./acre. Again, specific application rates will vary with site-specific considerations. Consequently, the current risk assessment considers the full range of labeled application rates for broadleaf weeds as well as all labeled application methods.

2.2. CHEMICAL DESCRIPTION AND COMMERCIAL FORMULATIONS

Aminopyralid is a new selective systemic herbicide that has been developed for the control of broadleaf weeds in rangeland, non-crop areas, and grazed areas. In addition to non-agricultural applications, aminopyralid is also registered for applications to wheat.

Aminopyralid is the common name for 4-amino-3,6-dichloro-pyridinecarboxylic acid. As illustrated in Figure 1, aminopyralid is a pyridine carboxylic acid, a class of herbicides that includes clopyralid, picloram, and triclopyr. The structure of aminopyralid is very similar to that of clopyralid with the only difference being an amino-group in the 4-carbon position of aminopyralid which is missing in clopyralid. Aminopyralid is also structurally similar to picloram, with the only difference being a chlorine in the 5-carbon position of picloram which is missing in aminopyralid.

The U.S. EPA issued a conditional registration for aminopyralid to Dow AgroSciences on August 10, 2005 as a *reduced risk* herbicide (U.S. EPA/OPPEPTS 2005). In other words, the U.S. EPA has concluded that the use of aminopyralid as a replacement for other herbicides will decrease risk to some nontarget species. The basis for this assessment is discussed further in Section 3 (Human Health Risk Assessment) and Section 4 (Ecological Risk

Assessment). While the U.S. EPA does not specifically address the herbicides that aminopyralid is likely to replace, an analysis by Dow AgroSciences indicates that aminopyralid is intended as an alternative to picloram, 2,4-D, dicamba, monosodium methanearsonate (MSMA), and metsulfuron methyl (Jachetta et al. 2004). Risk assessments have been prepared on all of these agents except MSMA: picloram (SERA 2003a), 2,4-D (SERA 2006a), dicamba (SERA 2004a), and metsulfuron methyl (SERA 2004b).

The NPS has indicated that aminopyralid could be used on some occasions as an alternative to picloram (Tordon formulations), clopyralid (Transline formulations), and glyphosate (Roundup formulations) and, to a lesser extent, as an alternative to dicamba and 2,4-D. In Region 6 of the Forest Service, the Pacific Northwest, it is anticipated that aminopyralid could replace up to 60% of the use of picloram (Bulkin 2007). In both the Forest Service and NPS, the selection of aminopyralid or an alternative herbicide will be made on a site-specific basis and will involve individual site-specific review.

The commercial formulations that contain aminopyralid and that are registered in the United States are summarized in Table 1. Two formulations, Milestone and Milestone VM, contain only the triisopropanolammonium salt of aminopyralid and these formulations are considered in this risk assessment. While Milestone and Milestone VM have separate labels as well as separate EPA registration numbers, both contain aminopyralid as the only active ingredient at 40.6% (w a.i./v) which is equal to an acid equivalent of 21.1% (w a.e./v) or 2 lbs a.e./gallon (Table 1).

Note that this risk assessment will refer to triisopropanolammonium salt of aminopyralid as *aminopyralid TIPA* and that aminopyralid TIPA will be considered as the active ingredient (a.i.). The aminopyralid anion will be referred to as the acid equivalent (a.e.) of aminopyralid TIPA. This is a standard distinction between a.i. and a.e. is that applied to weak acid pesticides. This distinction is noted because some of the DER's as well as many of the full studies on aminopyralid that are summarized in the appendices appear to use the terms a.i. and a.e. interchangeably.

Information on manufacturing processes, inerts, and impurities in aminopyralid and aminopyralid formulations has been submitted to the U.S. EPA (Ghaoui 2004; Jensen 2004a). These submission are classified as Confidential Business Information (CBI) and are not eligible for release under the Freedom of Information Act (FOIA). As noted in Section 1, the Ghaoui (2004) and Jensen (2004a) submissions were not available for or reviewed in the preparation the this risk assessment. Nonetheless, a review of the cleared Data Evaluation Records from the U.S. EPA (as discussed in Section 1) suggested that water is the only inert contained in the Milestone and Milestone VM formulations. Dow AgroSciences was queried on the inerts in Milestone and Milestone VM and confirmed that water is the sole inert in both formulations (Jachetta 2006). Thus, since both formulations contain the same amount of the triisopropanolammonium salt of aminopyralid and water as the only other component, Milestone and Milestone VM are identical.

As noted in Figure 1, aminopyralid is structurally very similar to both picloram and clopyralid. As noted in the previous risk assessment for picloram (SERA 2003a) and

clopyralid (SERA 2003b), technical grade picloram and clopyralid both contain hexachlorobenzene as well as other chlorinated benzenes as impurities. This is a concern in the risk assessments of these herbicides because hexachlorobenzene is a persistent carcinogen. Because of the structural similarities of aminopyralid to both picloram and clopyralid, Dow AgroSciences was queried on the occurrence of hexachlorobenzene and other chlorinated benzenes in technical grade aminopyralid. John Jachetta, the product manager for aminopyralid at Dow AgroSciences, provided the following clarification:

> *Aminopyralid contains no hexachlorobenzene or other chlorinated benzenes as contaminants. This was an early objective of the synthesis team when the manufacturing process was developed. The only components in technical aminopyralid (Reg No. 62719-518) are 95.3% aminopyralid itself, several closely related reaction products (all pyridine derivatives quite similar in structure to aminopyralid), and sodium chloride.*

Two other formulations that contain aminopyralid only as a minor component are registered in the United States: CleanWave (22.22% fluroxypyr and 1.92% aminopyralid) and ForeFront R&P (51.06% 2,4-D and 6.58% aminopyralid). CleanWave is registered only for use on wheat. ForeFront is labeled for uses on rangeland and pastures. These mixture formulations are not covered in the current risk assessment.

2.3. APPLICATION METHODS

The general use and application of herbicides in silviculture are discussed both in the available literature (e.g., Cantrell and Hyland 1985) and in Environmental Impact Statements (e.g., USDA/FS 1989a,b,c). This risk assessment focuses on the aspects of herbicide application that are most germane to the exposure assessments for human health and ecological effects (Sections 3.2 and 4.2).

In Forest Service and NPS programs, the most likely uses of aminopyralid will involve applications to forest and rangelands, rights-of-way, and developed recreational areas such as campgrounds, picnic areas and trails. Application methods may include backpack (selective foliar), hydraulic spray, and aerial applications. In general, areas that are relatively large will be treated by either hydraulic spray (typically broadcast sprays using truck mounted equipment) or aerial application. Smaller areas may be treated by backpack application (selective foliar application). Based on preliminary statistics from the NPS for applications made in 2006 to about 1500 acres, the most common method of application has been backpack (about 69%), followed by hydraulic ground spray (about 22%), and aerial spray (about 9%) (Beard 2007). Because aminopyralid is such a new herbicide, however, it is unclear if this pattern will continue. In both the Forest Service and NPS, decisions concerning application methods will be made on a case-by-case basis.

In backpack applications, the herbicide sprayer or container is carried by backpack and the herbicide is applied to selected target vegetation. Application crews may treat up to shoulder high brush, which means that chemical contact with the arms, hands, or face is plausible. To reduce the likelihood of significant exposures, application crews are directed not to walk

through treated vegetation. Usually, a worker treats approximately 0.5 acre/hour with a plausible range from 0.25 to1.0 acre/hour.

Broadcast ground applications involve spray equipment that may be mounted on all terrain vehicles or trucks and will typically occur in areas such as rights-of-way, along roadsides, or in other areas that are accessible to all terrain vehicles (ATVs). In truck-mounted applications, about 8 acres are treated in a 45-minute period (approximately 11 acres/hour) with approximately 200 gallons of the herbicide mixture (270 gallons/hour). Some special truck mounted spray systems may be used to treat up to 12 acres in a 35-minute period with approximately 300 gallons of herbicide mixture (approximately 21 acres/hour and 510 gallons/hour) (USDA/FS 1989b, p 2-9 to 2-10). Applications involving ATVs will tend to cover fewer acres per hour (Beard 2007). This is discussed further in Section 3.2.2 (Exposure Assessments for Workers).

Aerial applications of aminopyralid have been and are likely to continue to be used in both Forest Service and NPS programs. In aerial applications, liquid formulations are applied through specially designed spray nozzles and booms. The nozzles are designed to reduce turbulence and maintain a large droplet size, both of which contribute to a reduction in spray drift. Aerial applications may only be made under meteorological conditions that minimize the potential for spray drift. In aerial applications, approximately 40–100 acres may be treated per hour.

2.4. MIXING AND APPLICATION RATES

The application rates and mixing instructions for Milestone and Milestone VM are virtually identical. Both formulations are mixed with water and a non-ionic surfactant (80% a.i. at 0.25% to 0.5% by volume) is recommended. As with most herbicides and other pesticides, applications during temperature inversions is not recommended because of the high potential for drift.

In typical risk assessments, the range of application rates that are considered is based on both labeled rates as well as records of application rates in past programs taken from pesticide use reports (e.g., http://www.fs.fed.us/ foresthealth/pesticide/reports.shtml). Because aminopyralid has not had extensive use in past Forest Service or NPS programs, however, the use rates considered in this risk assessment are based on labeled application rates and preliminary comments for Forest Service and NPS personnel.

As summarized by the U.S. EPA (U.S. EPA/OPPEPTS 2005, p. 2), ground and aerial non-crop application rates range from 0.03 to 0.11 lb a.e./acre and no more than 0.11 lb a.e./acre may be applied in a single season. Lower application rates are used for wheat – i.e., a maximum of 0.009 lb a.e./acre. Applications to wheat, however, will not be conducted under either Forest Service or NPS programs and are not further considered. The current labels for Milestone and Milestone VM specify a minimum application rate of 3 fl oz formulation per year or about 0.047 lb a.e./acre.

The reason for the slight discrepancy between the lower rate of 0.03 lb a.e./acre used by the U.S. EPA and the rate of 0.047 lb a.e./acre from the current product labels is unclear. As

discussed further below, this discrepancy has little practical impact on the risk assessment and the application rate of 0.03 lb a.e./acre specified by the U.S. EPA (U.S. EPA/OPPEPTS 2005, p. 2) is considered as the lowest application rate in this risk assessment.

Aminopyralid may be applied in either broadcast or spot applications. Broadcast applications are conducted in a manner that is intended to result in a uniform coverage over a given area. Broadcast applications may be conducted with either ground equipment such as boom sprays or by aerial spraying. Spot applications, as the name implies, involve directed applications to target vegetation at specific spots in a treated area. These applications are typically made with backpack equipment. The labels for both Milestone and Milestone VM indicate that spot applications may be made at rates of up to 0.22 lb a.e./acre but that no more than 50% of a given acre may be treated in this manner. Thus, the net amount that may be applied to a given is acre is limited to 0.11 lb a.e./acre, the maximum labeled broadcast rate. Thus, the spot application of aminopyralid at rates of up to 0.22 lb a.e./acre over 50% of an acre are not considered separately in the exposure assessments used in this risk assessment (Section 3.2 and 4.2) because the resulting exposures are equivalent to the broadcast application rate of 0.11 lb a.e./acre.

As indicated in Table 1, the formulation of aminopyralid that will be used in weed control contains 2 lbs a.e./gallon or about 0.015625 lb/liquid ounce [1 gallon = 128 fluid ounces]. The applications rates specified above as the range considered in this risk assessment – i.e., 0.06 (0.03 to 0.11) lb a.e./acre – correspond to application rates of about 4 (2 to 7) fl oz formulation/acre. Based on limited reports of applications conducted in Forest Service programs (Kulla 2007), the highest labeled rate is likely to be commonly used for rhizomatous weeds and somewhat lower rates of about 5 oz formulation/acre (≈0.078 lb a.e./acre) will be used for non-rhizomatous weeds. This range of application rates is also consistent with application rates that have been used by the NPS (Beard 2007).

For this risk assessment, the typical application rate for aminopyralid will be taken as 0.078 lb a.e./acre or about 5 oz formulation/acre. This is somewhat above the average of the range of labeled application rates but is the application that might be used most often for non-rhizomatous weeds. The full range of the labeled rates – i.e., 0.03 to 0.11 lb a.e./acre – will be considered as the lower and upper bounds on application rates that might be used in Forest Service or NPS programs.

In addition to considering application rates, this risk assessment also considers specific application volumes – i.e., the number of gallons of material, including aminopyralid and the material (primarily water) in which the aminopyralid is mixed. For this risk assessment, the extent to which these formulations are diluted prior to application primarily influences dermal and direct spray scenarios, both of which are dependent on the 'field dilution' (i.e., the concentration of the pesticide in the applied spray). Because of the nature of these scenarios, estimates of risk are directly proportional to the concentration of the pesticide in the field solution.

Based on the information in the product labels for Milestone and Milestone VM formulations, recommended application volumes range from greater than 2 gallons per acre

(for aerial applications) to greater than 10 gallons per acre for ground broadcast applications. Much greater application volumes, 22 to 109 gallons per acre, are recommended for spot applications. For this risk assessment, 10 gallons per acre will be taken as the central estimate of the application volume. This rate is for ground broadcast application, which is the most likely application method for Forest Service programs. The range of application volumes is taken as 2 gallons per acre (the minimum volume that might be considered for aerial applications) to 20 gallons per acre (close to the minimum volume that might be used in spot applications). As noted above, higher application volumes, which are plausible in spot applications, would lead to lesser risks in some scenarios involving dermal contact and direct spray. This is considered further in the risk characterization sections.

It should be noted that the selection of application rates and dilution volumes in this risk assessment is intended to simply reflect typical or central estimates as well as plausible lower and upper bounds. In the assessment of specific program activities, the Forest Service and NPS may use program specific application rates in the worksheets that are included with this report to refine assessments of any potential risks for a specific application.

2.5. USE STATISTICS

Most Forest Service risk assessments attempt to characterize the use of a herbicide or other pesticides in Forest Service programs relative to the use of the herbicide or other pesticide in agricultural applications. The information on Forest Service use is typically taken from Forest Service pesticide use reports (http://www.fs.fed.us/foresthealth/pesticide/reports.shtml) and information on agricultural use is typically taken from use statistics compiled by the U.S. Geologic Survey (http://ca.water.usgs.gov/pnsp/pesticide_use_maps/) and/or detailed pesticide use statistics compiled by the state of California (http://www.calepa.ca.gov/).

Because aminopyralid is a new herbicide, these types of use statistics are not available. Preliminary reports from the NPS indicate that aminopyralid has been applied to over 1500 acres in 2006, primarily for the control of mullein, various types of knapweed and thistle, and poison hemlock (Beard 2007).

Dow AgroSciences has conducted market research and has projected the amount of aminopyralid that might be used in various types of applications. This information has been disclosed to the U.S. EPA in Confidential Attachment B to the report by Jachetta et al. (2004). While most of the Jachetta et al. (2004) report was provided by Dow AgroSciences for the current risk assessment, the Confidential Attachment were withheld because this type of information is classified as confidential under FIFRA and is typically regarded by pesticide registrants as proprietary.

For many pesticides, the amount of the pesticide used in forest, rangeland, and wilderness applications is very minor compared to agricultural applications. While quantitative estimates of the use of aminopyralid in forestry applications relative to agricultural applications cannot be made, it seems reasonable to assert that this general pattern may not be the case with aminopyralid. While aminopyralid is labelled for application to wheat, the narratives provided by both the U.S. EPA (U.S. EPA/OPPEPTS 2005) as well as available

sections of the aminopyralid review by Dow AgroSciences (Jachetta et al. 2004) suggest that non-agricultural uses of aminopyralid, such as those that might be conducted under Forest Service or NPS programs, are likely to be greater than agricultural uses.

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

Because aminopyralid is a new herbicide, no information is available in the published literature on the toxicity of aminopyralid to humans or other mammalian species. The only information on aminopyralid that is available for assessing potential hazards in humans is a series of toxicity studies that have been submitted to and evaluated by the U.S. EPA's Office of Pesticides in support of the registration for aminopyralid.

Although the mechanism of action of aminopyralid and other pyridine carboxylic acid herbicides is fairly well characterized in plants, the mechanism of action of aminopyralid in mammals is not well characterized. The weight-of-evidence suggests that aminopyralid may not have any remarkable systemic toxic effects. The effects that are most commonly seen involve effects on the gastrointestinal tract after oral exposure and these may be viewed as portal of entry effects rather than systemic toxic effects. The location of these effects within the gastrointestinal tract appears to vary among species with the ceca being the most common site of action in rats and the stomach being the most common site of action in dogs and rabbits. Mice do not seem to display any remarkable gastrointestinal effects after oral doses of aminopyralid. The reason for these differences among species is not clear but may simply reflect differences in methods of exposure (gavage versus dietary) and/or differences in anatomy.

In one acute oral toxicity study in rats using the aminopyralid TIPA formulation, lacrimation and cloudy eyes were noted in all test animals on the first day of the study. This is an unusual effect that has not been noted in other studies on aminopyralid, either the acid or the TIPA salt. The significance of this observation, if any, is unclear.

Aminopyralid is rapidly absorbed and excreted and is not substantially metabolized in mammals. As a consequence of rapid absorption and excretion, gavage and dietary exposures probably lead to very different patterns in the time-course of distribution in mammals. The oral LD_{50} of aminopyralid has not been determined because aminopyralid does not cause substantial mortality at the dose limits set by the U.S. EPA for acute oral toxicity studies – i.e., up to 5,000 mg/kg bw. Similarly, subchronic and chronic toxicity studies have failed to demonstrate any clear signs of systemic toxic effects. Developmental studies, however, have noted signs of incoordination in adult female rabbits. Two chronic oral bioassays have been conducted, one in mice and the other in rats, and a 1-year feeding study is available in dogs. Based on the results of the chronic bioassays as well as the lack of mutagenic activity in several mutagenicity screening assays, there is no basis for asserting that aminopyralid is a carcinogen. Similarly, based on the chronic bioassays and several additional subchronic bioassays in mice, rats, dogs, and rabbits, there is no basis for asserting that aminopyralid will cause adverse effects on the immune system or endocrine function. The potential for effects on the nervous system is less clear. Aminopyralid has also been

subject to several bioassays for developmental toxicity and one multi-generation study for reproductive performance. No adverse effects on offspring have been noted in these studies other than decreased body weight in offspring that is associated with decreased food consumption and decreased body weight in adult females.

3.1.2. Mechanism(s) of Action

Aminopyralid is a pyridine carboxylic acid herbicide as are other herbicides such as picloram and clopyralid (Section 4.1.2.3). While the general mechanism of toxicity of this class of herbicides to plants is reasonably characterized – i.e., the mimicking of the auxin plant growth hormone – the mechanism of toxicity to mammals has not been well-characterized.

3.1.2.1. Cecal Enlargement

The most typical response of rats to aminopyralid appears to be an increase in cecal weights after prolonged exposure to high doses of aminopyralid (Dryzga and Stebbins 2001; Johnson and Dryzga 2004; Marty et al. 2003; Liberacki et al. 2001a; Stebbins and Day 2000; Stebbins and Dryzga 2004). The specific observations are detailed in Appendix 3.

As illustrated schematically in Figure 2, the cecum is the first part of the large intestine relative to the stomach. Food passes from the stomach, then to the small intestine, and finally to the large intestine. The last part of the small intestine empties into the cecum at the base of the large intestine. In humans, the cecum a relatively small dead-end sac located at the base of the ascending colon near the appendix. The cecum of some carnivores such as the dog is similar to that of humans – i.e., a small dead-end sac (Yildiz et al. 2005) but the cecum is absent in other carnivores such as mink (Ahlstrom and Skrede 1998). While the function of the cecum in carnivores is unclear (it may be vestigial), the cecum is an important digestive organ in herbivores, particularly hindgut fermenters (as opposed to ruminants) and omnivores that consume large quantities of poorly digestible matter. In these animals, including rats and rabbits, the cecum is much larger and more elaborately structured and serves as an area where intestinal microflora break down cellulose (Kardong 2006). In the rat specifically, the cecum is similar to the schematic illustration in Figure 2 in that it consists of two pouches, a pouch-like cecal base and an elongated cecal apex.

Among the herbicides that are similar to aminopyralid, cecal enlargement has been noted in one study on picloram (Hayes et al. 1986). In this study, rats were administered potassium picloram by gavage for 14 days at doses of 60, 190, and 600 mg/kg body weight per day. Hayes et al. (1986) note that: *The incidence of caecal enlargement appeared to be dose related* (Hayes et al. 1986, pp. 467-468). No other details or comments on this effect are made. Based on other comments made by these investigators, it seems clear that the cecal enlargement is not regarded by Hayes et al. (1986) as toxicologically significant.

The mechanism of cecal enlargement has been characterized for poorly absorbed and osmotically active compounds. Bertram (1996) and Jachetta et al. (2004) note that compounds which are osmotically active and poorly absorbed may cause distension of the cecum by a purely physical mechanism – i.e., attraction of fluid into the lumen. The enlargement of the ceca would, in such a case, be a simple physical process. In some instances, however, the cecum remains enlarged and has a greater mass than normal even

when the lumen is emptied. In such instances, cecal enlargement may be accompanied by hypertrophy (enlarged cells) and hyperplasia (an increase in the number of cells) in the intestinal mucosa.

For aminopyralid, increased cecal weights have been noted based on both full and empty ceca (Marty et al. 2003). In addition, hyperplasia (but not hypertrophy) of epithelial cells has been noted in rats with cecal enlargement at high doses (Liberacki et al. 2001a; Dryzga and Stebbins 2001) and hyperplasia has also been observed in the ileum of rats (Dryzga and Stebbins 2001). At lower doses, however, cecal enlargement after exposure to aminopyralid has been observed in the absence of hyperplasia (Dryzga and Stebbins 2001).

The toxicological significance of cecal enlargement is unclear. None of the investigators involved in the studies on aminopyralid suggest that the cecal enlargement is toxicologically significant (Dryzga and Stebbins 2001; Johnson and Dryzga 2004; Marty et al. 2003; Liberacki et al. 2001a; Stebbins and Day 2000; Stebbins and Dryzga 2004). Nonetheless, Stebbins and Day (2000) did use cecal enlargement as the basis for proposing a NOAEL. In discussing cecal enlargement accompanied by hyperplasia, Stebbins and Dryzga (2004) note that: *These morphological changes were interpreted to represent an adaptive process since the changes were shown to be reversible when the diets were returned to normal.* (Stebbins and Dryzga 2004). Reversibility in itself, however, does not seem sufficient for determining adversity. Many toxicological conditions as well as many disease states that are generally recognized as adverse are also reversible – i.e., the animal may recover.

In a review of the two-generation reproduction study by Marty et al. (2003), the U.S. EPA addresses the toxicological significance of cecal enlargement in the absence of hyperplasia:

> *In the absence of any histopathological changes to the ceca, and in the absence of any other treatment-related parental findings, the cecal findings were considered to be adaptive changes and were not considered to be adverse* (U.S. EPA/OPP-HED 2005, p. 24).

This appears to be a reasonable position and this position will be adopted in the current risk assessment – i.e., in the absence of any pathological changes or other adverse effects, cecal enlargement will not be classified as a toxicologically significant adverse effect.

As reviewed by Bertram (1996), a number of different chemicals, many of which are constituents in food (e.g., starches), will lead to cecal enlargement in rodents. Based on the citations in Bertram (1996), citations in the Liu (2004), and a supplemental literature search in TOXLINE conducted for this current risk assessment, a summary of chemicals that have been associated with cecal enlargement is given in Table 3. The upper portion of Table 3 summarizes information on aminopyralid and picloram. The lower portion of Table 3 summarizes information on various other compounds.

One noteworthy difference among these studies involves the dose that is associated with cecal enlargement. Both aminopyralid and picloram induce cecal enlargement at relatively low doses – i.e., about 50 mg/kg bw/day. This is well-characterized for aminopyralid but

few details are given in the study by Hayes et al. (1986) on picloram. The only other compound in Table 3 that induces cecal enlargement at a comparably low dose is an antibiotic, josamycin. Kasahara et al. (2002) do not provide details on the magnitude of the cecal enlargement by this antibiotic other than to note that the enlargement is *mild*.

Most of the other compounds in Table 3 – i.e., starches, polyols including polyethylene glycol as well as mono- or polysaccharide sugar alcohols, and magnesium sulfate – cause cecal enlargement only at much higher doses that are in the range of about 50-fold [2500 mg/kg/day / 50 mg/kg/day] to 200-fold [10,000 mg/kg/day / 50 mg/kg/day] greater than the doses of aminopyralid. The study with modified starch at a daily dose of about 10,000 mg/kg bw/day (Leegwater et al. 1974) may provide the most direct comparison to aminopyralid at a dose of 218 mg/kg bw/day in terms of essentially equitoxic doses (a 38% increase in cecal weight) administered over a similar period of time (10 weeks vs 8 weeks). Based on this comparison, aminopyralid would be considered about 46 times more potent than the modified starch [10,000 mg/kg bw/day / 218 mg/kg bw/day].

A number the authors cited in Table 3 note that cecal enlargement is likely to have little if any toxicological significance and that the effect is likely to be adaptive to substantial amounts of poorly absorbed but osmotically active material accumulating in the ceca (De Groot et al. 1974; El-Harith et al. 1976; Leegwater et al. 1974; Lina and Bar 2004; Sinkeldam et al. 1992b; Waalkens-Berendsen et al. 2004).

Walker and Harith (1978) provide a somewhat more cautious interpretation, similar to that given above by U.S. EPA/OPP-HED (2005, p. 24):

> *…it is probably justified to attach toxicological significance to results only when gross effects are observed associated with secondary nutritional and toxicological changes…* (Walker and Harith, 1978, p. 677)

While this may be a reasonable interpretation of the effect a large amount of material that is known to be osmotically active in the dead-end cecum of the rat, it is less clear that this interpretation applies to aminopyralid or some of the other compounds.

For example, cefotaxime, another antibiotic, has been shown to cause dilation of cecum (not otherwise detailed) in the rat after both intravenous and subcutaneous injection (Doerr et al. 1992). Quinacrine, an antiprotozoal, also cause cecal dilation and enlargement after intraperitoneal injections by acting on the muscle layers of the cecum. This is not to suggest that aminopyralid will act by these other mechanisms but it does suggest that the cecal enlargement caused by aminopyralid may not be associated with the well-characterized mechanism of cecal enlargement by starches and other similar material.

In addition, the high osmotic activity of some of the substances noted in Table 3 has been measured and documented (e.g. Leegwater et al. 1974). It is less clear that aminopyralid has remarkable osmotic activity that would explain the low-dose enlargement of the ceca relative to other compounds.

Pharmacokinetic studies on aminopyralid clearly indicate that substantial amounts of aminopyralid are not retained in the body, including the gastrointestinal tract, after oral exposures (Section 3.1.3). This suggests that the mechanism of cecal enlargement by aminopyralid might not be related to the mechanism of cecal enlargement by starches. The pharmacokinetic studies on aminopyralid, however, all involve gavage rather than dietary administration. As discussed in Section 3.1.3.4, differences in the kinetics of aminopyralid after gavage and dietary exposures are substantial.

As discussed further in Section 3.1.5 (Subchronic and Chronic toxicity), cecal enlargement has been noted in rats but has not been observed in mice, dogs, or rabbits after exposure to aminopyralid. Some of the other agents listed in Table 3 that have been shown to cause cecal enlargement in rats, most probably through an osmotic mechanism, have also been shown to cause this effect in mice (Til et al. 1986) and dogs (Til and Bar 1998).

No information is available on other possible mechanisms for cecal enlargement – e.g., effects on smooth muscle or effects on the autonomic nervous system that controls cecal emptying times. The inability to more fully identify and characterize the mechanism by which cecal enlargement occurs after exposure to aminopyralid is a limitation in assessing the significance of this endpoint to humans as well as other species covered in the ecological risk assessment (Section 4.1) and the potential relationship of this endpoint to other effects caused by aminopyralid in mammals (Section 3.1.2.5).

3.1.2.2. Stomach

With the exception of cecal enlargement, there is very little indication that aminopyralid causes adverse effects in mammals that could be associated with a discrete mechanism of action. While cecal enlargement has not been observed in species other than rats, hyperplasia and hypertrophy of the mucous cells of the stomach have been observed in dogs (Stebbins and Baker 2002; Stebbins and Day 2003a). This may be associated with the tendency of weak acids to be absorbed in the acidic pH of the stomach (Bertram 1996).

3.1.2.3. Liver

While there is no clear indication that aminopyralid has a specific toxic effect on the liver, effects have been reported in a few studies. Many chemicals will cause an increase in liver weight due to the induction of liver enzymes or recovery from injury. Increased liver weight accompanied by the enlargement of some liver cells has been observed in one study in dogs (Stebbins and Day 2003a) but a dose-related decrease in liver weight has been reported in one study in rabbits (Liberacki et al. 2001b). In addition, liver congestion has been observed in some fatally exposed animals (Carney and Tornesi 2004b) but this effect is often observed in animals *in extremis* and does not suggest a specific toxic effect on the liver.

3.1.2.4. Kidney

Many weak acids are preferentially excreted by and accumulate in the kidney via an active transport mechanism (e.g., Durkin et al. 2004). This is likely to be the case with aminopyralid but no specific studies have been conducted to characterize or confirm active transport mechanisms. In a pharmacokinetic study in goats (Macpherson 2003), peak concentrations of aminopyralid where substantially higher in the kidney than in other organs

(see Appendix 3 for specifics). While somewhat speculative, this concentration of aminopyralid in the kidney is consistent with the clearance of aminopyralid from the plasma by the kidney via a specific active transport process.

Toxicity to the kidney may be indicated by changes in urine chemistry. At high doses, aminopyralid will cause a decrease in urinary pH – i.e., the urine will become more acidic (Dryzga and Stebbins 2001). This effect, however, is simply a reflection of the excretion of aminopyralid by the kidney into the urine. As noted in Section 2, aminopyralid is a weak acid. If an animal is given a sufficiently high dose of aminopyralid, the amount of aminopyralid in the urine will be sufficient to lower the pH of the urine. This effect is an indication of exposure but not of toxicity.

A somewhat more interesting observation in the study by Dryzga and Stebbins (2001) is the decrease in urinary protein and decrease in ketones. Toxicity to the kidneys is commonly indicated by an increase in urinary protein, ketones, or creatinine. These effects can be associated with damage to the glomeruli of the kidney (e.g., Fitzsimmons et al. 1994; Nagata et al. 1992). On the other hand, a decrease in urinary protein and ketones is unusual. While there are various mechanisms that can be associated with these decreases (Bachmann et al. 2001, 2005; Schoel and Pfleiderer 1987), there is no indication that these mechanisms apply to aminopyralid. In addition, aminopyralid does not affect urinary creatinine (Dryzga and Stebbins 2001), indicating no impact on glomerular filtration. In the absence of any other effects that might suggest specific damage to the kidney, the effects observed by Dryzga and Stebbins (2001) do not seem to suggest that aminopyralid is a kidney toxin.

3.1.2.5. Ocular Effects After Oral Exposures

Local effects on the eye after direct exposure of the eye to a chemical – i.e., ocular installation – are common and assays for ocular damage are required for pesticide registration. As discussed in Section 3.1.11.3, technical grade aminopyralid powder is classified as a severe eye irritant (Category I) and the TIPA liquid formulation is classified as a minimal eye irritant (Category IV).

While eye damage does occur after the oral administration of certain chemicals such as naphthalene and galactose (Potts 1996), effects on the eyes after oral administration of a toxicant are uncommon observations.

For aminopyralid, bilateral cloudy eyes and lacrimation were noted in all male and female Fischer 344 rats (five animals per sex) on Day 1 after a single gavage dose of the TIPA formulation of aminopyralid at 2000 mg formulation/kg bw, equivalent to 1090 mg a.e./kg bw (Wilson et al. 2003). The cloudy eyes and lacrimation were observed only on the day of dosing. This study is discussed further in Section 3.1.4 (Acute Oral Toxicity). Because the formulation contained TIPA, this study is also addressed in Section 3.1.14 (Inerts and Adjuvants).

The DER prepared by the U.S. EPA has classified this study as acceptable and this study is used in the human health risk assessment prepared by the U.S. EPA to classify the acute toxicity of the formulation as Category IV(U.S. EPA/OPP-HED 2004, p. 14). The limitation

of this study, however, is that no control group was used. While the lack of a control group is not uncommon in limit studies, it does impair the interpretation of the study by Wilson et al. (2003).

While the cloudiness of the eyes in all test animals is noted by Wilson et al. (2003), the study authors do not discuss the potential significance of this effect. This effect is also noted in the DER on this study but the implications of the effect are not discussed. This effect is not noted or discussed in either the human health or ecological risk assessments on aminopyralid that have been conducted by the U.S. EPA (U.S. EPA/OPP-HED 2004; U.S. EPA/OPP-EFED 2005).

Cloudiness of the eyes has not been noted in two other gavage studies using the TIPA formulation: rabbits at doses of up to 520 mg a.e./kg be (Carney and Tornesi 2004b) and rats at doses of up to 528 mg a.e./kg bw/day (Bjorn 2003). The doses used in these studies are below those of the gavage study by Wilson et al. (2003) by a factor of about 2.

At the end of a 90 day feeding study in Fischer rats with the aminopyralid-TIPA formulation (doses of 0, 92, 241, and 482 mg a.e./kg bw/day), corneal clouding was prevalent in both control and dosed rats but there was no dose-response relationship (Stebbins and Dryzga 2004). Citing the review by Yoshitomi and Boorman (1990), Stebbins and Dryzga (2004) note that the corneal clouding corresponds to corneal mineralization and that this is a common condition in Fischer 344 rats.

In other acute toxicity studies with GF-871 using non-oral routes of exposure, no cloudiness of the eyes has been noted (i.e., the dermal study by Wilson et al. 2002, Section 3.1.12; the inhalation study by Landry and Krieger 2002; the eye irritation study by Brooks and Radtke 2002a). All of these studies are summarized in Appendix 3 and are discussed further in the appropriate subsections below.

The available studies on technical grade aminopyralid (as opposed to the TIPA formulation) have not noted any pronounced occurrence of cloudiness of the eyes that can be associated with exposure to aminopyralid. The acute neurotoxicity study on technical grade aminopyralid (Marable et al. 2002) is similar to the study by Wilson et al. (2003) on the TIPA salt in which all animals evidenced cloudiness of the eyes. Marable et al. (2002) administered doses of technical grade aminopyralid by gavage to Fischer 344 rats at 0, 500, 1000, and 2000 mg a.e./kg bw. The mid- and high-dose groups in this study are comparable to or greater than the 1090 mg a.e./kg bw dose used in the study by Wilson et al. (2003) on the TIPA formulation. Based on gross pathology/necropsy at the end of the study by Marable et al. (2002), cloudy eyes were observed in one female rat (Animal No. 2657) in the 1000 mg a.e./kg bw dose group. During observations of the animals on Study Day 1, the right eye of one male rat (Animal No. 2573) in the 1000 mg a.e./kg bw dose group was enlarged and … *partially cloudy in about 30% of the clear part of the eye* (Study Table 8, p. 132).

Other gavage studies involving multiple doses of technical grade aminopyralid have not noted any cloudiness of the eyes in rabbits (Marty et al. 2002; Liberacki et al. 2001b) or rats

(Carney and Tornesi 2001; Tornesi et al. 2001). These multiple dose studies involved daily dose rates of up to 750 mg/kg bw/day (Marty et al. 2002) and 1000 mg a.e./kg bw/day (Liberacki et al. 2001b) in rabbits and up to 1000 mg a.e./kg bw/day in rats (Carney and Tornesi 2001; Tornesi et al. 2001). No effects on the eyes associated with exposure have been noted in any of the subchronic or chronic dietary studies on technical grade aminopyralid (Section 3.1.5).

The weight of evidence suggests that the cloudiness of the eyes observed in all 10 rats in the study by Wilson et al. (2003) may have been incidental because this effect was not observed in other similar studies. Nonetheless, some mechanistic considerations suggest a plausible association between the cloudiness of the eyes and exposure to the TIPA aminopyralid formulation.

While cloudiness of the eyes is a common condition in aging Fischer rats and may have several causes (Yoshitomi and Boorman 1990), the rats used in the study by Wilson et al. (2003) were only 51 days old when the aminopyralid-TIPA formulation was administered. In addition, the cloudiness of the eyes appeared only on the day of administration of the compound. The only instance of rapidly reversible cloudiness of the eyes in Fischer rats noted in the review by Yoshitomi and Boorman (1990) involves agents such as narcotics that may interfere with blinking. As noted by Yoshitomi and Boorman (1990, p. 257): *These opacities are reversible within a few hours*. As discussed further in Section 3.1.6, aminopyralid is not classified as a neurotoxin by the U.S. EPA but observations of incoordination in rabbits after gavage dosing (which is also a rapidly reversible effects) would be consistent with a neurologic effect.

An osmotic mechanism for the development of cataracts due to exposure to galactose has been proposed by Potts (1996). This mechanism, however, involves metabolic conversion of galactose to dulcitol which cannot be further biodegraded in the lens and which exerts a strong osmotic force there. There is no basis for suggesting this type of mechanism would apply to aminopyralid.

3.1.3. Pharmacokinetics and Metabolism

3.1.3.1. General Considerations

The pharmacokinetics of aminopyralid have been examined in rats (Liu 2004; Domoradzki et al. 2004), pregnant and non-pregnant rabbits (Hansen et al. 2005), lactating goats (Macpherson 2003), and cows (Rosser et al. 2004). These studies are detailed in Appendix 3. While these studies meet the requirements for pesticide registration by the U.S. EPA (U.S. EPA/OPP-HED 2004), the available studies on the pharmacokinetics of aminopyralid are fewer and less detailed than the pharmacokinetic studies on other similar herbicides that have been in use for many years – e.g., 2,4-D (SERA 2006a), clopyralid (SERA 2004c), picloram (SERA 2003a), and triclopyr (SERA 2003b).

Because aminopyralid is a weak acid similar to picloram, triclopyr, clopyralid, and 2,4-D, it would be anticipated that aminopyralid would be excreted by kidney via a well-characterized active transport mechanism. As discussed in the risk assessment on 2,4-D (SERA 2006a),

this mechanism involves active secretion of the acid by the proximal tubules of the kidney, in a manner similar to excretion of paraminohippuric acid (PAH). This active transport mechanism can be saturated. As a result, the pharmacokinetics of weak acids tend to display dose-dependent patterns in which concentration of the acid in blood and/or tissues increases disproportionately as the dose increases beyond the point at which excretion is saturated.

There is limited evidence for dose-dependent pharmacokinetics in the studies on aminopyralid In terms of urinary excretion, Liu (2004) did note a somewhat longer urinary halftime in rats, 3.78 hours, at a dose of about 1000 mg/kg bw relative to the halftime of 2.85 hours at a dose of about 50 mg/kg bw. These difference, however, was not statistically significant. In addition, the residues in the skin and gastrointestinal tract after administration of the high dose were higher than corresponding residues after the low dose by factors of 364 and 135, respectively. Under the assumption of linear pharmacokinetics, the ratios in all tissues would have been expected to be identical to the ratios of the doses – i.e., about 20 (1000 / 50). In the multiple dose kinetic study involving cows (Rosser et al. 2004), no dose-dependent pattern is apparent. In this study, however, all of the dose were relatively low – i.e., 1.1 mg/kg bw to 23.27 mg/kg bw – and it is likely that the highest dose tested was not sufficient to saturate the excretion of aminopyralid.

The pharmacokinetic study in rabbits (Hansen et al. 2005) involves both single doses of ^{14}C-labeled aminopyralid to pregnant rabbits (Day 7 of gestation) and non-pregnant rabbits as well as multiple doses of unlabelled aminopyralid on Days 7 to 20 of gestation followed by a single dose of ^{14}C-labeled aminopyralid. Modestly longer plasma halftimes were noted in pregnant rabbits receiving multiple doses of aminopyralid but the differences are not significant. Estimates of bioavailability – expressed as total absorbed dose normalized for differences in dosing and body weight – in pregnant rabbits on Day 20 of gestation after multiple doses were higher than those for single-dosed rabbits by factors of 1.75 (relative to non-pregnant rabbits) and 2.16 (relative to pregnant rabbits on Day 7 of gestation). These differences are probably due to both the multiple dosing schedule (where an increase in body burden would be expected) as well as physiological differences between early-stage and late-stage pregnant rabbits.

Another factor relating to saturable pharmacokinetics involves the potential sensitivity of dogs and possibly other canid species. As discussed in the risk assessments of 2,4-D (SERA 2006a) and triclopyr (SERA 2003a), dogs have an impaired capacity to excrete weak acids and this excretion can be saturated at lower doses than in other species. There are no studies on the pharmacokinetics of aminopyralid is dogs. As noted in Section 4.1.2.1, however, toxicity studies on dogs have been conducted and there is no indication that dogs are substantially more sensitive to aminopyralid than other mammals. This does not necessarily indicate that saturable excretion processes are unimportant for aminopyralid because the apparent lack of sensitivity of dogs to aminopyralid could simply reflect the low inherent toxicity of this compound.

3.1.3.2. Absorption

Oral Absorption – As noted in Section 3.1.2.1, the most common effect associated with oral dosing of aminopyralid in rodents involves enlargement of the ceca. Liu (2004) has noted

that a greater proportion of aminopyralid is excreted in the feces compared to other structurally similar compounds and has suggested that cecal enlargement could be associated with malabsorption – i.e., aminopyralid is not completely absorbed in the stomach and is transported to and accumulates in the ceca:

> *It is noted that a greater amount of the administered dose of XDE-750 was in the feces compared to the other oral studies with compounds structurally rated to XDE-750 as cited above (33-43 vs. 3-19%). … These data would be consistent with a large amount of unabsorbed XDE-750 detected in the feces*. (Liu 2004, p. 24)

The suggestion of malabsorption for aminopyralid is somewhat counterintuitive. Many weak acids are well-absorbed from the acid environment of the stomach (Bertram 1996). Neither the study by Liu (2004) on aminopyralid acid or the study by Domoradzki et al. (2004) on both aminopyralid acid and aminopyralid-TIPA involve estimates of oral absorption rates. The pharmacokinetic study by Domoradzki et al. (2004, p. 27) specifically notes that the oral absorption rate could not be estimated because the rapid oral absorption of aminopyralid precluded the measurements of concentration-time values that would be necessary to estimate the oral absorption rate. Similarly, rapid oral absorption appears to be evident in the pharmacokinetic study in rabbits (Hansen et al. 2005).

Nonetheless, there do appear to be differences between the handling – if not the absorption – of aminopyralid compared to other similar herbicides. This is evident in a comparison of the peak plasma data reported by Domoradzki et al. (2004) and the same values reported from a very similar pharmacokinetic study on 2,4-D. As noted in Appendix 3, Domoradzki et al. (2004) administered a single dose of 50 mg aminopyralid a.e./kg bw to rats. This is equivalent to about 242 μmoles/kg bw. Peak plasma concentration of about 26 μg a.e./mL plasma (equivalent to about 0.12 μmoles/mL plasma) were noted at 15 minutes.

By comparison, a similarly designed study was conducted by Smith et al. (1980) on 2,4-D in which rats were given gavage doses of 10 mg/kg bw, 50 mg/kg bw, and 150 mg/kg bw. The dose of 50 mg/kg bw of 2,4-D (226 μmoles/kg bw) resulted in peak plasma concentrations of about 800 μmoles/L or 0.8 μmoles/mL. While there was substantial scatter among individual rats (Durkin et al. 2005, Figure 5, p. 84), the 2,4-D plasma concentrations peaked over a period of about 1 to 4 hours.

Thus, the peak plasma concentration of aminopyralid is about a factor of 6.5 less than the peak plasma concentration of 2,4-D after comparable molar doses [0.8 μmoles 2,4-D/mL / 0.12 μmoles aminopyralid/mL = 6.66 ratio of 2,4-D to aminopyralid]. This difference could be attributed to factors such as plasma protein binding that would impact clearance from plasma.

Data are available on plasma protein binding for both aminopyralid and 2,4-D. Hansen et al. (2005) noted 43.1% to 72.3% at aminopyralid concentrations of 10 μg/mL to 154 μg/mL – corresponding to molar concentrations of 48 μM to about 744 μM in rats and rabbits. The rabbits included non-pregnant females, pregnant females on Day 7 of gestation (GD 7), and

pregnant females on Day 22 of gestation (GD 22). As illustrated in Figure 3, the proportion of aminopyralid bound to plasma followed a systematic pattern: rats > non-pregnant rabbit > Gestation Day 7 rabbit > Gestation Day 22 rabbit.

As also illustrated in Figure 3, the binding of aminopyralid to plasma protein is substantially less than the binding of 2,4-D to plasma protein in rats (Ylitalo et al. 1990) and goats (Orberg 1980) at comparable molar concentrations. The lesser binding of aminopyralid to plasma protein relative to 2,4-D would result in greater plasma clearance through increased glomerular filtration as well as other excretion processes and this could account for some of the differences seen in peak plasma concentrations between aminopyralid and 2,4-D at comparable doses.

Dermal Absorption – Most of the occupational exposure scenarios and many of the exposure scenarios for the general public involve the dermal route of exposure. For these exposure scenarios, dermal absorption is estimated and compared to an estimated acceptable level of oral exposure based on subchronic or chronic toxicity studies in animals. Thus, it is necessary to assess the consequences of dermal exposure relative to oral exposure and the extent to which aminopyralid is likely to be absorbed from the surface of the skin.

Two types of dermal exposure scenarios are considered: immersion and accidental spills. As detailed in SERA (2006a), the calculation of absorbed dose for dermal exposure scenarios involving immersion or prolonged contact with chemical solutions use Fick's first law and require an estimate of the permeability coefficient, Kp, expressed in cm/hour. For exposure scenarios like direct sprays or accidental spills, which involve deposition of the compound on the surface of the skin, dermal absorption rates (proportion of the deposited dose that is absorbed per unit time) rather than dermal permeability rates are used in the exposure assessment.

No studies are available on the kinetics of the absorption of aminopyralid following dermal exposure. Based on the lack of toxicity in a 28-day dermal toxicity study (Section 3.1.12), the U.S. EPA concluded that aminopyralid is *…not absorbed or poorly absorbed through the skin* (U.S. EPA/OPP-HED 2005, p. 28). Given what is known about the dermal absorption of structurally similar weak acids such as 2,4-D (SERA 2006a), it is likely that aminopyralid is absorbed through the skin but that the rate of absorption is low relative to non-polar compounds of similar size.

In the absence of experimental data, quantitative structure activity relationships, detailed in SERA (2006a), are employed to estimate dermal absorption rates. Using the method recommended by U.S. EPA (1992), the estimated dermal permeability coefficient for aminopyralid is 0.00000094 cm/hour with a 95% confidence interval of 0.000000225 to 0.0000039 cm/hour. These estimates are used in all exposure assessments that are based on Fick's first law. The calculations for these estimates are presented in Worksheet B05. The estimated first-order dermal absorption rate coefficient is 0.00046 $hour^{-1}$ with a 95% confidence interval of 0.000086 $hour^{-1}$ to 0.0025 $hour^{-1}$. The calculations for these estimates are presented in Worksheet B06.

For some compounds, acute dermal and oral LD_{50} values can be used to assess the plausibility of the estimated dermal absorption rates relative to oral absorption rates. This is not possible for aminopyralid because the available acute oral toxicity studies (Section 3.1.4) and acute dermal toxicity studies (Section 3.1.12) all failed to estimate an LD_{50} value because of the lack of mortality (i.e., low toxicity) of aminopyralid over the range of doses that have been tested.

3.1.3.3. Excretion

Aminopyralid is rapidly excreted after oral exposures. In both of the pharmacokinetic studies in rats (Domoradzki et al. 2004; Liu 2004), the excretion of aminopyralid followed a two-compartment model with an initial α-phase followed by a slower β-phase. In the study by Domoradzki et al. (2004), plasma halftimes are 0.338 hours for the α-phase and 8.8 hours for the β-phase and are similar to the halftimes in urine, 2.8 hours for the α-phase and 7.8 hours for the β-phase. In the study by Liu (2004), similar urinary halftimes are reported: α-phase halftime of 2.85 hours at a dose of about 50 mg/kg and 3.78 hours at a dose of about 1000 mg/kg and β-phase halftimes of 10.23 hours at a dose of 50 mg/kg and 10.88 hours at a dose of 1000 mg/kg. In rabbits (Hansen et al. 2005), urinary excretion is also rapid but appears to follow simple first-order (one-compartment) kinetics with halftimes of about 6.5 hours, indicating somewhat more rapid urinary excretion in rabbits relative to rats.

While excretion rates are not used directly in either the dose-response assessment or risk characterization, excretion halftimes can be used to infer the effect of longer-term exposures on body burden based on the *plateau principle* (e.g., Goldstein et al. 1974). The concentration of the chemical in the body after a series of doses (X_{Inf}) over an infinite period time can be estimated based on the body burden immediately after a single dose, X_0, by the relationship:

$$X_{Inf}/X_0 = 1 / (1- e^{-ke\ t^*})$$

where t* is the interval between dosing.

Taking the first-order urinary k_e of 0.107 hr^{-1} [ln(2)÷6.5 hr] or 2.55 day^{-1} from the halftime of about 6.5 hours in non-pregnant rabbits (Hansen et al. 2005) and setting the interval between doses to 1 day (i.e., daily dosing), the increased body burden with infinite exposure relative to the body burden after a single dose would be about 1.08.

While the plateau principle is based on simple first-order excretion, the terminal β-phase halftimes from a two-compartment model can be used to estimate relative body burdens over infinite dosing. Based on a urinary halftime of 11 hours in rats, somewhat higher than the range reported by Liu (2004), the first-order elimination rate constant can be estimated at 0.063 $hours^{-1}$ [ln(2)÷11 hours] or about 1.5 day^{-1}. Again setting the interval between doses to 1 day – i.e., daily dosing – and using the above equation with the halftime of 1.5 day^{-1}, the increase in body burden with daily exposure for an infinite period of time would be about a factor of 1.3.

This estimate of a 1.3 increase in body burden is somewhat lower than the relative bioavailability factors of 1.75 to 2.16 for pregnant rats after multiple doses of aminopyralid (Hansen et al. 2005). As discussed in Section 3.1.3.1, however, these bioavailability factors are based on comparisons of plasma concentrations in pregnant rats on Day 21 of gestation after multiple doses of aminopyralid to non-pregnant rats (a factor of 1.75) and pregnant rats on Day 7 of gestations (a factor of 2.16) after a single dose. Consequently, these factors may reflect differences based on both multiple dosing as well as physiological differences between late-term rabbits and early-terms or non-pregnant rabbits. In any event, aminopyralid has a very low potential to accumulate in the body. This is identical to the pattern seen for other structurally similar weak acid herbicides – e.g., picloram (SERA 2003a), triclopyr (SERA 2003b), clopyralid (SERA 2004c), and 2,4-D (SERA 2006a).

3.1.3.4. Gavage Versus Dietary Exposures

As indicated in the previous subsections, aminopyralid is rapidly absorbed and rapidly excreted after gavage dosing. As a consequence, the body burdens and tissue concentrations are likely to be substantially different after dietary administration – i.e., the mixing of the compound into the diet – compared to gavage administration – i.e., intubation of the compound into the stomach. This difference can impact the interpretation of the relative utility of dietary and gavage toxicity studies in this risk assessment.

The pharmacokinetics of aminopyralid have not been determined during dietary administration. To illustrated the potential differences, the pharmacokinetic study by Domoradzki et al. (2004) involving gavage dosing of rats is used to model plausible kinetic patterns that might be expected after dietary administration.

As discussed in Section 3.1.3.1 (General Considerations) and detailed in Appendix 3, Domoradzki et al. (2004) administered ^{14}C- labeled aminopyralid acid or aminopyralid TIPA salt to groups of four rats by gavage at a single dose of 50 mg a.e./kg bw and monitored plasma concentrations and urinary and fecal excretion over a 5-day period. The results of this study are discussed in Section 3.1.3.2 (Absorption) and Section 3.1.3.3 (Excretion) and additional details are given in Appendix 3. As noted by Domoradzki et al. (2004), the absorption phase could not be estimated directly because the first data point, taken at 15 minutes after dosing, was the maximum measured concentration in plasma. Thus, Domoradzki et al. (2004) appear to have based their analyses on a simple two-compartment open model with no absorption term (e.g., O'Flaherty 1981, p. 116 ff).

To assess plausible kinetic differences between gavage and dietary administration, some estimate of the oral absorption rate (k_a) is needed. As noted in Appendix 3, considerations of plasma volume in the rat and the gavage dose used by Domoradzki et al. (2004) suggest an apparent volume of distribution of about 189 mL. Using this as a starting value and the average plasma concentrations for the four rats used in the Domoradzki et al. (2004) experiment with ^{14}C-labeled aminopyralid acid, kinetic parameters were estimated for a two-compartment model with first-order absorption and first-order elimination (e.g., O'Flaherty 1981, p. 135 ff). This model is illustrated in Figure 4 along with an illustration of the data fit and the results of the analyses.

Figure 5 applies the model developed in Figure 4 to a dietary exposure. Rats will generally consume food over about a 12 hour period. In developing the data for Figure 5, it was assumed that the animals consume a uniform amount of food once every 15 minutes over a 12 hour period and that no food is consumed for the remaining 12 hour period in the day. The total daily dose to the animal was set to equal the 50 mg/kg bw dose from the study by Domoradzki et al. (2004). The kinetic parameters used to estimate plasma concentrations for the dietary exposure are identical to the parameters given in Figure 4. Based on this simulation, gavage administration leads to peak plasma concentrations (≈24.5 mg/L) that are a factor of about 6.1 greater than those after dietary administration (≈3.98 mg/L).

This ratio is a crude approximation at best. Conducting kinetic analyses on averaged data among individual animals and approximating the apparent volume of distribution based on the empirical peak plasma concentrations are not generally appropriate. Nonetheless, the consequence of using the empirical peak concentration will be to underestimate the peak – i.e., the true peak concentration almost certainly occurred before 15 minutes. Consequently, the ratio of about 6.1 given above is almost certainly an underestimate.

A more serious limitation in the simulation, however, involves the potential effect of mixing aminopyralid in food on the apparent first-order oral absorption rate. While speculative, it is likely that aminopyralid will bind to protein and perhaps other constituents in food. This, in turn, is likely to lead to decreases in the apparent first-order oral absorption rate of aminopyralid. As with the underestimate of time to peak plasma concentrations, using the k_a from a gavage study to simulate a dietary exposure will lead to an overestimate of peak plasma concentrations after dietary exposure. Thus, the plausible interpretation of the simulation illustrated in Figure 5 is that the ratio of peak concentrations after gavage relative to dietary exposure would be at least a factor of 6 and the actual ratio could be much higher.

3.1.4. Acute Oral Toxicity

As with all other types of toxicity studies, the only information on the acute oral toxicity of aminopyralid comes from studies that were conducted as part of the registration process. All of the studies discussed in this subsection as well as subsequent subsections to this hazard identification are detailed in Appendix 3.

Two acute oral toxicity studies have been conducted on technical grade aminopyralid (Brooks 2001a; Marable et al. 2002) and one study has been conducted on a formulation of the TIPA salt of aminopyralid (Wilson et al. 2003). The study by Marable et al. (2002) is a special type of acute toxicity study that is designed to assess neurotoxicity. This is discussed further in Section 3.1.6. The study on the formulation (Wilson et al. 2003) refers to the test material as GF-871. As noted in Table 1, GF-871 is identical to the Milestone formulation and contains only the TIPA salt of aminopyralid and water.

As detailed in SERA (2007, Section 3.1.4), the results of acute toxicity studies are usually expressed as LD_{50} values. Studies that are useful in estimating the LD_{50} involve testing at a number of different dose levels that result in mortality rates that bracket 50% of the treated animals. These data are then used to estimate the oral LD_{50} value. In the registration process, however, the U.S. EPA will accept limit tests in which the compound is tested at

only a singe high dose. If the compound does not cause substantial mortality – i.e., mortality rates of 50% or more – the requirement for a full study to determine the LD_{50} value may be waived.

This latter case applies to aminopyralid. Both the studies by Brooks (2001a) on aminopyralid and Wilson et al. (2003) on aminopyralid TIPA are limit tests and both of these studies have been accepted by the U.S. EPA (U.S. EPA/OPP-HED 2004). In the study on aminopyralid (Brooks 2001a), a single dose of 5000 mg a.e./kg bw in rats resulted in the death of only 1 of 10 animals. Signs of toxicity included decreased reactivity, loose or watery feces, and transient weight loss. In the one animal that died, observations included gas in the gastrointestinal tract and hemolyzed blood. These observations may simply reflect post-mortem changes. The study on the TIPA salt of aminopyralid (Wilson et al. 2003) yielded similar signs of toxicity at a dose of 2000 mg a.e./kg bw: loose/watery feces and transient weight loss. Unlike the study on the acid form of aminopyralid, however, observations in rats also included lacrimation and cloudy eyes in all animals on Day 1 of the study. The effect on the eyes is discussed further in Section 3.1.11.3.

Based on these studies, the U.S. EPA has classified aminopyralid as having low acute oral toxicity – i.e., Category IV (U.S. EPA/OPP-HED 2004, p. 12). The categorization scheme used by the U.S. EPA is used to designate the least toxic category for pesticides. As discussed in SERA (2007, Table 3-2), these classifications impact the labeling requirements of pesticides, with progressively less severe warning notices (referred to as signal words) going from Category I (*Danger*) to Category IV (no signal word required).

The ocular effects seen in the study of the formulation (Wilson et al. 2003) occurred at a dose of 5000 mg formulation/kg bw or 1090 mg a.e./kg bw. This dose is substantially lower than the limit study on technical grade aminopyralid by Brooks (2001a) – i.e., 5000 mg a.e./kg bw. Since the only difference between aminopyralid acid and the formulation is the TIPA cation and water contained in the formulation, the ocular effects seen in the formulation study by Wilson et al. (2003) may be associated with the TIPA cation. This is discussed further in Section 3.1.14 (Inerts and Adjuvants).

3.1.5. Subchronic or Chronic Systemic Toxic Effects

3.1.5.1. General Considerations

As discussed in SERA (2006a, Section 3.1.5), *subchronic* and c*hronic* are somewhat general terms that refer to studies that involve repeated dosing. Some studies are designed to detect special types of toxicities such as reproductive and neurologic effects. Except for some comments in this subsection on general signs of toxicity, these specialized studies are discussed in subsequent subsections of this hazard identification. The current subsection focuses on toxicity studies that are designed to detect more general signs of systemic toxicity and to quantify no-observable-effect levels (NOAELs) for the identified endpoints.

As summarized in Appendix 3, subchronic toxicity studies have been conducted in dogs (Stebbins and Baker 2000; Stebbins and Baker 2002), mice (Stebbins et al. 2001; Yano and Dryzga 2000), and rats (Liberacki et al. 2001a; Dryzga and Stebbins 2001; Stebbins and

Dryzga 2004; Stebbins and Day 2000). In addition to these subchronic studies, chronic studies are available in dogs (Stebbins and Day 2003a), mice (Stebbins and Day 2003b), and rats (Johnson and Dryzga 2004).

The 90-day toxicity study in rats by Stebbins and Dryzga (2004) is the only subchronic toxicity study that used the GF-871 formulation – i.e., the TIPA salt of aminopyralid in water. All other studies involved technical grade aminopyralid. The formulation study is discussed further below as well as in Section 3.1.14 (Inerts and Adjuvants).

With the exception of the subchronic study in rats by Liberacki et al. (2001a), all of these studies were submitted to and evaluated by the U.S. EPA and have been classified as *Acceptable* – i.e., the studies followed the guidelines established by the U.S. EPA and satisfy requirements for pesticide registration (U.S. EPA/OPP-HED 2004).

The study by Liberacki et al. (2001a) is a 13 week feeding study in rats that is characterized as a *probe* study – i.e., a preliminary study that is sometimes conducted prior to a full study to refine aspects of the experimental design. This study appears to have been submitted to the U.S. EPA – i.e., an MRID number was assigned to the study – but the U.S. EPA does not have a data evaluation record for this study (Bressant 2007) and this study is not cited in the risk assessment conducted by the Health Effects Division of OPP (U.S. EPA/OPP-HED 2004). While the results of this study are consistent with the results of other toxicity studies in rats, the study does have reporting deficiencies. For example, the study involved dietary exposures and reports intended or target doses of 0, 100, 500, and 1000 mg/kg bw/day. The study, however, fails to provide information on dietary concentration and food consumption that would permit a calculation of actual doses to the animals in terms of mg/kg bw/day. Thus, if the U.S. EPA had reviewed this study, the study would probably have not been classified as *Acceptable*. This limitation has no impact on the current risk assessment because of the other acceptable studies as well as the consistency of the Liberacki et al. (2001a) study with other studies on aminopyralid.

In the U.S. EPA review of the available toxicity studies, the Agency concluded that: *The toxicology database for aminopyralid is complete and there are no data gaps. The scientific quality of the database for aminopyralid is relatively high and the toxicity profile can be characterized for all effects…*(U.S. EPA/OPP-HED 2004, p. 12). Based on the review of these studies as well as the DERs for these studies in the preparation of the current risk assessment, this conclusion by the U.S. EPA seems appropriate. As discussed further in Section 3.3, the U.S. EPA has based the chronic RfD for aminopyralid on the two-year rat feeding study by Johnson and Dryzga (2004).

As discussed below (Section 3.1.5.2), aminopyralid also appears to effect the stomach in dogs and rabbits. While these gastrointestinal effects are considered under the general category of systemic toxic effects, they may be viewed more as portal of entry effects in that they have been demonstrated only after oral exposures. Other weak acid herbicides that are structurally similar to aminopyralid may damage both the liver and kidney – e.g., picloram (SERA 2003a), triclopyr (SERA 2003b), clopyralid (SERA 2004c), and 2,4-D (SERA

2006a). There is no clear indication, however, that aminopyralid causes specific damage to either the liver, kidney, or tissues other than those in the digestive tract (Section 3.1.5.3).

3.1.5.2. Stomach Lesions

Effects have been noted in the stomach of both dogs (Stebbins and Baker 2002; Stebbins and Day 2003a) and rabbits (Marty et al. 2002). In rabbits, effects in the stomach are characterized as erosions or ulcers of the glandular mucosa. These effects were seen in only 2 of 26 females at the highest dose of technical grade aminopyralid that was tested – i.e., 750 mg a.e/kg bw (Marty et al. 2002). This effect was not seen in the rabbit developmental study with the TIPA salt of aminopyralid (Carney and Tornesi 2004b). In dogs, frank stomach lesions have not been noted. Instead, the only observed effect is hyperplasia and hypertrophy at dietary concentration of 30,000 ppm for 13 weeks in male and female dogs (Stebbins and Baker 2002) and hyperplasia and hypertrophy of the stomach mucosa with slight inflammation in male (967 mg a.e./kg bw/day) and female (1030 mg a.e./kg bw/day) dogs after one year (Stebbins and Day 2003a).

No effects on the stomach of mice have been noted as remarkable in the subchronic or chronic studies in mice (Stebbins and Day 2003b; Stebbins et al. 2001; Yano and Dryzga 2000) or in the U.S. EPA reviews of these studies. As noted in Appendix 3, the subchronic toxicity studies in mice (Stebbins and Day 2003b; Stebbins et al. 2001; Yano and Dryzga 2000) do not indicate any effects on the stomach that can be attributable to exposure to aminopyralid. Similarly, a number of pathological lesions to the stomach were observed in the chronic study in mice but the incidence of abnormal lesions do not appear to be dose-related or statistically significant (Stebbins and Day 2003b).

The reason or reasons for the stomach ulcerations in dogs and rabbits and lack of stomach ulcers in mice and rats is unclear. Because aminopyralid is a weak acid, more acidic (lower pH) environments would tend to favor more rapid absorption because more of the aminopyralid would be protonated. The normal pH of the stomach in these species, however, is not remarkably different: 1.9 to 3 for rats, 3.1 to 4.5 for mice, 3.4 to 5.5 for dogs, and about 1.9 for rabbits (RIVM 2007).

3.1.5.3. Other Tissues

There is very little information indicating that aminopyralid affects tissues other than those in the digestive tract. As discussed in Section 3.1.9.3, some abnormalities have been noted in ovaries of mice during a chronic feeding study in mice (Stebbins and Day 2003b) but these effects do not appear to be associated with aminopyralid exposure.

In the high dose (1000 mg a.e./kg/day) females in the chronic feeding study in mice, Stebbins and Day (2003b) also noted increase mortality and associated this effect with nephropathy – i.e., kidney damage. The kidney damage itself, however, was not associated with exposure to aminopyralid. The U.S. EPA has reviewed this study and has agreed with the discussion in Stebbins and Day (2003b) that the effects on the kidney are not attributable to aminopyralid exposure. This conclusion appears to be based on kidney histopathology – i.e., a microscopic examination of the kidneys – in which the incidences of kidney damage are 35/50 (controls), 29/50 (50 mg/kg), 30/50 (250 mg/kg), and 33/50 (1000 mg/kg). This

clearly indicates the lack of any treatment related effect of aminopyralid on kidney histopathology.

Nonetheless, as also detailed in Appendix 3, the incidence of gross pathology of the kidney in female mice is 7/50 (controls), 7/50 (50 mg/kg), 10/50 (250 mg/kg), and 15/50 (1000 mg/kg). Using the Fisher Exact test, the incidence of kidney pathology in the high dose group relative to the controls is marginally significant (p=0.0448) but the incidence in the 250 mg/kg dose group is not (p=0.2977). Based on probit analysis using the U.S. EPA Benchmark Dose program (U.S. EPA/ORD 2001), the p-value for the goodness-of-fit for the regression is 0.8942 – i.e., the probit model fits the dose-response data. Thus, the gross kidney pathology in female rats could be interpreted as treatment related. Notwithstanding these relationships, the gross kidney pathology is not supported by histopathology and, in the absence of any confirming histopathology, the assessment by Stebbins and Day (2003b) and confirmed by the U.S. EPA/OPP that aminopyralid did not cause any treatment related adverse effects seems reasonable.

3.1.6. Effects on Nervous System

As discussed in Durkin and Diamond (2002), a neurotoxicant is a chemical that disrupts the function of nerves, either by interacting with nerves directly or by interacting with supporting cells in the nervous system. This definition of neurotoxicant distinguishes agents that act directly on the nervous system (direct neurotoxicants) from those agents that might produce neurological effects that are secondary to other forms of toxicity (indirect neurotoxicants). Virtually any chemical will cause signs of neurotoxicity in severely poisoned animals and can be classified as an indirect neurotoxicant. For aminopyralid, there is ample indication of indirect effects that might be associated with neurotoxicity but no indication of specific neurotoxicity.

In three developmental studies in rabbits, signs of incoordination have been noted after gavage administration of technical grade aminopyralid or the GF-871 formulation at doses of 78 mg a.e./kg bw/day (Carney and Tornesi 2004c, GF871), 1000 mg/kg bw/day (Carney and Tornesi 2004b, GF871), as well as 500 and 750 mg a.e./kg bw/day (Marty et al. 2002, technical grade aminopyralid). Other gavage studies in rabbits have failed to note incoordination at doses of up to 1000 mg a.e./kg bw (Liberacki et al. 2001b) and incoordination in rats has not been noted in gavage developmental studies at doses of up to 1000 mg a.e./kg bw/day (Bjorn 2003; Tornesi et al. 2001). In addition, no signs of incoordination have been noted in any dietary exposure studies or in single dose gavage administration in rats at doses of 5,000 mg a.e./kg bw (Brooks 2001a; Wilson et al. 2003). The gavage study in rats (Brooks 2001a) did note decreased reactivity in some rats but incoordination was not noted.

The U.S. EPA/OPP does have protocols for testing the effects of pesticides on the nervous system. Two such studies have been conducted on aminopyralid: an acute neurotoxicity study (Marable et al. 2002) and a neurologic evaluation after 12-months of dietary exposure (Maurissen et al. 2003) that was conducted as part of the 2-year feeding study in rats (Johnson and Dryzga 2004). These studies are summarized in Appendix 3.

The acute neurotoxicity study involved gavage administration to rats at doses of 0 (control), 500, 1000, and 2000 mg a.e./kg bw with a 14-day post-dosing observation period. The neurologic evaluations included the Functional Observation Battery (a series of standardized tests and observations relating to potential effects on the nervous system), tests of motor activity, and both gross and histological examinations of tissue. No adverse effects attributed to treatment were observed by the study authors (Marable et al. 2002) and the U.S. EPA concurred with the assessment that this study provided no indication of neurotoxicity (U.S. EPA/OPP-HED 2005).

The chronic neurotoxicity assay was conducted on animals at dietary doses of 0, 5, 50, 500, 1000 mg/kg bw/day. The assays in the Functional Observation Battery were conducted pre-exposure (baseline) and at 3, 6, 9, and 12 months after exposures were initiated. Both gross and histological examines of nerve tissue were conducted at 12 months. As in the acute neurotoxicity study, no adverse neurologic effects were noted.

Based on these studies, both of which were classified as *Acceptable* by the Agency, the U.S. EPA concluded that aminopyralid is not neurotoxic (U.S. EPA/OPP-HED 2005, p. 12). This conclusion seems warranted based on these two neurotoxicity studies and the failure to note other signs of neurologic effects in the other repeated dose studies on aminopyralid (Appendix 3).

The U.S. EPA does not specifically address the incoordination noted in the gavage studies in rabbits, which are discussed above. Given the lack of any signs of neurotoxicity in the acute gavage neurotoxicity study in rats at a dose of 2000 mg a.e./kg bw (Marable et al. 2002), there may be species differences in response to aminopyralid. This does not demonstrate that the incoordination in rabbits is attributable to a neurotoxic effect. Nonetheless, given the generally low toxicity of aminopyralid and the lack of any organ-specific toxicity in rabbits, it is difficult to propose an alternative mechanism for the incoordination seen in rabbits after gavage dosing.

As discussed in Section 4.1 (Hazard Identification for the Ecological Risk Assessment), incoordination has also been observed in birds at gavage doses as low as 63 mg a.e/kg bw/day (Gallagher et al. 2001a). As with mammals, however, no signs of incoordination were observed at much high doses in dietary studies in birds (Section 4.1.2.2). Thus, the incoordination appears to be limited to gavage studies in both rabbits and birds. As discussed in Section 3.1.3.4, peak concentrations of aminopyralid in plasma are likely to be substantially higher after gavage dosing relative to concentrations in plasma after dietary dosing. This suggests that incoordination may be dependent on peak concentrations rather than time-weighted average concentrations of aminopyralid in plasma.

3.1.7. Effects on Immune System

A variety of tests have been developed to assess the effects of chemical exposures on various types of immune responses, including assays of antibody-antigen reactions, changes in the activity of specific types of lymphoid cells, and assessments of changes in the susceptibility of exposed animals to resist infection from pathogens or proliferation of tumor cells (Durkin and Diamond 2002). Except for studies on skin sensitization (Section 3.1.11.2), specific

studies on the effects of pesticides on immune function are not required for pesticide registration and no such studies are available on aminopyralid. In the U.S. EPA human health risk assessment of aminopyralid (U.S. EPA/OPP-HED 2005), potential effects on immune function are not addressed.

While no specific studies are available on the immunologic effects of aminopyralid, limited information is available from the standard subchronic and chronic studies (Section 3.1.5). Typical subchronic or chronic animal bioassays conduct morphological assessments of the major lymphoid tissues, including bone marrow, major lymph nodes, spleen and thymus (organ weights are sometimes measured as well), and blood leukocyte counts. These assessments can detect signs of inflammation or injury indicative of a direct toxic effect of the chemical on the lymphoid tissue. Changes in morphology/cellularity of lymphoid tissue and blood, indicative of a possible immune system stimulation or suppression, can also be detected.

As noted in Section 3.1.5 and Appendix 3, remarkable effects in lymphoid tissue have not been noted in these standard toxicity studies on aminopyralid.

3.1.8. Effects on Endocrine System

Assessment of the direct effects of chemicals on endocrine function are most often based on mechanistic studies on estrogen, androgen, or thyroid hormone systems (i.e., assessments on hormone availability, hormone receptor binding, or post-receptor processing). In addition, changes in structure of major endocrine glands – i.e., the adrenal, hypothalamus, pancreas, parathyroid, pituitary, thyroid, ovary, and testis – may also be indicative of effects on the endocrine system. Disruption of the endocrine system during development may give rise to effects on the reproductive system that may be expressed only after maturation. Consequently, multigeneration exposures are recommended for toxicological assessment of suspected endocrine disruptors (Durkin and Diamond 2002). The one available multigeneration reproduction study on aminopyralid is discussed in Section 3.1.9.2 and the effects of aminopyralid on gonadal tissue are discussed in Section 3.1.9.3.

As noted in Appendix 3, there are several studies that report weight loss in experimental mammals after exposure to aminopyralid (Brooks and Yano 2001; Johnson and Dryzga 2004; Kiplinger 2001; Landry and Krieger 2002; Stebbins and Day 2003a; Wilson et al. 2002). While changes (increases or decreases) in body weights could be associated with effects on endocrine function, body weight loss is a very common observation in toxicity studies and could be due to a variety of other factors secondary to general adverse effects. In the absence of any indication of effects on endocrine tissue, there is no basis for asserting that decreases in body weights are associated with changes in endocrine function.

Although the U.S. EPA has yet to adopt standardized screen tests for endocrine disruptors, this endpoint is addressed in the U.S. EPA human health risk assessment of aminopyralid (U.S. EPA/OPP-HED 2005) and the U.S. EPA has concluded that: *In the available toxicity studies on aminopyralid, there was no estrogen, androgen, and/or thyroid mediated toxicity.* Based on the review of the toxicity studies summarized in Appendix 3, the current risk

assessment concurs with this conclusion with the qualification that no studies on binding to estrogen or androgen receptors are available.

3.1.9. Reproductive and Teratogenic Effects

3.1.9.1. Developmental (Teratology) Studies

Developmental studies are used to assess whether a compound has the potential to cause birth defects as well as other effects during development or immediately after birth. These studies typically entail gavage administration to pregnant rats or rabbits on specific days of gestation. Teratology assays as well as studies on reproductive function (Section 3.1.9.2) are generally required for the registration of pesticides. Very specific protocols for developmental studies are established by U.S. EPA/OPPTS and are available at http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized.

As detailed in Appendix 3, four developmental studies are available in rabbits: two studies on technical grade aminopyralid (Marty et al. 2002; Liberacki et al. 2001b) and two studies on the formulation (Carney and Tornesi 2004b; Carney and Tornesi 2004c). In addition, three developmental studies are available in rats – i.e., two studies on technical grade aminopyralid (Carney and Tornesi 2001; Tornesi et al. 2001) and one study on the formulation (Bjorn 2003).

With the exception of the study by Carney and Tornesi (2004c), all of the developmental studies have been classified as *Acceptable* by the U.S. EPA, full DERs are available, and all of the studies are cited in the U.S. EPA human health risk assessment of aminopyralid (U.S. EPA/OPP-HED 2005). The Carney and Tornesi (2004c) study appears to be supplemental to Carney and Tornesi (2004b), although the studies were assigned different MRID numbers by the U.S. EPA. Only a one-page and partial DER is available on Carney and Tornesi (2004c). Nonetheless, a full copy of this study was provided by Dow AgroSciences for the preparation of the current risk assessment.

The developmental studies in rats with both technical grade aminopyralid (Carney and Tornesi 2001; Tornesi et al. 2001) and the formulation (Bjorn 2003) are consistent and unremarkable. At doses of up to 1000 mg a.e./kg bw/day administered from Days 6 to Days 19 or 20 of gestation, no adverse effects were noted in dams or offspring.

In developmental studies using rabbits, however, adverse effects are noted in dams. At doses of 500 mg a.e./kg bw/day and 750 mg a.e./kg bw/day in the study on technical grade aminopyralid by Marty et al. (2002), decreased weight gain and incoordination were noted in dams. Dose-related incoordination was also noted in the formulation study by Carney and Tornesi 2004b – i.e., 1/26, 2/26, and 19/26 at doses of 104, 260, 520 mg a.e./kg bw/day. Incoordination is also reported in the formulation study by Carney and Tornesi (2004c) at a dose of 150 mg a.i./kg bw/day (equivalent to 78 mg a.e./kg bw/day). In the Carney and Tornesi (2004c) study, incoordination was observed in 3 of 21 female rats within 30 to 75 minutes after dosing and remained evident for several hours after dosing. In addition, these three rabbits evidenced this effect only on Days 14, 25, and 26 of gestation.

Carney and Tornesi (2004c) do not discuss the possible reasons why incoordination was seen in the same three rabbits but only on the same three days. Carney and Tornesi (2004c) do note that the rack order of each rabbit showing incoordination was changed each time that the incoordination was observed in order to prevent positional bias. That the same 3 rabbits displayed incoordination over the course of the study could be a matter of differences in individual sensitivities that would be expected to remain constant over the course of the study. The reason that the effects were seen only on the same 3 days of the 21 days of dosing, however, seems unusual. As indicated in Appendix 3, all rabbits were dosed once each day on 7-27 of gestation. If the incoordination was caused by exposure to aminopyralid, it would be reasonable to have expected to see incoordination in the three sensitive animals after each dosing.

No incoordination or other remarkable behavioral changes, however, are reported in the study by Liberacki et al. (2001b) using technical grade aminopyralid at doses up to 1000 mg a.e./kg/day on Day 7 to Day 27 of gestation. The reason or reasons for this discrepancy are not apparent. Both of the studies on technical grade aminopyralid appear to be virtually identical in design and both used the same vehicle (water with 0.5% methylcellulose and hydroxypropyl methylcellulose polymers).

The formulation study by Carney and Tornesi (2004b) also noted decreased maternal food consumption and severe weight loss at 520 mg a.e./kg bw/day and extreme weight loss in one doe at 260 mg a.e./kg bw/day. The only fetal effect was a decrease in fetal weight (which appears to be secondary to maternal weight loss) at 520 mg a.e./kg bw/day. Based on this study, the U.S. EPA set the maternal NOEL at 104 mg a.e./kg bw/day and the developmental NOAEL at 260 mg a.e./kg bw/day. This study is considered further in Section 3.3 in the derivation of an acute RfD.

Based on these studies and the single developmental study discussed in Section 3.1.9.2, the U.S. EPA has concluded that: *Developmental and reproduction studies show that there is no evidence of increased qualitative or quantitative susceptibility of the fetuses to aminopyralid* (U.S. EPA/OPP-HED 2005, p. 4). Since the decrease in body weight in fetuses is the only fetal effect that has been note and since this effect appears to be secondary to maternal weight loss, the conclusion reach by the U.S. EPA appears to be appropriate.

3.1.9.2. Reproduction Studies

Reproduction studies involve exposing one or more generations of the test animal to the compound. The general experimental method involves dosing the parental (P) generation (i.e., the male and female animals used at the start of the study) to the test substance prior to, during mating, after mating, and through weaning of the offspring (F1). In a 2-generation reproduction study, this procedure is repeated with male and female offspring from the F1 generation to produce another set of offspring (F2). During these types of studies, standard observations for gross signs of toxicity are made. Additional observations often include the length of the estrous cycle, assays on sperm and other reproductive tissue, and number, viability, and growth of offspring.

The U.S. EPA requires only one acceptable multi-generation reproduction study and only a single study (Marty et al. 2003) has been submitted. Unlike the developmental studies, which were all by gavage, the study by Marty et al. (2003) involved dietary exposures at concentrations of 0, 50, 250, 1000 ppm (mg a.e./kg diet) for 10 weeks. Over this period, the parental (P1) animals produced a single (F1) generation. Since the parental animals as well as the offspring grew over the period of exposure, food consumption was variable. As noted in Appendix 3, an examination of body weights and food consumption suggests that the maximum food consumption (as a fraction of body weight) was about 0.218 kg diet/kg bw. Thus, the dietary concentrations correspond to maximum daily doses of about 0 (control), 10.9 mg a.e./kg bw (50 ppm diet), 54.5 mg a.e./kg bw (250 ppm diet), and 218 mg a.e./kg bw (1000 ppm diet). The only effect noted was an increase in cecal weight. As discussed in Section 3.1.2.1, this is the most consistent effect seen in rats after the consumption of aminopyralid. This effect was classified by both the study authors (Marty et al. 2003) and the U.S. EPA (U.S. EPA/OPP-HED, 2005, p. 24) as being not toxicologically significant. Consequently, the U.S. EPA classified the 1000 ppm dietary concentration as a NOAEL for both parental, reproductive, and developmental effects.

3.1.9.3. Target Organ Toxicity

As noted in Section 3.1.8, damage to gonadal tissue (ovaries or testes) can suggest an effect on endocrine function and damage to these organs would clearly suggest a potential for adverse reproductive effects. With the exception of the chronic study in mice (Stebbins and Day 2003b), there is no indication that aminopyralid will damage gonadal tissue. In the female mice at the high dose group, Stebbins and Day (2003b) did note a statistically significant increase in absolute and relative ovary weights in the 50 mg/kg bw and 250 mg/kg target dose groups but not in the 1000 mg/kg target dose group. These data have been carefully reanalyzed by the U.S. EPA in the DER for this study and the Agency has concluded that the increased ovarian weights were related to the development of idiopathic ovarian cysts in the 50 mg/kg bw and 250 mg/kg target dose groups and that this effect was not associated with exposure to aminopyralid. Given the lack of a dose-response relationship and the association of the increased ovarian weights with ovarian cysts, the conclusion that the increased ovarian weights are not attributable to aminopyralid exposure seems reasonable.

3.1.10. Carcinogenicity and Mutagenicity

Aminopyralid has been tested for mutagenicity in a number of different test systems (Linscombe et al. 2001, 2002a,b, 2004; Mecchi 2004a; Spencer and Gorski 2002;) and has been assayed *in vivo* for carcinogenic activity in rats (Johnson and Dryzga 2004) and mice (Stebbins and Day 2003b). These studies are summarized in Appendix 3 and all studies have been classified by the U.S. EPA as *Acceptable*.

The only positive response from the *in vitro* mutagenicity studies involved chromosomal aberrations in cultured rat lymphocytes at concentrations of 1000, 1400 and 1700 μg/mL (Linscombe et al. 2002a). This response is characterized by the U.S. EPA as weak clastogenic activity secondary to cell toxicity. The U.S. EPA assessment states that the weak clastogenic activity occurred …*only at cytotoxic levels with metabolic activation* (U.S. EPA/OPP-HED 2004, p. 59). This statement in the HED risk assessment appears to be a

typographic error that is carried over from the DER. The data in the study (Linscombe et al. 2002a) as well as the data presented in the DER indicate a clastogenic effect only in the absence of metabolic activation.

In terms of a quantitative significance to the human health risk assessment, carcinogenicity is an issue only if the data are adequate to support the derivation of a cancer potency factor. Since neither of the *in vivo* bioassays (Johnson and Dryzga 2004; Stebbins and Day 2003b) noted any carcinogenic activity, no cancer potency factor has been derived.

Based on the results of the mutagenicity screening studies and the *in vivo* bioassays, the U.S. EPA has concluded that aminopyralid is… *"not likely" to be carcinogenic to humans* (U.S. EPA/OPP-HED 2004, p. 13). This conclusion is clearly supported by the available data.

3.1.11. Irritation and Sensitization (Effects on the Skin and Eyes)

3.1.11.1. Skin Irritation

Two dermal irritation studies in rats are available, one on technical grade aminopyralid (Brooks 2001c) and the other on the GF-871 formulation (Brooks and Radtke 2002b). Both of these follow the same very standard protocol that is required by the U.S. EPA for pesticide registration and both are classified by the U.S. EPA as *Acceptable*.

As summarized in Appendix 3, the study with technical grade aminopyralid involved 500 mg a.e. in a 0.3 mL aqueous suspension. The study on the formulation involved 0.5 mL of the formulation (about 109 mg a.e.). No dermal irritation was observed with the technical grade material (Brooks 2001c) but slight erythema on Days 1 and 3 were observed in the assay of the formulation (Brooks and Radtke 2002b).

Neither study evidenced marked irritation and both studies have resulted in a Category IV classification – i.e., the lowest classification used by the U.S. EPA. While somewhat speculative, the irritation observed in the formulation study may be attributable to TIPA rather than aminopyralid since the occurrence of TIPA in the formulation is the only difference between technical grade aminopyralid and the GF-871 formulation. This is discussed further in Section 3.1.14 (Inerts and Adjuvants).

3.1.11.2. Skin Sensitization

As with dermal irritation, two dermal sensitization studies are available, one on technical grade aminopyralid (Wilson 2001) and the other on the GF-871 formulation (Wilson 2002). These studies also follow a standardized protocol and both studies have been classified as *Acceptable* by the U.S. EPA. Both studies yielded the same result, no evidence of any dermal sensitization.

3.1.11.3. Ocular Effects

Two studies are also available on the ocular effects of aminopyralid, one on technical grade aminopyralid (Brooks 2001b) and the other on the GF-871 formulation (Brooks and Radtke 2002a). As with the studies on dermal irritation and sensitization, these studies follow very standard protocols and both are classified by the U.S. EPA as *Acceptable*.

The study on the technical grade aminopyralid involved placing 100 mg of the powder into the conjunctival sac of rabbits, 2 males and 1 female. Severe irritation with corneal damage was observed in all animals and these effects persisted throughout the 36 day post-exposure observation period. Consequently, the U.S. EPA classified technical grade aminopyralid as a severe eye irritant (Category I).

In the formulation study (Brooks and Radtke 2002a), 0.1 mL of GF-871 (about 28.8 mg aminopyralid TIPA in water) was applied to the conjunctival sac of rabbits (1 male and 2 females) and only slight redness of the conjunctiva was noted and this irritant effect lasted until only Day 2 of the study, by which time no irritant effects were evident. Consequently, the U.S. EPA classified this aminopyralid formulation as Category IV, the minimal classification for eye irritants.

3.1.12. Systemic Toxic Effects from Dermal Exposure

Two acute dermal toxicity studies, both of which are limit tests, are available in rats, one on technical grade aminopyralid (Brooks and Yano 2001) and the other on the GF-871 formulation (Wilson et al. 2002). In addition, a 28-day subchronic dermal toxicity study in rats is available on technical grade aminopyralid (Stebbins et al. 2002). These studies are summarized in Appendix 3 and all of these studies have been classified by the U.S. EPA as *Acceptable* (U.S. EPA/OPP-HED 2005).

The results of the acute toxicity studies are unremarkable. Both studies involved a single application of the test material to the skin for 24 hours at a dose of 5000 mg/kg bw. For the technical grade powder, this dose is equivalent to 5000 mg a.e./kg bw. For the formulation, the liquid dose was equivalent to 1090 mg a.e./kg bw. In both studies, treated animals displayed a transient loss of body weight, some signs of dermal irritation, and soiling (perineal for the formulation and periocular or perioral for the technical grade powder). Mortality or frank signs of toxicity were not observed in any of the animals in either study (Brooks and Yano 2001; Wilson et al. 2002). Based on these two acute dermal toxicity studies, the U.S. EPA classified aminopyralid (both the acid and formulation) as Category IV, the minimal classification for acute dermal toxicity.

The subchronic dermal study with technical grade aminopyralid was similarly unremarkable. No signs of frank toxicity were observed at dermal doses (6 hours/day) of 0, 100, 500, and 100 mg a.e./kg bw/day for 28 days. The only responses were slight epidermal hyperplasia in 2/10 males at 500 mg/kg and in 3/10 males at 1000 mg/kg.

3.1.13. Inhalation Exposure

As summarized in Appendix 3, two inhalation toxicity studies are available, one on technical grade aminopyralid (Kiplinger 2001) and the other on the GF-871 formulation (Landry and Krieger 2002). Both of these studies have been classified by the U.S. EPA as *Acceptable* (U.S. EPA/OPP-HED 2005).

As with the studies on acute dermal toxicity (Section 3.1.12), both of these studies are limit tests, each with a four hour period of exposure to a single concentration of the test substance

in air (5.5 mg a.e./L for technical grade aminopyralid and 1.26 mg a.e./L for the formulation). The study on the technical grade dust involved nose-only exposures – i.e., an inhalation tube connected to the nose of the exposed animal (Kiplinger 2001). The formulation assay involved whole body exposures – i.e., the animals were in an inhalation chamber. In both studies, the animals exhibited various signs associated with the very high exposures and the stress of the test – e.g., gasping and dropping eyes lids in the nose-only exposures and soiling of the fur in the whole body exposures. The only systemic effects were slight (1%-4.5%) and transient losses of body weight.

Based on these two acute inhalation toxicity studies, the U.S. EPA classified aminopyralid (both the a.e. and the formulation) as Category IV, the minimal classification for acute inhalation toxicity.

3.1.14. Inerts and Adjuvants

As noted in Section 2.2, the Milestone formulations covered in this risk assessment contain only the triisopropanolamine (TIPA) salt of aminopyralid and water. Inerts are classified by the U.S. EPA as inerts of toxicological concern (List 1), potentially toxic compounds (List 2), inerts of unknown toxicity (List III), inerts of minimal concern (List 4A), and other compounds that are not likely to be of concern based on use patterns (4B). A listing of all inerts is available at http://www.epa.gov/opprd001/inerts/lists.html. Triisopropanolamine (CAS No. 122-20-3) is classified by the U.S. EPA as a List 3 inert. In other words, the U.S. EPA judges that the available information on TIPA is not sufficient to determine whether or not the use of TIPA in pesticides poses a potential risk.

Consistent with the position taken by the U.S. EPA on the classification of TIPA as a List 3 inert, relatively little information is available on the toxicity of TIPA. Material Safety Data Sheets (MSDS) (e.g., ScienceLab 2005) are available and a very brief overview of the toxicity of TIPA is available from the World Health Organization (WHO 1997). The most detailed compilation on TIPA encountered in the literature is the summary in the Hazardous Substances Databank (HSDB 2003), a compendium of chemical information profiles maintained by the National Library of Medicine.

On the MSDS from ScienceLab (2005), the rat LD_{50} for TIPA is listed as 4730 mg/kg. Following the categorization system used by the U.S. EPA in human health risk assessments, TIPA would be classified marginally as Category III (*Caution*), which applies to compounds with oral LD_{50} values in the range of >500 to 5,000 mg/kg (SERA 2007A, Table 3-2). Following the classification system used by the U.S. EPA in ecological risk assessments, TIPA would be classified as *Practically Nontoxic* because the oral LD_{50} is >2000 mg/kg (see SERA 2007A, Table 4-1). The HSDB (2003) summary of TIPA indicates that this compound is approved as an indirect food additive for use only as a component of adhesives. TIPA, however, is not listed as an approved food additive in Clydesdale (1997).

The MSDS for TIPA also classifies TIPA as a moderate eye irritant that may cause corneal damage (ScienceLab 2005) and this classification is also given in the HSDB (2003) summary. The WHO (1997) summary on TIPA does not give a categorization for eye irritation but does indicate that TIPA can cause corrosive effects on the eyes including pain,

redness, severe deep burns, loss of vision. The MSDS also indicates that TIPA is a moderate skin irritant.

Two subchronic studies on TIPA have been conducted: a 2-week drinking water toxicity study in rats (McCollister et al. 1981) and a 13-week dietary toxicity study in dogs (Mullin 1987). These studies have not been reviewed by the U.S. EPA in the human health risk assessment for the registration of aminopyralid (U.S. EPA/OPP-HED 2005). Summaries of these studies, however, are contained in the 90-day subchronic study of the GF-871 formulation by Stebbins and Dryzga (2004).

In the subchronic drinking water study in rats by McCollister et al. (1981), the animals were dosed with 100, 300, 600, 1200 or 2000 milligrams TIPA/kg bw for 2 weeks. The only effect was increased kidney weight (but no organ pathology or changes in BUN values) at doses of 300 mg/kg bw and higher. In the 13-week dietary study by Mullin (1987), dogs were exposed to TIPA in the diet at doses equivalent to 0, 16.8, 71.2, and 272 mg TIPA/kg body weight/day for males and 0, 19.7, 78.3, and 288 mg/kg for females. The summary of this study by Stebbins and Dryzga (2004) indicates that: *There were no effects that were considered compound related or biologically significant in any of the parameters measured* (Stebbins and Dryzga 2004, p. 15).

It is not clear that TIPA plays a role in any of the toxicity studies conducted on the GF-871 formulation. As noted in Section 3.1.5.1, the 90-day toxicity study in rats by Stebbins and Dryzga (2004) is the only subchronic mammalian toxicity study that used the GF-871 formulation. Expressed as TIPA equivalents, the doses in this dietary study were 0, 92, 241, and 482 mg TIPA/kg bw/day. Based on the rat drinking water study by Mullin (1987) on TIPA, increased kidney weights in rats could have been expected in the Stebbins and Dryzga (2004) study. As noted in Appendix 3, however, increased kidney weights were noted only at the highest dose and the increases were slight – 2.4% in males and 3% in females – and were not statistically significant.

Several acute studies are available on the GF-871 formulation. As noted in Section 3.1.4, lacrimation and cloudy eyes have been noted in an acute oral limit test with the TIPA salt formulation of aminopyralid at a dose of 5000 mg formulation/kg bw (Wilson et al. 2003) and this effect was not noted in the acute oral limit test of technical grade aminopyralid at a dose of 5000 mg a.e./kg bw (Brooks 2001a). Conversely, technical grade aminopyralid powder caused severe and persistent eye damage in a standard eye irritation assay (Brooks 2001b) whereas the GF-871 formulation with TIPA caused only minimal eye irritation (Brooks and Radtke 2002a). Lastly, the skin irritation studies on both technical grade aminopyralid (Brooks 2001b) and the GF-871 formulation (Brooks and Radtke 2002b) noted little skin irritation. As discussed in Section 3.1.11.1, however, the formulation study did note slight erythema that was not seen in the study on technical grade aminopyralid.

Thus, it is not clear if any of these differences in the studies on technical grade aminopyralid and the GF-871 formulation can be associated with the presence of TIPA in the formulation. The ocular effects in the oral study of the formulation (Wilson et al. 2003) are unusual. Since the GF-871 formulation consists of 41.9% aminopyralid TIPA, the 5000 mg

formulation/kg bw corresponds to a dose of about 2095 mg aminopyralid TIPA/kg bw. Based on the molecular weights of aminopyralid TIPA (398.27 g/mole) and TIPA (191.27 g/mole) (Table 2), the formulation dose in the Wilson et al. (2003) study corresponds to a dose of about 1000 mg TIPA/kg bw. The available toxicity studies on TIPA, discussed above, are all substantially below this dose of 1000 mg TIPA/kg bw. It is unclear if the ocular effects observed in the study by Wilson et al. (2003) are attributable to TIPA or a simply an aberration. That the ocular effects are attributable to aminopyralid or TIPA is unclear. As detailed in Section 3.1.2.5, the study by Wilson et al. (2003) is the only oral study on aminopyralid in which ocular effects were noted and several other comparable studies have been conducted in which these ocular effects were not noted.

3.1.15. Impurities and Metabolites

3.1.15.1. Metabolites

As discussed in SERA (2007, Sections 3.1.3.1), two types of metabolites may be considered in a risk assessment, *in vivo* metabolites and environmental metabolites. *In vivo* metabolites, refer to the compounds that are formed within the animal after the agent has been absorbed. Environmental metabolites refer to compounds that may be formed in the environment by a number of different biological or chemical processes including breakdown in soil or water or breakdown by sunlight (photolysis).

As summarized in Appendix 3 and reviewed in further detail by the U.S. EPA (U.S. EPA/OPP-HED 2005, p. 9 ff), aminopyralid does not appear to be extensively metabolized by mammals – i.e., rats (Liu 2004), cows (Rosser et al. 2004) or goats (Macpherson 2003) – and the same pattern is seen in hens (Magnussen 2004a). In all of these organism, the major product that is excreted is the parent compound and this accounts for over 95% of the excreted material. Only one minor metabolite was detected in goats and this accounted for less than 0.2% of the administered dose (Macpherson 2003). As with many other pesticides, it seems reasonable to assert that the available *in vivo* toxicity studies will encompass the concerns with *in vivo* metabolites in both the human health and ecological risk assessments.

The occurrence and potential significance of the environmental metabolites of aminopyralid is a somewhat more complex issue. As detailed in Appendix 1, aminopyralid will degrade to a number of different metabolites via aqueous photolysis and two specific metabolites have been identified – i.e., oxamic acid and malonamic acid. Other unidentified metabolites include 2 or 3 carbon acid amides (Cook 2003b).

While there appears to be very little information on oxamic acid and malonamic acid and no inferences can be made on the potential risks of other unidentified metabolites, the U.S. EPA has clearly indicated that these metabolites are not of substantial concern in the human health risk assessment conducted by OPP:

> *The Health Effects Division (HED) has very low concern regarding the hazard associated with these environmental metabolites. Searches of various hazard databases (e.g., TOX.NET, MEDLINE, and others) did not reveal any cause for concern for either chemical. Both*

> *chemicals are small amino acid analogs. Following uptake, they are expected to be readily metabolized and/or rapidly excreted without any significant biological effects. Based on the available information, HED does not believe that it is appropriate to include residues of either oxamic acid or malonamic acid in dietary risk assessments; therefore, these compounds should not be included as residues of concern in drinking water.*. (U.S. EPA/OPP-HED 2005, p. 12)

The above paragraph appears to be more of a judgment than a conclusion reached by analysis. While this judgment may be correct, the U.S. EPA/OPP-HED (2005) assessment does not provide any additional data or rationale for their conclusion.

The Environmental Fate and Effects Division (EFED), the branch of the Office of Pesticide Programs that is responsible for the conduct of ecological risk assessments, has taken a somewhat different view on the environmental metabolism of aminopyralid:

> …*EFED is concerned about the lack of data covering the metabolites of aminopyralid. Aminopyralid is a dichlorinated pyridine and while the amino and carboxyl sidechains should be easily cleaved through chemical and microbial action, the remaining chlorinated ring structure maybe more resistant to further metabolic breakdown.* (U.S. EPA/OPP-EFED 2005, p. 51)

The importance of metabolites in the risk assessment of aminopyralid cannot be fully or well characterized with the information that is available. A search of the TOXLINE conducted for the current risk assessment did not yield any citations on either oxamic acid (n=144) or malonamic acid (n=16) that would appear to be adequate for more completely assessing either toxic potency or exposure.

Notwithstanding the limited information on the identity and toxicity of metabolites, some reasonable suppositions can be made. All of the aquatic toxicity studies that are discussed in Section 4 (Ecological Risk Assessment) involve exposures to organisms under conditions of natural lighting. Many of these exposures are static and cover a period of several days. If substantial quantities of toxic metabolites secondary to photodegradation were generated, it is plausible that observations would be available indicating that the toxicity of aminopyralid substantially increased as the duration of exposure increased. As detailed in Section 4, this is not the case. In one study in trout (Marino et al. 2001a), partial loss of equilibrium was observed in 2 of 30 organisms (6.66%) exposed to 100 mg/L at 96 hours but not at 24, 48, or 72 hours. This effect, however, is not statistically significant and does not provide substantial support for the assertion that metabolites formed by photolysis are likely to be toxicologically significant. While aquatic toxicity studies are not directly applicable to the assessment of human health risks, these studies do offer modest support to the judgment expressed by U.S. EPA/OPP-HED (2005) that the metabolites of aminopyralid are …*a very low concern*.

Other methods – e.g., bioassays on weathered pesticide residues – have been employed with some pesticides to address concerns for environmental metabolites (e.g., Gooch and Matsumura 1987; Lee et al. 1977). These types of studies, however, are not required for pesticide registration and have not been conducted on aminopyralid. Given the lack of any evidence that aminopyralid or its metabolites will persist in the environment and the lack of any indication that the environmental metabolites are toxic based on the studies discussed in Section 4, the need for such testing is questionable.

3.1.15.2. Impurities

Virtually no chemical synthesis yields a totally pure product. Technical grade aminopyralid, as with other technical grade products, undoubtedly contains some impurities. To some extent, concern for impurities in technical grade aminopyralid is reduced by the fact that the existing toxicity studies on aminopyralid were conducted with the technical grade product. Thus, if toxic impurities are present in the technical grade product, they are likely to be encompassed by the available toxicity studies on the technical grade product.

Dow AgroSciences has identified impurities in technical grade aminopyralid and these have been disclosed to the U.S. EPA (Ghaoui 2004). The Ghaoui (2004) submission, however, also contains information on production processes – i.e., methods of synthesis – and these are considered propriety. Thus, the Ghaoui (2004) submission is one of only two submissions to the U.S. EPA that were not provided by Dow AgroSciences for the preparation of the current risk assessment. Jachetta (2006), however, has indicated that the impurities in aminopyralid are … *several closely related reaction products (all pyridine derivatives quite similar in structure to aminopyralid), and sodium chloride*.

Impurities can be a substantial concern in a risk assessment if the impurities pose risks that are qualitatively different from active ingredient. For example, both picloram (SERA 2003a) and clopyralid (SERA 2004c) contain hexachlorobenzene as an impurity. Hexachlorobenzene is a concern in the risk assessments on picloram and clopyralid because hexachlorobenzene is a persistent carcinogen. Thus, full exposure assessments, dose-response assessments and risk characterizations are given for the hexachlorobenzene impurity in the risk assessments on picloram (SERA 2003a) and clopyralid (SERA 2004c). As noted in Section 2.2, aminopyralid does not contain hexachlorobenzene and the exclusion of hexachlorobenzene and other chlorinated benzenes was a major goal in the development process of aminopyralid (Jachetta 2006).

3.1.16. Toxicological Interactions

No information is available on the interactions of aminopyralid with other compounds and most inferences that can be made are speculative.

In terms of mechanism of action, it is likely that aminopyralid would influence and be influenced by other weak acids that are excreted by the kidney. These influences, however, would be significant only at relatively high doses that saturated the active transport processes involved in excretion by the kidney.

As discussed in Section 3.1.2.1, a number of other chemical agents result in cecal enlargement. There is no basis for anticipating any form of joint action other than additivity with these agents.

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview

All exposure assessments for aminopyralid are summarized in Worksheet E01 for workers and Worksheet E03 for the general public (Attachment 1: SERA EXWS 07-52-04-01c). For workers applying aminopyralid, three types of application methods are modeled: directed ground spray, broadcast ground spray, and aerial spray. In non-accidental scenarios involving the normal application of aminopyralid, central estimates of exposure for workers are approximately 0.001 mg/kg/day for aerial and backpack workers and about 0.002 mg/kg/day for broadcast ground spray workers. Upper ranges of exposures are approximately 0.012 mg/kg/day for broadcast ground spray workers and 0.006 mg/kg/day for backpack and aerial workers. All of the accidental exposure scenarios for workers involve dermal exposures. Except for the scenario involving a spill on the lower-legs for 1 hour (an upper bound dose of 0.003 mg/kg/event), the accidental exposures lead to dose estimates that are substantially lower than the general exposure levels estimated for workers. This is not uncommon and it reflects the fact that the general exposure estimates are based on field studies of workers in which accidental and/or incidental events such as spills probably occurred and in some cases were specifically noted to occur.

For the general public (Worksheet E03), acute levels of exposures range from minuscule (e.g., $1x10^{-8}$ mg/kg/day) to about 0.4 mg/kg bw at the typical application rate of 0.078 lb a.e./acre. The upper bound of exposure, 0.4 mg/kg bw, is associated with the consumption of contaminated water by a child shortly after an accidental spill. This exposure scenario is highly arbitrary. The upper bound of the dose associated with the consumption of contaminated vegetation, a more plausible but still extreme exposure scenario, is about 0.1 mg/kg bw. The other acute exposure scenarios lead to much lower dose estimates – i.e., ranging from near zero to about 0.042 mg/kg for the accidental direct spray of a child. The lowest acute exposures are associated with swimming in or drinking contaminated water.

The chronic or longer-term exposures are much lower than the estimates of corresponding acute exposures. The highest longer-term exposures are associated with the consumption of contaminated vegetation and the upper bound for this scenario is about 0.027 mg/kg/day. This is followed by the scenario for the longer-term consumption of contaminated fruit with an upper bound of 0.003 mg/kg/day. As with the acute exposures, the lowest longer-term exposures are associated with the consumption of surface water.

3.2.2. Workers

The exposure assessments used for workers in the current risk assessment are based on a standard set of exposure scenarios that have been used for other herbicides that have similar uses and application methods – i.e., 2,4-D (SERA 2006), clopyralid (SERA 2004c), picloram (SERA 2003a), and triclopyr (SERA 2003b). While these exposure assessments vary depending on the characteristics as well as the relevant data on the specific chemical, the organization and assumptions used in the exposure assessments are standard and consistent. All of the exposure assessments for workers as well as members of the general public are detailed in an EXCEL workbook that accompany this risk assessment (Attachment 1: SERA

EXWS 07-52-04-01b). This workbook contains a set of worksheets on aminopyralid that detail each exposure scenario discussed in this risk assessment and as well as summary worksheets for both workers and members of the general public that cover the range of application rates considered in this risk assessment. Documentation for these worksheets is presented in SERA (2005). This section on workers and the following section on the general public provide a plain verbal description of the worksheets and discuss the aminopyralid specific data used in the worksheets.

Exposure assessments for workers are summarized in Worksheet E01 of the EXCEL workbook. Two types of exposure assessments are considered: general and accidental/incidental. The term *general* exposure assessment is used to designate exposures involving absorbed dose estimates based on handling a specified amount of chemical during specific types of applications. The accidental/incidental exposure scenarios involve specific events that may occur during any type of application. The exposure assessments developed in this section as well as other similar assessments for the general public (Section 3.2.3) are based on the typical application rate of 0.078 lb a.e./acre (Section 2). The consequences of using different application rates in the range considered by the Forest Service are discussed further in the risk characterization (Section 3.4), and these risks are detailed in Worksheets E02a (central application rate), E02b (lower bound of application rate), and E02c (upper bound of application rate).

3.2.2.1. General Exposures

As described in SERA (2007), worker exposure rates are expressed in units of mg of absorbed dose per kilogram of body weight per pound of chemical handled. Based on analyses of several different pesticides using a variety of application methods, default exposure rates are estimated for three different types of applications: directed foliar (backpack), boom spray (hydraulic ground spray), and aerial.

The specific assumptions used for each application method are detailed in worksheets C01a (directed foliar), C01b (broadcast foliar), and C01c (aerial). The typical application rate is taken directly from the program description (Section 2.4). The central estimate of the amount handled per day is calculated as the product of the central estimate of the acres treated per day and the application rate.

As noted in the program description (Section 2.3), these three standard and general application methods may not reflect some of the specific methods that may be used in the application of aminopyralid. For example, ground applications are modeled in the worksheets for both directed foliar applications (backpack) and broadcast foliar applications (truck mounted boom spray). In some cases, however, all terrain vehicles (ATVs) may be used. Depending on the application site and target vegetation, the use of ATVs could mimic either backpack applications (direct wand spray) or truck mounted boom spray. In the former case, workers would likely be subject to exposures that are comparable to backpack application. In the latter case, the worker exposure rates would likely be comparable to truck mounted boom spray but fewer acres per hour would be treated (Paul Mistretta, USDA/Forest Service R8, personal communication). These types of site-specific considerations can be addressed in site-specific analyses by modifying the appropriate

worksheets in the EXCEL workbook that accompanies this risk assessment. The exposure assessments that are presented in Worksheets C01a to C01c, however, should encompass or exceed the range of worker exposures that are plausible in the application of aminopyralid.

No worker exposure studies with aminopyralid were found in the literature. Both the U.S. EPA/OPP-HED (2004) and Tiu and Selman (2004) have use the Pesticide Handlers Exposure Database (PHED) to estimate worker exposures. As detailed in SERA (2007, Section 3.2.2), PHED is a monitoring-based model in which the exposure rather than the absorbed dose is estimated from measurements of air concentrations and skin deposition of pesticides. As also discussed in SERA (2007), a different approach is taken in this risk assessments as well as other similar risk assessments that have been conducted as part of this series of risk assessments.

Rather than using data on deposition, worker exposure rates are expressed in units of mg of absorbed dose per kilogram of body weight per pound of chemical handled based on biomonitoring studies in workers. These exposure rates are based on worker exposure studies on nine different pesticides with molecular weights ranging from 221 to 416 and log $K_{o/w}$ values at pH 7 ranging from -0.75 to 6.50. The estimated exposure rates are based on estimated absorbed doses in workers as well as the amounts of the chemical handled by the workers. As summarized in Table 2 of this risk assessment, the molecular weight of the TIPA salt of aminopyralid is about 398 and the log $K_{o/w}$ at pH 7 is approximately -2.87. The log $K_{o/w}$ for aminopyralid is outside of the range of values on which the general worker exposure rates are based and this reduces confidence in the exposure assessments.

As described in SERA (2007), the ranges of estimated occupational exposure rates vary substantially among individuals and groups, (i.e., by a factor of 50 for backpack applicators and a factor of 100 for mechanical ground sprayers). It seems that much of the variability can be attributed to the hygienic measures taken by individual workers (i.e., how careful the workers are to avoid unnecessary exposure); however, pharmacokinetic differences among individuals (i.e., how individuals absorb and excrete the compound) also may be important.

An estimate of the number of acres treated per hour is needed to apply these worker exposure rates. These values are taken from previous USDA risk assessments (USDA 1989a,b,c) and are comparable to the values that are used by the U.S. EPA (e.g., Sandvig 2001). The number of hours worked per day is expressed as a range, the lower end of which is based on an 8-hour work day with 1 hour at each end of the work day spent in activities that do not involve herbicide exposure. The upper end of the range, 8 hours per day, is based on an extended (10-hour) work day, allowing for 1 hour at each end of the work day to be spent in activities that do not involve herbicide exposure.

It is recognized that the use of 6 hours as the lower range of time spent per day applying herbicides is not a true lower limit. It is conceivable and perhaps common for workers to spend much less time in the actual application of a herbicide if they are engaged in other activities. Thus, using 6 hours may overestimate exposure. In the absence of any published or otherwise documented work practice statistics to support the use of a lower limit, this approach is used as a protective assumption.

The range of acres treated per hour and hours worked per day is used to calculate a range for the number of acres treated per day. For this calculation as well as others in this section involving the multiplication of ranges, the lower end of the resulting range is the product of the lower end of one range and the lower end of the other range. Similarly, the upper end of the resulting range is the product of the upper end of one range and the upper end of the other range. This approach is taken to encompass as broadly as possible the range of potential exposures.

The central estimate of the acres treated per day is taken as the arithmetic average of the range. Because of the relatively narrow limits of the ranges for backpack and boom spray workers, the use of the arithmetic mean rather than some other measure of central tendency, like the geometric mean, has no marked effect on the risk assessment.

3.2.2.2. Accidental Exposures

Typical occupational exposures may involve multiple routes of exposure (i.e., oral, dermal, and inhalation); nonetheless, dermal exposure is generally the predominant route for herbicide applicators (Ecobichon 1998; van Hemmen 1992). Typical multi-route exposures are encompassed by the methods used in Section 3.2.2.1 on general exposures. Accidental exposures, on the other hand, are most likely to involve splashing a solution of herbicides into the eyes or contaminating the surface of the skin.

There are various methods for estimating absorbed doses associated with accidental dermal exposure (SERA 2007A). Two general types of exposures are modeled in this risk assessment: those involving direct contact with a solution of the herbicide and those associated with accidental spills of the herbicide onto the surface of the skin. Any number of specific exposure scenarios could be developed for direct contact or accidental spills by varying the amount or concentration of the chemical on or in contact with the surface of the skin and by varying the surface area of the skin that is contaminated.

For this risk assessment, two exposure scenarios are developed for each of the two types of dermal exposure, and the estimated absorbed dose for each scenario is expressed in units of mg chemical/kg body weight. Both sets of exposure scenarios are summarized in Worksheet E01, which references other worksheets in which the specific calculations are detailed.

Exposure scenarios involving direct contact with solutions of the chemical are characterized by immersion of the hands for 1 minute in a field solution of aminopyralid or wearing contaminated gloves for 1 hour. Generally, it is not reasonable to assume or postulate that the hands or any other part of a worker will be immersed in a solution of a herbicide for any period of time. On the other hand, contamination of gloves or other clothing is quite plausible. For these exposure scenarios, the key element is the assumption that wearing gloves grossly contaminated with a chemical solution is equivalent to immersing the hands in a solution. In either case, the concentration of the chemical in solution that is in contact with the surface of the skin and the resulting dermal absorption rate are essentially constant.

For both scenarios (hand immersion and contaminated gloves), the assumption of zero-order absorption kinetics is appropriate. Following the general recommendations of U.S. EPA/ORD (1992), Fick's first law is used to estimate dermal exposure. As discussed in Section 3.1.3, an experimental dermal permeability coefficient (Kp) for aminopyralid is not available. Thus, the Kp for aminopyralid is estimated using the algorithm from U.S. EPA/ORD (1992), which is detailed in Worksheet B05.

Exposure scenarios involving chemical spills onto the skin are characterized by a spill on to the lower legs as well as a spill on to the hands. In these scenarios, it is assumed that a solution of the chemical is spilled on to a given surface area of skin and that a certain amount of the chemical adheres to the skin. The absorbed dose is then calculated as the product of the amount of the chemical on the surface of the skin (i.e., the amount of liquid per unit surface area multiplied by the surface area of the skin over which the spill occurs and the concentration of the chemical in the liquid), the first-order absorption rate, and the duration of exposure.

For both scenarios, it is assumed that the contaminated skin is effectively cleaned after 1 hour. The specific equation used in these exposure assessments is specified in Worksheet B06.

Confidence in these exposure assessments is diminished by the lack of experimental data on the dermal absorption of aminopyralid. Nonetheless, the estimated dermal absorption rate for aminopyralid is very similar to the well-documented dermal absorption rate of 2,4-D, another weak acid herbicide with properties that are very similar to aminopyralid (SERA 2006). As detailed in Worksheet B06, the central estimate of the first-order dermal absorption rate for aminopyralid is about 0.00046 $hour^{-1}$. This estimate is very close to the first-order dermal absorption rate of 2,4-D, 0.00066 $hour^{-1}$. This rate is taken from a physiologically-based pharmacokinetic model for 2,4-D (Durkin et al. 2004) in which the first-order dermal absorption rate is derived from experimental data on dermal absorption in humans (Feldmann and Maibach 1974). In addition, the exposure scenario in which contaminated gloves are worn for 1 hour (Worksheet C02b) and the exposure scenario in which a chemical solution is spilled on to the skin surface of the hands and cleaned after 1 hour (Worksheet C03a) are also very similar. This also enhances confidence in the estimated dermal absorption rates by the fact that two similar scenarios based on different empirical relationships yield similar estimates of absorbed dose.

3.2.3. General Public

3.2.3.1. General Considerations

3.2.3.1.1. Likelihood and Magnitude of Exposure

The likelihood that members of the general public will be exposed to aminopyralid in Forest Service applications is highly variable. In some Forest Service applications and in virtually all NPS applications aminopyralid will be applied in recreational areas such as campgrounds, picnic areas and trails. In these instances, exposures to member of the general public are virtually certain. In some of these applications, large numbers of people may be exposed. For example, aminopyralid may be applied in Yellowstone National Park, Ellis Island, or the

Statue of Liberty National Monument. These areas are visited by millions of individuals each year.

Because of the conservative exposure assumptions that are used in the current risk assessment, neither the probability of exposure nor the number of individuals that might be exposed have a substantial impact on the characterization of risk that is presented in Section 3.4. As noted in Section 1 (Introduction) and detailed in SERA (2007, Section 1.2.2.2), the exposure assessments developed in this risk assessment are based on ***Extreme Values*** rather than a single value. Extreme value exposure assessments, as the name implies, bracket the most plausible estimate of exposure (referred to statistically as the central or maximum likelihood estimate) with extreme lower and upper bounds of plausible exposures.

This Extreme Value approach is essentially an elaboration on the concept of the *Most Exposed Individual* (MEI), sometime referred to a the M*aximum Exposed Individual.* As this name also implies, exposure assessments that use the MEI approach are based on an attempt to characterize the extreme but still plausible upper limit on exposure. This is common exposure assessment approach that used by the U. S. EPA, other governmental agencies, as well as the International Commission on Radiological Protection (e.g., ATSDR 2002; ICRP 2005; Payne-Sturges et al. 2004). In the current risk assessment, the upper bounds on exposure are all based on the MEI.

In addition to this upper bound MEI value, the Extreme Value approach used in this risk assessment also provides a central estimate of exposure as well as a lower bound on exposure. While not germane to the assessment of upper bound risk, it is worth noting that the use of the central estimate and especially the lower bound estimate is not intended to lessen concern. To the contrary, the central and lower estimates of exposure are used to assess the feasibility of mitigation – e.g., protective measures to limit exposure. If lower bound exposure estimates exceed a level of concern (which is not the case in the current risk assessment), this is strong indication that the pesticide cannot be used in a manner that will lead to acceptable risk.

In considering very high use sites, such as the Yellowstone National Park and the Statue of Liberty National Monument, where large numbers of people may be exposed, a related concern involves exposures of sensitive individuals. This concern is considered in the dose-response assessment (Section 3.3) in which exposures are based on the most sensitive endpoint in the most sensitive species and an uncertainty factor for sensitive individuals is used. Atypical sensitivities – i.e., special conditions that could increase the sensitivity of an individual to a particular agent – are also considered separately in the risk characterization (Section 3.4.4).

Thus, the Extreme Value approach in the exposure assessment is part of an integrated approach that is designed to encompass plausible upper limits of risk for the most exposed and most sensitive individuals regardless of the specific probabilities or number of exposures. In the event that an extreme value risk assessment triggers concern, probabilistic methods can be employed that deal more explicitly with probabilities of exposure, numbers of individuals exposed as well as a number of other quantitative considerations (e.g., SERA

2007A, Section 1.2.2.1). As detailed further in Section 3.4, however, substantial or even identifiable risk is not evident in this assessment on aminopyralid.

3.2.3.1.1. Summary of Assessments

The two types of exposure scenarios developed for the general public include acute exposure and longer-term or chronic exposure. All of the acute exposure scenarios are primarily accidental. They assume that an individual is exposed to the compound either during or shortly after its application. Specific scenarios are developed for direct spray, dermal contact with contaminated vegetation, as well as the consumption of contaminated fruit, water, and fish. Most of these scenarios should be regarded as extreme, some to the point of limited plausibility. The longer-term or chronic exposure scenarios parallel the acute exposure scenarios for the consumption of contaminated fruit, water, and fish but are based on estimated levels of exposure for longer periods after application.

The exposure scenarios developed for the general public are summarized in Worksheet E03. As with the worker exposure scenarios, details of the assumptions and calculations involved in these exposure assessments are given in the worksheets that accompany this risk assessment (Worksheets D01–D11). The remainder of this section focuses on a qualitative description of the rationale for and quality of the data supporting each of the assessments.

3.2.3.2. Direct Spray

Direct sprays involving ground applications are modeled in a manner similar to accidental spills for workers (Section 3.2.2.2). In other words, it is assumed that the individual is sprayed with a solution containing the compound and that an amount of the compound remains on the skin and is absorbed by first-order kinetics. Two direct spray scenarios are given, one for a young child (D01a) and the other for a young woman (D01b).

For the young child, it is assumed that a naked child is sprayed directly during a ground broadcast application and that the child is completely covered (that is, 100% of the surface area of the body is exposed). This is and is intended to be extreme. As discussed in Section 3.2.3.1.1, the upper limits of this exposure scenario is intended to represent the *Extreme Value* upper limits of exposure for the *Most Exposed Individual* (MEI).

The exposure scenario involving the young woman (Worksheet D01b) is somewhat less extreme but more plausible. In this scenario, it is assumed that the woman is accidentally sprayed over the feet and lower legs. A young woman rather than an adult male is used in many of the exposure assessments. This preference again relates to concerns for both the *Most Exposed Individual* (MEI) as well as the most sensitive individual. As detailed in Section 3.1.9, reproductive effects are a major concern in this risk assessment as well as other risk assessments on pesticides. Consequently, exposures for a young woman of reproductive age are used in order to better assess the potential for adverse effects in the population at risk from potential reproductive effects – i.e., the most exposed and the most sensitive individual.

For this exposure scenario, assumptions are made regarding the surface area of the skin and the body weight of the individual, as detailed in Worksheet A03. The rationale for and

sources of the specific values used in these and other exposure scenarios is given in the documentation for the worksheets (SERA 2005).

3.2.3.3. Dermal Exposure from Contaminated Vegetation

In this exposure scenario, it is assumed that the herbicide is sprayed at a given application rate and that a young woman comes in contact with sprayed vegetation or other contaminated surfaces at some period after the spray operation (D02). For these exposure scenarios, some estimates of dislodgeable residue (a measure of the amount of the chemical that could be released from the vegetation) and the rate of transfer of the chemical from the contaminated vegetation to the surface of the skin must be available.

No data are available on dermal transfer rates for aminopyralid. This is not a severe limitation in this risk assessment. As detailed in Durkin et al. (1995), dermal transfer rates are reasonably consistent for a number of different pesticides and the methods and rates derived in Durkin et al. (1995) are used as defined in Worksheet D02. Similarly, no data are available on dislodgeable residues for aminopyralid. This is a somewhat greater source of uncertainty. Dislodgeable residue rates, however, are available on 2,4-D. As noted in Section 3.2.2.2, aminopyralid and 2,4-D are similar in chemical and physical properties and the dislodgeable residue for aminopyralid is based on data from a field simulation study measuring dermal exposures in humans after the application of 2,4-D (Harris and Solomon 1992).

The exposure scenario assumes a contact period of one hour and assumes that the chemical is not effectively removed by washing for 24 hours. Other estimates used in this exposure scenario involve estimates of body weight, skin surface area, and first-order dermal absorption rates, as discussed in the previous section.

3.2.3.4. Contaminated Water

Water can be contaminated from runoff, as a result of leaching from contaminated soil, from a direct spill, from unintentional direct spray from aerial applications, or drift from either ground or aerial applications. For this risk assessment, the three types of estimates are made for the concentration of aminopyralid in ambient water: an accidental spill (Section 3.2.3.4.1), unintended direct spray or drift (Section 3.2.3.4.2), as well as both acute and longer-term exposures in ponds and streams that could be associated with the application of this compound (Section 3.2.3.4.3).

3.2.3.4.1. Accidental Spill

Two exposure scenarios are presented for the acute consumption of contaminated water: an accidental spill into a small pond (0.25 acres in surface area and 1 meter deep) and the contamination of a small stream by runoff or percolation.

The accidental spill scenario assumes that a young child consumes contaminated water shortly after an accidental spill of 200 gallons of a field solution into a small pond. The specifics of this scenarios are given in Worksheet D05. Because this scenario is based on the assumption that exposure occurs shortly after the spill, no dissipation or degradation is considered. This scenario is dominated by arbitrary variability and the specific assumptions

used will generally overestimate exposure. The actual concentrations in the water would depend heavily on the amount of compound spilled, the size of the water body into which it is spilled, the time at which water consumption occurs relative to the time of the spill, and the amount of contaminated water that is consumed. Based on the spill scenario used in this risk assessment, the concentration of aminopyralid in a small pond is estimated to range from about 0.36 mg/L to 3.6 mg/L with a central estimate of about 0.7 mg/L (Worksheet D05).

3.2.3.4.2. Accidental Direct Spray/drift for a Pond or Stream

These scenarios are less severe but more plausible than the accidental spill scenario described above. The U.S. EPA typically uses a 2 meter deep pond to develop exposure assessments (SERA 2004). If such a pond is directly sprayed with aminopyralid at the central estimate of the application rate (0.078 lb a.e./acre), the peak concentration in the pond would be about 0.0044 mg/L, equivalent to 4.4 µg/L or 4.4 ppb (Worksheet D10a). This concentration is a factor of about 820 below the upper bound of the peak concentration of 3.6 mg/L after the accidental spill of a liquid formulation (Section 3.2.3.4.1, Worksheets D05). Worksheet D10a also models concentrations at distances of 25-900 feet down wind based on standard values adapted from AgDrift (SERA 2007A). Based on these estimates, aminopyralid concentrations in a small pond contaminated by drift would range from about 0.000004 mg/L (4 part per trillion) to 0.0006 mg/L (60 parts per trillion).

Similar calculations can be made for the direct spray of or drift into a stream. For this scenario, the resulting water concentrations depend on the surface area of the stream and the rate of water flow in the stream. The stream modeled using GLEAMS (see below) is about 6 feet wide (1.82 meters) and it is assumed that the pesticide is applied along a 1038 foot (316.38 meters) length of the stream with a flow rate of 710,000 L/day. Using these values, the concentration in stream water after a direct spray is estimated at about 0.0071 mg/L (7.1 parts per billion). Much lower concentrations, ranging from about 0.000006 mg/L (6 part per trillion) to 0.001 mg/L (1 part per billion) are estimated based on drift at distances of 25-900 feet (Worksheet D10b).

3.2.3.4.3. Standard GLEAMS Modeling

For compounds like aminopyralid, which may be applied over a large proportion of a watershed, drift and even direct spray are not the only and may not be the greatest source of contamination of surface water. Water contamination may also occur from soil runoff (the pesticide dissolved in runoff water), sediment (pesticide adsorbed to soil in runoff water), or percolation (pesticides leaching into subsurface water). Depending on local conditions, these losses can lead to substantial contamination of ponds or streams.

This section describes the relatively standardized modeling approach has been used in risk assessments of herbicides – e.g., 2,4-D (SERA 2006), clopyralid (SERA 2004c), picloram (SERA 2003a), and triclopyr (SERA 2003b). This is followed by subsections on GLEAMS modeling at specific locations (Section 3.2.3.4.5), other modeling efforts(Section 3.2.3.4.6), and monitoring data (Section 3.2.3.4.7). The standard application of the GLEAMS model and the use of the output from this model to estimate concentrations in ambient water are detailed in SERA (2004d). The application site was assumed to consist of a 10-hectare square area that drained directly into a small pond or stream. As detailed in SERA (2004d),

the standard GLEAMS modeling encompasses rainfall rates of 5 to 250 inches per year, assuming that the rainfall occurs uniformly on every tenth day, with the first rainfall event occurring on the day after pesticide application. This approach to the use of GLEAMS will be referred to as *standard GLEAMS modeling*. More realistic rainfall patterns are in the location-specific modeling in Section 3.2.3.4.5.

Modeling of aminopyralid concentrations in stream water conducted for this risk assessment are based on GLEAMS (Groundwater Loading Effects of Agricultural Management Systems) modeling. GLEAMS is a root zone model that can be used to examine the fate of chemicals in various types of soils under different meteorological and hydrogeological conditions (Knisel and Davis 2000). As with many environmental fate and transport models, the input and output files for GLEAMS can be complex.

Both the standard GLEAMS modeling discussed in this section as well as the location-specific modeling in Section 3.2.3.4.5 are based on a common set of assumptions that are intended to be generally conservative. As detailed in SERA (2004d), all model runs are conducted at an application rate of 1 lb a.e./acre. This is done simply because GLEAMS outputs information in a fixed decimal format. This can result in the loss of information if the model is run at low application rates. Because pesticide losses in runoff, sediment, and percolation are all linearly related to application rate, the expected concentrations in water and soil based on the application rates that will be used in Forest Service and NPS programs can be calculated simply as the value from the GLEAMS modeling at 1 lb/acre multiplied by the application rate that will actually be used.

The standard GLEAMS modeling as well as the location-specific modeling (Section 3.2.3.4.5) are conducted for three types of soils: clay, loam, and sand. For clay, site conditions are assumed to favor runoff. For sand, site conditions are assumed to favor percolation. For loam, moderate assumptions are used in the modeling in terms of surface conditions. For all model runs, buffers are not considered – i.e., the applications are assumed to occur up to the edge of the water. A full description of the generic approach to GLEAMS modeling is given in SERA (2004d).

The chemical specific values as well as the details of the pond and stream scenarios used in the GLEAMS modeling are summarized in Table 4. For the most part, the chemical specific input values used in the GLEAMS modeling are similar to those used by the U.S. EPA (U.S. EPA/OPP-HED 2004; U.S. EPA/OPP-EFED 2004) as well as by Dow AgroSciences (Jachetta et al. et al. 2004). These other modeling efforts are discussed below (Section 3.2.3.4.4). The modeling input values are based on the environmental fate studies submitted to the U.S. EPA (Appendix 1) and the specific sources of information used in the GLEAMS modeling are given in the notes to Table 4.

The only exception to the similarity with the values used by the U.S. EPA and Dow AgroSciences involves water solubility. Both the U.S. EPA (U.S. EPA/OPP-EFED 2004, p. 23) and Dow AgroSciences (Jachetta et al. 2004, p. 81) use a water solubility of 2480 mg/L. As summarized in Appendix 1, this value for water solubility is taken from the submission by Ghaoui (2003, MRID 46235703) which includes the original study on water solubility which

was conducted by Nelson (2002, Study ID: FORO1015). This study conducted two different types of water solubility determinations, one in buffer solutions with nominal pH value of 5, 7, and 9 and the other in unbuffered water. As summarized in Appendix 1, the water solubility values in unbuffered water was 2.48 g/L or 2480 mg/L. The water solubility determinations in buffered solutions were about a factor of 100 higher: 212 g/L at pH 5, 205 g/L at pH 7, and 203 g/L at pH 9. Note that the order of the water solubility values for the buffered solutions is not intuitive. In general, the solubility of weak acids in water will decrease with decreasing pH (increasing acidity) – e.g., 2,4-D (SERA 2006), triclopyr (SERA 2003b) – because the protonated form of the weak acid is less soluble in water than the ionized form. The reason for the pattern seen with the buffer solutions is not discussed in the Nelson (2002) study. In any event, the relatively low solubility of aminopyralid in unbuffered water is probably the result of acidification of the water by aminopyralid. As illustrated in the discussion of this study in Appendix 1, the pH of a 2.48 g/L solution of compound with a pK_a of 2.56 will be about 2.33 – i.e., over half of the compound will be protonated. Thus, for the GLEAMS modeling, the water solubility at pH 7 was used – i.e., 205,000 mg/L.

Estimates of runoff, sediment, and percolation concentrations in a stream adjacent to a treated plot were determined by running the GLEAMS model, as discussed in Section 6.4 of SERA (2004d). The results of the GLEAMS modeling for the small stream are summarized in Table 5 and the corresponding values for the small pond are summarized in Table 6. These estimates are expressed both as average and peak concentrations in water. All of these GLEAMS runs were conducted at an application rate of 1 lb a.e./acre, the values given in Tables 5 and 6 are expressed as water contamination rates (WCR) – i.e., the concentration of the compound in water in units of ppb (µg/L) normalized for an application rate of 1 lb a.e./acre. In the worksheets that accompany this risk assessment, the WCR values are multiplied by the application rate to estimate concentrations in surface water.

Surface water contamination is not estimated for very arid regions – i.e., annual rainfall of 10 inches or less. It should be noted, however, that this result may be an artifact of the way the GLEAMS modeling is conducted. As noted above, the generic GLEAMS modeling is based on a rainfall pattern in which rainfall occurs every 10^{th} day and the amount of rainfall is uniform each day. Thus, for an annual rainfall of 10 inches per year, the amount of rainfall in each event is about 0.25 inches – i.e., 10 inches per year divided by 37 rainfall events per year.

At higher rainfall rates and the application rate of 1 lb a.e./acre, the modeled peak concentrations in streams range from about 22.6 ppb (loam at an annual rainfall rate of 15 inches) to about 240 ppb (sand at an annual rainfall rate of 50 inches) (Table 5). In Table 5, modeled concentrations in streams decrease at annual rainfall rates above 50 inches. This pattern of decreasing concentrations with increasing rainfall is not uncommon and indicates that virtually all of the aminopyralid could be transported with the stream via percolation or runoff at relatively low rainfall rates. With increasing rainfall, the concentrations decrease because of dilution. Average concentrations in the stream are modeled at about 0.33 ppb (clay at 15 inches per year) to 18.7 ppb (clay at 200-250 inches per year).

Modeled peak concentrations in a small pond (Table 6) are only somewhat lower than those modeled in the stream. As with the stream modeling, no surface water contamination is expected in very arid regions. For regions with annual rainfall rates of 15 inches or more, the modeled peak concentrations in ponds at an application rate of 1 lb a.e./acre range from about 6 ppb (clay at an annual rainfall rate of 15 inches) to about 180 ppb (sand at an annual rainfall rate of 15 inches). Average concentrations in the pond are estimated at about 3.3 ppb (loam at 15 inches per year) to 116 ppb (sand at 20 inches per year), substantially higher than those modeled for the stream.

3.2.3.4.4. GLEAMS Modeling At Specific Sites

The standard GLEAMS modeling discussed in the previous section has been used in many past pesticide risk assessments and incorporates a number of conservative assumptions (SERA 2004d). Nonetheless, a limitation in the standard approach to using GLEAMS to model concentrations in ambient water involves the assumption that rainfall is evenly distributed over an every 10th day interval. To address this limitation and to more generally facilitate site-specific assessments of pesticide applications, the Forest Service has developed Gleams-Driver, a computer program that serves as a preprocessor and postprocessor for GLEAMS (SERA 2006b). One feature of Gleams-Driver involves a utility for importing weather files from Cligen, a climate generator program that was developed and is maintained by the USDA Agricultural Research Service (http://horizon.nserl.purdue.edu/Cligen).

While the utility of Gleams-Driver for site-specific exposure assessments is still under evaluation, Gleams-Driver offers the option of conducting general exposure assessments identical to those described in the previous section but using site-specific weather files from Cligen rather than the every 10th rainfall files. To explore the potential impact of more realistic rainfall patterns on the estimates of the concentrations of aminopyralid in surface water, Gleams-Driver was used to model concentrations in a small stream and small pond using the same parameters specified in Table 4 as well as the characteristics of small stream and small pond that are used in the standard GLEAMS modeling (SERA 2004d).

The locations selected for modeling included a total of 12 sites as illustrated in Figure 6. As detailed in SERA (2006b), nine of the sites are standard test sites for Gleams-Driver that are intended to represent combinations of precipitation (dry, average, and wet) and temperature (hot, temperate, and cool). These standard test sites are designated by diamonds (♦) in Figure 6. The other three sites represent the locations of specific National Parks where aminopyralid is likely to be applied. These sites are represented by rectangles (▬) in Figure 6. For each site, Gleams-Driver was used to simulate 100 applications of aminopyralid at a unit application rate of 1 lb/acre to clay, loam, and sand soils and each of the simulations was followed over a 1½ year period after application.

The results of the Gleams-Driver simulations are given in Table 7 (peak concentrations) and Table 8 (one-year average concentrations) for a small stream and Table 9 (peak concentrations) and Table 10 (one-year average concentrations) for a small pond. As discussed in SERA (2007b), all values are expressed as the midpoint (median) with 95% empirical confidence intervals.

For the small stream, the peak concentrations based on Gleams-Driver simulations (Table 7) are similar to those based on standard GLEAMS modeling (Table 5). In arid regions, the lower ranges of estimated concentrations are zero or very close to zero and the central estimates of peak concentrations do not exceed 0.14 ppb. In areas with average to high rainfall rates, the maximum concentration in streams is about 140 ppb (Table 7), somewhat lower than the 240 ppb concentration based on standard GLEAMS modeling using an every 10^{th} day rainfall pattern. Typical peak concentrations in streams are about 30 ppb for regions with average rainfall and clay or loam soils. In regions with sandy soils, the typical peak concentrations in streams are somewhat higher, about 100 ppb.

The differences in average concentrations of aminopyralid in a small stream based on standard GLEAMS modeling (Table 5) and the Gleams-Driver simulations (Table 8) are similar to those based on peak exposures. The maximum average concentration in a small stream based on standard GLEAMS modeling is 18.7 ppb and the corresponding maximum from the Gleams-Driver simulations is 9.2 ppb.

For a small pond, the peak concentrations from Gleams-Driver (Table 9) are higher than those from standard GLEAMS modeling by a factor of about 3.3 (600 ppb vs 182 ppb). Based on central estimates of the peak concentrations from Gleams-Driver, the differences are relatively small – i.e., a factor of about 2.2 (400 ppb vs 182 ppb). Similar differences are evident between standard GLEAMS modeling (Table 6) and Gleams-Driver simulations (Table 10) based on average concentrations in a small pond – i.e., about a factor of 2.2 based on upper bounds from Gleams-Driver (260 ppb vs 116 ppb) and a factor of 1.7 based on central estimates from Gleams-Driver (200 ppb vs 116 ppb).

The differences between the standard GLEAMS modeling and the Gleams-Driver may be impacted by differences in rainfall patterns. In addition, it should be noted that the algorithms for evaporation are different. The standard GLEAMS modeling of a small pond uses a simple form of Penman's equation (Section 6.3 in SERA 2004d) while Gleams-Driver uses a more elaborate modification of the Penman equation more suited to use with Cligen weather simulations (Section 7.4 in SERA 2007b).

3.2.3.45. Other Modeling Efforts

A summary of the GLEAMS modeling discussed above as well as modeling of aminopyralid presented by the U.S. EPA/OPP (U.S. EPA/OPP-HED 2004; U.S. EPA/OPP-EFED 2004) and Dow AgroSciences (Jachetta et al. 2004) is given in Table 11. Table 11 includes a summary of both the standard GLEAMS modeling (Section 3.2.3.4.3) as well as the location specific modeling conducted with Gleams-Driver (Section 3.2.3.4.4). Because the location specific Gleams-Driver modeling involves Monte Carlo analysis whereas all of the other modeling is based on point estimates, the results of the Gleams-Driver modeling is not directly comparable to the other modeling efforts. Thus, the focus of this section is on a comparison of the standard GLEAMS modeling to the modeling conducted by the U.S. EPA/OPP and Dow AgroSciences. The Gleams-Driver simulations are discussed further in the selection of water contamination rate (WCR) values used in the current risk assessment (Section 3.2.3.4.7).

In the human health risk assessment of aminopyralid, U.S. EPA/OPP (U.S. EPA/OPP-HED 2004) used two water contamination models: PRZM/EXAMS and SCI-GROW. As discussed in SERA (2007a), PRZM/EXAMS is a model, or more accurately a system of linked models, that the U.S. EPA uses to assess plausible concentrations of pesticides in water after agricultural applications. Different types of PRZM/EXAMS scenarios can be conducted and the modeling summarized in Table 11 involved the use of an index reservoir (i.e., a standard reservoir) that is commonly used by the U.S. EPA/OPP. SCI-GROW is a Tier 1 screening model developed by the U.S. EPA to provide estimates of concentrations of a compound in groundwater based on a given application rate, number of applications, the interval between applications, and standard environmental fate parameters for a specific compound.

The U.S. EPA/OPP modeled concentrations of aminopyralid in water at the maximum labeled rate of 0.11 lb a.e./acre (U.S. EPA/OPP-HED 2004, Table 6.2, p. 36). In Table 11 of the current risk assessment, the reported concentrations are normalized to 1 lb a.e./acre by dividing the concentration reported by the U.S. EPA by the modeled concentration of 0.11 lb a.e./acre. The estimate of the peak concentration from PRZM/EXAMS is 91 ppb at an application rate of 1 lb a.e./acre. This peak concentration is only about a factor of 2 below the peak concentration based on standard GLEAMS modeling – i.e., 180 ppb vs 91 ppb. In comparisons of PRZM/EXAMS modeling conducted by the U.S. EPA to GLEAMS modeling conducted in this series of risk assessments, higher estimates are typically found using the standard GLEAMS modeling because of the conservative assumptions built into the standard GLEAMS modeling (SERA 2004c) – i.e., rainfall rates up to 250 inches/year with rainfall occurring on every 10^{th} day. This is another example of the attempt to assess exposures to the Most Exposed Individual, as discussed in Section 3.2.3.1.1.

A similar pattern is seen in the estimates of longer-term averages from the standard GLEAMS modeling in which the upper limit from the GLEAMS modeling is about a factor of 7 higher than the reported value from PRZM/EXAMS, again reflecting the extreme value approach taken in the exposure assessment. It should be noted, however, that the geometric mean of the range of values modeled using GLEAMS is about 23 ppb $[(4.9 \times 116)^{0.5}]$, very close to the value of 17.6 ppb from the PRZM/EXAMS modeling. As noted in SERA (2004d), PRZM and GLEAMS, both of which are root zone models, tend to yield comparable results when similar input values are used. For the GLEAMS modeling, the input parameters associated with central estimates of exposure (e.g., more typical rainfall rates) are more closely related to the implementation of PRZM/EXAMS used in the modeling by the U.S. EPA.

As also summarized in Table 11, the ecological risk assessment conducted by the U.S. EPA (U.S. EPA/OPP-EFED 2004) used GENEEC. As discussed in SERA (2004c), GENEEC simulates a farm pond identical to the pond scenario used with GLEAMS – i.e., a 1 ha pond that is 2 meters deep and fed by a 10 ha drainage area. GENEEC simulates runoff and drift as well as the standard degradation processes used in the GLEAMS modeling as well as many of the processes used in PRZM/EXAMS modeling. Specifically, GENEEC is designed to provide upper range estimates that would be obtained from using the PRZM/EXAMS approach with uniformly conservative assumptions. For aminopyralid, the peak

concentration modeled with GENEEC give a somewhat lower value than the corresponding value from the PRZM/EXAM run (58 ppb vs 91 ppb) or the GLEAMS run (58 ppb vs a maximum of 190). The longer-term average concentration from GENEEC is somewhat higher than the PRZM/EXAMS run (49 ppb vs 17.6 ppb) but about a factor of 2 below the upper range of the longer-term concentration from the standard GLEAMS runs (49 ppb vs 116 ppb).

The estimated peak concentration of aminopyralid in groundwater based on SCI-GROW (U.S. EPA/OPP-HED 2004) is very close to the lower bound of the range of concentrations modeled for a pond with standard GLEAMS modeling (5.7 ppb vs 6 ppb). This similarity is probably serendipitous. As discussed in SERA (2004b), SCI-GROW is a Tier 1 model designed specifically to estimate concentrations in ground water – i.e., wells that might be used as a source of drinking water by humans or livestock. As with GENEEC, SCI-GROW considers the application rate and a number of specific environmental fate properties – i.e., aerobic soil degradation and adsorption coefficient normalized for organic carbon in soil, and estimates ground water contamination for sites with sandy soils and shallow ground water. While SCI-GROW is a conservative model, it is common for estimates of concentrations in ground water from SCI-GROW to be substantially less than estimates from other models for surface waters, reflecting differences in the processes of surface water and ground water contamination.

Jachetta et al. (2004) also used both GENEEC and SCI-GROW to model concentrations of aminopyralid in surface and ground water. As summarized in Table 11, the estimates from Jachetta et al. (2004) are comparable to those from U.S. EPA/OPP. The only substantial difference between the modeling done by Jachetta et al. (2004) and the modeling done by the U.S. EPA/OPP involves the soil halftime. In doing SCI-GROW modeling, Jachetta et al. (2004) used two soil halftimes, 88.7 days and 30 days. The value of 88.7 days is taken as the mean of soil halftimes reported by Yoder and Smith (2002), which ranged from 5 days to 343 days (Appendix 1). The field halftime of 30 days appears to be taken from the field studies summarized in Appendix 2.

All of the modeling summarized in Table 11 is very sensitive to soil halftimes. The halftimes used by Jachetta et al. (2004) are substantially shorter than those used by the U.S. EPA or those used in the current risk assessment. As noted in Appendix 1, the 5 day halftime reported by Yoder and Smith (2002) was considered invalid in the review by U.S. EPA/OPP-HED (2004). In terms of using "field halftime" from Appendix 2, this is not appropriate for models such as PRZM, GLEAMS, or GENEEC because these models expect a soil degradation rate. Soil halftimes from field studies reflect both degradation and dissipation. Because the models considered in this section handle dissipation as a separate process, only degradation halftimes should be used.

In the GLEAMS modeling, this risk assessment uses the longest halftime reported in Yoder and Smith 2002, MRID 46235729 (Appendix 1) – i.e., 343 day. In GENEEC modeling, the U.S. EPA/OPP-EFED (2005) used a somewhat shorter soil half-time of 310.5 days. This is based on a reanalysis by EFED of the halftime in silt loam (Holdrege) soil from the study by Yoder and Smith (2002). EFED recalculated the halftime for this soil based on the

assumption that non-extractable residues consisted of non-extracted parent compound. Based on this assumption, EFED calculated a halftime of 103.5 days. EFED multiplied this halftime by 3 to account because EFED considered the 103.5 day value to be the only acceptable value. Other halftimes calculated by EFED ranged up to 533.2 days but these values were not considered valid by EFED because of variability and fluctuation in material balances in the Yoder and Smith (2002) study (EPA DER for MRID 46235729).

3.2.3.4.6. Monitoring Data

No surface water monitoring data are available on aminopyralid that could be used to assess the plausibly of the modeling discussed in the previous subsections. This is a limitation in this risk assessment and a source of uncertainty. As discussed in Section 1, the lack of monitoring data reflects the fact that aminopyralid is a relatively new herbicide.

As discussed in Section 4.2.3.3, field studies are available on concentration of aminopyralid in soil (Roberts and Schelle 2004a,b). GLEAMS simulations of one of the sites are consistent with the soil concentration data reported by Roberts and Schelle (2004a,b).

3.2.3.4.7. Concentrations in Water Used for Risk Assessment

Table 12 summarizes the concentrations of aminopyralid in water used for the current risk assessment. The upper part of this table gives the concentrations expected at the nominal application rate of 0.078 lb a.e./acre, in units of micrograms per liter or ppb. The lower part of this table gives the water contamination rates, the concentrations in water expected at a normalized application rate of 1 lb a.e./acre, converted to units of ppm or mg/L per lb a.e./acre. The conversion from ppb to ppm is made because these latter units – i.e., ppm or mg/L – are used in the EXCEL workbook in the various exposure scenarios involving contaminated water in both the human health and ecological risk assessments. The water contamination rates are entered in Worksheet B04 and links to these values are used in scenario specific worksheets in the EXCEL workbook.

The upper range of the expected peak WCR of aminopyralid in surface water is taken as 0.6 ppm per lb a.e./acre. This estimate is based on peak aminopyralid concentrations in ponds modeled in Gleams-Driver simulations as summarized in Table 11 and detailed in Table 9. The value of 0.6 ppm is the upper bound of concentrations modeled in ponds in areas with average rainfall, warm temperature, and predominantly sandy soils. As indicated in Table 11, this estimate is somewhat higher than the peak concentrations in streams or ponds based on standard GLEAMS modeling. As also noted in Table 11, this upper bound of the peak water contamination rate is likely to encompass accidental exposures, such as direct spray and drift. In other words, while accidental direct spray or inadvertent contamination due to drift might be considered an extreme or at least atypical exposure, higher concentrations in water could be associated with normal use of aminopyralid in some areas.

For the lower bound of the peak WCR, an argument may be made that concentrations of aminopyralid are likely to be essentially zero – i.e., applications at sites that are distant from open bodies of water and in areas in which runoff or percolation are not likely to occur. For this risk assessment, the lower range of the peak water contamination rate will be set at 2 ppb

or 0.002 ppm per lb/acre. This is in the lower range of non-zero concentrations modeled in streams at an annual rainfall rates of 150 to 250 inches in regions with predominantly sandy soils.

The central estimate for the peak WCR is set at 100 ppb or 0.1 ppm per lb a.e./acre. This central estimate is comparable to the central estimates of peak concentration modeled in a small pond using Gleams-Driver in regions with average rainfall and clay or loam soils (Tables 9) and is also very close to the peak concentration of 91 ppb or 0.091 ppm per lb/acre estimated by the U.S. EPA/OPP based on the Index Reservoir using PRZM/EXAMS (U.S. EPA/OPP-HED 2004; U.S. EPA/OPP-EFED 2004).

The water contamination rates for longer-term exposures are derived in a similar manner. At an application rate of 1 lb/acre, the highest longer-term concentration is taken as 260 ppb or 0.260 ppm per lb a.e./acre. This is the maximum longer-term concentration in ponds modeled using Gleams-Driver simulations (Table 10, sand, average rainfall in warm or temperate regions). The central estimate of the longer-term water contamination rate is taken as 40 ppb or 0.04 ppm per lb a.e./acre, which is near the average longer-term concentration modeled in ponds modeled using Gleams-Driver. This central estimate is very close to the longer-term average concentration modeled by U.S. EPA – i.e., 49 ppb per lb a.e./acre, as summarized in Table 11 of this risk assessment.

As with the lower bound estimates of peak concentrations, the lower bound of the longer-term concentration could be taken as zero. For the current risk assessment, the lower bound is taken as 1 ppb or 0.001 ppm per lb a.e./acre, which coincides approximately with the longer-term concentrations of aminopyralid ponds modeled using Gleams-Driver in arid areas (Table 10).

The judgmental and to some degree arbitrary nature of the selected water contamination rates and the assumptions used to derive these rates should be apparent and appreciated. GLEAMS as well as PRZM/EXAMS are highly parameterized models that are intended for site-specific exposure assessments. The generic applications of GLEAMS and Gleams-Driver in this current risk assessment are intended only to provide general estimates of plausible exposures in order to identify which exposure scenarios might present the greatest risk under a wide-ranging set of conditions and some very conservative assumptions. This is discussed further in the risk characterization (Section 3.4).

3.2.3.5. Oral Exposure from Contaminated Fish

Oral exposures associated with the consumption of contaminated fish are essentially identical to similar exposure scenarios used for other herbicides – e.g., 2,4-D (SERA 2006), clopyralid (SERA 2004c), picloram (SERA 2003a), and triclopyr (SERA 2003b). Two sets of exposure scenarios are presented: one set for acute exposures following an accidental spill (Worksheets D08a and D08b) and the other for chronic exposures based estimates of longer-term concentrations in water (Worksheets D09a and D09b). The two worksheets for each duration are intended to account for rates of caught consumption in the general population as well as subsistence populations. Details of this exposure scenario are provided in Section 3.2.3.5 of SERA (2007).

In addition to estimates of peak and longer-term term concentrations of the chemical in water, this exposure scenario requires information on the bioconcentration factor (BCF). The U.S. EPA has waived the requirement for a bioconcentration study in fish because the low octanol-water partition coefficient for aminopyralid (Table 2) suggest that aminopyralid will not bioconcentrate in fish. Consequently, for the contaminated fish scenarios used in this risk assessment, the assumption is made that bioconcentration will not occur and that the concentration in fish will be equivalent to the concentration in water (BCF = 1).

3.2.3.6. Dermal Exposure from Swimming in Contaminated Water

Some of the sites maintained by the Forest Service and NPS will contain surface water that is intended for or could be used for swimming by members of the general public. To assess potential risks associated with swimming, an exposure assessment is developed for a young woman swimming in surface water for one hour (Worksheet D11).

Conceptually and computationally, this exposure scenario is virtually identical to the contaminated gloves scenario used for workers (Section 3.2.2.2) – i.e., a portion of the body is immersed in an aqueous solution of the compound at a fixed concentration for a fixed period of time. The major differences in the two scenarios involve the concentration in water and the surface area of the body that is exposed. For the worker wearing contaminated gloves, the assumption is made that both hands are exposed to the field solution – i.e., the concentration of the compound in the solution that is being applied. For the swimmer, the assumption is made that the entire body surface area is exposed to the expected peak concentrations in ambient water (Table 12). While the swimmer will not be immersed for one hour, the entire body surface is used both as a conservative approximation (i.e., the MEI) and to consider intermittent episodes during which the whole body might be immersed or at least wet.

As with the corresponding worker exposure scenario, the one-hour period of exposure is somewhat arbitrary and longer periods of exposure are plausible. The one-hour period, however, is not completely arbitrary but is intended as a unit exposure estimate. In other words, the exposure and consequently the risk will increase linearly with the duration of exposure as indicated in Worksheet D11. Thus, a two hour exposure would lead to a hazard quotient that is twice as high as that associated with an exposure period of one hour. In cases in which this or other similar exposures approach a level of concern, further consideration is given to the duration of exposure in the risk characterization (Section 3.4).

3.2.3.7. Oral Exposure from Contaminated Vegetation

Although none of the Forest Service or NPS applications of aminopyralid will involve crop treatment, this series of risk assessments typically include standard exposure scenarios for the acute and longer-term consumption of contaminated vegetation. Two sets of exposure scenarios are provided: one for the consumption of contaminated fruit and the other for the consumption of contaminated vegetation. These scenarios are detailed in Worksheets D03a and D03b for acute exposure and Worksheets D04a and D04b for chronic exposure.

The concentration of the pesticide on contaminated fruit and vegetation is estimated using the empirical relationships between application rate and concentration on different types of vegetation (Fletcher et al. 1994). While human health risk assessment conducted by the U.S. EPA/OPP (U.S. EPA/OPP-HED 2004) does not consider this exposure scenario, the use of the residue rates recommended by Fletcher et al. (1994) both here and in the ecological risk assessment (Section 4.2) is identical to the approach used by U.S. EPA/OPP in their ecological risk assessment of aminopyralid (U.S. EPA/OPP-EFED 2004).

For chronic exposures, both initial concentrations and a halftime on vegetation are required to estimate the time-weighted average exposure (Worksheet D04a and D04b). These worksheets accommodate a central estimate as well as lower and upper bounds on the halftime. These are calculated from the halftimes reported in vegetation residue studies by Roberts et al. (2004) and McCormick et al. (2004) – i.e., a central estimate of 13.4 days with a 95% confidence interval of 10.5 days to 16.3 days. As noted in Appendix 6, the halftimes reported by Roberts et al. (2004) and McCormick et al. (2004) based on some data sets that are that are consistent with first-order dissipation and other data sets that poorly fit the first-order dissipation model. The most likely reason for the lack of fit is the intermittent nature of rainfall during some of the field studies by Roberts et al. (2004) and McCormick et al. (2004).

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

The Office of Pesticide Programs of the U.S. EPA has derived a chronic RfD of 0.5 mg/kg/day for aminopyralid. This RfD is based on a chronic rat NOAEL of 50 mg/kg/day. The Office of Pesticide Programs has also derived an acute RfD of 1 mg/kg bw/day based on a NOAEL from a reproduction study of 100 mg/kg/day. In deriving both of these RfD values, the U.S. EPA used an uncertainty factor of 100, a factor of 10 for extrapolating from animals to humans and a factor of 10 for extrapolating to sensitive individuals within the human population. Both of these RfD values are based on NOAELs for the most sensitive endpoint in the most sensitive species and studies in which LOAEL values were identified. In addition, both of the NOAEL values are supported by other studies. Thus, the RfD values recommended by the U.S. EPA are adopted directly in the current risk assessment.

3.3.2. Chronic RfD

The human health risk assessment prepared by the U.S. EPA (U.S. EPA/OPP-HED 2005) proposes a chronic RfD of 0.5 mg a.e./kg bw/day for the general population. This chronic RfD is based on a NOAEL of 50 mg a.e./kg/day from the 24-month feeding study in rats (Johnson and Dryzga 2004). As detailed in Appendix 3, this study involved dietary exposures equivalent to doses of 0, 5, 50, 500, 1000 mg a.e./kg bw/day over a 2-year period. No effects were observed in either of the two lower dose groups. At 500 mg a.e./kg bw/day, effects included a slight decrease in body weight with a slight increase in food consumption in male rats, a substantial increase in cecal weights – i.e., factors of 1.8 in males and 1.3 in females – as well as changes in urine chemistry – i.e., increases in urine volume and decreases in urine specific gravity, urinary protein, and urinary ketones. While these effects were used to classify the 500 mg a.e./kg bw/day exposure as a LOAEL, these effects not severe or substantial. As discussed in Section 3.1.2.1, the most substantial effect, cecal enlargement, may have very little relevance to potential effects in humans. The urinary effects may be attributable to exposure but these effects were not accompanied by any changes in kidney pathology and the decreases on urinary protein and ketones are not the types of effects that are typically associated with kidney damage.

The RfD of 0.5 mg a.e./kg/day was derived by dividing the NOAEL of 50 mg a.e./kg bw/day by an uncertainty factor of 100. This uncertainty factor consists of two components: a factor of 10 for extrapolating from animals to humans and a factor of 10 for extrapolating to sensitive individuals within the human population. Using the same conversion factor, the 500 mg a.e./kg bw/day dose corresponds to an estimated functional human LOAEL of 5 mg a.e./kg/day. At this functional LOAEL, moderately adverse effects might be anticipated.

The NOAEL of 50 mg a.e./kg bw/day in rats used by the U.S. EPA to derive the chronic RfD is supported by the chronic NOAEL of 50 mg a.e./kg bw/day in mice (Stebbins and Day 2003b) as well as the chronic NOAEL in dogs of about 100 mg a.e./kg bw/day (Stebbins and Day 2003a). Thus, the U.S. EPA has selected the most sensitive endpoint for the most

sensitive species and the chronic RfD developed by the U.S. EPA will be used directly in the current risk assessment.

The risks associated with longer-term exposures between the RfD of 0.5 mg a.e./kg bw/day and the functional LOAEL of 5 mg a.e./kg/day cannot be characterized. While this range of indeterminate dose is relatively large – i.e., a factor of 10 – this has no impact on the current risk assessment. As discussed further in Section 3.4.2., all of the estimated longer-term exposures to aminopyralid are substantially below the chronic RfD.

3.3.3. Acute RfD

For incidental (short-term and intermediate exposures), the U.S. EPA has proposed and RfD of 1.0 mg a.e./kg bw/day or incident. This RfD is based on the developmental study in rabbits by Carney and Tornesi (2004b) in which groups of 26 time-mated females per group were administered gavage doses of GF 871, the TIPA formulation that is equivalent to the Milestone formulations that are considered in this risk assessment (see Table 1). As detailed in Appendix 3, the rabbits were dosed at rates of 0, 484, 1211, and 2421 mg formulation/kg bw/day which is equivalent to doses of 0, 104, 260, 520 mg a.e./kg bw/day from days 7 to 21 of gestation. At the highest dose, effects included decreased maternal food consumption and body weight as well as a spontaneous abortion in 1/26 female rats. In addition, three adult females were euthanized due to extreme weight loss. Effects on the fetuses were limited to decreased fetal weight. At the dose of 260 mg a.e./kg bw/day, no fetal effects were noted but effects in the adult female rats included severe weight loss in 1/26 animals and incoordination in two other animals, one of which was not pregnant. No adverse effects that could be associated with treatment were noted the dose of 104 mg a.e./kg bw/day and this dose was accepted by the U.S. EPA as a NOAEL.

The selection of 104 mg a.e./kg bw/day as a NOEAL appears to be appropriate. As noted in Appendix 3, a case could be made for selecting the dose of 260 mg a.e./kg bw/day as a NOEAL because effects were noted in 3/26 animals compared to 0/26 in the control group. This difference is not significant using the Fisher Exact test (p-value of 0.117647). Using the higher NOAEL value, however, would not properly consider the biological significance of the effects or the dose/response relationship.

The dose response pattern for incoordination – i.e., 0/26, 1/26, 2/26, and 19/26 at 0, 104, 260, 520 mg a.e./kg bw/day – also supports the NOAEL of 104 mg a.e./kg bw/day. Using a standard benchmark response rate of 10% as a functional NOAEL, the lower limit on the dose is 184.88 mg a.e./kg bw/day. Thus, the NOAEL of 104 mg a.e./kg bw/day recommended by the study authors and confirmed by EFED seems reasonable -- i.e., it is somewhat below the lower limit using the benchmark dose approach. Lower benchmarks could, of course be used. Based on a benchmark dose of 0.01, the central estimate of the ED_{01} (the dose associated with a 1% response) is 93.83 mg a.e./kg bw/day and the lower limit on the dose is 40.48 mg a.e./kg bw/day. In general, however, benchmark doses are intended to represent estimates of response rates that incorporate the full dose-response relationship but as still within the observable range – i.e., interpolation rather than extrapolation. Thus, the default benchmark of 0.1 recommended by the U.S. EPA (U.S. EPA/ORD 2001) is an appropriate response rate for comparison to the empirical NOAEL.

The NOAEL of 104 mg a.e./kg bw/day is support by several other *Acceptable* developmental studies in rabbits (Marty et al. 2002; Liberacki et al. 2001b) and rats (Carney and Tornesi 2001; Bjorn 2003; Tornesi et al. 2001) in that all of these studies report NOAELs of greater than 104 mg a.e./kg bw/day and none of these studies report any effects at or below 104 mg a.e./kg bw/day.

Only one study, Carney and Tornesi (2004c), suggests a potential effect at a dose lower than 104 mg a.e./kg bw/day. As noted in Appendix 3, Carney and Tornesi (2004c) did note transient incoordination in 3 of 52 rabbits on 3 of 20 days during a developmental study with aminopyralid-TIPA at a dose of 150 mg a.i./kg bw/day, equivalent to 78 mg a.e./kg bw/day. This study is not addressed explicitly in the U.S. EPA human health risk assessment (U.S. EPA/OPP-HED 2005). While this is a concern, the incoordination noted by Carney and Tornesi (2004c) does not appear to be severe and the effect was reversible after several hours. Because this effect was noted only in the same three animals and only on 3 of the 20 days of treatment, it is not clear that this effect was attributable to aminopyralid. Given the other supportive studies in rabbits and rats, as cited above, the Carney and Tornesi (2004c) study does not provide a sufficient basis for deriving a lower acute RfD. Following standard practice in Forest Service risk assessments, the acute RfD of 1 mg a.e/kg bw/day derived by the U.S. EPA will be adopted in this risk assessment.

As with the chronic RfD (Section 3.3.2), the acute RfD of 1 mg a.e/kg bw/day or incident is derived by dividing the acute NOAEL of 100 mg/kg bw/day by an uncertainty factor of 100, the rational for which is the same as in the chronic RfD. Taking the acute dose of 260 mg a.e./kg bw/day as a minimal acute LOAEL and using the uncertainty factor of 100, the functional human acute LOAEL is estimated at 2.6 mg/kg/day. At this functional LOAEL, moderately adverse effects might be anticipated in human exposures.

There is a much narrower range between the acute RfD and the functional human acute LOAEL than for the corresponding chronic values. This reflects the narrow dose spacing used in the study by Carney and Tornesi (2004b). As with the corresponding chronic estimates, however, this positioning has little impact on the risk characterization because none of the acute exposure exceed either the acute or chronic RfD values.

3.4. RISK CHARACTERIZATION

3.4.1. Overview

The risk characterization for both workers and members of the general public is reasonably simple and unambiguous: based on a generally conservative and protective set of assumptions regarding both the toxicity of aminopyralid and potential exposures to aminopyralid, there is no basis for suggesting that adverse effects are likely in either workers or members of the general public even at the maximum application rate that might be used in Forest Service or NPS programs.

For workers, no exposure scenarios, acute or chronic, exceeds the RfD at the upper bound of the estimated dose associated with the highest application rate of 0.11 lb a.e./acre. The hazard quotients for directed ground spray, broadcast ground spray, and aerial applications are below the level of concern by factors of 33 to 200 over the range of application rates considered in this risk assessment.

For members of the general public, upper bounds of hazard quotients at the highest application rate are below a level of concern by factors of 100 to 125,000 for longer term exposures. For one accidental exposure scenario, the consumption of contaminated water by a child immediately after an accidental spill of aminopyralid into a small pond, the hazard quotient is 0.6, approaching the level of concern (1.0). This is an intentionally extreme exposure scenario that typically leads to the highest hazard quotient in pesticide risk assessments similar to the current assessment on aminopyralid. The upper bounds of acute exposure scenarios for contaminated vegetation or fruit are below the level of concern by factors of 10 to 50. Acute non-accidental exposure scenarios for members of the general public that involve contaminated water are below the level of concern by factors of about 50 to 500.

The risk characterization given in this risk assessment is qualitatively similar to that given by the U.S. EPA: no risks to workers or members of the general public are anticipated. The current risk assessment derives somewhat higher hazard quotients than those in the U.S. EPA human health risk assessment because the current risk assessment uses a number of extreme exposure scenarios that are not used by the U.S. EPA.

3.4.2. Workers

A quantitative summary of the risk characterization for workers associated with exposure to aminopyralid is presented in Worksheets E02a, E02b, and E02c. The quantitative risk characterizations for workers are expressed as the hazard quotients, the ratios of the estimated doses from Worksheet E01 to the RfD. For acute exposures – i.e., accidental or incidental exposures – the acute RfD of 1 mg/kg/day is used (Section 3.3.3). For general exposures – i.e., daily exposures that might occur over the course of an application season – the chronic RfD of 0.5 mg/kg/day is used (Section 3.3.2).

Worksheet E02a provides hazard quotients for the typical application rate of 0.078 lb a.e./acre. The hazard quotients for the lower bound (0.03 lb a.e/acre) and upper bound (0.11

lb a.e./acre) of the application rates considered in this risk assessment are given in Worksheets E02b and E02c, respectively.

In terms of general exposures – i.e., exposures that might be expected to occur over the course of each work day during a prolonged application program – the hazard quotients range from 0.005 (backpack or aerial spray at an application rate of 0.03 lb a.e./acre) to 0.03 (ground broadcast spray at an application rate of 0.11 lb a.e./acre). These are below the level of concern (1.0) by factors of about 33 to 200.

While the accidental exposure scenarios are not the most severe one might imagine (e.g., complete immersion of the worker or contamination of the entire body surface for a prolonged period of time) they are representative of reasonable accidental exposures. None of these hazard quotients for accidental exposures approach a level of concern even at the upper bounds. The highest hazard quotient is 0.004 – i.e., a spill on to the lower legs over a one-hour period at the highest application rate. This hazard quotient is below the level of concern (1.0) by a factor of 250.

The simple verbal interpretation of this quantitative characterization of risk is that under a protective set of exposure assumptions, workers would not be exposed to levels of aminopyralid that are regarded as unacceptable so long as reasonable and prudent handling practices are followed.

The risk characterization for workers given in this risk assessment is somewhat more severe quantitatively than that given by the U.S. EPA (U.S. EPA/OPP-HED 2005). For short- and intermediate-term worker scenarios, the U.S. EPA estimates daily exposures to workers using the Pesticide Handlers Exposure Database (PHED). Unlike the approach used in Section 3.2 of this risk assessment, PHED estimates deposited doses rather than absorbed doses. Using this approach, the U..S. EPA estimates doses in the range of 0.0000000042 mg/kg bw/day to 0.00264 mg/kg bw/day at the maximum application rate of 0.11 lb a.e./acre (U.S. EPA/OPP 2005a, Table 9, p. 44). These are below the dose estimates for workers given Worksheet E01 of this risk assessment – i.e., 0.0000187 mg/kg bw/day to 0.0118 mg/kg bw/day – at an application rate of 0.078 lb a.e./acre. Adjusted to an application rate of 0.11 lb a.e./acre, the dose estimates for workers derived in this risk assessment are about 0.0000264 to 0.0166 mg/kg bw/day. Notwithstanding these differences, the qualitative conclusions given in this risk assessment are consistent with those of the U.S. EPA – i.e., there is no basis for asserting that adverse effects in workers are plausible.

As discussed in Section 3.1.11.3., technical grade aminopyralid in powder form can cause severe eye irritation with corneal damage. All field applications considered in this risk assessment, however, involve the use of Milestone formulations – i.e., solutions of aminopyralid-TIPA in water. These formulations are much less irritating to the eyes than aminopyralid powder and the aminopyralid-TIPA formulation has been classified by the U.S. EPA as Category IV, the minimal classification for eye irritants(U.S. EPA/OPP-HED 2005). Similarly, technical grade aminopyralid and the liquid formulation of aminopyralid have been classified as minimal skin irritants (Section 3.1.11.1).

While it does not seem likely that applications of liquid formulations of aminopyralid-TIPA will lead to skin or eye irritation, the U.S. EPA has expressed concern eye irritation (because of the irritant effects of aminopyralid powder) in workers who may reenter treated fields (U.S. EPA/OPP-HED 2005, p. 5) and has recommended a restricted reentry interval of 48 hours. As with all pesticide applications, potential dermal and ocular effects can and should be minimized or avoided by prudent industrial hygiene practices during and after the application of aminopyralid formulations.

3.4.3. General Public

Quantitative summaries of the risk characterization for members of the general public associated with exposures to aminopyralid are presented in Worksheets E04a, E04b, and E04c. As with workers, the quantitative risk characterizations are expressed hazard quotients. Acute hazard quotients are based on the acute RfD of 1 mg/kg/day (Section 3.3.3) and longer-term hazard quotients are based on the chronic RfD of 0.5 mg/kg/day (Section 3.3.2). Also as with workers, the three summary worksheets correspond to the application rates explicitly considered in this risk assessment: the typical application rate of 0.078 lb a.e./acre (Worksheet E04a), the lower bound application rate of 0.03 lb a.e/acre (Worksheet E04b), and the upper bound application rate of 0.11 lb a.e/acre (Worksheet E04c).

As detailed in Section 3.2.3.1.1. (Likelihood and Magnitude of Exposure), all exposure assessments used for members of the general public are based on the Most Exposed Individual (MEI). Consequently, the corresponding risk characterizations detailed in this section will encompass the potential for adverse effects associated with recreational areas and other sites that may be used by large numbers of individuals.

Although there are several uncertainties in the longer-term exposure assessments for the general public, as discussed in Section 3.2.3, the upper bounds of hazard quotients associated with the longer-term exposures at the maximum application rate of 0.11 a.e. lb/acre are all below a level of concern. The highest longer-term hazard quotient is associated with the longer-term consumption of contaminated vegetation. This is a common pattern with herbicides or any pesticide applied directly to plants. The scenario for the longer-term consumption of contaminated vegetation is also an extremely conservative assumption in that most plants treated with a herbicide at the highest application rate would show some signs of damage and humans would not be likely to consume the plant over a prolonged period of time. The upper bound of this hazard quotient at the application rate of 0.11 lb a.e./acre is 0.08, below the level of concern by a factor of about 12. All of the other longer-term hazard quotients at the maximum application rate are in the range of 0.000008 (the consumption of contaminated fish by the general public) to 0.01 (the consumption of contaminated fruit). These hazard quotients are below the level of concern by factors of 100 to 125,000. This risk characterization is comparable to although somewhat broader than the risk quotients based on longer-term aggregate exposures that have been derived by the U.S. EPA – i.e., exposures that are below the level of concern by factors of 416 to 2174 (U.S. EPA/OPP-HED 2005, Table 7.2, p. 43).

Thus, the risk characterization for longer-term exposures is unambiguous: based on the available information and under the foreseeable conditions of application, there is no route of

exposure or scenario suggesting that the general public will be at any substantial risk from longer-term exposure to aminopyralid even when the compound is applied at the maximum labeled application rate.

As with chronic exposures, none of the hazard quotients associated with acute/accidental exposure scenarios exceed the level of concern even that the upper bounds of the hazard quotients at the maximum application rate (Worksheet E04c). Exposure resulting from the consumption of contaminated water after an accidental spill is of greatest concern. The estimate of the upper bound of exposure resulting from the consumption by a child of contaminated water from a small pond immediately after an accidental spill is 0.6, only modestly below the level of concern. As noted in 3.2.3.4.1, this accidental exposure scenario is dominated by arbitrary variability. The exposure scenario is used consistently in this series of risk assessments to provide a very general sense of the hazards that might be posed by a relatively serious accident. This is an extremely conservative scenario that typically results in an excursion above the RfD. This is not the case with aminopyralid. Nonetheless, the risk quotient approaches a level of concern. With aminopyralid as with all pesticides, prudent measures should be taken to limit exposure to members of the general public after any type of spill event.

Other more plausible exposure scenarios involve the acute consumption of or contact with contaminated vegetation or fruit as well as the consumption of contaminated water or fish and swimming in contaminated water. As with the chronic exposure scenarios, the consumption of contaminated vegetation or fruit lead to acute hazard quotients that are higher than those associated with the contamination of water. The upper bounds of the hazard quotients at the maximum application rate are 0.1 for vegetation and 0.02 for fruit, below the level of concern by a factors of 10 and 50, respectively. The non-accidental exposures involving ambient water are 0.007 (consumption of ambient water) and 0.00007 (swimming), which are below the level of concern by factors of about 140 to 14,000.

The risk characterization for acute exposures given in this risk assessment involves a somewhat broader range of hazard quotients than those given by the U.S. EPA in which the estimated total aggregate exposures are below the level of concern by factors of 320 to 400 (U.S. EPA/OPP-HED 2005, Table 7.1, p. 42). In making this comparison, it should be noted that the U.S. EPA provides aggregate margins of exposure (MOE) of 32,000 to 40,000 rather than risk quotients. The factors of 320 to 400 are derived by dividing the MOE by the uncertainty factor of 100.

These differences between the U.S. EPA risk assessment and the current risk assessment reflect the extreme value approach that is taken in the current risk assessment (Section 3.2.3.1.1). In other words, no fundamental differences exist in the conclusions reached by the U.S. EPA and those reached in the current risk assessment – i.e., no plausible risks associated with the use of aminopyralid are identified. The risk characterization given in current risk assessment is somewhat more severe than that given by the U.S. EPA simply because some of the exposure assessments given in the current risk assessment are much more extreme than those used by the U.S. EPA.

Each of the hazard quotients summarized in Worksheets E04a through E04c involves a single exposure scenario. In some cases, individuals could be exposed by more than one route and in such cases risk can be quantitatively characterized by simply adding the hazard quotients for each exposure scenario. For aminopyralid, considerations of multiple exposure scenarios have little impact on the risk assessment. For example, take a combined scenario where an individual is sprayed on the lower legs, stays in contact with contaminated vegetation, eats contaminated fruit, drinks contaminated ambient water, and consume contaminated fish at rates characteristic of subsistence populations. In such a case, the combined hazard quotient would be 0.0935 (0.006 + 0.0005 + 0.02 + 0.007 + 0.06), below the level of concern by a factor of about 10.6. Similarly, for all of the chronic exposure scenarios, the addition of all possible pathways at the maximum application rate leads to a combined hazard quotient of about 0.0884 which is below the level of concern by a factor of about 11.

3.4.4. Sensitive Subgroups

There is no information to suggest that specific groups or individuals may be especially sensitive to the systemic effects of aminopyralid. Due to the lack of data in humans, the critical effect of aminopyralid in humans, if any, cannot be identified.

As noted in Section 3.1, it is not clear that aminopyralid has any remarkable systemic toxic effects. The most common effects in experimental mammals involve effects on the gastrointestinal tract which may be viewed as portal of entry effects. These effects are variable among different species of mammals and appear to be associated with levels of exposure that are substantially higher than any likely human exposures. Thus, it would seem highly speculative to suggest that individuals with gastrointestinal diseases might be more susceptible than other individuals to aminopyralid.

Two components of the hazard identification, however, remain troubling: the ocular effects after oral exposure to aminopyralid-TIPA that were noted in a single study in rats after gavage dosing (Section 3.1.2.5) and the signs of incoordination noted in developmental studies with rabbits (Section 3.1.6).

While the quantitative risk characterization does not provide any basis for indicating that risks are plausible based on the information that is available at this time, aminopyralid is a new pesticide and the information that is available on this pesticide is limited to those studies that are required for pesticide registration. The ocular effects and the incoordination in rabbits are concerns simply because they are not well-understood. Hence, the implications (if any) for risks to humans cannot be well-articulated.

Nonetheless, these effects do not raise substantial concern at this time. The ocular effects after oral administration may be incidental – i.e., the effects were seen in the study but they may have been caused by some unidentified factor not associated with aminopyralid. This supposition is supported because the effect noted in this one study does not appear to be reproducible – i.e., the effect on the eyes has not been observed in other similar studies on aminopyralid and aminopyralid-TIPA. Lastly, both the ocular effects in rats and the incoordination in rabbits occurred after gavage exposures, which have limited relevance to any foreseeable human exposures. As detailed in Section 3.1.3.4, gavage exposures are

likely to lead to sharp spike in plasma concentrations that rapidly decline after dosing. This pattern of plasma concentration seems to be consistent with the time-course of incoordination in rabbits after gavage dosing – a rapid onset with rapid reversibility.

3.4.5. Connected Actions

The U.S. EPA does not specifically address connected actions in their human health risk assessment of aminopyralid (U.S. EPA/OPP-HED 2005). This is a very typical situation because pesticides are registered by the U.S. EPA under FIFRA (Federal Insecticide, Fungicide and Rodenticide Act) and considerations of connected actions are required under NEPA (National Environmental Policy Act).

The Council on Environmental Quality (CEQ), which provides the framework for implementing NEPA, defines connected actions (40 CFR 1508.25) as actions which occur in close association with the action of concern; in this case, the use of aminopyralid as proposed in Section 2. Actions are considered to be connected if they: (i) automatically trigger other actions which may require environmental impact statements; (ii) cannot or will not proceed unless other actions are taken previously or simultaneously, and (iii) are interdependent parts of a larger action and depend on the larger action for their justification. Within the context of this assessment of aminopyralid, "connected actions" include actions or the use of other chemicals which are necessary and occur ***in close association*** with use of aminopyralid.

As discussed in Section 2 and summarized in Table 1, aminopyralid may be formulated with other herbicides, specifically 2,4-D and fluroxypyr. These formulations, however, are not being proposed for use by the U.S. Forest Service or the NPS and are not considered in this risk assessment.

The use of inerts and adjuvants as well as the occurrence of impurities and metabolites would be classified as connected actions under the CEQ definition. As discussed in detail in Section 3.1.14 (Inerts and Adjuvants), the aminopyralid formulations covered in this risk assessment do not contain inerts other than water. As discussed in Section 3.1.15, there is no basis for asserting that the impurities in aminopyralid or the metabolites of aminopyralid are likely to result in effects that are not encompassed by the hazard quotients for human that are discussed in Sections 3.4.2 (workers) and the 3.4.2 (general public).

While the aminopyralid formulations do not contain adjuvants, the product labels for Milestone and Milestone VM indicate that non-ionic surfactants may be added to improve efficacy. The recommended surfactant concentrations are in the range of 0.25 to 0.5%. If surfactants are used in aminopyralid applications, the impact of the surfactant may need to be addressed in a project specific analysis.

3.4.6. Cumulative Effects

Cumulative effects may involve either repeated exposures to an individual agent or simultaneous exposures to the agent of concern (in this case aminopyralid) and other agents that may cause the same effect or effects by the same or a similar mode of action.

Cumulative effects, within the context of the Food Quality Protection Act (FQPA), are addressed by the U.S. EPA (U.S. EPA/OPP-HED 2005):

> *Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding for aminopyralid and any other substances. Furthermore, aminopyralid does not appear to have a toxic metabolite that is produced by other substances.* (U.S. EPA/OPP-HED 2005, p. 43)

As discussed in Section 3.1.2.1, a number of different agents, some of which are either food items or food additives, have been shown to induce cecal enlargement in rodents. It is not clear, however, that the mechanism of action of these food items or food additives are identical to the mechanism of action of aminopyralid in rodents. In addition, it is not clear that the effect on the rodent cecum cause by aminopyralid or these other agents is relevant to potential effects in humans.

In terms of repeated exposures, the current risk assessment does specifically consider the effect of repeated and longer-term exposures to aminopyralid for both workers and members of the general public. The chronic RfD is used as an index of acceptable longer-term exposures. Consequently, the risk characterizations presented in this risk assessment for longer-term exposures specifically address and encompass the potential impact of the cumulative effects of aminopyralid. As discussed in Sections 3.4.2 and 3.4.3, there is no basis for asserting that cumulative adverse effects associated with longer-term or repeated exposures to aminopyralid are plausible.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

The mammalian toxicity of aminopyralid is relatively well-characterized in experimental mammals in a series of toxicity studies that are required for pesticide registration. In standard experimental toxicity studies in rats, mice, rabbits, and dogs, aminopyralid has low acute and chronic oral toxicity. It seems reasonable to assume the most sensitive effects in wildlife mammalian species will be the same as those in experimental mammals (e.g., changes in the gastrointestinal tract, weight loss, and incoordination).

Results of acute exposure studies in birds indicate that avian species appear no more sensitive than experimental mammals to aminopyralid in terms of acute lethality. In terms of non-lethal effects, however, birds may be somewhat more sensitive than mammals to aminopyralid after gavage exposures. In developmental studies involving gavage dosing, NOAEL values for mammals are in the range of 200 mg a.e./kg bw/day. In birds, the single dose gavage NOAEL is 14 mg a.e/kg bw. Birds are much less sensitive to dietary exposures compared to gavage exposures with NOAEL values for 5-day dietary exposures of over 1000 mg a.e./kg bw/day. While chronic studies (i.e., those approach the lifespan of the animal) are not available in birds, two standard reproduction studies have been conducted in bobwhite quail and one standard reproduction study has been conducted in mallard ducks. One of the reproduction studies in bobwhite quail appears to be a failed study but the second study in bobwhites, although not yet reviewed by the U.S. EPA, appears to acceptable. The study in mallards yielded the lowest NOAEL, 184 mg a.e./kg bw/day, comparable to the reproductive NOAEL values in mammals.

A standard set of toxicity studies are also available on terrestrial plants. Dicots (i.e., broadleaf plants) are substantially more sensitive to aminopyralid than monocots (e.g., grasses). This is consistent with the proposed uses of aminopyralid and the quantitative aspects of this difference in sensitivity are discussed further in the dose-response assessment for terrestrial plants. Relatively little information is available on the toxicity of aminopyralid to terrestrial invertebrates or terrestrial microorganisms. Based on bioassays in honeybees, earthworms, and soil microorganisms, aminopyralid does not appear to be very toxic to terrestrial invertebrates or soil microorganisms.

There is no indication that aminopyralid is likely to be toxic to aquatic animals based on standard acute and chronic bioassays in fish and invertebrates as well as one acute toxicity study in a species of frog. As would be expected from a herbicide, some aquatic plants are more sensitive than aquatic animals to the effects of aminopyralid. Duckweed, the one macrophyte on which a bioassay of aminopyralid has been conducted, does not appear to be sensitive to aminopyralid.

4.1.2. Toxicity to Terrestrial Organisms

4.1.2.1. Mammals

There are several standard toxicity studies in experimental mammals that were conducted as part of the registration process. The most common effects noted in these study involve changes in the gastrointestinal tract and decreased body weight. Incoordination has been noted in gavage studies with rabbits. Other than these effects, aminopyralid does not appear to cause specific target organ toxicity in mammals.

No field studies are available in which the impact of aminopyralid applications were assessed on mammalian wildlife communities. In standard experimental toxicity studies, aminopyralid has low acute oral toxicity. A common measure of acute oral toxicity is the LD_{50}, the estimate of the dose that may be lethal to 50% of the exposed animals. As summarized in Section 3.1.4, in rats the acute oral LD_{50} is greater than 5,000 mg/kg for technical grade aminopyralid (Brooks 2001a) and for the TIPA formulation of aminopyralid (5000 mg formulation/kg bw = 1090 mg e.g./kg bw) (Wilson et al. 2003). Mortality was noted in only 1 of 10 rats in the study on technical grade aminopyralid (Brooks 2001a) and no mortality was noted in the formulation study (Wilson et al. 2003).

As also discussed in Section 3.1, a standard series of bioassays in mammals are available for subchronic and chronic toxicity (Section 3.1.5) as well as developmental and reproductive effects (Section 3.1.9). Because aminopyralid is a weak acid and because dogs are known to have a limited ability to excrete weak acids (Section 3.1.3.2), dogs and other canid species might be expected to be more sensitive to aminopyralid than rodents. Based on the available subchronic and toxicity studies, this does not appear to be the case for aminopyralid because the NOAEL/LOAEL values are virtually identical in mice, rats, and dogs in both subchronic and chronic studies. In subchronic studies, the NOAEL in dogs is in the range of about 500 mg a.e./kg/day and the only clear LOAEL is in the range of 1000 mg a.e./kg bw/day (effects on stomach cells from the study by Stebbins and Baker 2002). The NOAEL value in mice is about 500 mg a.e./kg bw/day with a corresponding LOAEL of about 1000 mg a.e./kg bw (increase hepatocytes size in the study by Yano and Dryzga 2000). Virtually identical values are evident in rats under the assumption the changes in cecal weights are not judged to be adverse in the absence of organ pathology (Section 3.1.2.1). Using this criteria, the NOAEL in rats is also about 500 mg a.e./kg bw/day with a corresponding LOAEL of about 1000 mg a.e./kg bw/day (Dryzga and Stebbins 2001). In chronic studies, the NOAEL/LOAEL values are also virtually identical: about 100/1000 mg a.e./kg bw/day in both dogs (Stebbins and Day 2003a) and mice (Stebbins and Day 2003b) and 50/500 mg a.e./kg bw/day in rats (Johnson and Dryzga 2004). Note that the different NOAEL/LOAEL values for chronic effects in rats may reflect a simple difference in the doses selected for study rather than a clear difference in species sensitivity. In any event, the available subchronic and chronic studies offer no indication that dogs are more sensitive to aminopyralid than other species of mammals. Thus, as discussed further in Section 4.3.2, no separate dose-response assessment is conducted for dogs and other canids.

4.1.2.2. Birds

A relatively standard set of toxicity studies required for pesticide registration have been submitted to the U.S. EPA: two acute gavage studies in bobwhite quail (Gallagher et al. 2001a; Gallagher et al. 2003), subacute dietary studies in bobwhite quail (Gallagher et al. 2001b) and mallard ducks (Gallagher et al. 2001c), and reproduction studies in bobwhite quail (Mach 2003a) and mallard ducks (Mach 2003b). All of these toxicity studies have been reviewed by the U.S. EPA during the registration of aminopyralid (U.S. EPA/OPP-EFED 2004). An additional reproduction study in quail has been conducted (Temple et al. 2007) and this study is included in the current risk assessment.

Gavage Studies: The initial gavage study by Gallagher et al. (2001a) in bobwhite quail used dose levels of 63 to 2250 mg a.e./kg bw and the study is classified as *Acceptable.* No animals died during the course of this study. Because the highest dose exceeded the limit dose of 2000 mg a.e./kg bw (SERA 2007A, Table 4-1) and no mortality occurred, aminopyralid is classified by the U.S. EPA as *…practically non-toxic to avian species by acute oral exposure* (U.S. EPA/OPP-EFED 2004, p. 31). This is the lowest toxicity category used by the U.S. EPA for classification of acute toxicity. Not withstanding this classification, the study by Gallagher et al. (2001a) noted sublethal but still adverse effects in the test animals over the complete range of doses tested – i.e., 63 to 2250 mg a.e./kg bw/day. These effects included decreased responsiveness, incoordination, lower limb weakness and other signs of toxicity (as detailed in Appendix 4) that were progressively more severe with increasing dose.

Because a NOEC was not identified for adverse sublethal effects in the Gallagher et al. (2001a) study, the study by Gallagher et al. (2003) was designed as a supplemental study using lower doses: 8 to 292 mg a.e./kg bw (see Appendix 3 for details). Because the doses were much lower than the limit dose of 2000 mg a.e./kg bw, this study was classified as *Supplemental* rather than *Acceptable* (a.k.a. *Guideline*) but this classification does not reflect on the merits of the study. This low dose study confirmed the results of the earlier study by Gallagher et al. (2001a), noting similar but less severe effects over the range of doses tested. While loss of coordination was observed in 1 of 5 males at 35 minutes after a dose of 8 mg a.e./kg/day, no effects were observed at the next higher dose, 14 mg a.e./kg bw. Consequently, the U.S. EPA classifies the dose of 14 mg a.e./kg bw as a NOEC. The LOEC was set at 23 mg a.e./kg bw/day based on abnormal (ruffled) appearance. At higher doses – i.e., 63 mg/kg bw and above – progressively more severe and frequent signs of incoordination were noted.

Acute Dietary Studies: The two subacute dietary studies (5-day exposure period followed by a 3-day recovery period) did not yield any remarkable results. No adverse effects were observed at any dietary concentration – 178 to 5620 ppm nominal – and the U.S. EPA classified the NOEC as 5620 ppm in both quail (Gallagher et al. 2001b) and mallards (Gallagher et al. 2001c). Based on measured dietary concentrations and measured food consumption values, the dietary concentration of 5620 ppm corresponds to doses of 1669 mg a.e./kg bw/day in quail and 2360 mg a.e./kg bw/day in mallards. These doses are substantially above the adverse effect levels noted in the gavage studies. This is a relatively common pattern and probably reflects the greater peak exposures following gavage exposure

relative to the more gradual consumption (with simultaneous excretion) in a dietary study (Section 3.1.3.4). These differences are discussed further in the dose-response assessment for birds (Section 4.3.2.2).

Reproduction Studies: Of the two reproductions studies submitted to the U.S. EPA in support of the registration of aminopyralid (Mach 2003a,b), the study in quail (Mach 2003b) appears to have been flawed. The quail study has been repeated and full copy of this study (Temple et al. 2007) has been provided by Dow AgroSciences. As discussed below, the study by Temple et al. (2007) is consistent with the study in mallards (Mach 2003b).

In the mallard study (Mach 2003a), no significant adverse effects were observed in adults or offspring at dietary concentrations of up to 2700 ppm. As with the multigeneration studies in mammals (Section 3.1.9.2), bird reproduction studies involve variable rates of food consumption over the course of the study. Based on average body weights during the exposure period and measured food consumption during exposure period, the dietary concentration of 2700 ppm corresponds to a dose of about 184 mg a.e./kg bw/day. This dose is substantially higher than the LOAEL of 23 mg a.e./kg bw from the gavage study in quail (Gallagher et al. 2003) and this difference is probably attributable to the inherent differences between gavage and dietary exposures as noted above and discussed further in Section 3.1.3.4. This study was classified as *Acceptable* by the U.S. EPA (U.S. EPA/OPP-EFED 2004).

The reproductive study in bobwhite quail appears to be flawed because of the failure during the study to turn on a brooder battery. As detailed in Appendix 4, the study author (Mach 2003b) cites this as the cause for the death of 14 hatchings – i.e., death due to cool temperatures – and these 14 hatchlings were removed from the study. In addition, the study author notes that 27 hatchlings died in another brooder and the author attributes the death of these animals to pecking.

In the EPA review of this study – i.e., the DER – the Agency classifies this study as *Supplemental* because … *raw data on hatchling weight not provided. Also, quantity and fate of acetone in diet not specified.* For the current risk assessment, the study by Mach (2003b) is considered a failed study – i.e., the cause of the adverse effects cannot be clearly determined. The Mach (2003b) study is not used in the EFED risk assessment on aminopyralid (U.S. EPA/OPP-EFED 2004) and it will not be used in the current risk assessment to assess reproductive effects.

A second reproduction study by quail was conducted by Temple et al. (2007). As summarized in Appendix 4, this study is very similar in design to the earlier study in quail (Mach 2003b) with only minor differences in the number of animals. No deviations from protocol or problems in the conduct of this study are reported by Temple et al. (2007). As in the mallard study (Mach 2003b), no signs of toxicity or effects on reproduction were noted in quail in the study by Temple et al. (2007). The NOAEL for the Temple et al. (2007) study is a dietary concentration of 2700 ppm, identical to that in the study on mallards. Based on measured body weights and measured food consumption, the dietary NOAEL in quail corresponded to daily doses in the range of 203 to 239 mg a.e./kg bw/day (Temple et al.

2007). As with the mallard study, this NOAEL for reproduction is substantially above the gavage LOAEL in quail of 23 mg a.e./kg bw (Gallagher et al. 2003) and this difference is probably due to the higher peak body burdens that would expected after gavage dosing relative to dietary dosing (Section 3.1.9.2).

Kinetics: In addition to the above toxicity studies, one metabolism study of aminopyralid has been conducted in hens (Magnussen 2004a) and this study is also summarized in Appendix 4. This is not a detailed pharmacokinetic study but simply a study to assess plausible residues in poultry. As would be expected from the pharmacokinetic studies in mammals, residues in tissue were very low.

4.1.2.3. Terrestrial Invertebrates

The registration requirements for testing the effects of herbicides on terrestrial invertebrates are relatively modest and only tests on honey bees are typically submitted. For aminopyralid, the standard contact bioassay in bees is available (Aufderheide 2001a) as well as an oral bioassay in bees (Aufderheide 2001b) and an acute toxicity study in earthworms (Ward and Boeri 2001). As with the other groups of organisms considered in this ecological risk assessment, no field studies are available. Field studies are not typically required for pesticide registration. Instead, field studies are most commonly conducted independently and are published in the open literature.

As with most herbicides, there is no indication that aminopyralid is toxic to honeybees. In the limit test for contact toxicity (Aufderheide 2001a), no mortality was observed at a dose of 0.1 mg/bee. This study does not specify the body weights of the bees used in this bioassay. Using a body weight of 0.093 g (0.000093 kg) for the honey bee (USDA/APHIS 1993), this dose per bee corresponds to a mg/kg bw dose of about 1075 mg a.e./kg bw [0.1 mg/0.000093 kg]. This is comparable to the subacute NOAEL values of about 500 mg a.e./kg bw in mammals (Section 4.1.2.1).

Very similar results for aminopyralid are evidenced in the 6-hour feeding study in honeybees, with a NOAEL of 0.12 mg/bee or about 1290 mg a.e./kg bw [0.12 mg/0.000093 kg] (Aufderheide 2001a). As noted in Appendix 5, sporadic mortality was observed in some control groups as well as in lower dose groups but this mortality is not dose-related or statistically significant.

The earthworm bioassay is also a single dose limit test in which the organisms were exposed to aminopyralid in soil at 5000 ppm (mg a.e./kg soil) over a 14-day period (Ward and Boeri 2001). While no statistically significant effects were observed, the control replicates averaged an increase of 3.35% in body weight and the exposed groups averaged a decrease of 1.3% in body weight. Qualitatively, no adverse effects (e.g., burrowing behavior) were noted. As discussed in Section 3.4, the 5000 ppm used in the Ward and Boeri (2001) study is orders of magnitude above any plausible concentrations of aminopyralid in soil.

4.1.2.4. Terrestrial Plants (Macrophytes)

4.1.2.4.1. Toxicity

While specific studies on the mechanism of action of aminopyralid in plants have not been encountered, the structural similarity of aminopyralid to auxin-mimicking herbicides such as clopyralid, picloram, and triclopyr (see Figure 1) suggests that aminopyralid acts in a manner similar to these other herbicides (U.S. EPA/OPP-EFED 2004, p. 8). As discussed in risk assessments on clopyralid, picloram, and triclopyr (SERA 2003a,b; SERA 2004c), the pyridine carboxylic acid herbicides mimic indole auxin plant growth hormones and cause uncontrolled growth in plants. These herbicides behave similarly to the chlorophenoxy acid herbicides such as 2,4-D. At sufficiently high levels of exposure, the abnormal growth is so severe that vital functions cannot be maintained and the plant dies.

The testing requirements for the effects of herbicides on terrestrial plants are relatively rigorous since terrestrial vegetation is the typical target group for herbicides. The testing requirements (U.S. EPA/OPPTS 2007c) involve bioassays for seedling germination and emergence (soil exposures) as well as vegetative vigor (foliar exposures) in several species of dicots and several species of monocots. Consistent with these requirements, a complete set of studies have been submitted on aminopyralid in two submissions: seedling germination and emergence (Aufderheide 2004a) and vegetative vigor (Aufderheide 2004b). As detailed in Appendix 6, each of these submissions consists of series of plant bioassays on six dicots (cucumber, lettuce, oilseed rape, radish, soybean, and sugar beet) and four species of monocots (barnyard grass, corn, onion, and wheat).

Both of these studies have been classified by the U.S. EPA (U.S. EPA/OPP-EFED 2004) as *Supplemental* rather than *Acceptable*. The rationale given by EFED in the DER for classifying the seedling germination and emergence study (Aufderheide 2004a) is as follows: *…because soil surface watering occurred without report of test substance mobility characteristics, and because Thiram was applied to sugar beet without further explanation.* Similarly, the vegetative vigor study (Aufderheide 2004b) is classified by EFED as supplemental because*…Thiram was applied to sugar beet without further explanation, and because both corn and radish were grown under very low light conditions, which may have affected the results*.

While these deviations from EPA protocol are sufficient to warrant the classification of these studies as *Supplemental*, the deviations themselves do not appear to substantially limit the utility of these studies. Thiram is the common name for tetramethylthiuram disulfide (CAS No. 137-26-8) that is used as a contact fungicide for treating various types of fungal diseases on a variety of plants (Tomlin 2004). There is no clear basis for asserting that thiram treatment would have substantially altered the response of sugar beet, corn, or radish to aminopyralid. The comment on substance mobility is difficult to interpret since all required studies on environmental fate and transport have been submitted to the U.S. EPA (Appendix 1). The comment on low light conditions is clearly relevant. Low light could enhance the toxicity of a herbicide to plants but would also impact the growth of control plants, possibly making it more difficult to detect a significant response due to the herbicide. The implicit position of EFED is that these studies are, on balance, useful in an ecological risk assessment in that EFED did use these studies quantitatively in their ecological risk assessment on

aminopyralid (U.S. EPA/OPP-EFED 2004). This position seems reasonable and these studies are used in this current risk assessment.

As detailed in Appendix 6, dicots (i.e., broadleaf plants) are substantially more sensitive to aminopyralid than monocots (e.g., grasses). This is consistent with the proposed uses of aminopyralid and the quantitative aspects of this difference in sensitivity are discussed further in Section 4.3.2.4.

4.1.2.4.2. Persistence

In addition to the standard toxicity studies, additional studies on the persistence of aminopyralid on vegetation have also been conducted (Roberts et al. 2004; McCormick et al. 2004). No DERs are available on these studies and these studies are not specifically used or cited in the U.S. EPA risk assessments (U.S. EPA/OPP-EFED 2004; U.S. EPA/OPP-HED 2004). As discussed in Section 3.2.3.6, these studies are used in the current risk assessment to estimate halftimes (under the assumption of first-order dissipation) on vegetation in both the human health and ecological exposure assessments. As noted in Appendix 6, some of the specific data sets on the decline in residues in vegetation offer a poor fit to the first-order model. This poor fit is probably attributable to the fact that both of the residue studies were conducted in the field, where varying patterns of precipitation and other factors may result in erratic patterns of dissipation. Notwithstanding this limitation, these studies yield a relatively narrow range of halftime values – i.e., 8 to 19 days.

4.1.2.5. Terrestrial Microorganisms

Studies on the toxicity of herbicides to terrestrial microorganisms are not generally required for registration and no such studies have been submitted to the U.S. EPA. Dow AgroSciences, however, has provided a copy of the study by McMurray (2002) which assayed the effects of aminopyralid on respiration (CO_2 evolution) and nitrogen metabolism on soil microflora following European guidelines from OECD. Two concentrations of aminopyralid in soil were used, 1.68 mg a.e./kg soil and 8.4 mg a.e./kg soil and effects on respiration and nitrogen metabolism were assayed at 0, 7, 14, and 28 day. No statistically significant or substantial effects were noted on CO_2 evolution. Nitrate and total mineral nitrogen concentrations were significantly elevated in treated soils on Day 0 but not during subsequent observation periods. The magnitude of the increases in nitrate and total mineral nitrogen concentrations based on mean measured values on Day 0 was in the range of 12% to 15%.

The only other information that might relate to microbial toxicity involves the observation in the aerobic soil degradation by Yoder and Smith (2002) that two of three soils used to assess the persistence of aminopyralid in soil had lower biomass at the end of the study than at the beginning and that the biological activity in these soils (measured by the degradation of dichlorobenzoic acid) decreased over time (Appendix 1). These effects were noted at aminopyralid concentrations in soil of 0.03 and 0.05 ppm. This study, however, does not permit the assessment of whether or not these effects were due to aminopyralid or simply reflected normal changes in biomass over the course of the study. Changes in soil biomass and biological activity are to be expected in soils over prolonged periods of incubation. The study by Yoder and Smith (2002) involved incubation periods of 1-year. In addition, this

study is not designed to measure microbial toxicity – i.e., no concentration-response relationship can be established and hence the observed effects cannot be attributed to aminopyralid.

4.1.3. Aquatic Organisms

4.1.3.1. Fish

Acute Studies: Standard toxicity bioassays to assess the effects of exposure of fish to aminopyralid are summarized in Appendix 7. Acute toxicity studies have been conducted in two species of freshwater fish – i.e., rainbow trout (Marino et al. 2001a) and bluegill sunfish (Machado 2003) – and one species of saltwater fish – i.e., sheepshead minnow (Machado 2002b). All of these acute bioassays as well as the longer-term study discussed below (Marino et al. 2003) have been conducted on technical grade aminopyralid. No toxicity studies in fish are available on the TIPA formulation of aminopyralid.

All of the acute toxicity studies were reviewed by the U.S. EPA (DERs are available) and all except the bluegill study by Machado (2003) were classified as *Acceptable* (U.S. EPA/OPP-EFED 2004). The bluegill study is classified as *Supplemental* because the size of some of the bluegills used in the Machado (2003) study were smaller than the sizes specified in the Guidelines for acute toxicity studies. Nonetheless, the study appears to have been well conducted and the results are useful in a risk assessment.

The acute toxicity studies in fish are all unremarkable. No mortality was observed at the maximum concentration tested in all of these assays, 100 mg a.e./L. The Marino et al. (2001a) study in trout is the only study that reports any sublethal effects – i.e., partial loss of equilibrium in 2 of 30 organisms (6.66%) exposed to 100 mg/L at 96 hours but not at 24, 48, or 72 hours. EFED has classified the 100 mg/L exposure as a NOEC. This seems reasonable because the incidence of the effect (2/30) in the 100 mg a.e./L group is not statistically significant relative to the incidence in the control group (0/30) using the Fisher Exact test ($p = 0.2457$). Based on the results of the acute toxicity studies, the U.S. EPA has classified aminopyralid as *practically non-toxic* to freshwater and saltwater fish (U.S. EPA/OPP-EFED 2004, p. 27).

Longer-Term Studies: As detailed in SERA (2007), two types of longer-term studies may be conducted in fish as part of the registration process: full life cycle studies (Guideline 850.1500) and early life stage studies (Guideline 850.1400). As the name implies, the full life cycle study is analogous to the lifetime chronic bioassays in rodents and fish are exposed over the course of a full life cycle – i.e., from egg to fry to adult to egg production. The early stage toxicity study, often referred to as an egg-and-fry study, involves exposure from the egg stage to the fry stage. The only study available on aminopyralid is the early life stage study in fathead minnow (Marino et al. 2003). This limitation is not uncommon in studies submitted for pesticide registration. Full life cycle studies are required by the U.S. EPA only if concern for longer-term exposures is triggered by the results of the egg-and-fry study. As detailed in Section 4.4.3.1 (Risk Characterization for fish), this is not the case for aminopyralid.

The early life stage study by Marino et al. (2003) has been reviewed by the U.S. EPA and classified as *Supplemental* (U.S. EPA/OPP-EFED 2004). This classification reflects the failure of the study to report all of the information required by Guidelines. In the case of the Marino et al. (2003) study, the following reporting deficiencies are specified by EFED: … *replicate data for the days-to mean hatch and sub-lethal effects were not submitted and could not be verified by EFED.* No flaws in the study design or execution, however, are noted in the EFED review and none have been found in the review of the Marino et al. (2003) study for the current risk assessment. It is likely that the Marino et al. (2003) study could be classified as *Acceptable* if the reporting deficiency was corrected.

As detailed in Appendix 7, the mean measured concentrations tested in the Marino et al. (2003) study were 0 (untreated control), 0 (solvent control), 0.0708, 1.36, 2.44, 3.89, 6.71, and 11.4 mg a.e./L. A noted above, the test material was technical grade aminopyralid. No larvae survived at the two higher concentrations. Based on reductions in fry weight, fry length, larval survival, and % normal larvae, the 2.44 mg a.e./L exposure was classified as the LOEC and the concentration of 1.36 mg a.e./L was classified as a NOEC by the U.S. EPA (U.S. EPA/OPP-EFED 2004).

4.1.3.2. Amphibians and Reptiles

One acute toxicity limit test on the northern leopard frog larvae (Henry et al. 2003a) has been submitted to the U.S. EPA and has been classified as *Supplemental* (U.S. EPA/OPP-EFED 2004). The classification of Supplemental is given because the U.S. EPA/OPPTS (2007c) does not have guidelines for amphibian toxicity testing. In this limit test, no mortality or sublethal effects were observed over a 96-hour exposure of the frog larvae to a mean measured concentration of 95.2. mg a.e./L (Appendix 7). Based on the results of this acute toxicity study, the U.S. EPA has classified aminopyralid as *practically non-toxic to aquatic-phase amphibians* (U.S. EPA/OPP-EFED 2004, p. 27).

4.1.3.3. Aquatic Invertebrates

Acute Studies: As with testing requirements in fish, a standard set of toxicity tests are required in aquatic invertebrates for pesticide registration (U.S. EPA/OPPTS 2007c). As detailed in Appendix 8, acute toxicity studies are available in one species of freshwater invertebrate, *Daphnia magna* (Marino et al. 2001b) and two species of saltwater invertebrates, oysters (Cafarella 2002) and shrimp (Machado 2002a). All of these acute toxicity studies were classified as *Acceptable* by the U.S. EPA (U.S. EPA/OPP-EFED 2004).

While the daphnid bioassay (Marino et al. 2001b) is a single submission, two studies were actually conducted. The first was a probe study in which no mortality and no other signs of toxicity were observed in groups of 10 organisms at concentrations of 25, 50, 75, and 100 mg a.e./L over a 48-hour exposure period. Because of the lack of toxicity in the probe study, the full study (3 replicates at 10 organisms/replicate) was conducted as a limit test at a single nominal test concentration of 100 mg a.e./L (measured value of 98.6 mg a.e./L) over a 48-hour observation period. Again, no mortality and no other signs of toxicity were observed.

Similar results were obtained in the studies on saltwater species. The study in mysid shrimp (Machado 2002a) led to essentially the same result as the daphnid study with no mortality

and no other signs of toxicity observed at measured average test concentrations of 14, 22, 36, 59, and 100 mg a.e./L over a 96 hour observation period. The study using eastern oysters (Cafarella 2002) involved 96-hour exposures to test concentrations of 12, 21, 31, 50, and 89 mg a.e./L. No adverse effects related to exposure were noted at any concentration. At 89 mg a.e./L, shell growth was reduced by 12% relative to controls but this difference was not statistically significant. EFED classified 89 mg a.e./L as a NOEC. Given the lack of any dose-related trends or growth inhibition at lower concentrations, the classification of 89 mg a.e./L as a NOEC is appropriate.

Based on the results of these acute toxicity studies, the U.S. EPA has classified aminopyralid as *practically non-toxic to freshwater invertebrates*, *practically non-toxic to the estuarine/marine mysids* and *slightly toxic to the estuarine/marine mollusks* (U.S. EPA/OPP-EFED 2004, p. 28). The different classification for mollusks is due to the 100 mg/L cutoff for classifying a compound as *practically non-toxic* to aquatic species (SERA 2007A, Table 4-1). In the bioassay on oysters, Cafarella (2002) tested only up to 89 mg a.e./L and thus the lowest classification that could be given in *slightly toxic*. This classification is thus an artifact of the experimental design and does not indicate that mollusks are any more sensitive to aminopyralid than any other group of aquatic invertebrates.

Longer-term Studies: Longer-term toxicity studies are available in both *Daphnia magna* (Henry et al. 2003b) and midge larvae (Putt 2002). Although the durations of the longer-term studies using invertebrates are relatively short – i.e. 14 days for the daphnid study and 28 days for the midge study – both of the longer-term toxicity studies are essentially full life-cycle toxicity studies in which the organisms are exposed from a very young age (<24 hours post-release for daphnids or post-hatch for midge) through to the production of the next generation of young.

Both of these longer-term studies were classified as *Supplemental* rather than *Acceptable* by the U.S. EPA (U.S. EPA/OPP-EFED 2004). The midge assay is classified as *Supplemental* simply because guidelines are not available for this assay. The daphnid study (Henry et al. 2003b) was classified as *Supplemental* because of minor deviation from Guideline protocols: *excessive water hardness, low dissolved oxygen (31 %), and reduced replicate size* (Study DER). The comment on replicates concerns the numbers of organisms per test chamber. The study authors used 1 organism per test chamber and 8 replicates per concentration. This is the general approach favored in OECD (i.e., the European *Organisation for Economic Co-operation and Development*) guidelines. The U.S. EPA prefers a somewhat more complex design with 22 organisms per concentration (7 replicates of 1 organism per replicate and 3 replicates of 5 organisms per replicate).

In the daphnid study, no adverse effects on adults, offspring, or reproductive parameters were noted over the range of test concentrations, 2.29 mg a.e./L to 102 mg a.e./L. The study authors proposed a NOEC of 102 mg a.e./L and this was confirmed and accepted by EFED (U.S. EPA/OPP-EFED 2004).

The midge assay was conducted at mean measured test concentrations of 58, 123, 247, 520, and 973 mg a.e./L and adverse effects were noted (Putt 2002). At 973 mg a.e./L, all

organisms died. At 520 mg a.e./L, a significant decrease was noted in male midge development rate based on 1-day inspection intervals and overall emergence was significantly decreased – i.e., 75% vs 94% in controls. At 250 mg a.e./L, the only effect noted was a lesser but still statistically significant decrease in overall emergence – i.e., 80% vs 94% in controls. The study authors proposed a NOEC of 130 mg a.e./L based on mean measured test concentrations. While the EPA did not dispute the selection of the experimental group on which the NOEC should be based, U.S. EPA/OPP-EFED (2004) set the NOEC at 82 mg a.e./L with an LOEC of 158 mg a.e./L based on pore water concentrations. This approach is sensible because midges are benthic species – i.e., they live in sediment at the bottom of water bodies. Consequently, it is reasonable to assume that it is the concentration of the chemical in the pore water (i.e., the free water between the soil particles or interstitial space in sediment) is the best measure of exposure to the organism.

4.1.3.4. Aquatic Plants

The toxicity of aminopyralid has been examined in a series of standard bioassays that are required for the registration of herbicides: three species of freshwater algae (Hoberg 2002a,c; Hoberg 2003b), one species of saltwater algae (Hoberg 2002b) and duckweed (Hoberg 2003a), an aquatic macrophyte. These studies are summarized in Appendix 9. With the exception of the study in blue-green algae (Hoberg 2002c), all studies were reviewed by and classified as *Acceptable* by the U.S. EPA (U.S. EPA/OPP-EFED 2004). As detailed below, the study by Hoberg (2002c) in *Anabaena flos-aquae* was classified by the U.S. EPA as *Unacceptable*. A new study has been conducted by Hancock et al. (2007) to address the deficiencies noted by the U.S. EPA. The Hancock et al. (2007) study is also summarized in Appendix 9 and is discussed further in this subsection.

The study by Hoberg (2002c) was conducted on *Anabaena flos-aquae*. This study is classified as *Unacceptable* because of *…high variability in the controls made interpretation of the data uncertain* (U.S. EPA/OPP-EFED 2004, p. 31). While abnormal variability is not discussed specifically in the report by Hoberg (2002c), substantial variability among replicates is apparent in both control groups as well as several of the treatment groups (Hoberg 2002c, Study Table 4, Study page 27). Hoberg (2002c) does note that the cells were dispersed by "rapid pipetting" prior to counting. *Anabaena flos-aquae* is a filamentous and motile cyanobacteria that forms long strands of connected cells (e.g., http://www.fytoplankton.cz/fytoatlas.php?show=9). While *Anabaena flos-aquae* is problematic as a test organism in determining concentration-response relationships (e.g., Abou-Waly et al. 1991a,b), the error that appears to have been made in the Hoberg (2002c) study involves the use of a pipette to break up the cells prior to counting. As noted in the EPA guidelines in using this species, *…the filaments are broken up and dispersed using a syringe, ultrasonic bath, or blender* (U.S. EPA/OPPTS 1996a, p. 3). Thus, the classification of the Hoberg (2002c) study as *Unacceptable* is appropriate and this study is not further considered in this risk assessment.

Hancock et al. (2007) have recently conducted another bioassay on *Anabaena flos-aquae*. This study employed a Multisizer 3 Coulter Counter, an instrument that is intended to permit simultaneous measurements of particle volumes, mass, and surface area (Beckman Coulter, Inc. 2007). As discussed by Hancock et al. (2007), this device was used to determine cell

volumes in the stock culture of *Anabaena flos-aquae*. These measurements were used to calculate nominal cell volumes which were in turn used to calculate growth rates. As noted by Hancock et al. (2007, Study page 17), this approach did not permit accurate measurements of cell counts because of the filamentous nature of *Anabaena flos-aquae*. This study has not yet been reviewed by the U.S. EPA. While the approach taken by Hancock et al. (2007) appears to be innovative and while the variability among replicates does not appear to be substantial, the method of estimating endpoints used by Hancock et al. (2007) is not part of the standard test guidelines for algae (U.S. EPA/OPPTS 1996a). Nonetheless, the study does appear to provide useful information that is consistent with the toxicity data in other species of algae. This is discussed further in Section 4.3.3.4 (Dose-Response Assessment for Aquatic Plants).

Studies on the mechanism of action of aminopyralid in aquatic plants were not identified. However, aminopyralid is assumed have the same mechanism in aquatic plants as in terrestrial plants (Section 4.1.2.4). As might be expected for a herbicide, aquatic plants are more sensitive than aquatic animals to the effects of aminopyralid. The most sensitive algal species in the acceptable studies is the diatom, *Navicula pelliculosa*, with a NOEC for cell density and biomass of 6 mg a.e./L. The least sensitive species of algae is the saltwater diatom, *Skeletonema costatum*. Similar to the NOEC values in fish, no adverse effects were noted in this species at the highest concentration tested, 120 mg a.e./L (Hoberg 2002b).

Toxicity data on aminopyralid are available for only one species of aquatic macrophyte, *Lemna gibba,* which evidenced a NOEC for frond density of 44 mg a.e./L (Hoberg 2003a). No toxicity tests are available on aquatic vascular plants that are prominently dependent on auxin growth regulators – e.g., aquatic dicots such as water milfoil and pond lilies. This may be an important limitation in assessing risk to aquatic plants given the pattern observed in terrestrial plants in which dicots are more sensitive to aminopyralid than monocots (Section 4.1.2.4.1).

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

Terrestrial animals might be exposed to any applied herbicide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation. The exposure scenarios for terrestrial species are summarized in Worksheet G01 of the EXCEL workbook that accompanies this risk assessment for the typical application rate of 0.078 lb a.e./acre. Other application rates are considered in the Risk Characterization worksheets (G02a through G07c).

In acute exposure scenarios, the highest exposure for terrestrial vertebrates involves the consumption of contaminated insects by a small bird, which could reach up to about 3 mg/kg. There is a wide range of exposures anticipated from the consumption of contaminated vegetation by terrestrial animals: central estimates range from 0.1 mg/kg for a small mammal consuming fruit to 2.1 mg/kg for a large bird with upper bound estimates of about 0.2 mg/kg for a small mammal consuming fruit and 6 mg/kg for a large bird consuming grasses. The consumption of contaminated water will generally lead to much lower levels of acute exposure – i.e., in the range of about 0.00002 to 0.007 mg/kg. A similar pattern is seen for chronic exposures. The central estimate for daily doses for a small mammal from the longer term consumption of contaminated vegetation at the application site is about 0.002 mg/kg/day, with an upper estimate of about 0.01 mg/kg/day. Dose estimates associated with the consumption of contaminated water are in the range of 0.00001 mg/kg bw/day to 0.003 mg/kg bw/day for a small mammal. Based on general relationships of body size to body volume, larger vertebrates will be exposed to lower doses than small vertebrates under comparable exposure conditions. Because of the apparently low toxicity of aminopyralid to animals, the rather substantial variations in the different exposure assessments have little impact on the assessment of risk to terrestrial animals.

For terrestrial plants, five exposure scenarios are considered quantitatively: direct spray, spray drift, runoff, wind erosion and the use of contaminated irrigation water. Unintended direct spray is expressed simply as the application rate – i.e., 0.078 lb a.e./acre for the typical application rate. For directed foliar applications, this scenario should be regarded as an extreme/accidental form of exposure that is not likely to occur in most applications. For broadcast applications, the direct spray scenario is much more plausible. Spray drift is based on estimates from AGDRIFT. The proportion of the applied amount transported off-site from runoff is based on standard GLEAMS modeling of clay, loam, and sand. The amount of aminopyralid that might be transported off-site from wind erosion is based on estimates of annual soil loss associated with wind erosion and the assumption that the herbicide is incorporated into the top 1 cm of soil. Exposure from the use of contaminated irrigation water is based on the same data used to estimate human exposure from the consumption of contaminated ambient water. All of these exposure scenarios are dominated by situational variability because the levels of exposure are highly dependent on site-specific conditions. Thus, the exposure estimates are intended to represent conservative but plausible ranges that could occur but these ranges may over-estimate or under-estimate actual exposures in some cases.

Exposures of aquatic plants and animals to aminopyralid are based on essentially the same information used to assess the exposure to terrestrial species from contaminated water. The peak estimated rate of contamination of ambient water associated with the application of aminopyralid is 0.1 (0.002 to 0.6) mg a.e./L at a normalized application rate of 1 lb a.e./acre. For longer-term exposures, estimated rate of contamination of ambient water is 0.04 (0.001 to 0.26) mg a.e./L at a normalized application rate of 1 lb a.e./acre. For the assessment of potential hazards to aquatic species, these water contamination rates are adjusted based on the application rates considered in this risk assessment.

4.2.2. Terrestrial Animals

Terrestrial animals might be exposed to any applied pesticide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation.

In the exposure assessments for the ecological risk assessment, estimates of oral exposure are expressed in the same units as the available toxicity data. As in the human health risk assessment, these units are usually expressed as mg of agent per kg of body weight and abbreviated as mg/kg for terrestrial animals. For dermal exposures to terrestrial animals, the units of exposure are expressed in mg of agent per cm^2 of surface area of the organism and abbreviated as mg/cm^2. In estimating dose, however, a distinction is made between the exposure dose and the absorbed dose. The *exposure dose* is the amount of material on the organism (i.e., the product of the residue level in mg/cm^2 and the amount of surface area exposed), which can be expressed either as mg/organism or mg/kg body weight. The *absorbed dose* is the proportion of the exposure dose that is actually taken in or absorbed by the animal. As in the human health risk assessment, all exposure scenarios for mammals are detailed in the EXCEL workbook for aminopyralid (Attachment 1: SERA EXWS 07-52-04-01b). The exposure assessments for terrestrial animals are summarized in Worksheet G01. The computational details for each exposure assessment presented in this section are provided as scenario-specific worksheets (Worksheets F01 through F16b).

Because of the relationship of body weight to surface area as well as to the consumption of food and water, small animals will generally receive a higher dose, in terms of mg/kg body weight, than large animals for a given type of exposure. Consequently, most general exposure scenarios for mammals and birds are based on a small mammal or a small bird. For small mammals, exposure assessments are conducted for direct spray (F01 and F02a), consumption of contaminated fruit (F03a, F04a, F04b), and contaminated water (F05, F06, F07). Generally, herbicide concentrations on grasses will be higher than concentrations on fruits and other types of vegetation (Fletcher et al. 1994). Although small mammals do not typically consume large amounts of grass over prolonged periods of time, small mammals like the meadow vole (*Microtus pennsylvanicus*) may consume grasses as a substantial proportion of their diet at certain times of the year. Consequently, the acute consumption of contaminated grass by a small mammal is considered in this risk assessment (F03b). Large mammals may consume grasses over a long period of time, and these scenarios are included both for acute exposures (Worksheet F10) and longer-term exposures (Worksheets F11a and F11b). Other exposure scenarios for mammals involve the consumption of contaminated

insects by a small mammal (Worksheet F14a) and the consumption of small mammals contaminated by direct spray by a large mammalian carnivore (Worksheet F16a). Exposure scenarios for birds involve the consumption of contaminated insects by a small bird (Worksheet F14b), the consumption of contaminated fish by a predatory bird (Worksheets F08 and F09), the consumption by a predatory bird of small mammals contaminated by direct spray (F16b), and the consumption contaminated grasses by a large bird (F12, F13a, and F13b).

Clearly, a very large number of other exposure assessments could be generated. The specific exposure scenarios outlined in this section are designed to identify the groups of organisms and routes of exposure of greatest concern and to serve as guides to more detailed site-specific assessments.

4.2.2.1. Direct Spray

The unintentional direct spray of wildlife during broadcast applications of herbicides is a plausible exposure scenario similar to the accidental exposure scenarios for the general public discussed in Section 3.2.3.2. In a scenario involving exposure to direct spray, the amount absorbed depends on the application rate, the surface area of the organism, and the rate of absorption.

For this risk assessment, three groups of direct spray or broadcast exposure assessments are conducted (Worksheets F01, F02a, and F02b). The first spray scenario, which is defined in Worksheet F01, involves a 20 g mammal that is sprayed directly over one half of the body surface as the chemical is being applied. This exposure assessment assumes first-order dermal absorption. The second exposure assessment (detailed in Worksheet F02a) assumes complete absorption over day 1 of exposure. This assessment is included in an effort to encompass the increased exposure due to grooming. The third exposure assessment is developed using the typical body weight of a honey bee, again assuming complete absorption of the compound. There are no exposure assessments for the direct spray of large mammals, principally because allometric relationships dictate that the amounts of a compound to which large mammal will be exposed on the basis of body weight as a result of direct spray is less than amount to which smaller mammals will be exposed on a body weight basis.

4.2.2.2. Contact with Contaminated Vegetation

As in the human health risk assessment (Section 3.2.3.3), the only approach for estimating the potential significance of dermal contact with contaminated vegetation is to assume a relationship between the application rate and dislodgeable foliar residue. Unlike the human health risk assessment in which transfer rates for humans are available, there are no transfer rates available for wildlife species. Wildlife, compared with humans, are likely to spend longer periods of time in contact with contaminated vegetation. It is reasonable to assume that for prolonged exposures an equilibrium may be reached between levels on the skin, rates of absorption, and levels on contaminated vegetation. No data regarding the kinetics of such a processes, however, are available. In the absence of such data, no quantitative assessments are made for this scenario in the ecological risk assessment.

4.2.2.3. Ingestion of Contaminated Vegetation or Prey

Since aminopyralid will be applied to vegetation, the consumption of contaminated vegetation is an obvious concern. Separate exposure assessments are developed for acute and chronic exposure scenarios involving a small mammal (Worksheets F03a, F03b, F04a and F04b), a large mammal (Worksheets F10, F11a, and F11b), and large birds (Worksheets F12, F13a, and F13b). Similarly, the consumption of contaminated insects is modeled for a small bird (Worksheet 14a) and a small mammal (Worksheet 14b). As with residues on vegetation and consistent with the approach taken by U.S. EPA/OPP-EFED (2004), the empirical relationships recommended by Fletcher et al. (1994) are used to estimate residues in contaminated insects (Worksheets F14a and F14b).

A similar set of scenarios is provided for the consumption of small mammals by either a predatory mammal (Worksheet 16a) or a predatory bird (Worksheet 16a). In addition to the consumption of contaminated vegetation, insects, and other terrestrial prey, aminopyralid may reach ambient water and fish. Thus, a separate exposure scenario is developed for the consumption of contaminated fish by a predatory bird in both acute (Worksheet F08) and chronic (Worksheet F09) exposures. Details of each scenario are given in the cited worksheets.

Since multi-route exposures (e.g., the consumption of contaminated vegetation and contaminated water) are likely, numerous exposure assessments could be developed to account for the various combinations. In the current risk assessment, such assessments are not included because, as illustrated in Worksheet G01, the predominant route of plausible exposure is the consumption of contaminated vegetation by herbivores or the consumption of prey by predators; therefore, explicit considerations of multiple routes of exposure would have no impact on the characterization of risk.

4.2.2.4. Ingestion of Contaminated Water

The methods for estimating aminopyralid concentrations in water are identical to those used in the human health risk assessment (Section 3.2.3.4). The only major differences in the estimates of exposure involve the weight of the animal and the amount of water consumed. These differences are detailed and documented in the worksheets regarding the consumption of contaminated water (F05, F06, F07).

Unlike the human health risk assessment, estimates of the variability of water consumption are not available. Thus, for the acute scenario, the only factors affecting the estimate of the ingested dose include the field dilution rates (i.e., the concentration of the chemical in the solution that is spilled) and the amount of solution that is spilled. As in the acute exposure scenario for the human health risk assessment, the amount of the spilled solution is taken as 200 gallons.

In the exposure scenario involving ponds or streams contaminated by runoff or percolation, the factors that affect the variability in exposure are the water contamination rates (Section 3.2.3.4.2) and the application rates.

4.2.3. Terrestrial Plants

In general, the primary hazard to nontarget terrestrial plants associated with the application of most herbicides is unintended direct deposition or spray drift. In addition, herbicides may be transported off-site by percolation or runoff or by wind erosion of soil.

4.2.3.1. Direct Spray

Unintended direct spray will result in an exposure level equivalent to the application rate. For many types of herbicide applications, it is plausible that some nontarget plants immediately adjacent to the application site could be sprayed directly. This type of scenario is modeled in the worksheets that assess off-site drift (see below).

4.2.3.2. Off-Site Drift

Because off-site drift is more or less a physical process that depends primarily on droplet size and meteorological conditions rather than specific properties of the compound being sprayed, estimates of off-site drift can be modeled using AgDrift. AgDrift is a model developed as a joint effort by the U.S. EPA, the Forest Service, and the Spray Drift Task Force, a coalition of pesticide registrants (SERA 2007a, Section 4.2.3.2).

For aerial applications, AgDrift permits very detailed modeling of drift based on the chemical and physical properties of the applied product (i.e., pesticide and carrier), the configuration of the aircraft, as well as wind speed and temperature. For ground applications, AgDrift provides estimates of drift based on distance downwind as well as the type of ground application: low boom spray, high boom spray, and orchard airblast. Representative estimates based on AgDrift (Version 1.16) are given in Worksheets G05a-c for low boom applications and Worksheets G06a-c for aerial applications. The estimates of drift should be regarded as little more than generic estimates similar to the water concentrations modeled using GLEAMS (Section 3.2.3.4). Actual drift will be depend on a large number of conditions depending on the site, weather, and formulation that is being applied. All of these factors cannot be considered in this general risk assessment.

While drift of droplets during backpack applications is likely to be less than any form of broadcast application, comparable methods of quantifying drift after backpack applications are not available.

4.2.3.3. Runoff and Soil Mobility

Any pesticide can be transported from the soil at the application site by runoff, sediment loss, or percolation. Runoff, sediment loss, and percolation are considered in estimating contamination of ambient water. Only runoff and sediment loss are considered in assessing off-site soil contamination. This approach is reasonable because off-site runoff and sediment transport will contaminate the off-site soil surface and could impact non-target plants. Percolation, on the other hand, represents the amount of the herbicide that is transported below the root zone and thus may impact water quality but should not affect off-site vegetation. The GLEAMS modeling used to estimate concentrations in water (Section 3.2.3.4.3) provides data on loss by runoff. These data are typically modeled for clay, loam, and sand at rainfall rates ranging from 5 inches to 250 inches per year. These data may be

used in addition to any available monitoring studies that provide estimates of runoff after defined applications.

For aminopyralid, the results of the standard GLEAMS modeling of runoff and sediment losses are summarized in Table 13. These values are also used in Worksheets G04a through G04c to estimate exposures to nontarget vegetation over the range of application rates considered in this risk assessment. As indicated in Table 13, runoff of about 1% to 5% of the applied aminopyralid from predominantly clay soils might be expected depending on rainfall rates. Much less runoff is expected from loam soils and virtually no runoff is expected from predominantly sand soils.

The amount of pesticide not washed off in runoff or sediment will penetrate into the soil column, and the depth of penetration will depend on the properties of the chemical, the properties of the soil, and the amount of rainfall. GLEAMS outputs concentrations in soil layers of varying depths. These concentrations are output by GLEAMS in mg pesticide/kg soil (ppm). The minimum non-zero value that GLEAMS will output is 0.000001 mg/kg, equivalent to 1 nanogram/kg soil or 1 part per trillion (ppt).

The deepest penetration of aminopyralid in clay, loam, and sand modeled using GLEAMS is summarized in Table 14. Based on the standard GLEAMS modeling, aminopyralid may penetrate to about 60 inches in all soil types at annual rainfall rates of 15 inches per year or more. It should be noted that the GLEAMS modeling is based on a 60 inch root zone. Thus, the actual soil penetration could be greater than 60 inches. This modeling is consistent with the assessment given in the EPA ecological risk assessment of aminopyralid: *Given its high mobility, and moderate persistence in soil, aminopyralid is likely to leach to ground water, irrespective of soil type* (U.S. EPA/OPP-EFED 2004, p. 19).

As summarized in Appendix 2, Roberts and Schelle (2004a) conducted studies on the degradation and transport of aminopyralid in soil at two sites in the United States: Greenville, Mississippi and Fresno, California. At both of these sites, very little soil penetration was noted: a maximum soil penetration to 15 inches in Mississippi over a 183 day observation period and a maximum soil penetration to about 30 inches over a 182 day observation period in California.

The U.S. EPA (U.S. EPA/OPP-EFED 2004, p. 19) has suggested that the failure to detect deeper soil penetration in the study by Roberts and Schelle (2004a) could have been due to the lack of sampling between the day of application and 8 to 9 days after application. As noted in the study by Roberts and Schelle (2004a), however, no or very little rainfall occurred over the initial 8 to 9 day period (Roberts and Schelle 2004a, Study Tables 1 and 2 in Appendix H). In addition, based on the standard GLEAMS modeling (Section 3.2.3.4.3), this sampling delay would not appear to be a plausible reason for failing to detect deeper leaching of aminopyralid. While not tabulated or otherwise detailed in this risk assessment, the standard GLEAMS modeling estimated concentrations in the lower 30 inches of the 60 inch soil column that would be in the range of about 4 ppb to 6 ppb over a six month period after the application aminopyralid under conditions similar to those in the study by Roberts and Schelle (2004a) – i.e., an application of 0.13 a.e./acre to loam at an annual rainfall rate of

50 inches. These modeled concentrations are somewhat above the limit of quantification (1.5 ppb) and well above the limit of detection (0.3 ppb) in the studies by Roberts (2004).

The most plausible sources of this inconsistency between the field data of Roberts and Schelle (2004a) and the standard GLEAMS modeling (Section 3.2.3.4.3) are the generic assumptions built into the standard GLEAMS modeling. As detailed in the documentation for standard GLEAMS exposure assessments (SERA 2004d), the standard GLEAMS runs are based on conservative assumptions of site conditions – e.g., soil characteristics, slope, and other parameters that impact runoff and percolation. In addition, the generic modeling assumes a uniform rainfall that occurs every tenth day.

To explore the potential impact of these factors on the apparent overestimates from the standard GLEAMS modeling relative to the field studies, Gleams-Driver was used to conduct simulations that might better approximate the field conditions reported by Roberts and Schelle (2004a). As discussed in SERA (2007b), GLEAMS does not precisely simulate the depth of penetration into the soil column. Instead, GLEAMS specifies concentrations in different computational soil layers to a minimum value of 0.000001 ppm (mg compound/kg soil). The maximum penetration is thus given by the deepest computational soil layer with a non-zero value.

In conducting the Gleams-Driver simulations, the aminopyralid specific properties were identical to those used in the standard GLEAMS simulations for loam soil textures (Table 4 of this risk assessment). The simulation was conducted for the Fresno, California site from the study by Roberts and Schelle (2004a) using soil specific characteristics given in Table 2 of the study by Roberts and Schelle (2004a) as well as other site specific characteristics – e.g., bare soil surface with a slope of <1%. In addition, Cligen 4.2 was used to generate site-specific weather files for Fresno, California and these were imported into Gleams-Driver and used in conducting 200 simulations at an application rate of 0.135 lb a.e./acre and an application date of April 15, identical to the application at the Fresno, California site. While Roberts and Schelle (2004a) report results to a depth of 90 cm (≈35 inches), the Gleams-Driver simulation modeled to a depth of 60 inches to detect instances of deeper soil penetration than those observed in the study by Roberts and Schelle (2004a).

The results of these simulations are illustrated in Figure 7. This figure has two components, a bar graph histogram and a line plot. The bar graph histogram gives the absolute number of simulations (left vertical axis) that resulted in the specified soil penetration depths – i.e., 0-6 inches, 6-12 inches, and so on to over 60 inches. For example, the first bar indicates that 5 of 200 simulations indicated soil penetration to no more than six inches. The line in Figure 7 indicates the cumulative relative frequency of the bars – i.e., the percent of simulations that indicated a penetration to the corresponding depth – as indicated on the right vertical axis.

As illustrated in Figure 7, the Gleams-Driver simulation estimated that the maximum depth of penetration would be no greater than 30 inches in about 40% of the simulations. These results would be consistent with the values monitored by Roberts and Schelle (2004a). Conversely, in 60% of the simulations, penetration could be deeper than 30 inches and over 20% of the simulations indicated that aminopyralid leached to or below 60 inches (the

maximum soil depth modeled in the simulation). These results are consistent with the standard GLEAMS runs and the assessment by the U.S. EPA, quoted above, that aminopyralid may be highly mobile in soil (U.S. EPA/OPP-EFED 2004, p. 19). In addition and as discussed further in Section 4.2.4 (Soil Organisms), the average soil concentrations estimated by the standard GLEAMS runs are reasonably consistent with those reported by Roberts and Schelle (2004a).

These comparisons suggest that the results of the standard GLEAMS runs are plausible and support the use of these results in this generic risk assessment. Nonetheless, these results also indicate the importance of using site-specific information to refine the exposure assessment in terms of the probability of different exposures depending on site-specific model input parameters. While it is plausible that aminopyralid may remain in the upper levels of the soil column, as observed in the study by Roberts and Schelle (2004a), it is also plausible that aminopyralid could evidence much deeper leaching into the soil column.

4.2.3.4. Contaminated Irrigation Water

Unintended direct exposures of nontarget plant species may occur through the use of contaminated ambient water for irrigation. The effects of exposure to contaminated irrigation water on nontarget vegetation have been observed for some herbicides (e.g., Bhandary et al. 1991).

The levels of exposure associated with this scenario will depend on the concentration of the pesticide in the ambient water used for irrigation and the amount of irrigation water that is applied. Concentrations in ambient water are generally based on the concentrations modeled in the human health risk assessment (Section 3.2.3.4). The amount of irrigation water that may be applied will be highly dependent on the climate, soil type, topography, and plant species under cultivation. Thus, the selection of an irrigation rate is somewhat arbitrary. Typically, plants require 0.1 to 0.3 inch of water per day (Delaware Cooperative Extension Service 1999).

In the absence of any general approach of determining and expressing the variability of irrigation rates, the application of one inch of irrigation water is used in this risk assessment. This is somewhat higher than the maximum daily irrigation rate for sandy soil (0.75 inches/day) and substantially higher than the maximum daily irrigation rate for clay (0.15 inches/day) (Delaware Cooperative Extension Service 1999).

4.2.3.5. Wind Erosion

Wind erosion is a major transport mechanism for soil (e.g., Winegardner 1996). Although no specific incidents of nontarget damage from wind erosion have been noted for aminopyralid, this mechanism is associated with the environmental transport of other herbicides (Buser 1990).

Wind erosion leading to off-site contamination of pesticides is likely to be highly site-specific. The amount of aminopyralid that might be transported by wind erosion depends on several factors, including the application, the depth of incorporation into the soil, the persistence in the soil, the wind speed, and the topographical and surface conditions of the

soil. Under desirable conditions, like relatively deep (10 cm) soil incorporation, low wind speed, and surface conditions that inhibit wind erosion, it is likely that wind transport of aminopyralid would be neither substantial nor significant.

For this risk assessment, the potential effects of wind erosion are estimated in Worksheets G07a-c. In these worksheets, it is assumed that aminopyralid is incorporated into the top 1 cm of soil. This is identical to the depth of incorporation used in GLEAMS modeling. Average soil losses are estimated to range from 1 to 10 tons/ha/year with a typical value of 5 tons/ha/year. These estimates are based on field studies conducted on agricultural sites that found that wind erosion may account for annual soil losses ranging from 2 to 6.5 metric tons/ha (Allen and Fryrear 1977).

As noted in Worksheets G07a-c, the offsite losses are estimated to reach up to about 0.014% of the application rate. Larney et al. (1999), however, report that wind erosion of other herbicides could be associated with losses up to 1.5% of the nominal application rate following soil incorporation or 4.5% following surface application. This difference appears to be at least partially due to the much higher soil losses noted by Larney et al. (1999) – i.e., up to 56.6 metric tons/ha from a fallow field. The losses reflected in Worksheets G07a-c may be somewhat more realistic for forest or rangeland applications, which will not generally be made to fallow areas. In any event, the higher offsite losses reported by Larney et al. (1999) are comparable to exposures associated with offsite drift at distances of 50-100 feet from the application site (G07a-c). All of these estimates, both for wind erosion and offsite drift, are likely to be highly variable based on site and weather conditions.

4.2.4. Soil Organisms

As discussed in Section 3.2.3.4.3, estimates of concentrations in soil as well as estimates off-site movement (runoff, sediment, and percolation) are output from GLEAMS. Based on the GLEAMS modeling, concentrations in clay, loam, and sand over a wide range of rainfall rates are summarized in Table 15 for the top 60 inches of soil and Table 16 for the top 1 foot of soil.

Peak modeled soil concentrations in the top 1 foot of soil at an application rate of 1 lb a.e./acre range from about 120 to 500 ppb. At the nominal application rate of 0.078 lb a.e./acre, the corresponding concentrations would be in the range of about 9 ppb to 39 ppb. The average modeled soil concentrations in the top 12 inches of soil at an application rate of 1 lb/acre range from about 2.6 ppb (sand at 250 inches of rainfall per year) to 320 ppb (clay at 10 inches of rainfall per year). At the nominal application rate of 0.078 lb a.e./acre, these concentration correspond to a range of about 0.2 ppb to 25 ppb.

The soil concentrations reported in the study by Roberts (2002) can be used as a crude check of these modeled estimates. As noted in Appendix 2, however, there is a relatively minor discrepancy in the reported initial concentrations in both the Mississippi and California studies – i.e., the reported initial peak concentrations are higher than the calculated nominal peak concentrations by about a factor of 2. Using the mid-point concentrations are average concentrations, Roberts (2002) reports concentrations of about 16.2 ppb for Mississippi and 1.4 ppb for California. Adjusting from the application rate 0.13 lb a.e./acre used by Roberts (2004) to the normalized application rate of 1 lb a.e./acre used in the GLEAMS model, the mid-point concentrations reported by Roberts (2002) correspond to concentrations of about 125 ppb (MI) and 10.8 ppb (CA) and these correspond to 0.125 ppm and 0.0108 ppm.

As noted in Table 16 – the concentration of aminopyralid modeled in top 12 inches of soil – the estimated concentrations in loam at an annual rainfall of 50 inches per year is about 0.033 ppm. This is almost exactly the geometric mean of the corresponding mid-point values from the study by Roberts (2002) – i.e., $(0.125 \text{ ppm} \times 0.0108 \text{ ppm})^{0.5} = 0.037$ ppm. While there conditions in the Roberts (2004) study do not precisely match those of the GLEAMS modeling, the correspondence of the modeled estimate to the measured values of aminopyralid concentrations in soil is noteworthy.

4.2.5. Aquatic Organisms

For the application of aminopyralid, the plausibility of effects on aquatic species is based on estimated concentrations of aminopyralid in water that are identical to those used in the human health risk assessment. These values are summarized in Table 12 and discussed in Section 3.2.3.4.7.

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview

The specific toxicity values used in this risk assessment are summarized in Table 17 and the derivation of each of these values is discussed in the various subsections of this dose-response assessment. The available toxicity data support separate dose-response assessments in eight classes of organisms: terrestrial mammals, birds, terrestrial invertebrates, terrestrial plants, fish, aquatic invertebrates, aquatic algae, and aquatic macrophytes. Different units of exposure are used for different groups of organisms depending on how exposures are likely to occur and how the available toxicity data are expressed. When possible, a range of toxicity values based on the most sensitive and most tolerant species within a given group of organisms are given.

For terrestrial mammals, the dose-response assessment for aminopyralid is based on the same data as the human health risk assessment (i.e., an acute gavage NOAEL of 104 mg/kg bw and a chronic dietary NOAEL of 50 mg/kg/day). In terms of acute toxicity, birds appear to be more sensitive than mammals to aminopyralid with an acute NOAEL of 14 mg a.e./kg/day from a gavage study. In terms of longer-term toxicity, however, the toxicity value for birds is 184 mg a.e./kg bw/day, somewhat higher than the corresponding value in mammals. It should be noted that the acute NOAEL for birds is lower than the chronic NOAEL for birds. This is an atypical situation. Birds appear to be much more sensitive to aminopyralid after gavage administration than after dietary administration. This difference in sensitivity results in the lower acute NOAEL (gavage) relative to the chronic NOAEL (dietary). Basing the acute NOAEL for birds on a gavage study is a conservative, and perhaps grossly conservative, approach. This is discussed further in the risk characterization.

For terrestrial invertebrates, no mortality would be expected following acute exposure to doses up to 1075 mg/kg based on direct spray studies in honey bees. Based on a single bioassay in earthworms, soil invertebrates do not appear to be sensitive to aminopyralid with a NOEC value of 5000 mg a.e./kg soil. Based on the results of a single bioassay of mixed microbial populations in soil – i.e., McMurray (2002) as discussed in Section 4.1.2.5 – no substantial effects on soil microorganisms would be expected at concentrations of up to 8.4 mg a.e./kg soil.

The toxicity of aminopyralid to terrestrial plants is relatively well-characterized. Aminopyralid is more toxic to dicots than monocots. The most sensitive species have a NOEC value of 0.00048 lbs a.e./acre based on seeding emergence studies (soil exposures) and a NOEC value of 0.0002 lb a.e./acre based on foliar exposure. Tolerant species have NOEC values of 0.11 lb a.e./acre for both soil and foliar exposures.

Aminopyralid has a low order of acute toxicity to aquatic animals, with acute NOEC values falling within a narrow range: 50 mg a.e./L for sensitive fish to 100 mg a.e./L for tolerant fish. Acute toxicity values for amphibians and aquatic invertebrates fall within this range. Algae and aquatic macrophytes are only somewhat more sensitive with NOEC values for algae in the range of 6 mg a.e./L to 23 mg a.e./L and a single NOEC of 44 mg a.e./L for an

aquatic macrophyte. The lowest aquatic toxicity value is 1.36 mg a.e./L from an egg-and-fry study in fathead minnow. Aquatic invertebrates are much less sensitive to longer-term exposures to aminopyralid with NOEC values in the range of 102 mg a.e./L to 130 mg a.e./L.

4.3.2. Toxicity to Terrestrial Organisms

4.3.2.1. Mammals

As summarized in the dose-response assessment for the human health risk assessment (Section 3.3), the Office of Pesticide Programs of the U.S. EPA used an acute NOAEL in rabbits of 104 mg a.e./kg/day (with a corresponding acute LOAEL of 260 mg a.e./kg/day based on weight loss and incoordination) as a NOAEL for deriving an acute RfD (Section 3.3.3) and a chronic NOAEL in rats of 50 mg a.e./kg/day (with a corresponding LOAEL of 500 mg/kg/day based on cecal enlargement with slight histopathology) as the basis of the chronic RfD (Section 3.3.2). For the current risk assessment, these NOAEL values are adopted as the toxicity values for mammalian wildlife.

For assessing longer-term exposures in mammalian wildlife, the Ecological Fate and Effects Division of the EPA Office of Pesticides (U.S. EPA/OPP-EFED 2005) uses dietary NOAEL of 1000 ppm of from the two-generation reproduction study in rats by Marty et al. (2003). As indicated in Appendix 3, the 1000 ppm dietary concentration corresponded to a maximum daily dose of about 218 mg/kg bw/day. While increased cecal weights were noted in this exposure, the increased weights were not accompanied by any pathological changes and were thus considered to be adaptive rather than adverse effects (Section 3.1.2.1).

The reproductive NOAEL of 218 mg/kg bw/day selected by EFED is about a factor of 4 above the chronic NOAEL in rats of 50 mg/kg bw/day. This reflects the practice in EFED risk assessments to use a reproductive NOAEL rather than a chronic NOAEL. This practice reflects the major concern in ecological risk assessment with populations rather than individuals. The NOAEL value identified by EFED is then applied only to peak exposures – i.e., it is treated as an acute NOAEL.

While the EFED approach is understandable, risk assessments conducted for the Forest Service typically take a more conservative position: The NOAEL values for both reproductive toxicity studies and chronic toxicity studies are considered and the lowest NOAEL is selected for longer-term exposures unless a compelling case can be made for doing otherwise. The reproductive NOAEL is typically used for peak exposures (as is the case in EFED risk assessments) but the chronic NOAEL is used for longer-term exposures if the chronic NOAEL is lower than the reproductive NOAEL. This is the case with aminopyralid and, for chronic exposures, the NOAEL of 50 mg/kg bw/day is used as the toxicity value.

As discussed in Section 4.1.2.1, dogs may be more sensitive to some weak acids because dogs do not excrete weak acids as efficiently as other mammals. For aminopyralid, however, the data are adequate to assert that dogs and presumably other canid species appear to be no more sensitive to aminopyralid than rodents. The subchronic NOAELs for dogs are about 177 to 282 mg a.e./kg bw/day (Stebbins and Baker 2000; Stebbins and Baker 2002 as detailed in Appendix 3). Thus, the NOAEL of 104 mg a.e./kg bw/day for rodents is applied to canids and a separate toxicity value for canids is not derived.

4.3.2.2. Birds

Acute Toxicity

For acute exposures to birds, the current risk assessment uses the acute gavage study in quail by Gallagher et al. (2003). The NOAEL from this study is taken as 14 mg a.e./kg bw. Abnormal (ruffled) appears was noted in some birds at the next higher dose, 23 mg/kg bw/day, and signs of incoordination were noted at doses of 63 mg/kg bw and above. As noted in Appendix 4 and in Section 4.1.2.2, loss of coordination was observed in one of five males at 35 minutes after a dose of 8 mg a.e./kg/day but this was not attributed to treatment by either the study authors or EFED.

This approach is substantially different from the approach taken by U.S. EPA/OPP-EFED (2005) in their ecological risk assessment of aminopyralid. For calculating acute *risk quotients* (a term used by EFED that is equivalent to *hazard quotients* in the current risk assessment), EFED used the 2250 mg a.e./kg bw dose from the Gallagher et al. (2003) as a $>LD_{50}$ value. As detailed in SERA (2007, Section 4.4 and Table 4-2), the use of LD_{50} values for risk characterization with variable levels of concern (LOC) is a common practice in ecological risk assessments performed by the U.S. EPA. For birds, the lowest LOC is 0.1 and this is applied to threatened and endangered species. In terms of the hazard quotient method used in the current risk assessment, this is equivalent to using a toxicity value of 225 mg a.e./kg bw [2250 mg a.e./kg x 0.1]. The EFED practice is not used in the current risk assessment or other risk assessments in this series because of concern for sublethal effects. For aminopyralid, basing the acute risk value on the NOAEL for sublethal effects is more conservative than the approach taken by EFED by a factor of about 16 [225 mg a.e./kg bw / 14 mg a.e./kg bw].

The route of exposure, however, is a more substantial concern in using any dose from the gavage study by Gallagher et al. (2003) for risk characterization. As noted in Section 4.2.2, all acute exposure assessments for birds involve dietary exposure – i.e., eating contaminated insects, vegetation, or fish. While there are no acute dietary studies in mammals, there are two subacute dietary studies in birds (Gallagher et al. 2001b,c) that indicate short-term oral NOAEL values of about 1669 mg a.e./kg bw/day in quail and 2360 mg a.e./kg bw/day in mallards (Section 4.1.2.2). These dose estimates from the dietary studies are based on measured food consumption values as well as measured concentrations in the diets. While there are no pharmacokinetic studies on dietary versus gavage exposure in either mammals or birds, the information that is available on the pharmacokinetics of aminopyralid in mammals strongly suggest that gavage exposures will lead to much higher peak plasma concentrations than dietary exposures (Section 3.1.3.4).

As noted in Appendix 4, the incoordination in the gavage study of aminopyralid in quail (Gallagher et al. 2003) has a rapid onset (inversely related to dose) and is rapidly reversible. This would be consistent with the incoordination being related more to peak plasma concentrations rather than average plasma concentrations (i.e., AUC or time-weighted average concentrations over the course of a day). Consequently, the use of a gavage study to characterize risks associated with acute dietary exposures could be viewed as leading to substantial (and perhaps gross) overestimates of risk.

On the other hand, dietary studies involve pre-mixing the compound in the diet prior to exposure of the test animals. The acute exposure scenarios for vegetation or contaminated insects that are used in this risk assessment, however, assume that the compound has been recently sprayed onto vegetation or insects and that the food items are rapidly consumed. While these scenarios are not equivalent to gavage, they could constitute a more severe exposure than if the compound were blended into the diet. Thus, as a conservative assumption, the gavage toxicity study will be used for the risk characterization for acute exposures.

Chronic Toxicity

The U.S. EPA (U.S. EPA/OPP-EFED 2005) used a dietary NOEC of 2523 ppm from the reproduction study in mallards by Mach (2003a). This corresponds to an approximate dose of 184 mg a.e./kg bw/day. U.S. EPA/OPP-EFED (2005) discusses concerns with the dietary reproduction study in quail (Mach 2003b) and classifies the lowest exposure level from this study, 640 ppm, as a LOAEL. The quail study is not used quantitatively by EFED, however, because the study is classified as *Supplemental* rather than *Acceptable*. A repeat of the reproduction study in quail (Temple et al. 2007) determined a nominal dietary NOEC of 2750 ppm corresponding to estimated daily doses of 203-239 mg a.e./kg bw.

As discussed in Section 4.1.2.2, the current risk assessment regards the study in quail by Mach (2003b) as a failed study. The study does not lead to a clear interpretation of whether the observed effects were caused by aminopyralid or by other uncontrolled conditions in the study. Consistent with the approach taken by U.S. EPA/OPP-EFED (2005), the study by Mach (2003b) is not used quantitatively in this risk assessment.

For the current risk assessment, the NOEC of 184 mg a.e./kg bw/day from the reproduction study in mallards is used as the toxicity value for assessing the consequences of longer-term exposures in birds. The study in mallard is selected over the repeated study in quail (Temple et al. 2007) because the NOEC from mallards is slightly lower than the NOEC from Temple et al. (2007) in quail.

This longer-term NOEC of 184 mg a.e./kg bw/day is a factor of about 13 above the acute NOEC of 14 mg a.e./kg bw that is used in this risk assessment. This is atypical. In general, acute NOEC values will be higher than longer-term NOEC. As discussed in the previous subsection, this situation arises from the very substantial differences between the results of acute toxicity values based on gavage exposures and those based on dietary exposures.

4.3.2.3. Terrestrial Invertebrates

As discussed in Section 4.1.2.3, very little information is available on the toxicity of aminopyralid to terrestrial invertebrates and the dose-response assessment for this group is uncomplicated. The NOEC of 1075 mg a.e./kg bw is honey bees is used to assess the consequence of direct spray of this species from the contact toxicity limit test by Aufderheide (2001a). A 14-day NOEC of 5000 ppm in soil is available for earthworms (Ward and Boeri 2001). As discussed further in Section 4.4.2.3, the limited information on toxicity of aminopyralid to terrestrial invertebrates limits the risk characterization for this group.

4.3.2.4. Terrestrial Plants (Macrophytes)

Aminopyralid is a herbicide and is designed to adversely affect plants, particularly dicots or broadleaf weeds. As with most herbicides, data are adequate for deriving toxicity values for both sensitive and tolerant species for both soil exposures (i.e., herbicide runoff to an untreated field) as well as for foliar exposures (direct spray, wind erosion, or drift). The available studies are discussed in Section 3.1.2.4 and summarized in Appendix 6.

For soil exposures, the most sensitive species/endpoint combination is shoot weight in soybeans (dicot) with a NOEC of 0.9 g a.i./ha, which corresponds 0.0008 lb a.i./acre or 0.00048 lb a.e./acre. The most tolerant species are all monocots (barnyard grass, corn, and wheat), all with a NOEC of 230.8 g a.i./ha, which corresponds to 0.206 lb a.i./acre or 0.11 lb a.e./acre. All data are from the seedling emergence study by Aufderheide (2004a). These values – i.e., 0.00048 lb a.e./acre for sensitive species and 0.11 lb a.e./acre for tolerant species – are used in Worksheets G04a to G04c to assess the risks to nontarget plant species from soil contamination associated with the runoff of aminopyralid from the application site.

For direct spray or drift scenarios, toxicity values are taken from the vegetative vigor study by Aufderheide (2004b). The most sensitive species/endpoint combination is shoot weight and shoot length in soybean (dicot) with a NOEC of 0.45 g a.i./ha, which corresponds to 0.0004 lb a.i./acre or 0.0002 lb a.e./acre. As with soil exposures, the most tolerant species are all monocots (barnyard grass, corn, and wheat), all with a NOEC of 230.8 g a.i./ha, which corresponds to 0.206 lb a.i./acre or 0.11 lb a.e./acre. These NOEC values are used in Worksheets G05a through G07c for characterizing risks associated with direct spray, off-site drift, and wind erosion of contaminated soil.

4.3.2.5. Terrestrial Microorganisms

As discussed in Section 4.1.2.5, the study by McMurray (2002) provides the only data that are directly useful in assessing the potential effects of aminopyralid on soil microorganisms. In this study, the only effects associated with aminopyralid concentrations of up to 8.4 mg a.e./kg soil were transient and modest increases in nitrate and total mineral nitrogen concentrations in soil. These increases were statistically significant only on Day 0 of the study – i.e., the day that the aminopyralid was applied – and no statistically significant effects were noted on Days 7, 14, and 28 of the study. As summarized in Section 4.2.4, the maximum concentrations of aminopyralid in the top 1 foot of soil at an application rate of 0.078 lb a.e./acre is about 25 ppb or 0.025 mg a.e./kg soil. This concentration is about a factor of 336 below the concentration of 8.4 mg a.e./kg soil in the study by McMurray (2002). Thus, there does not appear to be a basis for suggesting that adverse effects on soil

microorganisms are plausible. As with many other aspects of the ecological risk assessments, this risk characterization is based on only a single study for a limited number of endpoints.

4.3.3. Aquatic Organisms

4.3.3.1. Fish

Acute Toxicity Values

As discussed in Section 4.1.3.1, fish do not appear to be highly sensitive to aminopyralid and aminopyralid has been classified as practically nontoxic to fish by the U.S. EPA (U.S. EPA/OPP-EFED 2004, p. 27). In the U.S. EPA ecological risk assessment of aminopyralid, LC_{50} values of >100 mg/L in trout (Marino et al. 2001a) and >120 mg/L in sheepshead minnow (Machado 2002b) are used for risk characterization of acute exposures (U.S. EPA/OPP-EFED 2004, p. 37). The current risk assessment will use NOEC values rather than LC_{50} values for risk characterization. For aminopyralid, however, the resulting numbers are essentially identical to those of the U.S. EPA because most of the $>LC_{50}$ values used by U.S. EPA/OPP-EFED (2004) are actually NOEC values – i.e., no mortality or sublethal effects were observed.

The NOEC of 100 mg a.e./L for tolerant species is based on the acute toxicity study in bluegills (Machado 2003, MRID 46235815) and is supported by two acute toxicity studies in sheepshead minnow, one with a NOEC of 100 mg a.e./L (Machado 2002b, MRID 46235820) and the other with a NOEC of 120 mg a.e./L (Machado 2002b, MRID 46235820). The study in bluegills is classified by EFED as *Supplemental* rather than *Acceptable* because the fish used were smaller than guideline recommendations. This deviation is not very serious because using small fish would generally tend to result in lower (more conservative) rather than higher (less conservative) LD_{50} values. Both of the studies in sheepshead minnow are classified as *Acceptable*.

For sensitive species, the acute NOEC of 50 mg a.e./L is taken from the study in rainbow trout by Marino et al. (2001a, MRID 46235814) in which a partial loss of equilibrium was noted in 2/30 fish after 96 hours of exposure to 100 mg a.e./L. This effect was not seen at shorter periods of exposure (24, 48, and 72 hours) and was not seen in any control fish (n=30). The difference between control and exposed organisms is not significantly different (p-value = 0.245763 using the Fisher Exact Test) and this effect was not seen in the probe phase of the study by Marino et al. (2001a) which use small numbers of fish (5 per concentration) at concentrations of 0, 0.781, 1.56, 3.13, 6.25, 12.5, 25.0, 50.0, and 100 mg a.e./L. Both the study authors and EFED (U.S. EPA/OPP-EFED 2005) set the NOEC for the trout study at 100 mg a.e./L.

Nonetheless, the partial loss of equilibrium could be time-related. In addition, this sublethal effect could be biologically significant in salmonids and as well as other fish and incoordination has been noted in mammals after gavage dosing (Section 3.1.6). For the current risk assessment, the NOEC for this study is set at 50 mg a.e./L and this toxicity value is used for the risk characterization of sensitive species of fish after acute exposures to aminopyralid.

Longer-Term Toxicity Values

For fish, there appears to be a substantial difference between acute and chronic toxicity. The potential for chronic effects in fish is based on the available egg-and-fry/early life stage bioassay in fathead minnow by Marino et al. (2003). This is the only longer-term study in fish that is available. The U.S. EPA (U.S. EPA/OPP-EFED 2004) classified the concentration of 1.36 mg a.e./L from this study as a NOEC. This chronic value is a factor of about 37 less than the acute NOEC of 50 mg a.e./L in trout and a factor of about 73 less than the acute NOEC in bluegills.

The chronic NOEC of 1.36 mg a.e./L in fathead minnows will be applied to tolerant species of fish. Because Marino et al. (2003) is the only longer-term toxicity value that is available and because there does not appear to be any substantial differences between sensitivity in fish in acute toxicity studies, no chronic value for sensitive species is proposed. As discussed further in Section 4.4, this has no impact on the risk characterization.

4.3.3.2. Amphibians

As discussed in Section 4.1.3.2, only one study is available on amphibians, the acute limit test in the northern leopard frog larvae (Henry et al. 2003a). The NOEC from this study, 95.2 mg a.e./L, is used to assess the consequence of acute exposures to amphibians. There are no data for proposing a chronic toxicity value for amphibians. This is discussed further in the risk characterization (Section 4.4).

4.3.3.3. Aquatic Invertebrates

Acute Values

The acute toxicity values for aquatic invertebrates are similar to those of fish and amphibians and there is no indication of substantial differences in sensitivity among invertebrates based on acute toxicity. For the characterization of risk from acute exposures, mollusks are considered as the sensitive species based on a NOEC of 89 mg a.e./L (Cafarella 2002) and daphnids are considered as a tolerant species with a NOEC of 98.6 mg a.e./L (Marino et al. 2001b). As discussed in Section 4.1.3.3, these differences were sufficient for the U.S. EPA to give different toxicity classifications to mollusks (*slightly toxic*) and freshwater invertebrate (*practically non-toxic*). This difference, however, simply reflects the lower concentrations used in the mollusk study. While the difference between mollusks and daphnids is insubstantial, the different values for sensitive and tolerant invertebrates is maintained in this risk assessment to reflect the different classifications given by the U.S. EPA (U.S. EPA/OPP-EFED 2004). These differences have no impact on the risk characterization (Section 4.4).

Longer-Term Toxicity Values

Unlike the case with fish, there is no indication that aminopyralid is more toxic to aquatic invertebrates as the duration of exposure increases. Chronic reproduction studies are available in two species of aquatic invertebrates: daphnids (Henry et al. 2003b) and midges (Putt 2002). These studies yield similar results. The NOEC in the daphnid study was 102 mg a.e./L based on measured concentrations. The NOEC in the midge study is 130 mg a.e./L based on mean measured test concentrations in the water column and NOEC at 82 mg a.e./L based on concentrations in pore water – i.e., interstitial water in the sediment. In terms of

risk characterization, this risk assessment uses concentrations of aminopyralid in the water column and 130 mg a.e./L could be selected as the longer-term toxicity value for tolerant species. This is not substantially different, however, from the daphnid NOEC of 102 mg a.e./L. Consequently, the daphnid NOEC of 102 mg a.e./L will be the only toxicity value used for assessing longer-term risks to aquatic invertebrates.

It should be noted that the longer-term daphnid NOEC of 102 mg a.e./L is slightly greater than the acute daphnid NOEC of 98.6 mg a.e./L. This is just the consequence of different measured values for the same nominal concentration of 100 mg a.e./L.

4.3.3.4. Aquatic Plants

Aquatic plants are often much more sensitive to herbicides than aquatic animals. While aquatic plants do appear to be somewhat more sensitive than aquatic animals to aminopyralid in terms of acute toxicity values, the differences are not remarkable. Because of the short lifespan of aquatic algae, separate chronic values for this group are not derived. It is noteworthy, however, that the acute values for aquatic plants studies that the U.S. EPA classified as *Acceptable* are substantially above the chronic toxicity value for fish – i.e., 1.36 mg a.e./L as discussed in Section 4.3.3.1.

The lowest reported effect level for any aquatic plant is 1 mg a.e./L. This is from the study by Hoberg (2002c) in a blue-alga (*Anabaena flos-aquae*) in which the concentration of 1 mg a.e./L was associated with 47% inhibition in biomass. As discussed in Section 4.1.3.4, however, this study appears to be flawed (possibly due to the technique used to break cell filaments prior to counting) and the EFED DER classifies Hoberg (2002c) as ***Unacceptable*** because of high variability in the controls. Consequently, this study is not used in the EFED risk assessment (U.S. EPA/OPP-EFED 2005). This decision seems appropriate and the Hoberg (2002c) study is not used quantitatively in this current risk assessment.

Excluding the study by Hoberg (2002c), the most sensitive aquatic plant is taken as the diatom (*Navicula pelliculosa*), with a NOEC of 6 mg a.e./L for the most sensitive endpoint (cell density) (Hoberg 2002a). The NOEC of 23 mg a.e./L for tolerant species of algae is taken from the study by Hoberg (2003b) and is based on the NOEC for all endpoints (i.e., cell density, biomass, and growth rate) in the green alga, *Pseudokirchneriella subcapitata*. All of these studies were reviewed by the U.S. EPA and were classified as *Acceptable*. As discussed Section 4.1.3.4, a more recent study on *Anabaena flos-aquae* has been conducted by Hancock et al. (2007). This study has not yet been reviewed by the U.S. EPA. As indicated in Appendix 9, the NOEC value for *Anabaena flos-aquae* from the study by Hancock et al. (2007) is 11.6 mg/L for the most sensitive endpoint. This NOEC is between the NOEC values used for sensitive and tolerant species. Thus, the eventual status of this study in terms of the review by the U.S. EPA does not impact the selection of toxicity values that are used in the current risk assessment.

Only one study is available on aquatic macrophytes, the study on duckweed (*Lemna gibba*) by Hoberg (2003a). The NOEC of 23 mg a.e./L applies to all endpoints – i.e., 14-day cell density, 14-day biomass, and 7-day growth rate.

4.4. RISK CHARACTERIZATION

4.4.1. Overview

Aminopyralid is an effective herbicide that is designed to damage certain types of terrestrial plants, particularly broadleaf weeds. Consequently, nontarget plants that are similar to target species in sensitivity to aminopyralid may also be adversely affected by aminopyralid applications. Aminopyralid is selective to the extent that dicots (broadleaf plants) are much more sensitive to aminopyralid than monocots (e.g. grasses). Consequently, some nontarget dicots that are directly sprayed with aminopyralid at or near effective application rates are likely to be adversely affected. Direct spray scenarios for sensitive species of plants result in risk quotients in the range of 150 to 550 over application rates from 0.03 lb a.e./acre to 0.11 a.e./acre. For all forms of broadcast applications, the direct spray scenario seems plausible and relevant. The direct spray of nontarget species could be much less likely in directed foliar applications (e.g., backpack). Of the indirect exposure scenarios (i.e., drift, runoff, and wind erosion), drift appears to present the highest potential risks to sensitive species of plants. At distances from about 25 feet to about 300 feet downwind, hazard quotients for sensitive plant species are in the range of about 2 to 10 for ground applications and 2 to about 80 for aerial applications. Except in areas that are highly susceptible to runoff such as hard packed and predominantly clay soils, offsite losses associated with runoff do not appear to pose a substantial risk. Similarly, risks associated with transport of the herbicide by wind erosion appear to be insubstantial. All of the individual exposure scenarios for nontarget vegetation could be highly variable depending on a large number of site-specific considerations.

There is no indication that other groups of organisms will by adversely affected by aminopyralid. These groups include tolerant species of terrestrials plants (such a grasses), aquatic plants (algae or macrophytes), mammals, birds, aquatic or terrestrial invertebrates, terrestrial microorganisms, fish, and amphibians.

As with all ecological risk assessments, the current risk assessment is based on tests in only a limited number of species and under conditions that may not well-represent populations of free-ranging nontarget species. For some groups of organisms including soil microorganisms and amphibians, this limitation is severe in that the available information is sparse and not well-suited to quantitative risk assessment. In other groups of organisms, there are uncertainties in the application of the different types of information that are available for the characterization of risk. These uncertainties are particularly evident in the assessment of potential risks to birds in which the current risk assessment takes an extremely conservative approach in the application of gavage toxicity data to the assessment of risks from dietary exposures.

An additional factor in tempering the risk characterization for aminopyralid involves the nature of the available data. All of the information on the toxicity of aminopyralid comes from studies that have been submitted to the U.S. EPA in support of aminopyralid registration. While these studies have been reviewed and the bulk of these studies appear to have been appropriately designed, conducted and reported, the available information on

aminopyralid is much less diverse than the information that is available on herbicides that have been used for many years and for which the open literature is rich and varied. This situation will exist for any new herbicide.

4.4.2. Terrestrial Organisms

4.4.2.1. Mammals

The risk characterization for mammals is simple and unambiguous: there is no basis for asserting that adverse effects are plausible in large or small mammals. Over the range of application rates and over the range of the estimated exposures, the hazard quotients for mammals range from 0.00001 (the lower bound for direct spray of a small mammal assuming first-order absorption at an application rate of 0.03 lb a.e./acre) to 0.07 (the consumption of contaminated insects by a small mammal after an application of 0.11 lb a.e./acre). This range is below the level of concern (1.0) by factors of about 14 to 100,000.

Because all hazard quotients are well below the level of concern, this discussion will focus only on a comparison of the upper bound estimates of the hazard quotients at the highest application rate that might be used in Forest Service or NPS programs.

For acute exposure scenarios, the highest hazard quotients all involve the consumption of contaminated vegetation or prey and these range from 0.003 for the consumption of contaminated fruit by a small mammal to 0.07 for the consumption of contaminated insects by a small mammal. The hazard quotients for the direct spray scenarios range form 0.001 (assuming first-order dermal absorption) to 0.03 (assuming 100% absorption). Acute hazard quotients involving contaminated water are substantially lower, ranging from 0.00009 (expected peak concentrations) to 0.007 (accidental spill).

For chronic exposures, the highest hazard quotients involve the consumption of contaminated plant material and these hazard quotients range from 0.000006 (a small mammal consuming fruit sporadically in the treatment area) to 0.03 (a large mammal consuming grasses exclusively inside of the treatment area). These are below the level of concern by factors of about 33 to over 160,000. Also as in the acute exposure scenarios, the hazard quotient associated with the consumption of contaminated water is very low, 0.00008, and below the level of concern by a factor of 12,500.

This risk characterization for mammals is consistent with the risk characterization presented by the U.S. EPA, which found no basis for asserting that adverse effects in mammals are plausible. The maximum risk quotient derived by the U.S. EPA is 0.02 – a small mammal consuming short grasses (U.S. EPA/OPP-EFED 2004, Table 31, p. 39) – only modestly less than the maximum hazard quotient of 0.07 derived in the current risk assessment. This modest difference is due to the range of values for food consumption used in the current risk assessment.

The application of any effective herbicide, including aminopyralid, is likely to alter terrestrial vegetation. This alteration is likely to lead to some secondary changes that could impact

mammals – e.g., changes in food availability and habitat quality. These secondary effects are likely to vary over time and vary among different species of mammals.

4.4.2.2. Birds

The risk characterization for birds is similar to that of mammals in that no hazard quotients exceed the level of concern (1.0). Unlike the case with mammals, however, the upper bound of the acute hazard quotients approach a level of concern at the highest application rate – i.e., a hazard quotient of 0.6 for a large bird consuming contaminated grasses and a hazard quotient of 0.9 for a small bird consuming contaminated insects.

As discussed in Section 4.3.2.2, the acute NOEC for birds is 14 mg a.e./kg bw/day from the gavage study in quail by Gallagher et al. (2003) and this NOAEL is a factor of about 120 to 170 below dietary NOEC values in birds which are equivalent to short-term oral NOAEL values of about 1669 mg a.e./kg bw/day in quail and 2360 mg a.e./kg bw/day in mallards. In this situation, the use of a gavage toxicity value to assess hazards from dietary exposures may be considered extremely conservative.

While this conservative approach is acknowledged, the approach is maintained in this risk assessment because of the lack of any field studies on the potential effects of aminopyralid on birds. As discussed in Section 1, aminopyralid is a new herbicide with no published literature and no field studies. In addition, the laboratory dietary studies all involve pre-mixing aminopyralid in the diet of the birds prior to exposure. The acute scenarios considered in this risk assessment assume that the birds consume the dietary items on the day of application. While this is not intended as the sole rationale for the selection of a gavage toxicity value over a dietary value, it is a factor that should be considered.

Conversely, the available pharmacokinetic studies in mammals suggest differences of at least a factor of 6 between peak plasma levels after gavage exposure relative to dietary exposure (Section 3.1.3.4). Because of the way that the absorption coefficient was estimated in the analysis given in Section 3.1.3.4 – i.e., the use of the observed peak plasma concentration as the true peak plasma concentration – it is likely that the oral absorption rate and hence the differences in peak plasma concentrations were underestimated. Thus, it is plausible that factors of 120 to 170 noted above (i.e., the differences between dietary and gavage NOAEL values) would reflect both the plausible differences in kinetics and the impact of mixing. The use of the dietary NOEC values in deriving hazard quotients could represent a better estimate of plausible risk. While this is acknowledged, it does not offset the uncertainties associated with the lack of field studies in birds.

As with mammals, hazard quotients for the longer-term exposure scenarios for birds are very low: a maximum hazard quotient of 0.01 for a large bird consuming contaminated vegetation and 0.00001 for a fish-eating bird consuming contaminated fish.

Qualitatively, the risk characterization for birds given in this risk assessment is similar to that given by the U.S. EPA (U.S. EPA/OPP-EFED 2004, Table 29, p. 38) in that none of the hazard quotients (referred to as *risk quotients* by EFED) exceed a level of concern. The U.S. EPA, however, uses the gavage LD_{50} values rather than the acute gavage NOAEL values for

calculating acute risk quotients – i.e., the LD_{50} of >2250 mg a.e./kg bw rather than the NOAEL of 14 mg a.e./kg bw (Section 4.3.2.2). Thus, all of the risk quotients for birds calculated by the U.S. EPA are at or below 0.01.

As discussed above, the more substantial uncertainty in the risk characterization for birds is the use of any gavage toxicity values rather than dietary toxicity values for deriving hazard quotients.

As with mammals, secondary effects on some species of birds may occur through changes in vegetation that may impact food availability and habitat. These effects may be beneficial to some species of birds and detrimental to others. The magnitude of any secondary effects are likely to vary over time. Again, there are no field studies on aminopyralid that could be used to further characterize potential secondary effects.

4.4.2.3. Terrestrial Invertebrates

Information on the toxicity of aminopyralid to terrestrial invertebrates is limited to acute bioassays in honeybees (Aufderheide 2001a,b) and earthworms (Ward and Boeri 2001). Based on this information, there is no indication that adverse effects on terrestrial invertebrates are likely.

As indicated in Worksheet G03c, the highest hazard quotient for the honeybee is 0.02, below the level of concern by a factor of 50. The one bioassay on earthworms indicated a NOEC of 5000 ppm (mg a.e./kg soil). The maximum plausible concentration in the top 12 inches of soil at an application rate of 1 lb a.e./acre is about 0.5 ppm (Table 12). For the maximum application rate of 0.11 lb a.e./acre, this would correspond to a concentration of about 0.05 ppm. This is below the NOEC by a factor of about 100,000. This very large difference between the NOEC and plausible levels of exposure indicates inconsequential risks to earthworms.

As with most pesticide risk assessments and virtually all herbicide risk assessments, there is a great difference between the number nontarget species, in this case the number terrestrial invertebrate species, and the number of species on which data are available. This is true even for very well-studied herbicides such as 2,4-D (SERA 2006a). This places obvious limitations on the risk characterization for this group of organisms. Nonetheless, based on the information that is available, there is no basis for asserting that toxic effects in terrestrial invertebrates are likely based on plausible exposures to aminopyralid.

In addition to the above considerations, aminopyralid is an effective herbicides and applications of aminopyralid will affect vegetation, target species and possibly nontarget species (Section 4.4.2.3) and this may lead to secondary effects on terrestrial invertebrates. The extent with which secondary effects would be regarded as beneficial or detrimental is speculative and would probably vary among different groups and species of terrestrial invertebrates.

4.4.2.4. Terrestrial Plants

A quantitative summary of the risk characterization for terrestrial plants is presented in Worksheets G04a-c for runoff, Worksheets G05a-c for drift after low boom ground applications, Worksheets G06a-c for drift after aerial applications, and Worksheets G07a-c for off-site contamination due to wind erosion. As with the worksheets for terrestrial animals, the a-c designations represent groups of three worksheets for the typical application rate (a), the lowest anticipated application rate (b), and the highest anticipated application rate (c). Also analogous to the approach taken for terrestrial animals, risk in these worksheets is characterized as a ratio of the estimated exposure to a toxicity value (i.e., exposure associated with a defined response).

The toxicity values for aminopyralid are all NOAEC values, as derived in Section 4.3.2.4, for both sensitive and tolerant species. As noted in Section 4.3.2.4, aminopyralid is much more toxic to dicots (e.g., broadleaf plants) than to monocots (e.g., grasses) and these differences are reflected in the risk quotients for sensitive and tolerant plant species. For tolerant species such as grasses, no adverse effects would be anticipated even if the tolerant species are directly sprayed. Thus, no effects are anticipated in tolerant species in any of the exposure scenarios even at the highest application rates.

Nontarget species of dicots, however, are likely to evidence adverse effects over the entire range of application rates that are considered in this risk assessment. This is a very common pattern for herbicides. While some herbicides such as aminopyralid may be generally selective to different groups of plants such as monocots or dicots, herbicides that are effective in controlling a particular groups of weeds (target species) are likely to cause adverse effects in related nontarget plant species.

As indicated in Section 4.3.2.4, there is relatively little difference in the toxicity of aminopyralid to sensitive dicots in terms of soil exposures (i.e., a seedling emergence NOAEC of 0.00048 lb a.e./acre) versus foliar exposures (i.e., a vegetative vigor NOAEC of 0.0002 lb a.e./acre). Consequently, the differences in hazard quotients among the different exposure scenarios (runoff, drift, and wind erosion) reflect differences primarily in the amount of exposure.

Direct spray and spray drift appear to be the exposures of primary concern. Direct spray – i.e., a distance of zero from the treated field in Worksheets G05a-c and G06a-c – leads to risk quotients in the range of 150 to 550 over application rates from 0.03 lb a.e./acre to 0.11 a.e./acre. Based on the standard drift exposure assessments (Section 4.2.3.2), the hazard quotients exceed the level of concern (1.0) across the range of application rates considered in this risk assessment at distances from the application site of 100 feet for ground application (Worksheets G05a-c) and 300 feet for aerial application (Worksheets G06a-c). The exceedances range from modest (about a factor of 2) to substantial (about a factor of 10 to 80 at the highest application rate after ground and aerial application respectively). These risk quotients for direct spray are very similar to those developed by the U.S. EPA (U.S. EPA/OPP-EFED 2004, Table 33, p. 41) – i.e., risk quotients of about 20 to 528 for direct spray and <1 to about 50 for offsite drift.

Adverse effects on sensitive nontarget plant species associated with runoff appear to a concern only in areas with high runoff potential. Based on the standard GLEAMS modeling (Section 4.2.3.3), hazard quotients exceed the level of concern only for sites with clay soils and the exceedances are modest, factors of somewhat over 1 in relatively arid regions at the lowest application rate to about 11 in regions with heavy rainfall at the highest application rate (Worksheets G04a-c). The hazard quotients associated with erosion of contaminated soil by wind are all substantially below the level of concern with a maximum hazard quotient of 0.0001 (Worksheet G07c).

As stressed in the exposure assessment for terrestrial plants (Section 4.2.3), all of the exposure scenarios except for direct spray are generic and actual exposures would be influenced by site-specific conditions during or after application. This type of variability cannot be well encompassed by the general exposure assessments given in Section 4.2.3 and used here to characterize risk. Nonetheless, it seems likely that drift will be the most critical factor in controlling potential adverse effects on nontarget plant species.

4.4.2.5. Soil Microorganisms

As noted in Section 4.3.2.5, concentrations of aminopyralid in soil of up to 8.4 mg a.e./kg soil have not been associated with any significant effects in mixed soil microorganism other than 12% to 15% increases in nitrate and total mineral nitrogen on the first day of the 28-day study (McMurray 2002).

4.4.3. Aquatic Organisms

4.4.3.1. Fish

Based on the available toxicity studies and the worst-case exposure assessments developed in this risk assessment, there is no basis for suggesting that adverse effects in fish are plausible (Worksheets G03a-c). The highest hazard quotient is 0.1, below the level of concern by a factor of 10. The hazard quotient of 0.1 is the upper bound associated with sensitive species of fish after the scenario for an accidental spill of aminopyralid into a small pond (3.2.3.4.1) at the highest application rate (Worksheet G03c). The upper bounds of the hazard quotients for fish associated with expected (i.e., non-accidental) concentrations of aminopyralid in water after applications at the highest labeled rate are in the range of 0.002 to 0.02, below the level of concern by factors of about 50 to 500.

While the U.S. EPA derived somewhat different risk quotients based on somewhat different estimates of expected environmental concentrations and toxicity values (U.S. EPA/OPP-EFED 2004, Table 26, p. 37), the U.S. EPA reached a qualitatively similar conclusion: no risks to fish are identified and all risk quotients derived by EFED (equivalent to hazard quotients in this current risk assessment) are less than 0.01.

4.4.3.2. Amphibians

The only information on amphibians is a NOEC of 95.5 mg a.e./L from a single acute limit test on northern leopard frog larvae (Henry et al. 2003a). This value is very similar to the 100 mg a.e./L for tolerant species of fish and hence the hazard quotients for amphibians

given in Worksheets G03a-c are similar to those in fish and do not exceed or approach a level of concern.

As noted at the start of this risk characterization (Section 4.4.1), the number species on which toxicity data are available is very small relative to the number of nontarget species which might be exposed to aminopyralid. For amphibians, this limitation is severe. In addition, the nature of the data – i.e., a single acute limit test – is less substantial than the data that are available on fish. Lastly, it should be noted that the hazard quotients for longer-term exposures are calculated as the ratio of the estimated longer-term concentrations to acute NOEC because no longer-term data are available. While a *surrogate* longer-term NOEC could have been developed using the data on fish (SERA 2007A, Section 4.3.4), this would have no impact on the risk characterization in terms of the classification of hazard quotients.

For these reasons, the risk characterization for amphibians is particularly weak. In plain language, the most that can be said is that the very limited acute toxicity data on amphibians indicate that leopard frog larvae are no more sensitive to aminopyralid than fish.

4.4.3.3. Aquatic Invertebrates

The risk characterization for aquatic invertebrates is very similar to that for fish and is based on similar data – i.e., acute toxicity studies as well as longer-term toxicity studies. Based on acute toxicity values, invertebrates are about as sensitive to aminopyralid as are fish – i.e., invertebrate NOEC values of 89 to about 100 mg a.e./L compared to corresponding values in fish of 50 to 100 mg a.e./L. As discussed in Section 4.3.3.3, however, there is no indication that the duration of exposure impacts the toxicity of aminopyralid to aquatic invertebrates. In other words, the acute and longer-term toxicity values in aquatic invertebrates are virtually identical. Consequently, the longer-term risk quotients for aquatic invertebrates are lower than those for fish.

The highest hazard quotient for aquatic invertebrates is 0.06, below the level of concern by a factor of about 17. As with fish, this hazard quotient is associated with the accidental spill of aminopyralid into a small pond (3.2.3.4.1) at the highest application rate (Worksheet G03c). The upper bounds of the hazard quotients for aquatic invertebrates associated with expected (i.e., non-accidental) concentrations of aminopyralid in water after applications at the highest labeled rate are in the range of 0.0003 to 0.0007, below the level of concern by factors of about 1,400 to 3,300.

While many more species of aquatic invertebrates will be exposed to aminopyralid compared to the number of species on which data are available, the species that have been tested are at least somewhat varied and longer-term toxicity data are available on two species. Given the extremely large differences between plausible levels of exposure and NOEC values from well-conducted studies, concern for aquatic invertebrates is not substantial.

As with the risk characterization for fish, the risk characterization for aquatic invertebrates given in the current risk assessment is consistent with the conclusions of the EPA (U.S. EPA/OPP-EFED 2004, Table 27, p. 37) in which all risk quotients are below 0.01.

4.4.3.4. Aquatic Plants

The qualitative risk characterization for aquatic plants is not substantially different from that for fish or aquatic invertebrates. This follows directly from the toxicity values for aquatic plants, which are only slightly lower than those for fish and aquatic invertebrates (Section 4.3.3.4).

As with other aquatic organisms, none of the hazard quotients for aquatic plants exceed the level of concern (1.0). The upper bound of the risk quotient for sensitive species of algae is associated with the accidental spill scenario and is 0.8 at the highest application rate (Worksheet G03c). The upper bounds of the risk quotients for corresponding non-accidental scenarios – i.e., exposures based on concentrations in water that might be expected after application of aminopyralid at the maximum labeled rate – for sensitive species of algae are in the range of 0.005 to 0.01, below the level of concern by factors of about 100 to 200. The risk quotients for aquatic macrophytes are based on only a single bioassay. Nonetheless, these hazard quotients range from 0.0007 to 0.002, below the level of concern by factors of 500 to over 1,400. Thus, based on the available information, there is no basis for asserting that adverse effects in aquatic plants (algae or macrophytes) are likely.

This conclusion is concordant with the conclusions reached by the U.S. EPA in their ecological risk assessment of aminopyralid (U.S. EPA/OPP-EFED 2005, Table 28, p. 38). In their risk characterization, the U.S. EPA does note the unacceptable study by Hoberg (2002c) on *Anabaena flos-aquae* and further notes the important role of cyanobacteria in aquatic ecosystems (U.S. EPA/OPP-EFED 2005, Table 28, p. 42). The more recent study in *Anabaena flos-aquae* (Hancock et al. 2007), however, addresses many of the concerns with the Hoberg (2002c) study raised by the U.S. EPA (Section 4.1.3.4) and the newer study suggests that the sensitivity of *Anabaena flos-aquae* to aminopyralid is intermediate relative to the most sensitive and tolerant species.

As discussed in Section 4.1.3.4, the toxicity data on aquatic macrophytes is limited to the single bioassay on duckweed, a monocot. No data are available on the toxicity of aminopyralid to aquatic dicots. This limitation is noteworthy because of the pattern of toxicity in terrestrial plants in which terrestrial dicots are more sensitive to aminopyralid than terrestrial monocots.

5. REFERENCES

Abou-Waly H; Abou-Setta MM; Nigg HN; Mallory LL. 1991a. Growth response of freshwater algae, *Anabaena flos-aquae* and *Selenastrum capricornutum* to atrazine and hexazinone herbicides. Bull. Environ. Contam. Toxicol. 46: 223-229.

Abou-Waly H; Abou-Setta MM; Nigg HN; Mallory LL. 1991a. Dose-response relationship of *Anabaena flos-aquae* and *Selenastrum capricornutum* to atrazine and hexazinone using chlorophyll (a) content and 14C uptake. Bull. Environ. Contam. Toxicol. 46: 223-229.

Ahlstrom O; Skrede A. 1998. Comparative Nutrient Digestibility in Dogs, Blue Foxes, Mink and Rats. J Nutrition. 128(12): 2676S-2677S.

Allen RR; Fryrear DW. 1977. Limited tillage saves soil, water, and energy. ASAE Annual Meeting, NC State Univ., Raleigh, NC. June 26-29, 1977. 14 pp.

ATSDR (Agency for Toxic Substances and Disease Registry). 2002. Exposure Investigation Spring Valley Neighborhood. Available at: http://www.atsdr.cdc.gov/sites/ springvalley/mar02ei.html.

Aufderheide J. 2001a. XDE-750: Acute Contact Toxicity Test with the Honeybee, *Apis mellifera*. Project Number: 46595, 011044. Unpublished study prepared by Analytical Bio-Chemistry Labs, Inc. 17 p. MRID No. 46235831.

Aufderheide J. 2001b. XDE-750: Acute Oral Toxicity Test with the Honeybee (*Apis mellifera*). Project Number: 46596, 011045. Unpublished study prepared by Analytical Bio-Chemistry Labs, Inc. 20 p. MRID No. 46235832.

Aufderheide J. 2004a. Effect of GF-871 on Seedling Emergence and Growth of Selected Non-Target Terrestrial Plants (Tier II). ABC Laboratories, Inc., Columbia, Missouri. ABC Study No. 48322. Dow Study No. 030066. Report dated Jan. 21, 2004. (Not in bibliography from FOIA) MRID No. 46235824.

Aufderheide J. 2004b. Effect of GF-871 on Vegetative Vigor of Selected Non-Target Terrestrial Plants (Tier II). ABC Laboratories, Inc., Columbia, Missouri. ABC Study No. 48323. Dow Study No. 030066. Report dated Jan. 21, 2004. (Not in bibliography from FOIA) MRID No. 46235825.

Bachmann S; Dawnay AB; Bouby N; Bankir L. 1991. Tamm-Horsfall protein excretion during chronic alterations in urinary concentration and protein intake in the rat. Renal Physiol. 14: 236-245.

Bachmann S; Mutig K; Bates J; Welker P; Geirst B; Gross V; Luft FC; Alenina N; Bader M; Thiele BJ; Prasadan K; Raffi HS; Kumar S. 2005. Renal effects of Tamm-Horsfall protein (uromodulin) deficiency in mice. Am. J. Renal Physiol. 288: F559-F567.

Bargar E; Dybowski J. 2004. XDE-750 in or on Grass Forage, Grass Hay, Wheat Commodities, Milk, Cattle Meat and Meat By-Products: Section E, F, and G of the Petition for Permanent Tolerances and FQPA Assessment. Project Number: GH/C/5714. Unpublished study prep& by Dow AgroSciences LLC.79 p. MRID No. 46235724.

Beckman Coulter, Inc. 2007. Product Description for Multisizer 3 Coulter Counter. Available at: http://www.beckmancoulter.com/products/instrument/partChar/pc_multisizer3.asp

Beard R. 2007. Rita Beard, Invasive Plant Coordinator, National Park Service, Natural Resource Program Center, Fort Collins, CO. Comments on: SERA TR-052-04-01a: Aminopyralid, Human Health and Ecological Risk Assessment, (Introduction, Program Description, Citations), dated January 3, 2007. Comments and EXCEL spreadsheet on aminopyralid use dated 1/30/07.

Bertram TA. 1991. Gastrointestinal Tract, Chapter 12 in: Handbook of Toxicologic Pathology, W. M. Haschek and C. G. Rousseaux, eds., pp. 195-251. Academic Press, San Diego, CA.

Bhandary RM; Whitwell T; Briggs J. 1997. Growth of containerized landscape plants is influenced by herbicide residues in irrigation water. Weed Technol. 11 (4):793-797.

Bjorn AT. 2003. GF-871: An oral developmental toxicity study in Sprague Dawley rats. Charles River Laboratories, Inc. Discovery and Development Services, Spencerville, OH. Dow Study ID 031142. Charles River Study ID 3504.344. Unpublished. (Not in U.S. EPA/OPP bibliography from FOIA) MRID No. 46235631.

Boorman GA; Eustis SL; Elwell MR; Montgomery CA; MacKenzie WF. 1990. Pathology of the Fisher Rat. Academic Press, Inc. San Diego, CA. 580 pp.

Bressant J. 2007. Letter to Patrick Durkin, SERA Inc., in response to Freedom of Information Act Request HQ RIN 1454-06. Letter dated January 22, 2007.

Brooks K. 2001a. XDE-750: Acute Oral Toxicity Study in Fischer 344 Rats. Project Number: 011115. Unpublished study prepared by Dow Chemical Co. 47 p. MRID No. 46235603.

Brooks K. 2001b. DE-750: Acute Eye Irritation Study in New Zealand White Rabbits. Project Number: 011118.Unpublished study prepared by Dow Chemical Co. 17 p. MRID No. 46235609.

Brooks K. 2001c. XDE-750: Acute Dermal Irritation Study in New Zealand White Rabbits. Project Number: 011117. Unpublished study prepared by Dow Chemical CO.15 p. MRID No. 46235611.

Brooks KJ; Radtke BJ. 2002a. GF-871: Acute Eye Irritation Study In New Zealand White Rabbits. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 021098. (Not in EPA bibliography from FOIA.) MRID No. 46235610.

Brooks KJ; Radtke BJ. 2002b. GF-871: Acute Dermal Irritation Study In New Zealand White Rabbits. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 021099. (Not in EPA bibliography from FOIA.) MRID No. 46235612.

Brooks K; Yano O. 2001. XDE-750: Acute Dermal Toxicity Study in Fischer 344 Rats. Project Number: 011116 - Unpublished study prepared by Dow Chemical Co. 43 p. MRID No. 46235605.

Bulkin S. 2007. Steve Bulkin, R6 Regional Office, Team Leader, Invasive Plant FEIS/ROD Implementation, and Training Cadre, USDA/Forest, Medford, Oregon. Comments on: SERA TR-052-04-01a: Aminopyralid, Human Health and Ecological Risk Assessment, (Introduction, Program Description, Citations), dated February 13, 2007.

Buser HR. 1990. Atrazine and other s-triazine herbicides in lakes and in rain in Switzerland. Environ. Sci. Technol. 24(7): 1049-1058.

Cafarella M. 2002. XDE-750 - Acute Toxicity to Eastern Oysters (*Crassostrea virginica*) Under Flow-Though Conditions. Project Number: 12550/6189, 011268. Unpublished study prepared by Springborn Laboratories Inc. 53 p. MRID No. 46235818.

California EPA (California Environmental Protection Agency). 2006. Summary Of Toxicology Data on Aminopyralid. Chemical Code # 5927. Department Of Pesticide Regulation, Medical Toxicology Branch, Report dated May 3, 2006. Available at: http://www.cdpr.ca.gov/docs/toxsums/pdfs/5927.pdf

CambridgeSoft Corporation. 2007. ChemFinder.com Database. Available at: http://chemfinder.cambridgesoft.com.

Cantrell RL; Hyland JR. 1985. Application techniques. In: A Guide to Silvicultural Herbicide Use in the Southern United States. Auburn University School of Forestry, Alabama Agricultural Experiment Station. November 1985. 612 pp.

Carney E; Tornesi B. 2001. XDE-750: Oral Gavage Developmental Toxicity Study in CD Rats. Project Number: 011061. Unpublished study prepared by The Dow Chemical Co. 458 p. MRID No. 46235629.

Carney EW; Tornesi B. 2004a. GF-871: Oral gavage developmental toxicity study in New Zealand White rabbits. Toxicology and Environmental Research and Consulting. The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 031142; Unpublished. (Not in U.S. EPA/OPP bibliography from FOIA.) MRID No. 46235631.

Carney EW; Tornesi B. 2004b. GF-871: Oral Gavage Developmental Toxicity Study In New Zealand White Rabbits. Toxicology and Environmental Research and Consulting. The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 031142. Report dated March 5, 2004. (Not in U.S. EPA/OPP bibliography from FOIA) MRID No. 46235632.

Carney EW; Tornesi B. 2004c. GF-871: Oral Gavage Developmental Toxicity Study In New Zealand White Rabbits. Toxicology and Environmental Research and Consulting. The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 031142S. MRID No. 46284901 (Supplement to Carney and Tornesi 2004b. Only have one-page partial DER from EPA. Full Study Provided by Dow AgroSciences, February 28, 2007.)

Carrithers VF; Masters RA; Halstvedt MB; Duncan C: DiTomaso J; Wilson RG; Dewey S. 2006. Managing Rangeland Invasive Plants with Aminopyralid (Milestone™ Herbicide). Meeting the Challenge: Invasive Plants in PNW Ecosystems. Symposium held at the University of Washington, Sept. 19, 2006. Available at: http://depts.washington.edu/ urbhort/html/invasives/abstract_Carrithers.html.

Cathie C. 2003. Group B: Determination of Physical State, Colour, Odor, Oxidizing and Reduction Action, Flammability, Explodability, pH, Viscosity and Density of GF-871, a Liquid End-Use Product Containing XR-750 TIPA. Project Number: 02/070/L. Unpublished study prepared by Dow AgroSciences (New Zealand) Ltd. 17 p. MRID No. 46235704.

Cherwell Scientific. 2000. ModelMaker Reference and User Manual, Version 4. Cherwell Scientific Publishing Limited, Oxford Science Park, Oxford U.K., April 2000. 143+90 pages.

Clydesdale, FM. 1997. Food Additives: Toxicology, Regulation, and Properties. CRC Press, Boca Raton, Florida. CD-ROM Database.

Cohen SM; Ito N. 2002. A critical review of the toxicological effects of carrageenan and processed Eucheuma seaweed on the gastrointestinal tract. Crit Rev Toxicol. 32(5):413-44.

Cook W. 2003a. Hydrolysis of XDE-750 at pH 5, 7, and 9. Project Number: 020067- Unpublished Study prepared by Dow AgroSciences LLC. 45 p. MRID No. 46235726.

Cook W. 2003b. Aqueous Photolysis of XDE-750 in pH 5 Buffer Under Xenon Light. Project Number: 020066. Unpublished study prepared by Dow AgroSciences LLC. 92 p. MRID No. 46235727.

Davies B; Morris T. 1993. Physiological parameters in laboratory animals and humans. Pharma. Res. 10, 1093.

De Groot AP; Til HP; Feron VJ; Dreef-Van Der Meulen HC; Willems MI. 1974. Two-year feeding and multigeneration studies in rats on five chemically modified starches. Food Cosmet Toxicol. 12(5-6): 651-664.

Delaware Cooperative Extension. 1999. Agronomy Facts Series: AF-03. http://bluehen.ags.udel.edu/deces/af/af-03.htm.

DiSalvo C. 2007. Carol DiSalvo, IPM Coordinator, National Park Service, Washington, D.C. Comments on: SERA TR-052-04-01a: Aminopyralid, Human Health and Ecological Risk Assessment, (Introduction, Program Description, Citations), dated January 3, 2007. Comments dated 1/31/07.

Doerr BI; Glomot R; Kief H; Kramer M; Sakaguchi T. 1982. Cefotaxime toxicity studies: a review of preclinical studies and some clinical reports. Rev Infect Dis. 4 Suppl: S354-9.

Doherty M. 2005. Aminopyralid. Petition for the Establishment of Permanent Tolerances for Use of Aminopyralid on Grasses and Wheat. Summary of Analytical Chemistry and Residue Data. PP#4F6827. Memorandum dated July 12, 2005. Provided U.S. EPA/OPP via FOIA. [Annotation: Discusses a number of residue studies. DERs follow but are catalogued separately in this reference list.]

Domoradzki J; Rick D; Clark A. 2004. XDE-750, Triisopropanolamine Salt: Dissociation and Metabolism in Male Fischer 344 Rats. Project Number: 031129, 2004/8. Unpublished study prepared by The Dow Chemical Co. 57 p. MRID No. 46235833.

Dow AgroSciences LLC. 2004. Milestone MSDS. MSDS No. 007887 dated May, 18, 2004. Available at: http://www.lanecounty.org/RoadMaint/documents/MSDSMilestone.pdf

Dow AgroSciences LLC. 2004a. Submission of Residue and Toxicity Data in Support of the Applications for Registration of Aminopyralid Technical and GF-871 and the Petition for Tolerance of Aminopyralid on Grass Forage, Grass Hay, Wheat commodities, Milk, Cattle Meat and Meat By-Products. Transmittal of 37 of 108 Studies. MRID No. 46235600.

Dow AgroSciences LLC. 2004b. Submission of Product Chemistry, Residue and Environmental Fate Data in Support of the Applications for Registration of Aminopyralid Technical and GF-871and the Petition for Tolerance of Aminopyralid on Grass Forage, Grass Hay, Wheat Commodities, Milk, Cattle Meat and Meat By-Products. Transmittal of 35 of 108 Studies. MRID No. 46235700.

Dow AgroSciences LLC. 2004c. Submission of Toxicity, Fate, Risk, and Exposure Data in Support of the Applications for Registration of Aminopyralid Technical and GF-871 and the Petition for Tolerance of Aminopyralid on Grass Forage, Grass Hay, Wheat, Commodities, Milk, Cattle Meat and Meat By-Products. Transmittal of Studies 36 of 109 Studies. MRID No. 46235800.

Dow AgroSciences LLC. 2004d. Submission of Reduced Risk Data in Support of the Application for Registration of Aminopyralid Technical. Transmittal of 1 Study. MRID No. 46259600.

Dow AgroSciences LLC. 2005a. Milestone Specimen Label. Label Code: D02-879-001. LOES Number: 010-02112. Label Date: 08-29-05. Available at: www.co.lane.or.us/RoadMaint/ documents/lblMilestone.pdf

Dow AgroSciences LLC. 2005b. Dow AgroSciences Launches a New Weed Control Alternative for Rangeland and Pasture. From Around Dow, November 2005. Available at: http://news.dow.com/dow_news/arounddow/pdfs/162-02090.pdf.

Dow AgroSciences. 2006a. Dow AgroSciences (DAS) response to JMPR's [FAO/WHO Joint Meetings on Pesticide Residues]questions to aminopyralid (dated 4 October 2006). Unpublished document courtesy of John Jachetta, Dow AgroSciences.

Dow AgroSciences. 2006b. New CleanWave™ Herbicide Controls Tough Broadleaf Weeds in Dryland Wheat. Product Synopsis. Available at: http://www.dowagro.com/usag/resource/ smgrains/20060131a.htm

Dow AgroSciences Canada, Inc. 2006. Aminopyralid Herb Non-crop E 28137 Feb06f SPECIMEN Label. Label code: CN-28137-001-E. Dated 02/07/06. Available at: http://www.truenorthspecialty.com/english/Products/Labels/Milestone_Eng.pdf.

Dryzga M; Stebbins K. 2001. Revised Report for XDB-750: 13-Week Dietary Toxicity with 4-Week Recovery Study in Fischer 344 Rats. Project Number: 001221, 001221R. Unpublished study prepared by The Dow Chemical Co. 737 p. MRID No. 46235621.

Durkin PR; Diamond G. 2002. Neurotoxicity, Immunotoxicity, and Endocrine Disruption with Specific Commentary on Glyphosate, Triclopyr, and Hexazinone: Final Report. SERA TR 01-43-08-04a dated January 30, 2002. Available at www.fs.fed.us/foresthealth/pesticide/risk.htm.

Durkin PR; Rubin L; Withey J; Meylan W. 1995. Methods of assessing dermal absorption with emphasis on uptake from contaminated vegetation. Toxicol. Indust. Health. 11(1): 63-79.

Durkin P., Diamond G., Hertzberg R. 2004. Application of PBPK Model for 2,4-D to Estimates of Risk in Backpack Applicators. Environmental Toxicology and Pharmacology. 16: 73-91.

Ecobichon DJ. 1998. Occupational Hazards of Pesticide Exposure – Sampling, Monitoring, Measuring. Taylor & Francis, Philadelphia, PA. 251 pp.

El-Harith EA; Dickerson JW; Walker R. 1976. Potato starch and caecal hypertrophy in the rat. Food Cosmet Toxicol. 14 (2): 115-121.

EPI Suite 2004. Estimation Program Interface, Version 3.10. Syracuse Research Corporation, Syracuse, N.Y. for U.S. Environmental Protection Agency, Office of Pollution, Prevention and Toxics, Washington D.C. Downloadable copy of EPI-SUITE computer program available at: http://www.epa.gov/opptintr/exposure/docs/episuitedl.htm

Feldmann RJ; Maibach HI. 1974. Percutaneous penetration of some pesticides and herbicides in man. Toxicol. Appl. Pharmacol. 28: 126-132.

Feletti F; De Bernardi di Valserra M; Contos S; Mattaboni P; Germogli R. 1992. Chronic toxicity study on a new glucan extracted from *Candida albicans* in rats. Arzneimittelforschung. 42(11):1363-7. [This is in English]

Ferrell JA; Mullahey JA; Langeland KA; Kline WN. 2006. Control of Tropical Soda Apple (*Solanum viarum*) with Aminopyralid. Weed Technology. 20(2): 453-457.

Fitzsimmons ME; Baggs RB; Anders MW. 1994. Nephrotoxicity and Hepatotoxicity of 5,6-Dichloro-4-thia-5-hexenoic Acid: Evidence for Fatty Acid Beta-Oxidation-Dependent Bioactivation. Journal of Pharmacology and Experimental Therapeutics. 271(1): 515-523.

Fletcher JS; Nellessen JE; Pfleger TG. 1994. Literature review and evaluation of the EPA food-chain (Kenaga) nomogram, an instrument for estimating pesticide residues on plants. Environ. Toxicol. Chem. 13(9): 1383-1391.

Gajanayake 2006. Evaluation of the new active, AMINOPYRALID, in the product Hotshot Herbicide. Australian Pesticides and Veterinary Medicines Authority, Report dated February 2006, Canberra. Australia. National Registration Authority for Agricultural and Veterinary Chemicals 2006. ISSN1443-1335. Available at: http://www.apvma.gov.au/ publications/prs_aminopyralid.pdf.

Gallagher S; Grimes J; Beavers J. 2001a. XDE-750: An Acute Oral Toxicity Study with the Northern Bobwhite. Project Number: 379/106, 011046. Unpublished study prepared by Wildlife International, Ltd. 33 p. MRID No. 46235808.

Gallagher S; Beavers J.; Martin K. 2001b. XDE-750: A Dietary LC_{50} Study with the Northern Bobwhite. Project Number: 379/107, 011047. Unpublished study prepared by Wildlife International, Ltd. 51 p. MRID No. 46235810.

Gallagher S; Beavers J; Martin K. 2001c. XDE-750: A DietaryLC_{50} Study with the Mallard. Project Number: 379/108, 011048. Unpublished study prepared by Wildlife International, Ltd. 50 p. MRID No. 46235811.

Gallagher S; Grimes J; Beavers J. 2003. XDE-750 Technical: An Acute Oral Toxicity Study with the Northern Bobwhite. Project Number: 379/130, 031112. Unpublished study prepared by Wildlife International, Ltd. 34 p. MRID No. 46235809.

Ghaoui L. 2003. Group B: Physical and Chemical Properties of Aminopyralid (IEDE-750) Technical. Project Number: NAFST744, D08323/024653, FAPC/013053. Unpublished study prepared by Dow AgroSciences LLC, Dow AgroSciences (New Zealand) Ltd. and The Dow Chemical Co. 313 p. MRID No. 46235703.

Ghaoui L. 2004. Group A: Product Identity and Composition, Description of Materials Used to Produce the Product, Description of the Production Process, Discussion of Formation of Impurities, Certified Limits, Preliminary Analysis, and Enforcement Analytical Methods for Aminopyralid (XDE-750) Technical. Project Number: NAFST759, DECO/GL/AL/MD/2003/001969: DECO/GL/AL/MD/2003/001968/AI. Unpublished study prepared by Dow AgroSciences LLC and The Dow Chemical Co. 255 p. MRID No. 46235701.

Goldstein A; Aronow L; Kaman SM. 1974. Principles of Drug Action: The Basis of Pharmacology. 2nd ed. John Wiley and Sons, New York, NY. 854 p.

Gooch JW; Matsumura F. 1987. Toxicity of chlorinated bornane (toxaphene) residues isolated from Great Lakes lake trout (*Salvelinus namaycush*). Arch. Environ. Contam. Toxicol. 16(3): 349-355.

Graper L; Smith K; Hilla S. 2003. A Nature of the Residue Study with (Carbon 14)-Labeled XDE-750 Applied to Spring Wheat. Project Number: 020022. Unpublished study prepared by Dow AgroSciences LLC and Research for Hire. 197 p. MRID No. 46235709.

Greenbook 2006. Product Labels and Material Safety Data Sheets. Available at: www.GreenBook.net.

Grice HC; Goldsmith LA. 2000. Sucralose – An Overview of the Toxicity Data. Food Chem. Toxicol. 38(Suppl. 2): S1-S6.

ICRP (International Commission on Radiological Protection). 2005. 2005 Recommendations of the International Commission on Radiological Protection. Available at: http://www.icrp.org/docs/2005_recs_CONSULTATION_Draft1a.pdf.

Iijima T; Hasegawa K. 1985. Quinacrine-induced dilation of the rat cecum and regeneration of large granular vesicle-containing neurons in the myenteric plexus. Cell and Tissue Research. 241 (2): 383-389.

Hamilton T. 2004a. Amended Report for Dissociation of XDE-750 Tri-isopropanolamine Salt. Project Number: DECO/GL/AL/MS/2002/004418. Unpublished study prepared by The Dow Chemical Company. 21 p. MRID No. 46235706.

Hamilton T. 2004b. Amended Report for Dissociation of XDE-750 Potassium Salt. Project Number: GL/AL/MD/2002/004397. Unpublished study prepared by The Dow Chemical, Co. 21 p. MRID No. 46235707.

Hancock GA; Najar JR; McClymont EL. 2007. Aminopyralid: Growth Inhibition Test with the Blue-Green Alga, *Anabaena flos-aquae*. Dow Chemical Company Report No. DECO HET DR-0293-9028-083. Final report dated April 4, 2007. Copy courtesy of John Jachetta, Dow AgroSciences, on April 5, 2007.

Hansen SC; Mendrala AL; Markham DA; Saghir S. 2005. DE-750: Metabolism and Pharmacokinetics of 14C-DE-750 in Non-pregnant and Pregnant New Zealand White Rabbits Following Single and Repeated Oral Administration. Dow Chemical Company Study ID: 041109. 80 p. (No MRID number)

Harris SA; Solomon KR. 1992. Human exposure to 2,4-D following controlled activities on recently sprayed turf. J. Environ. Sci. Health. B27(1): 9-22.

Hartzler B. 2006. Aminopyralid - New herbicide for pastures, roadsides, etc., Iowa State University. Available at: http://www.weeds.iastate.edu/mgmt/2006/aminopyralid.shtml.

Hastings M. 2003. Method Validation Report for the Determination of Residues of. Aminopyralid in Water by Liquid Chromatography with Tandem Mass Spectrometry Detection Using Dow AgroSciences Method GRM 0132. Project Number: 01 159. Unpublished study prepared by Dow AgroSciences LLC. 48 p. MRID No. 46235601.

Havens P. 2004. Spray Drift Assessment for Terrestrial and Aquatic Non-Target Plants from the Use of Aminopyralid in Canada. Project Number: GH/C/5708. Unpublished study prepared by Dow AgroSciences LLC. 28 p. MRID No. 46235834.

Hayes JR; Condie LW; Borzelleca JF. 1986. Acute, 14-Day Repeated Dosing, and 90-Day Subchronic Toxicity Studies of Potassium Picloram. Fundamental and Applied Toxicology. 7(3): 464-470.

Henry K; McClymont E; Yaroch A; et al. 2003a. XDE-750: 96-h Acute Toxicity to Larval Amphibians Using the Northern Leopard Frog, *Rana pipiens*, as a Biological Model. Project Number: 031030. Unpublished study prepared by The Dow Chemical Co. 34 p. MRID No. 46235816.

Henry K; Marino T; Staley J; et al. 2003b. XDE-750: 21-Day Chronic Toxicity with the Daphnid, *Daphnia magna* Straus. Project Number: 021085. Unpublished study prepared by The Dow Chemical Co. 51 p. MRID No. 46235822.

Hoberg J. 2002a. XDE-750 - Acute Toxicity to the Freshwater Diatom (*Navicula pelliculosa*). Project Number: 12550/6198, 011278. Unpublished study prepared by Springborn Laboratories Inc. 61 p. MRID No. 46235827.

Hoberg J. 2002b. XDE-750 - Growth Inhibition Test with Marine Diatom (*Skeletonema costatum*). Project Number: 12550/6200, 011280. Unpublished study prepared by Springborn Laboratories Inc. 62 p. MRID No. 46235828.

Hoberg J. 2002c. XDE-750 - Growth Inhibition Test with Freshwater Blue-green Alga (*Anabaena flos-aquae*). Project Number: 12550/6199, 011279. Unpublished study prepared by Springborn Laboratories Inc. 57 p. MRID No. 46235829.

Hoberg J. 2003a. XDE-750 - Toxicity to Duckweed, *Lemna gibba*. Project Number: 12550/6160, 011223. Unpublished study prepared by Springborn Laboratories Inc. 64 p. MRID No. 46235826.

Hoberg J. 2003b. XDE-750 - Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata*. Project Number: 12550/6161, 011222. Unpublished study prepared by Springborn Laboratories Inc. 66p. MRID No. 46235830.

HSDB (Hazardous Substances Data Bank). 2003. Triisopropanolamine, Hazardous Substances Databank Number 5593. Available at: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~tBQaBi:1.

Jachetta J. 2006. Response to Query on Aminopyralid Impurities and Inerts. E-mail to Patrick Durkin (SERA, Inc) from John Jachetta, Dow AgroSciences, dated December 27, 2006.

Jachetta J. 2007. Response to Queries On Aminopyralid . E-mail correspondence with Patrick Durkin (SERA, Inc). John Jachetta, Dow AgroSciences.

Jachetta J; Havens P; Dybowski J; et al. 2004. Reduced-Risk Pesticide Rationale for Aminopyralid Technical. Project Number: J042004. Unpublished study prepared by Dow AgroSciences LLC. 523 p. MRID No. 46259601.

Jensen J. 2004a. Group A - Product Identity, Composition, and Analysis for GF-871; an End Use Product Containing Aminopyralid. Project Number: NAFST763, DAS/AM/03/002. Unpublished study prepared by Dow AgroSciences LLC and Dow AgroSciences (New Zealand) Ltd. 69 p. MRID No. 46235702.

Johnson K; Dryzga M. 2004. XDE-750: Two-Year Chronic Toxicity/Oncogenicity and Chronic Neurotoxicity Study in Fischer 344 Rats. Project Number: 011040. Unpublished study prepared by The Dow Chemical Co. 2865 p. MRID No. 46235615.

Kardong KV. 2006. Vertebrates: Comparative Anatomy, function, evolution. 4th Edition. McGraw-Hill, New York, New York. 782 pp.

Kasahara K; Nishikawa A; Furukawa F; Ikezaki S; Tanakamaru Z; Lee IS; Imazawa T; Hirose M. 2002. A chronic toxicity study of josamycin in F344 rats. Food Chem Toxicol. 40(7):1017-22. [This is in English]

Keigwin T. 2004. Review of Acute Toxicity in Support of Aminopyralid Technical (XDE-750), EPA File Symbol: 62719-LRI. Includes One-Liners. Memorandum Dated 9/20/2004. Provided U.S. EPA/OPP via FOIA.

Kiplinger G. 2001. XDE-750: Acute Inhalation Toxicity Study in the Fischer 344 Rat: Final Report. Project Number: 011097, WIL/406012. Unpublished study prepared by WIL Research Laboratories, Inc. 106 p. MRID No. 46235607.

Knisel WG; Davis FM. 2000. GLEAMS (Groundwater Loading Effects of Agricultural Management Systems), Version 3.0, User Manual. U.S. Department of Agriculture, Agricultural Research Service, Southeast Watershed Research Laboratory, Tifton, GA. Pub. No.: SEWRL-WGK/FMD-050199. Report Dated May 1, 1999 and revised August 15, 2000. 194pp.

Konishi F; Oku T; Hosoya N. 1984. Hypertrophic effect of unavailable carbohydrate on cecum and colon in rats. J Nutr Sci Vitaminol (Tokyo). 30(4):373-9. [This is in English]

Kulla A. 2007. Andy Kulla, Resource Staff Officer, Missoula Ranger District, Weed Program Leader, Lolo National Forest, Fort Missoula, Missoula, MT 59804. Comments on: SERA TR-052-04-01a: Aminopyralid, Human Health and Ecological Risk Assessment, (Introduction, Program Description, Citations), dated January 3, 2007. Comments dated 1/28/07.

Lala M; Mollica J; West S. 2002. PAM I Multiresidue Protocol Testing for XDE-750: Final Report. Project Number: 021197, DOW/1413. Unpublished study prepared by Pyxant Labs Inc. 267 p. MRID No. 46235718.

Landry TD; Krieger SM. 2002. GF-871: Acute Liquid Aerosol Inhalation Toxicity Study In Fischer 344 Rats. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 021061. (Not in EPA bibliography from FOIA.) MRID No. 46235608.

Larney FJ; Cessna AJ; Bullock MS. 1999. Herbicide transport on wind-eroded sediment. Journal of Environmental Quality. 28(5): 1412-1421.

Lee GF; Hughes RA; Veith GD. 1977. Evidence for partial degradation of toxaphene [pesticide] in the aquatic environment. Water Air Soil Pollut. 8(4): 479-484.

Leegwater DC; De Groot AP; Van Kalmthout-Kuyper M. 1974. The etiology of caecal enlargement in the rat. Food Cosmet Toxicol. 12(5-6): 687-698.

Liberacki A; Marty M; Thomas J. 2001a. DE-750: 13-Week Dietary Reproduction Study in CD Rats. Project Number: 011046. Unpublished study prepared by The Dow Chemical Co. 277 p. MRID No. 46235619.

Liberacki A; Marty M; Thomas J. 2001b. XDE-750: Oral Gavage Developmental Toxicity Probe Study in New Zealand White Rabbits. Project Number: 001235. Unpublished study prepared by The Dow Chemical Co. 136 p. MRID No. 46235634.

Lina BA; Baumlr A. 2004. Subchronic (13-week) oral toxicity study of alpha-cyclodextrin in dogs. Regul Toxicol Pharmacol. 39 (Suppl 1): S27-33.

Lina BA; Baumlr A. 1998. Subchronic oral toxicity studies with gamma-cyclodextrin in rats. Regul Toxicol Pharmacol. 27(2):178-88

Lina BA; Bos-Kuijpers MH; Til HP; Baumlr A. 1996. Chronic toxicity and carcinogenicity study of erythritol in rats. Regul Toxicol Pharmacol. 24(2 Pt 2):S264-79.

Lindsay D. 2004a. Frozen Storage Stability of XDB-750 in Range Land and Pasture Grass and Hay and Wheat Straw and Wheat Grain - Interim Report. Project Number: 030004. Unpublished study prepared by Dow AgroSciences LLC. 50 p. MRID No. 46235719.

Lindsay D. 2004b. Frozen Storage Stability of XDE-750 in Soil--Interim Report. Project Number: 030002. Unpublished study prepared by Dow AgroSciences LLC. 41 p. MRID No. 46235720.

Lindsey A. 2004d. Method Validation Report for the Determination of Residues of Aminopyralid in Soil by Liquid Chromatography with Tandem Mass Spectrometry Detection Using Dow AgroSciences Method GRM 01.32. Project Number: 021295. Unpublished study prepared by Dow AgroSciences LLC. 56 p. MRID No. 46235602.

Linscombe V; Schisler M; Beuthin D. 2001. Evaluation of XDE-750 in the Chinese Hamster Ovary Cell/Hypoxanthine-Guanine-Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay. Project Number: 011037. Unpublished study prepared by The Dow Chemical Co. 26 p. MRID No. 46235801.

Linscombe V; Jackson K; Schisler M.; et. al. 2002a. Evaluation of FE-750 in an *In Vitro* Chromosomal Aberration Assay Utilizing Rat Lymphocytes. Project Number: 011040. Unpublished study prepared by The Dow Chemical Co. 40 p. MRID No. 46235802.

Linscombe VA; Jackson KM; Schisler MR.. 2002b. Evaluation of GF-871 in an *In Vitro* Chromosomal Aberration Assay Utilizing Rat Lymphocytes. Toxicology and Environmental Research and Consulting, The Dow Chemical Co., Midland, MI. Laboratory Project Study ID 031134. Unpublished. (Not in EPA/OPP bibliography from FOIA.) MRID No. 46235803.

Linscombe VA; Schisler MR; Seidel SD.. 2004. Evaluation of GF-871 in the Chinese hamster ovary cell/hypoxanthine-guianine-phosphoribosyl transferase (CHO/HGPRT) Forward mutation assay. Toxicology and Environmental Research and Consulting, The Dow Chemical Co., Midland, MI. Laboratory Project Study ID 031134. Unpublished. (Not in EPA/OPP bibliography from FOIA.) MRID No. 46235804.

Liu J. 2004. C^{14}-XDE-750: Absorption, Distribution, Metabolism, and Excretion in Male Fischer 344 Rats: Amended Final Report. Project Number: 47456,021200. Unpublished study prepared by Analytical Bio-Chemistry Labs, Inc. 134 p. MRID No. 46235807.

Mach J. 2003a. Avian Reproduction Study with XDE-750 in Mallards (*Anas platyrhynchos*): Amended Final Report. Project Number: 011272, 02002. Unpublished study prepared by Genesis Laboratories, Inc. 124 p. MRID No. 46235813.

Mach J. 2003b. Avian Reproduction Study with XDE-750 in Northern Bobwhite (*Colinus virginianus*): Final Report. Project Number: 011271, 02001. Unpublished study prepared by Genesis Laboratories, Inc. 122 p. MRID No. 46235812.

Machado N. 2002a. XDE-750 - Acute Toxicity to Mysids (*Americamysis bahia*) Under Static Conditions. Project Number: 12550/6190, 011269. Unpublished study prepared by Springborn Laboratories Inc. 46 p. MRID No. 46235819.

Machado M. 2002b. XDE-750 - Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Static Acute Conditions. Project Number: 12550/6191, 011270. Unpublished study prepared by Springborn Laboratories Inc. 47 p. MRID No. 46235820.

Machado M. 2003. XDE-750 - Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Static Conditions. Project Number: 12550/6162, 011225. Unpublished study prepared by Springborn Laboratories Inc. 47 p. MRID No. 46235815.

Macpherson D. 2003. The Distribution and Metabolism of (Carbon 14)-XDE-750 in the Lactating Goat. Project Number: 201893. Unpublished study prepared by Inveresk Research International. 11l p. MRID No. 46235708.

Magnussen J. 2004a. ^{14}C XDE-750 Poultry Nature of the Residue Study. Project Number: 030009,379/131. Unpublished study prepared by Dow Agro Sciences LLC and Wildlife International, Ltd. 117 p. MRID No. 46235711.

Magnussen J. 2004b. A Confined Rotational Crop Study with ^{14}C XDE-750. Project Number: 030008. Unpublished study prepared by Dow AgroSciences LLC and Research for Hire. 142 p. MRID No. 46235725.

Magnussen J; Balcer J. 2004. 14^{C} XDE-750 Grass Nature of Residue Study. Project Number: 010071. Unpublished study prepared by Dow AgroSciences LLC. 139p. MRID No. 46235710.

Mann SW; Yuschak MM; Amyes SJG; Aughton P; Finn JP. 2000. A Combined Chronic Toxicity/Carcinogenicity Study of Sucralose in Sprague-Dawley Rats. Food Chem. Toxicol. 38 (Suppl. 2): S71-S89.

Marable B; Andrus A; Stebbins K. 2002. Revised Report for: XDE-750: Acute Neurotoxicity Study in Fischer Rats. Project Number: 011073R,011073. Unpublished study prepared by The Dow Chemical Co. 404 p. MRID No. 46235616.

Marino T; McClymont E; Yaroch A; et al. 2001a. XDE-70 Herbicide: An Acute Toxicity Study with the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Project Number: 011078. Unpublished study prepared by The Dow Chemical Co. 36 p. MRID No. 46235814.

Marino T; Hales C; McClymont E; et al. 2001b. XDE-750 Herbicide: An Acute Toxicity Study with the Daphnid, *Daphnia magna* Straus. Project Number: 011079. Unpublished study prepared by The Dow Chemical Co. 35 p. MRID No. 46235817.

Marino T; McClymont E; Yaroch A; et al. 2003. Revised Report for XDE-750: Toxicity to the Early Life Stages of the Fathead Minnow, *Pimephales promelas* Rafinesque. Project Number: 021029. Unpublished study prepared by The Dow Chemical Co. 55 p. MRID No. 46235821.

Marty M; Liberacki A; Thomas J. 2002. XDE-750: Oral Gavage Developmental Toxicity Study in New Zealand White Rabbits. Project Number: 01 1047, 011047A. Unpublished study prepared by The Dow Chemical Co. 755 p. MRID No. 46235630.

Marty M; Zablatny C; Thomas J. 2003. XDE-750: Two-Generation Dietary Reproduction Toxicity Study in CD Rats. Project Number: 011205. Unpublished study prepared by The Dow Chemical Co. 1373 p. MRID No. 46235635.

Masters RA; , Burch PL; Breuninger JM; Carrithers VF; Jachetta JJ; Kline WN; , Hare DD; Chemello AA; Troth JL; Schultz. RD. 2005. Aminopyralid: a New Herbicide for Pasture Vegetation Management. The ASA-CSSA-SSSA International Annual Meetings (November 6-10, 2005). Poster 315-6. Dow AgroSciences, Indianapolis, IN.

Maurissen J; Andrus A; Johnson IC; et al. 2003. XDE-750: Chronic Neurotoxicity Study in Fischer 344 Rats. Project Number: 011049N. Unpublished study prepared by The Dow Chemical Co. 495 p. MRID No. 46235617.

McAndrew E. 2005. Review of Acute Toxicity Data for GF-982. Includes One-Liners. Memorandum dated 1/31/2005. Provided U.S. EPA/OPP via FOIA.

McCollister SB; Quast JF; Hermann EA; Pugh LA; Dittenber DA; Gorzinski SJ. 1981. Triisopropanolamine: Results of a Two-Week Toxicity Study in the Drinking Water of CDF Fischer 344 Rats. Report of Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.

McCormick R; Schelle G; Dolder S. 2004. Magnitude of Residue of XDE-750 and 2,4-D in Rangeland and Pasture Grasses. Project Number: 020018. Unpublished study prepared by Dow AgroSciences LLC. 202 p. MRID No. 46235722.

McFarlane J. 2003. Group B – Physical/Chemical Properties for OF-871, A Liquid End Use Product Containing XR-75 0TIPA. Project Number: NAFST788: GHF/P/2709. Unpublished study prepared by Dow AgroSciences LLC. 5 p. MRID No. 46235705.

McMurray A. 2002. A laboratory assessment of the effects of XDE-750 on soil microflora respiration and nitrogen transformation according to OECD guidelines. Chemex reference: ENV5579. Unpublished study prepared by Chemex Environmental International Ltd., Cambridge, England for Dow AgroSciences, Oxon, England. 65 p. Copy courtesy of .

Mecchi M. 2004a. *Salmonella - Escherichia coli* Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay with XDE-750: Second Amended Final Report. Project Number 22338/0/4220ECD,011058,2001/113. Unpublished study prepared by Covance Laboratories, Inc. 37 p. MRID No. 46235636.

Mecchi MS. 2004b. *Salmonella - Escherichia coli* Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay with GF-871. Covance Laboratories Inc. (Covance), Vienna, VA. Covance Study No. 25552-0-422OECD. Dow Study ID: 031150. Unpublished. (Not listed in U.S. EPA/OPP bibliography from FOIA.) MRID No. 46235637.

Mullin LS. 1987. Subchronic Oral Toxicity: 90-Day Study with TIPA Feeding Study in Dogs. Medical Research Project No. 8086-001. E. I. du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware. As summarized in Stebbins and Dryzga 2004 MRID 46235622.

Nagata T; Kono I; Masaoka T; Akahori F. 1992. Subacute Toxicity of Paraquat in Beagle Dogs: Clinicopathology and Pathologic Examinations. Veterinary and Human Toxicology. 34(1): 15-20.

Nelson J; Jachetta J; Halstvedt M.; et al. 2004. Public Interest Document for Aminopyralid. Project Number: PID/DOW03JN01. Unpublished study prepared by ABG, Inc. 144p. (Have only non confidential appendices in copy from Dow.) MRID No. 46235835.

Newberne PM; Conner MW; Estes P. 1988. The influence of food additives and related materials on lower bowel structure and function. Toxicol Pathol. 16(2):184-97.

Olberding E; Hastings M. 2004. Validation Report for Method GRM 02.31 - Determination of Residues of Aminopyralid in Agricultural Commodities by Liquid Chromatography with Tandem Mass Spectrometry Detection. Project Number: 021310, GRM/02/3 1. Unpublished study prepared by Dow AgroSciences LLC. 61 p. MRID No. 46235717.

O'Flaherty EJ. 1981. Toxicants and Drugs: Kinetics and Dynamics. John Wiley and Sons, New York, NY. 398 pp.

Orberg J. 1980. Observations on the 2,4-diclorophenoxyacetic acid (2,4-D) excretion in the goat. Acta Pharmacol. Toxicol. 46, 78.

Payne-Sturges DC; Burke TA; Breysse P; Diener-WestD; Buckley T. 2004. Personal Exposure Meets Risk Assessment: A Comparison of Measured and Modeled Exposures and Risks in an Urban Community. Environmental Health Perspectives. 112(5): 589-598.

Potts AM. 1996 Toxic Responses of the Eye. In: Cassarett and Doull's Toxicology: The Basic Science of Poisons. 5th ed., CD Klaassen, MO Amdur, J Doull, Eds. McGraw-Hill, New York, NY. pp. 583-615.

Putt A. 2002. XDE-750 - The Full Life-Cycle Toxicity to Midge (*Chironomus riparius*) Under Static Conditions. Project Number: 12550/6195, 011277. Unpublished study prepared by Springborn Laboratories Inc. 88 p. MRID No. 46235823.

Reed R. 2004a. Independent Laboratory Validation of Dow AgroSciences LLC Method GRM 02.3 1 - Determination of Residues of Aminopyralid in Agricultural Commodities by Liquid Chromatography with Tandem Mass Spectrometry Detection. Project Number: 020157, ML03/1110/DOW. Unpublished study Prepared by Morse Laboratories. 147 p. MRID No. 46235712.

Reed R. 2004b. Independent Laboratory Validation of Dow AgroSciences Method GRM 01.32 - Determination of Residues of Aminopyralid in Water by Liquid Chromatography with Tandem Mass Spectrometry. Project Number: 030039, ML03/111 I/DOW. Unpublished study prepared by Morse Laboratories. 113 p. MRID No. 46235713.

Reed R. 2004c. Independent Laboratory Validation of Dow AgroSciences LLC Method GRM 03.18 - Determination of Residues of Aminopyralid in Bovine Tissues by Liquid Chromatography with Tandem Mass Spectrometry. Project Number: 030098, ML03/1122/DOW. Unpublished study prepared by Morse Laboratories. 13 0 p. MRID No. 46235714.

Reed R. 2004d. Independent Laboratory Validation of Dow AgroSciences Method GRM 02.34 - Determination of Residues of Aminopyralid in Soil by Liquid Chromatography with Tandem Mass Spectrometry. Project Number: 020158, ML03/1102/DOW. Unpublished study prepared by Morse Laboratories. 125 p. MRID No. 46235715.

RIVM (National Institute for Public Health and the Environment, Netherlands). 2007. Interspecies Information. Available at: http://www.rivm.nl/interspeciesinfo/intra/.

Roberts D; Schelle G. 2004a. Terrestrial Field Dissipation of XDE-750 in the USA. Project Number: 020032. Unpublished study prepared by Dow AgroSciences LLC. 158 p. MRID No. 46235734.

Roberts D; Schelle G. 2004b. Terrestrial Field Dissipation of XDE-750 in Canada. Project Number: 020031, Unpublished study prepared by Dow AgroSciences LLC. 302 p. MRID No. 46235735.

Roberts D; Schelle G; Knuteson J. 2004. Magnitude of the Residues for XDE-750 in Wheat Agricultural Commodities: Amended Report. Project Number: 030042. Unpublished study prepared by Dow AgroSciences LLC. 232 p. MRID No. 46235721.

Rosser S; Rutherford L; McFarlane J. 2004. Magnitude ofXDE-750 Residues in Bovine Tissues and Milk from a 28-Day Feeding Study. Project Number: 030061, 208/001/10. Unpublished study prepared by Dow AgroSciences LLC and Genesis Midwest Laboratories. 259 p. MRID No. 46235723.

Rutherford L. 2002. Soil Batch Equilibrium Adsorption/Desorption of XDE-750. Project Number: 010064. Unpublished study prepared by Dow AgroSciences LLC. 128 p. MRID No. 46235732.

Rutherford L. 2004. Photodegradation of XDE-750 on Soil. Project Number: 020080. Unpublished study prepared by Dow AgroSciences LLC. 80p. MRID No. 46235728.

Rutherford L; Hastings M. 2003. Method Validation Report for the Determination of Aminopyralid in Bovine Tissues by Liquid Chromatography with Tandem Mass Spectrometry Using Dow AgroSciences LLC Method GRM 03.18. Project Number: 021327, GRM/03/18. Unpublished study prepared by Dow AgroSciences LLC. 53 p. MRID No. 46235716.

Rutherford L; Meitl T. 2004. Anaerobic Aquatic Metabolism of XDE-750. Project Number: 020052. Unpublished study prepared by Dow AgroSciences LLC. 73 p. MRID No. 46235730.

Sandvig R. 2001. Policy 9.1. Policy Science Advisory Council for Exposure, U.S. EPA/OPP HED. Standard Values for Daily Acres Treated in Agriculture. Document dated September 25, 2001. Copy courtesy of Dr. Henry Appleton, USDA/FA, Forest Health Protection, Washington, DC.

Schoel B; Pfleiderer G. 1987. The amount of Tamm-Horsfall protein in the human kidney, related to its daily excretion. J. Clin. Chem. Clin. Biochem. 25: 681-682.

ScienceLab. 2005. Triisopropanolamine Material Safety Data Sheet. Available at: http://www.sciencelab.com/xMSDS-Triisopropanolamine-9925326.

SERA (Syracuse Environmental Research Associates, Inc). 2003a. Picloram - Human Health and Ecological Risk Assessment - Final Report. SERA TR 03-43-16-01b. Report dated June 30, 2003. Available at: http://www.fs.fed.us/foresthealth/pesticide/risk.shtml

SERA (Syracuse Environmental Research Associates, Inc). 2003b. Triclopyr - Human Health and Ecological Risk Assessment - Final Report. SERA TR 02-43-13-03b. Report dated March 15, 2003. Available at: http://www.fs.fed.us/foresthealth/pesticide/risk.shtml

SERA (Syracuse Environmental Research Associates, Inc). 2004a. Dicamba - Human Health and Ecological Risk Assessment - Final Report. SERA TR 04-43-17-06d. Report dated November 24, 2004. Available at: http://www.fs.fed.us/foresthealth/pesticide/risk.shtml

SERA (Syracuse Environmental Research Associates, Inc). 2004b. Metsulfuron Methyl - Human Health and Ecological Risk Assessment - Final Report. SERA TR 04-43-17-01c. Report dated December 9, 2004. Available at: http://www.fs.fed.us/foresthealth/pesticide/risk.shtml

SERA (Syracuse Environmental Research Associates, Inc). 2004c. Clopyralid - Human Health and Ecological Risk Assessment - Final Report. SERA TR 04-43-17-03c. Report dated December 5, 2004. Available at: http://www.fs.fed.us/foresthealth/pesticide/risk.shtml

SERA (Syracuse Environmental Research Associates, Inc). 2004d. Documentation for the Use of GLEAMS (Version 3) and Auxiliary Programs in Forest Service Risk Assessments (Version 2.04). SERA TD 2004-02.04b. Report dated February 10, 2004. Available at: www.sera-inc.com

SERA (Syracuse Environmental Research Associates, Inc.). 2005. Documentation for Worksheets Version 4.01 - Human Health and Ecological Risk Assessments. Report dated May 4, 2005. Available at: www.sera-inc.com.

SERA (Syracuse Environmental Research Associates, Inc.). 2006a. 2,4-D - Human Health and Ecological Risk Assessment - Final Report. SERA TR 06-43-29-02b. Report dated September 30, 2006. Available at: http://www.fs.fed.us/foresthealth/pesticide/risk.shtml

SERA (Syracuse Environmental Research Associates, Inc.). 2006b. Modifications to Gleams-Driver Version 1: Expanded Weather Files. SERA TR TR-052-05-04a. Report dated December 17, 2006. Available at: www.sera-inc.com.

SERA (Syracuse Environmental Research Associates, Inc.). 2007b. Preparation of Environmental Documentation and Risk Assessments, SERA MD 2007-01a, draft dated January 21, 2007. Syracuse Environmental Research Associates, Inc., Fayetteville, NY. Available at www.sera-inc.com.

SERA (Syracuse Environmental Research Associates, Inc.). 2007b. Gleams-Driver User Guide (Version 1.3). SERA TR 07-52-05-05a. Report dated March 17, 2007. Available at: www.sera-inc.com. On Peer Review CD.

Sethi P. 2006. Aminopyralid. Commonwealth of Australia Gazette. No. APVMA 1, 3 January 2006. Available at: www.apvma.gov.au/gazette/gazette0601p11.pdf.

Sinkeldam EJ; Hollanders VM; Woutersen RA; Koeumlter HB; Baumlr A. 1992a. Multigeneration reproduction study of lactitol in rats. J Am Coll Toxicol. 11(2): 233-48.

Sinkeldam EJ; Woutersen RA; Hollanders VMH; Til HP; Van Garderen-Hoetmer A; Bar A. 1992b. Subchronic and chronic toxicity/carcinogenicity feeding studies with lactitol in rats. J Am Coll Toxicol. 11(2): 165-188.

Smith, F.A., et al., 1980. Pharmacokinetics of 2,4-Dichlorophenoxyacetic Acid in Fisher 344 Rats. Dow Chemical USA, R&D Report HET K-002372-(24) dated 9 December 1980, Copy courtesy of Dr. Joseph Dybowski, Dow Chemical. As cited in Durkin et al. 2004.

Spencer P; Gorski T. 2002. Evaluation of XDE-750 in the Mouse Bone Marrow Micronucleus Test. Project Number: 011125. Unpublished study prepared by The Dow Chemical Co. 46 p. MRID No. 46235805.

Spencer PJ; Linscombe AV; Grundy J. 2004. Evaluation of GR-871 in the Mouse Bone Marrow Micronucleus Test. Project Number: 011125. Unpublished study prepared by The Dow Chemical Co. 46 p. (Not in EPA/OPP bibliography from FOIA.) MRID No. 46235806.

Stebbins K; Baker P. 2000. XR-750: 4-Week Dietary Toxicity Study in Beagle Dogs. Project Number: 001030. Unpublished study prepared by The Dow Chemical Co. 141 p. MRID No. 46235620.

Stebbins K; Baker P. 2002. Revised Report for: XDE-750: 13-Week Dietary Toxicity Study in Beagle Dogs – Project Number: 001239R, 001239. Unpublished study prepared by The Dow Chemical Co. 261 p. MRID No. 46235623.

Stebbins K; Day S. 2000. XR-750: 4-Week Repeated Dose Dietary Toxicity Study in Fischer 344 Rats. Project Number: 001031. Unpublished study prepared by The Dow Chemical Co. 313 p. MRID No. 46235625.

Stebbins K; Day S. 2003a. XDE-750: One-Year Dietary Toxicity Study in Beagle Dogs. Project Number: 021027. Unpublished study prepared by The Dow Chemical Co. 285 p. MRID No. 46235627.

Stebbins K; Day S. 2003b. XDE-750: Oncogenicity Dietary Study in CD-I Mice. Project Number: 011163. Unpublished study prepared by The Dow Chemical Co. 1416 p. MRID No. 46235628.

Stebbins KE; Dryzga MD. 2004. GF-871: 90-Day dietary toxicity study in Fischer 344 rats. February 25, 2004. Toxicology and Environmental Research and Consulting, Midland, MI. Study ID 031140. Unpublished. [Did not appear in listing of studies from OPP. Have DER.] MRID No. 46235622.

Stebbins K; Day S; Thomas J. 2001. XDE-750: 13-Week Dietary Toxicity Study in CD-I Mice. Project Number: 001240. Unpublished study prepared by The Dow Chemical Co. 470p. MRID No. 46235618.

Stebbins K; Thomas K; Day S. 2002. XDE-750: 28-Day Dermal Toxicity Study in Fischer Rats. Project Number: 011219. Unpublished study prepared by The Dow Chemical Co. 297 p. MRID No. 46235626.

Temple DL; Martin KH; Beavers JB; Jaber M. 2007. XDE-750: A Reproduction Study with the Northern Bobwhite Final Report, Wildlife International, Ltd. Project Number 379-176, Dow AgroSciences Study Number 060091.

Til HP; Feron VJ; Immel HR; Vogel WF. 1986. Chronic 89-Week Feeding Study with Hydroxypropyl Distarch Phosphate Starch Acetate Lactose and Sodium Alginate in Mice. Food Chem Toxicol. 24(8): 825-834.

Til HP; Kuper CF; Falke HE; Baumlr A. 1996. Subchronic oral toxicity studies with erythritol in mice and rats. Regul Toxicol Pharmacol. 24(2 Pt 2):S221-31.

Til HP; Baumlr A. 1998. Subchronic (13-week) oral toxicity study of gamma-cyclodextrin in dogs. Regul Toxicol Pharmacol. 27(2): 159-65.

Tiu C.; Selman F. 2004. Estimation of Exposure and Risk to Workers from the Use of Aminopyralid Herbicide on Range and Pasture, Industrial Vegetation Management and Cereal Crops in Canada. Project Number: GH/C/5715. Unpublished study prepared by Dow AgroSciences LLC. 27 p. MRID No. 46235836.

Tomlin C. 2004. The e-Pesticide Manual, Thirteenth Edition, Crop Protection Publications; British Crop Protection Council. Available at: http://www.bcpcbookshop.co.uk.

Tornesi B; Carney E; Thomas J. 2001. XDE-750: Developmental Toxicity Probe Study in CD Rats. Project Number: 001234. Unpublished study prepared by The Dow Chemical Co. 134p. MRID No. 46235633.

U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 2004a. Aminopyralid; Notice of Filing a Pesticide Petition to Establish a Tolerance for a Certain Pesticide Chemical in or on Food. Federal Register. 69(106): 31106-31110.

U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 2004b. Aminopyralid: Pesticide Tolerance. Federal Register. 70(153): 46419- 46428.

U.S. EPA/OPP 2005b. (U.S. Environmental Protection Agency/Office of Pesticide Programs). Pesticide Product Registrations; Conditional Approval. Federal Register. 70(225): 70837-70838.

U.S. EPA/OPP-EFED (U.S. Environmental Protection Agency/Office of Pesticide Programs – Environmental Fate and Effects Division). 2004. EFED Science Chapter: Environmental Fate and Ecological Risk Assessment for the Registration of Aminopyralid.

U.S. EPA/OPP-EFED (U.S. Environmental Protection Agency/Office of Pesticide Programs – Health Effects Division). 2004. HED Science Chapter on Aminopyralid.

U.S. EPA/OPPEPTS (U.S. Environmental Protection Agency/Office of Prevention, Pesticides Environmental Protection and Toxic Substances). 2005. Pesticide Fact Sheet: Aminopyralid. Available at: http://www.epa.gov/opprd001/factsheets/aminopyralid.pdf.

U.S. EPA/OPPTS (U.S. Environmental Protection Agency/Office of Pollution Prevention and Toxic Substances). 2007a. OPPTS Series 835 (Health Effects) Test Guidelines (Master List). Copies of all protocols available at: http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/835_Fate_Transport_and_Transformation_Test_Guidelines/index.html.

U.S. EPA/OPPTS (U.S. Environmental Protection Agency/Office of Pollution Prevention and Toxic Substances). 2007b. OPPTS Series 870 (Health Effects) Test Guidelines (Master List). Copies of all protocols available at: http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/index.html.

U.S. EPA/OPPTS (U.S. Environmental Protection Agency/Office of Pollution Prevention and Toxic Substances). 2007c. Ecological Effects Guidelines (Master List). Copies of all protocols available at: http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/index.html.

U.S. EPA/OPPTS (U.S. Environmental Protection Agency/Office of Prevention, Pesticides and Toxic Substances). 1996a. Ecological Effects Test Guidelines, OPPTS 850.5400 Algal Toxicity, Tiers I and II. EPA712–C–96–164 dated April 1996. Available at: http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-5400.pdf. **Copy on Peer-Review CD.**

U.S. EPA/OPPTS (U.S. Environmental Protection Agency/Office of Prevention, Pesticides and Toxic Substances). 1996b. Ecological Effects Test Guidelines, OPPTS 850.4400 Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II. EPA712–C–96–156 dated April 1996. Available at: http://www.epa.gov/opptsfrs/publications/ OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-4400.pdf. **Copy on Peer-Review CD.**

U.S. EPA/OPPTS (U.S. Environmental Protection Agency/Office of Prevention, Pesticides and Toxic Substances). 1996c. Health Effects Test Guidelines, OPPTS 870.1100 Acute Oral Toxicity. EPA 712–C–98–190 dated August 1996. Available at: http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/. **Copy on Peer-Review CD.**

U.S. EPA/ORD (U.S. Environmental Protection Agency/Office of Research and Development). 1992. Dermal Exposure Assessment: Principles and Applications. EPA/600/8-91/011B. Interim Report. Exposure Assessment Group, Office of Health and Environmental Assessment, Washington, DC. Available at: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=12188

U.S. EPA/ORD (U.S. Environmental Protection Agency/Office of Research and Development). 2001. Help Manual for Benchmark Dose Software Version 1.3. Office of Research and Development, U.S. EPA, Washington, DC. EPA/600/R-00/014F. Report dated March 2001. Available at: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=20167.

USDA/APHIS (U.S. Department of Agriculture Animal and Plant Health Inspection Service). 1993. Nontarget Risk Assessment for the MEDFLY Cooperative Eradication Program. USDA Animal and Plant Health Inspection Service. February 1993.

USDA/FS (USDA Forest Service). 2006. Final Environmental Impact Statement, Weed Management, Custer National Forest. Record of Decision dated November 1, 2006. Available at: http://www.fs.fed.us/r1/custer/projects/Planning/weedwebdocs/

USDA/FS (U.S. Department of Agriculture/Forest Service). 1989a. Final Environmental Impact Statement: Vegetation Management in the Coastal Plain/Piedmont, Management Bulletin R8-MB-23, dated January, 1989. 1213 pp.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1989b. Draft Environmental Impact Statement: Vegetation Management in the Ozark/Ouachita Mountains, Management Bulletin R8-MB-23, dated June, 1989. 499 pp.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1989c. Final Environmental Impact Statement: Vegetation Management in the Appalachian Mountains, Management Bulletin R8-MB-38, dated July, 1989. 1104 pp.

van Hemmen JJ. 1992. Agricultural pesticide exposure data bases for risk assessment. Rev. Environ. Contam. Toxicol. 126: 1-85.

Waalkens-Berendsen DH; Kuilman-Wahls ME; Baumlr A. 2004. Embryotoxicity and teratogenicity study with neohesperidin dihydrochalcone in rats. Regul Toxicol Pharmacol. 40(1): 74-9.

Walker R; El Harith EA. 1978. Nutritional and Toxicological Properties of Some Raw and Modified Starches. Ann Nutr Aliment. 32(2/3): 671-679.

Ward T; Boeri R. 2001. XDE750: 14 Day Soil Exposure Acute Toxicity to the Earthworm, *Eisenia foetida*. Project Number: 2219/DO, 011049. Unpublished study prepared by T.R. Wilbury Laboratories, Inc. 34 p. MRID No. 46235733.

Wilson C. 2001. XDE-750: A Dermal Sensitization Study in Hartley Albino Guinea Pigs: Maximation Design: Final Report. Project Number: 3504/155,011034. Unpublished study prepared by Springborn Laboratories, Inc. 77p. MRID No. 46235613.

Wilson CF. 2002. GF-871: A Dermal Sensitization Study In Hartley Albino Guinea Pigs, Maximization Design. Springborn Laboratories, Inc. (SLI), Ohio Research Center, Spencerville, Ohio. SLI Study No. 3504.250. Dow Study No. 021071. Report dated June 18, 2002. (Not in EPA bibliography from FOIA.) MRID No. 46235614.

Wilson D; Brooks K; Radtke B. 2002. GF-871:Acute Oral Toxicity Study in Fischer 344 Rats. Project Number: 021096. Unpublished study prepared by Dow Chemical Co. 44 p. MRID No. 46235604.

Wilson DM; Brooks KF; Radtke BJ. 2002. GF-871: Acute Dermal Toxicity Study in Fischer 344 Rats. Project Number: 021097. Unpublished study prepared by Dow Chemical Co. dated Dec. 2, 2002.

Winegardner DL. 1996. An Introduction to Soils for Environmental Professionals. CRC Press, Boca Raton, Florida. 270 pp.

Wood A. 2006. Compendium of Pesticide Common Names: Aminopyralid. Available at: http://www.alanwood.net/pesticides/index_cn_frame.html.

Yano B; Dryzga M. 2000. XR-750:4-Week Repeated Dose Dietary Toxicity Study in CD-1 Mice. Project Number: 001048. Unpublished study prepared by The Dow Chemical Co. 289 p. MRID No. 46235624.

Yildiz J; Arslan K; Coskun I; Yildiz B. 2005. A Geometric Modeling of Dog Intestine. Jurk. J Vet Anim Sci. 30: 483-488.

Ylitalo P; Narhi U; Elo HA. 1990. Increase in the acute toxicity and brain concentrations of chlorophenoxyacetic acids by probenecid in rats. Gen. Pharma. 21, 811.

Yoder R; Smith K. 2002. Aerobic Soil Degradation of XDE-750 in Five North American Soils. Project Number: 010091. Unpublished study prepared by Dow AgroSciences LLC. 106 p. MRID No. 46235729.

Yoder R; Smith K. 2003. Degradation of XDE-750 in 2 European and 1 US Sediment and Pond Water Systems. Project Number: 020062. Unpublished study prepared by Dow AgroSciences LLC. 88 p. MRID No. 46235731.

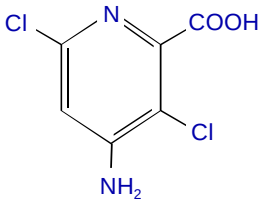
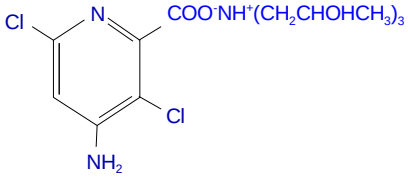
Yoshitomi K; Boorman GA. 1990. Eye and Associated Glands. Chapter 16 in Pathology of the Fisher Rat. Edited by: Boorman GA; Eustis SL; Elwell MR; Montgomery CA; MacKenzie WF. Academic Press, Inc. San Diego, CA. pp. 239-259.

Table 1: Commercial Formulations Containing Aminopyralid

Formulations [1]	Dow Code(s)	Active Ingredients	Inerts/Impurities
Aminopyralid formulations covered by risk assessment			
Milestone [EPA Reg. No. 62719-519]	GF-871	Aminopyralid, Triisopropanol-ammonium salt (40.6 % w a.i./v) Equivalent to 21.1% a.e. or 2 lbs a.e./gal.	Water, sole inert (Jachetta 2006)
Milestone VM [EPA Reg. No. 62719-537]	GF-871	Identical to Milestone	Water, sole inert (Jachetta 2006)
Mixture formulations not covered by risk assessment			
CleanWave [EPA Reg. No. 62719-525]	GF-982	Aminopyralid (1.92%) Fluroxypyr methylheptyl ester (20.22 %)	Aromatic solvents including naphthalene and dipropylene glycol methyl ether
ForeFront R&P [EPA Reg. No. 62719-524]	GF-1004	Aminopyralid (6.58%) 2,4-D (51.06%)	Polyglycol 26-2 Triisopropanolamine

[1] All information from the product labels and material safety data sheets from Greenbook (2006) unless otherwise specified.

Table 2: Selected Physical and Chemical Properties of Aminopyralid

Property	Value	Reference
Structure, acid		
Structure, TIPA Salt		
Appearance/state, ambient	Brown liquid	Greenbook 2006
Bioconcentration	None anticipated.	U.S. EPA/EFED 2005
	3.162 (QSAR estimate)	EPI Suite 2004
CAS number	150114-71-9	Jachetta et al. 2004
Conversion factor, TIPA salt to aminopyralid acid	0.519748 [207/398.27]	See molecular weights below
Conversion factor, TIPA salt to TIPA	0.48276 [191.27/398.27]	See molecular weights below
Density	1.14 g/mL (20 °C)	Greenbook 2006
Foliar halftimes	13.4 days with 95% confidence interval of 10.5 days to 16.3 days	Roberts et al. 2004 [2]
Henry's law constant	9.30 x 10^{-12} Pa m^3 mol^{-1}	EPI Suite 2004
Kd (Koc), soil type	0.22 (average) 0.13 (median) 0 - 39 (range)	Jachetta et al. 2004
Koc, mL/g (soil type)	0.81 (clay) 0.87 (clay) 6.2 (clay) 7.96 (silty clay) 23.69 (clay loam) 27.24 (clay loam) 4.52 (silt) 5.51 (silty loam) 5.31 (silty loam) 8.91 (loam) 11.62 (loam) 28 (loamy sand)	Jachetta et al. 2004

Property	Value	Reference
log K_{ow}	-1.75 (pH 5, 19 °C) -2.87 (pH 7, 19 °C) -2.96 (pH 9, 19 °C) 0.201 (unbuffered, 19 °C)	Jachetta et al. 2004
Melting point	139.27 °C (QSAR Estimate)	EPI Suite 2004
Metabolites, environmental	$C0_2$, NH_3, and chloride ion	Jachetta et al. 2004
Metabolites, aqueous photolysis	Oxamic acid and malonamic acid as well as four or more unidentified acid amides	U.S. EPA/EFED 2005
Molecular formula, acid	$C_6H_4Cl_2N_2O_2$	EPI Suite 2004
Molecular formula, salt	$C_6H_4Cl_2N_2O_2$,*$[CH_3CH(OH)CH_2]_3N$	CambridgeSoft 2007[3]
Molecular weight, acid	207 g/mole	Jachetta et al. 2004
Molecular weight, TIPA salt	398.27 g/mole (MW TIPA = 191.2698)	CambridgeSoft 2007[3]
pH	7.33 (19.8 °C as a 1% solution)	Greenbook 2006
pK_a	2.56 (20 °C)	Jachetta et al. 2004
Sediment-Water halftimes	272 (127 - 447) days (water phase) 759 (533 - 999) days (total system)	Jachetta et al. 2004
SMILES Notation	c1c(nc(c(c1N)Cl)C(=O)O)Cl	Jachetta et al. 2004
Soil halftimes (NOS)	130.4 days (upper 90th percentile)	Jachetta et al. 2004
Soil halftimes, field dissipation (range)	30 (25 - 35) days, U.S. sites (n=3) 38 (6 - 74) days, Canadian sites (n=5) 25 (8 – 35) days, European sites (n= 4)	Jachetta et al. 2004
Soil halftimes (aerobic)	88.7 days (N.S.) 60, 48, 59, and 46 days (silty loam) 25, 49, 34 days (loam) 14, 21, 14 days (sandy loam) 266, 341, 343 days(clay loam) 5, 5 days (clay)	Jachetta et al. 2004
Soil halftimes (anaerobic)	Stable	Jachetta et al. 2004
Soil photolysis	61 days	Jachetta et al. 2004
Synonyms, general	4-amino-3,6-dichloro-2-pyridinecarboxylic acid (IUPAC) 4-amino-3,6-dichloro-pyridinecarboxylic acid (CAS)	Jachetta et al. 2004
Synonyms, Dow Codes	XDE-750, XR-750, DE-750 [all refer to the acid] GF-871: TIPA salt of aminopyralid	

Property	Value	Reference
U.S. EPA Docket Number	OPP-2004-0139	
Vapor pressure	7.14×10^{-11} mm Hg	Jachetta et al. 2004
Water halftime (NOS)	447 days (upper limit)	Jachetta et al. 2004
Water hydrolysis halftime	Stable	Jachetta et al. 2004
Water, aquatic metabolism	462-990 days	U.S. EPA/EFED 2005
Water photolysis halftime	0.6 days	Jachetta et al. 2004
Water solubility (mg/L)	248,000 mg/L (pH 5, 20 °C) 205,000 mg/L (pH 7, 20 °C) 203,000 mg/L (pH 9, 20 °C) 2,480 mg/L (Unbuffered)	Ghaoui 2003

[1] Specific environmental fate parameters used in modeling are discussed in Section 3.2.
[2] Data from Roberts et al. (2004) summarized in Appendix 6.

Table 3: Agents causing cecal enlargement in rats

Agent	Exposure [1,2]	Relative Increase in Cecal Weight	Reference
Herbicides			
Aminopyralid	Dietary: ≈54.5 mg/kg bw/day x 10 wks Dietary: ≈218 mg/kg bw/day x 10 wks	10% M; 9.5% F, no pathology. 38% M; 33% F, hyperplasia.	Stebbins and Day 2000
Picloram	Gavage: 60, 190, or 600 mg/kg/day x 14 days	Dose-dependent increase (NOS)	Hayes et al. 1986
Other compounds			
γ-Cyclodextrin (cyclic polysaccharide)	Diet: 10% (≈5,000 mg/kg bw/day) x 13 wks	≈30% M; 33%F – NS [3]	Lina and Bar 2004
Erythritol (polyol)	Diet: 10% (≈5,000 mg/kg bw/day) x 13 wks	≈50% M [3]	Til et al. 1996
Erythritol (polyol)	Dietary: 5% (≈2,500 mg/kg bw/day) x 2 yr	≈4% M– NS; 16%F [3]	Lina et al. 1996
Glucomannan (polysaccharide)	Diet: 20% (≈10,000 mg/kg bw/day) x 2 wks	≈300%	Konishi et al. 1984
Josamycin (antibiotic)	Diet: 0.1% (50 mg/kg bw/day) x 52 wks	*mild* NOS. No pathology.	Kasahara et al. 2002
Lactitol (disaccharide)	Diet: 5% (≈2,500 mg/kg bw/day) x 4 wks after weaning	Cecal enlargement (NOS), no pathology	Sinkeldam et al. 1992
Mannitol (polyol)	Diet: 10% (≈5,000 mg/kg bw/day) x 2 yr	≈35% M; 42%F [3]	Lina et al. 1996
$MgSO_4$	Diet: 3% (≈1,500 mg/kg bw/day) x 20 days	≈27% [3]	Leegwater et al. 1974
Modified starch	Diet: 20% (≈10,000 mg/kg bw/day) x 8 wks	≈38% [3]	Leegwater et al. 1974
Modified starches (4/5)	Diet: 10% diet (≈5,000 mg/kg bw/day) x 2 years	Cecal enlargement (NOS) with no pathology	De Groot et al. 1974
Neohesperidin dihydrochalcone (disaccharide)	Diet: 5% (≈2,500 mg/kg bw/day) x 21 days (gestation period)	≈25% F [3]	Waalkens-Berendsen et al. 2004
Polyethylene glycol	Diet: 4% (≈2,000 mg/kg bw/day) x 20 days	≈37% [3]	Leegwater et al. 1974
Raw potato starch	Diet: 16% (≈8,000 mg/kg bw/day) x 21 days	≈100% [3] Up to 800% enlargement at higher doses.	El-Harith et al. 1978

[1] Unless otherwise specified, the minimum dose associated with cecal enlargement.
[2] Doses calculated from dietary studies assuming 5% daily food consumption relative to body weight.
[3] Calculated from tables in publication using relative empty cecal weights.
NOS = not otherwise specified; NS=not statistically significant; wks=weeks; yr=years

Table 4: Chemical and site parameters used in GLEAMS modeling for aminopyralid

Parameter	Clay	Loam	Sand	Note/ Reference
Halftimes (days)				
Aquatic Sediment		1073.6		Note 1
Foliar		19		Note 2
Soil		343		Note 3
Water		1000		Note 4
Soil $K_{o/c}$, mL/g	0.87	8.91	4.52	Note 5
Sediment K_d, mL/g	0.63	0.55	0.39	Note 5
Water Solubility, mg/L		205,000		Note 7
Foliar wash-off fraction		0.95		Note 6
Fraction applied to foliage		0.5		Note 6

Note 1 Value used by EFED based on the upper 90th percentile of DT_{50} values in sand, silt loam and sandy loam taken from Yoder and Smith 2003, MRID 46235731. See Appendix 1.

Note 2 This is the highest value reported (i.e., the halftime on forage reported in Roberts et al. 2004, MRID 46235721). It is somewhat higher than the upper 95th percentile of halftimes on hay and forage given in Roberts et al. 2004 (MRID 46235721) and McCormick et al. 2004 (MRID 46235722) – i.e., 16.3 days. See Appendix 6. The first-order dissipation model did not fit the data well, probably due to intermittent rainfall.

Note 3 This is the longest halftime reported in Yoder and Smith 2002, MRID 46235729 (Appendix 1). In GENEEC modeling, the U.S. EPA/OPP-EFED (2005) used a somewhat shorter soil half-time of 310.5 days. This is based on a reanalysis of the halftime on silt loam (Holdrege) soil of 103.5 day from the study by Yoder and Smith 2002. EFED multiplied this halftime by 3 to account for using only a single value because they considered the 103.5 day value to be the only acceptable value. See comments in Appendix 1.

Note 4 The hydrolysis rate of aminopyralid is negligible (Cook 2003a, MRID 46235726). To be conservative, aqueous photolysis is not considered.

Note 5 The values are taken from Rutherford 2002, MRID 46235732 (Appendix 1). As discussed by U.S. EPA/OPP-EFED (2005), soil binding is variable and is not closely related to the organic carbon content of the soil. U.S. EPA/OPP-EFED (2005) used a Kd of 0.03. Assuming a 1% OC, this corresponds to a Koc of 3. Jachetta et al. (1974) used a Koc of 7.1 for SCI-GROW and 0.81 for GENEEC2.

Note 5 Based on Rutherford and Meitl 2004, MRID 46235730 (Appendix 1). Reported mean value for sand is used directly. Reported mean and upper range of values for sandy loam are used for loam and clay, respectively.

Note 6 The foliar washoff fraction not available for aminopyralid. Two closely related herbicides (triclopyr and clopyralid) have reported foliar washoff fractions of 0.95 (Knisel and Davis 2000). The fractional application to foliage is a default for liquid formulations.

Note 7 Water at pH 7 from Table 2. See Section 3.2.3.4.3 for discussion.

Table 5: Summary of modeled concentrations in streams based on standard GLEAMS modeling

(all concentrations are in µg/L or ppb per lb/acre applied)

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0.331	65.7	0.576	22.6	7.71	184
20	3.58	92.4	4.26	93.1	11.3	158
25	7.54	98.4	6.14	95.3	11	204
50	15.1	71.4	5.76	101	7.61	241
100	17.9	43	3.68	98.5	4.05	222
150	18.5	33	2.58	84.9	2.74	202
200	18.7	31	1.97	72.8	2.07	180
250	18.7	31.6	1.59	64.1	1.66	170

Table 6: Summary of modeled concentrations in ponds based on standard GLEAMS modeling

(all concentrations are in µg/L or ppb per lb/acre applied)

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	4.89	6.15	3.34	16.8	85.4	182
20	10.3	30.4	27.7	67.1	116	160
25	17.2	40.5	42.8	73.1	110	139
50	20.1	30.4	46.5	59.2	70.5	130
100	13.2	17.5	30.5	55.9	40.8	126
150	9.65	12.3	22.3	49.8	29.3	120
200	7.6	11.3	17.5	44.3	23	114
250	6.3	11.7	14.5	39.7	19	110

Table 7: Peak concentrations in a small stream based on Gleams-Driver simulations

(all concentrations are in µg/L or ppb per lb/acre applied)

Site	Clay	Loam	Sand
Dry and Warm	0.14 (0 - 0.51)	0 (0 - 0.016)	0 (0 – 6.2)
Dry and Temperate	0.087 (0 - 0.33)	0 (0 - 0.41)	0 (0 - 49)
Dry and Cold	0.096 (0.00027 – 3.6)	0 (0 - 0.03)	0 (0 – 4.1)
Average Rainfall and Warm	28 (12 - 44)	30 (13 - 60)	120 (90 - 140)
Average Rainfall and Temperate	27 (16 – 38)	32 (17 – 46)	100 (79 - 120)
Average Rainfall and Cool	30 (24 – 35)	33 (26 - 40)	100 (84 - 120)
Wet and Warm	42 (39 - 44)	49 (46 - 52)	130 (110 - 140)
Wet and Temperate	43 (40 – 45)	49 (46 – 51)	120 (110 - 130)
Wet and Cool	49 (47 – 52)	59 (52 - 61)	130 (120 - 140)
Badlands NP, SD	2.1 (0.39 – 5.8)	0.36 (0.0008 – 5.4)	58 (3.7 - 130)
Theodore Roosevelt NP, ND	1.8 (0.37 – 8.9)	0.2 (0.0009 - 10)	51 (1.9 - 98)
Glacier Bay NP, AK	45 (41 – 48)	50 (43 - 55)	130 (110 - 140)

Table 8: One-year average concentrations in a small stream based on Gleams-Driver simulations

(all concentrations are in µg/L or ppb per lb/acre applied)

Site	Clay	Loam	Sand
Dry and Warm	0.00062 (0 - 0.0021)	0 (0 - 0.00007)	0 (0 - 0.035)
Dry and Temperate	0.00042 (0 - 0.0018)	0 (0 - 0.0013)	0 (0 - 0.33)
Dry and Cold	0.00029 (7.5E-07 - 0.01)	0 (0 - 0.000083)	0 (0 - 0.043)
Average Rainfall and Warm	1.2 (0.29 - 2.4)	1.3 (0.28 - 2.7)	4.1 (2.9 – 6.1)
Average Rainfall and Temperate	1.8 (0.53 - 2.7)	1.7 (0.4 - 2.6)	4.7 (3.3 - 7)
Average Rainfall and Cool	2.3 (1.5 - 3)	2.1 (1.4 - 2.8)	4.7 (3.8 – 6.8)
Wet and Warm	4.2 (3.3 – 5.8)	3.9 (3.1 – 5.7)	5 (3.6 – 7.6)
Wet and Temperate	3.8 (2.9 – 5.6)	3.7 (2.8 – 5.3)	4.5 (3.4 – 7.1)
Wet and Cool	5.8 (4.4 – 6.7)	5.9 (4.4 – 6.6)	6.4 (3.6 – 8.1)
Badlands NP, SD	0.013 (0.003 - 0.1)	0.0028 (0.0000064 - 0.061)	0.87 (0.043 - 2.5)
Theodore Roosevelt NP, ND	0.013 (0.0028 - 0.13)	0.0016 (0.000003 - 0.13)	0.78 (0.015 - 2.8)
Glacier Bay NP, AK	4.6 (3.8 – 6.6)	4.5 (3.7 – 6.5)	6.2 (4.9 – 9.2)

Table 9: Peak concentrations in a small pond based on Gleams-Driver simulations

(all concentrations are in µg/L or ppb per lb/acre applied)

Site	Clay	Loam	Sand
Dry and Warm	0.05 (0 - 0.19)	0 (0 - 0.007)	0 (0 - 3)
Dry and Temperate	0.03 (0 - 0.19)	0 (0 - 0.24)	0 (0 - 60)
Dry and Cold	0.024 (0.00006 - 1.1)	0 (0 - 0.018)	0 (0 - 3)
Average Rainfall and Warm	90 (24 - 210)	110 (25 - 260)	400 (240 - 600)
Average Rainfall and Temperate	80 (31 - 150)	100 (28 - 180)	310 (210 - 500)
Average Rainfall and Cool	100 (70 - 160)	110 (70 - 170)	300 (260 - 500)
Wet and Warm	150 (90 - 230)	160 (100 - 260)	400 (240 - 500)
Wet and Temperate	70 (60 - 110)	80 (60 - 120)	200 (140 - 300)
Wet and Cool	190 (130 - 260)	210 (140 - 290)	210 (130 - 400)
Badlands NP, SD	1 (0.23 - 8)	0.29 (0.0003 - 7)	80 (2.2 - 300)
Theodore Roosevelt NP, ND	1.1 (0.18 - 17)	0.15 (0.0005 - 15)	70 (1.3 - 260)
Glacier Bay NP, AK	210 (140 - 250)	230 (160 - 270)	400 (240 - 500)

Table 10: One-year average concentrations in a small pond based on Gleams-Driver simulations

(all concentrations are in µg/L or ppb per lb/acre applied)

Site	Clay	Loam	Sand
Dry and Warm	0.01 (0 - 0.05)	0 (0 - 0.002)	0 (0 - 0.5)
Dry and Temperate	0.007 (0 - 0.05)	0 (0 - 0.06)	0 (0 - 19)
Dry and Cold	0.005 (0.000012 - 0.23)	0 (0 - 0.004)	0 (0 - 1.1)
Average Rainfall and Warm	30 (6 - 60)	40 (7 - 90)	200 (120 - 260)
Average Rainfall and Temperate	40 (12 - 70)	40 (10 - 90)	180 (140 - 260)
Average Rainfall and Cool	50 (31 - 70)	50 (30 - 80)	180 (150 - 220)
Wet and Warm	60 (30 - 100)	70 (40 - 120)	130 (80 - 200)
Wet and Temperate	30 (14 - 60)	31 (13 - 60)	40 (26 - 90)
Wet and Cool	80 (60 - 110)	90 (60 - 110)	110 (50 - 190)
Badlands NP, SD	0.3 (0.07 - 2.6)	0.08 (0.00015 - 2.1)	30 (0.9 - 100)
Theodore Roosevelt NP, ND	0.29 (0.05 - 4)	0.05 (0.00014 - 4)	26 (0.5 - 110)
Glacier Bay NP, AK	100 (80 - 110)	110 (90 - 130)	200 (120 - 230)

Table 11: Estimated water contamination rates (WCR) of aminopyralid in surface and groundwater based on modeling

(all concentrations are in µg/L or ppb per lb/acre applied)

Scenario	Peak	Long-Term Average
MODELING FOR THIS RISK ASSESSMENT (1 lb a.e./acre)		
Direct Spray of Pond (Section 3.2.3.4.2) [a]	56	N/A
Pond, drift at 25 feet (Section 3.2.3.4.2) [a]	7.7	N/A
Direct Spray of Stream (Section 3.2.3.4.2) [a]	91	N/A
Stream, drift at 25 feet (Section 3.2.3.4.2) [a]	12.8	N/A
GLEAMS Stream, Section 3.2.3.4.3	22.6 – 240	0.33 – 18.7
GLEAMS, Pond, Section 3.2.3.4.3	6 – 180	3.3 – 116
Gleams-Driver, Stream, Section 3.2.3.4.4	30 (0.1 – 14)	2 (0.01 – 9.2)
Gleams-Driver, Pond, Section 3.2.3.4.4	110 (0.05 – 600)	40 (0.01 – 260)
OTHER MODELING		
U.S. EPA		
PRZM/EXAMS, Index Reservoir [b]	91	17.6
GENEEC [c]	58	49
SCI-GROW [b]	5.7	N/A
Jachetta et al. 2004		
GENEEC, ground [d]	56	36
GENEEC, aerial [d]	58	37
SCI-GROW [e]	15	N/A
SCI-GROW [f]	1.1	N/A

[a] Section 3.2.3.4.2 discusses expected concentrations in terms of the nominal application rate of 0.078 lb a.e./acre. The concentrations in Section 3.2.3.4.2 are divided by the application rate of 0.078 lb a.e./acre to get the water contamination rates given in this table.

[b] From U.S. EPA/OPP-HED 2004, Table 6.2, p. 36. Values in Table 6.2 from this EPA report adjusted to WCR values by dividing by the modeled application rate of 0.11 lb a.e./acre used by EPA.

[c] From U.S. EPA/OPP-EFED 2004, Table 6, p. 24. Values in Table 6 from this EPA report adjusted to WCR values by dividing by the modeled application rate of 0.11 lb a.e./acre used by EPA. The longer term value is the maximum 60 day time-weighted average concentration.

[d] From Jachetta et al. (2004). Input values in Table 43 (p.81) and output values in Table 44 (p. 82). Reported concentrations adjusted to WCR by dividing by the application rate, 0.107 lb a.e./acre. The longer term values are based on the maximum 90 day time-weighted average concentration.

[e] From Jachetta et al. (2004). Input values in Table 45 (p. 84) using a soil halftime of 88.7 days. Output values in Table 64, p. 84. Reported concentrations adjusted to WCR by dividing by the application rate, 0.107 lb a.e./acre.

[f] From Jachetta et al. (2004). Input values in Table 45 (p. 84) using a soil halftime of 30 days. Output values in Table 64, p. 84. Reported concentrations adjusted to WCR by dividing by the application rate, 0.107 lb a.e./acre.

Table 12: Concentrations of aminopyralid in surface water used in this risk assessment.
(see Section 3.2.3.4.7 for discussion)

Typical Application Rate:	**0.078**	**lb/acre**
	Peak Concentration (ppb or µg/L)	**Longer-term Concentration (ppb or µg/L)**
Central	7.8	3.12
Lower	0.16	0.078
Upper	46.8	20.3
Water contamination rate [a]	**mg/L per lb/acre applied**	
	Peak Concentration (mg/L or ppm per lb/acre)	**Longer-term Concentration (mg/L or ppm per lb/acre)**
Central	0.1	0.04
Lower	0.002	0.001
Upper	0.6	0.260

[a] Water contamination rates – concentrations in units of mg a.e./L expected at an application rate of 1 lb a.e./acre. Units of mg a.e./L are used in the EXCEL workbook that accompanies this risk assessment.

Table 13: Summary of the cumulative loss from soil runoff and sediment as a proportion of the application rate

Annual Rainfall (inches)	Clay	Loam	Sand
5	0	0	0
10	0	0	0
15	0.0119	0	0
20	0.0155	0	0
25	0.0179	0	0
50	0.0234	5.06E-06	0
100	0.03	9.21E-06	0
150	0.0365	1.14E-06	0
200	0.0432	1.97E-07	0
250	0.0498	4.79E-08	0

Table 14: Maximum depth (inches) of penetration into soil.

Annual Rainfall	Clay	Loam	Sand
5	6.5	6.5	6.5
10	6.5	6.5	6.5
15	60	60	60
20	60	60	60
25	60	60	60
50	60	60	60
100	60	60	60
150	60	60	60
200	60	60	60
250	60	60	60

Table 15: Summary of modeled concentrations in the entire 60 inch soil column

(all units are mg/kg soil or ppm per lb/acre applied)

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0.0576	0.0953	0.0529	0.0879	0.0543	0.0898
10	0.0636	0.105	0.0609	0.102	0.0543	0.09
15	0.0679	0.116	0.0653	0.111	0.0559	0.0872
20	0.0716	0.12	0.0621	0.0982	0.0383	0.0619
25	0.0691	0.11	0.0532	0.0823	0.0286	0.0495
50	0.0559	0.0861	0.0284	0.0491	0.0128	0.0455
100	0.0472	0.074	0.0161	0.0455	0.00595	0.0444
150	0.044	0.0694	0.0123	0.0455	0.00388	0.0418
200	0.0423	0.0668	0.0104	0.0454	0.00289	0.0387
250	0.0412	0.0651	0.00931	0.0453	0.00232	0.0357

Table 16: Summary of modeled concentrations in the top 12 inches of the soil column

(all units are mg/kg soil or ppm per lb/acre applied)

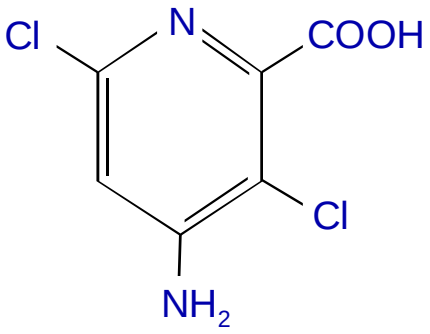
Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0.288	0.476	0.264	0.439	0.271	0.449
10	0.318	0.525	0.305	0.508	0.272	0.45
15	0.215	0.363	0.171	0.301	0.0901	0.234
20	0.155	0.294	0.107	0.243	0.0497	0.22
25	0.125	0.268	0.0778	0.229	0.034	0.212
50	0.0802	0.243	0.0329	0.212	0.0135	0.171
100	0.0621	0.237	0.017	0.185	0.00623	0.118
150	0.0565	0.235	0.0127	0.168	0.00414	0.116
200	0.0537	0.234	0.0107	0.157	0.00315	0.116
250	0.0519	0.232	0.00961	0.149	0.00258	0.116

Table 17: Summary of toxicity values used in ecological risk assessment

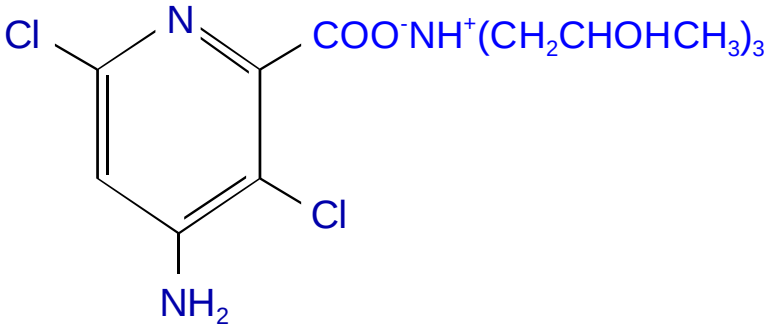
Group/Duration		Endpoint	Toxicity Value	Source
Terrestrial Animals				
Acute				
Non-canine Mammals		Rabbit NOAEL	104 mg a.e./kg bw/day	Section 4.3.2.1
Canine Mammals		Surrogate NOAEL	104 mg a.e./kg bw/day	Section 4.3.2.1
Birds[1]		Quail NOAEL	14 mg a.e./kg bw/day	Section 4.3.2.2
Soil Invertebrates		Earthworm NOAEL	5000 mg a.e./kg soil	Section 4.3.2.3
Other Invertebrates		Honey Bee NOAEL	>1075 mg a.e./kg bw	Section 4.3.2.3
Longer-term				
Non-canine Mammals		Rat NOAEL	50 mg a.e./kg bw/day	Section 4.3.2.1
Canine Mammals		Surrogate NOAEL	50 mg a.e./kg bw/day	Section 4.3.2.1
Birds [1]		Surrogate NOAEL	184 mg a.e./kg bw/day	Section 4.3.2.2
Terrestrial Plants				
Soil exposure	Sensitive	NOEC, dicot	0.00048 lb a.e./acre	Section 4.3.2.4
	Tolerant	NOEC, monocot	0.11 lb a.e./acre	Section 4.3.2.4
Foliar exposure	Sensitive	NOEC, dicot	0.0002 lb a.e./acre	Section 4.3.2.4
	Tolerant	NOEC, monocot	0.11 lb a.e./acre	Section 4.3.2.4
Aquatic Animals				
Acute				
Amphibians	Sensitive	N/A	N/A	Section 4.3.3.2
	Tolerant	NOEC, *Rana pipiens*	95.2 mg a.e./L	Section 4.3.3.2
Fish	Sensitive	NOEC, trout	50 mg a.e./L	Section 4.3.3.1
	Tolerant	NOEC, bluegill	100 mg a.e./L	Section 4.3.3.1
Invertebrates [2]	Sensitive	NOEC, mysid shrimp	89 mg a.e./L	Section 4.3.3.3
	Tolerant	NOEC, daphnid	98.6 mg a.e./L	Section 4.3.3.3
Longer-terms				
Amphibians	Sensitive	N/A	N/A	Section 4.3.3.2
	Tolerant	N/A	N/A	Section 4.3.3.2
Fish	Sensitive	N/A	N/A	Section 4.3.3.1
	Tolerant	NOEC, fathead minnows	1.36 mg a.e./L	Section 4.3.3.1
Invertebrates [2]	Sensitive	N/A	N/A	Section 4.3.3.3
	Tolerant	NOEC, daphnid	102 mg a.e./L	Section 4.3.3.3
Aquatic Plants				
Algae	Sensitive	NOEC, diatom	6 mg a.e./L	Section 4.3.3.4.
	Tolerant	NOEC, green algae	23 mg a.e./L	Section 4.3.3.4.
Macrophytes	Sensitive	N/A	N/A	Section 4.3.3.4.
	Tolerant	NOEC, duckweed	44 mg a.e./L	Section 4.3.3.4.

[1]Note that the longer-term NOEC for birds is greater than the acute NOEC for birds. As discussed in Section 4.3.2.2, this is due to the use of a gavage NOEC value for acute exposures and a dietary NOEC value for longer-term exposures.

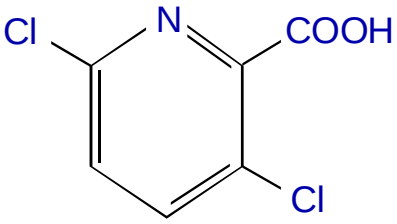
[2]Note that the acute NOEC for daphnids is somewhat less than the chronic NOEC. This is an artifact of differences in measured concentrations. The nominal exposures were both 100 mg a.e./L.



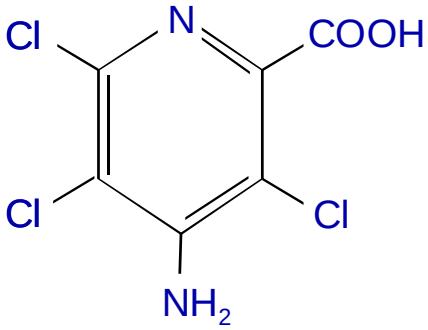
Aminopyralid



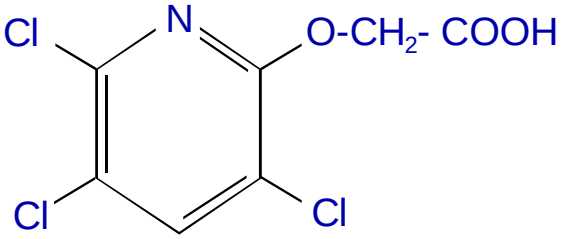
Aminopyralid, TIPA Salt



Clopyralid



Picloram



Triclopyr

Figure 1: Structures of Aminopyralid, Clopyralid, Picloram, and Triclopyr

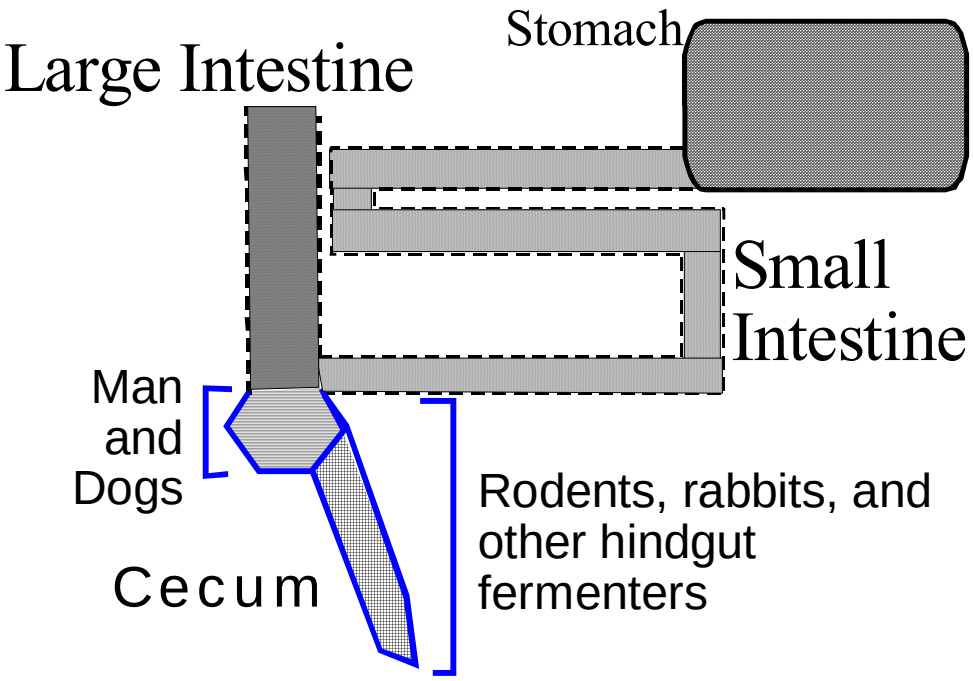


Figure 2: Schematic diagram of position of cecum in mammals

Parallel line area only: Simple cecum of man, dogs, and most other carnivores.

Parallel and hatched line area: Elaborated cecum of rodents, rabbits and other hindgut fermenters.

Adapted from illustrations in Kardong (2006, Figure 13.28), Yildiz et al. (2005) and various illustrations on Internet (e.g., http://www.utm.edu/departments/cens/biology/rirwin/ratAbdAnsw.htm and http://www.wsu.edu/~rlee/biol103/lect06/sld001.htm, http://www.chemistry.ucsc.edu/teaching/Spring97/BIOC150L/rat1.html)

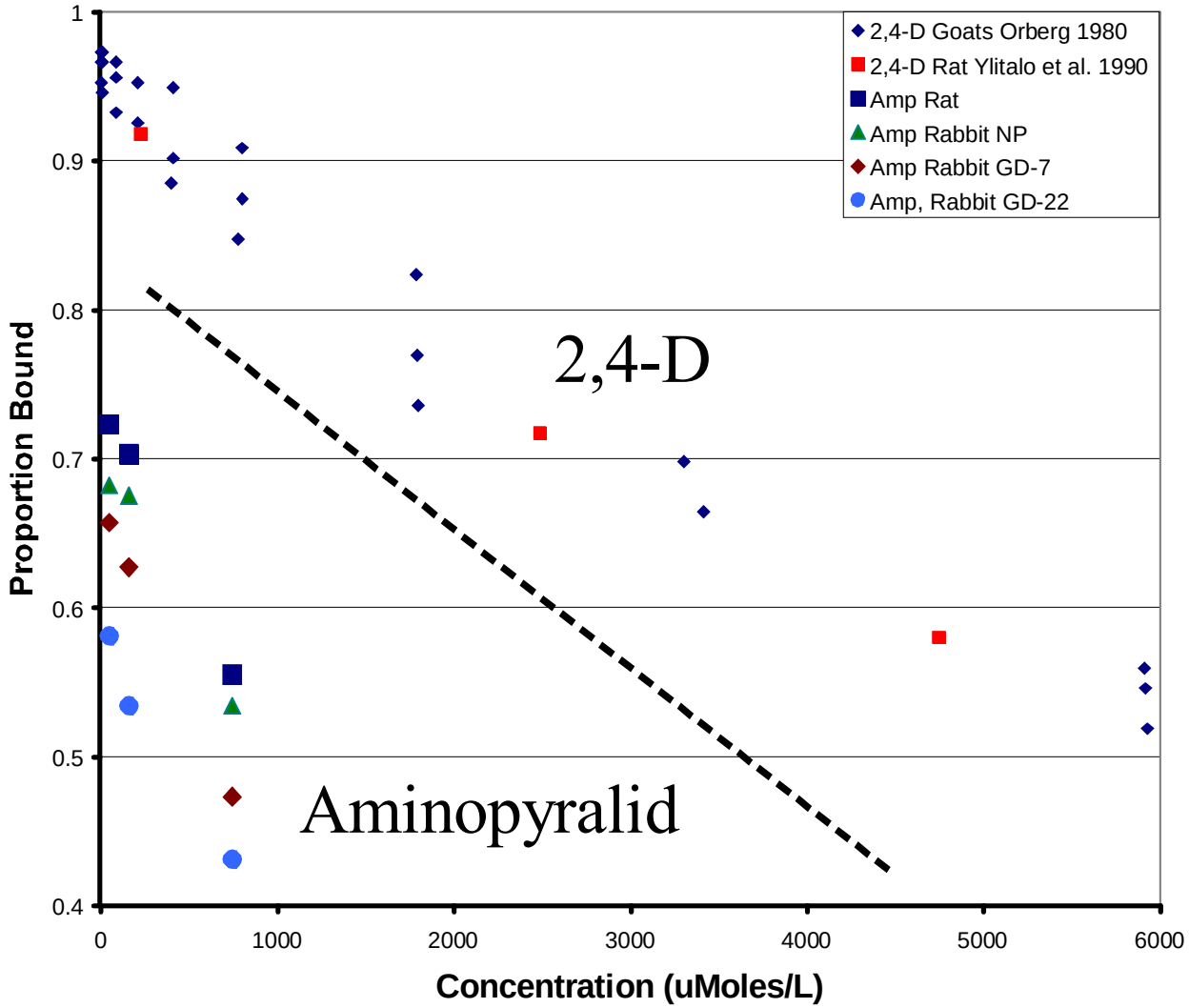


Figure 3: Comparative binding of aminopyralid and 2,4-D to plasma protein

Key:

Amp: Aminopyralid

GD: Gestation Day for pregnant rabbits.

NP: Not pregnant.

See Section 3.1.3.2 for discussion.

Diagonal dashed line added only to emphasize the data areas for 2,4-D and aminopyralid.

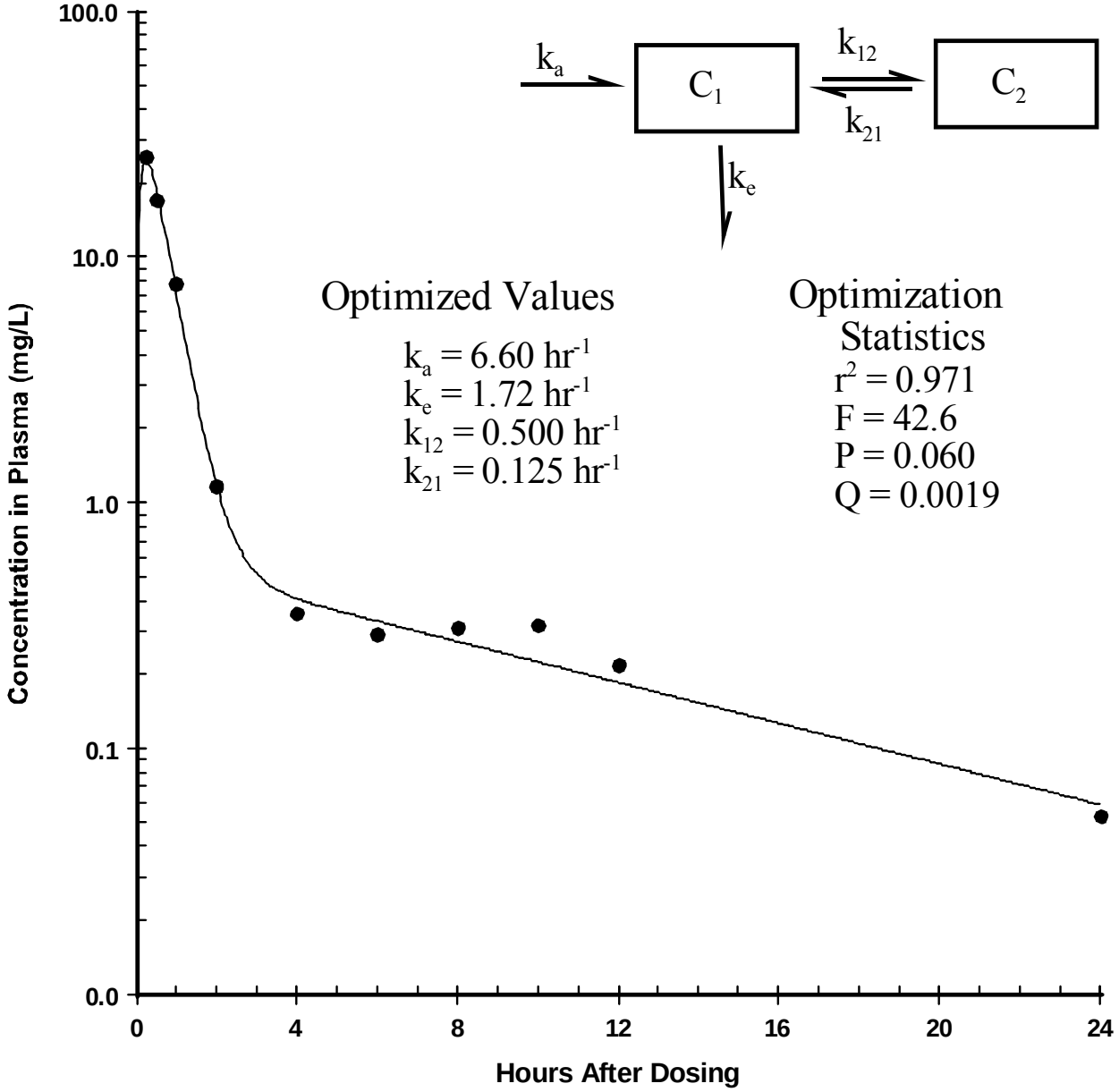


Figure 4: Plasma kinetics of aminopyralid acid in rats after a single gavage dose of 50 mg/kg

Data from Domoradzki et al. (2004).
Kinetics analyzed in ModelMaker 4 (Cherwell Scientific 2000).

Key to Optimization Statistics (Cherwell Scientific 2000, pp. 138-139) give in Figure 4:

r^2: Standard squared correlation coefficient.

F: Variance ratio, Model Mean Error/Residual Mean Square Error

P: Probability that the model explained the variation by chance.

Q: Probability that the differences between the model and the data occurred by chance.

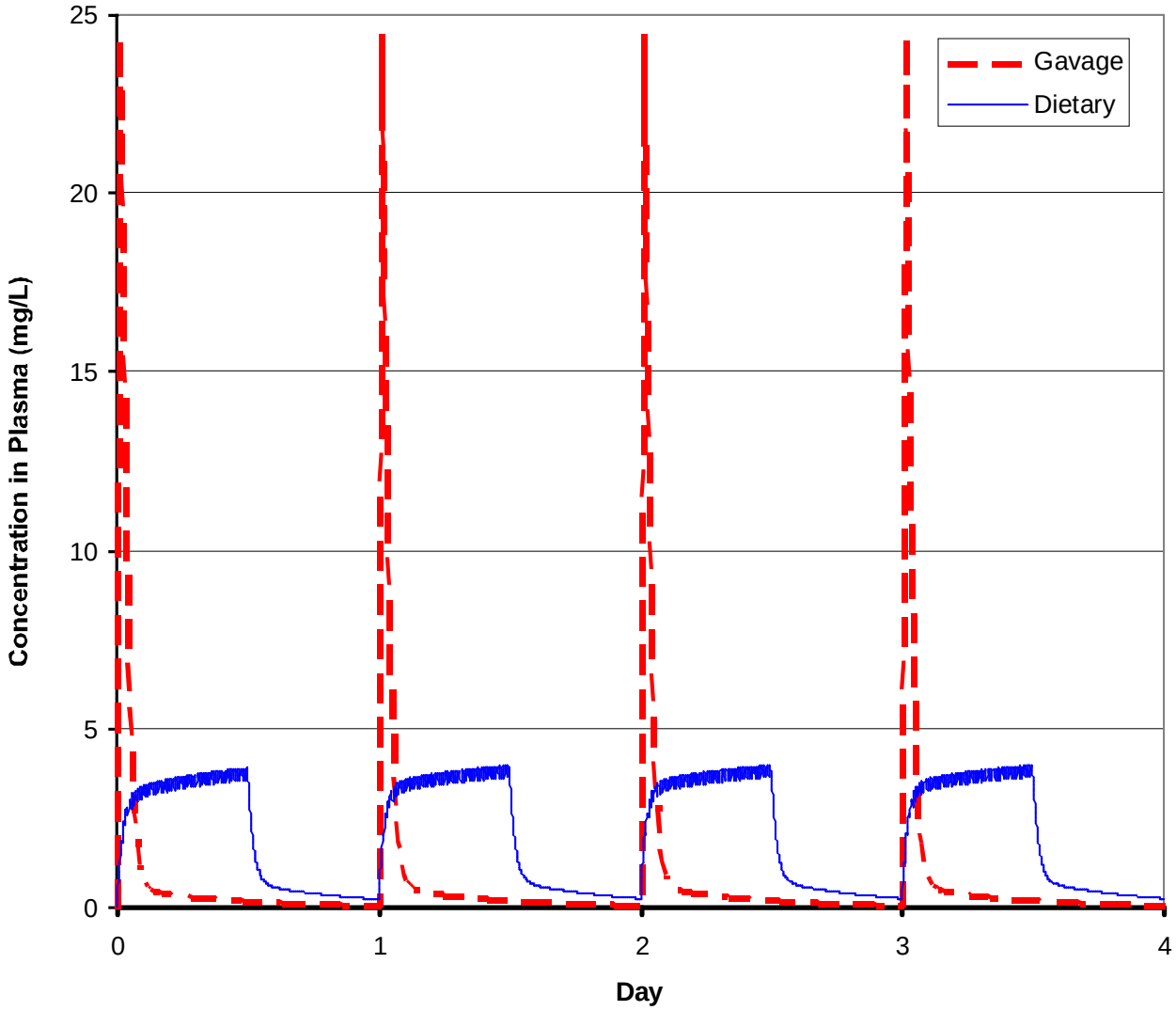


Figure 5: Modeled estimates of plasma kinetics after gavage and dietary administration to rats at a total dose of 50 mg/kg bw.

Model based on data from Domoradzki et al. (2004). See Figure 4 for kinetic analyses of gavage administration. Dietary estimates based on a 12 hour feeding cycle with 4 uniform feeding events per hour. See Section 3.1.3.4 for discussion.

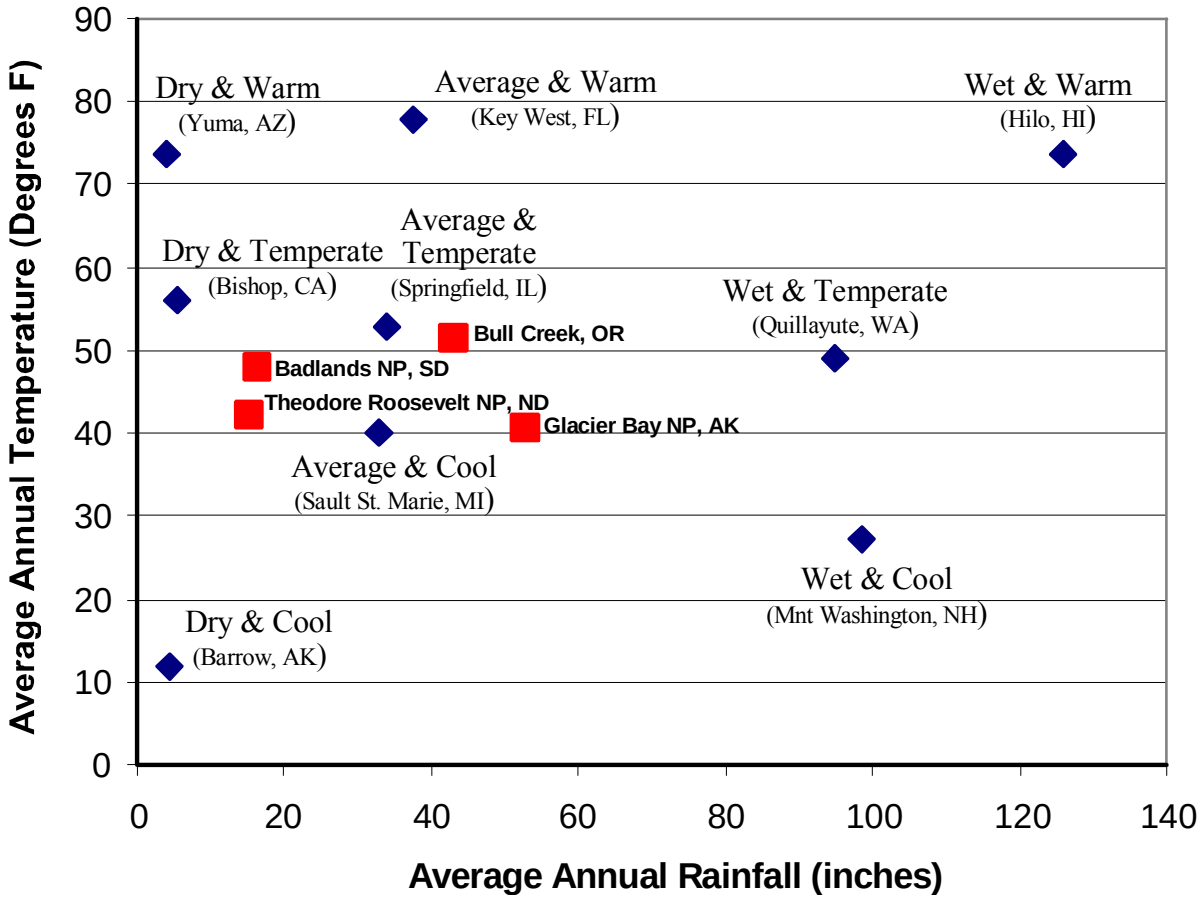


Figure 6: Specific Sites Used in Gleams-Driver Modeling

Blue diamonds are standard Gleams-Driver test sites. Red rectangles are sites specified by the NPS. See Section 3.2.3.4.4 for discussion.

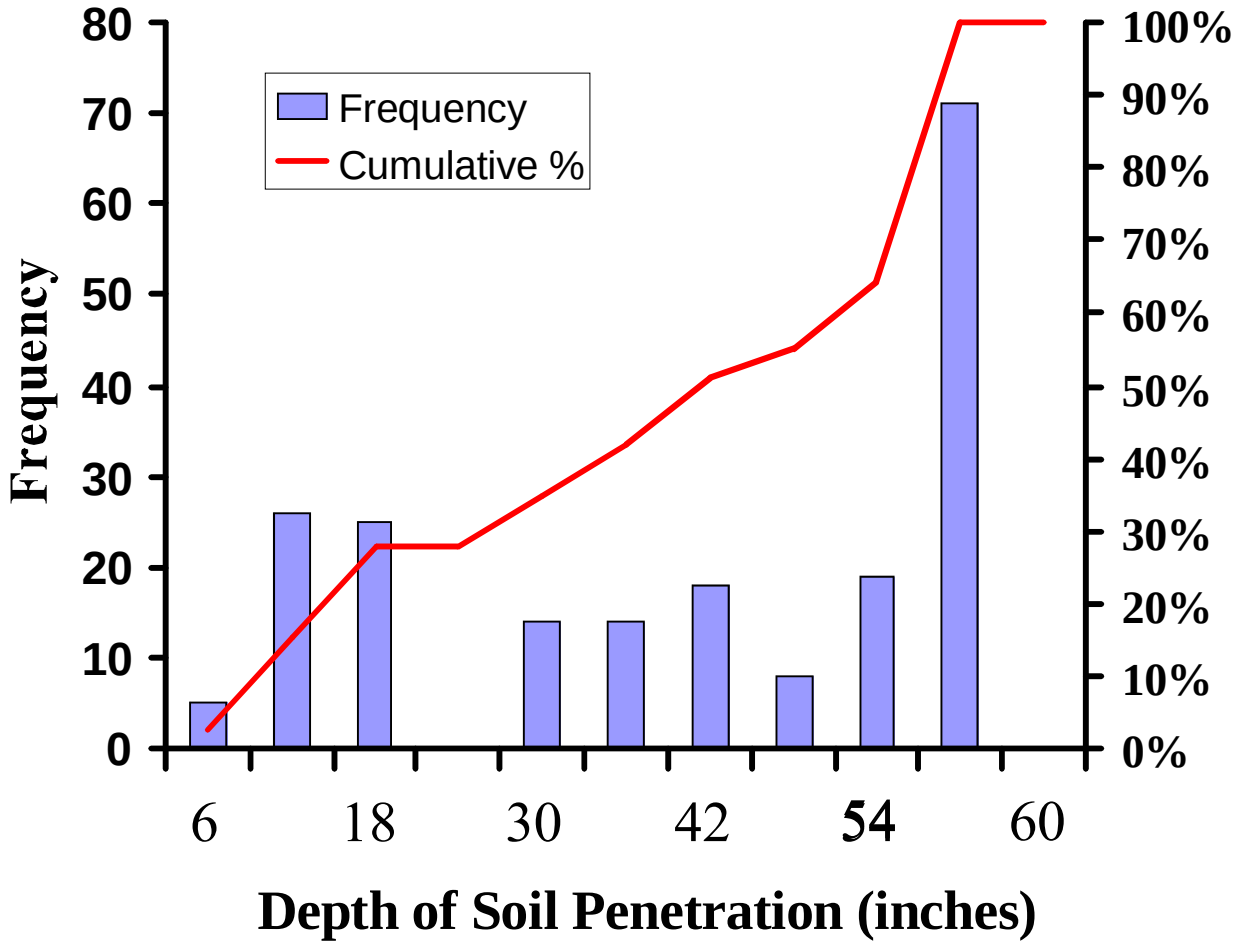


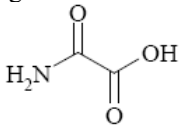
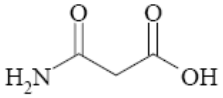
Figure 7: Simulation of Depth of Soil Penetration for CA Site in Roberts and Schelle (2004a).

Based on Gleams-Driver simulation (n=200) of Fresno, CA from Roberts and Schelle (2004a) using aminopyralid specific parameters from general GLEAMS modeling (Table 4) with site-specific soil data from Study Table 2 and site-specific weather data from Cligen (see SERA 2006b). See Section 4.2.3.3. for discussion.

Appendix 1: Laboratory Studies on Physical Chemical Properties and Environmental Fate.

Property	Data Summary	Reference, MRID, OPP Classification
Aerobic Aquatic Metabolism	Three sediment/pond water (3:5) systems with 4 ppm C^{14}-aminopyralid in aquatic phase at start: Sand (France), Silt Loam (Italy), Sandy Loam (North Dakota): Incubated in dark for 101 days. Average Kd of 1 mL/g at study termination. About 95% unchanged aminopyralid at end of study. Metabolites not identified. DT_{50} from system of 819 days (sand), 458 days (silt loam), and 999 days (sandy loam). Metabolism halftimes >450 days. **Note for Forest Service risk assessment: Reported halftimes are much greater than observation times and could not have been very accurate.** **EFED DER recalculated halftimes and reports values of 866.4 days (sand), 462.1 days (silt loam), and 990.2 days (sandy loam) for a mean value of 772.9 days.**	Yoder and Smith 2003, MRID 46235731 Supplemental (6/1/05), *three test systems were anaerobic throughout most of the study*. Cited in EFED risk assessment without comment
Aerobic Soil Degradation	Degradation in silt loam, loam, sandy loam, clay loam, and clay. Initial soil concentrations of 0.03, 0.05, or 0.06 ppm soil. The concentration is 0.06 ppm is reported to be equivalent to 0.11 lb a.e./acre). Incubation periods of up to 1 year and samples taken at 0, 1, 4, 8, 14, and 22 days and 1, 2, 3, 4, 6, 9 and 12 months. First order halftimes: silt loam (46 to 59 days), loam (34 to 49 days), sandy loam (14 to 21 days), clay loam (341 to 343 days), and clay (5 days). No apparent relationship between initial concentration and degradation rate. The only degradation products were CO2 and non-extractable residue. Overall average DT_{50} of 89 days.	Yoder and Smith 2002, MRID 46235729 Supplemental (5/9/05), *extraction was insufficient and material balances were low or variable in four of the five soils.* In addition, the results for clay are invalid.

Appendix 1: Laboratory Studies on Physical Chemical Properties and Environmental Fate.

Property	Data Summary	Reference, MRID, OPP Classification
Yoder and Smith 2002, MRID 46235729 *(continued)* In all soils except clay, the biological activity (measured by the degradation of dichlorobenzoic acid of the soils decreased over time. *Of the three soils where biomass was measured at study initiation and termination, two showed a decrease in biological activity while the third, the Houston Black clay actually had a higher biomass value at study termination than at initiation.*(Study p. 39). Apparent Kd (non-equilibrium in units of mL/g) values Day 0: silt loam (0.7 to 0.8 mL/g), loam (0.6-1 mL/g), sandy loam (0.1 to 0.4 mL/g), clay loam (0.9 to 1.2 mL/g), and clay (0.2). 14 Days: silt loam (3.5 to 4.9), loam (2.5-2.8), sandy loam (0.8-0.9), clay loam (4 to 4.4), and clay (1.7 to 2.3). 1 Month: silt loam (3.7 to 3.9), loam (3.9-6.6), sandy loam (1.2-1.4), clay loam (3.8 to 4), and clay (not able to quantify). EFED Notes: Clay values not valid. The apparent Kd values increase over time by factors of 3 to 10 indicating that contact time increases sorption. Halftimes unreliable due to extraction procedures. *Half-lives in the four invalid soils ranged from 3 1.5 to 533.2 days when calculated by adding non-extractible residue amounts in with parent concentrations.*		
Anaerobic Aquatic Metabolism	No measurable degradation in anaerobic systems at 20°C or 25°C. Aquatic sediment Kd values ranged from 0.27 to 0.48 with a mean of 0.39 (sand, 1.3%OC) to 0.45 to 0.63 with a mean of 0.55 (sandy loam, 4.9% OC). See unnumbered tables on p. 73 of study.	Rutherford and Meitl 2004, MRID 46235730 Acceptable (5/12/05)
Aqueous Photolysis	A 0.2 ppm concentration of aminopyralid exposed to artificial sunlight equivalent to 38 days in summer at 40° North. Degradation halftime of 0.6 days. Degradates included:  oxamic acid (CAS 471-47-6) and  malonamic acid (CAS No. 2345-56-4). No degradation in dark controls – i.e., no indication of hydrolysis or biological degradation.	Cook 2003b, MRID 46235727 Supplemental (2/1/05), lack of identification and quantification of all major degradates

Appendix 1: Laboratory Studies on Physical Chemical Properties and Environmental Fate.

Property	Data Summary		Reference, MRID, OPP Classification
Basic Chemical and Physical Properties	Ghaoui (2003) is a collection of individual studies of basic chemical and physical properties that was submitted as a single document to the U.S. EPA and assigned a single MRID number. These studies were conducted by different individuals at different times. To maintain consistency with the EPA records, all of this information is cited as Ghaoui 2003. The individual studies are summarized in the following rows.		Ghaoui 2003, MRID 46235703 DER not available (Bressant, 2007)
	Color, physical state, odor, pH, and density	White powder, no odor, 1.72 g/mL @ 20°C. pH of 1% w/w aqueous solution is 2.31 @ 23.4°C	Report No. FAPC012087, MW Russell, March 26, 2003
	Melting point	165.2 °C	Report No. FAPC 013053, MW Russell, February 7, 2003
	Dissociation Constant	2.56±0.03	Study ID: 01-822-AG, Cheryl Cathie, August 29, 2001
	Octanol-water partition coefficient	Unbuffered: 1.59 Log Kow = 0.201 pH 5: 0.0172 Log Kow = -1.76 pH 7: 0.00134 Log Kow = -2.87 pH 9: 0.00109 Log Kow = -2.96	Study ID: FORO1009, S. Madsen, February 5, 2003
	Water solubility	Unbuffered: 2.48 g/L @ 18°C (2,480 mg/L) pH 5: 212 g/L @ 20°C (212,000 mg/L) pH 7: 205 g/L @ 20°C (205,000 mg/L) pH 9: 203 g/L @ 20°C (203,000 mg/L)	Study ID: FORO1015, Rose M. Nelson, May 15, 2002
	Note on water solubility in unbuffered water: A 2,480 mg/L solution of aminopyralid is equivalent to [2.48 g/L / 207 g/mole = 0.0112 moles/L]. Using the pKa of 2.56 and the definition of K_a, the pH can be calculated from the definition of K_a (e.g., O'Flaherty 1992 or any elementary chemistry text): $K_a = [H^+] [A^-] / [HA]$ Since $[H^+] = [A^-]$, substitute [X] for each term. The total concentration of the acid C = HA + A^+. For [HA] above, substitute C – X. Substitute 10^{-pKa} for K_a. $10^{-pKa} = [X]^2 / [C] – [X]$ Setting C = 0.0122 mole/L, $10^{-2.56} = [X]^2 / (0.0112 - [X])$ Solve for X= 0.00458 M in Mathematica 5.2 with Solve[] function. Thus, the pH of the solution is 2.33.		
	Vapor Pressure	1.94×10^{-10} mm Hg @ 25°C (equivalent to 2.59×10^{-8} Pascals (Newtons per m^2)	Study ID: NAFST744, K.A. Griffin, May 15, 2001

Appendix 1: Laboratory Studies on Physical Chemical Properties and Environmental Fate.

Property	Data Summary	Reference, MRID, OPP Classification
Dissociation Constant (pK_a)	Aminopyralid TIPA Salt: Complete dissociation. pKa for aminopyralid: 2.56	Hamilton 2004a, MRID 46235706. DER not available (Bressant, 2007)
Dissociation Constant (pK_a)	Aminopyralid, Potassium Salt: Complete dissociation pKa for aminopyralid: 2.56	Hamilton 2004b, MRID 46235707 DER not available
Hydrolysis	Aminopyralid at 0.4 mg a.i./L, pH 5, 7, and 9 and 20 °C over a 30 day period of observation. No detectable degradation.	Cook 2003a, MRID 46235726 Acceptable (5/12/05)
Soil Adsorption/ Desorption	Adsorption Kd: 0.22 ± 0.28 mL/g (max = 1.04 mL/g). Adsorption Koc:, 10.0 mL/g. (max = 38.9 mL/g). Desorption Kd: 13.96 ± 54.36 mL/g (max = 405.94 mL/g). Desorption Koc: 1301 mL/g (max = 40,594 mL/g). Kf 1/n values of 0.61 to 1.00. Sorption increases with contact time. **Adsorption/Desorption Study, Adsorption Kd/Koc** Silt loam (pH 7.8, OC 1%): 0.053 (0.019-0.087)/5.31(1.92-8.67) Clay (pH 7.5, OC 3.2%): 0.036 (0.000-0.064)/ 0.87(0.00-1.99) Silty clay loam (pH 7.8, OC 3.9%): 0.308 (0.207-0.406)/ 7.96 (5.34-10.50) Sand (pH 6.6, OC 1.6%): 0.072 (0.027 - 0.118)/ 4.52(1.72-7.37) Loam (pH 6.1, OC1.0%): 0.089 (0.037-0.176)/ 8.91(3.75-17.62) Clay (pH 6.9, OC 1.5%): 0.024 (0.000- 0.039)/ 0.81(0.00-2.58) Clay loam (pH 4.8, OC 3.6%): 0.853 (0.613- 1.035)/ 23.69(17.03-28.75) Loamy sand (pH 4.5, organic carbon 0.6%): 0.162 (0.093-0.223)/ 27.00(15.56-38.87) **Adsorption Study, Adsorption Kd/Koc** Clay loam (pH 4.6, OC 1.5%): 0.409/ 27.24 Loam (pH 7.5, OC3.4%): 0.395/11.62 Sandy loam (pH 7.3, organic carbon 1.2%): 0.066/5.51 Clay (pH 7.5, OC 3.4%): 0.211/6.21 EFED Note: Soil binding not related to OC, pH, or cation exchange capacity. …*in general, aminopyralid is likely to be highly mobile in most soils in the environment.*	Rutherford 2002, MRID 46235732 Supplemental, *none of the test soils had an organic matter content of greater than or equal to 1%* Note: EFED appears to be in error: OM = OC * 1.724 (e.g., Winegardner, 1996, p. 117). All of the test soils appear to have an organic matter content greater than 1.%.

Appendix 1: Laboratory Studies on Physical Chemical Properties and Environmental Fate.		
Property	**Data Summary**	**Reference, MRID, OPP Classification**
Soil Photolysis	5.2 ppm in silt loam with irradiation equivalent to 28 days of summer sun at 40° north latitude. Ke value of 0.013 $days^{-1}$ (halftime = 53.3 days). Degradates not identified.	Rutherford 2004, MRID 46235728 Supplemental (5/14/05), *loss of material balance …and variability in material balance in the dark samples.*

Appendix 2: Field Studies on the environmental fate (all studies used XDE-750, the TIPA salt of aminopyralid unless otherwise specified as the formulation – i.e., aqueous solution)

Application/Field Conditions	Results	Reference
Limit of Detection: 0.3 µg/kg (0.3 ppb) Limit of Quantification: 1.5 µg/kg (1.5 ppb) Greenville, MS: 150 g a.e./ha (0.13 lbs a.e./acre) to bare ground with tractor mounted broadcast boom. Rainfall 30" rainfall over 6-month period. Soil sampled to 90 cm (35.4"). Soil: Silty loam, silty Clay, and silty clay-loam, 0.6-1, 1% OM, pH 6-6.7. [See Table 1 of study, p. 38 for additional details.] Sampling Schedule (DAT): -11, 0, 8, 15, 29, 57, 93, 122, 183	First-order soil dissipation halftime of 34 days. No detectable residues below 15 cm. Soil concentrations dropped from an initial concentration of about 150 ppb to about 2.5 ppb by DAT 183. The 3-month (mid-point) concentration was about 16 ppb. (see detailed Study Table 8, pp. 45-53 and summary Study Table 10, p. 55) EFED DER Note: *run-off of bound or unbound residues, and volatilization were not measured. …uneven application, temporally variable concentrations, and questionably adequate sampling schedules.* U.S. EPA/OPP-EFED 2004, p. 60: *Aminopyralid did not appear to leach below the 15-30 cm soil depth, although lack of sampling between 0 and 8 days may not have allowed for detection of leaching during the first week post application.*	Roberts and Schelle 2004a, MRID 46235734 Supplemental (5/11/05), *data variability make the half-lives of questionable value*

Roberts and Schelle 2004a, MRID 46235734, Mississippi Site *(continued)*

Notes for this risk assessment:

The peak concentration of about 150 ppb in the top 15 cm (about 6 inches) of Mississippi soil immediately after an application of aminopyralid at 0.13 lb a.e./acre. This application rate corresponds to about 1.46 µg/cm^2 [1 lb/acre = 11.21 µg/cm^2]. In Study Table 1 (p. 38), the bulk density of this soil in the top 15 cm is given as 1.18 g/cm^3. Thus, the expected nominal average concentration in the top 15 cm of soil immediately after application would be 0.082 µg/g [1.46 µg/cm^2 / 15 cm x 1.18 g/cm^3] which corresponds to 0.082 ppm or 82 ppb. This is a factor of about 2 below the reported concentration. The same pattern is seen in California soil in the next entry.

Appendix 2: Field Studies on the environmental fate (all studies used XDE-750, the TIPA salt of aminopyralid unless otherwise specified as the formulation – i.e., aqueous solution)

Application/Field Conditions	Results	Reference
LOC and LOD as above. Fresno, CA: 150 g a.e./ha (0.13 lbs a.e./acre) to bare ground with tractor mounted broadcast boom. Rainfall/irrigation of 26" over 6-month period. Soil sampled to 90 cm (35.4"). <1% slope. Soil: Silty loam, 0.2-1.3 % OM, pH 7.2-7.7. [See Table 2 of study, p. 39 for additional details.] Sampling Schedule (DAT): -2, 0, 9, 15, 22, 65, 91, 126, 182	First-order soil dissipation halftime of 26 days. Most residues in the top 60 cm (36 inches) of soil and no residues detected below 75 cm (29.5 inches). Soil concentrations dropped from an initial concentration of about 146 ppb to <1 ppb by DAT 182. The 3-month concentration (mid-point) was about 2 ppb. (see detailed Study Table 11, pp. 56-64 and summary Study Table 13, p.66). EFED Note: Identical to note on Greenville, MS, above.	Roberts and Schelle 2004a, MRID 46235734
Roberts and Schelle 2004a, MRID 46235734, California Site *(continued)* **Notes for this risk assessment:** The peak concentration of about 146 ppb in the top 15 cm (about 6 inches) of California soil immediately after an application of aminopyralid at 0.13 lb a.e./acre. This application rate corresponds to about 1.46 µg/cm^2 [1 lb/acre = 11.21 µg/cm^2]. In Study Table 2 (p. 39), the bulk density of this soil in the top 15 cm is given as 1.15 g/cm^3. Thus, the expected nominal average concentration in the top 15 cm of soil immediately after application would be 0.085 µg/g [1.46 µg/cm^2 / 15 cm x 1.15 g/cm^3] which corresponds to 0.085 ppm or 85 ppb.		
Six sites in Canada (see column 2) using a common study design. Applications at 150 g a.e./ha (0.13 lb a.e./acre). Soil samples taken over a 450 day post-application period. Limit of Detection: 0.3 µg/kg (0.3 ppb) Limit of Quantification: 1.5 µg/kg (1.5 ppb)	New Brunswick: DT_{50} = 21 days. Quantifiable residues to 45 cm (18 in). Initial samplings at 6 DAT. Ontario: DT_{50} = 31 days. Quantifiable residues to 45 cm (18 in). Initial samplings at 9 DAT. Manitoba: DT_{50} = 30 days. Quantifiable residues to 45 cm (18 in). Initial samplings at DAT. Saskatchewan: DT_{50} = 9 days. Quantifiable residues to 45 cm (18 in). Initial samplings at 7 DAT.	Roberts and Schelle 2004b, MRID 46235735 DER not available (Bressant, 2007) Not cited in EFED risk assessment.

Appendix 2: Field Studies on the environmental fate (all studies used XDE-750, the TIPA salt of aminopyralid unless otherwise specified as the formulation – i.e., aqueous solution)

Application/Field Conditions	Results	Reference
	Montana: DT_{50} = 35 days. Quantifiable residues to **90 cm** (36 in). Initial samplings at 7 DAT.	
	Alberta: DT_{50} = 54 days. Quantifiable residues to 45 cm (18 in). Initial samplings at 7 DAT.	
Notes for Forest Service Risk assessment: The study states in several places (e.g., Study p. 15) that the primary route of degradation is likely to be microbial. This is probably correct but the experimental support for this statement in this study is not apparent. No degradation assays with sterile soils were done. Extensive data are given in appendices and some statistical analyses were done fitting the dissipation rates to a first-order model (e.g., Figures 5, 7, 9, 11, 13, 15). While r^2 values are given, the confidence intervals on the dissipation rates, however, do not appear to be in the report. Note that the r^2 values are very high for most sites. Most are over 0.9 and all are over 0.88.		
Modeling using AgDRIFT Version 2.0.05. Fixed-wing and helicopter aerial applications. Application rate of 120 g a.e./ha (0.11 lb a.e./acre).	Estimated peak water concentrations of 88 mg/L for a 15 cm deep body of water. **Note on EFED drift assessment** (U.S. EPA/OPP-EFED 2004): Used AgDRIFT Version 2.01. Fractional drift: Ground: 0.025 (100'), 0.0038 (500'), 0.0014 (900'). Aerial: 0.096 (100'), 0.018 (500'), 0.012 (900').	Havens 2004, MRID 46235834 DER not available (Bressant, 2007) Not cited in EFED risk assessment.

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
ORAL – ACUTE			
Rats, Fischer 344, 5 male, 5 female, 0.13-0.291 kg bw	Limit Test: XDE-750 (aminopyralid acid), 94.5%, 5000 mg/kg bw, gavage in water with 0.5% methyl cellulose. 14-day post-dosing observation period. No controls.	One male died on Day 3 post-dosing. Pathology included hemolyzed blood and gas in GI tract. Observations included decrease reactivity, loose/watery feces, staining. Symptoms diminished with time after dosing. Transient weight loss in 4/9 surviving animals.	Brooks 2001a MRID 46235603 Acceptable (9/2/04)
Rats, Fischer 344, 5 males (≈0.15 kg bw), 5 females (≈0.1 kg bw)	Limit Test: **GF-871 (41.9% aminopyralid TIPA, equiv to 21.8% a.e.)**. Single gavage dose Formulation: 5000 mg formulation/kg Acid equivalent: 1090 mg a.e./kg bw. 14-day post-dosing observation period. No control group.	No mortality. Observations included loose/watery feces, staining, lacrimation, and cloudy eyes in all rats on Day 1 (the day of administration). No symptoms by Day 4. Transient weight loss on Day 1 but all animals gained weight over course of study. No abnormal findings on necropsy.	Wilson et al. 2003, MRID 46235604 Acceptable (9/28/04)
Wilson et al. 2003, MRID 46235604 *(continued)* The rats were 51 days old on the day that the doses were administered. The cloudiness of the eyes was bilateral in all animals and was observed only on day of dosing.			

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Rats, Fischer 344, 10 males (≈0.11 kg bw), 10 females (≈0.088 kg bw)	Acute Neurotoxicity: XDE-750 (aminopyralid acid), 94.5%. Dose: 0, 500, 1000, and 2000 mg a.e../kg bw, gavage in water with 0.5% Methocel [2]. 14-day post-dosing observation period.	No effects on body weights, neuropathic effects, or motor activity. At 2000 mg/kg, higher incidence of soiling: fecal in males and urinary in females for up to 8 days after dosing.	Marable et al. 2002, MRID 46235616 Acceptable (9/1/05) DER concurs with interpretation of study authors.
Marable et al. 2002 *(continued)* **Note for Risk Assessment:** Based on gross pathology/necropsy at the end of the study, cloudy eyes were observed in one female rat (Animal No. 2657) in the 1000 mg a.e./kg bw dose group. During the FOB observations on Study Day 1, the right eye of one male rat (Animal No. 2573) was enlarged and … *partially cloudy in about 30% of the clear part of the eye* (Study Table 8, p. 132).			
ORAL – DEVELOPMENTAL STUDIES			
Rabbits, New Zealand White, 26 time-mated females per group, ≈3.2 kg	XDE-750 (aminopyralid acid), 94.5% by gavage *Phase I:* Doses: 0, 25, 100, and 250 mg a.e./kg bw/day on Days 7-20 of gestation, gavage in water with 0.5% Methocel [2].	No treatment related effects on dams, embryos, or offspring.	Marty et al. 2002, MRID 46235630 Acceptable (9/1/05)
	Phase II: Doses: 0, 500 and 750 mg a.e./kg bw/day on Days 7-20 of gestation, gavage in water with 0.5% Methocel [2].	500 mg/kg: Decreased body weight gain and transient lack coordination in adults. No effects on offspring. 750 mg/kg: Incoordination, decreased food consumption and decreased body weights in adults. Two does had erosions or ulcers of the glandular mucosa of the stomach.	

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Rabbits, New Zealand White, 26 time-mated females per group, ≈3.1 kg bw	**GF-871 (41.9% aminopyralid TIPA, equiv to 21.8% a.e.)**. Formulation Doses: 0, 484, 1211, and 2421 mg formulation/kg bw/day. XDE-750 TIPA Doses: 0, 200, 500, and 1000 mg XDE-750 TIPA/kg bw/day Acid Doses: 0, 104, 260, 520 mg a.e./kg bw/day. Duration: Day 7 to Day 27 of gestation. Gavage, no vehicle.	**Adult Effects** 1000 mg a.i./kg: decrease maternal food consumption and body weight. Spontaneous abortion in 1/26 does. 3 other does euthanized due to extreme weight loss. 500 mg a.i./kg: One doe euthanized due to extreme weight loss. Mild incoordination in 1/26, 2/26, and 19/26 at doses of 200, 500, and 1000 mg a.i./kg. **Fetal Effects** Decreased fetal weights at 1000 mg a.i./kg.	Carney and Tornesi 2004b, MRID 46235632 Acceptable (10/5/05)

Carney and Tornesi 2004b, MRID 46235632 *(continued)*

Notes from Study Authors:

The authors suggest a maternal NOAEL of just slightly below 200 mg a.i./kg bw/day (104 mg a.e./kg bw/day) and a developmental NOAEL of 500 mg a.i./kg bw/day (260 mg a.e./kg bw/day). As noted below, EFED concurs.

Notes from EFED DER:

Maternal NOAEL: 104 mg a.e./kg bw/day
Maternal LOAEL: 520 mg a.e./kg bw/day (incoordination)
Developmental NOAEL: 260 mg a.e./kg bw/day
Developmental LOAEL: 520 mg a.e./kg bw/day (decreased body weight)

Notes for this risk assessment:

The maternal NOAEL and LOAEL are based on incoordination and weigh loss, both of which occurred at 520 mg a.e./kg bw/day. At 260 mg a.e. (500 mg a.i.)/kg bw/day, however, one doe (1/26) was euthanized for severe weight loss and two does (2/26) experienced incoordination (Animal Nos. 5261 and 5265). Animal 5261 is marked as being NP (not pregnant) as distinct from AB (aborted) and data from this animal was not included in the analysis.

Notes continued on next page

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Carney and Tornesi 2004b, MRID 46235632 *(continued)* The animal that was euthanized due to extreme weight loss in the 260 mg a.e./kg bw/day dose groups appears to be Animal No. 5252. This animal is reported to have had liver congestion and a hairball in the stomach (Study p. 21). Clinical observations are reported on this animal up to Day 17 with the notation "*Disposition, Other – Unscheduled*". On Day 16, the animals body weight was 2990.1 g, representing a 9.15% weight loss from the Day 0 value of 3268 g (Study page 74). The animal was reported as being *Moribund*. At sacrifice, this animal was pregnant with 9 normal fetuses and 1 partially resorbed implant. At the dose of 260 mg a.e./kg bw/day, effects were noted in 3/26 animals compared to 0/26 in the control group. This difference is not signification using the Fisher Exact test (*p*-value of 0.117647). In this sense, the NOAEL of 104 mg a.e./kg bw/day could be viewed as somewhat overly conservative – i.e., a case could be made for using a maternal NOAEL of 260 mg a.e./kg bw/day. Doing this, however, would not properly consider the biological significance of the effects or the dose/response relationships. Mild incoordination The 0/26, 1/26, 2/26, and 19/26 at 0, 104, 260, 520 mg a.e./kg bw/day is clearly dose related. Using a benchmark of 10% as a functional NOAEL, the lower limit on the dose is 184.88 mg a.e./kg bw/day. Thus, the NOAEL of 104 mg a.e./kg bw/day recommended by the study authors and confirmed by EFED seems reasonable. Using a benchmark dose of 0.01, the benchmark dose (MLE) is 93.83 mg a.e./kg bw/day and the lower limit on the dose is 40.48 mg a.e./kg bw/day. **Eyes**: No remarkable effects. No clouding of the eyes.			
Rabbits, New Zealand White, groups of 26 mated females per dose.	**GF-871 (41.9% aminopyralid TIPA, equiv to 21.8% a.e.)**. Doses a.i.: 0, 50, 150 mg a.i./kg bw/day on days 7-27 of gestation. Acid Equivalent Doses: 0, 26, 78 mg a.e./kg bw/day. Gavage in deionized water.	Dams: 150 mg/kg: Incoordination at 30-75 minutes post-dosing in each of 3 rabbits. In two of these rabbits, displayed repetitive chewing behavior	Carney and Tornesi 2004c, MRID 46284901 Only a 1-page partial DER. Have full study. Not cited in HED risk assessment. Appears to be supplemental to Carney and Tornesi 2004b, , MRID 46235632.
Carney and Tornesi 2004c, MRID 46284901 *(continued)* DER cites a transient decrease in body weight a 1000 mg/kg. This appears to refer to the 1000 mg/kg/day dose group from Carney and Tornesi 2004b. Offspring: No effects. Spontaneous abortion specified in one 1000 mg/kg bw animal. Again, this seems to refer to the 1000 mg/kg bw/day study by Carney and Tornesi 2004b. *Notes continued on next page*			

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Carney and Tornesi 2004c, MRID 46284901 *(continued)* The incoordination was observed in the same three rabbits (Animal Nos. 992, 1011, and 1015) and the incoordination was observed in all three of these rabbits only on Days 14, 25, and 26. Incoordination took about 0.5 to 1.25 hours to develop and lasted for several hours. EFED Evaluation: NOEC: 50 mg a.i./kg for maternal toxicity. LOEC: 150 mg a.i./kg (see above)			
Rabbits, New Zealand White, 7 time-mated females per group, ≈3.2 kg bw	XDE-750 (aminopyralid acid), 94.5%. Doses: 0, 250, 500, 750, or 1000 mg a.e./kg/day on Day 7 to Day 27 of gestation. Gavage in 0.5% Methocel [2].	750 and 1000 mg/kg: Substantial decrease in body weights in dams. Groups taken off study on Days 10 and 17, respectively. Decreased body weight gains at 250 mg/kg/day (53%) and 500 mg/kg/day (33%). Relative Liver weight: 14% decrease at 500 mg/kg/day (statistically significant) and 7% (not statistically significant) at 250 mg/kg/day.	Liberacki et al. 2001b, MRID 46235634 Acceptable (9/1/05)
Rats, CD, 25 time-mated females, ≈0.22 kg	XDE-750 (aminopyralid acid), 94.5%. Doses: 0, 500, 100, and 1000 mg a.e./kg bw/day on Days 6-20 of gestation, gavage in water with 0.5% Methocel [2].	No effects at any dose on dams and no embryotoxic or fetotoxic effects.	Carney and Tornesi 2001, MRID 46235629 Acceptable (9/1/05)

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	**Dose/Exposure**	**Response**	**Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)**
Rats, Sprague-Dawley, 25 time-mated females per group,	**GF-871 (41.9% aminopyralid TIPA, equiv to 21.8% a.e.)**. Gavage, no vehicle. Formulation Doses: 0, 484, 1211, and 2421 mg formulation/kg bw/day TIPA Equivalent: 0, 200, 500, and 1000 mg XDE-750 TIPA (a.i.)/kg bw/day) Acid Equivalent: 0, 105, 264, and 528 mg a.e./kg bw/day) Duration: Day 6 to Day 19 of gestation.	No dose-related adverse effects in adults. No fetotoxic or embryotoxic effects and no effects on offspring. Each animal examined daily and examination included the eyes. No effects noted.	Bjorn 2003, MRID 46235631 Acceptable (9/1/05)
Rats, CD, 8 time-mated females per group, ≈0.22 kg bw	XDE-750 (aminopyralid acid), 94.5%. Doses: 0, 250, 500, 750, or 1000 mg a.e./kg/day on Day 6 to Day 20 of gestation. Gavage in 0.5% Methocel [2].	No effects on dams or offspring.	Tornesi et al. 2001, MRID 46235633 Acceptable (9/1/05)

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
ORAL – REPRODUCTION STUDY			
Rats, CD. (≈0.11 to 0.12 kg)	XDE-750 (aminopyralid acid), 94.5%. Dietary concentrations: 0, 50, 250, 1000 ppm (mg a.e./kg diet) for 10 weeks. Based on body weights and food consumption (Study Tables 12 to 30), the maximum food consumption as a fraction of body weight was 0.218 and occurred in P2 females for Day 21 of lactation – i.e., an average food consumption of 72.8 g (Table 19, p. 80) and an average body weight of 333.6 g (Table 30, p. 93). Based on this proportion, the maximum daily dose was 218 mg/kg bw/day [0.218 kg diet/kg bw x 1000 mg/kg diet]. The 250 ppm dietary group corresponded to maximum daily doses of about 54.5 mg/kg bw/day [0.218 kg diet/kg bw x 250 mg/kg diet]	1000 ppm: Significant increase in full and empty cecal weights. 38% in increase males and 33% in females based on empty ceca. No histopathology in ceca (Study Table 40, p. 141) 250 ppm: Significant increase in full and empty cecal weights. 10% increase in males and 9.5% in females based on empty ceca. Changes in cecal weight were not regarded by investigators as toxicologically significant. No other effects on adults and no effects on reproductive parameters.	Marty et al. 2003, MRID 46235635 Acceptable (9/1/05)

Marty et al. 2003, MRID 46235635 *(continued)*
OPP/HED Assessment:
NOAEL: 1000 mg/kg/day for both parental, reproductive, and developmental effects.
LOAEL not identified.
EPA concurs with study authors that the effects seen in the ceca were not toxicologically significant (U.S. EPA/OPP-HED, 2005, p. 24).

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
ORAL – SUBCHRONIC DIETARY			
Dogs, beagles, 2 females and 2 males per group. About 6 to 8.3 kg bw.	XDE-750 (aminopyralid acid), 94.5%. Exposure period: 4 weeks. Dietary concentrations: 0, 0.15%, 0.45% and 1.5% (1500 ppm, 4500 ppm, 15,000 ppm). Resulting average doses reported in study based on individual food consumption: Males: 0, 62, 193, 543 mg/kg bw/day. Females: 0, 62, 177, 556 mg/kg bw/day.	Females: No statistically significant effects. Males: Statistically significant decrease in food consumption (≈29%) in high dose group only. No dose-trend at lower doses.	Stebbins and Baker 2000, MRID 46235620 Acceptable/Non-guideline (9/1/05)
Dogs, beagles, 4 females and 4 males per group. About 6 to 8.3 kg bw, 5-6 months old, 6.1-9.3 kg males and 6.2-7.4 kg females.	XDE-750 (aminopyralid acid), 94.5%. Exposure period: 13 weeks. Dietary concentrations: 0, 0.15%, 0.75% and 3% (1500 ppm, 7500 ppm, 30,000 ppm). Resulting average doses reported in study based on individual food consumption: Males: 0, 54.5, 282, 1070 mg/kg bw/day. Females: 0, 52.7, 232, 929 mg/kg bw/day.	High Dose males and females, hyperplasia and hypertrophy of mucous cells in stomach and hyperplasia of chief cells in gastric mucosa.	Stebbins and Baker 2002, MRID 46235623 Acceptable (9/1/05)

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Mice, CD-1, 6-7 weeks old at start, 10 male and 10 female per group	XDE-750 (aminopyralid acid), 94.5%. Dietary exposure for 13 weeks Intended Doses: 0, 10, 100, 500, 1000 mg/kg bw/day Concentrations in Diet (ppm): Males: 0, 65.1, 632, 3160, 6350. Females: 50.2, 471, 2440, 4840 Actual Doses: Males: 10.2, 101, 502, 1020 mg/kg bw/day Females: 10.2, 103, 515, 1020 mg/kg bw/day	No effects attributable to treatment. Stomach: Only controls and high dose group examined. No abnormalities in female mice. In males, lesions observed in 1/10 controls and 2/10 high dose animals.	Stebbins et al. 2001, MRID 46235618 Acceptable (9/1/05)
Mice, CD-1, 5 males and 5 females per group, 7 weeks old.	XDE-750 (aminopyralid acid), 94.5%. Exposure Period: 4 weeks Intended Doses: 0, 10, 100, 500, 1000 mg/kg bw/day. Concentrations in Diet (%) Males: 0, 0.00648, 0.0542, 0.306, 0.545 Females: 0.00478, 0.05, 0.268 0.496 Actual Doses: Males: 11, 102, 524.7, 1038 mg/kg bw/day Females: 10.8, 105, 530, 1058 mg/kg bw/day	High Dose: Increase in size of hepatocytes in 2/5 males. Stomach: Examinations in only controls and high dose animals (n=5). No abnormalities noted.	Yano and Dryzga 2000, MRID 46235624 Acceptable (9/1/05)

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Rats, Sprague-Dawley, 10 males and 10 females per group, 6 weeks old at start.	XDE-750 (aminopyralid acid), 94.5%. Dietary Exposure for 13 weeks Intended Doses: 0, 100, 500, 1000 mg/kg bw/day	Increased cecal weights in males and females at 500 and 1000 mg/kg dose groups. Increased weight of ileum in males only at 1000 mg/kg group. Increase organ weights associated with hyperplasia of epithelial cells. No stomach lesions.	Liberacki et al. 2001a, MRID 46235619 DER not available (Bressant 2007). Study not used in HED risk assessment. *Notes continued on next page*
Liberacki et al. 2001a, MRID 46235619 *(continued)* Note for Forest Service risk assessment: Cannot find the dietary concentrations used or the calculated actual doses. Very odd. This study may have been rejected or withdrawn.			
Rats, Fischer 344, 10 per group, 6 weeks old	XDE-750 (aminopyralid acid), 94.5%. Exposure Period: 13 weeks with 4 week post-exposure observation period in control and highest dose group. Target Doses: 0, 10, 100, 500, or 1000 mg/kg bw/day.	Cecum and Ileum 1000 mg/kg group, males: Hyperplasia of cecum and ileum epithelial cells and increase in ceca weights. 1000 mg/kg, females and 500 mg/kg group, males and females: Increased cecal weights but no pathology. *Continued on next page*	Dryzga and Stebbins 2001, MRID 46235621 Acceptable (9/1/05)

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Dryzga and Stebbins 2001, MRID 46235621, *continued* **Urinary pH** Decreased in both sexes in 500 mg/kg (pH 6-7.5) and 1000 mg/kg (pH 5-6.5) groups relative to controls (pH 7-8). Typical of acidification of urine by weak acid. In recovery groups, urinary pH is normal in 1000 mg/kg/day group, again as would be expected with excretion of aminopyralid. **Urinary protein and ketones** In discussion [p. 14 and elsewhere], study authors note a decrease in urinary protein and ketones in males and females in 1000 mg/kg group. Study authors indicate that they cannot associate these decreases with any pathology and do not consider them to be treatment related. *Urinary protein* Protein and ketones not quantified. Tables use on +, ++, TRC (trace), and NEG. Effects at end of study are as reported. Effects are not apparent in recovery group at 1000 mg/kg/day. **Note:** Decreases in urinary pH, urinary protein, and urinary ketones. No change in creatinine (i.e., impact on GFR not likely). HED DER notes but does not comment on decreases in urinary pH, protein, and ketones. **Stomach Lesions**: Only controls and high dose groups examined. Focal glandular submucosal lesion noted in only 1/10 high dose males.			
Rats, Fischer 344, 10 male and 10 female per group, six weeks old	**GF-871 (41.9% aminopyralid TIPA, equiv to 21.8% a.e.)**. Target Dietary Doses: Formulation: 0, 465, 1211, and 2421 mg/kg bw/day Aminopyralid TIPA salt: 0, 192, 500, and 1000 mg/kg bw/day. Acid equivalent: 0, 100, 260, and 520 mg/kg bw/day. TIPA equivalent: 0, 92, 241, and 482 mg/kg bw/day.	**Cecal Weights**: Significant increase in both sexes at two higher dose groups. **Spleen Weights**: A small (6%) but statistically significant increase in relative kidney weights in low dose males. No increase at higher doses and no increases in females. Effect does not appear to be compound related.	Stebbins and Dryzga 2004, MRID 46235622 Acceptable (9/1/05)

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Stebbins and Dryzga 2004, MRID 46235622 *(continued)* **Kidney Weights**: Slight increases in relative weights in males (2.4%) and females (3%) at the highest dose. Not statistically significant and does not appear to be compound related. [Study Tables 32 and 33, pp. 80-81] **Urine Volume**: Increased in males at two higher doses (9% and 30%) and females (27%) at highest dose. Except for urine volume in high dose males, the changes were not statistically significant. [Study Table 3, p. 32] **Urine Density**: Slight decrease in high dose females only (1.049 vs 1.059). **Stomach**: No gross of histopathological lesions. **Eyes**: Detailed eye examinations conducted before study initiation and prior to necropsy (Study Tables 10 and 11). Corneal clouding seen in both control and exposed animals at the end of study. (Common condition in Fischer rats) Periocular soiling in 3/10 high dose females and in 2/10 (low dose), 1/10 (mid-dose), and 2/10 (high dose) males. No periocular soiling in control animals. Daily examinations of the eyes included palpebral closure, pupil size, and lacrimation, and observable abnormalities in the eye. No abnormalities noted.			
Rats, Fischer 344, 5 per sex per dose group, 7 weeks old	XDE-750 (aminopyralid acid), 94.5%. Exposure Period: 4 weeks Intended Doses: 0, 10, 100, 500, 1000 mg/kg bw/day.	Increased cecal weight: 1000 mg/kg: all animals. 500 mg/kg: 3/5 males and 2/10 females. Stomach: No gross of histopathological lesions.	Stebbins and Day 2000, MRID 46235625 Acceptable (9/1/05)
ORAL – CHRONIC			
Dogs, Beagle, 4 per sex per dose, 7 months old	XDE-750 (aminopyralid acid), 94.5%. Exposure Period: 12 months Concentrations: 0, 0.03%, 0.3%, and 3%. Doses: Males: 0, 9.9, 99.2, and 967 mg/kg bw/day Females: 0, 9.2, 93.2, and 1030 mg/kg bw/day	Body Weight: High dose: decreased body weight (9%) and lower body weight gains (58%) in females. Liver Weight: High dose: Increase in mean absolute and relative liver weights. Increases in relative liver weights were statistically significant (21% in males and 11% in females).	Stebbins and Day 2003a, MRID 46235627 Acceptable (9/1/05)

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Stebbins and Day 2003a, MRID 46235627 *(continued)* **Liver pathology:** High dose: slight midzonal hypertrophy in 2 males and 2 females. **Stomach:** Hyperplasia and hypertrophy of mucosa with slight inflammation in all high dose males and females. **Lymphoid Tissue:** No pathology or other effects on the thymus, bone marrow, lymph nodes, spleen pathology or weight, or leukocyte counts. **Notes from EFED DER:** NOAEL: 99 (M) and 93 (F) mg a.e./kg bw/day. LOAEL: 976 (M) and 1038 (F) mg a.e./kg bw/day. Gastric effects taken as basis for LOAEL.			
Mice, CD-1, 50 per sex per dose, six weeks old	XDE-750 (aminopyralid acid), 94.5%. Exposure Period: 18 months Target Doses: 0, 50, 250, and 1000 mg/kg/day. Average Actual Doses: Males: 0, 50.2, 251, and 1000 mg/kg bw/day Females: 0, 50.9, 252, and 1010 mg/kg bw/day	Mortality High Dose: Females only, increased mortality attributed to nephropathy. Studies authors state (Study p. 11): *the overall incidence and severity of nephropathy was not increased in males or females from any dose group. Therefore, the increased number of high-dose females that died or were euthanized moribund due to nephropathy was interpreted to be unrelated to treatment.*	Stebbins and Day 2003b, MRID 46235628 Acceptable (9/1/05)

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Stebbins and Day 2003b *(continued)* **Kidney:** **Notes from OPP DER:** Cites gross pathology incidence for bilateral pale kidneys: 5/50 (controls), 6/50 (50 mg/kg), 7/50 (250 mg/kg), and 13/50 (1000 mg/kg). Data cited to "pages 118 to 134 of study report". This specific data set is from Table 27, p. 123 of the full study. The DER goes on to state that no adverse effects were observed in males or females at any dose. ***Notes for this risk assessment:*** No effects on the kidney in males. Using the Fisher Exact Test and the data above cited by OPP, the incidence of bilateral pale kidneys in female rats at the high dose group is statistically significant (p-value = 0.000423). Based on Table 27, p. 122 of the study report, the incidence of all forms of kidney gross pathology in female rats is 7/50 (controls), 7/50 (50 mg/kg), 10/50 (250 mg/kg), and 15/50 (1000 mg/kg). Using the Fisher Exact test, the incidence in high dose group relative to the controls is statistically significant (p=0.0448) but the incidence in the 250 mg/kg dose group is not (p=0.2977). Based on probit analysis using benchmark dose, the p-value for the goodness-of-fit for the regression is 0.8942 – i.e., the probit model fits the dose-response data. Thus, the kidney pathology in female rats appears to be related to exposure. The 1000 mg/kg dose appears to be a LOAEL for female rats. Based on histopathological examinations of the kidneys in female rats (Table 28, p. 143 of Study), the incidence of abnormal histopathology in the kidney is 35/50 (controls), 29/50 (50 mg/kg), 30/50 (250 mg/kg), and 33/50 (1000 mg/kg). This clearly indicates the lack of any treatment related effect on kidney histopathology.			
Stomach: In the individual pathology reports, ulcers to the stomach, generally characterized as multifocal ulcers of the glandular mucosa were noted in 8 of 50 female mice (Animal Numbers 8840, 8841, 8848, 8849, 8857, 8859, 8860, 8861), both in the high dose group. In the pathology summary table (Study Table 28, p. 166), it is noted that histological observations of stomachs of high dose female mice were within normal limits for 39/50 animals (76%). At next lower dose, stomach pathology was normal in 12/15 animals (80 %). In the low dose group and control groups, stomach pathology was normal in 11/17 (64%) and 40/50 (80%), respectively. None of these differences appear to be dose related and none are statistically significant. **Ovaries:** Significant increase in absolute and relative ovary weights at 50 mg/kg and 250 mg/kg but not at 1000 mg/kg. As reviewed in the OPP DER, the increased ovarian weights were related to ovarian cysts in the 50 mg/kg and 250 mg/kg dose groups and were not considered treatment related. **Lymphoid Tissue:** No pathology or other effects on the thymus, bone marrow, lymph nodes, spleen pathology or weight, or leukocyte counts.			

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Rats, Fischer 344, 65 animals per sex per group, 7 weeks old.	XDE-750 (aminopyralid acid), 94.5%. Exposure Period: 24 weeks (50 per group for oncogenicity and chronic toxicity) 12 months (5 per group for interim pathology; 5 per groups for chronic neurotoxicity evaluation) Doses: 0, 5, 50, 500, 1000 mg a.e./kg bw/day.	**1000 mg/kg** Body Weights: Decrease in males (5%) with increase in food consumption. Decrease in females (2-3%) with no change in food consumption. AST: Increases in females only at 3 months (30%), 6 months (61%), and 12 months (46%, NS). No significant differences at 18 months (-13%) or 24 months (28%).	Johnson and Dryzga 2004, MRID 46235615 Acceptable (9/1/05) Neurotoxicity detailed in Maurissen et al. 2003, MRID 46235617, see below.

Johnson and Dryzga 2004, MRID 46235615 (*continued)*

1000 mg/kg *(continued)*

Cecal weights: Increase (x4 in males and x3 in females) with slight hyperplasic of mucosa.
Urine: Increase in volume with decrease in specific gravity. Decreased urine protein and ketones.
Histopathology: None

Notes continued on next page.

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Johnson and Dryzga 2004, MRID 46235615 (*continued)* **500 mg/kg** Body Weights: Decrease in males (3%) with slight increase in food consumption. Cecal weights: Increase (x1.8 in males and x1.3 in females) with slight hyperplasic of mucosa in 3/10 males at 10 months only. No histopathology at 24 months. Urine: Increase in volume with decrease in specific gravity. Decreased urine protein and ketones. **Notes for Forest Service risk assessment** Increases in urine volumes and corresponding decreases in urine specific gravity (Text Tables 4 and 5, p57) are clear at 500 mg/kg and 1000 mg/kg dose groups. Temporal relationships, however, are not strong. Urine protein not quantified – i.e., +, ++, +++ etc. dip stick classifications (Text Table 8, p. 49) or for ketones (Text Table 9, p. 50). Nonetheless, the decreases are clear but again there is not strong temporal relationship. For both effects, 50 mg/kg seems to be a clear NOEL. **Stomach Lesions:** 50 animals at each dose group examined at the end of the study. Various ulcerations noted in about 10% of the animals. No dose-response relationship. This effect cannot be associated with treatment. **Lymphoid Tissue:** Decreased cellularity on the thymus in 4/10 females high dose females but also in 8/10 control females at 12 months. No pathology or other effects on the thymus at 24 months. No remarkable changes in bone marrow, lymph nodes, spleen pathology or weight, or leukocyte counts. **U.S. EPA/OPP-HED DER** Females: NOAEL 500 mg/kg bw/day. LOAEL 1000 mg/kg bw/day based on increased cecal weights. Males: NOAEL 50 mg/kg bw/day. LOAEL 500 mg/kg bw/day based on slight body weight decrease.			
Rats, Fischer 344, 10 animals per sex per group, 7 weeks old.	XDE-750 (aminopyralid acid), 94.5%. Exposure Period: 12 months. Evaluations: 1, 3, 6, 9, and 12 months. Intended Doses: 0, 5, 50, 500, 1000 mg/kg bw/day.	No signs of neurotoxicity.	Maurissen et al. 2003, MRID 46235617 Acceptable (9/1/05) Animals from Johnson and Dryzga 2004, see above

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
DERMAL – ACUTE			
Guinea Pig, Hartley Albino, 10 each sex per group, ≈0.35 to 0.43 kg bw.	XDE-750 (aminopyralid acid), 94.5%. **Dermal Sensitization** Dosing: Intradermal injections of 5% (w/w) aminopyralid with injections of FCA Emulsion (Freund's Complete Adjuvant). After 1 week, topical application of aminopyralid. Positive Controls: 1-chloro-2,4-dinitrobenzene and α-hexylcinnamaldehyde	No sensitization response to aminopyralid. Expected sensitization responses in positive controls.	Wilson 2001 MRID 46235613 Acceptable (8/31/04)
Guinea Pig, Hartley Albino, 10 each sex per group, ≈0.36 to 0.45 kg bw.	**GF-871 (41.9% aminopyralid TIPA, equiv to 21.8% a.e.)**. **Dermal Sensitization** Dosing: Intradermal injections of 1% (w/w) with injections of FCA Emulsion (Freund's Complete Adjuvant). After 1 week, topical application of GF-871. Positive Controls: 1-chloro-2,4-dinitrobenzene and α-hexylcinnamaldehyde	No sensitization response to aminopyralid. Expected sensitization responses in positive controls.	Wilson 2002, MRID 46235614 Acceptable (10/5/04)
Rabbits, New Zealand White, 1 male and 2 female , ≈2.5 kg bw	XDE-750 (aminopyralid acid), 94.5%. **Dermal Irritation** Dosing: 500 mg a.e. (0.3 mL aqueous in 0.5%methylcellulose) Duration: 4 hours with gauze patch Observation Period: 72 hours	No dermal irritation.	Brooks 2001c, MRID 46235611 Acceptable (8/31/04)

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Rabbits, New Zealand White, 1 male and 2 female , ≈2.8-3.5 kg bw	**GF-871 (41.9% aminopyralid TIPA, equiv to 21.8% a.e.)**. **Dermal Irritation** Dosing: 0.5 mL GF-871 to intake skin (≈109 mg a.e.). Duration: 4 hours with gauze patch. Observation Period: 7 Days	Females: Slight erythema on Days 1 and 3 days. Normal by Day 7. No edema. Male: No effects.	Brooks and Radtke 2002b, MRID 46235612 Acceptable (10/4/04)
Rats, Fischer 344, 5 per group per sex.	XDE-750 (aminopyralid acid), 94.5%. Limit Test: 5000 mg a.e./kg bw in 0.5% methylcellulose for 24 hours. Observation Period: 2 weeks	No mortality. All animals lost weight between Day 1 and Day 2 (≈6% in males and ≈8% in females). Abrasion at test site in 1 male and 1 female due to removal of test material stuck to skin after 24 hours. Soiling (periocular or perioral) in 2 males and 2 females over Days 1 and 2.	Brooks and Yano 2001, MRID 46235605 Acceptable (8/31/04)
Rats, Fischer 344, 5 per group per sex.	**GF-871 (41.9% aminopyralid TIPA, equiv to 21.8% a.e.)**. Limit Test: 5000 mg neat/kg bw for 24 hours. Equivalent to 1090 mg a.e./kg bw. Observation Period: 2 weeks	No mortality. Weight loss on Day 2 relative to Day 1. Not apparent over rest of study. Perineal soiling in one male and reddened skin at application site in 2 males.	Wilson et al. 2002. MRID 46235606. Acceptable (9/28/04)
DERMAL – SUBCHRONIC			
Rats, Fischer 344, 10 per sex per group, ≈0.12 kg bw.	XDE-750 (aminopyralid acid), 94.5%. Duration: 4 weeks Dosing: 0, 100, 500, and 100 mg a.e./kg bw/day, 6 h/d, 7d/w.	Slight epidermal hyperplasia, 3/10 males at 1000 mg/kg and 2/10 males at 500 mg/kg. No effects in females.	Stebbins et al. 2002, MRID 46235626 Acceptable (9/1/05)

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
EYES			
Rabbits, New Zealand White, 2 male and 1 female , ≈2.7 to 2.8 kg bw	XDE-750 (aminopyralid acid), 94.5%. Dosing: 100 mg powder in conjunctival sac Observation Periods: 36 days	Moderate to marked irritation with corneal opacity and vascularization. Irritation persisted over 36 days in 2/3 animals.	Brooks 2001b, MRID 46235609 Acceptable (8/31/04)
Rabbits, New Zealand White, 1 male and 2 female , ≈2.5 to 2.2 kg bw	**GF-871 (41.9% aminopyralid TIPA, equiv to 21.8% a.e.)**. Dosing: 0.1 mL in conjunctival sac Observation Periods: 72 hours (3 days).	Slight conjunctival redness in treated eye of 2/3 animals by 1 hour after exposure. No signs of irritation by Day 2. OPP Note: No signs of corneal opacity. Note: Dosing corresponds to ≈28.8 mg a.e.	Brooks and Radtke 2002a, MRID 46235610 Acceptable (10/4/04)
INHALATION			
Rats, Fischer 344, 5 per sex, ≈0.12 kg bw.	XDE-750 (aminopyralid acid), 94.5%. Exposure: Nose only, dust (2.5 µm), 5.5 mg/L for 4 hours. Observation Period: 2 weeks	Gasping in 1 animal and dropping eyelids in 9 animals immediately after exposure. "dried red material around the nose", dropping or closed eyes, and yellow material around the urogenital region of one female during the first week post-exposure. OPP Comment: Yellow material is deposited test substance. Not toxicologically significant. Note: Study authors note a slight body weight loss from Day 0 to Day 1: 2% in males and 1% in females.	Kiplinger 2001, MRID 46235607 Acceptable (no date)

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Rats, Fischer 344, 5 per sex, ≈0.17 kg bw for males and ≈0.13 kg bw for males.	**GF-871 (41.9% aminopyralid TIPA, equiv to 21.8% a.e.)**. Exposure: 5.79 mg formulation/L (1.26 mg a.e./L) for 4 hours. Observation Period: 2 weeks	Soiling of haircoat in 3/5 males and 5/5 females during exposure. Extensive body soiling post-exposure. No effects by Day 4 after exposure. Body weight losses of 3.4% in males and 4.5% in females over 24-hours post-exposure. Body weight gains thereafter.	Landry and Krieger 2002, MRID 46235608 Acceptable (no date)
METABOLISM and PHARMACOKINETICS			
Rats, Fischer 344, 11 males in single high dose and multiple low dose groups, 4 males per group, 10 weeks old, 0.2 to 0.23 kg bw.	[^{14}C]XDE-750 (aminopyralid acid), 99.5% chemical purity, 98.6% radiolabel purity. Vehicle: 0.5% methyl cellulose in distilled water. Dosing: Single oral gavage nominal (measured) doses of 50 (52.1) mg/kg bw and 1000 (1174.4) mg/kg bw. Observation Period: 168 hours (7 days)	Excretion: Urine (≈41-49%) and feces (≈43%). No significant dose-dependency. <0.01% ^{14}C in expired air. ≥96% excreted in urine as parent compound. 100% excreted in feces as parent compound.	Liu 2004, MRID 46235807 Acceptable (10/20/05) Single dose exposures

Liu 2004, MRID 46235807, Single dose exposures *(continued)*

Urinary kinetics: Apparent two-compartment urinary elimination: α-phase $T_{½}$: 2.85 h (low dose) and 3.78 h (high dose). β phase $T_{½}$: 10.23 h (low dose) and 10.88 h (high dose). Difference between doses not statistically significant.

Tissue Residues: Highest concentrations in skin (0.074 ppm at low dose and 27 ppm at high dose). This is clearly dose-dependent. Apparent dose-dependence in GI tract residue (0.019 ppm vs 2.56 ppm) and carcass (0.03 ppm vs 4.45 ppm). Lowest measured residue in fat (0.004 ppm at low dose and 0.072 ppm at high dose) with no dose-dependency (0.072/0.004 = 18). Concentrations in the kidney were virtually identical to the concentrations in the blood and liver (0.02 to 0.026 ppm at the low dose and 0.54 to 0.61 ppm at the high dose).

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Rats, Fischer 344, 4 males, 10 weeks old, 0.2 to 0.23 kg bw.	[^{14}C]XDE-750 (purity and vehicle as detailed above) Dosing: 50 (51.8) mg/kg bw/day for 14 days by gavage. Observation Period: 168 hours (7 days) after final dose.	Excretion: Urine (≈59%) and feces (≈33%). <0.01% ^{14}C in expired air. ≥96% excreted in urine as parent compound. Urinary kinetics Apparent two-compartment urinary elimination: α-phase $T_{½}$: 3.27 h; β phase $T_{½}$: 12.25 h. Not significantly different from single dose.	Liu 2004, MRID 46235807 Acceptable (10/20/05) Repeated dose exposure
Liu 2004, MRID 46235807, Multiple dose exposure *(continued)* Tissue Residues (tabular summary on Study page 14): Highest concentrations in skin (0.148 ppm) and carcass (0.032 ppm). Most of tissue concentrations in the range of 0.01 to < 0.03 ppm). Concentrations in the kidney were virtually identical to the concentrations in the blood and liver. Very little residue in fat (0.004 ppm), identical to residue in single-dose 50 mg/kg bw group.			
Rats, Fischer 344, Male, 4 animals, ≈0.18 kg.	[^{14}C] Aminopyralid acid, 94.5% chemical purity, 98.25% radiolabel purity. Dosing: 50 mg a.e./kg bw by gavage in 0.5% aqueous methyl cellulose. Sampling from 15 minutes to 5 days.	Peak plasma concentration of 26 µg a.e./mL plasma at 15 minutes (Study p. 34, Figure 1. Exact mean of 25.834 µg/g plasma, Table 5, p. 40). Peak plasma concentrations ranged from about 17.5 µg/g to 31.6 µg/g.	Domoradzki et al. 2004, MRID 46235833 Acceptable/ non-guideline (9/1/05)
Domoradzki et al. 2004, MRID 46235833 *(continued)* Absorption rate coefficient too rapid to estimate with the time-course data. Plasma halftimes: α-phase, 0.338 h; β phase: 8.8 h. Urinary excretion: 38.3% in six hours. α-phase $T_{½}$: 2.8 h; β phase $T_{½}$: 7.8 h. % dose in urine at 120 hours: 46.3% 24 h post-dosing excretion: 93.5% (44.7% urine and 48.8% feces). All excretion as parent compound. Notes for Forest Service Risk Assessment: The peak plasma halftime of 26 µg a.e./mL is equivalent to 26 mg/L or 0.126 millimoles/L [26 mg / 207 mg/millimole = 0.126 millimole] or 126 µmoles/L. The dose of aminopyralid 50 mg/kg bw/day is equivalent to 242 µmoles/kg bw [50 mg / 207 mg/millimole = 0.242 millimole = 242 µmoles].			

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Rats, Fischer 344, Male, 4 animals, ≈0.18 kg.	[^{14}C] Aminopyralid TIPA salt, 94.5% chemical purity, 98.25% radiolabel purity. Dosing: 96 mg a.i./kg bw (equivalent to 50 mg a.e./kg bw)	Peak plasma concentration of 16 µg a.e./mL plasma at 15 minutes. Peak plasma concentrations ranged from about 12.6 µg/g to 19.6 µg/g. Plasma halftimes: α-phase, 0.509 h; β phase 13 h.	Domoradzki et al. 2004, MRID 46235833 Acceptable/ non-guideline (9/1/05)
Domoradzki et al. 2004, MRID 46235833 *(continued)* Urinary excretion: 34.6% in six hours. α-phase $T_{½}$: 2.5 h; β phase $T_{½}$: 10.7 h. % absorption based on urinary recovery at 120 hours: 42.5% 24 h post-dosing excretion: 93.5% (41.5% urine and 51.8% feces) 99.66% excretion as parent compound.			
Rabbits, New Zealand White, females, non-pregnant, n=3 x, bw≈3.2 kg .	Group 1 DE-751 (aminopyralid acid) Single Dose: [^{14}C] labeled 371 mg a.e./kg bw by gavage.	Peak plasma concentration (≈32-61 µg/g) in 1-2 hours of dosing. Residue in GI tract at 72 hrs post dosing = % excreted in urine: 77% % excreted in feces: 20% Plasma k_{el}: 0.073-0.16 h^{-1} Urinary t1/2: 6.47±0.98 hr	Hansen et al. 2005. No MRID number. No DER.
Rabbits, New Zealand White, females, pregnant, n=3 x, bw≈3.3 kg	Group 2 Single Dose: [^{14}C] labeled 362 mg a.e./kg bw by gavage on Day 7 of gestation.	Peak plasma concentration (≈36-65 µg/g) in about 1.5-1.7 hours of dosing. Residue in GI tract at 72 hrs post dosing = % excreted in urine: 83% % excreted in feces: 16% Plasma k_{el}: 0.13-0.28 h^{-1} Urinary t1/2: 5.96±0.41 hr	Hansen et al. 2005. No MRID number. No DER.

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Rabbits, New Zealand White, females, pregnant, n=3 x, bw≈3.4 kg	Group 3 Multiple Dose: unlabeled 279 mg a.e./kg bw/day by gavage on Days 7-20 of gestation. [^{14}C] labeled 279 mg a.e./kg bw/day by gavage on Day 21 of gestation	Kinetic observations only on Day 21 after administration of [^{14}C] labeled aminopyralid. Peak plasma concentration (≈65-74 μg/g) in 0.3 to 1 hour of dosing. Residue in GI tract at 72 hrs post dosing = % excreted in urine: 86% % excreted in feces: 8% Bioavailability 75% to 115% higher than single dose groups. Plasma k_{el}: 0.15-0.21 h^{-1} Urinary t1/2: 6.77 ±0.4 hr	Hansen et al. 2005. No MRID number. No DER.
Hansen et al. 2005 *(continued)* Total %excretion given above recorded at 72 hours post-dosing. In all groups, >99% excreted with no detectable metabolism at 72 hours post-doing. **Notes for Risk Assessment**: Bioavailability based on plasma AUC normalized for dose. Group 1, Non-pregnant: 0.000902 AUCinf / Dose (hr*kgbw/ml) Group 2, Pregnant, Single dose: 0.00073 AUCinf / Dose (hr*kgbw/ml) Group 3, Pregnant, Day 22 dose: 0.00158 AUCinf / Dose (hr*kgbw/ml) *Bioavailability* in Group 3 a factor of 1.75 high than non-pregnant rabbits (Group 1) and 2.16 higher than single dose pregnant rabbits (Group 2).			
Goat, British Toggenburg, females, (n=2), lactating, 71 and 77.5 kg, 2.5 years old	[^{14}C]XDE-750 (aminopyralid acid), 100% radiolabel purity. Dosing: Target dose of 21.33 mg in gelatin capsule for 5 days and 19.68 mg in gelatin capsule for 1 additional day.	Excretion: ≈46% in both urine and feces. 0.1% in milk. Concentration in milk: ≈0.005 ppm (w/w).	Macpherson 2003, MRID 46235708 Acceptable (6/13/05)
Macpherson 2003, MRID 46235708 *(continued)* Tissue levels: kidney (71 ppb), liver (8 ppb), milk (6-7 ppb) fat (1 ppb). No detectable residue in muscle. Concentration in feces was 1.31 **ppm** at 20 hours and 9.70 **ppm** at 120 hours. One minor metabolite (not identified) was detected an accounted for 0.2% of administered dose). Note: Skin residues were not assayed.			

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	**Dose/Exposure**	**Response**	**Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)**
Cows, Holstein, 4 in control group, 3 each in T1 to T3 groups, and 9 in T4 group, 513 to 712 kg	XDE-750 (aminopyralid acid), 94.5% unlabelled. Dosing: Capsules, 1.1 (0.5X), 2.48 (1X), 6.40 (3X), and 23.27 mg a.e./kg bw/day Duration of Dosing: 28 days. Sacrifice within 24 hours after last dose except for 6 cows in high dose. Two each sacrificed at 3, 7, and 14 days after last dose.. Dietary Equivalents: Intended to be equivalent to feed concentrations of 38.5 ppm (0.5X), 75 ppm (1X), 225 ppm (3X) and 750 ppm (10X).	No signs of toxicity in any animals. Highest concentrations in kidney followed by liver ≈ fat, >milk, > muscle. Concentrations in tissue appear to be approximately linearly related to dose.	Rosser et al. 2004, MRID 46235723 Acceptable (6/7/05)
MUTAGENCITY SCREENING ASSAYS			
Organism	Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification
Chinese hamster ovary cells	XDE-750 (aminopyralid), purity 94.5%, in DMSO 0, 31.25, 62.5, 125, 250, 500, 1000, 1500, and 2070 µg/mL with and without Aroclor 1254-stimulated rat liver metabolic activation system.	No indication of gene mutation.	Linscombe et al. 2001, MRID 46235801 Acceptable
Chinese hamster ovary cells	**GF-871 (41.9% aminopyralid TIPA, equiv to 21.8% a.e.)** 0, 250, 500, 1000, 2000, and 400 µg a.i./mL with and without Aroclor 1254-stimulated rat liver metabolic activation system.	No indication of gene mutation.	Linscombe et al. 2004, MRID 46235804 Acceptable

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Mice, CD-1, Male, 6 per dose level. (bone marrow micronucleus assay)	XDE-750 (aminopyralid), purity 94.5% Dosing: 0, 500, 1000, and 2000 mg a.e./kg bw/day for 2 days.	No effect on number of abnormal red blood cells in bone marrow.	Spencer and Gorski 2002, MRID 46235804 Acceptable (09/01/05)
Rat lymphocyte cultures (chromosome aberration assay)	XDE-750 (aminopyralid), purity 94.5%, in DMSO Assay 1: 0, 32.3, 64.7, 129.4, 258.8, 517.5, 1035, 2070 µg a.e./mL with and without Aroclor 1254-stimulated rat liver metabolic activation system Assay 2: 0, 125, 250,500,750,1000, 1400, 1700 or 2070 µg a.e./mL without activation. 0, 62.5, 125, 500, 1000, and 2070 a.e./mL with activation. Assay 3: 0, 400, 600, 800, 1000, 1200, 1400, 1600, 1700, 1800 or 2070 a.e./mL without activation.	Significant increases in chromosomal aberrations (mostly chromosome breaks) at 1000, 1400 and 1700 µg/mL without metabolic activation. EFED Review: *XDE-750 is not a clastogenic agent in the presence of metabolic activation but induced a weak clastogenic effect only at cytotoxic levels with metabolic activation*. (U.S. EPA/OPP-HED 2004)	Linscombe et al. 2002a, MRID 46235802 Acceptable, (09/01/05)
Linscombe et al. 2002a, MRID 46235802 *(continued)* **Note for Forest Service Risk Assessment:** The above appears to be a typographical error in the DER that was carried over to U.S. EPA/OPP-HED (2004). It appears that the latter part of the above sentence should read: *only at cytotoxic levels **without** metabolic activation*.			
Salmonella typhimurium strains TA98, TAl00, TA1535 and TA1537 and *Escherichia coli* strain WP2uvr	XDE-750 (aminopyralid), purity 94.5%, in DMSO 0, 100, 333, 1000, 3300, and 5000 µg a.e./plate with and without Aroclor 1254-stimulated rat liver metabolic activation system	No evidence of mutagenic activity.	Mecchi 2004a, MRID 46235636 Acceptable (8/31/05)

Appendix 3: Toxicity to and kinetics in experimental mammals (formulation studies using **GF-871** in **bold type** for emphasis only).

Animal, number, initial body weight or age	Dose/Exposure	Response	Reference, MRID No., U.S. EPA-HED Classification [1] (date of review)
Salmonella typhimurium strains TA98, TAl00, TA1535 and TA1537 and *Escherichia coli* strain WP2uvr	**GF-871 (41.9% aminopyralid TIPA, equiv to 21.8% a.e.)** 0, 100, 333,1000, 3300 or 5000 μg a.i./plate with and without Aroclor 1254-stimulated rat liver metabolic activation system	No evidence of mutagenic activity.	Mecchi 2004b, MRID 46235637 Acceptable (8/31/05)

[1] Classifications give by the Health Effect Division (HED) Data Evaluation Records unless otherwise noted. The date following the classification refers to the date of the last signature on the DER. Most DERs in this appendix have at least 2 signatures, the initial HED reviewer and the HED supervisor who approved the DER. Notes on the HED risk assessment of aminopyralid refer to U.S. EPA/OPP-HED (2004).

[2] Methocel: water-soluble methylcellulose and hydroxypropyl methylcellulose polymers. See http://www.dow.com/methocel/

Appendix 4: Toxicity to birds.

Animal	Dose	Response	Reference, EFED Classification [1]
Acute, Gavage			
Bobwhite Quail (*Colinus virginianus*), 19 weeks old at start, 5 per dose per sex.	XDE-750 (aminopyralid acid) Nominal doses: 0, 63, 292, 486, 810, 1350 and 2250 mg a.e./kg bw. Deionized water used as vehicle. Administered by intubation (gavage). 14-day post-exposure observation period. Note: The EFED DER shows adjustments on doses downward to account for impurities. This is not done in Forest Service risk assessments. The dose is assumed to be a mixture of the acid and impurities – i.e., the mixture of concern. In identifying effect and no effect levels, EFED uses the unadjusted doses – i.e., identical to approach in Forest Service risk assessments.	No mortality. Body Weights: Reduced in males and females at 1350 and 2250 mg/kg. Reduced food consumption at 2250 mg/kg. **Sublethal effects** Time to onset of sublethal effects inversely related to dose. Recovery time directly related to dose. 63 mg/kg: decreased responsiveness in 2/5 females on Day 0 only. 292 mg/kg: decreased responsiveness in 10/10 birds (both sexes) on Day 0 to Day 3. 486 mg/kg: *lethargy, ruffled appearance, loss of coordination, and lower limb weakness.* All organisms normal by Day 5. 810 mg/kg: *decreased reaction to external stimuli (sound and movement), ruffled appearance, lethargy, wing droop, loss of coordination, lower limb weakness, prostrate posture, lower limb rigidity, minor muscle fasciculation, convulsions and loss of righting reflex*. All animals normal by Day 7. 1350 and 2250 mg/kg: similar to 810 mg/kg group. Normal by Day 8. No effects at necropsy appear to be related to treatment.	Gallagher et al. 2001a, MRID 46235808 Acceptable (6/16/05) EFED DER concurs with the assessment with that a NOEC for sublethal effects is not identified.

Appendix 4: Toxicity to birds.

Animal	Dose	Response	Reference, EFED Classification [1]
Bobwhite Quail (*Colinus virginianus*), 24 weeks old at start, 5 per dose per sex.	XDE-750 (aminopyralid acid) Nominal doses: 0, 8, 14, 23, 38, 63 and 292 mg a.e./kg bw. Deionized water used as vehicle. Administered by intubation (gavage). 14-day post-exposure observation period.	No mortality in any group (controls or exposed). No effects on body weights and food consumption **Sublethal effects** 8 mg/kg: loss of coordination in 1/5 males at 35 minutes after dosing. At 1-hour, the appearance of the bird was abnormal (ruffled) and the bird was panting. No effects in any other bird. 14 mg/kg: No effects in any birds. **Accepted by EFED in DER as the NOEC.** 23 mg/kg: abnormal (ruffled) appearance after 1-hour in one bird. Recovery by 5 hours. No effects in other birds 38 mg/kg: In one male, slight loss of coordination at 2 hours after dosing and reduced reaction to stimuli at 3 hours Recovery by 5 hours after dosing. (Lesion on foot of one female from Day 4 to Day. Does not appear to be related to treatment.) 63 mg/kg: Signs of toxicity in 4 birds (3 male and 1 female) within 20 minutes and continued during Day 0. Signs in males included wing droop, loss of coordination, lethargy, and neck curl. Female evidenced only decreased response to stimulus. 292 mg/kg: Signs of toxicity within 25 minutes and continuing over Day 0 in 3 males and 3 females. Signs included loss of coordination, prostrate posture, and lower limb weakness. Ruffled appearance in 2 birds on Day 1.	Gallagher et al. 2003, MRID 46235809 Supplemental (6/16/05), The study is scientifically sound and does not contain flaws. The study is not considered *Acceptable* simply because it was not designed to fulfill guideline requirements – i.e., the highest dose is substantially below the limit dose of 2000 mg a.i./kg bw that must be used in a guideline study. This study, however, was designed to be supplemental to the study by Gallagher et al. 2001a.

Appendix 4: Toxicity to birds.

Animal	Dose	Response	Reference, EFED Classification [1]
Acute, Dietary			
Bobwhite Quail (*Colinus virginianus*), 5 per replicate and 6 replicates for controls, 5 per replicate and 2 replicates for exposed groups. Sexes not determined in juvenile birds. All birds 17 to 25 grams bw and 10 days old at start at start.	XDE-750 (aminopyralid acid), 94.5%. Nominal Dietary Concentrations: 0, 178, 316, 562, 1000, 1780, 3160 and 5620 ppm a.e. Measured Dietary Concentrations: 0, 185, 309, 548, 979, 1720, 3053 and 5556 ppm a.e. Dietary exposure for 5 days with a 3 day post-exposure observation period.	No mortality or signs of toxicity in any groups. No effect on body weight (Study Table 2 and Appendix VI) or food consumption (Study Table 3 and Appendix VII). Based on average body weights during exposure period (Study Table 2) and food consumption during exposure period(Study Table 3), the birds appear to have consumed about 25% to 35% of their body weight per day in food. Using 30%, the measured dietary concentrations corresponded to doses of about 0, 56, 93, 164, 294, 516, 916, and 1669 mg a.e./kg bw/day. Notes on EFED DER: NOEC: 5556 ppm LOEC: not determined	Gallagher et al. 2001b, MRID 46235810 Acceptable (2/2/05)

Appendix 4: Toxicity to birds.

Animal	Dose	Response	Reference, EFED Classification [1]
Mallard Duck (*Anas platyrhynchos*), 5 per replicate and 6 replicates for controls, 5 per replicate and 2 replicates for exposed groups. Sexes not determined in juvenile birds. All birds 150 to 209 grams bw and 10 days old at start at start.	XDE-750 (aminopyralid acid) , 94.5%. Nominal Dietary Concentrations: 0, 178, 316, 562, 1000, 1780, 3160 and 5620 ppm a.e. Mean Measured Dietary Concentrations: 0, 172, 309, 548, 979, 1720, 3053 and 5496 ppm a.e. Dietary exposure for 5 days with a 3 day post-exposure observation period. Dietary exposure for 5 days with a 3 day post-exposure observation period.	No mortality or signs of toxicity in any groups. No effect on body weight (Study Table 2 and Appendix VI) or food consumption (Study Table 3 and Appendix VII). Based on average body weights during exposure period (Study Table 2) and food consumption during exposure period(Study Table 3), the birds appear to have consumed about 41% to 43% of their body weight per day in food. Using 42%, the dietary concentrations corresponded to doses of about 0, 75, 133, 236, 420, 748, 1327, and 2360 mg a.e./kg bw/day. Notes on EFED DER: NOEC: 5496 ppm LOEC: not determined	Gallagher et al. 2001c, MRID 46235811 Acceptable (6/16/05)

Appendix 4: Toxicity to birds.

Animal	Dose	Response	Reference, EFED Classification [1]
Reproduction – Dietary			
Bobwhite Quail(*Colinus virginianus*), 21 weeks old at start of study, 20 pairs in control group and 15 pairs in each treatment group.	XDE-750 (aminopyralid acid) , 94.5%. Nominal Dietary Concentrations: 0 (VC), 675(T1), 1350(T2), 2700(T3) ppm a.e. Mean Measured Concentrations: 0, 640, 1270, and 2610 ppm a.e. Dietary exposure to adult birds for 20 weeks: 10 week pre-egg laying and 10 week post-egg laying. Acetone used in preparation of diets. Based on measurements in individual pens (i.e., pairs of animals), the dietary groups reportedly correspond to approximate average doses at week 8 of 0, 0.045, 0.094, 0.185 mg a.e./kg bw/day. See Study Table XXI. **See check of these calculations on next page.**	No mortality or effects on reproductive parameters associated with treatment (eggs laid, eggshell thickness, cracked eggs, viability, and embryo development). **Sublethal effects in Adults**: Observations included disorientation, decreased reactivity, immobility, ataxia and mortality. Do not appear to be dose/related. **Sublethal effects on hatchlings**: Dose related decrease in hatching success: 90.1%, 85.2%, 79.5%, and 78.2%. **Note for Forest Service:** The study authors suggest an NOAEL of 2700 ppm nominal.	Mach 2003b, MRID 46235812 Supplemental, *raw data on hatchling weight not provided. Also, quantity and fate of acetone in diet not specified.* **The DER from OPP is badly damaged and is incomplete.**

Appendix 4: Toxicity to birds.

Animal	Dose	Response	Reference, EFED Classification [1]

Mach 2003b, MRID 46235812 *(continued)*

Notes from Study Authors on Hatchling Survival: *None of the chicks showed any test substance-related toxicological symptoms during the 14-day observation periods, except for observations of hyporeactivity, ataxia, and moribundity. However, these observations were limited and there appeared to be no dose-response. Therefore, we do not classify these observations as test substance-related toxicosis. … The percent survivability out of the number of normal hatchlings for each group in the test were 89.0%, 65.7%, 84.5%, and 82.0% in the VC, T1, T2, and T3 groups, respectively*. …[Acknowledgement that survivability in the low dose group was statistically significant] …

The difference in the hatchling survival may be attributed to the following circumstances. During week 19 (T1), a brooder battery was not turned on, that resulted in the death of 14 hatchlings due to cool temperatures. These 14 hatchlings were removed from the calculations for appendices C10 and C11.

During the same week, yet in a separate brooder, pecking was attributed to the death of at least 15 hatchlings. A total of 27 hatchlings died in this one brooder, most likely attributable to pecking. In addition, during week 20, another 12 bird deaths can be attributed to pecking. Pecking may attribute to as many as 22 bird deaths in this brooder. This totals 49 birds that died from causes not common in any of the other brooders. The statistical difference identified above may have been avoided had these hatchlings not suffered these abnormal fates. (Study page 23)

EFED DER Assessment: NOAEC not determined. EFED did not use this study in the risk characterization for birds. See discussion in Section 4.3.2.2 of risk assessment.

Note from DER: *There were statistically significant differences found in the lowest dose tested for two survival endpoints (hatchling survival per eggs set and 14-day hatchling survival), but it is unclear whether these were treatment-related effects. Together with apparent downward trends in hatchling per live embryos and hatchlings per pen, it is uncertain that the authors conclusion that these effects are not treatment related can be supported. At the very least, the husbandry during the study can be called into question. Therefore, the study did not determine a NOEC for these endpoints* (p. 2/46).

(Notes continued on next page)

Appendix 4: Toxicity to birds.

Animal	Dose	Response	Reference, EFED Classification [1]
Mach 2003b, MRID 46235812 *(continued)* Note from EFED Risk Assessment: *…there were statistically significant differences found in the lowest dose tested for two survival endpoints: hatchling survival per eggs set and 14 day hatchling survival), but it is unclear whether these were treatment-related effects. … The differences appear to be artifacts of poor husbandry during the study. A new study should be submitted to clarify potential toxicity.* **Check on calculation of average daily doses from Table XXI** Appendix C1 (Study pages 77-80) gives food consumption rates for each pen in units of g food/bird/day. There is very little variability with the rates ranging from 18 to 26 g food/bird/day. Based on reported means per dose group, the average value is 21.5 g food/bird/day (22 g food/bird/day in the control and high dose groups and 21 g food/bird/day in the low and mid dose groups). The average body weights are given in Appendix C2 (Study pages 81-84). Taking the Week 8 values as an approximate average, the body weights are 0.305 kg (control), 0.31 kg (low dose), 0.30 kg (mid dose), and 0.36 (high dose). Thus, the food consumption values, as a proportion of body weight (kg food/kg bw), are: 0.072 (control), 0.068 (low dose), 0.07 (mid dose), and 0.061 (high dose). Based on the reported mean measured concentrations (0, 640, 1270, and 2610 ppm or mg a.e./kg diet), the estimated average daily doses would be: 0 mg a.e/kg bw (control), 43.52 mg a.e./kg bw/day (640 ppm x 0.068), 88.9 mg/kg bw/day (1270 ppm x 0.07), and 159.2 mg a.e./kg bw/day (2610 ppm x 0.061). These calculated doses are far greater than the doses reported in Study Table XXI (Study page 45): week 8 doses of 0, 0.045, 0.094, 0.185 mg a.e./kg bw/day. Note: It appears that the calculations given in Table XXI of the study are based on an error in reading Appendix C1 and the assumption that the food consumption values were expressed in units of mg rather than grams. This error has been confirmed by Dow AgroSciences (Jachetta 2007).			

Appendix 4: Toxicity to birds.

Animal	Dose	Response	Reference, EFED Classification [1]
Bobwhite Quail(*Colinus virginianus*), 21 weeks old at start of study, 16 pairs in control group and 16 pairs in each treatment group.	XDE-750 (aminopyralid acid), 94.5%. Nominal Dietary Concentrations: 0 (Group 1), 675 (Group 2), 1350 (Group 3), 2700 (Group 3) ppm a.e. Average Daily Doses based on measured concentrations in diet and food consumption (Study p. 23): 0, 50-65, 102-129, and 203-239 mg a.e./kg bw. Dietary exposure to adult birds for 20 weeks: about 10 week pre-egg laying and 10 week post-egg laying. No solvent/vehicle specified.	**Mortality**: 1 male in control group and 1 male in 2700 ppm group. Food Consumption: Slight but statistically significant reductions in 675 ppm group only during Week 7. Does not appear to be treatment related. No treatment related effects on body weights, reproductive parameters, body weight of offspring, egg shell thickened, behavior, or gross pathology.	Temple et al. 2007 Repeat of Mach 2003a. Not yet reviewed by the U.S. EPA.

Appendix 4: Toxicity to birds.

Animal	Dose	Response	Reference, EFED Classification [1]
Temple et al. 2007 *(continued)* Mean Measured Concentrations (midpoint (range): 0, 662 (642-681), 1395 (1360-1430), and 2715 (2660-2770) ppm a.e. Initial mean body weights of birds were about 207 g (males and females). At the termination of the study the mean body weights were about 228 g (males) and 248 g (females) (Study Table 1, p. 26). At Week 8, the mean body weights were about 222 g (males and females). No dose-related changes in body weights. The mean food consumption at Week 10 (start of egg laying) was about 17 g/bird/day. (Study Table 2, p. 29). The approximate food consumption (as a proportion of body weight) will be based on Week 8 weights and Week 10 food consumption: 17 g food /bird/day / 222 g bw = 0.0766 g food/g bw. Taking the mid-point of the ranges for measured concentrations in diet, the estimated doses are: 0, 662 mg a.e./kg food x 0.0766 kg food/kg bw = 50.7 mg a.e./kg bw 1395 mg a.e./kg food x 0.0766 kg food/kg bw = 107 mg a.e./kg bw 2715 mg a.e./kg food x 0.0766 kg food/kg bw = 208 mg a.e./kg bw The above a very similar to doses calculated by study authors (Study p. 23)			
Reproduction – Dietary *(continued)*			
Mallard Duck(*Anas platyrhynchos*), about 18 weeks old, 13 pairs per group.	XDE-750 (aminopyralid acid) , 94.5%. Nominal Dietary Concentrations: 0 (VC), 675(T1), 1350(T2), 2700(T3) ppm a.e. Dietary exposure to adult birds for 20 weeks. Mean Measured Concentrations: 0, 642, 1287, and 2623 ppm.	No significant adverse effects on adults or offspring in any exposed group relative to controls. Based on average body weights during exposure period (Study Table III) and food consumption during exposure period(Study Table IV), the birds appear to have consumed about 6% to 8% of their body weight per day in food. Taking a proportion of 0.07 as an average, the measured dietary concentrations would correspond to dose levels of 0, 44.94, 90.7, and 184 mg a.e./kg bw/day.	Mach 2003a, MRID 46235813 Acceptable (6/16/05)

Appendix 4: Toxicity to birds.

Animal	Dose	Response	Reference, EFED Classification [1]
Mach 2003a, MRID 46235813 *(continued)* Using an estimate of 7%, the concentrations would correspond to average daily doses of 0, 45, 91, and 184 mg a.e./kg bw/day. Note on EFED DER: The DER did a complete statistical reanalysis or the raw data. The basic conclusions reached by EFED are consistent with those reported in study. **EFED Classifications NOAEC: 2623 ppm; LOAEC: >2623 ppm**			
Kinetics			
Hens (*Gallus gallus domesticus*), 45 weeks old, 10 per group, 1.793 kg for treated group	[^{14}C]XDE-750 (aminopyralid acid), radiochemical purity >95%. Doses: 0 and 1.7 mg/per bird/day (target equiv. to 10 ppm dietary). [Note: Average body weight over study of about 1.7 kg. Average dose of 1 mg/kg bw/day.] Duration of dosing: 7 days Observation Period: Sacrificed about 24 hours after last dose.	No signs of toxicity. No effect on egg production Residues in fat and muscle not detectable. Detectable residues in skin (0.0029 ppm) and liver (0.0024 ppm).	Magnussen 2004a, MRID 46235711 DER does not classify study. No deficiencies noted. Not cited in EFED risk assessment. Information (but not study) is cited in HED risk assessment.

[1] Classifications give by the Environmental Fate and Effects Division (EFED) in their risk assessment of aminopyralid (U.S. EPA/OPP-EFED 2004).

Appendix 5: Toxicity to terrestrial Invertebrates.

Animal	Dose/Exposure	Response	Reference, EFED Classification [1]
Honey Bee – Contact Bioassay			
Honeybee (*Apis mellifera*), 10 organisms per group, no replicates	Preliminary Study: ADE 750 (aminopyralid acid). 0 and 100 µg/bee, 48 hour observations period.	No mortality in any group.	Aufderheide 2001a, MRID 46235831 Acceptable (6/16/05)
Honeybee (*Apis mellifera*) 10 organisms per replicate, three replicates per group.	Full Study: ADE 750 (aminopyralid acid). Doses: 0 (untreated), 0 (solvent) and 100 µg a.e./bee, 48 hour observations period. Solvent Control: Acetone, 100% -- i.e., test material dissolved in acetone. Positive Toxic Agent: dimethoate.	No mortality or signs of toxicity in control groups or aminopyralid group. LD_{50} for dimethoate consistent with historical values. Note: Body weights are not given in the study.	
Honey Bee – Oral Bioassay			
Honeybee (*Apis mellifera*), 10 organisms per group, no replicates	Preliminary Study: ADE 750 (aminopyralid acid). Doses: 0, 0.1, 1.0, 10, and 120 µg a.e. /bee. 6 hour feeding period and 48 hour observations period. Administered in a sucrose solution.	From Study p.11: *Mortality after 48 hours was 0% in the treatments and in the control.*	Aufderheide 2001b, MRID 46235832 Supplemental, non-guideline (6/7/05)

Appendix 5: Toxicity to terrestrial Invertebrates.

Animal	Dose/Exposure	Response	Reference, EFED Classification [1]
Honeybee (*Apis mellifera*), 10 organisms per replicate, three replicates per group.	Full Study: XDE 750 (aminopyralid acid). Doses: 0, 5.8, 6.4, 13, 15, 16, 23, 30, 31, 33, 49, 110, and 120 µg a.e./bee. 6 hour feeding period and 48 hour observations period. Administered in a sucrose solution. Positive Toxic Agent: dimethoate.	Bees consumed from 25% to 100% of test material. See Study Table 1, p. 15. Mortality in groups ranged from 0% to 10%. Clearly NOT dose-related. 10% mortality in 1 of 3 control groups, 5.8 µg/bee, and 16 µg/bee. No mortality at doses of 6.4, 13, or 23 µg/bee and higher. LD_{50} estimated as >120 µg/bee. 120 µg/bee is a NOEC.	
Earthworm			
Eisenia foetida, 10 organisms per group.	Range-finding study. XDE 750 (aminopyralid acid), 94.5% purity. Concentration in Soil: 0 and 1,000 mg a.e./kg soil (70% sand, 20% kaolin clay, and 10% peat moss). 14 day exposure. Positive control: 2-chloracetamide.	Identical mortality in control and exposed groups: no mortality on Day 7 and 1/40 on Day 14.	Ward and Boeri 2001, MRID 46235733 No DER. Cited but not discussed in U.S. EPA/OPP-EFED 2004
Eisenia foetida, 10 organisms per replicate, 4 replicates.	Definitive test. XDE 750 (aminopyralid acid), 94.5% purity. Concentrations in Soil: 0 and 5,000 mg a.e./kg soil (70% sand, 20% kaolin clay, and 10% peat moss). 14 day exposure. Positive control: 2-chloracetamide.	No significant differences in body weights but the control replicates averaged an increase of 3.35% and the exposed group averaged a decrease of 1.3%. No sublethal effects (e.g., burrowing behavior). Normal result for positive control (i.e., LD_{50} of 17 mg/kg).	Ward and Boeri 2001, MRID 46235733 No DER. Cited but not discussed in U.S. EPA/OPP-EFED 2004

[1] Study classification given in EFED risk assessment (U.S. EPA/OPP-EFED 2004) and in DERs unless otherwise specified.

Appendix 6: Toxicity to Terrestrial Plants. (All studies used formulation GF-871, 40.6% aminopyralid TIPA salt)

Plant	Response	Reference
Seedling Germination and Emergence Assays of GF-871 formulation (TIPA salt of aminopyralid, a.i.): Assays conducted at 0.028, 0.056, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.43, 28.9, 57.7, 115.8, and 230.8 g **a.i.**/ha. All units below given in g **a.i.**/ha. Conversions to lbs a.e./acre are discussed in Section 4.3.		Aufderheide 2004a, MRID 46235824
Dicots		Supplemental, *…classified as supplemental because soil surface watering occurred without report of test substance mobility characteristics, and because Thiram was applied to sugar beet without further explanation.* Data on monocots given on next page.
Cucumber	All Endpoints: NOEC, EC_{25}, and EC_{50} all ≥57.7 (the highest rate tested).	
Lettuce	Emergence: NOEC: 57.7 ; EC_{25}: 76.4; EC_{50}: 132 Shoot Length: NOEC: 28.9 ; EC_{25}: 36.8; EC_{50}: 50.9 Shoot Weight: NOEC: 28.9 ; EC_{25}: 23.8; EC_{50}: 31.2	
Oilseed rape	NOEC, EC_{25}, EC_{50} for emergence and shoot length all ≥230.8 (the highest rate tested) Shoot Weight: NOEC: 57.7 ; EC_{25}: ≥57.7; EC_{50}: ≥230.8	
Radish	NOEC, EC_{25}, and EC_{50} all ≥230.8 (the highest rate tested.	
Soybean ***Most sensitive species. Shoot weight most sensitive endpoint.***	Emergence: NOEC: 7.21 ; EC_{25}: 16.3; EC_{50}: 33.7 Shoot Length: NOEC: 3.6 ; EC_{25}: 5.63; EC_{50}: 10.0 Shoot Weight: NOEC: 0.90 ; EC_{25}: 2.62; EC_{50}: 5.74	
Sugar beet (Seeds pre-treated with Thiram, a fungicide used on seeds)	Emergence: NOEC, EC_{25}, and EC_{50} all ≥57.7. Shoot Length: NOEC: 7.21 ; EC_{25}: 23.0; EC_{50}: 59.5 Shoot Weight: NOEC: 14.4 ; EC_{25}: 16.2; EC_{50}: 29.9	

Appendix 6: Toxicity to Terrestrial Plants. (All studies used formulation GF-871, 40.6% aminopyralid TIPA salt)

Plant	Response	Reference
Monocots		Aufderheide 2004a, MRID 46235824
Barnyard grass	All Endpoints: NOEC, EC_{25}, and EC_{50} all ≥230.8.	
Corn	All Endpoints: NOEC, EC_{25}, and EC_{50} all ≥230.8.	
Onion ***Most sensitive monocot***	Emergence: NOEC: 57.7 ; EC_{25}: 24.4; EC_{50}: 57.0 Shoot Length: NOEC: 28.9 ; EC_{25}: 46.5; EC_{50}: 103 Shoot Weight: NOEC: 57.7 ; EC_{25}: 50.7; EC_{50}: 166	
Wheat	All Endpoints: NOEC, EC_{25}, and EC_{50} all ≥230.8.	
Vegetative Vigor Assays of GF-871 formulation (TIPA salt of aminopyralid, a.i.): Assays conducted at 0.028, 0.056, 0.11, 0.23, 0.45, 0.90, 1.80, 3.61, 7.21, 14.43, 28.9, 57.7, 115.8, and 230.8 g **a.i.**/ha. All units below given in g **a.i.**/ha. Conversions to lb a.e./acre discussed in Section 4.3.		Aufderheide 2004b, MRID 46235825 Supplemental, *… classified as supplemental because Thiram was applied to sugar beet without further explanation, and because both corn and radish were grown under very low light conditions, which may have affected the results.*
Dicots		
Cucumber	Emergence: NOEC: 28.85 ; EC_{25}: NC[a]; EC_{50}: 46.4 Shoot Length: NOEC: 7.21 ; EC_{25}: 11.1; EC_{50}: 18.5 Shoot Weight: NOEC: 7.21 ; EC_{25}: 12.4; EC_{50}: 23.4	
Lettuce	Emergence: NOEC: 28.85 ; EC_{25}: NC[a]; EC_{50}: 42.3 Shoot Length: NOEC: 3.61; EC_{25}: 7.10; EC_{50}: 10.8 Shoot Weight: NOEC: 1.8 ; EC_{25}: 3.64; EC_{50}: 5.67	*Dicots continued on next page.*

Appendix 6: Toxicity to Terrestrial Plants. (All studies used formulation GF-871, 40.6% aminopyralid TIPA salt)

Plant	Response	Reference
Oilseed rape	NOEC, EC_{25}, EC_{50} for all endpoints ≥230.8 (the highest rate tested).	
Radish	Emergence: NOEC: 115.4 ; EC_{25}: ≥115.4; EC_{50}: ≥115.4 Shoot Length: NOEC: 57.7; EC_{25}: ≥115.4; EC_{50}: ≥115.4 Shoot Weight: NOEC: 14.43 ; EC_{25}: 28.0; EC_{50}: ≥115.4	
Soybean ***Most sensitive species. Shoot weight most sensitive endpoint.***	Emergence: NOEC: 28.85 ; EC_{25}: NC[a]; EC_{50}: ≥57.7 Shoot Length: NOEC: 0.45 ; EC_{25}: 1.31; EC_{50}: 7.4 Shoot Weight: NOEC: 0.45 ; EC_{25}: 1.97; EC_{50}: 4.53	Aufderheide 2004b, MRID 46235825 *continued*
Sugar beet (Seeds pre-treated with Thiram, a fungicide used on seeds)	Emergence: NOEC: 28.85 ; EC_{25}: NC[a]; EC_{50}: 53.7 Shoot Length: NOEC: 28.85 ; EC_{25}: 70.6; EC_{50}: ≥57.7 Shoot Weight: NOEC: 28.85 ; EC_{25}: 20.1; EC_{50}: 33.1	
Monocots		
Barnyard grass	All Endpoints: NOEC, EC_{25}, and EC_{50} all ≥230.8.	
Corn	All Endpoints: NOEC, EC_{25}, and EC_{50} all ≥230.8.	
Onion ***Most sensitive monocot***	Emergence: All endpoints ≥230.8 Shoot Length: NOEC: 57.7 ; EC_{25}: >230.8; EC_{50}: >230.8 Shoot Weight: NOEC: 57.7 ; EC_{25}: 78.2; EC_{50}: >230.8	
Wheat	All Endpoints: NOEC, EC_{25}, and EC_{50} all ≥230.8.	

Appendix 6: Toxicity to Terrestrial Plants. (All studies used formulation GF-871, 40.6% aminopyralid TIPA salt)

Plant	**Response**		**Reference**
Residues/Kinetics			
Plant	Treatment	Residues	Reference
Forage and hay	GF-871 (TIPA salt of aminopyralid, a.i) 0.01 g a.e./ha (0.0892 lb a.e./acre) with 28 day post-application sampling period. Note for this risk assessment: Generally poor fit to 1st order model probably due to rainfall. These are field trials.	Residue halftimes Hay: 15 days (k_e value of 0.0468), good fit to first-order decay. Forage: 19 days (k_e value of 0.0372), poof fit to first-order decay. Note: Raw data not included in report.	Roberts et al. 2004, MRID 46235721 No DER
Grasses, hay and forage, 4-30 inches high	GF-871 (TIPA salt of aminopyralid, a.i.), 0.12 g a.e./ha (0.11 lb a.e./acre). 20 different sites Note for this risk assessment: Generally poor fit to 1st order model probably due to rainfall. These are field trials.	Forage halftimes of 8, 9, 17, and 18 days (Study Fig 3, p. 50-51, r^2 ≈0.4 to 0.66). Hay halftimes of 9, 10, 14, and 15 days (Study Fig 4, p. 52-53, r^2 0.53 to 0.76). Raw data is in Appendix D.	McCormick et al. 2004, MRID 46235722 No DER

[a] NC: Value could not be calculated from the data.

Appendix 7: Toxicity to fish and amphibians.

Animal	Dose/Exposure	Response	Reference, EFED Classification [1]
FISH			
Freshwater Fish – Acute			
Trout, Rainbow (*Oncorhynchus mykiss*), juveniles, 5 fish per concentration. No replicates.	Probe Studies: XDE-750 (aminopyralid acid), 94.5%. 0, 0.781, 1.56, 3.13, 6.25, 12.5, 25.0, 50.0, and 100 mg a.e./L for 96 hours. No solvent used.	No mortality or sublethal effects observed in any organisms.	Marino et al. 2001a, MRID 46235814 Acceptable (6/16/05)
Trout, Rainbow (*Oncorhynchus mykiss*), juveniles, 5 fish per replicate, 6 replicates per concentration	Limit Test: XDE-750 (aminopyralid acid), 94.5%. Concentrations: 0 and 100 mg a.e./L (nominal), 100 mg a.e./L measured for 96 hours (static). Use of solvent not specified. Note on EFED DER: EFED states that 0.1 ppm dimethylformamide was used. This is not stated in the full study.	No mortality observed in any organisms. Partial loss of equilibrium in 2 of 30 organisms (6.66%) exposed to 100 mg/L at 96 hours but not at 24, 48, or 72 hours. No other signs of toxicity based on gross observations of behavior and pathological conditions. Study asserts a NOEC: >100 mg a.e./L because the sublethal effects occurred in <10% of the organisms. Using Fisher Exact Test on 0/30 vs 2/30, *p*-value = 0.2457 – i.e., not a significant difference. EFED NOEC: 100 mg/L.	Marino et al. 2001a, MRID 46235814 Acceptable (6/16/05)
Bluegill Sunfish (*Lepomis macrochirus*), 10 fish per replicate, 3 replicates per concentration including blank and solvent controls	Limit Test: XDE-750 (aminopyralid acid), 94.5%. 0 and 100 mg a.e./L (nominal), 100 mg a.e./L measured for 96 hours (static). Solvent, dimethylformamide (DMF) at 0.1 mL DMF/L	No mortality and no signs of sublethal effects in any fish in any of the groups (blank control, solvent control, and treated).	Machado 2003, MRID 46235815 Supplemental (6/16/05), *the size of fish (0.18-0.92 g) used was less than the recommended range of 0.5 to 5 g.*

Appendix 7: Toxicity to fish and amphibians.

Animal	Dose/Exposure	Response	Reference, EFED Classification [1]
Freshwater Fish – Early Life-Stage / Chronic			
Fathead Minnow (*Pimephales promelas*), eggs 17 to 24 hours old, 4 replicates for all control and test concentrations.	XDE-750 (aminopyralid acid), 94.5%. Duration of Exposure: 36 days (egg stage to fry stage) Nominal text concentrations: 0.780 1.30, 2.16, 3.60, 6.00, and 10.0 mg a.e./L. Measured test concentrations: 0.0708, 1.36, 2.44, 3.89, 6.71, and 11.4 mg a.e./L. Separate solvent and untreated controls. Solvent control: Dimethylformamide, 0.085 mL/L. No probe/range-finding study appears to have been conducted.	No larvae survived at 6 and 10 mg/L. Decreases in % normal at test termination, %larval survival, and overall survival are apparent at 1.3 mg/L but not at 0.78 mg/L. **NOEC and LOEC values reported in study**: 1.36 mg a.e./L and 2.44 mg a.e./L based on weight, length, larval survival, and % normal larvae.	Marino et al. 2003, MRID 46235821 Supplemental (6/16/05), *replicate data for the days-to mean hatch and sub-lethal effects were not submitted and could not be verified by EFED*

Marino et al. 2003, MRID 46235821 *(continued)*
No effects on % hatched of days to mean hatch at 11.4 mg a.e./L.

Abnormalities in larvae included (p. 20): pale coloration, immobility, deformed/underdeveloped body, and scoliosis. The abnormalities do not appear to be dose/related.

Notes from EFED DER:
Hatching, time to hatch:
NOEC: 11.4 ppm. LOEC: Not determined.
Post-hatch larval survival, wet weight, length, and % normal larvae:
NOEC: 1.36 ppm. LOEC: 2.44 ppm

Notes continued on next page

Appendix 7: Toxicity to fish and amphibians.

Animal	Dose/Exposure	Response	Reference, EFED Classification [1]
Marino et al. 2003, MRID 46235821 *(continued)* **Notes for Forest Service Risk Assessment:** Appendix F gives data on number of abnormal larva in each of 4 replicates. These values are not tabulated in Study Table 7. Most larvae that are classified as abnormal are dead. The number of abnormal larvae (out of 4 replicates of 25 per replicate) are: water control (1) ; solvent control (0), 0.0706 mg/L (2), 1.36 mg/L (2), 2.44 mg/L (2), 3.89 mg/L (0), 6.71 mg/L (0), and 11.4 mg/L (0). Out of surviving larvae, the incidence of abnormality is: 0 mg/L 1/78 (1.28%) 0 mg/L 0/87 (0%) -- Solvent control 0.706 mg/L 2/87 (2.3%) 1.36 mg/L 2/81 (2.47%) 2.44 mg/L 2/53 (3.77%) [Fisher Exact *p*-values: 0.565312 relative to untreated control, 0.141624 relative to solvent control, and 0.147266 relative to untreated and solvent controls combined.] The abnormalities do not appear to be treatment related.			
Saltwater Fish – Acute			
Sheepshead Minnow (*Cyprinodon variegatus*), 0.2 to 0.58 g, 28 to 32 mm, 10 fish per group, no replicates	Preliminary Study: XDE-750 (aminopyralid acid), 94.5%. Test concentrations of 0, 0.1, 1.0, 10.0, and 100 a.e./L.	No mortality or sublethal effects in any organisms.	Machado 2002b, MRID 46235820 Acceptable (6/16/05)
Sheepshead Minnow (*Cyprinodon variegatus*), 0.2 to 0.58 g, 28 to 32 mm, 30 organisms per group. No replicates.	Definitive Test: XDE-750 (aminopyralid acid), 94.5%. Nominal Concentrations: 0, 0 (solvent control), 13, 22, 36, 60, and 100 mg a.e./L. Measured Test Concentrations: 13, 22, 36, 60 and 120 mg a.e./L. 94 hour exposures. Solvent control: Dimethylformamide, 0.5 mL/L.	No mortality or sublethal effects in any organisms at any concentration.	Machado 2002b, MRID 46235820 Acceptable (6/16/05)

Appendix 7: Toxicity to fish and amphibians.

Animal	Dose/Exposure	Response	Reference, EFED Classification [1]
AMPHIBIANS			
Northern Leopard Frog (*Rana pipiens*) larvae (7-days post-hatch), 10 organisms per replicate, 3 replicates per group including controls	XDE-750 (aminopyralid acid), 94.5%. Nominal Concentrations: 0 mg/L (water control) and 100 mg a.e./L. Measured Test Concentration: 95.2 mg a.e./L Duration of Exposure: 96 hours	No mortality or sublethal effects in any organisms.	Henry et al. 2003a, MRID 46235816 Supplemental (6/16/05), non-guideline [2]

[1] Classifications given are based on a review of the DER and/or the U.S. EPA/EFED (Environmental Fate and Effects Division) in their risk assessment of aminopyralid (U.S. EPA/OPP-EFED 2004). When the DER was reviewed in the preparation of this Forest Service risk assessment, the last review date give in the DER is specified in parenthesis after the classification. Otherwise, the EFED classification is taken solely from the EFED risk assessment (U.S. EPA/OPP-EFED 2004).

[2] The classification of *Acceptable* is limited to studies for which guidelines have been written – i.e., the study is acceptable under or fulfills the guideline requirement (see http://www.epa.gov/opptsfrs/home/guidelin.htm). All non-guideline studies that are considered scientifically valid are classified as *Supplemental* rather than *Acceptable*.

Appendix 8: Toxicity to Aquatic Invertebrates.

Animal	Dose/Exposure	Response	Reference, EFED Classification [1]
Freshwater – Acute			
Daphnia magna, <24 hours old. 3 replicates at 10 organisms/replicate	Limit Test: XDE-750 (aminopyralid acid), 94.5%. 0 and 100 mg a.e./L (nominal), 98.6 mg a.e./L measured for 48 hours (static). No solvent used.	NOEC: > 98.6 mg a.e./L. No effects observed in any organisms. In a separate probe study, no mortality (0/10) observed at nominal concentrations of 25, 50, 75, and 100 mg a.e./L.	Marino et al. 2001b, MRID 46235817 Acceptable (6/16/05)
Freshwater – Chronic			
Daphnia magna, **Probe Study**. 1 daphnid per replicate, 8 replicates per concentration.	XDE-750 (aminopyralid acid). 21-Day exposure. Static renewal. Concentrations: 0, 0.185, 0.410, 0.911, 2.02, 4.50, and 10.0 mg a.e./L.	At 4.5 mg a.e./L, 6/8 daphnids dead by end of study. Mortality in all other groups <20%. No effects on reproduction at any concentration.	Henry et al. 2003b, MRID 46235822 Supplemental, see entry below
Daphnia magna, **Definitive Study**. 1 daphnid per replicate, 8 replicates per concentration. Organisms <24 hours old (post-realse) at start of study.	XDE-750 (aminopyralid acid). 21-Day exposure. Static renewal. Nominal Test Concentrations: 3.13, 6.25, 12.5, 25, 50, and 100 mg a.e./L. Measured Test Concentrations: 0, 2.29, 6.16, 12.5, 25.5, 49.8, and 102 mg a.e./L.	No effects observed in any treated group relative to controls. NOEC for survival, growth and reproduction: 102 mg a.e./L. EFED DER: Confirms and accepts NOEC of 102 mg/L.	Henry et al. 2003b, MRID 46235822 Supplemental, *excessive water hardness, low dissolved oxygen (3 1 %), and reduced replicate size.*

Appendix 8: Toxicity to Aquatic Invertebrates.

Animal	**Dose/Exposure**	**Response**	**Reference,** EFED **Classification** [1]
Midge (*Chironomus riparius*). 8 replicates (egg masses) per treatment level. Organisms <24 hours post hatch from egg mass Sediment: 77% sand, 6% silt, and 17% clay, 1.8% OC, pH 7.5	XDE-750 (aminopyralid acid), 94.5%. Exposure Period: 28 days. Nominal Concentrations: 63, 130, 250, 500 and 1000 mg a.e./L. Mean Measured Concentrations: 58, 123, 247, 520, and 973 mg a.e./L.	NOEC: 130 mg a.e./L LOEC: 250 mg a.e./L. 1000 mg a.e./L: No emergence (i.e., 100 %mortality) *Continued below*	Putt 2002, MRID 46235823 Supplemental, non-guideline [2] (6/16/05)

Putt 2002, MRID 46235823 *(continued)*

500 mg a.e./L: significant decrease in male midge mean development rate based on 1-day inspection intervals – i.e., 0.0582 day^{-1} vs 0.0625 day^{-1} in controls for a decrease of about 7%. In addition, percent emergence was significantly decreased (75% vs 94% in controls)

250 mg a.e./L: percent emergence was significantly decreased (80% vs 94% in controls).

EFED DER: Concentrations reported as pore water. NOEC: 82 ppm, LOEC: 158 ppm

EC_{50}:
4032 (200 – 210,000) mg a.e./L,
Slope: 0.77±0.46

[Note: EFED slopes are usually based on Log10 transform of concentration]

Saltwater – Acute

Eastern Oysters (*Crassostrea virginica*), 20 organisms per replicate, 2 replicates per treatment level, mean valve height: 39±4 mm.	XDE-750 (aminopyralid acid), 94.5%. Flow-through Mean Measured Concentrations: 0, 12, 21, 31, 50, and 89 mg a.e./L. Untreated control and solvent control (dimethylformamide, 0.5 mL/L). Duration: 96 hours	NOEC: 89 mg a.e./L. No mortality at any treatment level. At 89 mg a.e./L, 12% reduction in shell growth. Not statistically significant.	Cafarella 2002, MRID 46235818 Acceptable (6/16/05)

Cafarella 2002, MRID 46235818 *(continued)*

Noted from EFED DER:
NOEC for mortality: 89 ppm a.e.
Shell Deposition: EC_{50} > 89 ppm. *Reductions in shell deposition did approach 50%, so the EC_{50} value was visually determined to be greater than the highest treatment concentration.* (p. 10).

Notes for Forest Service Risk Assessment
The above sentence from the DER appears to be a typographical error. The sentence should read that *shell deposition did* **not** *approach 50%*. The reduction at 89 mg/L is only 12% and is not statistically significant.
Shell deposition decreased only at the highest (89 mg a.e./L) concentration. There were no decreases or dose-related trends at lower concentrations. Nothing approached a 50% reduction.

Appendix 8: Toxicity to Aquatic Invertebrates.

Animal	**Dose/Exposure**	**Response**	**Reference,** EFED **Classification** [1]
Mysid Shrimp (*Americamysis bahia*), < 24 hours old, 10 organisms per replicate, 2 replicates per treatment or control group.	XDE-750 (aminopyralid acid), 94.5% Measured Test Concentrations: 14, 22, 36, 59 and 100 mg a.e./L. Untreated control and Solvent control (dimethylformamide, 0.1 mL/L). Duration: 96 hours.	NOEC: 100 mg a.e./L. No mortality in any control or treated group.	Machado 2002a, MRID 46235819 Acceptable (6/16/05)

[1] Classifications given are based on a review of the DER and/or the U.S. EPA/EFED (Environmental Fate and Effects Division) in their risk assessment of aminopyralid (U.S. EPA/OPP-EFED 2004). When the DER was reviewed in the preparation of this Forest Service risk assessment, the last review date give in the DER is specified in parenthesis after the classification. Otherwise, the EFED classification is taken solely from the EFED risk assessment (U.S. EPA/OPP-EFED 2004).

[2] The classification of *Acceptable* is limited to studies for which guidelines have been written – i.e., the study is acceptable under or fulfills the guideline requirement (see http://www.epa.gov/opptsfrs/home/guidelin.htm). All non-guideline studies that are considered scientifically valid are classified as *Supplemental* rather than *Acceptable*.

Appendix 9: Toxicity to Aquatic Plants.

Organism	Dose/Exposure	Response	Reference/ Classification [1]
Freshwater – Algae			
Diatom (*Navicula pelliculosa*)	XDE-750 (aminopyralid acid), 94.5%. 6.0, 12, 23, 48 and 100 mg a.e./L (measured) for 120 hours (static). Including solvent control, dimethylformamide, at 0.1 mL/L.	Cell Density: 120 hour EC_{50} = 22 (6.0 - 81) mg a.e./L; NOEC = 6 mg a.e./L. Biomass: 72 hour EC_{50} = 18 (5.4 - 59) mg a.e./L; NOEC = 6 mg a.e./L. Growth Rate: 72 hour EC_{50} = 21 (3.7 - 140) mg a.e./L; NOEC = 23 mg a.e./L.	Hoberg 2002a, MRID 46235827 Acceptable (6/16/25)
Blue-green Alga (*Anabaena flos-aquae*)	XDE-750 (aminopyralid acid), 94.5%. 0, 0.39, 1.0, 2.5, 6.2, 16, 38, and 100 mg a.e./L (measured) for 120 hours (static). Including solvent control, dimethylformamide, at 0.1 mL/L.	Cell Density: 120 hour EC_{50} = 15 (5.0 - 40) mg a.e./L; NOEC = 16 mg a.e./L. Biomass and growth rate not determined by investigators because of poor concentration-response relationship. At 72 hours, there was a 47% inhibition of biomass at 1.0 mg/L.	Hoberg 2002c, MRID 46235829 ***Unacceptable***, high variability in the controls made interpretation of the data uncertain. (6/16/05)

Hoberg 2002c, MRID 46235829 *(continued)*

See Section 4.1.3.4 of this risk assessment for a discussion of study quality. Note that biomass is calculated from cell counts (AUC). Thus, the problems in the study with cell counts impacts the utility of the estimates of biomass.

Appendix 9: Toxicity to Aquatic Plants.

Organism	Dose/Exposure	Response	Reference/ Classification [1]
Blue-green Alga (*Anabaena flos-aquae*)	XR-750 (aminopyralid acid), 94.5%. Mean Measured Concentrations: 0 (medium control), 3.1, 5.62, 11.6, 23.3, 47.2, and 94.7 mg a.e/L for 120 hours (static)	Reported NOEC values for most sensitive endpoints: 11.6 mg/L. Applies to72-hour cell volume and 72-hour and 96-hour biomass. Cell counts were approximated. Morphologic abnormalities only in the two highest test concentrations. See Section 4.1.3.4 for discussion.	Hancock et al. 2007 **This study is available in draft form and has not yet been submitted to or reviewed by the U.S. EPA. A DER for this study is not available.**
Hancock et all, 2007 *(continued)* Table 8 of study indicates a statistically significant inhibition (14%) of biomass at 120-hours and a concentration of 23.3 mg/L. This is consistent with replicate data (Study Appendix H). A 10% decrease in biomass is evident at 3.1 mg/L but no decrease in biomass is apparent at 5.62 and 11.6 mg/L. For the current risk assessment, the 120-hour NOEC for biomass is taken as 11.6 mg/L. These values do not impact the dose-response assessment for algae (Section 4.3.3.4).			
Green Alga (*Pseudokirchneriella subcapitata*)	XDE-750 (aminopyralid acid), 94.5%. 5.6, 12, 23, 50, and 100 mg a.e./L (measured) for 120 hours (static). Including solvent control, dimethylformamide, at 0.1 mL/L.	Cell Density: 120 hour EC_{50} = 32 (9.4 - 100) mg a.e./L; NOEC = 23 mg a.e./L. Biomass: 72 hour EC_{50} = 32 (1.6 - 130) mg a.e./L; NOEC = 23 mg a.e./L. Growth Rate: 72 hour EC_{50} = 30 (11 - 79) mg a.e./L; NOEC = 23 mg a.e./L.	Hoberg 2003b, MRID 46235830 Acceptable (6/16/05)
Freshwater – Macrophytes			
Duckweed (*Lemna gibba*)	XDE-750 (aminopyralid acid), 94.5%. 5.2, 11, 21, 44, and 88 mg a.e./L (measured) for 14-days (static). Including solvent control, dimethylformamide, at 0.1 mL/L.	Frond Density: 14-day LOEC of 88 mg a.e./L and NOEC of 44 mg a.e./L. EC_{50} >88 mg/L. Biomass: 14-day LOEC >88 mg a.e./L and NOEC of 88 mg a.e./L. EC_{50} >88 mg a.e./L. Growth Rate: 7-day LOEC >88 mg a.e./L and NOEC of 88 mg a.e./L. EC_{50} >88 mg a.e./L.	Hoberg 2003a, MRID 46235826 Acceptable (6/16/05)

Appendix 9: Toxicity to Aquatic Plants.

Organism	Dose/Exposure	Response	Reference/ Classification [1]
Hoberg 2003a, MRID 46235826 *(continued)* Notes for Forest Service risk assessment: The statistical analysis of 7-day growth rates are summarized on Study page 62. This page appears to be a copy from another document. A handwritten note on this page indicates that*: "raw data entry for growth rate is on page 82 of this log*". This appears to be initialed JRH, the initials of the study author. There is, however, no page 62 in the copy of the study that is available. The EFED DER for this study, however, indicates that the raw data were reanalyzed and EFED confirms the statistical analyses of both the growth rates and other endpoints.			
Saltwater – Algae			
Diatom (*Skeletonema costatum*)	XDE-750 (aminopyralid acid), 94.5%. 6.2, 13, 25, 50, and 100 mg a.e./L (measured) for 120 hours (static). Including solvent control, dimethylformamide, at 0.1 mL/L.	Cell Density: 120 hour EC_{50} = >120 mg a.e./L. NOEC = 100 mg a.e./L. Biomass: 72 hour EC_{50} = 77 (13 - 1000) mg a.e./L; NOEC = 13 mg a.e./L. Growth Rate: 72 hour EC_{50} >100 mg a.e./L; NOEC = 13 mg a.e./L.	Hoberg 2002b, MRID 46235828 Acceptable (6/16/05)

[1] Classifications given are based on a review of the DER and/or the U.S. EPA/EFED (Environmental Fate and Effects Division) in their risk assessment of aminopyralid (U.S. EPA/OPP-EFED 2004). When the DER was reviewed in the preparation of this Forest Service risk assessment, the last review date give in the DER is specified in parenthesis after the classification. Otherwise, the EFED classification is taken solely from the EFED risk assessment (U.S. EPA/OPP-EFED 2004).