



**Australian Pesticides &
Veterinary Medicines Authority**

**The reconsideration of approvals of the
active constituent 2,4-D, registrations of products containing
2,4-D and their associated labels.**

Preliminary Review Findings (Environment)

**Part 1:
2,4-D Esters**

Volume 2: Technical Report

APRIL 2006

**Australian Pesticides &
Veterinary Medicines Authority**

**Canberra
Australia**

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2,4-D Review – Preliminary Review Findings

This preliminary review findings (environment) Part1: 2,4-D esters, report for products containing 2,4-D is published by the Australian Pesticides & Veterinary Medicines Authority. For further information about this review or the Review Program, contact:

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FOREWORD

The Australian Pesticides & Veterinary Medicines Authority (APVMA) is an independent statutory authority with responsibility for the regulation of agricultural and veterinary chemicals in Australia. Its statutory powers are provided in the Agvet Codes scheduled to the *Agricultural and Veterinary Chemicals Code Act 1994*.

The APVMA can reconsider the approval of an active constituent, the registration of a chemical product or the approval of a label for a container for a chemical product at any time. This is outlined in Part 2, Division 4 of the Agvet Codes.

The basis for the current reconsideration is whether the APVMA is satisfied that continued use of the active constituent 2,4-D and products containing 2,4-D in accordance with the instructions for their use:

- would not be an undue hazard to the safety of people exposed to it during its handling; and
- would not be likely to have an effect that is harmful to human beings; and
- would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment.

The APVMA also considered whether product labels carry adequate instructions and warning statements.

A reconsideration may be initiated when new research or evidence has raised concerns about the use or safety of a particular chemical, a product or its label.

The reconsideration process includes a call for information from a variety of sources, a review of that information and, following public consultation, a decision about the future use of the chemical or product.

In undertaking reconsiderations (hereafter referred to as reviews), the APVMA works in close cooperation with advisory agencies including the Office of Chemical Safety, the Department of the Environment and Heritage, and state departments of agriculture as well as other expert advisers as appropriate.

The APVMA has a policy of encouraging openness and transparency in its activities and community involvement in decision-making. The publication of review reports is a part of that process.

The APVMA also makes these reports available to the regulatory agencies of other countries as part of bilateral agreements. The APVMA recommends that countries receiving these reports not utilise them for registration purposes unless they are also provided with the raw data from the relevant applicant.

2,4-D Review – Preliminary Review Findings

This document sets out the preliminary review findings and proposed recommendations relating to 2,4-D esters only. A subsequent document (Preliminary Review Findings (Environment) Part 2: 2,4-D Acids, Salts and Low Volatile Esters) will be published in due course, following refinement of the risk assessment. The preliminary review findings and proposed recommendations are based on information collected from a variety of sources. The information and technical data required by the APVMA to review the safety of both new and existing chemical products must be derived according to accepted scientific principles, as must the methods of assessment undertaken.

The review summary (Volume 1) and the technical reports (Volume 2) for all registrations and approvals relating to 2,4-D esters are available from the APVMA web site: <http://www.apvma.gov.au/chemrev/chemrev.html>.

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ACRONYMS AND ABBREVIATIONS

ae	Acid equivalent
AR	Applied Radioactivity
BCF	Bioconcentration Factor
CEC	Cation Exchange Capacity
DEH	Department of the Environment and Heritage
dw	Dry weight
Koc	Organic carbon adsorption coefficient
Kow	Octanol-Water partition coefficient
LCx/ECx	The concentration of a substance that will be lethal (L) or induce an effect (E) to x% of the test population
LDx	The dose of a substance that will be lethal to x% of the test population
Log	Logarithm to the base 10.
MATC	Maximum Acceptable Toxicant Concentration
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Level
OM	Organic Matter
Pa	Pascals
PEC	Predicted Environmental Concentration
pH	Potential hydrogen – <i>logarithm</i> (to the base 10) of the hydrogen ion concentration (H ⁺)
ppb	Parts per billion
ppm	Parts per million
Q	Quotient value
WHC	Water Holding Capacity

This Preliminary Review Findings (Volume 2 Technical Report) has been provided to the APVMA from the Department of Environment and Heritage (DEH).

This volume contains the full environmental assessment for all forms of 2,4-D (acid, salts and esters) from all environmental data and information provide for the review.

In addition to the assessment of the data package this volume contains a refined environmental risk assessment for the high volatile esters 2,4-D ethyl ester, 2,4-D butyl esters, 2,4-D isobutyl ester ONLY.

This report contains a recommendation to the APVMA for 2,4-D ethyl ester, 2,4-D butyl esters, 2,4-D isobutyl ester and products containing these 2,4-D esters ONLY.

The *Preliminary Review Findings (Environment) Part 2: 2,4-D Acids, Salts and Low Volatile Esters* report, will be published once the refined risk assessment of the low volatile esters, acid and salts is completed.

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1 Introduction

In June 2003, the APVMA initiated its reconsideration of the approvals of the active constituent 2,4-D, the registrations of products containing 2,4-D, and the approvals of associated labels. While the scope document states that approvals of the active constituent are being reconsidered because of toxicological concerns, it is also stated that products containing 2,4-D and all associated labels are being reviewed because of environmental, toxicological and occupational health and safety concerns.

The environmental aspects for consideration as outlined in the scope document are:

- potential for contamination of waterways; and
- potential hazard to non-target animals and plants

In order to assist the environmental risk assessment, large quantities of data have been provided to the APVMA. These data were primarily generated as part of the 2,4-D reregistration process required by the US EPA. Through this process, the Industry Task Force II on 2,4-D Research Data, and registrant companies, conducted soil dissipation studies during 1993 and 1994 on amine and ester formulations. Several laboratory data were also provided.

Prior to this research, in 1989 the World Health Organization published the IPCS Environmental Health Criteria (number 84) on the environmental aspects of 2,4-D (WHO, 1989). This is considered to be an acceptable internationally peer reviewed document and DEH has used this document to consider relevant 2,4-D data available prior to 1989. Further to this document, the US EPA has made available their environmental assessment of 2,4-D that includes reviews of available literature. In addition, there are several studies summarised in the US EPA report that were not made available to the APVMA. These studies are relevant in terms of confidently bridging gaps in information on various ester or amine salt forms of 2,4-D. It is unclear why these data were not provided to the APVMA. In this assessment, the US EPA review is relied on for access to this information, where it is only discussed in summary form.

Due to the large number of test studies reviewed in undertaking the assessment, this report is structured in the form of an overview report where study and literature data are summarised and the risk assessment is provided, and a series of technical reports addressing the test data. Readers should refer to the respective technical reports to obtain fuller information on test conditions and findings. A number of metabolites are identified throughout the report. For representative structures, readers should refer to Attachment 1.

2,4-D belongs to the phenoxy or phenoxyacetic acid family of herbicides. The US EPA explain the 2,4-D mode of action as follows:

2,4-D, a synthetic auxin herbicide, causes disruption of plant hormone responses. Endogenous auxins are plant growth regulator hormones. These growth-regulating chemicals cause disruption of multiple growth processes in susceptible plants by affecting proteins in the plasma membrane, interfering with RNA production, and changing the properties and integrity of the plasma membrane. The plant's vascular system becomes blocked due to excessive cell division and the resulting growth crushes the vascular transport system. The most susceptible tissues are those that are undergoing active cell division and growth.

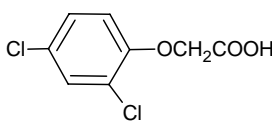
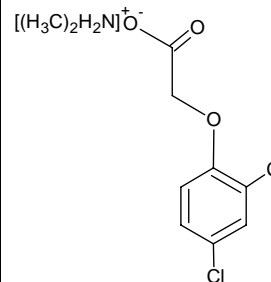
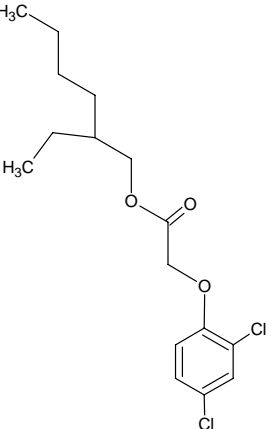
Plant injuries include growth and reproduction abnormalities, especially on new growth. Stem and petiole twisting (epinasty), leaf malformations (parallel

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venation, leaf strapping, and cupping), undifferentiated cell masses and adventitious root formation on stems, and stunted root growth is experienced by broadleaf plants. Rolled leaves (onion leafing), fused brace roots, leaning stems, and stalk brittleness are observed on grass plants. Disruption of reproductive processes may occur resulting in sterile or multiple florets and nonviable seed production. Symptoms may appear on young growth almost immediately after application, but death may not occur for several weeks.

2 Identity, Physical and Chemical Properties

The following table summarises the properties for 2,4-D and its main ester (2-EHE) and acid (DMA) forms. Readers should consult the relevant technical reports for further information on these and other ester and salt forms.

Common name:	2,4-D	2,4-D DMA	2,4-D 2-EHE
Chemical name:	acetic acid, (2,4-dichlorophenoxy)	(2,4-dichlorophenoxy) acetic acid dimethylamine salt)	(2,4-dichlorophenoxy) acetic acid, 2-ethylhexyl ester
CAS Number:	94-75-7	2008-39-1	1928-43-4
Molecular formula:	C ₈ H ₆ Cl ₂ O ₃	C ₁₀ H ₁₃ Cl ₂ NO ₃	C ₁₆ H ₂₂ Cl ₂ O ₃
Molecular weight:	221.0	266.13	333.26
Structure:			
Relative Density	bulk density: 0.66 g/mL tap density: 0.81 g/mL	pure a.s.: 1.1502 technical a.s.: 1.1527	pure a.s.: 1.1502 technical a.s.: 1.1527
Vapour pressure:	1.86×10 ⁻⁵ Pa at 25°C	Dissociates rapidly to 2,4-D acid	4.80×10 ⁻⁴ Pa at 25°C
Henry's Law:	3.54x10 ⁻⁸ atm.m ³ /mol	Not reported - Dissociates rapidly to 2,4-D acid	1.82x10 ⁻⁵ atm.m ³ /mole
Solubility:	(mg/L at 25°C) pH 1 buffered: 311 pH 5 buffered: 20031 pH 5 unbuffered: 29934 pH 7 buffered: 23180 pH 7 unbuffered: 44558 pH 9 buffered: 34196 pH 9 unbuffered: 41314	729 g/L @ 20 °C	86.7 µg/L (pH effect not investigated as, the compound is neither acidic nor basic). 32.4±3.2 µg/L in industrial water ¹ .
Log K _{ow} :	pH 1: logKow = 2.70 at 25°C pH 5: logKow = 0.18 at 25°C pH 7: logKow = -0.83 at 25°C pH 9: logKow = -1.01 at 25°C	Not reported - Dissociates rapidly to 2,4-D acid	logKow= 5.78 at 25°C (pH not stated)

- 1) The industrial water supply was pumped from the upper Saginaw Bay of Lake Huron. It was then limed and flocculated with ferric chloride at a water treatment plant. The water was sand filtered, pH adjusted with CO₂ to around pH 8, carbon filtered and UV irradiated prior to use in the study.

3 Regulatory Activity Overseas

The APVMA Scoping Paper for the 2,4-D assessment summarises the international regulatory activity as follows.

United States

The United States Environmental Protection Agency (US EPA) has considered 2,4-D for review under its Special Review Program. The consideration of 2,4-D for review related principally to concerns of carcinogenicity. 2,4-D was classified as Category D carcinogen (agents with inadequate human and animal evidence of carcinogenicity for which no data are available). Following an initial assessment, it was determined that the risk of carcinogenicity was lower than originally believed and therefore in 1998 the US EPA proposed to not initiate the Special Review.

In addition to the Special Review Program, the US EPA has considered the eligibility of all chemicals registered before November 1984, for re-registration under the Federal Insecticides, Fungicides and Rodenticides Act 1998. The Reregistration Eligibility Document (RED) for 2,4-D (salts and low-volatile esters) was released in 2005. The US EPA determined that all products containing 2,4-D as the active ingredient are eligible for reregistration, provided changes specified in the 2,4-D RED are incorporated into the labels. The US EPA has also called for further data in relation to the Agency's Endangered Species Protection Program. There will therefore be a further review of 2,4-D in the near future in relation to risks to specified endangered species.

Canada

The Pesticide Management Regulatory Authority (PMRA) re-evaluation of 2,4-D (salts and low volatile esters) has been split into two parts: review of the turf uses, which was announced in 2000, and review of the agricultural uses, which was targeted for completion in 2005. The PMRA has not yet finalised its review of 2,4-D of agricultural uses. The re-evaluation of lawn and turf uses of 2,4-D has determined that 2,4-D can be used safely on lawn and turf when label directions are followed, and is therefore proposing continued registration of 2,4-D for these uses.

UK

In 1993, the Pesticides Safety Directorate released a review report on 2,4-D and its salts and esters. It is evident from the report recommendations that significant gaps existed in the evaluated data. The report recommended additional data requirements, including toxicity/operator exposure, methods of analysis, physical properties, storage stability, monitoring of toxic impurities (dioxin), residues in food and crop metabolism, as well as studies on environmental fate and impacts on wildlife. Despite the deficiencies in the data, it was recommended that all approvals be allowed to continue while data requirements were generated and evaluated. Currently, the Pesticides Safety Directorate has national regulations that implement the directives and Maximum Residue Limits (MRLs) set by the European Commission in 2002.

Europe

The European Commission Standing Committee on Plant Health completed a re-evaluation of the active 2,4-D in October 2001. The effects of 2,4-D on mammalian toxicology, environment fate and behaviour, ecotoxicology and residues were assessed as a part of the re-evaluation process. The evaluation concluded that it may be expected that plant protection products containing 2,4-D will satisfy the safety

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requirements of the Council Directive. The commission concluded that residues arising from the proposed uses have no harmful effect on human or animal health and no unacceptable effects on the environment subject to conditions outlined in its re-evaluation. The review also identified several acceptable exposure scenarios for operators, workers and bystanders. The findings of the review are subject to the condition that active constituents are manufactured to a standard where the manufacturing impurities dioxins and furans are kept below detectable levels. The use of 2,4-D in Denmark has been restricted for environmental reasons. The registrations for the use of 2,4-D in Sweden and Norway were withdrawn as the market size did not warrant further data investment.

JMPR

The Joint WHO/FAO Meeting on Pesticides Residues (JMPR) conducted extensive reviews of 2,4-D in the 1970s, 1984, 1989 and more recently in 1996, 1997 and 1998. The 1996 review concluded that the toxicity of the salts and esters of 2,4-D was comparable to that of the acid. Accordingly, the ADI and NOEL reflected a combined exposure to the analogues. It also concluded that 2,4-D and its salts and esters do not appear to be genotoxic. The WHO concluded in 1998 that the intake of residues of 2,4-D resulting from the uses considered by JMPR is unlikely to present a public health concern in the long term. The 2000 JMPR meeting concluded that the intake of 2,4-D residues is unlikely to present a risk to consumers in the short-term.

4. Environmental Exposure

The modes of action, uses, properties and toxicities of herbicides vary between the different classes of herbicides. Phenoxy herbicides like 2,4-D act like plant hormones, disrupting normal cell growth and the plant's water and nutrient transport system.

The quantity of 2,4-D used in Australia annually is not known. However, it was reported in 2002 that over 1,000 tonnes of plant cell growth disrupting group of chemicals including phenoxys (of which 2,4-D is a member), benzoic acids and pyridines are used annually. The herbicides 2,4-D and its derivatives, along with MCPA, are the major chemicals used in this group (Radcliffe, 2002).

4.1 End use products

For this risk assessment, 2,4-D comes in multiple chemical forms and is found in numerous end use products intended for use in a wide range of use patterns. 2,4-D is an ingredient in several agricultural and home use products, primarily as a sole active ingredient but also in conjunction with other active ingredients. The compound is formulated primarily as an amine salt in an aqueous solution or as an ester in an emulsifiable concentrate. Chemical forms covered by this risk assessment are as follows:

Abbreviated name	Full name	Volatility
2,4-D	2,4-D acid	Essentially non-volatile
2,4-D DMA	2,4-D Dimethylamine salt	Essentially non-volatile
2,4-D IPA	2,4-D Isopropylamine salt	Essentially non-volatile
2,4-D TIPA	2,4-D Triisopropanolamine salt	Essentially non-volatile
2,4-D EHE	2,4-D Ethylhexyl ester ¹	Low volatile
2,4-D EE	2,4-D Ethyl ester	High volatile
2,4-D BE	2,4-D Butyl ester	High volatile
2,4-D IBE	2,4-D Isobutyl ester	High volatile

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2,4-D DEA	2,4-D Diethanolamine	Essentially non-volatile
2,4-D TEA	2,4-D Triethanolamine	Essentially non-volatile
2,4-D Sodium salt	2,4-D Sodium salt	Essentially non-volatile

- 1) 2-ethylhexyl ester is considered one of three isooctyl esters. For this assessment, labels using the active constituent 2,4-D Isooctyl Ester are all referred to as EHE.

Target pests include a wide variety of broadleaf weeds and aquatic weeds. 2,4-D may be applied with a wide range of application equipment including aircraft (both fixed wing and helicopter), backpack sprayer, band sprayer, boom sprayer and injection equipment. Methods of application of 2,4-D may include aerial and ground broadcast application, band treatment, basal spray treatment, spot treatment, stump treatment, tree injection treatment, and water related surface treatment. Timing of 2,4-D application can include pre-plant, pre- and post-emergence, before bud break, dough and established plantings.

4.2 Application rates and use patterns

DEH reviewed 122 separate product labels currently registered with the APVMA containing 2,4-D in various amine salt or ester forms, and some with mixtures of other pesticide active ingredients. Three products used 2,4-D in its sodium salt form. The break-up of this is as follows:

	Form	Number of products
Esters	Ethylhexyl ester	6
	Ethyl ester	13
	Ethyl/Butyl ester	7
	Butyl ester	1
	Isobutyl ester	6
Salts	Dimethylamine	27
	Isopropyl amine	28
	Dimethylamine/Diethanolamine	17
	Diethanolamine	6
	Diethanolamine/Triethanolamine	1
	Sodium salt	3
Acid	(Active identified only as 2,4-D)	7

Except for two granule products containing 2,4-D registered for home lawn use, there appear to be no other commercial granular formulations registered in Australia. The sodium salt formulations are in solid form, however, they are mixed with water prior to application..

Of the ethyl ester formulations, 2 are co-formulated with ioxynil. Of the diethanolamine formulation, 2 are co-formulated with dicamba and clopyralid. Of the acid formulations, 5 are co-formulated with mecoprop and dicamba.

2,4-D is registered for a very large range of uses. Within uses, several forms of application may exist including aerial, ground or spot treatments. The following table provides a distilled version of the registered uses of 2,4-D in Australia. Application rates are referred to as acid equivalents (ae) which is the equivalent application rate of 2,4-D acid regardless of the parent active constituent:

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Situation	Representative crops	Maximum registered rate (g acid equivalent/ha)
Winter cereals	Wheat, barley, cereal rye, triticale, (oats included on amine labels only)	1220
Fallow, stubble spray prior to direct drilling or sowing	Winter cereals, maize, sweetcorn, potatoes, (canola on some ester labels only)	1063
Summer cereals	Maize, sweetcorn, sorghum, millet	880
Sugar cane		3640
Peanuts		2250
Harvest aid or salvage spray	Winter cereals, maize, sorghum	1140
Pastures and non-agricultural	Amine labels	2000 ¹
Improved pasture containing legumes	Ester labels	560 ²
Pastures, rights of way and industrial		2750 ³ /4500 ⁴
Pastures, conservation tillage – direct drilling, surface sowing or fallow maintenance		1040 ⁵
Pastures, spray graze techniques	Amine labels.	1040
Lawns/turf	Includes domestic, golf fairways, commercial etc.	2500
Forage crops	Common or Carribean Stylo forage or seed crops	1040
Rice	Some ester labels only	800
Preparatory spray ⁶	Isopropylamine salt labels only	540
Bananas	Injection or spot spray	10 g ae/L water
Potatoes	Two ester labels only.	1620
Pears	One label only. Uses 2.3 g ae in 450 L water and applied as spray to individual trees with thorough coverage. No indication of a per hectare rate.	
Citrus	One label only. 0.1-0.2 ppm ae for tree spraying; 5 ppm ae for dipping.	
Timber regrowth control	One label only. Stem injection or cut stump.	15-200 g ae/L water
Spot spraying	All weeds – weed table provided with labels.	2240 ⁷
Aquatic	Control of water hyacinth	3300

- One product (2,4-D Acid) has an approved rate of 3000 g ae/ha.
- Two labels (isobutyl ester active) have registered rates of 3520-4480 g ae/ha for use in Victoria only.
- The rate is for aerial control of one weed species only. All other weeds are controlled through ground application with a maximum rate of 40 g ae/L of spray mix (hectare rate not always clear)
- Ester labels have much higher rates than amine labels. The highest rate of 4500 g is found on several EHE labels for boom spray in Victoria only.
- Ester labels with this use pattern carry an additional application rate of 3200-3360 g ae/ha for aerial or ground application to control one weed species (St. Johns Wort).
- Preparatory Spray for fallows and seed beds or prior to sowing of the following crops: Balansa clover, Barley, Canola, Chickpeas, Cotton, Faba beans, Field peas, Lentils, Linseed, Lucerne, Lupins, Narbon beans, Navy beans, Oats, Perennial ryegrass, Persian clover, Phalaris, Rice, Safflower, Sorghum, Soybeans, Subterranean clover, Sunflower, Triticale, Vetch, Wheat, White clover.
- Spot spraying may use high volume spraying where 1/10th of the rate on the labels weed table is added to 150 L water, expected to cover 1000 m², or knapsack application where 1/100th of the rate is added to 10 L and expected to cover 100 m². The maximum per hectare rate of application based on the weed tables is expected to be 1050 g ae/ha. Significantly different rates may be found for the same weed depending on the form of 2,4-D used in the formulation.

4.3 Monitoring data

No monitoring data were provided to the APVMA and no recent data have been found. In their 2002 review of pesticide use in Australia, the Australian Academy of Technological Sciences and Engineering (ATSE) report on several results of monitoring studies as follows (Radcliffe, 2002):

Irrigation areas in South Western NSW: Supply water from rivers was of high quality and generally few pesticides were detected. Just a few channels contained low levels of atrazine, endosulfan sulfate, molinate and 2,4-D. In their report on pesticide monitoring in the irrigation areas of south western NSW over the 1990-1995 period, Bowmer *et al* (1998) note only one detection of 2,4-D in the Murray River at a concentration of 0.5 ppb (which was the detection limit). Over 1994-95, 3 detections of 2,4-D in Murrumbidgee Irrigation Area (MIA) surface waters were found, ranging from 2-5.7 ppb (this is assumed to be in drainage channels). Over the same period, 2 detections of 2.1 ppb were found in surface waters of the Coleambally Irrigation Area (CIA - again assumed to be drainage channels).

Queensland waterways: 2,4-D was reported as being detected in between 9 and 27% of samples from the Johnstone and Daintree rivers. The concentration in the water was not reported.

Also in Queensland, 2,4-D is reported as being found in trace amounts in 3 of 52 groundwater samples from the Bundaberg region. Again, the concentration was not reported

South Australia: Several pesticides including 2,4-D were reported in surface waters around the Mount Lofty Ranges region at levels of 0.04-0.6 ppb.

Western Australia: Following claims of damage to tomato crops, 2,4-D was monitored in air around Geraldton over the 1979-1982 period. While the monitoring was not able to distinguish sources of separate “pulse” and “press” events, it did establish that damage may have occurred from short-distance drift of droplets of 2,4-D amine.

Sediment: Radcliffe (2002) reports on monitoring results from several sampling studies in sediments where various organochlorine pesticides were detected. 2,4-D is not reported as being detected, although presumably it was looked for.

5. Environmental Fate

In support of the assessment for the review of 2,4-D applicants submitted a full range of laboratory environmental fate data for 2,4-D acid and some bridging laboratory data for the 2-ethylhexyl ester and DMA forms of 2,4-D. In addition a full suite of field dissipation studies were provided for 2,4-D 2-EHE and 2,4-D DMA. No field data were provided for any other ester or amine salt form of 2,4-D. It is known from the US EPA report that several laboratory and field environmental fate data are available for other ester and amine salt forms of 2,4-D. These are discussed below, where available, based on the US EPA report.

There is a significant weight of evidence from literature and registrant sponsored data supporting the conclusion that 2,4-D amine salts and 2,4-D esters are not persistent under most environmental conditions including those associated with most sustainable agricultural conditions. 2,4-D amine salt dissociation is expected to be instantaneous

(< 3 minutes) under most environmental conditions. While the data reviewed tend to show de-esterification of 2,4-D esters to 2,4-D acid under all conditions is not instantaneous they do tend to demonstrate that 2,4-D esters in normal agriculture soil and natural water conditions are short-lived compounds (<1 week). Under these conditions, the environmental exposure from 2,4-D esters and 2,4-D amines is expected to be minimal in both terrestrial and aquatic environments.

There are exceptions to these statements. The US EPA notes persistence of 2,4-D Butoxyethyl ester (BEE – not registered in Australia) in sediments from aquatic field studies that were not provided to the APVMA. Additionally, 2,4-D 2-EHE was shown to persist on foliage and in leaf litter from registrant submitted forest field dissipation studies. No field dissipation data (terrestrial, forest, or aquatic) have been submitted for the amine salts, 2,4-D IPA, 2,4-D TIPA, and 2,4-D DEA, or for the esters 2,4-D BEE and 2,4-D Isopropyl Ester (IPE) to confirm their persistence under field conditions. Additionally, no data on the other esters used in Australia, namely the ethyl, butyl and isobutyl esters, have been provided by applicants.

5.1 *Stability in Water*

Direct evidence of the stability of 2,4-D amine salts in soil and aquatic environments is difficult due to the lack of analytical methods. The US EPA point out in their report that based on maximum application rates for 2,4-D amine salts (@ 4.5 kg ai/ha), 2,4-D amine salts are expected to fully dissociate in soil environments because their theoretical concentrations in soil solution does not exceed water solubilities. Additionally, dissociation studies indicate the time for complete dissociation is rapid (< 3 minutes).

Reim (1989) considered the dissociation of 2,4-D and 2,4-D DMA in water. Analytical grade 2,4-D and 2,4-DMA, in HPLC grade water had dissociation times of ≥ 120 minutes and <1 minute, respectively. Complete dissociation was determined through a comparison of theoretical and estimated electrical conductance measurements at infinite dilution. For 2,4-D DMA, plots of equivalent conductance vs. concentration^{1/2} (Onsager equation) are linear indicating it is a strong electrolyte while that for 2,4-D is non-linear indicating it is a weak electrolyte. Conductivity data are consistent with the premise that 2,4-D DMA completely dissociates in aqueous solution to form dimethylammonium ion and the conjugate base of 2,4-D.

Regarding the esters of 2,4-D, the US EPA (2005) reports that de-esterification of 2,4-D esters is more difficult to generalize because it is dependent on heterogenous hydrolysis (microbial-mediated and surface-catalyzed hydrolysis) and homogenous hydrolysis (alkaline catalyzed). The de-esterification of 2,4-D ester leads to formation of 2,4-D acid and an associated alcohol moiety. Unlike the physical dissociation mechanism of 2,4-D amine salts, the de-esterification of 2,4-D esters is dependent on abiotic and microbial-mediated processes. Any environmental variable influencing microbial populations or microbial activity could theoretically influence the persistence of the 2,4-D ester. Soil properties including clay mineralogy, organic carbon content, temperature, and moisture content are known to influence hydrolysis rates.

Test data were received addressing the hydrolytic stability of 2,4-D and 2,4-D 2-EHE. The former was stable to hydrolysis at environmentally relevant pH and temperature conditions. However, 2,4-D 2-EHE was hydrolytically unstable in sterile, basic conditions with a half-life of 52.2 hours at pH 9 (compared to 78.3 days and 99.7 days

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at pH 7 and 5 respectively). Additionally, when this compound underwent hydrolysis testing in natural water (pH 7.8), hydrolysis was rapid with a half-life of 6.2 hours demonstrating the importance of microbial activity in aiding hydrolysis. Hydrolytic rates in soil slurries were even faster with half lives of 1.22-1.45 hours in two soils with pH values of 6.7-6.9.

The US EPA assessed hydrolysis data for 2,4-D BEE and 2,4-D IPE (tests not supplied to the APVMA). They report that in sterile systems, radiolabeled 2,4-D BEE had first-order half-life of 196 days in pH 5 buffer solution, 47.5 hours in pH 7 buffer solution, and 55 minutes in pH 9 buffer solution. The major degradation product was 2,4-D acid. Additionally, the dissipation of 2,4-D IPE appears to be dependent on de-esterification through alkaline-catalyzed abiotic hydrolysis and microbial-mediated or soil surface catalyzed de-esterification processes. The abiotic hydrolysis half-life of 2,4-D IPE was >30 days at pH 5, 89.2 days at pH 7, and 22.4 hours at pH 9. However, in microbially active systems, Isopropyl 2,4-dichlorophenoxyacetate was rapidly de-esterified ($t_{1/2}$ <13 hours) in an aerobic sandy loam soil and aerobic sediment-water test system. The de-esterification of 2,4-D IPE will form 2,4-D and isopropanol.

Modelled data suggest hydrolytic rates for the ethyl, butyl and isobutyl esters would be similar under sterile conditions with predicted half-lives of 1.9, 2.25 and 3.5 days at pH 8, and 19.1, 22.5 and 35 days at pH 7. It could be expected that de-esterification would be more rapid in microbially active systems based on results discussed above for other longer chain esters.

5.2 Photodegradation

Air

The issue of volatility of esters is discussed below. Photodegradation rates in air for the various esters and 2,4-D, based on reaction with hydroxyl radicals, have been calculated using AOPWIN Version 1.91.

The diurnally and seasonally averaged concentration of tropospheric hydroxyl radicals used by the AOP program is $1.5 \times 10^6 \text{ cm}^{-3}$. Outputs from the modelling were as follows based on a 12 h:12 h light:dark day:

Ester	Smiles String	Rate Constant ¹	Half-life (hours)
2,4-D 2-EHE	<chem>Clc1cc(Cl)ccc1OCC(=O)OCC(CC)CCCC</chem>	14.5058	17.69
2,4-D EE	<chem>Clc1cc(Cl)ccc1OCC(=O)OCC</chem>	5.2610	48.72
2,4-D BE	<chem>CCCCOC(=O)COc1ccc(Cl)cc1Cl</chem>	8.1666	31.44
2,4-D IBE	<chem>Clc(cc(Cl)c1OCC(=O)OCC(c)C)cc1</chem>	8.1582	31.44
2,4-D BEE ²	<chem>CCCCOCCOC(=O)COc1ccc(Cl)cc1Cl</chem>	27.79	10.34
2,4-D IPE ²	<chem>CC(C)OC(=O)COc1ccc(Cl)cc1Cl</chem>	7.0379	36.48
2,4-D	<chem>OC(=O)COc1c(Cl)cc(Cl)cc1</chem>	6.6262	38.74

1) Rate constant, K_{OH} ($\times 10^{-12} \text{ cm}^3/\text{molecule}/\text{second}$)

2) Not registered in Australia

The US EPA report that the non-volatile nature of 2,4-D BEE prevented an estimation of the photodegradation rate in air (where less than 1.4% of the applied 2,4-D BEE volatilized). No photodegradates were identified.

Water

Test data were received addressing the photodegradation of 2,4-D and 2,4-D 2-EHE in water. In a sterile aqueous solution with pH of 7, 2,4-D photodegraded with a half-life of 13 days (12:12 hour light:dark regime). This compared to negligible photodegradation where direct photolysis in water was considered based on quantum efficiency. In this test, 2,4-D was stable in water for the 7 day test period.

Photolysis was shown to not be environmentally relevant for the removal of 2,4-D 2-EHE from water bodies. When exposed to natural sunlight in an aqueous pH 5 buffer solution for up to 31 days, less than 15% degradation was observed. The extrapolated photolysis half-life was determined to 128.2 days and the major identified degradation products were 2,4-D and 2,4-DCP.

The US EPA report a study where radiolabeled 2,4-D BEE had a half-life of 74 days in both irradiated and dark control samples. The major degradate identified was 2,4-D acid at less than 17% of applied 2,4-D BEE. The data indicate that 2,4-D BEE does not photodegrade in slightly acid aqueous environments. APVMA did not receive this study.

Aqueous photodegradation of 2,4-D amine salts were not investigated due to the rapid dissociation of these compounds to 2,4-D acid.

Soil

Data indicate 2,4-D is stable to photodegradation on soils with a theoretically calculated half-life of 69 calendar days using linear regression. However, this calculated half-life was not statistically reliable, as degradation did not follow first-order kinetics. The major photodegradate was identified as CO₂ (5% of applied). Many unidentified degradates (<10% of applied) also were separated by TLC.

5.3 Volatility

Volatility of 2,4-D 2-EHE was tested in the laboratory following application to soil pans. The soil was classified as a sandy loam with a pH of 6.5 and 4.17% moisture content at 1/3 bar. The highest losses occurred during the first day. Average volatilisation rates for the 100 and 300 mL/minute air flow rates were 8.06×10^{-4} and 3.45×10^{-3} µg/cm³/hour respectively with average air concentrations at these rates of 34.84 and 46.06 µg/m³ respectively. The conclusion was that volatilisation of 2,4-D 2-EHE from the light textured soils was low.

2,4-D esters with short-chain alcohols are highly volatile. This influences the effectiveness of their application to target crops, their effects on neighbouring crops, and the degree of contamination of the atmosphere. 2,4-D alkali salts or amine salts are much less volatile than esters, and these products are to be preferred when the use of 2,4-D esters might lead to evaporative 2,4-D losses and to crop damage or damage to the surrounding environment (WHO, 1989). As shown in Section 4.2 above, the majority of ester formulations registered for use in Australia are still in the short chain forms of ethyl, butyl or isobutyl esters of 2,4-D.

It is further stated in WHO, 1984 (EHC 29) that the drifting of vapours of the more volatile short-chain 2,4-D esters may result in air pollution and crop damage, and these products are being replaced by less volatile long-chain esters or by amine salts. However, the report does not state the countries where such replacement is occurring.

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Details of technical compositions, impurities, and analytical methods can be found in Environmental Health Criteria 84: 2,4-Dichlorophenoxyacetic acid (WHO, 1989).

Table 1. Vapour pressure and solubility of 2,4-D salts and esters

Compound	Vapour pressure ^a	Solubility
2,4-D free acid	0.4 mmHg (160°C)	0.09% in water (25 °C), 85% in acetone (25 °C)
dimethylamine salt		300% in water (20 °C), soluble in acetone
isopropyl ester	1.4 x 10 ⁻³ mmHg ^b 4.6 x 10 ⁻⁵ mmHg ^b	insoluble in water, soluble in most organic solvents
butoxyethanol ester	4.5 x 10 ⁻⁶ mmHg ^b	insoluble in water, soluble in most organic solvents
ethylhexyl ester	2.0 x 10 ⁻⁶ mmHg ^b	insoluble in water, soluble in organic solvents
isooctyl ester	2.0 x 10 ⁻⁶ mmHg ^b	insoluble in water, soluble in organic solvents
propyleneglycol butyl ether ester	3.0 x 10 ⁻⁶ mmHg ^b	insoluble in water, soluble in organic solvents
methyl ester	2.3 x 10 ⁻³ mmHg ^b	
ethyl ester	1.1 x 10 ⁻³ mmHg ^b	
butyl ester	3.97 x 10 ⁻⁴ mmHg ^b	

^a 1 mmHg = 0.133 kPa.

^b Vapour pressures of esters were determined at high temperatures by gas-liquid chromatography, and these values are the result of extrapolation to 25 °C. Values vary considerably between authors as a result of this extrapolation; original values at high temperatures agree. Results are presented here as an indication of relative vapour pressure at working temperature.

According to the scale of Mensink *et al* 1995, substances with vapour pressures of 7.5 X 10⁻⁶ – 7.5 X 10⁻³ mm Hg are slightly to moderately volatile, that indicates several of the above esters may volatilise following application. This appears particularly true for the ethyl and butyl esters used in Australia.

This statement is further supported by German research. Guth *et al* 2004 state that, based on direct measurements, no noticeable volatility can be expected from compounds with a vapour pressure below 10⁻³ Pa from soil and 10⁻⁴ Pa from crops, and this is fully confirmed by indirect measurements. Converting the above vapour pressures for the EHE, ethyl and butyl esters to Pa gives results of 2.67X10⁻⁴, 1.47X10⁻¹ and 5.29X10⁻² Pa respectively, indicating the latter two may be expected to volatilise from both soils and crops while EHE is marginal but may volatilise from crop surfaces (supported by data described in the Technical Report – Appendix II).

5.4 Biodegradation

Based on the premise that the 2,4-D esters hydrolyse to 2,4-D and the amine salts of 2,4-D rapidly dissociate to form 2,4-D and the relevant conjugate base, almost all laboratory biodegradation data have been provided for the 2,4-D acid only. It is noted some data are available and have been assessed by the US EPA that were not provided to the APVMA. Where relevant, these will be noted in the following discussion.

5.4.1 Aerobic Soil Metabolism

Two studies using 2,4-D in aerobic soils resulted in conflicting results. Both followed the same guidelines and microbial viability of the soil was established. In the first study the soil was a silty clay loam with pH 6.9, CEC 10.08 meq/100 g, 3.87% OM

and a water holding capacity of 27.16% at 1/3 bar. Degradation was rapid with a half-life of 1.7 days ($r^2 = 0.98$). This compared with the second study where a silty clay loam was also used (pH 7.4, CEC 9.8 meq/100 g, 1.4% OM but no WHC provided). In this study the half-life was much longer at 66.2 calendar days ($r^2 = 0.83$). In both studies, the major degradate was CO_2 with significant amounts of bound residues being found at the end of the respective study periods. 2,4-DCP and 2,4-DCA were found at residues of 3.5% AR or less throughout the first study, but were not identified in the second.

The US EPA report a study where radiolabelled 2,4-D IPE degraded with a first-order degradation half-life of 0.9 hours in a sandy loam soil. The major degradate was identified as 2,4-D (60 to 96% of applied ^{14}C -2,4-D IPE). The APVMA did not receive this study, and the US EPA report does not provide any information on corresponding degradation of 2,4-D in this study.

Aged residue column leaching studies discussed below are supportive of fast degradation in aerobic soils.

5.4.2 Anaerobic Soil Metabolism

No data were provided for any form of 2,4-D.

5.4.3 Aerobic Aquatic Metabolism

Three studies were assessed for the degradation of 2,4-D acid in water sediment systems. In two of these studies, degradation appeared to be biphasic in nature. Total system 2,4-D dissipation in the first phase was relatively slow with calculated half-lives in both of 71.5 days. The first phase continued for 20-25 days. The second phase half-life was considerably faster with calculated half-lives in both systems of 2.9-3.3 days. The fast degradation in the second phase was accompanied by significant production of CO_2 . Minor amounts of 2,4-DCP were found in both systems while 4-CPA and 4-CP were found in minor amounts in one system and chlorohydroquinone was found in minor amounts in the other system.

The third study was conducted for a 30 day period. In this case, 2,4-D did not significantly degrade with only around 5% degradation in the 30 days and only a small amount of CO_2 produced. This test was not conducted long enough to determine any sort of breakdown pattern. The biphasic patterns of degradation observed in the previous two studies indicate that once acclimatised, microbial degradation should occur quickly with a corresponding increase in CO_2 production, but no conclusions to this effect can be drawn from this test.

The US EPA report a study where radiolabelled 2,4-D IPE degraded with a first-order degradation half-life 13 hours in a sediment-water environment. The major degradate was identified as 2,4-D (60 to 96% of applied ^{14}C -2,4-D IPE). No other details of this study are provided and it was not made available to the APVMA for review.

5.4.4 Anaerobic Aquatic Metabolism

Two studies were provided addressing the anaerobic metabolism of 2,4-D acid in water/sediment systems. In the first test, 2,4-D metabolised significantly during incubation with an observed half-life of 41.1 days (based on first order kinetics). The major metabolite 4-CP, increased to 20.6% AR by day 70 of the study before declining to 0.5% AR by day 224. By the end of the study, CO_2 accounted for around 21% AR but a significant amount may not have been trapped. The degradation

appeared to follow a bi-phasic pattern of decline, and re-plotting the residue data demonstrated good correlation and showed a first phase half-life in water, sediment and the whole system of around 53, 64 and 57 days respectively, and a second phase half-life of around 17, 27 and 22 days respectively.

The second study showed much less degradation. By the end of the 365 day experiment, parent 2,4-D still accounted for around 39% AR of which 25.9% was found in the water and 13.2% in the sediment. CO₂ was the major metabolite accounting for around 22.1% AR at study end. 2,4-DCP was found at a maximum level of 21.6% at 30 days and then gradually declined to around 4% at study end with the major portion of this metabolite being found in sediment at any given sampling point. Linear regression of the log-transformed data describing 2,4-D degradation with time resulted in an approximate half-life of 312 days for the whole system. Half-lives for the separate water and sediment compartments have been calculated to be 273 and 334 days respectively.

No data for anaerobic aquatic degradation of ester forms of 2,4-D were provided for review. However, the US EPA report a study where radiolabelled 2,4-D BEE, degraded with a first-order half-life of 14.4 hours in a strongly acidic, rice paddy water and sediment test system. The major degradation product was 2,4-D, which was stable during a 12 month incubation period. Unidentified residues were also detected (<4% of applied) in sediment and water samples. No other details are provided.

5.5 *Mobility*

5.5.1 **Adsorption/Desorption**

Three batch equilibrium studies were provided measuring the adsorption/desorption characteristics of 2,4-D in 9 different soil types.

2,4-D was applied at nominal concentrations of 1.0, 2.5, 5.0 and 10.0 ppm to sand, sandy loam, loam and silty clay loam soil:solution slurries that were equilibrated for 24 hours at 25±1°C. For adsorption, Freundlich K_{ads} values were 0.36, 0.17, 0.28 and 0.52 with corresponding K_{oc} values were 76, 70, 117 and 59 mL/g respectively. For desorption, Freundlich K_{des} values determined following a 24-hour equilibration period were 1.16, 0.81, 1.48 and 1.9 with corresponding K_{oc} values were 247, 338, 617 and 216 mL/g respectively. These results indicate 2,4-D can be classed as having high mobility in all four soils. The main factors impacting mobility from this study were cation exchange capacity and clay content.

In a second study, 2,4-D was applied at nominal concentrations of 0.2, 1.0, 2.5 and 5.0 ppm to silt loam, sandy loam, loam and clay soil:solution slurries that were equilibrated for 24 hours. For adsorption, Freundlich K_{ads} values were 0.94, 0.08, 1.1 and 1.0 with corresponding K_{oc} values were 42, 34, 36 and 79 mL/g respectively. Desorption was not measured. These results indicate 2,4-D can be classed as having high mobility in the silty clay loam and very high mobility in the other three soils.

The third test measured adsorption and desorption of uniformly phenyl ring labelled 2,4-D in a rice paddy sediment classified as clay. Five concentrations were used of 0.1, 0.51, 1.0, 2.47 and 5.02 ppm and equilibrated for 24 h at around 22°C. The Freundlich K_{ads} value was 1.22 indicating a significant amount of chemical being adsorbed to the sediment. However, the corresponding K_{oc} of 58.1 indicated the

chemical was not tightly bound. This was supported by the K_{des} value of 1.64 (corresponding K_{oc} of 78.1). The chemical was classed as having high mobility.

An attempt was made to measure the adsorption/desorption of 2,4-D 2-EHE and 2,4-D Butyl Ester in four soils. However, due to hydrolysis, the esters were not stable enough to allow a batch equilibrium study to be performed for determination of K_d values. Hydrolysis to 2,4-D acid suggests that the behaviour of the esters in the environment can be modelled by using the parameters found for 2,4-D acid.

5.5.2 Leaching potential

5.5.2.1 Column Leaching Studies

No column leaching data were provided to the APVMA for unaged 2,4-D residues. One study was assessed in the European assessment for 2,4-D. EC (2001) reports that a loamy sand soil with higher organic matter (1.85%), was much more effective in reducing the mobility of 2,4-D compared to a sandy soil with lower organic matter (0.12%). After 48 mm of percolating water about 70% of the initial amount of 2,4-D was recovered in the first 5 cm increment of the loamy sand soil and 1.4 % between 10 and 15 cm, whereas in the sandy soil most of the 2,4-D (73%) was found between 10-15 cm. It is further stated in the EC report that the persistence of 2,4-D was considerably more important in loamy sand soil. Application of 200 mm of percolating water resulted in the presence of less than 15% of the herbicide below the 25 cm soil depth.

5.5.2.2 Aged Column Leaching Studies

Two studies were provided assessing mobility of aged 2,4-D residues through a loamy sand soil with mild humus content. The same soil and similar test system were used with one study performed to US EPA guidelines (test 1) and the other to German BBA guidelines (test 2). The ageing period was 28 and 30 days in tests 1 and 2 respectively. Following the respective ageing periods, in test 1, 89.05% AR was recovered of which only 5.39% was extractable. 69.15% was as CO_2 and the rest was bound. A similar situation was observed in test 2 where 87.84% AR was recovered 4.08% was extractable, 71.57% was as CO_2 and the remainder was bound. Less than 1% AR was attributable to parent product. Following leaching of the columns, 0.27% AR and 0.34% AR were found in leachates in tests 1 and 2 respectively. For 2,4-D itself, <0.1 ppb was found in leachates in both tests indicating that 2,4-D is unlikely to leach to groundwater when residues are aged. In both studies, the 2,4-DCP metabolite was not detectable on chromatograms from leachate residues.

5.5.2.3 Field Lysimeter Studies

One outdoor lysimeter study was undertaken on 2,4-D in its DMA form. The study was performed on a sandy soil with an application rate of 750 and 1500 g ai/ha. The metabolites 2-CP, 4-CP and 2,4-DCP were tested for but these along with 2,4-D were not detected in any of the analysed leachates. In the soil, total recovered radioactivity amounted to around 21% and 17% in the two lysimeters, with only around 0.1% found in the soil profile below 57 cm. The results indicated that 2,4-D is not mobile in the sandy soil tested.

5.6 Behaviour in the Field

5.6.1 Soil

Application of 2,4-D in its ethylhexyl ester form

Based on 16 field dissipation studies submitted for 2,4-D applied in the ester form, 2-EHE (see Appendix II – Technical report for Esters of 2,4-D), a total of 34 half-lives for the dissipation of the parent (2,4-D 2-EHE) were available. These covered many situations including forestry, pasture, wheat, turf and several different bare ground studies. Generally, 2 applications were made although with two of the bare ground studies where application was undertaken according to a corn use pattern, 4 applications were made. Two of the studies involved application of the ester in a granular form while the others applied it in an EC formulation.

The soil half-life results for 2,4-D 2-EHE are summarised in Table 2 below.

Table 2: Soil Half lives of 2,4-D 2-EHE from the various field studies discussed in Appendix II.

	App 1 (kg ae/ha)	Half life (Days)	App 2 (kg ae/ha)	Half life (Days)	App 3 (kg ae/ha)	Half life (Days)	App 4 (kg ae/ha)	Half life (Days)
Forestry	4.5	1						
Pasture	2.25	1.8	2.25	4.7				
Pasture	2.5	1.4	2.5	0.5				
Wheat	1.4	4	1.4	1.8				
Wheat	1.4	3.4	1.4	2				
Turf	2.24	0.34	2.24	-				
Turf	2.46	2.1	2.46	-				
Granules ¹	2.46	14.3	2.46	10.9				
Granules ²	2.46	7.4	2.46	10.3				
Bare Ground ³	2.46	2.7	2.46	2.6				
Bare Ground	1.4	1.7	1.4	1.4				
Bare Ground	2.24	0.9	2.24	0.5				
Bare Ground	1.54	4.4	1.54	3.6				
Bare Ground	1.4	12.9	1.4	5.2				
Bare Ground	2.46	2.9	1.23	2.6	0.62	2.9	1.85	4.1
Bare Ground	2.46	10.9	1.23	4.6	0.62	1.2	1.85	2.9

1) Applied to turf

2) Applied to bare soil

3) Bare ground studies were performed with application rates and timing between applications undertaken according to several different use patterns.

Results were largely consistent in all studies. Of the 34 half-lives generated, 29 were less than one week. Of the 6 half-lives that were in excess of 1 week, four of them were attributable to the granular applications. For the whole data set, the range of half-lives was 0.34 days to 14.3 days. The average half-life was 3.87 days, standard deviation of 3.54 days and a 90th percentile of 9.85 days.

If the longer half-lives resulting from granular application are removed from the data set so only spray applications using an EC formulation are considered, the data set is reduced to 30 half-lives of which 28 are 5.2 days or less. The mean half-life is reduced to 3.09 days and a smaller standard deviation of 2.75 days is calculated. The 90th percentile half-life is significantly reduced to 4.75 days.

Half-lives for 2,4-D acid were also determined in all field studies. On four occasions (two with granular applications), the half-life could not be calculated due to

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increasing residues over the period between applications. This period of increasing residue formation could last up to 2-3 weeks (granular formulation) or 0.5-2 weeks (EC formulation). Half-life results are summarised in Table 3 below.

Table 3: Soil half lives of 2,4-D following application in its 2-EHE form for the various field studies discussed in Appendix II.

	App 1 (kg ae/ha)	Half life (Days)	App 2 (kg ae/ha)	Half life (Days)	App 3 (kg ae/ha)	Half life (Days)	App 4 (kg ae/ha)	Half life (Days)
Forestry	4.5	4		1				
Pasture	2.25	11	2.25	27.3				
Pasture	2.5	4.2	2.5	13.1				
Wheat	1.4	9.3	1.4	6.2				
Wheat	1.4	6.5	1.4	2.8				
Turf	2.24	4.4	2.24	2.2				
Turf	2.46	7.9	2.46	9.7				
Granules ¹	2.46	-	2.46	13				
Granules ²	2.46	-	2.46	10.3				
Bare Ground	2.46	4.4	2.46	15				
Bare Ground	1.4	6	1.4	2				
Bare Ground	2.24	2.7	2.24	1.8				
Bare Ground	1.54	6.1	1.54	5.6				
Bare Ground	1.4	6	1.4	3.2				
Bare Ground	2.46	42.3	1.23	3.7	0.62	-	1.85	4.4
Bare Ground	2.46	-	1.23	5.5	0.62	1.2	1.85	7

1) Applied to turf

2) Applied to bare soil

3) Bare ground studies were performed with application rates and timing between applications undertaken according to several different use patterns.

In general, half-lives for 2,4-D were longer than the 2-EHE parent compound. Of the 32 half-lives obtained through these field trials, the range was from 1.2-42.3 days. Of these, only two half-lives were longer than 15 days. The mean half-life was 7.89 days with a large spread (standard deviation of 8.11 days). The 90th percentile was calculated to be 13.1 days. The longest half-life of 42.3 days was found following application to bare soil. A very poor correlation coefficient of 0.08 was determined for this half-life and was due to a period of residue increase in the soil following application that lasted until 3-7 days after application. As expected, data tend to show that after residues peak, the time for dissipation is significantly less than if the period of residue increase is shown in the half-life calculation. If the 42.3 day half-life is removed from the data set, then the spread of the half-life distribution is reduced (standard deviation of 5.2 days), but the 90th percentile half-life remains at 13 days.

The analytes 2,4-DCP and 2,4-DCA were generally only found in the top 15 cm soil horizons. In some studies, these analytes were only sporadically detected, while in two studies, 2,4-DCA was not detected at all. Generally speaking, 2,4-D 2-EHE and 2,4-D were retained in the top 15 cm of soil. However, movement did vary quite considerably between tests. For example, in the bare ground study in Colorado (sandy clay loam, 1.2% OM), 2,4-D was only found in one replicate at one sampling point in the 15-30 cm layer throughout the study while 2,4-D 2-EHE was not found below 15 cm at any time. This contrasted to the bare ground study in California (loamy sand, %OM 1.2) where quite significant movement of all analytes except 2,4-DCP was found over the course of the study, and at some sampling events, 2,4-D was found throughout the soil profile down to 120 cm.

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2,4-D acid will be considered further where the half-lives resulting from application of the DMA form are considered.

Application of 2,4-D in dimethylamine salt form

In the large number of field studies for 2,4-D DMA, analytical methods were not capable of separating and identifying 2,4-D DMA from 2,4-D acid, the most conservative half-lives of 2,4-D DMA would be equivalent to the 2,4-D acid half-lives in field studies. Table 4 contains a summary of half life values.

Table 4: Half-lives of 2,4-D based on application in its DMA form for the various field studies discussed in Appendix III.

	App 1 (kg ae/ha)	Half life (Days)	App 2 (kg ae/ha)	Half life (Days)	App 3 (kg ae/ha)	Half life (Days)	App 4 (kg ae/ha)	Half life (Days)
Forestry ¹	4.5	38.7						
Pasture	2.25	5.7	2.25	30.5				
Pasture	2.5	7.9	2.5	10.2				
Wheat	1.4	5.5	1.4	2.7				
Wheat	1.4	9.4	1.4	9.6				
Turf	2.24	3.3	2.24	2.3				
Turf	2.46	21.9	2.46	7.5				
Granules ²	2.46	10.3	2.46	5.1				
Granules ³	2.46	16.7	2.46	14.7				
Bare Ground	2.46	6.8	2.46	7.6				
Bare Ground	1.4	3.4	1.4	5.8				
Bare Ground	2.24	5.6	2.24	5.1				
Bare Ground	1.54	6.5	1.54	4.5				
Bare Ground	1.4	2.9	1.4	3				
Bare Ground	2.46	8.6	1.23	3.9	0.62	1.1	1.85	4.1
Bare Ground	2.46	26.1	1.23	5.9	0.62	1.3	1.85	16

- 1) In protected soil, the half-life was calculated to be 80.8 days.
- 2) Applied to turf
- 3) Applied to bare soil
- 4) Bare ground studies were performed according to several different use patterns to dictate application rates and timing between applications.

Based on 16 field dissipation studies submitted for 2,4-D applied in the amine salt form, DMA (see Appendix III – Technical report for Amine salts of 2,4-D), a total of 36 half-lives were calculated. The data set for statistical calculation was reduced to 35 as one of the half-lives related to protected soil in the forestry field trial that was significantly longer (half-life of 80.8 days) than any other 2,4-D half-life found in the field.

In some instances, DEH has re-calculated half-lives from those available in test reports. Reasons are documented in the study results provided in the technical report (Appendix III). The spread of half-lives in the field from various cropping situations including bare ground application ranged from 1.1-38.7 days. Of these, 28 half-lives were 10.3 days or less. Generally speaking, the persistence of 2,4-D following application of granules was greater with three of the four half-lives obtained from granule application being 10.3, 14.7 and 16.7 days. Considering all data for the application of 2,4-D in the DMA form, the mean dissipation half-life was 9.1 days with a large spread (standard deviation = 8.5 days) and a 90th percentile dissipation half-life of 19.8 days.

Mobility of all analytes tested was generally retained in the top 15 cm of soil although on occasions, significant movement through the soil column was observed with 2,4-D. The analytes 2,4-DCP and 2,4-DCA were often only sporadically detected and out of all 7 studies, only 2,4-DCA was found once below 15 cm where it was detected at a mean residue level of 0.002 ppm in the 15-30 cm layer in the Ohio study. In one study (Colorado), 2,4-D did not move beyond the top 15 cm. In three studies (North Carolina – turf use pattern, North Carolina – wheat use pattern and Ohio – corn use pattern), some measurements of 2,4-D residues were made in the 15-30 cm layer but none was found below this. There was one study where leaching was apparent. In the Nebraska study (corn use pattern, silt loam, 3.5% OM), 2,4-D was found throughout the soil profile down to 105 cm (at a mean residue level of 0.018 ppm in the 90-105 cm layer 7 days after the first application). A later sampling event (3 days after the second application) found 2,4-D distributed down to 60 cm.

5.6.2 Other environmental media

In addition to dissipation from soil, some of the field studies considered dissipation from other environmental media such as leaves, grass, thatch and water.

In the forestry field dissipation study, residue decline on foliage and leaf litter was considered. Where application was in the 2-EHE form of 2,4-D, half-lives of both the parent and 2,4-D were significantly longer than those for soil. 2,4-D 2-EHE had a field half-life on foliage and leaf litter of 7.2 days and 51.0 days respectively while corresponding 2,4-D half-lives were 23.5 and 52.2 days respectively. The long half-lives of 2,4-D were supported by the forest field dissipation studies where 2,4-D applied in its DMAS form declined on foliage and leaf litter with half-lives of 37.8 and 65.7 days respectively.

Turf trials considered dissipation on grass and thatch. Ignoring results from application of granule formulations, out of 4 studies (2 with 2,4-D 2-EHE and 2 with 2,4-D DMA), all used two applications and considered dissipation in grass while two also considered dissipation in thatch. The mean half-lives in thatch and grass were calculated to be 10.1 days (n = 4) and 7.67 days (n = 8) respectively. Due to the small sample sizes, 99th percentile half-lives were calculated and these were determined to be 15.6 and 9.3 days in thatch and grass respectively.

No aquatic dissipation data were received for ester forms of 2,4-D. Three studies were provided for application of 2,4-D to water in its DMA form. Two of these were to natural ponds (two applications each) while the third was done on a rice paddy (single application). In the natural pond systems, dissipation from both water and sediment was much slower than in the rice bays. Following the first application in one study, residues continued to increase over the three weeks following application and no half-life calculation was possible. Where half-lives could be calculated in the sediment, they ranged from 2.1 to almost 30 days. This compared with a half-life in rice bay sediment of 2.3 days (only one sample). A similar situation was observed in water dissipation with half-lives in the water column ranging from 2.7 to 19.7 days in the pond systems compared to the single half-life calculated in the rice paddy of 1.1 days. However, the latter may reflect the overall low residue levels.

The aquatic dissipation studies are useful to consider movement to sediment from water as application was made to the water column (unlike laboratory sediment/water tests described above where mixtures were shaken). While the test reports did not state the volume of water, the sediment characteristics are available, and

concentrations found in the sediment may be compared to maximum theoretical values to obtain an indication of the extent of movement from water to soil. Based on application of 46.85 kg ae/ha, the maximum theoretical concentration in the top 5 cm sediment (assuming all retained here) is 91 and 151 ppm at the North Carolina and North Dakota sites respectively. This compares with a maximum concentration found in this layer of sediment after the first application of 1.45 ppm (day 7, North Carolina study) and 1.22 ppm (day 14, North Dakota trial). These results indicate a rough measure of movement from water to sediment of <2% acid equivalent, suggesting the chemical will remain predominantly in the water compartment.

5.7 Accumulation

Given the properties of 2,4-D (high water solubility and low octanol-water partition co-efficient), it would not be expected to accumulate in biota to any extent. One literature report detailed in Appendix I describing a study performed to measure accumulation of 2,4-D in fish and water hyacinth confirms this theory. BCF values (calculated as radioactivity in water/radioactivity in fish) ranged between 11.8 to 45.4 in carp where exposure was to 0.5ppm 2,4-D and 6.2 to 26.7 in carp) in the 0.05 ppm 2,4-D exposure group.

2,4-D was absorbed by water hyacinth, and a part of the chemical transported to leaf and stem from the root. Exposure was to either 0.01 or 0.001 ppm 2,4-D. BCF values were not calculated by the study authors, but appear to be between 7.9-14.3 (residues in water/residues in plant).

6. Environmental Toxicity

In support of the assessment, significant data were provided to consider the toxicity of 2,4-D to environmental organisms. In addition, a large number of studies were reported in the US EPA 2,4-D assessment (US EPA, 2005), and further results obtained through the literature. Readers should refer to the relevant technical reports (Appendices I, II and III) to obtain study details where reviewed by DEH. The following information in the overview report is presented in summary form only.

Where tests were performed using formulated products, the toxicity has been assumed as coming from the active constituent only, and results are reported as such within the table.

Where the reference is stated as “ECOTOX Database” or “US EPA, 2005”, studies were not provided to the APVMA, hence the data have not been reviewed by DEH.

6.1 Avian Toxicity

6.1.1 Acute

Table 5. Summary of Acute Bird Toxicity Results for 2,4-D Acid and its Various Forms

Test substance	Species	LD50 (mg ae/kg bw)	Reference
2,4-D Acid ¹	Mallard duck	>5620	US EPA, 2005
2,4-D Acid ²	Domestic chicken	1064	Chittibabu, 2002a
2,4-D Acid ²	Pigeon	999	Chittibabu, 2002b
ESTERS			
2,4-D EHE	Mallard duck ³	423	Beavers, 1984a
2,4-D EHE ¹	Mallard duck	>1980	US EPA, 2005
2,4-D EHE ¹	Mallard duck	>3062	US EPA, 2005
2,4-D BEE ¹	Bobwhite quail	>1380	US EPA, 2005
2,4-D IPE ¹	Bobwhite quail	1578	US EPA, 2005
2,4-D BE	Mallard duck	>4640	ECOTOX Database ⁴
2,4-D EE ²	Domestic chicken	1759	Chittibabu, 2002c
2,4-D EE ²	Pigeon	346	Chittibabu, 2002d
SALTS			
2,4-D DMA ¹	Mallard duck	>3851.2	US EPA, 2005
2,4-D DMA	Bobwhite quail	415	Hoxter <i>et al</i> 1990
2,4-D DMA ²	Domestic chicken	1671	Chittibabu, 2002e
2,4-D DMA ²	Pigeon	334	Chittibabu, 2002f
2,4-D DEA ¹	Bobwhite quail	404.6	US EPA, 2005
2,4-D IPA ¹	Mallard duck	>314.4	US EPA, 2005
2,4-D TIPA ¹	Bobwhite quail	>218.7	US EPA, 2005
2,4-D Sodium salt ²	Domestic chicken	1834	Chittibabu, 2002g
2,4-D Sodium salt ²	Pigeon	1307	Chittibabu, 2002h

1) Data not reviewed by DEH

2) Non-Standard test.

3) US EPA, 2005 notes this study (based on the reported MRID number) being with Bobwhite Quail.

4) <http://www.epa.gov/ecotox/>

Based on the US EPA classification scale for avian toxicity

(http://www.epa.gov/oppefed1/ecorisk_ders/toera_analysis_eco.htm) 2,4-D in its acid, ester or salt forms is generally considered slightly to practically non-toxic to birds when exposed acutely through the oral route (that is, LD50 values >500 mg ae/kg). There is no evidence based on the above results to suggest ester forms (or formulations) exhibit any different toxicity characteristics to salt forms (or formulations).

Some results are suggestive of moderate toxicity (LD50 range of 51-500 mg ae/L). The most sensitive species tested appeared to be the pigeon with LD50s of 346 and 334 mg ae/kg from 2,4-D Ethyl Ester and 2,4-D DMA respectively. Both these tests followed non-standard methodology. However, results of a similar magnitude were found for mallard duck (exposed to 2,4-D 2-EHE) and bobwhite quail (exposed to 2,4-D DMA and 2,4-D DEA).

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6.1.2 Short term

Table 6. Summary of Short-Term Bird Toxicity Results for 2,4-D Acid and its Various Forms

Test substance	Species	LD50 (mg ae/kg diet)	NOEC	Reference
2,4-D Acid	Mallard duck	>5620	1000	Culotta <i>et al</i> , 1990a
2,4-D Acid	Bobwhite quail	>5620	1000	Culotta <i>et al</i> , 1990b
2,4-D Acid	Japanese quail	>5000	5000	Chittibabu, 2002i
ESTERS				
2,4-D EHE	Mallard duck	>3588	1136	Beavers, 1984b
2,4-D EHE	Bobwhite quail	>3588	2017	Beavers, 1984c
2,4-D EHE ¹	Bobwhite quail	>6600	-	US EPA, 2005
2,4-D EHE ¹	Mallard duck	>6600	-	US EPA, 2005
2,4-D BEE ¹	Bobwhite quail	>3878	-	US EPA, 2005
2,4-D BEE ¹	Mallard duck	>3866	-	US EPA, 2005
2,4-D IPE ¹	Bobwhite quail	>4583	-	US EPA, 2005
2,4-D IPE ¹	Mallard duck	>4383	-	US EPA, 2005
2,4-D BE ¹	Mallard duck	10000 (ac)	-	ECOTOX database
2,4-D BE ¹	Bobwhite quail	12979 (ac)	-	ECOTOX database
2,4-D BE ¹	Bobwhite quail	10000 (ac)	-	ECOTOX database
SALTS				
2,4-D DMA	Mallard duck	>4665	468	Long <i>et al</i> , 1990a
2,4-D DMA	Bobwhite quail	>4665	2623	Long <i>et al</i> , 1990b
2,4-D DMA ¹	Bobwhite quail	>8300	-	US EPA, 2005
2,4-D DMA ²	Mallard duck	>1899	1899	Fink, 1974a
2,4-D DMA ²	Bobwhite quail	>1899	879	Fink, 1974b
2,4-D DEA ¹	Bobwhite quail	>3821.6	-	US EPA, 2005
2,4-D DEA ¹	Mallard duck	>3821.6	-	US EPA, 2005
2,4-D IPA ¹	Bobwhite quail	>4440	-	US EPA, 2005
2,4-D IPA ¹	Mallard duck	>4440	-	US EPA, 2005
2,4-D TIPA ¹	Bobwhite quail	>3035	-	US EPA, 2005
2,4-D TIPA ¹	Mallard duck	>3035	-	US EPA, 2005

1) Data not reviewed by DEH

2) Non-standard test

Based on the US EPA classification scale for avian toxicity (http://www.epa.gov/oppefed1/ecorisk_ders/toera_analysis_eco.htm) 2,4-D in its acid, ester or salt forms is considered slightly to practically non-toxic to birds when exposed through the diet (that is, LD50 values >1000 mg ae/kg). There is no evidence based on the above results to suggest ester forms (or formulations) exhibit any different toxicity characteristics to salt forms (or formulations).

Based on the NOEC, the most sensitive species tested appeared to be the mallard duck with a NOEC of 468 ppm 2,4-D in the diet, based on a reduction in body weight gain at concentrations tested higher than this. Of the available studies reviewed, and those reported in US EPA, 2005, no definitive LD50 was derived as 50% mortality was not found at any of the rates tested.

6.1.3 Reproduction

Test substance	Species	NOEC	Endpoint	Reference
2,4-D Acid	Bobwhite	1000 ppm		Mitchell <i>et al</i> , 2000

No other reproduction data were available for any other species or using any other form of 2,4-D. The need for such data will depend on the outcome of the risk assessment and the judgement from this as to the likelihood of chronic exposure to birds.

6.1.4 Neurotoxicity

A non-standard test was performed to assess neurotoxic impacts of 2,4-D exposure to domestic egg-laying chickens (Chittibabu, 2002j). Birds were administered multiple doses for 21 days and observed for a further 21 days. Three doses of 25, 50 and 100 mg/kg bw were selected. Although haematological, biochemical and histopathological evaluations did not reveal any abnormality, based on observations of overt signs of toxicity in the highest treatment level (lethargy and incoordination), the NOEC of 2,4-D Acid to chickens under the conditions of this study was considered to be 50 mg/kg bw.

6.2 Aquatic Toxicity

6.2.1 Fish – Acute

Table 7. Summary of Acute Fish Toxicity Results for 2,4-D Acid

Test species	System	LC50 (mg/L)	Reference
Rainbow trout (<i>S. gairdneri</i>)	96 h flow-through	320	Alexander <i>et al</i> , 1983a
Bluegill sunfish (<i>L. macrochirus</i>)	96 h flow-through	263	Alexander <i>et al</i> , 1983a
Fathead minnow (<i>P. promelas</i>)	96 h flow-through	358	Alexander <i>et al</i> , 1983a
Tidewater silverside (<i>M. beryllina</i>)	96 h flow-through	175 (m)	Vaishnav <i>et al</i> , 1990a
Guppy (<i>P. reticulata</i>)	96 h static	>105	Chittibabu, 2002k
Zebra fish (<i>B. rerio</i>)	96 h static	>105	Chittibabu, 2002k
Mozambique tilapia (<i>T. mossambica</i>) ¹	96 h static	>100 ¹	Chittibabu, 2002l

1) non-standard test; (m) = measured concentration

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Table 8. Summary of Acute Fish Toxicity Results for 2,4-D Esters

Test species	System	LC50 (mg ae/L) ¹	Reference
2,4-D 2-EHE			
Rainbow trout (<i>S. gairdneri</i>)	96 h flow-through	>3.19	Alexander <i>et al</i> , 1983b
Bluegill sunfish (<i>L. macrochirus</i>)	96 h flow-through	>3.19	Alexander <i>et al</i> , 1983b
Fathead minnow (<i>P. promelas</i>)	96 h flow-through	>3.19	Alexander <i>et al</i> , 1983b
Fathead minnow (<i>P. promelas</i>)	8 d flow though	>15.88 (m)	Mayes <i>et al</i> , 1990a
Tidewater silverside (<i>M beryllina</i>)	96 h flow-through	>0.16 (m)*	Ward and Boeri, 1991a
Tidewater silverside (<i>M beryllina</i>)	96 h flow-through	>0.46 (m)*	Ward and Boeri, 1991b
Rainbow trout (<i>O. mykiss</i>)	96 h flow-through	3.12 (m)	Mayes <i>et al</i> , 1990b
Rainbow trout (<i>O. mykiss</i>)	8 d flow-through	2.03 (m)	Mayes <i>et al</i> , 1990b
2,4-D Ethyl Ester			
Mozambique tilapia (<i>T mossambica</i>) ²	96 h static	0.63 ²	Chittibabu, 2002m
Medaka (<i>Oryzias latipes</i>)	48 h static	2.84	ECOTOX Database
Goldfish (<i>Carassius auratus</i>)	48 h static	2.84	ECOTOX Database
Carp (<i>Cyprinus carpio</i>)	48 h static	0.89	ECOTOX Database
Bluegill sunfish (<i>L. macrochirus</i>)	48 h static	1.24	ECOTOX Database
2,4-D Butyl Ester			
Bluegill sunfish (<i>L. macrochirus</i>)	96 h static	0.23	ECOTOX Database
Bluegill sunfish (<i>L. macrochirus</i>)	96 h static	0.24	ECOTOX Database
Cutthroat trout (<i>O. clarki</i>)	96 h static	0.39-2.26; average = 0.81 ³	ECOTOX Database
Rainbow trout (<i>O. mykiss</i>)	96 h static	0.32	ECOTOX Database
Rainbow trout (<i>O. mykiss</i>)	96 h static	0.64	ECOTOX Database
Lake trout, siscowet (<i>S. namaycush</i>)	96 h static	0.40-2.23; average = 0.83 ⁴	ECOTOX Database
2,4-D BEE			
Rainbow trout (<i>O. mykiss</i>)	96 h Static	1.44 (m)	US EPA, 2005
Rainbow trout (<i>O. mykiss</i>)	96 h Static	0.45 (m)	US EPA, 2005
Bluegill Sunfish (<i>L. macrochirus</i>)	96 h	0.428 (m)	US EPA, 2005
Bluegill Sunfish (<i>L. macrochirus</i>)	96 h	0.69 (m)	US EPA, 2005
Fathead minnow (<i>P. promelas</i>)	96 h	1.79 (m)	US EPA, 2005
2,4-D IPE			
Rainbow trout (<i>O. mykiss</i>)	96 h Static	0.58 (m)	US EPA, 2005
Rainbow trout (<i>O. mykiss</i>)	96 h Static	0.66 (m)	US EPA, 2005
Bluegill Sunfish (<i>L. macrochirus</i>)	96 h Static	0.26 (m)	US EPA, 2005
Bluegill Sunfish (<i>L. macrochirus</i>)	96 h	0.26 (m)	US EPA, 2005

* very low recovery compared to nominal concentrations.; m = measured concentrations.

- 1) All results have been converted to acid equivalent values.
- 2) Non-standard test.
- 3) Total of 18 separate results from database.
- 4) Total of 14 separate results from database.

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Table 9. Summary of Acute Fish Toxicity Results for 2,4-D Salts

Test species	System	LC50 (mg ae/L)	Reference
2,4-D DMA			
Fathead minnow (<i>P. promelas</i>)	96 h flow-through	186	Alexander <i>et al</i> , 1983c
Bluegill sunfish (<i>L. macrochirus</i>)	96 h flow-through	293	Alexander <i>et al</i> , 1983c
Rainbow trout (<i>S. gairdneri</i>)	96 h flow-through	140	Alexander <i>et al</i> , 1983c
Rainbow trout (<i>O. mykiss</i>)	96 h static	>100	Memmert, 1997a
Tidewater silverside (<i>M beryllina</i>)	96 h flow-through	389 (m)	Scott Ward, 1991a
Rainbow trout (<i>S. gairdneri</i>)	96 h static	314	Bentley, 1974
Bluegill sunfish (<i>L. macrochirus</i>)	96 h static	322	Bentley, 1974
Carp (<i>C. carpio</i>)	96 h static	560-1000	Nigitz, 1990a
Rainbow trout (<i>S. gairdneri</i>)	96 h static	240	Nigitz, 1990b
Mozambique tilapia (<i>T mossambica</i>)	96 h static	35.6 ¹	Chittibabu, 2002n
Rainbow trout (<i>O. mykiss</i>)	96 h	>830 (m)	US EPA, 2005
Bluegill sunfish (<i>L. macrochirus</i>)	96 h	>100 (m)	US EPA, 2005
Bluegill sunfish (<i>L. macrochirus</i>)	96 h	>830 (m)	US EPA, 2005
Fathead minnow (<i>P. promelas</i>)	96 h	264 (m)	US EPA, 2005
Sheepshead minnow (<i>C. variegates</i>)	96 h	465 (m)	US EPA, 2005
2,4-D DEA			
Rainbow trout (<i>O. mykiss</i>)	96 h static	>81.6 (m)	US EPA, 2005
Bluegill sunfish (<i>L. macrochirus</i>)	96 h	>82.3 (m)	US EPA, 2005
Fathead minnow (<i>P. promelas</i>)	96 h	234 (m)	US EPA, 2005
Bluegill sunfish (<i>L. macrochirus</i>)	96 h	101 (m)	US EPA, 2005
Tidewater silverside (<i>M beryllina</i>)	96 h	>80.24 (m)	US EPA, 2005
2,4-D IPA			
Rainbow trout (<i>O. mykiss</i>)	96 h	2244 (m)	US EPA, 2005
Bluegill sunfish (<i>L. macrochirus</i>)	96 h	1343 (m)	US EPA, 2005
Fathead minnow (<i>P. promelas</i>)	96 h	1722 (m)	US EPA, 2005
Tidewater silverside (<i>M beryllina</i>)	96 h	187 (m)	US EPA, 2005
2,4-D TIPA			
Rainbow trout (<i>O. mykiss</i>)	96 h	162 (m)	US EPA, 2005
Bluegill sunfish (<i>L. macrochirus</i>)	96 h	217 (m)	US EPA, 2005
Tidewater silverside (<i>M beryllina</i>)	96 h	203 (m)	US EPA, 2005
2,4-D Sodium salt			
Rainbow trout (<i>O. mykiss</i>)	96 h Static	>91	US EPA, 2005
Mozambique tilapia (<i>T mossambica</i>)	96 h static	>80 ¹	Chittibabu, 2002o

1) Non-standard test; (m) = measured concentrations.

2,4-D in its free acid form may be considered practically non-toxic to fish. This is also the case for 2,4-D salts. However, one non-standard test using 2,4-D DMA showed this chemical to be slightly toxic to a non-standard species, Mozambique tilapia. In this test, there was only one replicate for each test treatment and no analytical measurement of exposure concentrations were performed. However, based on nominal concentrations, this result is still considered valid as there was no mortality in the control group of fish, and a very clear dose-response was established for the exposure concentrations.

2,4-D Esters, both in their technical form and in formulations, were much more toxic to fish. Tests reviewed for 2,4-D 2-EHE were indicative of moderate toxicity where a defined LC50 could be determined. Generally, however, this compound was not toxic up to its limit of solubility. In several cases, there was very low recovery of test substance and results were somewhat difficult to interpret. The majority of results

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available for esters were not reviewed by DEH, so end results only have been reported and are assumed to be accurate. These results show that the ethyl and butyl esters used in Australia are highly toxic to fish. One non-standard test using 2,4-D EE was reviewed by DEH and supports the other reported data, showing this chemical to be highly toxic to a non-standard species, Mozambique tilapia. In this test, there was only one replicate for each test treatment and no analytical measurement of exposure concentrations were performed. However, based on nominal concentrations, this result is still considered valid as there was no mortality in the control group of fish, and a very clear dose-response was established for the exposure concentrations.

6.2.2 Fish - Chronic/Sub-Chronic

Table 10. Summary of Chronic/Sub-Chronic Fish Toxicity Results (Flow-through conditions)

Test species	Test system	NOEC/MATC (mg ae/L)	Reference
2,4-D Acid Fathead minnow (<i>P. promelas</i>)	32 day ELS	63.4/80.4 (m)	Mayes <i>et al</i> , 1990c
2,4-D 2-EHE Fathead minnow (<i>P. promelas</i>)	32 day ELS	0.079/0.106 (m)	Mayes <i>et al</i> , 1990a
2,4-D BEE Fathead minnow (<i>P. promelas</i>)		0.056/0.066 (m)	US EPA, 2005
2,4-D DMA Rainbow trout (<i>O. mykiss</i>)	28 d	NOEC = 100	Monk, 1990
Fathead minnow (<i>P. promelas</i>)	31 day ELS	14.2/18.3 (m)	Dill <i>et al</i> , 1990
2,4-D DEA Fathead minnow (<i>P. promelas</i>)		19.8/36.3 (m)	US EPA, 2005

(m) = measured concentration

No data were available for the registered ethyl ester and butyl ester forms of 2,4-D. MATC values as modelled through the US EPA ECOSAR v0.99g model are calculated to be 1.024 and 0.173 mg/L respectively. It should be noted that ECOSAR predicted fish 96 h LC50 values of around 6.8 and 2.2 mg/L respectively, both of which are around an order of magnitude higher than the lowest experimental results shown above in Table 10. If this underestimation of the MATC were also true with the predicted values, actual MATCs for 2,4-D EE and 2,4-D BE could be around 0.1 and 0.02 ppm respectively.

6.2.3 Amphibians

2,4-D in both its free acid form and salt form (using DMA as a surrogate for all other salts), appears to be practically non-toxic to frog tadpoles. It is difficult to reach a conclusion on ester toxicity as only one results is available and no dose-response mortality was observed at the highest measured exposure concentration of 0.5 mg ae/L.

Table 11. Summary of Amphibian Toxicity Results

Test species	Test system	LC50 (mg ae/L)	Reference
2,4-D Acid Leopard frog tadpoles (<i>R. pipiens</i>)	96 h static	359	Palmer and Krueger, 1997a
2,4-D 2-EHE Leopard frog tadpoles (<i>R. pipiens</i>)	96 h static	>0.50	Palmer and Krueger, 1997b
2,4-D DMA Leopard frog tadpoles (<i>R. pipiens</i>)	96 h static	188	Palmer and Krueger, 1997c

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In addition to the above results, WHO (1989) reports that tadpoles of the Indian toad are particularly susceptible to 2,4-D. The result reported for the free acid was a 96 h LC50 of 8.3 mg/L. It is also reported that 2,4-D, at 5 g/litre, prevented development of the eggs of the common frog (*Rana temporaria*). At doses between 500 mg/litre and 4 g/litre, there was some development that decreased with increasing dose.

6.2.4 Invertebrates – Acute

Table 12. Summary of Acute Aquatic Invertebrate Toxicity Results for 2,4-D Acid

Test species	System	LC50 (mg/L)	Reference
<i>Daphnia magna</i>	48 h static	247.2	McCarty and Batchelder, 1977
<i>Daphnia magna</i>	48 h static	74.4	Chittibabu, 2002p
<i>Daphnia magna</i>	48 h static	262	McCarty, 1979
<i>Daphnia magna</i>	48 h static	25.0	Alexander <i>et al</i> , 1983c
<i>Daphnia magna</i>	48 h static	36.4	Alexander <i>et al</i> , 1983c
Eastern oyster (<i>C. virginica</i>)	96 h flow-through	EC50 = 57	Ward <i>et al</i> , 1993
Eastern oyster (<i>C. virginica</i>)	96 h flow-through	EC50 = 146	Wade and Overman, 1990
Pink shrimp (<i>P. duorarum</i>)	96 h flow-through	554	Vaishnav <i>et al</i> , 1990

Table 13. Summary of Acute Aquatic Invertebrate Toxicity Results for 2,4-D Esters

Test species	System	LC50 (mg ae/L)	Reference
2,4-D 2-EHE			
<i>Daphnia magna</i>	48 h static	12.38	McCarty, 1979
<i>Daphnia magna</i>	48 h static	2.92	Alexander <i>et al</i> , 1983b
Grass shrimp (<i>P. pugio</i>)	96 h flow-through	>0.09 (m)*	Ward and Boeri, 1991c
Eastern oyster (<i>C. virginica</i>)	96 h flow-through	NOEC = 0.14 (m)*	Ward and Boeri, 1991d
Eastern oyster (<i>C. virginica</i>)	96 h flow-through	NOEC = 0.32 (m)*	Ward and Boeri, 1991e
Grass shrimp (<i>P. pugio</i>)	96 h flow-through	NOEC = 0.65 (m)*	Ward and Boeri, 1991f
2,4-D Ethyl Ester			
<i>Daphnia magna</i>	96 h static	>8.9	ECOTOX Database
Water flea (<i>Moina macrocopa</i>)	3 h static	>8.9	ECOTOX Database
2,4-D Butyl Ester			
<i>Daphnia magna</i>	48 h static	8.44	McCarty, 1979
<i>Daphnia magna</i>	96 h static	EC50 = 2.23	ECOTOX Database
Stonefly (<i>Pteronarcella badia</i>)	96 h static	1.18	ECOTOX Database
Stonefly (<i>Pteronarcella badia</i>)	96 h static	1.04	ECOTOX Database
2,4-D BEE			
<i>Daphnia magna</i>	48 h static	4.97 (m)	US EPA, 2005
Eastern Oyster (<i>C. virginica</i>)	96 h	1.8 (m)	US EPA, 2005
Pink Shrimp (<i>Panaeus duorarum</i>)	96 h	3.8 (m)	US EPA, 2005
2,4-D IPE			
<i>Daphnia magna</i>	48 h static	2.2 (m)	US EPA, 2005

* very low recovery compared to nominal concentrations.; m = measured concentrations.

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Table 14. Summary of Acute Aquatic Invertebrate Toxicity Results for 2,4-D Salts

Test species	System	LC50 (mg ae/L)	Reference
2,4-D DMA			
<i>Daphnia magna</i>	48 h static	103	Alexander <i>et al</i> , 1983c
<i>Daphnia magna</i>	48 h static	NOEC = 102	Memmert, 1997b
Pink shrimp (<i>Panaeus duorarum</i>)	96 h flow-through	150 (m)	Scott Ward, 1991b
Eastern oyster (<i>C. virginica</i>)	96 h flow-through	113 (m)	Scott Ward, 1991c
Eastern oyster (<i>C. virginica</i>)	96 h flow-through	EC50 = 85-131	Heitmuller, 1975
Fiddler crabs (<i>Uca pugilator</i>)	96 h flow-through	>410.8	Heitmuller, 1975
Pink shrimp (<i>Panaeus duorarum</i>)	96 h flow-through	>410.8	Heitmuller, 1975
<i>Daphnia magna</i>	48 h	642.8 (m)	US EPA, 2005
Mysid shrimp	96 h	152.7	US EPA, 2005
Pink shrimp (<i>Panaeus duorarum</i>)	96 h	>82.7	US EPA, 2005
Fiddler crabs (<i>Uca pugilator</i>)	96 h	830	US EPA, 2005
Grass shrimp (<i>P. pugio</i>)	96 h	104.5	US EPA, 2005
2,4-D DEA			
<i>Daphnia magna</i>	48 h static	>68 (m)	US EPA, 2005
Eastern oyster (<i>C. virginica</i>)	96 h	>76.16 (m)	US EPA, 2005
Pink Shrimp (<i>Panaeus duorarum</i>)	96 h	>67.73 (m)	US EPA, 2005
2,4-D IPA			
<i>Daphnia magna</i>	48 h static	461 (m)	US EPA, 2005
Eastern oyster (<i>C. virginica</i>)	96 h	49.6 (m)	US EPA, 2005
Pink Shrimp (<i>Panaeus duorarum</i>)	96 h	478 (m)	US EPA, 2005
2,4-D TIPA			
<i>Daphnia magna</i>	48 h static	340.2 (m)	US EPA, 2005
Eastern oyster (<i>C. virginica</i>)	96 h	89.1 (m)	US EPA, 2005
Pink Shrimp (<i>Panaeus duorarum</i>)	96 h	401.8 (m)	US EPA, 2005

m = measured concentrations.

Results for 2,4-D in its free acid form and various salt forms (including formulations), show the compound to be practically non-toxic to a range of aquatic invertebrates, with some results being indicative of moderate toxicity. No tests for the more sensitive mysid shrimp have been reviewed. The US EPA reports one result for this species for 2,4-D DMA where it was shown to be practically non-toxic.

Esters again were more toxic in both their technical form and in formulation. LC/EC50 results show the various ester forms where results are available, can be classified as moderately toxic to aquatic invertebrates. Some results are difficult to interpret due to extremely low and variable recovery during analysis of exposure concentrations.

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6.2.5 Chronic

Table 15. Summary of Chronic Aquatic Invertebrate Toxicity Results

Test species	Test system	NOEC/MATC (mg ae/L)	Reference
2,4-D Acid <i>Daphnia magna</i>	21 d flow-through	79/109 (m) EC50 = 235 (m)	Ward and Boeri, 1991g
2,4-D 2-EHE <i>Daphnia magna</i>	21 d flow-through	0.01/0.013 (m)* EC50 = 0.086 (m)	Ward and Boeri, 1991h
2,4-D BEE <i>Daphnia magna</i>	21 d	0.20/0.311 (m)	US EPA, 2005
2,4-D DMA <i>Daphnia magna</i>	21 d semi-static	46.2/73.1	Mark and Hantink-de Rooy, 1989
<i>Daphnia magna</i>	21 d flow-through	22.8/33.6 (m)	Scott Ward, 1991d
2,4-D DEA <i>Daphnia magna</i>	21 d	16.05/>16.05 (m)	US EPA, 2005

* very low recovery compared to nominal concentrations.; m = measured concentrations.

Limited chronic results again show ester forms of 2,4-D to be significantly more toxic than free acid or salt forms of the chemical.

6.2.6 Algae and Aquatic Plants

Table 16. Summary of Algae/Aquatic Plant Toxicity Results for 2,4-D Acid

Test species	System	EC50 (mg ae/L)	Reference
Green alga (<i>S. capricornutum</i>)	120 h	EC25 = 29.0 EC50 = 33.2	Hughes, 1990a
Green alga (<i>C. vulgaris</i>)	72 h	101.5	Chandrasehar, 2001
Duckweed (<i>L. gibba</i>)	Assumed 14 d	EC50 = 0.695 NOEC = 0.0581	US EPA, 2005

Table 17. Summary of Algae/Aquatic Plant Toxicity Results for 2,4-D Esters

Test species	Test duration	EC50 (mg ae/L)	Reference
2,4-D 2-EHE Duckweed (<i>L. gibba</i>)	14 days	0.33*	Hughes, 1990b
Green alga (<i>S. capricornutum</i>)	120 h	>1.06 (m)*	Hughes, 1990c
Marine diatom (<i>S. costatum</i>)	120 h	0.15*	Hughes, 1990d
Blue green alga (<i>A. flos-aquae</i>)	120 h	>0.21 (m)*	Hughes, 1990e
Freshwater diatom (<i>N. pelliculosa</i>)	120 h	2.7*	Hughes, 1990f
2,4-D BEE Duckweed (<i>L. gibba</i>)		0.40	US EPA, 2005
Green alga (<i>S. capricornutum</i>)		17.14	US EPA, 2005
Marine diatom (<i>S. costatum</i>)		1.02	US EPA, 2005
Freshwater diatom (<i>N. pelliculosa</i>)		1.28	US EPA, 2005
Blue green alga (<i>A. flos-aquae</i>)		4.2	US EPA, 2005

* very low recovery compared to nominal concentrations.; m = measured concentrations.

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Table 18. Summary of Algae/Aquatic Plant Toxicity Results for 2,4-D Salts

Test species	Test duration	EC50 (mg ae/L)	Reference
2,4-D DMA			
Duckweed (<i>L. gibba</i>)	14 days	0.48 (m)	Hughes, 1990g
Green alga (<i>S. capricornutum</i>)	120 h	55.2 (m)	Hughes, 1990h
Marine diatom (<i>S. costatum</i>)	120 h	129*	Hughes, 1990i
Blue green alga (<i>A. flos-aquae</i>)	120 h	127	Hughes, 1990j
Freshwater diatom (<i>N. pelliculosa</i>)	120 h	4.38	Hughes, 1990k
Green alga (<i>S. subspicatus</i>)	72 h	89	Memmert, 1997c
Green alga (<i>S. capricornutum</i>)	96 h	550	Schoot Uiterkamp, 1989
2,4-D DEA			
Duckweed (<i>L. gibba</i>)		0.30	US EPA, 2005
Green alga (<i>S. capricornutum</i>)		7.48	US EPA, 2005
Marine diatom (<i>S. costatum</i>)		>64.6	US EPA, 2005
Freshwater diatom (<i>N. pelliculosa</i>)		>66	US EPA, 2005
Blue green alga (<i>A. flos-aquae</i>)		>65.3	US EPA, 2005
2,4-D IPA			
Green alga (<i>S. capricornutum</i>)		34.3	US EPA, 2005
2,4-D TIPA			
Duckweed (<i>L. gibba</i>)		1.3	US EPA, 2005
Green alga (<i>S. capricornutum</i>)		40.9	US EPA, 2005
Marine diatom (<i>S. costatum</i>)		38.3	US EPA, 2005
Freshwater diatom (<i>N. pelliculosa</i>)		51.0	US EPA, 2005
Blue green alga (<i>A. flos-aquae</i>)		71.8	US EPA, 2005

* DEH calculated result; (m) = measured concentration

The vascular aquatic plant, duckweed, was the most sensitive organism from algae and aquatic plants tested. There was no significant difference between toxicity of the free acid, ester or salt forms of 2,4-D to this organism, and all can be considered highly toxic to duckweed.

Simpler freshwater and marine algae tests showed the free acid and salt forms of 2,4-D to be generally slightly to practically non-toxic. There were two exceptions with 2,4-D DMA being moderately toxic to the freshwater diatom, *N. pelliculosa* and 2,4-D DEA being moderately toxic to the green alga, *S. capricornutum*. The two ester forms with results available (2,4-D 2-EHE and 2,4-D BEE) generally show moderate (approaching high) toxicity to simpler freshwater and marine algae. 2,4-D 2-EHE did exhibit high toxicity to the marine diatom, *S. costatum*. Some of the results for this compound are difficult to interpret due to very low and variable recoveries during testing for exposure concentrations.

6.3 *Terrestrial Toxicity*

6.3.1 **Non-Target Invertebrates**

Bees

Table 19. Summary of Toxicity to Bees for 2,4-D in Various Forms

Test species	Test duration	LD50 (µg ae/bee)	Reference
2,4-D Acid			
Worker bee (<i>A. indica</i>)	24 h contact	>5% w/v*	Jeyalakshmi, 2002a
2,4-D 2-EHE			
Honey bee (<i>A. mellifera</i>)	72 h oral	>62.9	Hoxter <i>et al</i> , 1997a
Honey bee (<i>A. mellifera</i>)	48 h contact	>64.3	Palmer and Krueger, 1997d
2,4-D Ethyl Ester			
Worker bee (<i>A. indica</i>)	24 h contact	0.013% v/v*	Jeyalakshmi, 2002b
2,4-D DMA			
Honey bee (<i>A. mellifera</i>)	72 h oral	78	Hoxter <i>et al</i> , 1997b
Honey bee (<i>A. mellifera</i>)	48 h contact	>83.3	Palmer and Krueger, 1997e
Worker bee (<i>A. indica</i>)	24 h contact	0.136% v/v*	Jeyalakshmi, 2002c
2,4-D Sodium salt			
Worker bee (<i>A. indica</i>)	24 h contact	>3% w/v*	Jeyalakshmi, 2002d

* Non-standard test

No standard oral or contact toxicity tests were provided for the free acid form of 2,4-D. However, based on the US EPA classification system, results for 2,4-D 2-EHE and 2,4-D DMA indicate that through both contact and oral routes, ester and salt forms of the chemical would be practically non-toxic to bees.

WHO, 1989 states that esters are less toxic to insects than are salts or the free acid. Non-standard testing for bees reviewed by DEH suggests that 2,4-D EE would be more toxic than salts or the free acid, although the results of this test can't be correlated with a dose rate per bee, or an application rate per hectare. Nonetheless, based on results of the negative and positive controls run in this non-standard test, the test system did seem to work, and results indicate that 2,4-D EE is some 10 times more toxic than 2,4-D DMA to worker bees.

Other Arthropods

Table 20. Summary of Toxicity to Terrestrial Arthropods for 2,4-D in Various Forms

Test species	Test system	IOBC Classification	Reference
2,4-D Acid No data provided			
2,4-D 2-EHE Predatory mite (<i>T. pyri</i>)	14 d laboratory	Harmless up to 564 g ae/ha	Kuhner, 1998a
Parasitic wasp (<i>A. rhopalosiphi</i>)	14 d laboratory	Harmful at 564 g ae/ha; Harmless at 28 g ae/ha	Kuhner, 1998b
2,4-D DMA Predatory mite (<i>T. pyri</i>)	14 d laboratory	Harmless up to 1800 g ae/ha	Kuhner, 1998c
Predatory mite (<i>T. pyri</i>)	14 d laboratory	Harmless up to 1000 g ae/ha	Goßmann, 1997a
Parasitic wasp (<i>A. rhopalosiphi</i>)	14 d laboratory	Harmless up to 1800 g ae/ha	Kuhner, 1998d
Spiders (<i>Pardosa</i> sp.) (7 species)	14 d laboratory	Harmless up to 1056 g ae/ha	Schmitzer and Breitwieser, 1997
Rove beetle (<i>A. bilineata</i>)	28 d laboratory	Harmless up to 1000 g ae/ha	Goßmann, 1997b

Results for terrestrial arthropod toxicity further suggest that the ester forms are more toxic to insects than salt forms of 2,4-D. The standard parasitic wasp species was tested using the same test system for both 2,4-D DMA and 2,4-D 2-EHE and maximum label application rates for their respective formulations. For 2,4-D DMA, reduction in beneficial capacity was such that this chemical is considered harmless up to its use rate of 1800 g ae/ha. However, the maximum rate of 564 g ae/ha for 2,4-D 2-EHE resulted in 100% mortality of adult wasps prior to the reproductive phase of the test.

2,4-D EHE was harmless to the predatory mite at its maximum application rate for the test of 564 g ae/ha. 2,4-D DMA was harmless to the predatory mite, spiders and the rove beetle up to 1800, 1056 and 1000 g ae/ha respectively.

6.3.2 Earthworms

Table 21. Summary of Toxicity to Earthworms for 2,4-D in Various Forms

Test species	Test system	LD50 (mg ae/kg dw soil)	Reference
2,4-D Acid Earthworm (<i>Lampito mauritii</i>)	14 d	327.2	Kumar, 2001
2,4-D Esters No data provided			
2,4-D DMA Earthworm (<i>E. fetida</i>)	14 d	350	Adema and Roza, 1989
Earthworm (<i>E. fetida</i>)	14 d	>500	Goßmann, 1997c

Earthworms were insensitive to 2,4-D exposure as both the free acid and in its DMA salt form with LD50s in excess of 300 mg ae/kg dw soil. According to the classification scale of Mensink *et al*, 1995, 2,4-D can be considered only slightly toxic to earthworms. No data were provided for toxicity of ester forms of 2,4-D to

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earthworms. However, field data indicate that these will hydrolyse relatively quickly in viable soils to form the 2,4-D acid, and prolonged exposure should not occur.

6.3.3 Soil Micro-organisms

No data have been provided for 2,4-D acid or any of its ester forms. Two studies, one addressing soil respiration and the other addressing soil nitrification, were provided for 2,4-D DMA when applied in a formulation containing 500 g/L 2,4-D acid. In these tests, two applications were made, one at the highest rate of that particular formulation and one at 10 times that rate, resulting in soil concentrations of 1.33 and 13.3 mg ae/kg soil. The outcomes of these tests were that 2,4-D should have no significant impact on soil respiration or ammonification and nitrification of soil when applied at a rate up to 13.3 mg ae/kg soil.

6.3.4 Non-Target Terrestrial Vegetation

Non-target toxicity data provided to the APVMA included tier II studies for 2,4-D Acid, 2,4-D DMA, 2,4-D EHE and 2,4-D BEE. Both seedling emergence and vegetative vigour studies were received for all except 2,4-D DMA where only seedling emergence data were available.

In addition, the US EPA (2005) received data in the form of seedling emergence and vegetative vigour studies 2,4-D IPE and 2,4-D DEA.

Table 22. Most Sensitive Plant EC25 Results (g ae/ha) for 2,4-D in Various Forms

	Seedling Emergence		Vegetative Vigour	
	Monocot	Dicot	Monocot	Dicot
2,4-D Acid	1640	33.6	<8.4	8.4
2,4-D DMA	50.7	22.3	No data available	
2,4-D DEA ¹	426	50	44.8	3.4
2,4-D EHE	270	33.6	244	11.2
2,4-D BEE	434 ²	237 ²	212	24
2,4-D IPE ¹	11.2	0.9	226	1.4

- 1) The test reports for these chemicals have not been provided to the APVMA.
- 2) The definitive test also tested seed germination and effects on radicle length. These were *in vitro* studies not considered representative of real exposure. Nonetheless, effects were severe with EC25 values for monocots and dicots <1 g ae/ha. The relevance of these effects is discussed in the refined assessment below.

Several of the above seedling emergence studies (2,4-D Acid, 2,4-D EHE and 2,4-D BEE) performed a separate seedling germination study *in vitro*. In all cases, preliminary testing showed the seed radicle for both monocots and dicots to be a very sensitive indicator to 2,4-D, with defined NOELs for most species of 0.07-0.67 g ae/ha. Only the 2,4-D BEE study considered this end point in definitive testing with EC25 values for both monocots and dicots ranging from 0.33 to 17.6 g ae/ha.

2,4-D is a plant growth regulator that is absorbed through the roots and foliage within 4-6 hours and distributed throughout the plant via the xylem and phloem. Once in the plant it selectively eliminates broadleaf plants by mimicking the effect of plant growth regulating hormones. This action stimulates growth, which leads to an abnormal growth pattern and death in some plants. Therefore, if even a portion of the surface area of a non-target plant comes into contact with 2,4-D there is a possibility that the plant may be severely damaged or dies as a result. Even if the plant only exhibits minor damage, the damage may be sufficient to prevent the plant from reproducing or

competing successfully or with other plants for resources, including water (US EPA, 2005).

Based on the above results, terrestrial vegetation may be highly sensitive to 2,4-D exposure. While these results are for food crops, in lieu of test results on Australian native species they need to be extrapolated to non-target native vegetation. Dicots generally appear more sensitive than monocots. The issue of off-target movement will need to be carefully evaluated through the risk assessment.

6.4 Conclusions on Environmental Toxicity

2,4-D Acid:

When tested in its acid form, 2,4-D was not toxic to birds based on acute oral exposure from one standard test (not reviewed) and two non-standard tests. Short term toxicity was not evident with three tests all resulting in an undefined LD50 due to a lack of mortality at up to 5620 ppm (although 40% mortality at this level was found for the bobwhite quail). A single reproduction test showed no effects on reproductive parameters for the bobwhite quail at 1000 ppm. There was no corresponding reproduction study for the mallard duck. However, given the lack of effects through dietary exposure for both species, and the lack of effects on bobwhite quail through the reproduction study, this test is not considered necessary.

Acute testing on fish showed a range of freshwater and marine fish species to be insensitive to 2,4-D in its acid form. The most sensitive species for which a defined end-point was obtained in acute testing was a 96 h LC50 of 175 mg/L for the marine tidewater silverside. A single acute amphibian test on leopard frog tadpoles resulted in a 96 h LC50 of 359 mg/L. One chronic study on fathead minnow showed a NOEC of 63.4 mg/L.

Aquatic invertebrates (water fleas, pink shrimp and eastern oysters) were variable in their sensitivity to 2,4-D. The most sensitive animal appeared to be *Daphnia magna* with a lowest 48 LC50 value of 25.0 mg/L (slight toxicity). A single chronic test on daphnids gave a 21 day NOEC of 79 mg/L (corresponding EC50 of 235 mg/L).

Two tests on different green algae species showed relatively low toxicity of 2,4-D acid to algae with EC50s between 30-100 mg/L. However, the duckweed *Lemna gibba* appears much more sensitive, and 2,4-D is highly toxic with a reported EC50 of 0.695 mg/L, from a study not assessed within this review.

No standard toxicity tests were performed on terrestrial organisms using 2,4-D in its acid form. This is acceptable as tests were performed with 2,4-D in various ester or salt forms that are used in formulations, and therefore are of more relevance. Data provided for 2,4-D in its acid form to non-target vegetation through seedling emergence and vegetative vigour studies demonstrated that plants were more sensitive in their growth stage rather than at emergence. Monocots tended to be less sensitive than dicots. EC25 values for dicot in the vegetative vigour studies ranged from 8.4 to 25.7 g/ha..

Esters:

Acute oral toxicity results were available for 2,4-D EHE, 2,4-D IPE, 2,4-D BEE, 2,4-D BE and 2,4-D EE, although only studies for 2,4-D EHE and 2,4-D EE were reviewed. Of the available results, mallard duck (LD50 = 423 mg ae/kg bw) and the pigeon (LD50 = 346 mg ae/kg bw from a non-standard test) were the most sensitive. Other results where data were not reviewed indicate the 2,4-D esters are moderately to

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practically non-toxic to birds, with results provided for mallard duck, bobwhite quail and the domestic chicken.

Only two short term toxicity tests were reviewed, both for 2,4-D EHE, with no defined short term LC50 able to be derived up to 3588 ppm ae for either the mallard duck or the bobwhite quail. In addition to these reviewed data, nine other results were reported, confirming this lack of toxicity through the diet. These results covered the 2,4-D EHE, 2,4-D BEE, 2,4-D IPE and 2,4-D BE esters and indicate that in general, 2,4-D esters will not be toxic to birds when consumed in the diet.

No avian reproductive tests were available for any of the 2,4-D esters.

Several acute fish toxicity results were reviewed for 2,4-D EHE and one for 2,4-D EE. Results for the former were often difficult to interpret due to inconsistent recoveries of the test substance and the main conclusion is that 2,4-D EHE is unlikely to be toxic up to its level of solubility. In one test where the ester was apparently kept in solution at sufficient quantities to obtain a result, rainbow trout appeared the most sensitive with a 96 h LC50 of 3.12 mg ae/L. The more soluble ethyl ester (2,4-D EE) was highly toxic to the non-standard species Mozambique tilapia with a 96 h LC50 of 0.63 mg ae/L. Several non-reviewed results for 2,4-D EE, 2,4-D BE, 2,4-D BEE and 2,4-D IPE confirm the highly toxic nature of the 2,4-D esters to fish, both freshwater and marine. A single acute amphibian test on leopard frog tadpoles for 2,4-D EHE again resulted in a conclusion that this substance was not toxic up to its limit of solubility. One chronic study on fathead minnow with 2,4-D EHE showed a NOEC of 0.079 mg ae/L, indicating moderate toxicity.

Several acute aquatic invertebrate toxicity results were reviewed for 2,4-D EHE and one for 2,4-D BE. Again, results for the former were often difficult to interpret due to inconsistent recoveries of the test substance and the main conclusion is that 2,4-D EHE is unlikely to be toxic up to its level of solubility. Non-reviewed results for other esters included 2,4-D EE, 2,4-D BE, 2,4-D BEE and 2,4-D IPE and covered a range of aquatic invertebrates. Results for 2,4-D BE, 2,4-D BEE and 2,4-D IPE indicate these esters are moderately (approaching highly) toxic with the most sensitive result being a 96 h LC50 of 1.04 mg ae/L for 2,4-D BE to the stonefly. Two chronic test results, both to *Daphnia magna*, are available. The reviewed study for 2,4-D EHE resulted in a MATC of 0.013 mg ae/L with a 21 d EC50 of 0.036 (moderate toxicity), while the non-reviewed result for 2,4-D BEE showed this ester to be less sensitive with a MATC of 0.31 mg ae/L.

Five standard tests for different algae and the duckweed *Lemna gibba* were available for 2,4-D EHE. There were significant problems interpreting the obtained test results due to very low recoveries of test material. The marine diatom, *S. costatum*, and duckweed appeared to be the most sensitive to this ester with EC50s of 0.15 and 0.33 mg ae/L respectively (highly toxic). Generally, 2,4-D EHE did not appear toxic up to its limit of solubility. The same species were tested with 2,4-D BEE (reports not reviewed), but showed this ester to be less toxic to algae. Again, the marine diatom, *S. costatum* was the most sensitive algae with an EC50 of 1.02 mg ae/L. Duckweed was the most sensitive species overall with an EC50 of 0.4 mg ae/L, indicating it to have around the same level of toxicity to this species as 2,4-D EHE.

2,4-D EHE was at worst, slightly toxic to bees through either contact or oral exposure routes with LD50 values of >64.3 and >62.9 µg/bee respectively. A non-standard test using 2,4-D EE to an Indian worker bee show this chemical to have an LD50 of

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0.013% v/v, but this value can not readily be translated to a rate per bee or a rate per hectare. No other bee toxicity data were available for any other esters. 2,4-D EHE was harmless to the predatory mite up to the highest rate tested of 564 g ae/ha. However, at this rate it was harmful to the parasitic wasp, causing 100% mortality of adults. It was harmless to this species at the next lowest rate tested of 28 g ae/ha. No other ester form of 2,4-D was tested on any other beneficial insect. Additionally, no ester toxicity data to earthworms or soil micro-organisms were available.

Some data were provided for 2,4-D in its EHE and BEE ester forms to non-target vegetation through seedling emergence and vegetative vigour studies with other results reported but not reviewed for this assessment. Terrestrial plants were shown to be very sensitive to 2,4-D. In *in vitro* seedling germination studies, radicle length was the most sensitive indicator. In soil assays, dicots were more sensitive, and plants were generally more sensitive in the actively growing phase (vegetative vigour studies). Results for vegetative vigour studies, particularly for 2,4-D EHE, indicate 2,4-D esters will have significant impacts on actively growing plants, often at relative low rates (<30 g ae/ha).

Salts:

Acute oral toxicity results were available for 2,4-D DMA, 2,4-D DEA, 2,4-D IPA, 2,4-D TIPA and 2,4-D sodium salt, although only studies for 2,4-D DMA and 2,4-D sodium salt were reviewed. The only standard test was with 2,4-D DMA to bobwhite quail and showed this chemical to be moderately toxic with an LD₅₀ of 415 mg ae/kg bw. The other tests reviewed were non-standard and conducted on the domestic chicken and pigeon. The pigeon was most sensitive to 2,4-D DMA with an LD₅₀ of 334 mg ae/kg bw, but the sodium salt was practically non-toxic to both these non-standard species. Other results where data were not reviewed indicate the 2,4-D DMA is practically non-toxic to the mallard duck and only threshold concentrations were obtained for the IPA and TIPA salts to mallard duck (LD₅₀ >314 mg ae/kg bw) and bobwhite quail (LD₅₀ >219 mg ae/kg bw) respectively.

Four short term toxicity tests were reviewed for 2,4-D DMA salt, with no defined short term LD₅₀ able to be derived up to 1899 ae ppm for either species in old, non-standard tests or >4655 ae ppm to either species in standard short-term tests. In addition to these reviewed data, seven other results were reported confirming this lack of toxicity through the diet. These results covered the 2,4-D DMA, DEA, IPA and TIPA salts, and indicate that in general, 2,4-D salts should not be toxic to birds when consumed in the diet.

No avian reproductive tests were available for any of the 2,4-D salts.

Several acute fish toxicity results were reviewed for 2,4-D DMA salt and one for 2,4-D sodium salt. Results were in good agreement that both these salts were practically non-toxic to fish (96 h LC₅₀s >100 mg ae/L) with the exception of a non-standard test using 2,4-D DMA salt on the non-standard species, Mozambique tilapia with a 96 h LC₅₀ of 35.6 mg ae/L (slightly toxic). A total of eighteen other non-reviewed results for the 2,4-D DMA, DEA, IPA, TIPA and Sodium salts all added to the weight of evidence that 2,4-D in its salt forms will not be toxic to fish with all definitive results showing 96 h LC₅₀s >80 mg ae/L. A single acute amphibian test on leopard frog tadpoles for 2,4-D DMA salt again resulted in a conclusion of low toxicity with the 96 h LC₅₀ of 188 mg ae/L. Two chronic studies reviewed for 2,4-D DMA resulted in a 28 d NOEC of 100 mg ae/L to rainbow trout and a 31 d MATC of 18.3 mg ae/L to

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fathead minnow while results of one non-reviewed test showed a MATC of 36.3 mg ae/L. Both indicate very slight toxicity.

Several acute aquatic invertebrate toxicity results were reviewed for 2,4-D DMA salt and all results tended to support a conclusion of this compound being practically non-toxic to aquatic invertebrates with defined LC50s >100 mg ae/ha. One older study resulted in an EC50 somewhere between 85-131 mg ae/L. In addition, fourteen non-reviewed tests provided results generally supportive of limited toxicity. The most sensitive species tested appeared to be the eastern oyster with a 96 h LC50 of 49.6 mg ae/L with 2,4-D IPA and 89.1 mg ae/L for 2,4-D TIPA (both slightly toxic).

Generally, however, defined LC50s were >100 mg ae/L for non-reviewed studies for 2,4-D DMA, DEA, IPA and TIPA salts. Two chronic test results, both to *Daphnia magna*, were reviewed resulting in MATC values of 73.1 and 33.6 mg ae/L. This very slight toxicity was supported with a non-reviewed test result of an MATC >16.05 mg ae/L for 2,4-D DEA salt to *Daphnia magna*.

Algae and aquatic plants were the most sensitive aquatic organisms to 2,4-D salts. Seven standard tests for different algae and the duckweed *Lemna gibba* were available for 2,4-D DMA along with supporting results for the 2,4-D DEA, IPA and TIPA salts. Generally, these salts were slightly to practically non-toxic to the simpler freshwater and marine algae. The most sensitive algae species appeared to be the freshwater diatom *N. pelliculosa* with a (unreviewed) EC50 of 4.38 mg ae/L from 2,4-D DMA salt, and the green alga *S. capricornutum* with an EC 50 of 7.48 mg ae/L to the 2,4-D DEA salt (both moderately toxic). The vascular aquatic plant, duckweed (*L. gibba*) was more sensitive with 2,4-D DMA and DEA salts being highly toxic to this plant with EC50s of 0.48 and 0.30 mg ae/L respectively. The only other salt for which a result is available to duckweed was 2,4-D TIPA with an EC50 of 1.3 mg ae/L.

2,4-D DMA was slightly toxic to bees through either contact or oral exposure routes with LD50 values of >83.3 and 78 µg/bee respectively. Non standard tests using 2,4-D DMA and 2,4-D sodium salt to an Indian worker bee showed these chemicals to have an LD50 of 0.14% v/v and >3% w/v respectively, but these values can not readily be translated to a rate per bee or a rate per hectare. No other bee toxicity data were available for any other salts.

2,4-D DMA salt was harmless to a range of non-target terrestrial arthropods up to the highest rates tested, which in all cases was at least 1000 g ae/ha. Similarly, earthworms were not affected at rates up to 500 mg ae/kg soil when exposed to 2,4-D DMA salt, making this chemical at worst, slightly toxic to earthworms. 2,4-D DMA salt was shown to have no significant impact on soil respiration or ammonification and nitrification of soil when applied at a rate up to 13.3 mg ae/kg soil. No other salt form of 2,4-D was tested on any other beneficial insect or soil micro-organisms.

Data were provided for the 2,4-D DMA salt with testing on seedling emergence, and other data for 2,4-D in various amine salt forms to non-target vegetation through seedling emergence and vegetative vigour studies are reported but were not reviewed for this assessment. Terrestrial plants were shown to be very sensitive to 2,4-D. In soil assays, dicots were more sensitive, and plants were generally more sensitive in the actively growing phase (vegetative vigour studies) with a non-assessed study for the 2,4-D DEA salt reporting EC25 values ranging from 3.4-50.4 g ae/ha.

7 Preliminary Risk Assessment

When assessing risk, every case cannot be accounted for, so DEH follows an iterative process by considering:

- a ‘worst case’ scenario, and, if needed,
- a series of refinements which account for other factors and results in setting more realistic scenarios at each step.

The worst case estimate is performed in this preliminary risk assessment and should identify the sensitive environmental compartment(s) most at risk from exposure to the chemical. If these environmental compartments are not at risk (ie the Q-value is acceptable), then no further assessment is needed. If at the preliminary stage, insufficient data are available to characterise risk, then no refined risk assessment has been conducted.

DEH uses a quotient approach. In general, where there are sufficient data and acute toxicity endpoints are used for the Q-value, values of 0.1 or less are considered acceptable. Where chronic toxicity values are used, Q-values of 1 or less are considered acceptable. In the case of terrestrial plants, a Q-value of 0.1 or less based on EC25 data will be considered in the preliminary assessment. Where further refinement is required, a representative NOEL will be determined based on the range of data, and a Q-value of 1 or less will be considered acceptable.

7.1 Terrestrial Preliminary Risk Assessment

7.1.1 Avian Toxicity

Birds did not appear to be sensitive to 2,4-D through either oral or dietary exposure. Considering the latter route, no definitive LC50 was determined at highest concentrations tested in any test reviewed by DEH or cited in US EPA, 2005. In terms of acid equivalent dose, all birds tested through dietary studies had an LC50 >1899 ppm. The lowest NOEC was 466 ppm to Mallard duck when exposed to 2,4-D in its DMA salt form, but generally, NOECs obtained were 1000 ppm or greater.

Non standard tests conducted on the pigeon showed this bird to be the most sensitive from acute oral exposure with acute LD50s of around 350 mg ae/kg bw for esters and salts, and around 1000 mg ae/kg bw for 2,4-D acid.

Given that the most probable route of exposure to birds will be through dietary uptake of 2,4-D residues contained in treated plant material or prey, and due to extensive data available for dietary exposure, dietary endpoints will be used in the risk assessment.

Dietary Q values for a single application of 2,4-D at the maximum registered label use rate of 4500 g ae/ha estimated by the updated Kenaga nomogram (Pfleeger *et al*, 1996) have been determined. This nomogram is based on a review of the highest levels of pesticide residues reported from spray application and represents an upper limit to residues likely to be found on various substrates.

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Environmental Compartment	Concentration fresh weight (mg ae/kg)
short grass	964.3
leaves and leafy crops	542.4
forage crops	542.4
small insects	542.4
grain/long grass	441.0
Pods with seeds	60.27
large insects	60.27
fruit	60.27

Quail total residues are based on a diet of 30% small insects and 70% grain. Mallard total residues are based on a diet of 30% grain (same as long grass) and 70% large insects.

Based on the application and diet described here, the Kenaga nomogram predicts residues in quail and mallard duck diets of 471.4 and 174.5 mg/kg fresh weight respectively. The dietary LC50s return acute Q values of 0.25 and 0.09 respectively. This indicates a low risk to mallard ducks and a potential risk to bobwhite quail. However, the dietary LC50 used to base this on was a threshold value of >1899 ppm in the diet (2,4-D DMA in the product DMA-4). These were the lowest threshold concentrations obtained from an old study where the test material was not characterised and acid equivalent concentrations have essentially been estimated based on information in other studies using the same product name. The corresponding NOEC from this study was 879 ppm in the diet (again estimated from other information). The Q value using the 5 day NOEC is 0.54. Even though this is not a chronic NOEC, it is still <1. The NOEC was based on observations of wing droop and lethargy at 1899 ppm (ae), the next highest concentration tested. This was the highest concentration and resulted in 20% mortality.

Corresponding tests on bobwhite quail and mallard duck where the test material (2,4-D DMA) was well characterised showed dietary LC50s >4665 ppm for both species. Using these more valid results for the DMA salt, dietary Q values for quail and mallard are 0.1 and 0.04 respectively. Other forms of 2,4-D for which test results are available all show that no defined LC50 could be calculated due to a lack of mortality at the highest tested rates, all >3000 ppm acid equivalent.

The very high rates of application are prescribed for use in pasture situations including rights of way and industrial areas with ester products. With the exception of one product registered for sugar cane at 3640 g ae/ha, field crop application rates are usually below 2500 g ae/ha including sugar cane with all other products. At this application rate, residues in quail and mallard diets are reduced to around 262 and 97 mg/kg fresh weight respectively. Using the threshold dietary LD50 of 1899 ppm results in Q values of 0.14 and 0.05 respectively. Further, it is unlikely that birds would be obtaining all their diet from the treated area consistently and a conclusion of low acute risk to birds is considered acceptable.

This conclusion is further supported when considering the reproduction NOEC to bobwhite exposed to 2,4-D acid was 1000 ppm.

Australian labels are generally silent on the issue of repeat applications. There are likely situations where repeat applications may occur, eg, pastures. However, the

short half-lives of 2,4-D and its various forms in the environment should preclude the opportunity for chronic exposure. Field half-lives from application to pastures and turf averaged 10 days or less with longer half-lives usually attributed to the use of granule formulations. In this regard it is important to note that no commercial granule formulations are registered in Australia. Two granule products containing 2,4-D are registered for home lawn use. Application rates of these products are low (equivalent to 165 g ae/ha) and instructions are to apply to wet foliage then water in the product after 24 h. Exposure to birds is expected to be limited.

The risk to birds through chronic exposure is therefore predicted to be acceptable.

7.1.2 Bees and other terrestrial invertebrates

No standard oral or contact toxicity tests were provided for the free acid form of 2,4-D. Standard oral and contact toxicity tests were performed on the 2-EHE and DMA salt forms of 2,4-D with all results showing that 2,4-D is likely to not be toxic to bees. The highest defined toxicity was exhibited through the oral route of 2,4-D DMA to bees with a 72 h LC50 of 78 µg ae/bee. The more relevant contact route did not result in defined toxicity values. 2,4-D EHE and 2,4-D DMA were both tested through contact routes to bees with LD50's >64.3 and >83.3 µg ae/bee respectively. In the 2,4-D EHE study, at the highest rate of 64.3 µg ae/bee, 35% mortality was found. Based on the dose/response observed in this study, plotting dose vs mortality, a linear relationship was found ($r^2 = 0.92$), and an LD50 of around 98 µg ae/bee can be extrapolated.

In the 2,4-D DMA experiment, the only mortality observed in the treatment groups was 3% in the 83.3 µg ae/bee group. With the exception of one bee in this group that was immobile on day 0, all other surviving bees in the five treatment groups were normal in appearance and behaviour throughout the test period. This suggests the LD50 will be well in excess of 83.3 µg ae/bee.

Given the insensitivity of bees to the 2,4-D DMA salt through the contact route, the extrapolated value of 98 µg ae/bee from the EHE test will be used as the end point for the risk assessment.

Where the quotient is <1, risk to bees and pollinating insects is considered to be acceptable. Assuming that a honeybee is approximately 1 cm² in surface area (Davis and Williams 1990), an application rate of 4500 g ae/ha is equivalent to 45.0 µg/cm². This results in a Q value of 0.46. This very high rate is prescribed for ester formulations used in non-legume pastures, rights of way and industrial areas. Table 29 below provides maximum registered broadcast application rates for other 2,4-D forms. With the exception of improved pastures, sugar cane, lawns/turf and pre-harvest potatoes, application rates tend to be around 1100 g ae/ha or less. The predominant registered rate for improved pastures, sugar cane and lawns/turf are between 2000-2750 g ae/ha (1620 g ae/ha for pre-harvest potatoes). These rates result in a deposition rate of 20-27 µg/cm² and give Q values of 0.27-0.20 further indicating an acceptable risk to bees. The lower rates for field crops of 1100 g ae/L or less result in deposition of 11 µg/cm² or less, and Q values of ≤0.11.

Results for terrestrial arthropod toxicity tests suggest that the ester forms are more toxic to insects than salt forms of 2,4-D. The standard parasitic wasp species was tested using the same test system for both 2,4-D DMA and 2,4-D 2-EHE and maximum label application rates for their respective formulations (not Australian). For 2,4-D DMA, reduction in beneficial capacity was such that this chemical is

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considered harmless up to its use rate of 1800 g ae/ha. However, the maximum rate of 564 g ae/ha for 2,4-D 2-EHE resulted in 100% mortality of adult wasps prior to the reproductive phase of the test. Unfortunately, this was a tier I test performed in the laboratory with exposure from application to glass plates. No higher tier testing was performed to establish the effect mitigating factors in the field may diminish toxicity (eg, interception, conversion of the ester to 2,4-D acid through hydrolysis, degradation or dissipation).

2,4-D EHE was harmless to the predatory mite at its maximum application rate for the test of 564 g ae/ha. 2,4-D DMA was harmless to the predatory mite, spiders and the rove beetle up to 1800, 1056 and 1000 g ae/ha respectively.

Clearly, application rates in Australia can be significantly higher than those tested in laboratory non-target invertebrate tests. Using 2,4-D DMA as a surrogate for all other salt forms of 2,4-D, it can be stated that no in-field adverse impacts on non-target invertebrates should be expected when the application rate of 2,4-D in its salt forms is 1000 g ae/ha or less, which would be the case for the majority of broadcast use patterns in Australia (see Table 29 below).

Very limited testing is available for the 2,4-D EHE and no tests on non-target arthropods are available for other ester forms. At broadcast application rates for 2,4-D EHE uses in Australia, adverse effects in-field to terrestrial arthropods can not be ruled out. However, limited results in this area are likely to overestimate toxicity and effects in the field are not expected to be as severe, particularly due to the rapid conversion of 2,4-D esters to the acid form in soils. Nonetheless, where non-target arthropods are exposed through plant surfaces rather than from soil exposure, this conversion may not occur and effects may be expected. Without higher tier testing, risks can not be quantified further.

Off-target adverse effects on non-target arthropods are not expected. The maximum registered application rate of 2,4-D salts is 3640 g ae/ha in sugar cane. Even drift of 10% (highly unlikely) would result in deposition of 360 g ae/ha, well below levels likely to cause adverse effects.

Off-target effects of 2,4-D esters again can not be ruled out due to the low tier level testing and limited results available. However, mitigation factors found in the field as discussed above are expected to limit any effects from drift to non-target areas. A deposition rate of 28 g ae/ha equates to 0.6% drift at the highest ground application rate of 4500 g ae/ha, or 0.9% for the maximum registered aerial application rate of 3200 g ae/ha. Downwind buffer zones are prescribed in the refined risk assessment below for other environmental compartments that will result in drift significantly less than these values. However, without higher tier testing, risks can not be quantified further for the ester formulations.

The risk to off-target adverse effects to non-target terrestrial arthropods is considered acceptable. There are insufficient data for ester formulations to be able to quantify risks to non-target arthropods and adverse effects to these organisms can not be ruled out.

7.1.3 Soil-dwelling invertebrates

Earthworms were insensitive to 2,4-D exposure as both the free acid and in its DMA salt form with LD50s in excess of 300 mg ae/kg dw soil.

An application rate of 4500 g/ha results in residues of 3.46 mg/kg soil if distributed through the top 10 cm of soil (density 1.3 g/cm²).

The lack of persistence of 2,4-D in soils indicates accumulation will not be an issue. The short term concentration of 3.46 mg/kg results in Q values of 0.01 for 2,4-D acid and salts (using DMA salt as a surrogate).

There are no data for toxicity of ester forms of 2,4-D to earthworms. However, studies assessed in this report indicate that in soils, 2,4-D EHE will hydrolyse rapidly with a half-life of <1.5 hours (Conch *et al.*, 1993c; McCoy and Lehman, 1998). Field half-lives for 2,4-D EHE, while not as quick as the laboratory values, were still fast. Of the 29 soil results available (not counting granule formulations) for forestry, pasture, wheat, turf and bare ground field dissipation studies, half-lives of 2,4-D EHE ranged from 0.34-12.9 days with an average of 3.1 days. Only two half-lives were >5.2 days. Conversion was primarily to the 2,4-D acid.

7.1.4 Soil Microorganisms

No data have been provided for 2,4-D acid or any of its ester forms. Two studies, one addressing soil respiration and the other addressing soil nitrification, were provided for 2,4-D DMA when applied in a formulation containing 500 g/L 2,4-D acid and demonstrated that 2,4-D should have no significant impact on soil respiration or ammonification and nitrification of soil when applied at a rate up to 13.3 mg ae/kg soil.

DEH has used these results as a surrogate for 2,4-D in both its acid and ester forms. 2,4-D DMA along with other 2,4-D salts dissociates very quickly to form 2,4-D acid, so the toxicological properties are expected to be similar. Similarly, the 2,4-D esters have been shown to rapidly breakdown in soils (not as quickly as the salt forms, but still with rapid half-lives as discussed in soil-dwelling invertebrates above), again producing the 2,4-D acid.

As shown with soil dwelling invertebrates above, the maximum application rate of 4500 g ae/ha results in a theoretical concentration in the top 10 cm of soil of 3.46 mg ae/L. This is almost 4 times lower than the maximum rate tested that was shown not to impact soil microorganisms. This along with the lack of persistence of 2,4-D in soil indicates the risk to soil microorganisms is low.

7.1.5 Non-Target Terrestrial Plants

Seedling emergence test data were received for 2,4-D acid, 2,4-D EHE, 2,4-D BEE and 2,4-D DMA. Vegetative vigour test data were received for 2,4-D acid, 2,4-D EHE and 2,4-D BEE. In addition, the US EPA reports data for 2,4-D IPE and 2,4-D DEA. No data are available for the EE and BE forms.

The most sensitive EC25 (expressed as g/ha) are as follows:

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Table 23: Most Sensitive Terrestrial Plant Toxicity Results (EC 25, g ae/ha).

	Seedling Emergence		Vegetative Vigour	
	Monocot	Dicot	Monocot	Dicot
2,4-D Acid	1640	33.6	<8.4	8.4
2,4-D DMA	50.7	22.3	No data available	
2,4-D DEA ¹	426	50	44.8	3.4
2,4-D EHE	270	33.6	244	11.2
2,4-D BEE	434 ²	237 ²	212	24
2,4-D IPE ¹	11.2	0.9	226	1.4

- 1) The test reports for these chemicals have not been provided to the APVMA.
- 2) The definitive test also tested seed germination and effects on radicle length. These were *in vitro* studies not considered representative of real exposure. Nonetheless, effects were severe with EC25 values for monocots and dicots <1 g ae/ha. The relevance of these effects is discussed in the refined assessment below.

By far the most sensitive result in the above table is that for seedling emergence from the 2,4-D IPE test where an EC25 of 0.9 g/ha to the lettuce was found based on shoot length.

These values have to be used as surrogates for EE and BE in the absence of test data. With a maximum ground application rate of 4500 g/ha in pastures, a drift or volatilization rate of 5% moving to a neighbouring field or stand of native vegetation would result in an amount available for deposition of 250 g/ha. This would clearly result in an unacceptable risk and risk to non-target terrestrial plants will be considered in the refined risk assessment.

7.2 Aquatic Preliminary Risk Assessment

The following is for non-aquatic uses. Aquatic uses are considered separately.

7.2.1 Fish

NB This is considered protective of amphibians also based on reviewed data relating to effects on tadpoles.

Table 24. Acute Fish Toxicity Endpoints For Risk Assessment.

Test species	System	LC50 (mg ae/L)
2,4-D Acid Tidewater silverside (<i>M beryllina</i>)	96 h flow through	175 (m)
2,4-D Esters Bluegill sunfish (<i>L. macrochirus</i>) ¹	96 h static	0.23
2,4-D Salts Mozambique tilapia (<i>T mossambica</i>) ²	96 h static	35.6

- 1) This is the lowest definitive result obtained through the ECOTOX data base for the butyl ester. Even though the study has not been reviewed by DEH, there is strong weight of evidence from other results from this data base. The lowest definitive result of a study reviewed by DEH was an LC50 of 0.63 ppm (nominal) for ethyl ester, which although obtained from a non-standard test, is still considered valid due to no control mortality and a clear dose-response curve. 2,4-D in its butyl ester and iso-butyl ester form are well represented amongst registered products in Australia. Therefore the use of this value is considered appropriate.
- 2) Definitive results for 2,4-D salts from tests reviewed by DEH overwhelmingly showed these forms to be practically non-toxic to fish. This is supported by weight of evidence from US EPA, 2005. However, the result used for the risk assessment showed higher than usual toxicity, and although the test was performed using non-standard guidelines, it is considered scientifically valid as no control mortality and a clear dose-response curve were observed.

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Worst case exposure assumptions include application to a standard pond of 1 ha surface area and average depth of 15 cm. Based on direct overspray, the following Q-values are determined:

2,4-D Acid. For an acceptable Q value of 0.1 or less, exposure from 2,4-D acid can not result in a concentration of more than 17.5 mg/L. The maximum registered rate of 2,4-D in acid equivalent terms resulting from broadcast use for non-ester labels is 3640 g/ha in sugarcane.

Direct overspray: 2.43 mg/L; Q value = 0.01 (Acceptable risk)

2,4-D Esters: For an acceptable Q value of 0.1 or less, exposure from 2,4-D esters can not result in a concentration of more than 0.023 mg/L. The maximum registered rate of 2,4-D in acid equivalent terms resulting from broadcast use for ester labels is 4500 g/ha in pasture.

Direct overspray: 3 mg/L; Q value = 13 (Unacceptable risk)

Acceptable drift: 0.77% drift would result in an exposure concentration of 0.023 mg/L resulting in an acceptable Q value of 0.1.

2,4-D Salts. For an acceptable Q value of 0.1 or less, exposure from 2,4-D salts can not result in a concentration of more than 3.56 mg/L. The maximum registered rate of 2,4-D in acid equivalent terms resulting from broadcast use for non-ester labels is 3640 g/ha in sugarcane.

Direct overspray: 2.43 mg/L; Q value = 0.07 (Acceptable risk)

CONCLUSION: The fish risk assessment will focus on ester forms of 2,4-D as currently registered broadcast applications using salt forms or labelled as 2,4-D acid result in an acceptable acute risk to fish under worst-case exposure calculations.

7.2.2 Invertebrates – Acute

Table 25. Acute Aquatic Toxicity Endpoints For Risk Assessment.

Test species	System	LC50 (mg ae/L)
2,4-D Acid		
<i>Daphnia magna</i>	48 h static	25.0
2,4-D Esters		
Stonefly (<i>Pteronarcella badia</i>) ¹	96 h static	1.04
2,4-D Salts		
Eastern oyster (<i>C. virginica</i>) ²	96 h	49.6 (m)

- 1) This is the lowest definitive result obtained through the ECOTOX data base for the butyl ester. Even though the study has not been reviewed by DEH, there is strong weight of evidence from other results from this data base. While there were several studies reviewed by DEH where lower results were obtained, these were all threshold values where no impacts were found at the highest rate tested and can not realistically be applied in the risk assessment. The lowest definitive LC50 in a test reviewed by DEH was 2.92 mg/L to *Daphnia magna* when exposed to 2,4-D 2-EHE. 2,4-D in its butyl ester and iso-butyl ester form are well represented amongst registered products in Australia and therefore, the use of this value is considered appropriate.
- 2) DEH reviewed several studies where definitive LD/EC50 concentrations were obtained for 2,4-D DMA salt. These showed this chemical to be practically non-toxic to aquatic invertebrates. The end-point chosen for the risk assessment is reported in US EPA, 2005 and was obtained for exposure to the IPA salt. Based on available data, it is considered a worst case for acute aquatic invertebrate exposure.

Worst case exposure assumptions include application to a standard pond of 1 ha surface area and average depth of 15 cm. Based on direct overspray, the following Q-values are determined:

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2,4-D Acid. For an acceptable Q value of 0.1 or less, exposure from 2,4-D acid can not result in a concentration of more than 2.5 mg/L. The maximum registered rate of 2,4-D in acid equivalent terms resulting from broadcast use for non-ester labels is 3640 g/ha in sugarcane.

Direct overspray: 2.43 mg/L; Q value = 0.097 (Acceptable risk)

2,4-D Esters: For an acceptable Q value of 0.1 or less, exposure from 2,4-D esters can not result in a concentration of more than 0.1 mg/L. The maximum registered rate of 2,4-D in acid equivalent terms resulting from broadcast use for ester labels is 4500 g/ha in pasture.

Direct overspray: 3 mg/L; Q value = 3 (Unacceptable risk)

Acceptable drift: 3.3% drift would result in an exposure concentration of 0.1 mg/L resulting in an acceptable Q value of 0.1.

2,4-D Salts. For an acceptable Q value of 0.1 or less, exposure from 2,4-D salts can not result in a concentration of more than 4.96 mg/L. The maximum registered rate of 2,4-D in acid equivalent terms resulting from broadcast use for non-ester labels is 3640 g/ha in sugarcane.

Direct overspray: 2.43 mg/L; Q value = 0.05 (Acceptable risk)

CONCLUSION: The aquatic invertebrates risk assessment will focus on ester forms of 2,4-D as currently registered broadcast applications using salt forms or labelled as 2,4-D acid result in an acceptable acute risk to aquatic invertebrates under worst-case exposure calculations.

7.2.3 Algae and Aquatic Plants

Table 26. Acute Algae/Aquatic Plant Toxicity Endpoints For Risk Assessment.

Test species	System	EC50 (mg ae/L)
2,4-D Acid Duckweed (<i>L. gibba</i>) ¹	Assumed 14 d	EC50 = 0.695
2,4-D Esters Duckweed (<i>L. gibba</i>) ²	14 days	0.33
2,4-D Salts Duckweed (<i>L. gibba</i>) ³		0.3

- 1) The APVMA was not provided with this study, reviewed and reported in US EPA, 2005. The result is significantly more sensitive than other algal results reviewed by DEH so will be used as the endpoint for 2,4-D Acid in the risk assessment.
- 2) The APVMA received several studies for algae and duckweed for 2,4-D 2-EHE. In all tests, there was extremely low recovery of test material and often the NOEC was greater than the EC50 making the results questionable. The US EPA reported a further duckweed result for 2,4-D BEE that approximates that for 2,4-D 2-EHE. Therefore, the 2,4-D 2-EHE result has been chosen as the endpoint for the risk assessment (result is as nominal concentration).
- 3) The lowest definitive result reviewed by DEH was a 14 d EC50 of 0.48 ppm (measured) for 2,4-D DMA to duckweed. However, the DEA salt is registered in Australia, and the result chosen for the risk assessment is reported in US EPA, 2005.

Given the similarities in worst case results for the 2,4-D acid, esters and salts, a single EC50 of 0.3 mg/L will be used to assess risk. The difference in risk will therefore be a function of exposure.

Worst case exposure assumptions include application to a standard pond of 1 ha surface area and average depth of 15 cm. Based on direct overspray, the following Q-values are determined:

2,4-D Acid and salts. For an acceptable Q value of 0.1 or less, exposure from 2,4-D acid can not result in a concentration of more than 0.03 mg/L. The maximum registered rate of 2,4-D in acid equivalent terms resulting from broadcast use for non-ester labels is 3640 g/ha in sugarcane.

Direct overspray: 2.43 mg/L; Q value = 8.1 (Unacceptable risk)

Acceptable drift: 1.25% drift would result in an exposure concentration of 0.03 mg/L resulting in an acceptable Q value of 0.1.

2,4-D Esters: For an acceptable Q value of 0.1 or less, exposure from 2,4-D acid can not result in a concentration of more than 0.1 mg/L. The maximum registered rate of 2,4-D in acid equivalent terms resulting from broadcast use for ester labels is 4500 g/ha in pasture.

Direct overspray: 3 mg/L; Q value = 10 (Unacceptable risk)

Acceptable drift: 1% drift would result in an exposure concentration of 0.03 mg/L resulting in an acceptable Q value of 0.1.

CONCLUSION: The algae/aquatic plants risk assessment will focus on all 2,4-D forms.

8 Refined Risk Assessment

Based on results from the preliminary risk assessment, the refined assessment will focus on non-target vegetation and aquatic organisms from exposure to the ester formulations.

Statement of uncertainties

There are areas of uncertainty in the terrestrial and the aquatic risk assessments that could potentially cause an underestimation of risk.

1. Several products contain 2,4-D present as a mixture, for example, formulated products may include 2,4-D with other active constituents such as ioxynil, dicamba and/or mecoprop. The risk assessment is only focussed on 2,4-D and any additive or synergistic effect from the mixture, or individual toxicity characteristics of other active constituents have not be considered.
2. This assessment accounts only for exposure to 2,4-D, but not to its metabolites. In the event that metabolites are also toxic, the risk may be underestimated.
3. Full data sets are not available for all forms of 2,4-D. While significant information exists on the 2,4-D acid, 2,4-D DMA and 2,4-D EHE, outside these there are significant data gaps in many areas, eg, non-target terrestrial arthropods. While the chemicals tested have been used as surrogates for non-tested 2,4-D forms, this is an area of uncertainty where the risk may be underestimated.
4. For non-target terrestrial plants, a single value has been chosen to be protective of 95% of species, based on analysis of the full terrestrial plant data set including acid, salt and ester formulations. The validity of this assumption may be questioned, particularly for run-off exposure where the extent of run-off is a function of solubility. However, the view has been taken that esters, while significantly less soluble than 2,4-D acid, will hydrolyse quickly to the acid form in moist soils. Where application is made to dry soils, this assumption may be incorrect although it is largely supported by field evidence where soil half-lives for 2,4-D EHE ranged from 0.34-12.9 days with an average of 3.1 days (29 observations). The majority of results (27 observations) resulted in half-lives of 5.2 days or less, and an average of 2.5 days.

The aquatic refined assessment is conducted first as there are worst case spray drift calculations performed in this section that will be used and refined further in the terrestrial assessment.

8.1 Aquatic Refined Risk Assessment

The refined aquatic risk assessment will consider exposure to fish and aquatic invertebrates from ester formulations only, and exposure to algae and aquatic plants from all types of 2,4-D formulations. For the acute assessment, the most sensitive endpoints will be maintained:

Esters – fish most sensitive endpoint = 0.23 mg/L

Esters – aquatic invertebrates most sensitive endpoint = 1.04 mg/L

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All forms – algae/aquatic plants most sensitive endpoint = 0.3 mg/L

The current Australian freshwater trigger value for 2,4-D acid for the protection of aquatic ecosystems is 0.28 mg/L. This moderate-reliability trigger value, which was calculated using a statistical distribution approach with an application factor of 10.2, is based on aquatic animal data as no acceptable aquatic plant toxicity data were identified (ANZECC/ARMCANZ, 2000). The data available in this assessment indicate close agreement with the established ANZECC trigger value.

Exposure will be considered from two routes, aerial and ground based application. For algae/aquatic plants, while the endpoint is the same for both esters and salt formulations, application rates may vary depending on the type of application. Therefore, risk to algae/aquatic plants will be considered based on exposure from the maximum ester application rate.

Registered uses of 2,4-D products and application rates for the various formulations are summarised in Tables 28 and 29 respectively below. Table 29 shows a maximum ground broadcast field rate for ester formulations of 4500 g ae/ha (pastures – non legumes, rights of way, industrial) with a corresponding aerial rate of 3180 g ae/ha.

With the exception of turf/pasture, sugar cane and peanuts, broadcast application rates are 1620 g ae/ha (potatoes, next is 1140 g ae/ha for harvest aid) or less for 2,4-D ester labels.

In the first instance, the predicted environmental concentrations (PEC) will be based on these maximum application rates, with exposure to be considered through spray drift. To predict spray drift, AgDrift will be used for aerial application, and drift data from Rautmann *et al*, (2001) used for ground based equipment.

The following assumptions have been used in running this model based on the label information and in accordance with the work of Woods and Dorr (2000):

Table 27: AgDrift Input Parameters

Aircraft type	AT502
Boom height	3 m
Flight lines	25
Swath width	20 m
Swath displacement fraction	0.5
Wind speed	4.2 m/s (15 km/h)
Wind direction	90°
Temperature	30°C
Relative humidity	50%
Spray volume	20 L/ha
Surface roughness	0.0075
Droplet size	BCPC Fine – Dv _{0.5} 183.09 µm

Labels for the ester formulations suggest 10-90 L water/ha for aerial application. A value of 20 L/ha has been chosen for modelling purposes, and a fine droplet size is expected.

Drift exposure to a stream/water body 3 m wide with a depth of 15 cm is assumed.

Table 28: Registered Use Patterns by Formulation Type.

	2,4-D Ester Products	
	EHE	Short Chain ¹
Broadcast Use Patterns		
Preparatory spray – fallows & seed beds	No	No
Cereals (Barley, Wheat, Cereal rye, Triticale)	Yes	Yes
Oats	No	Yes
Harvest Aid ²	Yes	Yes
Stubble/Fallow ³	Yes	Yes
Maize, sweetcorn, sorghum, saccaline, broom millet, millet, grain sorghum	No	No
Improved Pastures containing clover	Yes	Yes
Pasture – Non-legume, Rights of Way, Industrial	Yes	Yes
Pasture – direct drilling or surface sowing	Yes	Yes
Pasture – spray graze techniques	No	No
Sugar cane – post emergence	Yes	Yes
Sugar cane – pre emergence	No	Yes
Peanuts	No	No
Rice	No	Yes
Carribbean and Common Stylo forage and seed crops	No	Yes
Potatoes – Pre-harvest	Yes	No
Citrus	No	No
Pears	No	No
Turf, Lawns, Playing fields	No	No
Aquatic	No	Yes
Other Use Patterns		
Spot spraying – all situations	Yes	Yes
Bananas	No	No
Timber regrowth control	No	No
Home lawn	No	No

1. Includes 2,4-D Ethyl Ester; Butyl Ester; Ethyl/Butyl Ester mixtures and Isobutyl Ester.
2. On Ester labels, includes winter cereals only. Includes maize and sorghum on amine salt labels.
3. On Ester labels, includes winter cereals, Maize, Peanuts, and Sweetcorn.

Table 29: Maximum Registered Application Rate for Broadcast Use Patterns by Formulation Type (g ae/ha)

	2,4-D Ester Products	
	EHE	Short Chain ¹
Broadcast Use Patterns		
Preparatory spray – fallows & seed beds		
Cereals (Barley, Wheat, Cereal rye, Triticale)	540 ²	560
Oats		376
Harvest Aid	1140	1120
Stubble/Fallow spray	540	540
Maize, sweetcorn, sorghum, saccaline, broom millet, millet, grain sorghum		
Improved Pastures containing clover	540	560
Pasture – Non-legume, Rights of Way, Industrial	4500 ³	4500 ^{3,4}
Pasture – direct drilling or surface sowing	3180	3200
Pasture – spray graze techniques		
Sugar cane – post emergence	1620	2400
Sugar cane – pre emergence		3200
Peanuts		
Rice		800
Carribbean and Common Stylo forage and seed crops		1040
Potatoes – Pre-harvest	1620	
Citrus – to avoid early fruit fall		
Pears – to avoid early fruit fall		
Turf, Lawns, Playing fields		
Aquatic		3200

1. Includes 2,4-D Ethyl Ester; Butyl Ester; Ethyl/Butyl Ester mixtures and Isobutyl Ester.
2. Three labels (56117, 53817 and 54813) have a maximum rate of 2220 g ae/ha while 2 labels (55923 and 42229) have the maximum rate above. The lower value in this case was chosen as the three labels with the higher rate appear less consistent to the majority of labels with this use pattern.
3. This is the maximum rate for ground application. The maximum rate for aerial spraying is 3200 g ae/ha.
4. A higher rate of 3520 g ae/ha is registered for use in Victoria only on some labels.

Based on calculations for both ester and salt formulations with the parameters from Table 27, AgDrift calculates drift at 20, 50 and 100 m downwind to be around 21, 12 and 7% respectively.

8.1.1 Risk from Drift

8.1.1.1 Acute, Aerial Application

Ester Formulations

The following Q values can be obtained for ester formulations:

Table 30: Q values to aquatic organisms from exposure to ester formulations at the highest aerial field rate of 3200 g ae/ha

Buffer zone	Drift (%)	PEC (mg/L)	Q _{fish}	Q _{aquatic invertebrates}	Q _{algae/aquatic plants}
20	20.1	0.44	1.91	0.42	1.47
50	12.0	0.26	1.13	0.25	0.87
100	7.2	0.15	0.65	0.14	0.50
200	3.7	0.08	0.35	0.08	0.27
300	2.0	0.043	0.19	0.04	0.14
400	1.18	0.025	0.11	0.02	0.08

Fish are the most sensitive aquatic organisms. AgDrift predicts that an acceptable Q value to fish will be achieved with a 400 m buffer zone where drift will be around 1.18%, the PEC will be around 0.025 mg/L, and the Q value around 0.11.

Impact on reducing application rates

The next highest application rates for ester formulations potentially applied by air (apart from aquatic uses that are considered separately) are 1620 g ae/ha for pre-harvest preparation for potatoes and 1140 g ae/ha for harvest aid uses.

AgDrift predicts that as the application rate reduces, the level of drift (all other things being equal) increases. For example, at 400 m downwind, the level of drift from the peanuts and the harvest aid/salvage spray rate are 2.1% (PEC 0.023 mg/L) and 2.5% (PEC 0.019 mg/L) respectively. Corresponding Q_{fish} values are 0.1 and 0.08 respectively, suggesting that at application rates above 1000 g ae/ha, a buffer of 400 m would be needed.

Impact of droplet size

Increasing droplet size in the AgDrift Model gives a good indication of reduction in drift rate with buffer zones. Revised drift values have been calculated by changing the parameters to a BCPC medium droplet (median diameter 300 µm) and a BCPC coarse droplet (median diameter 402 µm) with application in 30 L water/ha. The following results were obtained for fish:

Table 31: Q values to aquatic organisms from exposure to ester formulations at the highest aerial field rate of 3200 g ae/ha with medium and coarse droplet sizes.

Buffer zone	Medium Droplets			Coarse droplets		
	Drift (%)	PEC (mg/L)	Q _{fish}	Drift (%)	PEC (mg/L)	Q _{fish}
50 m	4.26	0.091	0.4	1.91	0.041	0.18
100 m	2.4	0.051	0.22	0.93	0.020	0.09
150 m	1.64	0.035	0.15			
200	1.14	0.024	0.10			

These results suggest the best way to reduce drift is to increase droplet size. A Q value to aquatic organisms is within acceptable limits at 100 m downwind with a median droplet size of 400 µm at the highest aerial application rate of 3200 g ae/ha.

Modelled values should only be used as a guide. With this type of screening level assessment, alteration of a number of input parameters will impact the result of the model. For example, the water body characteristics used are not representative of

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water bodies exposed from all the different terrestrial cropping situations 2,4-D is registered for. If an exposed stream 3 m wide had a depth of 50 cm, the PEC with a coarse droplet size at 100 m would be 0.006 mg/L (Q = 0.03).

Further mitigation arguments

It is worth considering the end-point used for fish toxicity was a non-reviewed result for the butyl ester form of 2,4-D. Based on several non-reviewed results and one non-standard test for the ethyl ester, the BE and EE forms appear much more toxic to fish than the 2,4-D EHE. The latter compound generally showed no treatment related effects up to its level of solubility. One study run for 8 days determined a 96 h LC50 of 3.12 mg ae/L and an 8 day LC50 of 2.03 mg ae/L. Using the 8 day value, the Q-value to fish would be acceptable at 100 m with a fine droplet spray. The PEC of 0.21 mg/L and the most sensitive endpoint for fish from 2,4-D EHE of 2.02 mg/L results in a Q-value of 0.1.

Nonetheless, the 100 m buffer with coarse droplets would still be necessary to protect algae/aquatic plants based on the derived endpoint of 0.3 mg/L.

Conclusion

To mitigate potential risk to algae/aquatic plants from exposure resulting from spray drift of 2,4-D from ester and salt formulations, products should be applied with a median droplet diameter of 400 µm or more, corresponding to a BCPC Coarse droplet size, and a buffer zone of 100 m between field edges and downwind water bodies should be maintained.

This is considered to be protective to amphibians and sediment dwelling organisms as well as those exposed only through the water column.

8.1.1.2 Acute, Ground Application

Ester Formulations

There are German studies (Rautmann *et al*, 2001) of drift trials carried out in field crops (including some vegetables) and orchards. Some of these were done on bare ground with other on cereals during late growth stages. In previous trials of this nature (Ganzelmeier *et al*, 1995), the applied amount of fluid was adjusted to be 300 L/ha at a driving speed of 6 km/h and pressure ranging from 2.4-2.5 bar (240-250 kPa). This is assumed to be the case for the latest trials included in this paper.

Estimates for drift are given as the 90th percentile of mean values, quoted as % of the application rate. In the following table these estimated drift percentages are shown at given distances from field crops. The PEC values have been calculated based on the predicted drift entering a water body of surface area 1 ha and depth 15 cm. The rate of application has been defined as 4500 g ac/ha. Table 32 provides drift values along with corresponding PECs and Q-values for a range of buffer zones:

Table 32: Q values to aquatic organisms from exposure to ester formulations at the highest field rate of 4500 g ae/ha

Buffer zone (m)	Drift (%)	PEC (mg/L)	Q _{fish}	Q _{aquatic invertebrates}	Q _{algae/aquatic plants}
5	0.41	0.012	0.05	0.01	0.04
10	0.20	0.006	0.03	0.006	0.02

The risk to aquatic organisms from 2,4-D Ester formulations resulting from drift from ground based application equipment is acceptable without a need for a buffer zone.

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Aquatic Use Pattern

Several products using the short chain ester forms of 2,4-D are registered for aquatic use. The ester labels have this use pattern; it is labelled in the Directions for Use as a specific use pattern “Aquatic Areas” and is for control of water hyacinth. The maximum registered rate is 3200 g ae/ha.

Direct Application

Direct application at the highest rate to water bodies 15 cm deep will result in water concentrations up to 2.2 mg/L.

Using the endpoints for aquatic organisms in Tables 24, 25 and 26, the following Q values are obtained:

Table 33: Aquatic Q-values for direct application to water at 3300 g ae/ha.

	Fish	Aquatic Invertebrates	Algae/Aquatic Plants
2,4-D Esters	9.6	2.11	7.3

These results show that 2,4-D esters will pose an unacceptable risk to these organisms.

8.1.1.3 Non Broadcast Use Patterns

Application in these situations is unlikely to involve significant exposure to aquatic areas. Some drift exposure may occur. However, this is unlikely to be at levels resulting in adverse impacts to nearby aquatic areas.

8.1.2 Risk from Run-Off

8.1.2.1 Broadcast Use Patterns

Risks from run-off will be considered for 2,4-D acid due to the rapid conversion of 2,4-D esters to the acid in moist soils through hydrolysis. The amount of run-off is difficult to predict as it is dependent on many factors such as meteorological conditions, agricultural practices, hydrological aspects, rainfall intensity, formulation type and infiltration capacity.

In a review of pesticides in run-off and surface waters, Burgoa and Wauchope (1995) concluded that pesticide losses under “normal” rainfall conditions account for 0.5% of the amount applied. However, in this review, they point to literature showing that while losses are typically 0.5% or less, they can be up to 5% or more under worst case conditions.

Table 29 above lists the maximum registered use rates for 2,4-D, and depending on the form of 2,4-D and use pattern, applications range from 300 to 4500 g ae/ha. Q-values based on runoff of 5 and 1% from a ten hectare catchment area to a pond with a surface area of one hectare and depth of 15 cm for algae/aquatic plants are shown below:

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Table 34: Q values to Algae and Aquatic Vegetation from Run-off of 2,4-D

Application rate (g ae/ha)	5% run-off		1% run-off	
	EEC (mg/L)	Q	EEC (mg/L)	Q
500	0.17	0.57	0.03	0.1
1000	0.33	1.1	0.07	0.23
2000	0.66	2.2	0.13	0.43
2200	0.73	2.4	0.15	0.5
2750	0.92	3.1	0.18	0.6
3200	1.1	3.7	0.22	0.73
4500	1.5	5.0	0.30	1.0

It is clear from these results that even at 1% run-off, a potential risk exists to aquatic organisms from exposure to 2,4-D in run-off. Work has been done to try and quantify the amounts of chemical in run-off. The model described by Birkved and Hauschild (2003) has been chosen for this risk assessment as it considers the Koc of the chemical and calculates the fraction of applied chemical likely to run-off with consideration of soil type (based on sand content), slope of fields and rainfall. An additional formula is provided to account for the dissolved fraction of pesticide in run-off waters. Applying this model using the lowest Koc (34) found for 2,4-D, rainfall of 50 mm, a slope of 3%, a medium soil type (sand content between 45-85%) and 1% OC, the model predicts 0.57% will run-off. The majority of this (75%) is predicted to be in solution.

The model uses a 10:1 catchment area (10 ha running off to 1 ha). The volume of receiving water was set at 4500 m³ (4.5 ML) to account for excess runoff entering the previously existing shallow water body. With a maximum application rate of 4500 g ae/ha, the model predicts the concentration in receiving waters will be 0.042 mg/L, resulting in a Q value of 0.14. This is approaching acceptable limits.

Within the confines of this model, application rates of 3200 g ae/ha or less would result in an acceptable Q-value to aquatic organisms. The only use patterns registered at rates higher than this are for the 2,4-D EHE products with application to pastures at 4500 g ae/ha and the 2,4-D Na salt product registered for use on sugar cane at 3640 g ae/ha. These two uses would result in Q values based on the above model of 0.14 and 0.11 respectively, both being considered a marginal risk only.

This model is sensitive to changes in factors such as slope and soil type. For example, a flatter slope of 1% results in total predicted run-off of 0.19% (0.14% in solution). This low level would result in an EEC of 0.014 mg/L and an acceptable Q value (0.05) even at the highest application rate of 4500 g ae/ha.

Donald *et al* 2005 describes mobilization of pesticides on an agricultural landscape flooded by a torrential storm. The Vanguard area of southeastern Saskatchewan, Canada, was subjected to a torrential storm in July 2000 that produced as much as 375 mm rain in an 8-hour period. In the previous 45 days, some 96 mm had been recorded, so chances were the soil was fairly moist thereby reducing capacity for infiltration. The majority of herbicides would have been applied to crops in this area during the four weeks preceding the storm. After the storm, 19 herbicides and insecticides were detected in flooded wetlands, with 14 of the detected in 50% or more of wetlands (2,4-D was found in 100% of wetlands tested). Average concentrations of 2,4-D were 362 ng/L with the authors stating that the pesticides were probably from long-range transport, followed by deposition in rain and from

application to crops within the area subjected to the storm (1700 km²). Interestingly, in the following year when only 62 mm of rain fell in the same 45 day period, only five pesticides were detected in 50% or more of wetlands. One of these was 2,4-D, found in 100% of wetlands at an average concentration of 535.6 ng/L (0.00054 mg/L).

The higher levels of 2,4-D detection with the much lower rainfall is possibly due to much less dilution than from the large storm event. It is difficult to correlate these values to a percentage of run-off. However, if these values are taken as indicative of concentration levels in Australian receiving waters after run-off events, then an acceptable risk to aquatic organisms is predicted (Q-values to algae/aquatic plants of <0.01 based on a water concentration of 0.00054 mg/L)

Modelling predictions supported by field measurements following storm events indicate that risk to aquatic organisms through run-off is acceptable.

8.1.2.2 Aquatic Use Patterns

Not applicable in this case.

8.1.2.3 Non Broadcast Use Patterns

Non broadcast uses for 2,4-D include (from Table 28 above), spot spraying in all situations.

Application in these situations is unlikely to involve significant exposure to aquatic areas through run-off.

8.2 Terrestrial Refined Risk Assessment

The refined risk assessment for the terrestrial environment will focus on risk to non-target terrestrial plants.

8.2.1 Discussion on end point to be used in the assessment.

Test data indicate a wide range in sensitivity of plants to 2,4-D in its various forms. The general trend for all studies was that monocots were less susceptible than dicots, although in some cases, monocot species were also extremely sensitive.

As explained in the US EPA report (US EPA 2005), 2,4-D is a plant growth regulator that is absorbed through the roots and foliage within 4-6 hours and distributed throughout the plant via the xylem and phloem. Once in the plant it selectively eliminates broadleaf plants by mimicking the effect of plant growth regulating hormones. This action stimulates growth, which leads to an abnormal growth pattern and death in some plants. Therefore, if even a portion of the surface area of a non-target plant comes into contact with 2,4-D there is a possibility that the plant may be severely damaged or dies as a result. Even if the plant only exhibits minor damage, the damage may be sufficient to prevent the plant from reproducing or competing successfully with other plants for resources, including water. The use of 2,4-D could apply selective pressure against dicots along field edges resulting in changes in species composition.

Data available for plant germination

Seedling emergence studies provided for 2,4-D DMA, 2,4-D EHE and 2,4-D BEE tested seed germination by *in vitro* testing where germination was considered as a

function of the radicle length. Only the 2,4-D BEE study went on to measure effects on radicle length in the definitive study (still through *in vitro* exposure).

This end-point proved extremely sensitive. Preliminary results from the 2,4-D DMA and 2,4-D EHE test showed both monocots and dicots to have NOELs of 0.07-0.67 g ae/ha, with the exception of two monocot species with NOELs equal or greater to the highest tested rate of 6.7 g ae/ha. The definitive testing for 2,4-D BEE showed radicle length to be affected in both monocots (EC25 values ranging from 0.33-16.8 g ae/ha) and dicots (EC25 values ranging from 0.8-17.6 g ae/ha). For all monocots and dicots, the NOELs were 1 g ae/ha or less with radish being the most sensitive with a NOEL of 0.016 g ae/ha.

Impacts on radicles can potentially have serious consequences for plants. In most dicots the root develops from the radicle (lower end of the embryo). The radicle gives rise to an apical meristem, which continues to produce root tissue for much of the plant's life. By contrast, the radicle aborts in monocots, and new roots arise adventitiously from nodes in the stem.

The first indication that the processes of germination have begun is generally the swelling of the radicle. In all cases, the radicle imbibes water rapidly and, bursting the seed coats and other coverings that may be present, starts to grow downward into the soil. This helps to assure that the young seedling has an adequate supply of water and nutrients when the shoot breaks through the surface of the soil (Weier *et al*, 1982). An adverse impact on the radicle could be decisive over longer periods for susceptible plants. For example, more susceptible plants would likely develop weaker initial root systems possibly impacting on their competitiveness and altering population dynamics.

Conversely, it is recognised that the nature of the seed germination tests exaggerates the activity of 2,4-D. The seeds were germinated *in vitro* and exposed constantly to the chemical. Since 2,4-D is taken up by roots and moves systemically in the plant, exposure to a continuous low concentration of the compound can be accumulated and cause plant growth effects. It is therefore accepted that these effects will probably not be as pronounced in the field due to greater chance for dissipation and hence non-continuous exposure. However, drift/volatilisation exposure to non-target susceptible native vegetation is of concern and these effects should not just be ignored.

Given the uncertainty associated with extrapolating the *in vitro* results to a realistic exposure concentration in the field, the refined assessment will not include the available data on radicle length. However, the conclusions of the refined assessment will take into account any possible effects on this end-point.

Considering the range of data

In the preliminary assessment, only the most sensitive results were considered along with a single high drift rate of 5% from the maximum application rate of 4500 g ae/ha. For the refined assessment, given the wide range of data available and the wide range of application rates possible, this is not an appropriate method.

Use of a single value

In total, 110 NOEL values were used for the analysis. Data were log transformed. In many instances, NOELs could not be defined either because there were no effects at the highest treatment rate (17 observations, or 15.5% of observations), or because

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effects statistically different to the controls were found at the lowest rate tested (9 observations, or 8.2% of observations).

These censored data were dealt with as follows:

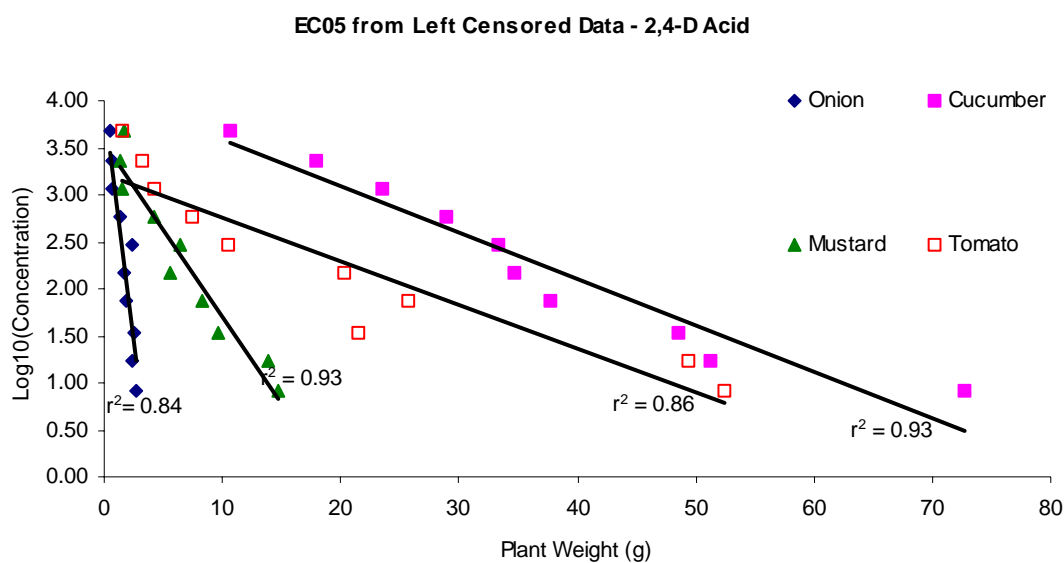
Left censored data

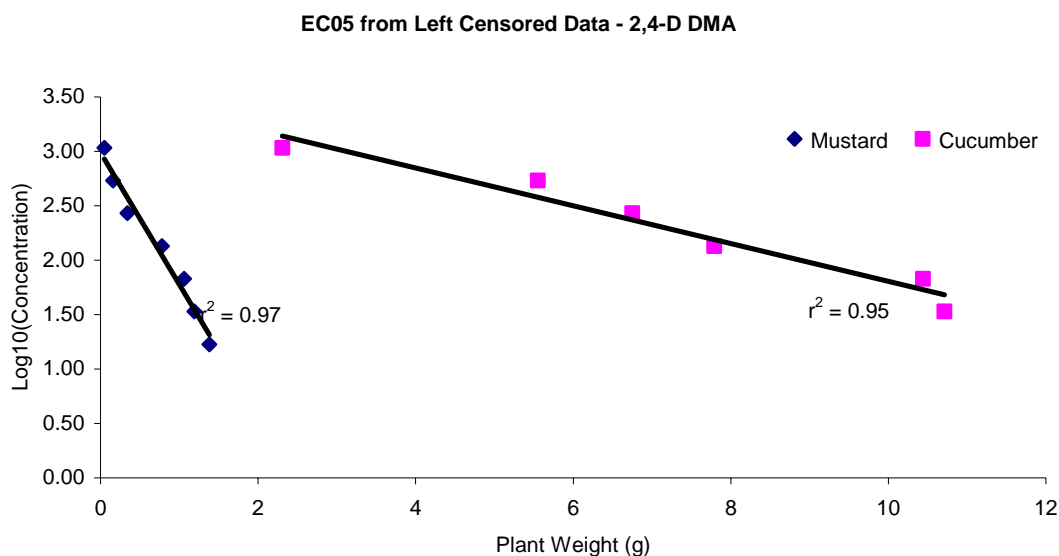
Vegetative vigour tests with 2,4-D acid resulted in four NOEL results being left censored data, that is, a NOEL was unable to be determined due to significant effects even at the lowest tested concentration.

To try and use more meaningful values in the risk assessment for these data, DEH has further analysed the raw data results. In many cases, values were difficult to read in the test report. However, best efforts were made to use the data where possible.

Onion (a monocot), cucumber (dicot), mustard (dicot) and Tomato (dicot) all had NOELs lower than the lowest treatment rate. Similarly for the 2,4-D DMA vegetative vigour studies, results for mustard and cucumber were left censored and were further analysed by considering the raw data.

In all cases, plotting Log[concentration] against weight (g) resulted in good linear dose-response relationships. The pooled control values were used for comparison, and an EC05 value used as the NOEL. The following data plots were obtained:





Based on the regression equations, the NOELs were determined as follows.

Table 35: Extrapolated EC05 values for Left Censored Data

	Onion	Cucumber	Mustard	Tomato	Mustard	Cucumber
Pooled control (Weight – g)	4.05	85.02	17.83	69.9	2.28	11.5
EC05 (g)	3.85	80.77	16.94	66.41	2.17	10.93
Concentration (g ae/ha)	1.3	1.2	2.8	1.3	2.3	44.1¹
r^2	0.84	0.92	0.93	0.86	0.97	0.95

1) This result ignored the apparent outlying results from the 16.8 g ae/ha group. Where these data were included, the NOEL was predicted to be 32.5 g ae/ha, but a much lower correlation co-efficient ($r^2 = 0.54$) was observed.

Additional left censored data were reported for the DEA salt with 2 values from the seedling emergence study and 1 value from the vegetative vigour study.

Unfortunately, no further analysis of these data were possible as the test reports were not provided. All three of these results appeared to have defined EC25 values. The two values from the seedling emergence study were significantly lower than other results available in this study and therefore, should be represented in the overall data set. A representative NOEL for these three results was arbitrarily set at 10% of the value of the defined EC50. This resulted in seedling emergence NOELs of 5 g ae/ha for mustard and buckwheat, and a vegetative vigour NOEL of 0.9 g ae/ha for cucumber.

Right Censored Data

A total of 17 NOEL results (15.5%) from the whole data set were right censored data, that is, no effects were found up to the highest level tested. It is not appropriate to disregard the right censored data as they represent the less sensitive results of the distribution. As no definitive value could be assigned to them, it was decided to treat the highest tested rate in each case as the NOEL.

Analysis of Data Sets

Data were analysed for the whole data set, and the sub-sets such as plant type, chemical type and test type.

The statistical analysis was performed using Microsoft EXCEL to determine the mean and standard deviation of each data set. The NOEL was then determined assuming

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the data were normally distributed around the mean, and using the 95th percentile as the NOEL (ie, the EC05 of each data set – see Attachment 2). This was based on the formula:

$$\text{LOG}_{10}\text{NOEL} = (z \times \text{Std. dev.}) + \text{mean}; \quad \text{where } z = -1.645(95\% \text{ confidence level}).$$

Table 36: 95th Percentile NOELs by Data Set (results provided in terms of g ae/ha).

	Whole data set	Test Type		Chemical Type		Plant Type	
		Seedling emergence	Vegetative vigour	Acids and Salts	Esters	Monocots	Dicots
No. obs.	110	60	50	50	60	40	70
Mean	71.4	149.7	29.3	88.1	59.9	343.8	29.0
Std dev	9.9	8.0	9.4	15.2	6.5	6.0	7.9
NOEL	1.6	4.9	0.73	1.0	2.7	18.2	0.97

On the basis of this analysis, plants were more susceptible in their post-emergent growth stage. The analysis confirmed the previous observation that dicots were significantly more sensitive than monocots and showed that acid/salt forms of 2,4-D were more toxic to plants at the lower end of the toxicity spectrum than ester formulations.

For the risk assessment from exposure to spray drift, a single representative value will be used. A representative NOEL of 0.73 g ae/ha will be used based on the sensitivity of plants in the vegetative vigour studies. Due to the use of a statistically derived representative NOEL, a Q value (PEC/NOEL) of 1 is considered acceptable. Therefore, an environmentally acceptable level of deposition from drift exposure is 0.73 g ae/ha.

This level is not considered too conservative for the following reasons:

1. Full life-cycles of plants including reproductive phases have not been tested. 2,4-D is a plant growth regulator and will therefore impact on the reproductive phase of plants.
2. Potential impacts to seedling germination at levels less than 1 g ae/ha should not be ignored. *In vitro* testing showed NOELs to several species of monocots and dicots of 0.67 and 0.07 g ae/ha based on radicle length. Where seeds are not incorporated into soil (such as expected in native stands of vegetation where seeds more likely remain on the soil surface), impacts on root development can not be ruled out.

8.2.2 Risk from Volatilisation

8.2.2.1 Broadcast Use Patterns

Off target movement from areas to 2,4-D application to non-target crops was a major issue raised in submissions received by the APVMA for this assessment. While there was little in the way of information received for off-target damage to native vegetation, it is unlikely such damage has ever actually been monitored. The reality is that with obvious non-target crop damage, damage to exposed native vegetation may also be expected.

Ester forms of 2,4-D

While public submissions to the APMVA seem to indicate the damage is through spray drift, it is quite likely that significant damage could actually occur due to

volatilization particularly where the ethyl ester, butyl ester and isobutyl ester formulations are used. These shorter chain esters are much more volatile than the longer chain esters and salt forms of 2,4-D. In addition, they persist in the atmosphere for long periods thereby increasing time and area of exposure.

Guth *et al* (2004) state that, based on direct measurements, no noticeable volatility can be expected from compounds with a vapour pressure below 10^{-3} Pa from soil and 10^{-4} Pa from crops, and this is fully confirmed by indirect measurements. The vapour pressures for EHE, EE and BE are 4.8×10^{-4} , 1.47×10^{-1} and 5.29×10^{-2} to 8.2×10^{-3} Pa respectively. This suggests that EHE may not volatilise from soil surfaces but may to some extent from crops, while the short chain EE and BE forms are expected to volatilise from both soil and crop surfaces. Further, the calculated Henry's Law Constants indicate the three esters may be moderately volatile to volatile from water bodies.

Australia has no formal model for quantifying volatilization of pesticides from soil or plant surfaces for regulatory use. However, this is an important issue for the short chain volatile esters. There are a number of models available to assess this endpoint. One such model is the EVA (Exposure Via Air) 2.0 model from Europe, and may have potential for use in a regulatory environment. This model is currently being evaluated through the European Union FOCUS air workgroup, and advice on this model is being sought.

One recent and publicly available model from the Netherlands can be used to give an indication of likely volatilization.

Volatilization from Crop Surfaces

In this model, described in van der Linden *et al*, 2004, volatilization from plant leaves is calculated as a function of the percentage of the application rate and the fraction intercepted by the crop. Calculations are as follows:

$$\text{Log}(CV_{\text{crop}}) = 1.661 + \log(P_{\text{sat}})$$

Where:

CV_{crop} = the cumulative volatilization, (% of amount reaching the crop);

P_{sat} = the saturated vapour pressure of the substance, (mPa), $P_{\text{sat}} \leq 11.8$ mPa.

Where $P_{\text{sat}} > 11.8$ mPa, the cumulative volatilization is taken to be 100%; substances having such a high P_{sat} are not likely to be sprayed on crops.

From this calculation, the total amount of substance volatilized from the crop is calculated according to:

$$E_{\text{crop, air}} = (CV_{\text{crop}} \times f_1 \times A)/100$$

Where:

$E_{\text{crop, air}}$ = the total amount volatilised from the crop (g/ha);

A = the nominal rate for a single application (g/ha)

f_1 = the fraction intercepted by the crop, (-); and

100 = factor to convert from % to fraction.

Vapour pressures for the ester forms registered in Australia are as follows:

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2,4-D EHE	2,4-D EE	2,4-D BE
0.48 mPa	147 mPa	52.9 mPa

Therefore, CV_{crop} for both EE and BE will be 100% for the calculations.

Calculations on PECs have been performed with the following assumptions. These assumptions are not scientifically based. The results will be used for comparative purposes only between the different ester forms.

- 1 Crop interception is 50%;
- 2 The amount of chemical volatilised will be “diluted” in the environment. In this regard, lift-off from one hectare will be assumed, to deposit over 10 ha of non-target area.

Based on the above calculations, the CV_{soil} for EHE was calculated to be 7% (100% for EE and BE). The following table shows $E_{\text{crop, air}}$ values based on different application rates and crop interception of 50%.

Table 37: Volatilisation from Crops Based on Ester Type and Application Rate.

Application rate (g ae/ha)	$E_{\text{crop, air}}$ - EHE	$E_{\text{crop, air}}$ - EE	$E_{\text{crop, air}}$ - BE
4500 (pastures)	158 g/ha	2250 g/ha	2250 g/ha
3200 (sugar cane)	112 g/ha	1600 g/ha	1600 g/ha
1620 (potatoes)	57 g/ha	810 g/ha	810 g/ha
1140 (harvest aid/salvage spray)	40 g/ha	570 g/ha	570 g/ha
1040 (forage crops)	37 g/ha	520 g/ha	520 g/ha
800 (winter cereals)	28 g/ha	400 g/ha	400 g/ha
560 (winter/summer cereals)	20 g/ha	280 g/ha	280 g/ha

Volatilization from Soil Surfaces

2,4-D will often be applied to bare soil. However, evidence indicates the esters will rapidly hydrolyse to 2,4-D acid (half-lives <1.5 hours in laboratory testing) thereby limiting their availability for volatilization from this route.

Given this, only exposure from volatilization from crop surfaces will be considered in more detail below.

Calculation of Risk Quotients

From the calculations in Table 37 above, a Q value for non-target plants can be derived by “diluting” these values over a 10:1 area ratio, and comparing to the generic endpoint of 0.73 g ae/ha discussed above.

The following Q values are derived:

Table 38: Q values for Different 2,4-D Esters to Non-Target Terrestrial Plants

Application rate (g ae/ha)	Q values- EHE	Q values - EE	Q values - BE
4500 (pastures)	21.6	308	308
3200 (sugar cane)	15.3	219	219
1620 (potatoes)	7.8	111	111
1140 (harvest aid/salvage spray)	5.5	78	78
1040 (forage crops)	5.1	71	71
800 (winter cereals)	3.8	55	55
560 (winter/summer cereals)	2.7	38	38

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These calculations demonstrate the much greater risk posed to non-target vegetation from the shorter chain esters. Of further concern is their persistence with modelling suggesting the atmospheric half-life in air of 2,4-D ethyl ester is in the order of 2 days. The atmospheric half-life in air for 2,4-D BE is around 1.7 days. For a chemical that migrates significantly through air, its half-life in air should be greater than 2 days (POPs screening criteria). Therefore, both these chemicals may be anticipated to travel long distances in the environment through air.

Characteristic travel distance (CTD) is a useful way of predicting the distance a chemical may travel in an environmental medium (water or air). It uses the concept of overall persistence in the environment (P_{ov}), a parameter that needs to be modelled, and considers the fraction of the chemical in the medium of interest. Absolute values are not considered appropriate in this case due to the large number of assumptions made in the modelling. However, the outcome in terms of ranking the chemicals relative to each other for their potential to move through the atmosphere is considered useful. Beyer and Matthies (2002) describe methodology for calculating CTD as:

$$CTD = \mu \times M_m/M_{tot} \times P_{ov}$$

Where:

μ = flow velocity of the medium (eg, wind speed);

M_m = mass of chemical in the medium (in this case air);

M_{tot} = total mass of chemical;

P_{ov} = overall persistence in the environment.

The velocity μ is simply a linear factor transforming the residence time into a measure in units of a length. The aim of this exercise is to demonstrate the propensity of the short chain esters to travel in relation to the longer chain, less volatile ester.

Therefore, μ will simply be taken as 1 in the equation as it is the ranking of the chemicals, not absolute values that are of interest.

For illustrative purposes, the overall persistence of 2,4-D EE, BE and EHE have been modelled by the Level III fugacity model from the US EPA PBT Profiler (www.pbtprofiler.net). Fractions of chemical in the air (M_m/M_{tot}) are taken from calculations already described above. Based on this, the following results are found:

Chemical	2,4-D EHE	2,4-D EE	2,4-D BE
P_{ov} (days)	168	59	65
M_{air}/M_{tot}	0.07	1	1
CTD (km)	11.8	59	65

In terms of ranking, it is predicted that 2,4-D EE and 2,4-D BE will move through the atmosphere more than five times the distance of the longer chain, less volatile 2,4-D EHE.

This is of particular concern given the very sensitive nature of non-target terrestrial plants (including both native vegetation and non-target crops) to these substances. The WHO review of 2,4-D (IPCS, 1989) states that the highly volatile ethyl, isopropyl, and butyl esters are being replaced by low-volatile esters or by amine salts to reduce crop damage resulting from 2,4-D vapour drift, and to decrease atmospheric pollution. This does not appear to have happened in Australia, however, the evidence from this risk assessment suggests this is a highly appropriate course of action. The

issue of persistence in the atmosphere, high volatility and potential to travel long distances in the environment along with their toxicity to non-target vegetation makes it very difficult to mitigate the risk from these compounds.

8.2.2.2 Aquatic Use Patterns

A measure of a chemical's propensity to move from water to the surrounding air may be made by its Henry's Law Constant. Based on the scale provided by Mensink *et al*, 1995, chemicals are moderately volatile from water where their Henry's Law Constant is between 2.45×10^{-7} – 7.34×10^{-4} atm.m³/mol, and highly volatile at $>7.34 \times 10^{-4}$ atm.m³/mol.

Henry's Law Constants for the short chain esters are 3.25×10^{-7} , 4.88×10^{-7} and 1.25×10^{-7} atm.m³/mol for ethyl ester, butyl ester and isobutyl ester respectively indicating they may be moderately volatile from water bodies. The Henry's Law Constant for 2,4-D EHE is 1.82×10^{-5} atm.m³/mol, a value indicative of moderate volatility from water bodies. This is a concern for the use of esters in aquatic situations, including the 2,4-D EHE, although in Australia, this chemical is not registered for aquatic uses.

8.2.2.3 Non Broadcast Use Patterns

No further assessment has been undertaken for this aspect. Volatility aspects of the chemicals discussed above for broadcast use patterns are transferable to non-broadcast use patterns.

8.3 Conclusions

Preliminary calculations using worst-case exposure showed that risks to birds, terrestrial invertebrates (2,4-D salt forms), soil dwelling invertebrates and soil microorganisms were acceptable with the registered uses and forms of 2,4-D. Risks to fish and aquatic invertebrates were considered acceptable when exposed to 2,4-D in its acid or salt forms under worst case exposure conditions. There were insufficient data to adequately characterise risk to terrestrial invertebrates from 2,4-D esters.

The preliminary assessment showed an unacceptable risk to non-target terrestrial vegetation and to fish and aquatic invertebrates through exposure to ester forms of 2,4-D. Additionally, risk to aquatic plants and algae was shown to be unacceptable to 2,4-D in all its forms based on worst case exposure. These end points were therefore considered in more detail in the refined risk assessment.

The refined aquatic risk assessment showed an acceptable risk to fish and aquatic invertebrates to ester forms of 2,4-D and algae/aquatic plants to ester forms of 2,4-D where aerial application used coarse spray droplet sizes (median droplet diameter of 400 µm) and a buffer zone of 100 m between treated areas and downwind water bodies was maintained for terrestrial use patterns. This was considered to be protective to amphibians and sediment dwelling organisms as well as those exposed only through the water column. Using ground application, risk to aquatic organisms was considered acceptable without the need for downwind buffer zones.

The refined aquatic risk assessment for aquatic uses identified an unacceptable risk to all aquatic organisms from use of ester products.

The refined risk assessment for the terrestrial compartment focussed only on non-target vegetation. A significant data base of effects to field crops through seedling emergence and vegetative vigour studies were available, and statistical analysis of

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these data determined a representative NOEL of 0.73 g ae/ha should be protective of 95% of species. This value was used in the risk assessment. Risk to non-target terrestrial vegetation was considered through several routes including volatilisation, spray drift (ground application) and run-off for three use pattern types, broadcast, aquatic and non-broadcast.

An assessment of volatilisation determined that the short chain esters may result in a high risk to non-target terrestrial vegetation through long range transport and their persistence in the atmosphere. The longer chain EHE is less of a risk through this route. Further, the ester forms are expected to be moderately volatile (short chain) to volatile (2,4-D EHE) from water.

Ground application for broadcast uses of 2,4-D were modelled and demonstrated that buffer zones of 100 m with the use of coarse droplet sizes should be protective for non-target vegetation for the majority of the use patterns.

Risks from run-off were difficult to model in any meaningful way given the range of possible application rates, use patterns and potentially different site characteristics. However, it was demonstrated that a potential risk to non-target plants from this exposure route could exist in the wrong conditions. For example, sites with larger slopes, less sandy soils or soils with lower organic carbon contents will have more run-off than flatter, sandier and higher organic carbon soils. Some of these may result in sufficient run-off to cause adverse effects in stands of non-target vegetation.

Risks to non-target terrestrial plants and aquatic organisms from non broadcast use patterns are considered acceptable.

9 Recommendations

Short chain ester forms. Due to the propensity of the short chain esters to volatilise and persist in the environment thereby causing off target damage, DEH recommends to the APVMA that they NOT be satisfied that continued use of or any other dealings with the active constituents 2,4-D Ethyl Ester, 2,4-D Butyl ester or 2,4-D isobutyl ester or products containing these active constituents in accordance with the instructions for use, would not be likely to have an unintended effect that is harmful to animals, plants or thing or to the environment.

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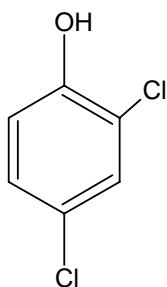
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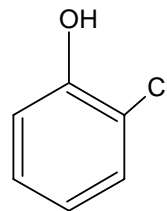
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Attachment 1 – Metabolite Chemical Structures

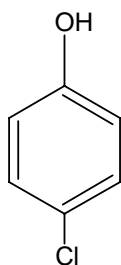
2,4-Dichlorophenol (**2,4-DCP**)



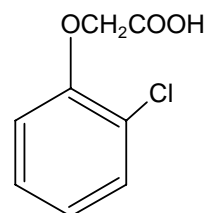
2-Dichlorophenol (**2-DCP**)



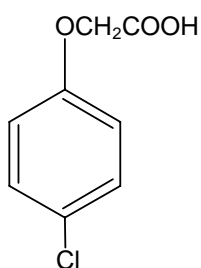
4-Dichlorophenol (**4-DCP**)



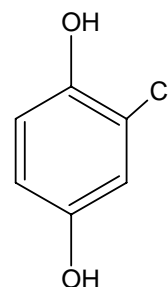
2-Chlorophenoxyacetic Acid (**2-CPA**)



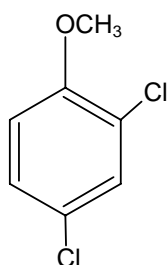
4-Chlorophenoxyacetic Acid (**4-CPA**)



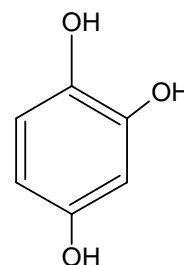
2-Chlorohydroquinone (**CHQ**)



2,4-Dichloroanisole (**2,4-DCA**)



1,2,4-Benzenetriol



2,4-D Review – Preliminary Review Findings

Attachment 2 – Plant Toxicity Data – Full Data Sets and Sub-Sets

LOG₁₀(NOEL) values for plant toxicity data set (reported to 4 decimal places):

Full Data Set	Results by Test Type		Results by Chemical Type		Results by Plant Type	
	Seedling Emergence	Vegetative Vigour	Acid/Salts	Esters	Monocots	Dicots
-0.3010	-0.3010	-0.0458	1.2253	-0.3010	1.5263	1.2253
-0.0458	0.0000	0.0792	1.2253	0.0000	2.4814	1.2253
0.0000	0.3617	0.1139	1.2253	0.7993	2.6232	1.2253
0.0792	0.6990	0.1139	1.5263	0.8195	2.7324	2.0043
0.1139	0.6990	0.3424	2.0043	1.0864	2.9243	2.1644
0.1139	0.7993	0.4472	2.1644	1.2253	2.9243	2.3284
0.3424	0.8195	0.4472	2.3284	1.2304	3.3711	2.3284
0.3617	1.0864	0.4472	2.3284	1.4216	3.3711	2.4314
0.4472	1.2253	0.4472	2.4314	1.5263	3.3711	2.4314
0.4472	1.2253	0.6021	2.4314	1.5441	3.3711	2.7024
0.4472	1.2253	0.7482	2.4814	2.0170	3.0334	3.0700
0.4472	1.2253	0.7482	2.6232	2.0531	3.0334	3.0700
0.6021	1.2304	0.7482	2.7024	2.1303	1.0492	1.6444
0.6990	1.4216	0.8274	2.7324	2.1303	2.3284	0.3617
0.6990	1.5263	0.8388	2.9243	2.1461	2.7738	0.6990
0.7482	1.5263	0.8751	2.9243	2.4314	2.9243	0.6990
0.7482	1.5441	0.8751	3.0700	2.4314	0.1139	3.0334
0.7482	1.6444	0.9243	3.0700	2.4314	3.2253	3.6721
0.7993	2.0043	0.9243	3.3711	2.4472	3.6721	0.3424
0.8195	2.0170	0.9243	3.3711	2.4472	3.6721	0.4472
0.8274	2.0531	1.0492	3.3711	2.4472	0.7993	0.4472
0.8388	2.1303	1.2253	3.3711	2.4472	2.0170	0.7482
0.8751	2.1303	1.2253	1.6444	2.4472	2.1303	0.7482
0.8751	2.1461	1.2279	0.3617	2.7324	2.1303	0.9243
0.9243	2.1644	1.2279	0.6990	3.0334	2.4314	0.9243
0.9243	2.3284	1.4502	0.6990	3.0334	2.4472	1.2253
0.9243	2.3284	1.4654	3.0334	3.0334	2.4472	-0.0458
1.0492	2.4314	1.5263	3.0334	2.7482	2.4472	0.1139
1.0864	2.4314	1.5441	3.0334	2.7482	3.0334	0.0792
1.2253	2.4314	1.5441	3.6721	2.7482	3.0334	0.4472
1.2253	2.4314	1.5441	0.3424	0.4472	2.7482	-0.3010
1.2253	2.4314	1.5441	0.4472	0.6021	1.4502	0.0000
1.2253	2.4472	1.5441	0.4472	0.7482	1.5441	0.8195
1.2253	2.4472	1.5441	0.7482	0.8274	1.8293	1.0864
1.2253	2.4472	1.5441	0.7482	0.8388	2.4314	1.2253
1.2279	2.4472	1.8062	0.9243	0.8751	2.4472	1.2304
1.2279	2.4472	1.8293	0.9243	0.8751	3.0334	1.4216
1.2304	2.4814	2.0531	1.0492	0.9243	3.0334	1.5263
1.4216	2.6232	2.3284	1.2253	1.2253	2.7482	1.5441
1.4502	2.7024	2.4314	2.3284	1.2279	2.7482	2.0531
1.4654	2.7324	2.4472	2.7738	1.2279		2.1461
1.5263	2.7324	2.7482	2.9243	1.4502		2.4314

2,4-D Review – Preliminary Review Findings

Full Data Set	Results by Test Type		Results by Chemical Type		Results by Plant Type	
	Seedling Emergence	Vegetative Vigour	Acid/Salts	Esters	Monocots	Dicots
1.5263	2.7482	2.7482	-0.0458	1.4654		2.4314
1.5263	2.7482	2.7738	0.1139	1.5263		2.4472
1.5441	2.7482	2.9243	0.0792	1.5441		2.4472
1.5441	2.9243	3.0334	0.4472	1.5441		2.7324
1.5441	2.9243	3.0334	0.1139	1.5441		3.0334
1.5441	3.0334	3.2253	3.2253	1.5441		2.7482
1.5441	3.0334	3.6721	3.6721	1.5441		2.7482
1.5441	3.0334	3.6721	3.6721	1.5441		0.4472
1.5441	3.0334			1.5441		0.6021
1.5441	3.0334			1.8062		0.7482
1.6444	3.0334			1.8293		0.8274
1.8062	3.0700			2.0531		0.8388
1.8293	3.0700			2.4314		0.8751
2.0043	3.3711			2.4472		0.8751
2.0170	3.3711			3.0334		0.9243
2.0531	3.3711			3.0334		1.2253
2.0531	3.3711			2.7482		1.2279
2.1303	3.6721			2.7482		1.2279
2.1303						1.4654
2.1461						1.5263
2.1644						1.5441
2.3284						1.5441
2.3284						1.5441
2.3284						1.5441
2.4314						1.5441
2.4314						1.5441
2.4314						1.8062
2.4314						2.0531
2.4314						
2.4472						
2.4472						
2.4472						
2.4472						
2.4472						
2.4472						
2.4814						
2.6232						
2.7024						
2.7324						
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2.7482						
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2.7482						
2.7482						
2.7482						

2,4-D Review – Preliminary Review Findings

Full Data Set	Results by Test Type		Results by Chemical Type		Results by Plant Type	
	Seedling Emergence	Vegetative Vigour	Acid/Salts	Esters	Monocots	Dicots
2.7738						
2.9243						
2.9243						
2.9243						
3.0334						
3.0334						
3.0334						
3.0334						
3.0334						
3.0334						
3.0334						
3.0700						
3.0700						
3.2253						
3.3711						
3.3711						
3.3711						
3.3711						
3.6721						
3.6721						
3.6721						

The statistical analysis was performed using Microsoft EXCEL to determine the mean and standard deviation of each data set. The NOEL was then determined assuming the data were normally distributed around the mean, and using the 95th percentile as the NOEL (ie, the EC05 of each data set). This was based on the formula:

$$\text{LOG}_{10}\text{NOEL} = (z \times \text{Std. dev.}) + \text{mean}; \quad \text{where } z = -1.645(95\% \text{ confidence level}).$$

The results, expressed in terms of g ae/ha, were found as follows:

	Whole data set	Test Type		Chemical Type		Plant Type	
		Seedling emergence	Vegetative vigour	Acids and Salts	Esters	Monocots	Dicots
No. obs.	110	60	50	50	60	40	70
<i>Results – Log10(g ae/ha):</i>							
Mean	1.8534	2.1753	1.4672	1.9448	1.7773	2.5363	1.4632
Std dev	0.9967	0.9031	0.9741	1.1817	0.8141	0.7761	0.8967
<i>Results – g ae/ha</i>							
Mean	71.4	149.7	29.3	88.1	59.9	343.8	29.0
Std dev	9.9	8.0	9.4	15.2	6.5	6.0	7.9
NOEL	1.6	4.9	0.73	1.0	2.7	18.2	0.97