Australian Pesticides & Veterinary Medicines Authority

The reconsideration of approvals of the active constituent atrazine, registrations of products containing atrazine, and their associated labels.

SECOND DRAFT FINAL REVIEW REPORT

Including additional assessments

October 2004

Australian Pesticides &Veterinary Medicines Authority

Canberra
Australia

This review report for products containing atrazine is published by the Australian Pesticides and Veterinary Medicines Authority. For further information about this review, or the Pesticides Review Program, contact:

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FOREWORD

The 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 Code 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 Code.

The basis for the reconsideration is whether the APVMA is satisfied that continued use of the active constituent atrazine and products containing atrazine 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; and
- would not unduly prejudice trade or commerce between Australia and places outside Australia.

The requirements for continued approval of a label for containers for a chemical product are that the label contains adequate instructions. Such instructions include:

- the circumstances in which the product should be used;
- how the product should be used;
- times when the product should be used;
- frequency of the use of the product;
- the withholding period after the use of the product;
- disposal of the product and its container;
- safe handling of the product.

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

In undertaking reconsiderations (also known as reviews), the APVMA works in close cooperation with advisory agencies including the Office of Chemical Safety (OCS), the Department of Environment and Heritage (DEH), and State Departments of Agriculture as well as other expert advisors, 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. Under this program it is proposed that countries receiving these reports will not utilise them for registration purposes unless they are also provided with the raw data from the relevant applicant.

This document is Part 1 of '*The reconsideration of approvals of the active constituent atrazine, registrations of products containing atrazine and their associated labels*' and relates to all products containing atrazine that have been nominated for review by the APVMA. The review's findings and 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 draft review report containing the APVMA's preliminary assessments and the technical reports from its advisory agencies for all registrations and approvals relating to atrazine are available from the APVMA website:

http://www.apvma.gov.au/chemrev/chemrev.shtml.

COMMENT FROM THE PUBLIC IS INVITED

The APVMA invites persons and organisations to submit their comments and suggestions on this draft review report directly to the APVMA. Your comments will assist the APVMA in preparing the final report.

The draft review report contains a review summary which outlines the APVMA review process, gives information to the public about how to respond to the review, summarises the technical assessments from the reviewing agencies and outlines the proposed regulatory action to be taken in relation to the continued registration of atrazine products. It also contains the full technical assessment reports from the Office of Chemical Safety (OCS), the Department of Environment and Heritage (DEH) and the Residues section of the APVMA.

In most cases the review summary should provide sufficient detail to enable response to the review. However, further details are available in the full technical reports if required.

PREPARING YOUR COMMENTS FOR SUBMISSION

You may agree or disagree with or comment on as many elements of the report as you wish.

When making your comments:

- clearly identify the issue and clearly state your point of view;
- give reasons for your comments supporting them, if possible, with relevant information and indicate the source of the information you have used;
- suggest to the APVMA any alternative solution you may have for the issue.

Please try to structure your comments in point form referring each point to the relevant section in the Review Summary or the technical report. This will help the APVMA assemble and analyse all of the comments it receives.

Finally please tell us whether the APVMA can quote your comments in part or in full.

THE CLOSING DATE FOR SUBMISSIONS IS: 25 February 2005

Your comments should be mailed to:

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ACRONYMS AND ABBREVIATIONS

ACPH	Australian Committee for Pesticides and Health
ADI	Acceptable Daily Intake
ai	active ingredient
ANZECC	Australian and New Zealand Environment and Conservation Council
ARfD	acute reference dose
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
BMP	Best Management Practices
bw	body weight
CAR	Catchment Area Ratio
Codex	FAO/WHO Codex Alimentarius Commission
DALA	Days After Last Application
DEA	Desethylatrazine
DEH	Department of Environment and Heritage
DIA	Desisopropylatrazine
ESI	Export Slaughter Interval
FAO	Food and Agriculture Organisation of the United Nations
FHRMG	Forest Herbicide Research Management Group
GAP	Good Agricultural Practice
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LC50	The concentration at which 50% of a test population dies
LOD	Limit of Detection
LOQ	Limit of analytical Quantitation, also referred to as limit of determination
LOR	Limit of Reporting
µg	microgram
mg	milligram
MRL	Maximum Residue Limit
NEDI	National Estimated Dietary Intake
NESTI	National Estimated Short-Term Intake
NH&MRC	National Health and Medical Research Council
NOEL	No Observed Effect Level
OCS	Office of Chemical Safety
OH&S	Occupational Health and Safety
OECD	Organisation for Economic Cooperation and Development
PACSC	Pesticide and Agricultural Chemical Standing Committee
PMRL	Provisional MRL
PPE	Personal Protective Equipment
ppm	parts per million
SAP	Scientific Advisory Panel
TMRL	Temporary MRL
US EPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WHO	World Health Organisation
WHP	withholding period

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EXECUTIVE SUMMARY

Introduction

Atrazine is a selective systemic herbicide that can be used both pre- and post-emergence for the control of grass and broadleaf weeds. It is mainly absorbed through the roots of plants and then transported to the actively growing tips and leaves, although some foliar absorption occurs. Atrazine kills the plant by inhibiting photosynthesis.

In Australia, atrazine is used to control weeds in summer crops such as sorghum, maize and sugarcane, and it is also widely used in Western Australia for control of weeds in lupins. Other uses include control of weeds in lucerne, grass seed, pasture and potatoes. Atrazine is also important in the establishment of pine and eucalypt plantations and for control of Parthenium weed in Queensland, Northern Territory and northern parts of New South Wales. A relatively new and major use pattern for atrazine is the application to triazine tolerant (TT) canola.

There are currently 35 registered products and six active constituent approvals (refer Appendices 1 & 2).

The review of atrazine was announced in December 1995 as part of the APVMA's first cycle of review chemicals. The active constituent atrazine, products containing atrazine, and their product labels were placed under review due to concerns over:

- human and animal carcinogen claims;
- moderate potential chronic toxicity risk;
- potential to contaminate ground and surface water;
- absence of maximum residue limits (MRLs) for major commodities; and
- reported breakdown in efficacy.

While these were the major reasons why a high priority had been given to the review of atrazine, the scope of the review covered all aspects for continued registration and approval of atrazine as prescribed by the Agvet Code.

There was a high level of public interest in the APVMA's review, with over 150 submissions received in response to the review announcement and call for information. The APVMA released an interim report in November 1997.

The interim report concluded that there were no major toxicological concerns relating to the use of atrazine and moreover, that atrazine poses no undue hazard to most users. As well, new conditions for use of atrazine were implemented in order to reduce chemical handling by workers, and reduce drift and runoff into water bodies. However, additional environmental and residue data were required to address remaining concerns related to the potential risk its use poses to the environment and the validity of a number of maximum residue limits (MRLs). Maximum residue limits are the maximum concentration of a chemical residue that is permitted in or on a food or food commodity.

Registrants were given up to three years to generate the required environmental and residue data. Assessment of these data led to the development of a draft final report, which was released for public comment in April 2002.

At the time of the release of the draft final report, a number of new overseas studies were published or foreshadowed that raised additional concerns that atrazine might cause adverse developmental and reproductive effects in frogs, such as endocrine disruption. Because of these new concerns, the APVMA delayed finalisation of the review and revisited the toxicological and environmental risks of using atrazine.

This current report discusses the action taken since the release of the 1997 interim report recommendations and the assessment of new information, including the required residues and environmental data, together with the new assessments centred on concerns raised by the frog studies. These assessments have not altered the conclusions of the April 2002 draft final report. Final recommendations are proposed regarding the registration and approval of atrazine in Australia.

Residues Assessment

The 1997 interim report required forage and fodder residue data consistent with Australian use patterns in order to determine withholding periods for grazing on sorghum, pastures and lucerne, and to confirm primary animal feed commodity MRLs. These parameters are required to assess residue levels on treated crops and therefore subsequent residues in animal commodities through use of crops for animal feed. This in turn allows an estimate of potential risks to human health through consumption of such commodities, and of potential risks to trade.

As an associated outcome of the review, new residue data for forage sorghum, grain sorghum and maize allowed the confirmation of MRLs to cover residues in primary animal feed and animal commodities. In addition, a new 28 day grazing withholding period is recommended for approved crop uses (except canola). Grazing and harvesting withholding periods for canola remain unchanged at 15 weeks when applied pre-emergence and six weeks post-emergence.

The residues assessment concluded that when atrazine is used according to the revised label directions, residues are unlikely to pose an unacceptable risk to human health.

Environmental Assessment

The environmental assessment considered the potential for atrazine to contaminate water bodies and its effects on the environment. It reviewed data from forestry industry studies on contamination of groundwater and surface water, and monitoring activities in annual cropping areas, and evaluated the environmental significance of atrazine residues in water. The potential effects of atrazine on amphibian development and sexual differentiation were also assessed.

It appears unlikely that atrazine, when used in accordance with the label recommendations, will contaminate waterways to any extent likely to present a hazard to the environment, or to human beings through the consumption of contaminated drinking water. Although levels of atrazine in water that increase during storms events may temporarily exceed the Australian and New Zealand Environment and Conservation Council (ANZECC) guidelines, long-term contamination at levels above the ANZECC guideline is unlikely.

Leaching studies at forestry sites and groundwater monitoring results indicate a low likelihood of groundwater contamination from atrazine use, even in areas of sandy soils. However, careless handling of atrazine concentrate and working solutions near bores or over permeable recharge areas could result in incidents of local groundwater contamination. This was highlighted in the 1997 interim report, with current atrazine labels already containing a restraint forbidding mixing, loading or application within 20 metres of a well, sinkhole, intermittent or perennial stream, or river. Best Management Practices (BMPs) play a key role in reducing chemical losses in runoff, especially in forestry production.

The key to minimising off-site transport of atrazine is to avoid use and handling on hard impermeable surfaces or in areas such as ephemeral drainage lines where water may flow. Accordingly, additional label statements are recommended in order to reduce runoff. If label directions are followed for atrazine use, atrazine concentrations in rivers and in groundwater aquifers should be below the relevant water quality guidelines.

Currently, there is widespread disagreement on whether atrazine affects amphibian development, and if so, the levels of exposure at which such effects may occur. Therefore, a conclusive risk assessment of the effects of atrazine in amphibians is not possible. Despite the reported developmental effects, there are inconsistencies between studies, and difficulty in independently replicating the low dose effects of atrazine in amphibians. It is also problematical to differentiate between the effects of atrazine and the likely influence of other stressors. Furthermore, it appears that healthy amphibian populations occur at sites where atrazine is present.

The US EPA has noted an inconsistency and lack of reproducibility across studies, and an absence of a dose-response relationship. The US EPA has sought additional data to reduce any uncertainty regarding the potential risk of atrazine to amphibians. The issue of atrazine and amphibians may be revisited if these additional data demonstrate that atrazine is likely to impact on frog populations at realistic levels of exposure. However, such outcomes are not considered likely.

Taken together, these data indicate that it is unlikely that atrazine is impacting adversely on populations of Australian amphibians at current levels of exposure.

Toxicological Assessment:

The original toxicology assessment was contained in the 1997 interim report and was not re-visited in the April 2002 draft final report. This additional assessment considered whether recent epidemiological and environmental reports on the carcinogenic, amphibian development and endocrine disruption potential of atrazine would change the human health assessment and recommendations of the 1997 interim report.

The 1997 interim report identified that atrazine caused neuroendocrine disruption in Sprague-Dawley (SD) rats, but that it did not bind to the oestrogen receptor or have any oestrogenic activity. Therefore, atrazine is unlikely to be an endocrine disruptor in humans, based on the known mechanism of action in SD rats. The latest assessment has not changed this conclusion.

It was concluded that the epidemiological data provided support for the absence of a carcinogenic potential for atrazine. The environmental reports are considered by the agencies that provide expert advice to the APVMA as unlikely to have a direct relevance to human health.

No changes to the existing health standards for atrazine are recommended.

Proposed Final Review Recommendations

After consideration of all data including the additional assessments, the APVMA accepts the recommendations of the DEH and the OCS, and the following regulatory actions are proposed:

a) Active constituent approvals are to be affirmed.
b) Existing label instructions are deemed to be inadequate and the most recently approved labels are to be amended as follows:
   - Buffer zone and precaution statements on labels are to be amended;
   - Withholding period instructions are to be amended;
   - Herbicide resistance reporting details are to be added to labels;
c) These variations to label instructions would then satisfy the requirements for continued registration of products; and so
d) Product registrations are to be affirmed.
e) Old approved labels are deemed not to contain adequate instructions and are to be cancelled.
f) As an associated outcome of the review, changes are to be made to the MRL standard.

1. INTRODUCTION

1.1 Regulatory status of atrazine in Australia

Atrazine is a triazine herbicide used for the control of grass and broadleaf weeds in crops such as sorghum, maize, sugarcane, and triazine tolerant (TT) canola. In addition, atrazine is widely used on lupins in Western Australia. Minor uses include control of weeds in lucerne, grass seed, pasture and potato crops. Atrazine is also important in the establishment of pine and eucalypt plantations, and for control of Parthenium weed in Queensland, the Northern Territory and northern parts of New South Wales.

Atrazine is one of the most widely used herbicides in the country. There are currently six active constituent approvals for atrazine (refer Appendix 1), 37 registered products containing the active constituent atrazine and 15 registrants (refer Appendix 2).

Formulation types include dry flowable, liquid, liquid concentrate, granular, wettable powder, water dispersible granule, and suspension concentrate.

1.2 Mode of action and toxicity of atrazine

Atrazine is mainly absorbed through the roots of plants and then translocated upward to the actively growing tips and leaves, although some foliar absorption occurs. In susceptible plant species, atrazine inhibits photosynthesis, while it is metabolised in tolerant plants.

Atrazine is slightly hydrophilic, with a water solubility of about 30 mg/L. It is moderately to highly mobile in soils with low clay or organic matter content. Because it does not adsorb strongly to soil particles and has a half-life ranging from 60 to greater than 100 days, atrazine has a high potential for water contamination, despite its moderate solubility in water.

Atrazine is highly persistent in soil, and can exist for longer than a year under dry or cold conditions. The primary breakdown route of atrazine is via chemical hydrolysis, followed by degradation by soil microorganisms. Atrazine can also have residual soil activity, which has the potential to cause toxicity to rotational crops if planted at an incorrect interval. Soybeans, vegetable crops, cereal grains, peanuts and potatoes are very sensitive to atrazine.

Atrazine is practically non-toxic to birds, slightly toxic to fish and some aquatic invertebrates, and moderately toxic to marine copepods and shrimp. It is highly toxic to some algae and aquatic vascular plants (e.g. duckweed). Atrazine is readily absorbed through the gastrointestinal tract and also through the lungs or the skin. The World Health Organisation (WHO) classifies it as a mild skin irritant and a severe eye irritant (WHO 1996). Overall, it is considered slightly to moderately toxic to humans and other mammals. At high doses, atrazine can cause neuro-muscular effects in laboratory animals, such as motor incoordination, limb paralysis, respiratory distress and hypothermia. The full toxicological assessment is contained in the 1997 interim report.

1.3 Reasons for review of atrazine

The review of the chemical atrazine was announced in December 1995 as part of the APVMA's first cycle of review chemicals. The active constituent atrazine, products containing atrazine, and their product labels, were placed under review due to concerns over:

- human and animal carcinogen claims;
- moderate potential chronic toxicity risk;
- potential to contaminate ground and surface water;
- absence of MRLs for major commodities; and
- reported breakdown in efficacy.

The scope of the review covered these specific issues as well as all aspects for continued registration and approval of atrazine as prescribed by the Agvet Code Regulations.

1.4 Scope of the review

The scope of this review was to determine whether the APVMA could be satisfied that the continued use of products containing atrazine in accordance with the instructions for their use would be unlikely to adversely affect human health, the environment, or trade.

1.5 Regulatory options

The basis for a reconsideration of the registrations and approvals for a chemical is whether the APVMA is satisfied that the requirements for continued registration and approval are being met, as specified by the Agvet Code. There can be three possible outcomes to the reconsideration of the registration of products containing atrazine and their labels. Based on the information reviewed, the APVMA may be:

- satisfied that the products and their labels continue to meet the prescribed requirements for registration and approval and therefore affirms the registrations and approvals.
- satisfied that the conditions to which the registration or approval is currently subject can be varied in such a way that the requirements for continued registration and approval will be complied with and therefore varies the conditions of registration or approval.
- not satisfied that the requirements for continued registration and approval continue to be met and suspends or cancels registration and/or approval.

2. INTERIM REPORT RECOMMENDATIONS

An interim report of the APVMA's findings from its review of existing data for atrazine was released in November 1997. The conclusions were as follows:

- non-agricultural/home garden uses were to be cancelled as they posed an undue risk to the environment;
- product labels were to be modified to include suitable warnings to protect the environment and worker safety; and

- modifications to recommended personal protective equipment were required to increase protection of users;
- approvals of extensions of some uses then under permit, such as TT canola and parthenium weed, were to be considered;
- MRLs for which there were no associated registered uses were to be deleted.

The interim report also identified that additional studies and information were required to alleviate remaining environmental and human health concerns, as follows:

- residue data were required to confirm animal feed commodity MRLs;
- information on annual sales were to be provided to the APVMA;
- incidents of herbicide resistance were to be reported to the APVMA; and
- additional water monitoring studies were to be conducted to determine whether the levels of atrazine in the environment were above or below the level that would impact on the environment.

These dot points are elaborated upon below.

2.1 Previous reconsideration action following the interim review report

2.1.1 Cancellation of home garden/non-agricultural use patterns

The potential for atrazine to contaminate ground and surface water was one of the key reasons for its review. When the review commenced, atrazine products could be applied to lawns, golf courses, irrigation channels, drains, roadsides, industrial premises and other non-agricultural areas. It was concluded that these uses contributed significantly to the total environmental load of atrazine and thus the continuation of such uses could not be maintained (excluding the control of parthenium weed on roadsides). As an outcome of the interim report, all homegarden/non-agricultural use patterns were cancelled in December 1998.

2.1.2 Label changes

The interim report made recommendations intended to reduce the overall load of atrazine in the environment, especially its presence in water. Changes to label statements for this purpose included limitations on the quantities that could be used, buffer zones, and restraint statements relating to spray drift, weather conditions, and application to waterlogged soil.

The interim review report also recommended changes to safety directions in order to protect workers. The changes included additional requirements for Personal Protective Equipment (PPE) and restrictions on application methods.

2.1.3 Cancellation of product label approvals

In March 2001 the APVMA cancelled the approvals of all labels approved prior to November 1997, to ensure that all labels were in line with the recommendations of the 1997 interim review outcomes.

2.1.4 Extensions of use

Subsequent to the interim report, data packages were assessed for extensions of use of atrazine to control weeds in triazine tolerant (TT) canola and chickpeas. These uses have now been added to a number of atrazine products. In addition, uses have been extended to include the control of parthenium weed in New South Wales and the Northern Territory.

2.1.5 Maximum residue limits

As an associated outcome of the interim review report, MRLs for citrus fruits, grapes and pineapples were deleted because there were no use patterns on labels. In addition, new MRLs were established for primary animal feed commodities, edible offal and milks.

Additional forage and fodder residue data for sorghum, pasture and lucerne were required to confirm residue levels for primary animal feed commodity MRLs and those of animal commodities. These data are assessed in Section 10 of this report.

2.1.6 Reporting of information on annual sales and herbicide resistance

Registrants were required to provide the APVMA with information on the amounts of atrazine products sold over one year. A total of 2100 tonnes of active ingredient were sold in the financial year 1997/1998.

Registrants were also required to report any incidents of herbicide resistance to atrazine to the APVMA and any follow-up investigations conducted by the registrant. Registrants advised that no reports of herbicide resistance have been received. However, it has been noted that current labels do not provide users with an address or contact to report resistance incidents to registrants. To ensure that an appropriate mechanism for reporting herbicide resistance is available for users, a recommendation to modify labels will be made (refer Section 6).

2.1.7 Additional water monitoring requirements

The interim review report required certain measures to reduce the overall load of atrazine on the environment, and also recommended that water monitoring be conducted to determine the effect of these measures. Monitoring would also provide information on the trends in atrazine contamination in both ground and surface water.

For cropping situations, initial investigations found that a number of water monitoring programs were already established in various areas of Australia and that these programs included records for the detection of atrazine. The principal registrant, Syngenta Crop Protection Pty Ltd., collated information from many of these programs. As sufficient information was available from these surveys, no additional studies were required.

In 1994, the APVMA issued a provisional label for use of atrazine in forestry. This label was issued on the understanding that the forestry industry would undertake a nationwide series of trials to evaluate the effects of atrazine, applied at the nominated rates, on water quality in forestry use situations.

The Forest Herbicide Research Management Group (FHRMG) was formed to establish research proposals for design, assessment and management of field trials. The final report from the FHRMG was presented to the APVMA in May 2000. Together with the collection of information from around Australia, this report forms the basis for the environmental assessment, found in Sections 11 & 12 of this second draft final review report.

2.1.8 Water quality guidelines

Drinking water

The interim review report concluded that exposure of people to atrazine in food was very unlikely, although concerns were raised over the potential for exposure from drinking water. Because atrazine is both mobile in soil and reasonably stable in the environment, exposure of the human population would most likely occur from contamination of drinking water. It was therefore recommended that consideration be given to updating the Australian Drinking Water Guidelines for atrazine, and to include the atrazine specific metabolites, desethylatrazine and hydroxyatrazine with atrazine in the definition for the guideline value.

The Joint Committee of the National Health and Medical Research Council (NHMRC) and the Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ) has now finalised the drinking water guidelines for atrazine.

Although atrazine should not be detected in drinking water, if present, atrazine would not be a health concern unless concentrations exceeded 0.04 mg/L. If atrazine is detected, then remedial action should be taken to stop contamination. The practical limit of determination of atrazine is 0.0001 mg/L.

Aquatic ecosystems

In 1997, Australia had yet to establish a water quality guideline for protection of aquatic ecosystems. A guideline value for atrazine of 2 µg/L was employed overseas and had been proposed for local application.

Guidelines for Fresh and Marine Water Quality were published in April 2001. The water quality guidelines are estimates of concentrations at which individual chemicals should not cause direct toxic effects in the environment. If the guideline value for a chemical is exceeded, there is a potential risk of an environmental impact. The freshwater moderate reliability trigger value for atrazine was set at 13 µg/L. The values apply to the overall or surrounding quality of water and they do not apply to a point of discharge or mixing zone.

3. ASSESSMENT SUMMARIES

The 1997 interim report required additional environmental data to address issues relating to the potential risk atrazine use poses to the environment, and residue data to affirm a number of MRLs. Assessments of these additional data were discussed in the draft final report, released for public comment in April 2002.

Following release of the draft final report, a number of new overseas studies were published or foreshadowed that raised additional concerns that atrazine might cause developmental and reproductive problems in frogs. It was also reported that the US EPA was sufficiently concerned that it was considering adding an additional 10-fold safety factor because of uncertainty over this new atrazine-cancer link. In light of these new concerns, the APVMA revisited the toxicological and environmental risks of atrazine use in Australia.

Summaries of the additional assessments are below, with the detailed technical assessment reports found in Section 10 (residues), Sections 11 & 12 (environment) and Section 13 (toxicology) of this document.

3.1 Residues assessment summary

The animal transfer studies evaluated as part of the 1997 interim review indicated that measurable residues of atrazine were unlikely to occur in animal commodities. However, in 1997, no Table 4 entries for atrazine existed in the MRL Standard. Consequently, information on group residues, including forage and fodder residue data for sorghum, pastures and lucerne, were necessary to set animal feed commodity MRLs. These data were also needed to confirm or change withholding periods for grazing such crops.

As described in the April 2002 draft final report, the new residue data for forage sorghum, grain sorghum and maize enabled confirmation of MRLs to cover residues in primary animal feed and animal commodities. A new 28 day grazing withholding period is recommended for approved crop uses, except canola. Grazing and harvesting withholding periods for canola remain unchanged at 15 weeks when applied pre-emergence and six weeks post-emergence.

The residues assessment concluded that when atrazine is used according to label directions, residues are unlikely to pose an unacceptable risk to human health.

3.2 Environmental assessment summary

Atrazine monitoring data from the both the FHRMG and from consolidated information from water monitoring programs in Australia showed that the key risk factors affecting the potential for water contamination following atrazine application were:

- vulnerability of soil to surface runoff;
- treatment of ephemeral drainage lines;
- treatment of runoff water – the need to channel it into areas other than directly into waterways;
- careless handling of atrazine near water or where soil surface runoff is likely;
- careless handling of atrazine near bore sites or recharge areas where water tables are shallow and soils are permeable such as sandy soils or in areas of cracking clay soil that may permit rapid bypass;
- site preparation practices (especially forestry) – mounding perpendicular to contour banks increases the rate of transport from crop areas to waterways; and
- runoff from storm events.

Leaching studies at forestry sites and groundwater monitoring results indicate a low likelihood of groundwater contamination from atrazine use, even in areas of sandy soils. However, careless handling of atrazine near bores or over permeable recharge areas could result in incidents of local groundwater contamination. The current atrazine label already contains a restraint prohibiting mixing, loading or application within 20 metres of a well or sinkhole as well as intermittent or perennial streams and rivers. This restraint remains appropriate.

The pattern of atrazine contamination in Australian surface waters indicates that safety margins continue to be narrow in some areas, both for timber plantations and annual cropping. The key factor that determines the likelihood of aquatic contamination appears to be the vulnerability of the soil to surface runoff. Avoidance of use and handling on hard impermeable surfaces, and in areas such as ephemeral drainage lines where water may flow, are essential to minimise off-site transport of atrazine. Accordingly, additional label statements are proposed in order to reduce runoff.

Best Management Practices (BMPs) play a key role in reducing chemical losses in runoff, especially in forestry production. If BMPs are followed for atrazine use, atrazine concentrations in rivers and in groundwater aquifers should be below the relevant water quality guidelines.

Storms events may cause atrazine levels to temporarily exceed ANZECC guidelines, but long-term contamination at levels above the ANZECC guideline is unlikely. Based on these data, it appears unlikely that atrazine use in accordance with the label recommendations will be an undue hazard to the environment or to human beings through the consumption of contaminated drinking water.

There are potential future environmental concerns associated with use of atrazine on TT canola, particularly associated with raised bed cropping practices. Raised bed cropping is often employed in areas where soil tends to become waterlogged, thereby killing crops. The practice therefore adds value to what would otherwise be low production land. Nevertheless, use on TT canola has substantially increased the amount of atrazine used in Australia, particularly in very wet areas. Because the primary problem with atrazine is its potential to run off and contaminate waterways, there are implications for greater ecosystem load of atrazine in these wet regions.

If TT canola were to become the dominant land use in such regions, then there is a risk of greater or more persistent atrazine burdens in catchments, particularly in wet years. As yet, however, there is no evidence that this is occurring. Therefore, at this stage, there are no specific concerns to recommend changes to use patterns.

3.2.1 Atrazine and amphibians

The main unintended effect of atrazine considered in this report is disruption of sexual differentiation. Some studies have reported such effects at low exposure levels typical of those that may occur in the Australian environment. However, these studies were conducted under laboratory conditions where other stressors such as poor water quality and high population densities may adversely affect amphibian development.

A dose response relationship between exposure to atrazine and alterations to sexual differentiation has not been established. The raw data for these studies are unavailable, and there are inconsistencies in the results reported (for example, the incidence of abnormalities in a sample of 20 frogs is reported to be 92%). It has not been possible to independently reproduce these effects at the same low exposure levels, although they have been replicated in the laboratory at higher exposures (25 µg/L). Such higher exposures could potentially give rise to indirect effects through reduced primary productivity.

In the absence of any dose-response relationship, it would appear more likely that the reported effects reflect impaired development under less than optimal rearing conditions, rather than chemical exposure. Based on currently available evidence, the likelihood that atrazine is disrupting sexual differentiation in Australian frogs at current exposure levels (peak concentrations typically 1-10 µg/L in Australian surface waters) is considered low.

One study identified delayed metamorphosis and reduced metamorphic size as potential unintended adverse effects of exposure to atrazine at concentrations of 40 and 320 µg/L, under laboratory conditions. However, an earlier study found no such effects at concentrations to 200 µg/L. Given that atrazine levels in the Australian environment are unlikely to exceed 1 µg/L over extended periods, the likelihood that atrazine is delaying metamorphosis or reducing metamorphic size in Australian frog populations is considered low.

Reduced immune function has also been reported in laboratory amphibians exposed to 3 µg/L and 30 µg/L atrazine. Similar effects have been reported in the field where there was no evidence of atrazine exposure, and in another laboratory study where tadpoles were exposed to a mixture of pesticides including atrazine, at concentrations well above those that would be expected to occur in the environment. Based on this limited evidence, the likelihood that atrazine is reducing immune function in Australian frogs is also considered low.

Continued registration of atrazine products depends not only on whether unintended effects are likely to occur, but also on whether these unintended effects will have adverse consequences for populations. Extrapolation from laboratory effects to population impacts in the field can be difficult. The researchers that report disruption of gonadal differentiation in laboratory amphibians at very low atrazine exposure levels (0.1 µg/L) have claimed that atrazine could be likely to have a significant impact on amphibian populations. However, these same researchers have reported that frogs were easily sampled from apparently healthy populations at sites reported as contaminated by atrazine.

The US EPA has conducted a detailed ecological risk assessment of atrazine, which concluded that atrazine is likely to result in community and population level risk at 10-20 µg/L. Detailed consideration of recent amphibian findings has not altered this conclusion. The US EPA has noted the inconsistency and lack of reproducibility across studies and an absence of a dose-response relationship, and will seek additional data to reduce any uncertainty regarding the potential risk of atrazine to amphibians. The issue of atrazine and amphibians should be revisited if these additional data demonstrate that

atrazine may be likely to impact on frog populations at realistic levels of exposure, but such outcomes are not considered likely.

Taken together, the inconsistencies between studies, difficulty in independently replicating the low dose effects of atrazine in amphibians, likely influence of other stressors, and occurrence of healthy amphibian populations at sites in the USA where atrazine is present, indicate that it is unlikely that atrazine is adversely impacting upon populations of Australian amphibians at current levels of exposure.

The APVMA accepts the recommendations of the DEH assessment and therefore proposes the regulatory actions outlined in Section 6.

3.3 Toxicological assessment summary

The additional toxicology assessment considered whether recent published reports on amphibian development, carcinogenicity, and endocrine disruption associated with atrazine would change the toxicological recommendations of the 1997 interim review report. Such recent published reports included epidemiological studies and environmental studies. The epidemiological studies considered a possible link between atrazine exposure and human cancer, and the environmental studies investigated possible effects on frog development. These environmental studies were included because of possible links to the endocrine disrupting potential of atrazine.

The epidemiological studies provided support for the absence of a carcinogenic potential for atrazine. The toxicological assessment of the interim review report evaluated a range of studies conducted in mice, rats and rabbits, which examined the ability of atrazine to perturb normal reproduction and development. These studies indicated that atrazine is not a reproductive or developmental toxicant.

There is currently no validated test method for the use of amphibians (or reptiles) in assessing the hazard to human health from chemical exposure. Therefore, the OCS considers that the environmental studies are unlikely to have a direct relevance to human health.

The interim review report identified that atrazine caused neuroendocrine disruption in Sprague-Dawley (SD) rats, but did not bind to the oestrogen receptor or have any oestrogenic activity. Therefore, it is unlikely to be an endocrine disruptor in humans based on the known mechanism of action in SD rats. No changes to the existing health standards for atrazine are recommended.

4. SUMMARY OF PUBLIC SUBMISSIONS & CONCLUSIONS

Approximately thirty public responses on the draft final report were received. Most of these responses were form letters from a community network. The network's main concerns related to persistence of atrazine in the environment, possible drinking water contamination, potential for endocrine disruption, allergenic potential, and potential links to diseases such as breast, ovarian and uterine cancers, leukaemia, tumours, and non-Hodgkin's lymphoma. Such issues have been considered in the toxicological and environmental reports.

The form letters also remarked that to continue to claim safe use of any registered chemical without tangible proof as taken from that chemicals use performance record is not acceptable to the community and is now widely considered to be fraud practice.

The APVMA garners data from the Adverse Experience Reporting Program and advice from various states and other regulatory and advisory bodies. The APVMA makes its assessments based on scientific data and takes a weight of evidence approach.

Also raised by the community network, along with a separate individual response, was the concern that mixtures of two triazine herbicides (or other mixtures of agricultural chemicals) may have greater toxic effects than a single compound. Such proposed interactions are beyond the scope if the current review. Nevertheless, the APVMA recognises this as a complex potential issue, which is being considered by regulatory authorities around the world. The US EPA is undertaking a cumulative risk assessment for the triazines. However, due to the complexity of the assessment, it will be several years before the results will be available.

Comments were also received from forestry representative groups who were of the view that the forestry industry had been unfairly targeted in the draft report. The APVMA met with these groups, noting that although forestry had been identified as a high-risk industry, the issues were relevant to all industries. Suggestions were also made to do with wording of label statements, some of which have been adopted by this report

Submissions from canegrowers indicated that they thought the APVMA had undertaken a thorough and balanced review of the chemical and its application in Australia.

An individual response expressed misgiving about a 'threshold for ecological effects' and postulated that the APVMA had increased in the Water Quality Guideline Trigger Value (from a draft value of 0.5 μg/L to a final value of 13 μg/L) because the lower level was often exceeded.

The Guidelines for Fresh and Marine Water Quality are prepared by Australia's National Water Quality Management Strategy (NWQMS). The NWQMS was jointly developed, and is jointly run, by two Ministerial Councils - the Australian and New Zealand Environment and Conservation Council (ANZECC) and the Agriculture and Resources Management Council of Australia and New Zealand (ARMCANZ). The Australian National Health and Medical Research Council (NHMRC) is involved in aspects relating to public health. The APVMA is not involved in the NWQMS, nor in setting the guidelines.

When the APVMA's interim report for atrazine was released in 1997, Australia had yet to establish a water quality guideline for atrazine, for protection of aquatic ecosystems. The draft guidelines were released for public comment in July 1999, and the final guidelines were published in April 2001. The draft guidelines set a level 1 trigger value of 0.5 μg /L in 1999, while final guidelines in 2001 set a freshwater moderate reliability trigger value for atrazine of 13 μg/L. This adjustment was the result of the adoption of a more robust statistical procedure. The final value for atrazine was derived using the statistical distribution method with 95% protection and an acute-to-chronic toxicity ratio (ACR) of 20.2.

The seed industry commented that atrazine is an essential chemical in their industry with no efficient, cost effective alternative chemical available. Changes to current rates per application and to buffer zones were also requested. These comments have been considered. The APVMA considers that the health risks to humans and the environment are paramount in its considerations, and therefore its recommendations are made on this basis. The APVMA also needs to be satisfied that changes to the existing buffer statements would not pose an undue hazard to the environment, and would require data to conduct a risk assessment of these new suggested changes. These data have not been provided and thus the existing buffer statements will remain.

5. OVERSEAS REGULATORY STATUS

5.1 United States of America

Currently, the US EPA March 2004 amendments to the January 2003 Atrazine Interim Reregistration Eligibility Decision (IRED) regards atrazine as not likely be a human carcinogen. Assessment of further data on workers is expected to be complete in mid 2005. At that time, the EPA will convene another Science Advisory Panel (SAP) meeting concerning atrazine and its possible association with carcinogenic effects. The Agency is also seeking additional data to reduce uncertainty regarding the potential risk to amphibians. The January 31, 2003 IRED required ecological monitoring and mitigation of atrazine in watersheds in order to address concerns regarding aquatic ecosystems. Such programs have now been designed and the US EPA expects that when implemented, the programs will effectively lower the level of atrazine below the level of concern. The EPA concluded that atrazine products are eligible for reregistration, provided these data gaps and conditions are met. The US EPA March 2004 amendments to the January 2003 Atrazine IRED considers that label amendments will adequately address drinking water, worker and residential concerns. Atrazine can continue to be used to control weeds in crops such as sugarcane, corn, guava, wheat stubble, commercial and residential lawns, bermudagrass, forest plantings, and golf courses. In addition, the EPA will be conducting a cumulative risk assessment for triazines, which may also impact on the registration status of atrazine.

5.2 Canada

The re-evaluation of atrazine was released in November 2003. Atrazine is used in Canada for control of weeds in corn, blueberries and TT canola. Registrants do not wish to generate the data to support the latter two uses, which are therefore being phased out. However, uses in corn have been retained, with restrictions on application rates, and no aerial application allowed. The document concluded that atrazine was of low to slight acute toxicity and that its primary mode of action is via impairment of hypothalamic-pituitary function in the rat. The ARfD was set at 0.04 mg/kg bw, based on a 4 day rat study (NOAEL 12.5 mg/kg bw/day, with an uncertainty factor of 10 x 10 x 3). The PMRA has not completed the environmental assessment, but has required additional water monitoring data.

5.3 European Union

In March 2004, the Commission of the European Communities decided that atrazine should not be included in Annex I to directive 91/414/EEC, meaning that authorisation

for its use would be withdrawn by September 2004 in the EU. However, limited uses have been retained until 2007 in some of the member states, such as Ireland, the UK, Spain and Portugal. The reason for this decision was that available monitoring data did not allay concerns regarding contamination of groundwater. Specifically, the Scientific Committee for Plants did not accept the reported calculations of the environmental concentrations in groundwater. It also determined that available monitoring data were insufficient to demonstrate that in large areas, concentrations of the active and its breakdown products would not exceed 0.1 μg/L in groundwater.

5.4 Joint FAO/WHO Meeting on Pesticide Residues

Atrazine is not currently on the Priority List of Compounds Scheduled for Evaluation or Reevaluation.

6. PROPOSED REVIEW RECOMMENDATIONS

6.1 Assessment outcomes

After consideration of all data including the additional assessments, the APVMA accepts the recommendations of the DEH, CRP and the OCS, and therefore the following regulatory actions are proposed:

a) Active constituent approvals are to be affirmed.

b) Existing label instructions are deemed to be inadequate and the latest approved labels are to be amended as follows:

- Add: *Do NOT apply product to any drainage line. Drainage lines show evidence of the action of periodically flowing water (for example, gravel, pebble, rock or sand bed, scour hole or nick point) and/or an incised channel at least 30 cm deep*;
- Add: *Do NOT handle, mix, apply or conduct testing operations to areas susceptible to runoff where drainage results in rapid entry into waterways. These areas include roads, access tracks, snig tracks and compacted log dumps where no specific action has been taken to prevent runoff into waterways, or areas mounded perpendicular to the contour*.
- Remove the current Protection of Livestock label statement: "Where treating native pasture, keep stock off for 14 days while Product X takes effect" (due to inconsistency with the new grazing withholding period).
- Add: *Grazing (except canola):Do NOT apply to areas that will or may be grazed or cut for stockfood within 28 days after application.*
- In order to ensure that any incidents of resistance following use of atrazine come to the attention of the APVMA, the following label statement is required: *Any incidents of resistance must be reported to [the company name and contact details]*.

c) These variations to label instructions would then satisfy the requirements for continued registration of products; and so

d) Product registrations are to be affirmed;

e) The following label approvals are deemed not to contain adequate instructions and thus are to be cancelled (Table 1). Products with these labels attached are to be used in accordance with the latest amended labels.

Table 1 Label approvals to be cancelled

Product Number	Label Approval	Product Name	Company Name
45774	45774/01	ATRADEX WG HERBICIDE	CROP CARE AUSTRALASIA PTY LTD
	45774/0101		
	45774/0399		
	45774/0898		
50243	50243/0101	ATRAGRANZ HERBICIDE	
	50243/0301		
	50243/0498		
52584	52584/0100	CROP CARE ATRAZINE FLOWABLE HERBICIDE	
	52584/0301		
	52584/1000		
45178	45178/01	FARMOZ FARMOZINE 500 FLOWABLE HERBICIDE	FARMOZ PTY LIMITED
	45178/0898		
46810	46810/00	FARMOZ FARMOZINE 900 WDG HERBICIDE	
	46810/0501		
	46810/1098		
48252	48252/01	FARMOZ AA COMBI 500 FLOWABLE HERBICIDE	
40411	40411/00	MACSPRED FOREST MIX GRANULAR HERBICIDE	MACSPRED PTY LTD
	40411/0399		
	40411/4321		
51532	51532/0799	MACSPRED FOREST MIX WATER DISPERSIBLE HERBICIDE	
	51532/0999		
51538	51538/0599	MACSPRED FOREST MIX SPECIAL BLEND GRANULAR HERBICIDE	
45370	45370/1098	ATRANEX ATRAZINE WETTABLE POWDER HERBICIDE	MAKHTESHIM-AGAN (AUSTRALIA) PTY LIMITED
46526	46526/1098	ATRAMET COMBI SC HERBICIDE	
47324	47324/0599	ATRANEX 500 SC HERBICIDE	
	47324/1098		
51091	51091/1098	ATRANEX 900 WG HERBICIDE	
31237	31237/0899	NUFARM FLOWABLE NU-ZINOLE AA LIQUID HERBICIDE	NUFARM AUSTRALIA LIMITED

Product Number	Label Approval	Product Name	Company Name
31586	31586/0199	NUFARM FLOWABLE NU-TRAZINE LIQUID HERBICIDE	
	31586/02		
	31586/0301		
31589	31589/01	NUFARM NU-TRAZINE 900 DF HERBICIDE	
	31589/0199		
	31589/0200		
	31589/0202		
	31589/4287		
50472	50472/100	ATRAMAX FLOWABLE HERBICIDE	
	50472/301		
	50472/499		
50527	50527/0300	ATRAMAX GRANULES 900 WG HERBICIDE	
	50527/0301		
	50527/0399		
	50527/1001		
50164	50164/0301	SIPCAM PACIFIC MAIZINA 500 FLOWABLE HERBICIDE	SIPCAM PACIFIC AUSTRALIA PTY LTD
	50164/0998		
50456	50456/1198	SIPCAM PACIFIC MAIZINA 900 WDG HERBICIDE	
49547	49547/01	SUMMIT ATRAZINE 900DF HERBICIDE	SUMMIT AGRO AUSTRALIA PTY LTD
	49547/0102		
	49547/0499		
49548	49548/01	SUMMIT ATRAZINE 500 HERBICIDE	
	49548/0499		
51814	51814/0699	SUMMIT COMBO SC HERBICIDE	
47615	47615/0398	FLOWABLE GESAPRIM 500 SC LIQUID HERBICIDE	SYNGENTA CROP PROTECTION PTY LIMITED
	47615/0600		
47616	47616/01	GESAPAX COMBI 500 SC LIQUID HERBICIDE	
	47616/0398		
47928	47928/0398	GESAPAX COMBI 800 WG HERBICIDE GRANULES	
	47928/0699		
	47928/1199		
49552	49552/01	GESAPRIM GRANULES 900 WG HERBICIDE	
	49552/0201		
	49552/0398		
	49552/0599		
	49552/0702		
	49552/0899		
	49552/1201		
50885	50885/501	PRIMEXTRA GOLD HERBICIDE	

Product Number	Label Approval	Product Name	Company Name
	50885/599		
53892	53892/0301	FLOWABLE GESAPRIM 600 SC LIQUID HERBICIDE	
	53892/0302		
	53892/0802		

7. AMENDMENTS TO STANDARDS

As an associated outcome of the review, changes are to be made to the MRL standard (Tables 2 & 3)**.**

Amendments to the *MRL Standard*

Table 2

Compound		Food	MRL (mg/kg)
Atrazine			
Delete:	MO 0105	Edible offal (mammalian)	T*0.1
	MM 0095	Meat [mammalian]	T*0.01
	ML 0106	Milks	T*0.01
Add:	MO 0105	Edible offal (mammalian)	*0.1
	MM 0095	Meat [mammalian]	*0.01
	ML 0106	Milks	*0.01

* set at or about the limit of analytical quantitation

Table 3

Compound	Animal feed	MRL (mg/kg)
Atrazine		
Delete:	Primary feed commodities	T40
Add:	Forage and fodder derived from cereals, pastures, legumes, sweet corn and sugar cane	40

8. CONCLUSION

Based on the outcomes of the initial review, subsequent assessment of the required supplementary information and the variation to conditions of label approval ensuring the requirements for continued approval or registration will be complied with, the APVMA is satisfied that the continued use of products containing atrazine meets the criteria for continued registration and label approval as prescribed by the Agvet Codes.

9. REFERENCES

WHO (1996) WHO/FAO data sheets on pesticides No. 82 ATRAZINE WHO/PCS/DS/96.82

Appendix 1 Active Constituents included in the review

Approval Number	Active Constituent	Company Name
44047	ATRAZINE	MAKHTESHIM-AGAN (AUSTRALIA) PTY LIMITED
44367	ATRAZINE	SYNGENTA CROP PROTECTION PTY LIMITED
45076	ATRAZINE	DOW AGROSCIENCES AUSTRALIA LIMITED
48797	ATRAZINE	SIPCAM PACIFIC AUSTRALIA PTY LTD
57454	ATRAZINE	KENSO CORPORATION (M) SDN BHD
57911	ATRAZINE	AGROGILL CHEMICALS PTY LTD

Appendix 2 Products included in the review

Product Number	Label Approval	Product Name	Company Name
52674¥	56274/0200Φ	4FARMERS ATRAZINE 500 SC	4FARMERS PTY LTD
55093¥	55093/0403Φ	COUNTRY ATRAZINE 900 WG HERBICIDE	A & C RURAL PTY LTD
56276¥	56276/1002Φ	ATRAQUEST 900 WG HERBICIDE	CONQUEST AGROCHEMICALS PTY LTD
45774	45774/01	ATRADEX WG HERBICIDE	CROP CARE AUSTRALASIA PTY LTD
	45774/0101		
	45774/0301Φ		
	45774/0399		
	45774/0898		
50243¥	50243/0101	ATRAGRANZ HERBICIDE	
	50243/0301		
	50243/0498		
	50243/0802Φ		
52584¥	52584/0100	CROP CARE ATRAZINE FLOWABLE HERBICIDE	
	52584/0301		
	52584/1000		
	52584/1101Φ		
51630¥	51630/1299Φ	DOW AGROSCIENCES ATRAZINE 500 FLOWABLE HERBICIDE	DOW AGROSCIENCES AUSTRALIA LIMITED
45178	45178/01	FARMOZ FARMOZINE 500 FLOWABLE HERBICIDE	FARMOZ PTY LIMITED
	45178/0801Φ		

Product Number	Label Approval	Product Name	Company Name
	45178/0898		
46810	46810/00	FARMOZ FARMOZINE 900 WDG HERBICIDE	
	46810/0103[Φ]		
	46810/0501		
	46810/1098		
48252[¥]	48252/01[Φ]	FARMOZ AA COMBI 500 FLOWABLE HERBICIDE	
	48252/1298		
56298[¥]	56298/1002[Φ]	KENSO AGCARE ATRAZINE 500 SC HERBICIDE	KENSO CORPORATION (M) SDN BHD
40411	40411/00	MACSPRED FOREST MIX GRANULAR HERBICIDE	MACSPRED PTY LTD
	40411/0399		
	40411/0701[Φ]		
	40411/4321		
51532[¥]	51532/0104[Φ]	MACSPRED FOREST MIX WATER DISPERSIBLE HERBICIDE	
	51532/0799		
	51532/0999		
51538[¥]	51538/0599[Φ]	MACSPRED FOREST MIX SPECIAL BLEND GRANULAR HERBICIDE	
45370	45370/0499[Φ]	ATRANEX ATRAZINE WETTABLE POWDER HERBICIDE	MAKHTESHIM-AGAN (AUSTRALIA) PTY LIMITED
	45370/1098		
46526	46526/0599[Φ]	ATRAMET COMBI SC HERBICIDE	
	46526/1098		
47324	47324/0502[Φ]	ATRANEX 500 SC HERBICIDE	
	47324/0599		
	47324/1098		
51091[¥]	51091/0702[Φ]	ATRANEX 900 WG HERBICIDE	
	51091/1098		
31237	31237/00[Φ]	NUFARM FLOWABLE NU-ZINOLE AA LIQUID HERBICIDE	NUFARM AUSTRALIA LIMITED
	31237/0899		
31586	31586/0199	NUFARM FLOWABLE NU-TRAZINE LIQUID HERBICIDE	
	31586/02		
	31586/0301		
	31586/0902[Φ]		
31589	31589/01	NUFARM NU-TRAZINE 900 DF HERBICIDE	
	31589/0199		
	31589/0200		
	31589/0202		
	31589/0802[Φ]		
	31589/4287		
50472[¥]	50472/100	ATRAMAX FLOWABLE HERBICIDE	

Product Number	Label Approval	Product Name	Company Name
	50472/301		
	50472/0402[Φ]		
	50472/499		
50527[¥]	50527/0300	ATRAMAX GRANULES 900 WG HERBICIDE	
	50527/0301		
	50527/0399		
	50527/0402[Φ]		
	50527/1001		
50164[¥]	50164/0301	SIPCAM PACIFIC MAIZINA 500 FLOWABLE HERBICIDE	SIPCAM PACIFIC AUSTRALIA PTY LTD
	50164/0402[Φ]		
	50164/0998		
50456[¥]	50456/0301[Φ]	SIPCAM PACIFIC MAIZINA 900 WDG HERBICIDE	
	50456/1198		
49547[¥]	49547/01	SUMMIT ATRAZINE 900DF HERBICIDE	SUMMIT AGRO AUSTRALIA PTY LTD
	49547/0102		
	495470402[Φ]		
	49547/0499		
49548[¥]	49548/01	SUMMIT ATRAZINE 500 HERBICIDE	
	49548/0403[Φ]		
	49548/0499		
51814[¥]	51814/0699	SUMMIT COMBO SC HERBICIDE	
	51814/0803[Φ]		
55066[¥]	55066/0302[Φ]	SUMMIT RELIEF HERBICIDE	
47615	47615/0201[Φ]	FLOWABLE GESAPRIM 500 SC LIQUID HERBICIDE	SYNGENTA CROP PROTECTION PTY LIMITED
	47615/0398		
	47615/0600		
47616	47616/01	GESAPAX COMBI 500 SC LIQUID HERBICIDE	
	47616/02[Φ]		
	47616/0398		
47928	47928/01[Φ]	GESAPAX COMBI 800 WG HERBICIDE GRANULES	
	47928/0398		
	47928/0699		
	47928/1199		
49552[¥]	49552/01	GESAPRIM GRANULES 900 WG HERBICIDE	
	49552/0201		
	49552/0398		
	49552/0599		
	49552/0702		
	49552/0802[Φ]		

Product Number	Label Approval	Product Name	Company Name
	49552/0899		
	49552/1201		
50885¥	50885/501	PRIMEXTRA GOLD HERBICIDE	
	50885/599		
	50885/801Φ		
53892¥	53892/0301	FLOWABLE GESAPRIM 600 SC LIQUID HERBICIDE	
	53892/0302		
	53892/0703Φ		
	53892/0802		
58450¥	58450/0304Φ	TRADEWYNS ATRAZINE 900WG HERBICIDE	TRADEWYNS PTY LTD
58456¥	58456/0204Φ	UNITED FARMERS ATRAZINE 900 WG HERBICIDE	UNITED FARMERS COOPERATIVE COMPANY LTD

¥ Product registered after commencement of the review but registration conditional on the outcomes of the review.

Φ Latest label approval to be varied.

Appendix 3 Status of protected information

The APVMA operates a program of data protection that provides compensation to those who submit data for a review and which meets the criteria specified in the Agvet Codes. The objectives of the program are:

- to provide an incentive for the development of products and data applicable to Australian or local conditions
- to encourage the availability of overseas products and data; and
- to provide reciprocal protection for Australian products and data under overseas' data protection systems.

In general the APVMA designates information as protected registration information for a protection period of two to seven years if the information:

- is requested by the APVMA for the purposes of a review; and
- relates to the interaction between the products and the environment of living organisms or naturally occurring populations in ecosystems, including human beings.

If the APVMA proposes to use the same information to determine whether to register or continue registration of another chemical product, the APVMA must not use the information until the parties come to an agreement as to terms for compensation, unless the protection period has expired or the APVMA is satisfied that it is in the public interest to use the information.

At the completion of the interim review in November 1997, there were a number of studies submitted for the review where the protection period had not elapsed. As at October 2004, no studies remain protected.

The supplementary environmental and toxicology data submitted for atrazine is not eligible for protection under the above scheme. The residue data (forage and fodder data) is eligible for protection and relevant to the review. However, the protection period has now expired.

10. RESIDUES ASSESSMENT REPORT

10.1 History of the atrazine residue assessment

The following recommendations were made in the 1997 interim report and are relevant to the subsequent consideration of residues issues:

Table 4 Changes to the MRL Standard

Commodity	***Existing MRL, mg/kg***	***Proposed MRL, mg/kg***
Table 1		
Citrus fruits	*0.1	Deleted
Grapes	*0.1	Deleted
Pineapple	*0.1	Deleted
Edible offal (mammalian)	*0.1	T*0.1
Meat (mammalian)	*0.01	T*0.01
Milks	*0.01	T*0.01
Table 4		
Primary animal feed commodities	-	T40

* set at or about the limit of analytical quantitation

10.1.1 Requirement for further data

Applicants were required to provide the APVMA with forage and fodder residue data for sorghum, pastures and lucerne to confirm the primary animal feed commodity MRL and those of animal commodities. These data were required to permit confirmation or appropriate change to withholding periods for grazing these crops. The trial data was required to be consistent with Australian use patterns for agricultural products that contain atrazine.

10.2 Basis for the original recommendations

The MRLs for citrus, grapes and pineapple were recommended for deletion due to lack of registered use patterns.

Residue data for animal forage and fodder were not available. The recommended temporary MRL for primary animal feed commodities was based on the highest feeding level administered in a dairy cattle transfer study. Residues of atrazine were not observed in tissues or milk following continuous feeding at doses equivalent to 3.75, 11.25 and 37.5 ppm in the diet. Metabolites of atrazine (particularly 2-chloro-4,6-diamino-s-triazine) were present at levels above the Limit of Quantitation (LOQ) of the analytical method, however, it was determined that the residue definition should remain as parent compound only.

The existing animal commodity MRLs were maintained as temporary MRLs subject to provision of residue data for primary animal feed commodities.

10.3 Summary of the current residue assessment

Syngenta Crop Protection P/L (formerly Novartis Crop Protection) provided new Australian residue data for forage sorghum, grain sorghum and maize. Assessment of these data has confirmed the following.

- These data are considered adequate to allow confirmation of the MRLs for primary animal feeds, edible offal, meat and milk. These data are adequate to allow the establishment of a grazing withholding period for forage and fodder crops, and therefore the outstanding residue data requirements are fulfilled.
- Dietary exposure to atrazine from residues arising from good agricultural practice would not pose an unacceptable risk to human health.

The following section summarises the relevant information and discusses the basis for the recommendations.

10.4 Discussion

10.4.1 Current relevant MRLs and toxicological information

Table 5: Australian MRLs[Ψ] for atrazine

Commodity			***MRL (mg/kg)***
MO	0105	Edible offal (mammalian)	T*0.1
VD	0545	Lupin (dry)	*0.02
GC	0645	Maize	*0.1
MM	0095	Meat [mammalian]	T*0.01
ML	0106	Milks	T*0.01
VR	0589	Potato	*0.01
SO	0495	Rape seed	*0.02
GC	0651	Sorghum	*0.1
GS	0659	Sugar cane	*0.1
VO	0447	Sweet corn (corn-on-the-cob)	*0.1
Animal feed			
Primary animal feed commodities			T40
Rape seed forage			10
Rape seed straw or fodder			0.5

* set at or about the limit of analytical quantitation

The prefix "T" denotes a MRL associated with a temporary use. It may also be used when an MRL is being phased out. The prefix "*" denotes an MRL set at or about the limit of analytical determination (also referred to as limit of quantitation or LOQ).

The Australian residue definition is:

Atrazine Atrazine

[Ψ] *MRL Standard*, as at 10 September 2001.

Atrazine has an Acceptable Daily Intake (ADI) of 0.005 mg/kg bw/day based on a no observable effect level (NOEL) of 0.5 mg/kg bw/day.

The Office of Chemical Safety in the Department of Health and Aging has determined that the establishment of an acute reference dose (ARfD) for atrazine is not necessary (Advisory Committee on Pesticides and Health, Background Paper, 2 May 2001, Agenda item 8).

10.4.2 Maximum treatment regime

The Syngenta product Flowable Gesaprim 500 SC Liquid Herbicide (NCRIS No. 47615) can be used as a herbicide in the following situations where forage or fodder would be produced: lucerne, grass pastures, lupins, maize, sweet corn, sorghum, broom millet, saccaline, forage sorghum, sugar cane and canola (triazine tolerant). Canola forage and fodder were considered in a registration application following release of the 1997 interim report. Suitable "Table 4" entries have already been established for this use and it will not be considered further in this assessment.

As a result of the interim review, the maximum application rate of atrazine in crop situations was fixed at 3 kg ai/ha per year. Depending on the situation, applications can be made: (i) pre-plant followed by post-emergence; (ii) at sowing followed by post-emergence; (iii) at sowing only; or (iv) post-emergence only. Where two applications are made, the total application rate must be less than 3 kg ai/ha.

There are currently no grazing withholding periods established other than for canola crops.

The Flowable Gesaprim 500 SC product currently has the following statement on the label under the heading "Protection of Livestock": *Where treating native pasture, keep stock off for 14 days while Gesaprim 500 SC takes effect.*

10.4.3 Residues in animal feed commodities

A total of three Australian residue trials were provided for evaluation. The 1997 interim report required provision of residue data for sorghum, pastures and lucerne. The applicant provided data for two sorghum crops and a maize crop. Although this deviates from the original requirement, the data are considered satisfactory for the intended purpose of confirming the "primary animal feed" MRL.

The treatment regime addressed in the trials was considered to adequately reflect the maximum label use pattern for Flowable Gesaprim 500 SC. The product was applied post-emergence of the crop at the maximum yearly label rate for crops (3 kg ai/ha). Residues of atrazine in foliage were expressed on a dry weight basis and are shown below.

Table 6 Summary of Australian residue trials in forage crops

Location, year, reference, crop	*Rate, kg ai/ha*	*No.*	*Volume, L/ha*	*PHI*, days*	*Atrazine, mg/kg*
Tamworth, NSW, 1998, forage sorghum	3	1	167	-0 0 3 7 14 28 42	<0.04, <0.04 2127, 2532 1392, 1278 42, 21 0.35, 0.67 0.30, 9.3 0.70, <0.04
Dalby, Qld, 1999, grain sorghum	3	1	140	-0 0 3 7 14 28 42	123, 0.06 3202, 6101 981, 2108 218, 351 27, 60 0.53, 0.11 0.54, <0.04
Millthorpe, NSW, 1998, forage maize	3	1	155	-0 0 3 7 14 28 41	<0.04, <0.04 1142, 1597 731, 649 190, 384 12, 20 0.10, <0.04 <0.04, <0.04

*PHI – post harvest interval

The highest feeding level investigated in the dairy cattle transfer study was approximately 40 ppm in the diet (37.5 ppm). The MRLs for animal commodities (currently temporary) have been established on the basis of a maximum feeding level of 40 ppm. Since the magnitude of residues in animal tissues and milk cannot be reliably estimated at feed levels greater than 40 ppm, the grazing withholding period for forage crops needs to reflect this point.

The applicant proposed a grazing withholding period of 28 days for forage crops other than canola where separate withholding periods have been established. At 28 days after post-emergence application at 3 kg ai/ha atrazine, residues in foliage were <0.04, 0.10, 0.11, 0.53, 0.3 and 9.3 mg/kg. All results were therefore less than 40 mg/kg. At the earlier sampling point of 14 days, residues of atrazine were up to 60 mg/kg.

General MRL entries such as "primary animal feed commodities" are no longer recommended by the APVMA on a routine basis. There is a clear preference to establish MRLs to cover narrower groups of commodities according to the Codex classification system. In this case, the confirmation of the general animal feed MRL was a specific recommendation of the interim review report. It is also apparent that detectable residues of atrazine are unlikely to occur in animal tissues or milk. The absence of residues probably extends to feeding levels higher than 40 ppm, although there are no data to confirm this. In the circumstances, the establishment of a permanent MRL for "primary animal feed commodities" is acceptable. The temporary MRL should be converted to a standard MRL at 40 mg/kg. The commodity description will be changed to "forage and fodder derived from cereals, pastures, legumes, sweet corn and sugar cane".

The following grazing withholding period should be established in conjunction with the animal feed MRL:

> **Grazing (except canola)-** DO NOT graze treated area or cut for stock food for 28 days after application.

Note that specific withholding period statements have previously been established for canola.

10.4.4 Residues in animal commodities

Since the magnitude of atrazine residues in animal feed commodities have now been confirmed, the animal commodity MRLs can also be confirmed.

Detectable residues of atrazine are not expected to occur in meat, edible offal or milk following continuous feeding at up to 40 ppm in the diet. The temporary MRLs for animal commodities should be converted to standard MRLs. No change to the magnitude of the MRLs are required.

10.4.5 Dietary risk assessment

- *Chronic dietary exposure*

The chronic dietary risk is estimated by the National Estimated Daily Intake (NEDI) calculation encompassing all registered/temporary uses of the chemical and dietary consumption data from the 1995 National Nutrition Survey of Australia. The NEDI calculation is made in accordance with the *Guidelines for Predicting Dietary Intake of Pesticide Residues (revised)* (WHO, 1997).

The NEDI for atrazine is equivalent to 4% of the ADI. It is concluded that when atrazine is used according to good agricultural practice, the chronic human dietary exposure is small and the risk to human health is acceptably low. The calculation is shown in Table 7.

- *Acute dietary exposure*

Acute dietary exposure to atrazine does not require further consideration. The establishment of an acute reference dose was not considered necessary.

10.5 Conclusions

The outstanding residue data requirements from the APVMA Review of Atrazine are considered to be fulfilled.

Adequate residue data were provided to allow the establishment of permanent MRLs to cover residues in primary animal feed commodities and animal commodities when atrazine is used according to good agricultural practice. Residues of atrazine in the diet do not pose an unacceptable risk to human health.

It is recommended that:

1. Changes are made to the *MRL Standard*

2. The following withholding period is required:

Grazing (except canola): DO NOT graze treated area or cut for stock food for 28 days after application.

Note that grazing and harvest withholding periods have been established for triazine tolerant canola crops, subsequent to the Review. These withholding periods will remain unchanged.

3. The current Protection of Livestock label statement ("Where treating native pasture, keep stock off for 14 days while Product X takes effect") must be removed because they are inconsistent with the new grazing withholding period.

4. The residue data supported a maximum total application rate of 3 kg ai/ha, consistent with the maximum in-crop application rate recommended in the Review Report.

10.6 References

McKee, K., Residue Report, residues of atrazine in forage sorghum, grain sorghum and forage maize following a single post-emergent application of Gesaprim 500 SC, Study No. P98/51, 3 May 2000, Novartis Crop Protection.

Table 7 Dietary exposure calculations

Calculation of NEDI
Atrazine
ADI for atrazine 0.005 mg/kg of body weight

Commodity	Food Consumption g/kg bw/day		MRL mg/kg	NEDI mg/kg bw/day	
Edible offal (mammalian)	0.0151	*	0.1	0.00000151	
Lupin (dry)	0.0001	*	0.02	0.000000002	default intake
Maize	0.0522	*	0.01	0.000000522	maize flour
Meat (mammalian)	1.7276	*	0.01	0.000017276	
Milks	8.9933	*	0.01	0.000089933	
Potato	0.9821	*	0.01	0.000009821	
Rape seed	0.001	*	0.02	0.00000002	
Sorghum	0.0001	*	0.1	0.00000001	default intake
Sugar cane	0.7328	*	0.1	0.00007328	DM0659
Sweet corn (corn-on-the-cob)	0.0881	*	0.1	0.00000881	
Total				0.000201184	mg/kg bw/day

Equivalent to 4 % of the ADI

These calculations have been made in accordance with 'Guidelines for Predicting Dietary Intake of Pesticide Residues' (World Health Organization)
MRL - Maximum Residue Limit
* - Denotes MRL set at or about the limit of analytical determination
NEDI - National Estimate of Dietary Intake
ADI - Acceptable Daily Intake
Food consumption data from 1995 National Nutrition Survey of Australia

11. ENVIRONMENTAL ASSESSMENT REPORT

11.1 Introduction

Atrazine was one of the first chemicals to be reviewed under the APVMA's Review Program. This triazine herbicide is widely used in Australia for control of grass and broadleaf weeds in a variety of crops, including maize, sorghum, sugarcane, timber plantations (pines and eucalypts), established lucerne, grass seed crops and potatoes. It is also used for weed control in conservation tillage farming systems, for seed bed establishment prior to planting sorghum, or for fallow maintenance prior to wheat, peas or lupins.

Atrazine is a slightly hydrophilic (water solubility about 30 mg/L) and persistent herbicide that can be transported in surface and groundwaters. Because of these properties and its widespread use, atrazine is a common contaminant of Australian surface waters, and is also often found in groundwater aquifers at low levels. The most recent Australian State of the Environment Report (Ball *et al*, 2001) notes that diffuse pesticide contamination of groundwater resources in some areas is significant, with pesticides detected in over 20% of samples from aquifers beneath intensively cropped land. Concentrations in surface water mostly remain below the threshold for ecological effects, generally accepted to be about 13 µg/L, but safety margins can be narrow in some areas. Accordingly, it is important to reduce levels of aquatic contamination by atrazine, particularly where levels detected breach water quality guidelines.

Atrazine mostly enters aquatic ecosystems in the dissolved phase of surface runoff. The risk of surface water contamination via runoff declines with time after application. Risks can be mitigated by techniques that improve water infiltration and retention. The APVMA's risk assessment resulted in label restrictions regarding application to waterlogged soil or where heavy rain is expected and recommended that monitoring of atrazine levels in Australian surface waters should continue in order to determine the effectiveness of these and other restrictions.

This further report briefly reviews recent literature data on monitoring of atrazine.

The report then evaluates the environmental significance of atrazine residues in water and describes the results obtained from:

- Forestry industry studies on contamination of groundwater and surface water;
- Monitoring activities in annual cropping areas.

11.2 Previous Australian regulatory actions

The APVMA announced in July 1994 that previous uses in non-crop situations such as fencelines, rights of way and irrigation channels would be discontinued by December 1995 because of concerns for aquatic contamination. These discontinued uses generally involved much higher rates of application in situations conducive to off-target movement of water. Maximum application rates were reduced, and no-spray buffer

zones were introduced around wells, sink holes, intermittent or perennial streams (20 m) and impounded waters (60 m).

To retain forestry uses, the APVMA introduced restrictions to use patterns and agreed to the establishment of a broadly based taskforce (the Forest Herbicide Research Management Group, FHRMG) whose role was to determine the effectiveness of these restrictions in reducing contamination of water.

Atrazine use in forestry mainly occurs during the establishment and early growth of timber plantations. Plantation establishment activities are regulated by State governments under various regulations, codes of practice and similar instruments.

The 1992 National Forest Policy Statement (NFPS) provides agreed objectives and policies for the future of Australia's public and private forests and a framework for achieving balanced returns to the community from the forest estate. The NFPS aims to achieve sustainable forest management through various tools including management plans and codes of practice. An undertaking was given in the NFPS to produce a companion volume to the Australian Forestry Council's 1991 Forest Practices Related to Wood Production in Native Forests: National Principles.

National principles for forest practices related to wood production in plantations (the national plantation principles) were endorsed by the Ministerial Council for Forestry, Fisheries and Agriculture in November 1995, and provide a framework for codes of practice for plantation management that exist in all States. Codes of practice take local environmental requirements into account and are reviewed and revised periodically in response to developments in knowledge and technology. The national plantation principles are posted on the Agriculture, Fisheries and Forestry Australia website (http://www.affa.gov.au/corporate_docs/publications/pdf/forestry/sustainability/national/principles_wood_production.pdf - accessed 27 November 2003).

Best practice site preparation techniques minimise the risk that herbicide residues will contaminate water draining plantation sites. Best management practices are not uniform across forestry areas and need to reflect local conditions. This is achieved through flexible codes of practice that can be tailored to local hydrology.

The national plantation principles include requirements that water quality (physical, chemical and biological) be protected by measures controlling change resulting from plantation activities, that soil stability be protected by measures that regulate site disturbance, and that soil and water catchment values be protected by the careful location, construction and maintenance of roads and tracks, and regulation of their use. Intensive management practices such as site preparation and weed control are to be carried out in accordance with codes of practice and be consistent with the above principles. Chemicals are to be used in accordance with State policies and procedures.

To comply with codes of practice, plantation managers must carefully plan all aspects of each operation from location and land selection through management for a decade or more to harvest. Plantation growers are required to comply with a variety of State laws regulating such matters as soil conservation and safe use of approved crop protection chemicals, including the avoidance of chemical runoff into watercourses.

Interpretation of the national plantation principles in different jurisdictions is outlined below, based on NSW, Tas and SA, with a focus on measures that help avoid chemical contamination of watercourses.

In NSW, the Plantations and Reafforestation (Code) Regulation 2001 establishes standards for protection of soil and water quality. Note that these standards mainly relate to avoidance of soil erosion and stream turbidity, and may be less effective against dissolved contaminants such as atrazine. For example, buffer zones within which activities are restricted apply to drainage features. Slope limits are set for site preparation activities (mounding, line ripping and ploughing) according to erosion hazard, as determined based on rainfall erosivity and class of soil regolith stability. Road drainage and runoff water flowing from mounds must be directed onto a stable area, or a structure, capable of filtering runoff water and trapping sediment. Machinery must not be operated on a site being prepared when the soil is saturated or surface runoff is occurring.

State Forests of NSW manages its entire estate for catchment protection. The NSW Forest Practices Code specifies operational standards for delivery of clean water, including protection measures such as undisturbed streamside filter strips, silt fencing and road drainage.

In Tasmania, Forest Practices Plans are required for plantation establishment. Forest Practices Plans must comply with the Forest Management Code (http://www.fpb.tas.gov.au/docs/code_contents.htm - accessed 27 November 2003) issued by the Forest Practices Board (FPB), an independent statutory authority. Plantation treatments that need to be carefully considered and appropriately prescribed in the Plan include site cultivation method and direction of cultivation, slope limits, erosion control measures, water quality protection measures, drainage and weed control. Runoff water should be dispersed as much as possible, with culvert outlets directed onto stable ground, preferably vegetated or covered with slash. Drainage depressions should not be cultivated, particularly on erodible soils.

The Forest Practices Code applies to public and private tenures. In order to secure the commitment of private landowners to legally enforceable provisions, it is based on a philosophy of cooperation and trust. The forest practices system is essentially one of self regulation by the forest sector, with oversight and independent enforcement by the government through the FPB. A commitment to this self regulatory approach allows the forest sector to strive for best practice, rather than attempting to meet minimum standards imposed by government. Self regulation allows the Code to be continually updated and improved based on research, operational experience and social expectations. The forest industry has greater autonomy and flexibility, allowing it to deliver improved environmental performance while avoiding unnecessary bureaucratic costs that may arise under a more adversarial system. Tasmania's effective and efficient forest practices system is actively supported by government, private landowners, the forest industry and the broader community (Wilkinson, 2001).

Timber production in SA is restricted to plantations, which are mainly based on softwood (pines). Environmental Management Guidelines for Plantation Forestry in South Australia, developed by Forestry SA, are based on the concept of land capability. The guidelines provide a framework for management with a focus on outcomes rather

than methodology, and are not prescriptive. Land capability is the ability of land to support a particular use with minimum risk of permanent damage to soil resources. It is based on soil characteristics, topography, rainfall and slope. The key factors for plantations, in order of importance, are water erosion potential, drainage and soil depth, degree of rockiness, soil fertility and wind erosion potential. Standard management practices, such as minimisation of disturbance to watercourses and drainage lines and careful management of runoff water, apply to higher capability land. Variations such as strip cultivation may be used on lower capability land. Land in the lowest capability classes is not suitable for commercial plantation establishment but may require that timber be planted for protection or conservation. Some areas where plantations were established on steep country are now being replanted with hardwoods or reverted to native forest.

Non-forestry uses of atrazine were evaluated in the NRA's existing chemicals review of November 1997, which concluded that improved management was also required in annual cropping situations in order to reduce the risk of contaminated runoff entering waterways. Further monitoring was recommended in order to confirm that safety margins are maintained or improved. Canola production was identified as an issue needing attention, as triazine tolerant varieties have allowed considerable expansion of this crop and an associated increased need for atrazine.

The principal registrant, Novartis, now Syngenta Crop Protection P/L, has provided data on monitoring activities in ground and surface waters from various locations within Australia. Collaborative projects are underway on the Atherton Tablelands, Darling Downs, Liverpool Plains, Lachlan River near Cowra, Naracoorte, and several sites in Western Australia. The FHRMG has also reported on its intensive program of monitoring activities in timber plantations.

11.3 Australian water quality guidelines

Redrafted Guidelines for Fresh and Marine Water Quality, prepared under the auspices of Australia's National Water Quality Management Strategy, were released for public comment in July 1999. The water quality guidelines are estimates of concentrations at which individual chemicals should not cause direct toxic effects in the environment. "The guidelines are the recommended limits to acceptable changes in water quality that will continue to protect the associated environmental values, but it should not be reasoned that water quality can be degraded to these levels". The values are ambient, ie apply to the overall or surrounding quality of water and they do not apply to a point of discharge or associated mixing zones.

The final guidelines were published in April 2001. The freshwater moderate reliability trigger value for atrazine is 13 µg/L. This value compares with a level 1 trigger value of 0.5 µg/L in the 1999 draft guidelines and is the result of the adoption of a more robust statistical procedure. It should be noted that the final value for atrazine was derived using the statistical distribution method with 95% protection and an acute-to-chronic toxicity ratio (ACR) of 20.2. Moderate reliability trigger values are calculated from acute data and the application of an acute-to-chronic (ACR) ratio.

If the guideline value for a chemical is exceeded, there is a potential risk of an environmental impact. In such a case, further assessment, using a hierarchical decision

framework, should be carried out to determine if that risk is reduced by the interaction of the toxicant with other site-specific environmental factors that can modify its toxicity or bioavailability.

The current (updated September 2001) NH&MRC Australian Drinking Water Guideline value for atrazine is 0.1 µg/L, and the health value is 40 µg/L. The guideline value has been lowered from 0.5 µg/L and the health value has been increased from 20 µg/L. This decrease in the guideline value is due to better detection methods. The higher health value is due to an increase in the proportionality factor of the ADI and is based on the assumption that at least 50% of the ADI will arise from the consumption of drinking water. Atrazine has not been found in the Australian food supply.

The guidelines assume that if a pesticide is detected at or above the guideline value, steps should be taken to determine the source and stop further contamination.

The health guidelines are set to assist health authorities in managing the health risks associated with inadvertent exposure such as a spill or mis-use of a pesticide.

Atrazine has rarely been found in Australian reticulated supplies. It has been reported in groundwater supplies at concentrations up to 2 µg/L in an area where atrazine was used to suppress weed growth in irrigation channels (NH&MRC, 1996).

11.4 International perspective

Reviews of Aquatic Risk

A review of the aquatic ecotoxicology of atrazine concluded that no permanent damage will be caused to aquatic ecosystems at concentrations up to 20 µg/L (Huber, 1993).

Another such review used a probabilistic approach, based on acute toxicity data for 52 species. The LC50 of the tenth percentile of species sensitivity was determined to be 37 µg/L. Affected species at this concentration were all plants. It was assumed that protecting 90% of species would also protect the ecosystem as a whole. This assumption was shown to be conservative. A similar analysis of chronic NOECs found a tenth percentile of 3.7 µg/L. A review of more than 20 microcosm and mesocosm studies found that exposures below 20 µg/L generally caused no effects on aquatic plants, and that occasional effects were always followed by recovery. Effects that sometimes occurred at exposures between 10 and 100 µg/L were similarly followed by recovery. It was concluded that atrazine exposures up to 20 µg/L caused no lasting harm to aquatic plant communities, even when exposure was maintained for extended periods. The lowest effect concentration was conservatively estimated to be 50 µg/L, with any effects followed by recovery (Giddings and Hall, 1998).

A detailed probabilistic risk assessment, focusing on watersheds in the Midwest of the USA where most use occurs, concluded that atrazine does not pose a significant risk to that aquatic environment. Ecological risks were considered highest in the midwest because of heavy use and high rainfall across this region during the critical growing season, which washes atrazine into surface water. The greatest frequency of elevated concentrations was associated with low-order streams, and associated small impoundments with limited outflow.

Risk was assessed against 90th percentile exposure values determined from probability distributions of monitoring data for rivers in Ohio, Iowa, Illinois and Nebraska. The 4 day average concentrations (0.56-9.0 µg/L) were very similar to the 90th percentile instantaneous concentrations. The analysis found that 4 day average concentrations rarely exceeded 20 µg/L, with frequencies of 0-3%. Effects on biomass and primary productivity in microcosm and mesocosm studies were only significant at concentrations above 50 µg/L. Some taxonomic shifts were evident at these concentrations, with a tendency for resistant species to expand into niches vacated by sensitive species. The analysis concludes that there is only a low probability that atrazine concentrations in US surface waters will exceed the tenth percentile of the sensitivity distribution (37 µg/L) and that this concentration does not represent an ecologically significant risk to the aquatic environment. Risk was found to be highest in some small watersheds with extensive pesticide use, and in reservoirs receiving drainage from these watersheds. In these higher risk situations, site specific risk assessments should be conducted, bearing in mind the use to which the ecosystem is likely to be put, and the effectiveness and cost-benefit aspect of any risk mitigation measures that may be applied (Solomon *et al*, 1996).

A simplistic approach to determining safe levels of exposure to a toxicant involves application of an assessment factor to laboratory toxicity data. A recent paper using such an approach predicted no effect concentrations of 0.074 or 0.37 µg/L by application of assessment factors to the most sensitive laboratory NOEC, 3.7 µg/L for growth inhibition in the unicellular alga *Chlamydomonas reinhardi*. More refined methods based on the distribution of toxicities across various species, predicted no effect concentrations in the order of 0.8-0.9 µg/L. A comprehensive approach based on stream and pond mesocosm experiments yielded a predicted no effect concentration of < 3 µg/L, based on chlorophyll *a* concentration in periphyton (Girling *et al*, 2000).

Water quality guidelines

A guideline of 2.0 µg/L has been developed in Canada for protection of aquatic life (CCREM, 1989). The final guideline was derived by application of an assessment factor of 10 to the most sensitive of several comparable MATCs, 17.9 µg/L in freshwater microbial communities.

The US EPA has recently proposed an acute criterion of 1500 µg/L (1 hour average) for protection of freshwater aquatic life (US EPA, 2003). In developing this proposal, the US EPA noted that atrazine toxicity to aquatic plants, both algae and macrophytes, commonly occurs at concentrations of 10 µg/L and above, with several reports of toxicity to specific plant taxa at concentrations below 10 µg/L (primarily freshwater plant species). Effects are thought to be algistatic rather than algicidal at these lower concentrations, with recovery occurring once the atrazine is removed. The lowest EC50 values for freshwater green algae with exposure durations of 4 days or longer were 10.2 and 4 µg/L for *Chlamydomonas reinhardtii* and *Selenastrum capricornutum*, respectively. Mean EC50 values for these species would be considerably higher.

Aquatic ecosystem structural and functional parameters have most frequently been observed to be adversely affected by atrazine concentrations of 10 µg/L and above. Ecosystem effects have been shown to occur at atrazine concentrations less than 5-10 µg/L, but data are limited. Several microcosm and mesocosm studies ranging from

7 days to 2 months report no effect of atrazine on community structure, composition and functionality at atrazine concentrations as low as 5 µg/L. The ecosystem effects that do occur below 5 µg/L are generally transient and not well established. Recovery is quite rapid and functionality is generally not compromised until much higher concentrations are reached. It appears that for effects at concentrations up to 15 µg/L, the communities can recover quite rapidly following dissipation of the atrazine concentration. The median LOEC from 65 community studies using multiple endpoints, excluding those studies where recovery was known to occur, is 60 µg/L, and the 5th percentile LOEC is 10 µg/L. The observed effects have been on both the plant and animal communities, with the effects upon the animal community being secondary in nature, mainly a result of decreased availability of shelter and plant matter for food. Thus, permanent ecosystem effects should only occur at atrazine concentrations greater than 10 µg/L.

It can be seen from the above that a range of water quality criteria can be developed for atrazine, depending on the approach taken. Use of assessment factors tends to favour conservative outcomes. The key determinant, however, of acceptable levels of atrazine in water, is the selection of the attribute to be protected. Criteria are much more conservative where the effect against the protection sought is a subtle sub-lethal parameter such as reversible suppression of chlorophyll *a* rather than measures of ecosystem function such as total biomass or primary productivity. The ANZECC Water Quality Guideline is 13 µg/L, which is in line with the above considerations.

Reregistration in the US

The US EPA published a notice of initiation of special review in November 1994, because of concerns over human cancer risks. Concerns were also expressed over ecological risks, but these were not used as a special review trigger. A qualitative assessment raised serious concerns about the ecological risks of continuing to apply such massive quantities of toxic chemicals across ecosystems and watersheds. The notice included some details of atrazine levels in the US, including reports of 480 µg/L in runoff entering Chesapeake Bay and 1000 µg/L leaving treated areas in Colorado and Kansas. Streamwater contamination was noted as a problem, with levels of 5-10 µg/L not uncommon in streamwater during the peak use period (late April to early July) and one sample recording 245 µg/L.

The preliminary ecological risk assessment (environmental fate and effects chapter dated 26 January 2001) confirmed the widespread presence of atrazine and its degradates in both surface and ground water. An initial screening-level risk assessment based on model exposure estimates combined with laboratory toxicity data (the quotient method) found that direct acute effects on birds, mammals, fish and aquatic invertebrates were not expected, even at maximum use rates, but that chronic effects on mammals, birds, fish and aquatic invertebrates were possible at maximum and typical use rates. A refined aquatic risk assessment based on monitored exposure levels eased concerns regarding direct effects on aquatic fauna. However, the revised assessment raised concerns for adverse toxicological effects on freshwater and estuarine plants and their communities as well as indirect adverse effects on aquatic invertebrate and fish populations resulting from disruption of habitat.

The revised ecological risk assessment (environmental fate and effects chapter dated 22 April 2002) found that, in areas of high atrazine use, there was widespread environmental exposure that:

- has resulted in direct acute effects on many terrestrial plant species at both maximum and typical use rates;
- may have caused direct effects on aquatic non-vascular plants which in turn could have caused reductions in primary productivity;
- may have caused reductions in populations of aquatic macrophytes, invertebrates and fish; and
- may have caused indirect effects on aquatic communities due to loss of species sensitive to atrazine and resulting in changes in structure and functional characteristics of the affected communities.

Based on laboratory data and simulated field studies, it was considered that potential adverse effects on sensitive aquatic plants and other nontarget aquatic organisms, as well as their populations and their communities, were likely to be greatest where atrazine concentrations in water equalled or exceeded approximately 10 to 20 µg/L on a recurrent basis or over a prolonged time period. Stream monitoring data from agricultural areas included maximum concentrations exceeding this threshold at 11-35% of sites sampled.

The revised ecological risk assessment also noted that atrazine had been reported to cause endocrine effects in frogs at 0.1 µg/L, and that "atrazine effects on tadpoles are a concern, because atrazine use coincides with spring rains and the breeding season for amphibians. While the gonadal abnormalities and laryngeal alterations raise concerns about adverse effects on amphibian reproduction, there is no conclusive evidence that these changes have an adverse effect on amphibian reproduction, and healthy juvenile frog populations occur at sites where atrazine is said to be causing gonadal abnormalities. Additional testing with atrazine-treated tadpoles and adult frogs should be conducted to determine what, if any, effects occur on reproduction." The US EPA discussed this issue further in its response of 10 April 2002 to public comments on the preliminary assessment, noting that "unless these effects on amphibians are shown to have adverse effects on reproduction and the population, it is unlikely that the Agency would regulate atrazine on these effects on gonads and larynges."

The risk assessment was revised further to support the US EPA's interim decision on the reregistration eligibility of and risk management decision for the current uses of atrazine and associated human health and environmental risks. The revised assessment (Interim Reregistration Eligibility Decision (IRED) for Atrazine, dated 31 January 2003) represents the conclusion of the ecological risk assessment, with the exception of the potential atrazine effects on amphibian endocrinology and reproductive and developmental responses. It was noted that studies were underway that may reduce some of the uncertainties in understanding potential atrazine effects on amphibian endocrinology and reproductive and developmental responses. These studies and associated information were scheduled for external scientific review by the Federal Insecticide, Fungicide and Rodenticide Act Science Advisory Panel at a public meeting in June 2003. It was anticipated that the results from this meeting would provide significant input to enable publication by 31 October 2003 of an amendment to the

interim decision document which would address the issue of the potential effects of atrazine on amphibian endocrinology and development.

The US EPA issued a media release on 31 October 2003, indicating that studies regarding possible developmental effects on amphibians exposed to low doses of atrazine had been carefully evaluated and had received scientific peer review. These data do not provide evidence to show that atrazine produces a consistent, reproducible effect on amphibian development. Further data are being generated, and ecologically vulnerable watersheds are to be monitored. The amphibian issues are discussed in more detail in Section 4 of this report.

The revised atrazine IRED does not change the conclusions reached in the 31 January 2003 IRED regarding atrazine's effects on amphibians, because of the inconsistency and lack of reproducibility across studies and the absence of a dose-response relationship. Additional data are to be generated to clarify the issue. The revised IRED confirms that change to the structure and function of aquatic primary producers is the most sensitive endpoint for the ecological risk assessment. It estimates a level of concern (LOC) using an ecological food chain model (CASM, Comprehensive Aquatic Systems Model) that predicts changes in aquatic communities in streams based on a community similarity index (CSI) that quantifies the average changes in plant biomass. The LOC is based on analysis of reported effects and the atrazine exposure profiles in 25 microcosm and mesocosm studies. These analyses established the LOC as any measured atrazine exposure profile obtained through a monitoring study that would result in a predicted 5% or greater average change in the CSI through CASM. The ecological monitoring program will focus in its initial year on streams in 40 small watersheds representative of those predicted to be potentially most vulnerable to atrazine contamination.

The revised IRED is not the final reregistration eligibility decision for atrazine as a cumulative risk assessment and risk management decision for the triazines remain outstanding. Atrazine remains eligible for reregistration, provided that the measures outlined in the revised IRED are adopted, but a final decision will not be made until the cumulative assessment is complete.

The draft aquatic life criterion for atrazine (US EPA, 2003) was issued for public comment at the same time as the revised IRED. The draft aquatic life criterion and the LOC were derived in an identical manner. The freshwater criterion is a less than 5% change in the average primary producer Steinhaus similarity index (based on species specific daily biomass) as determined by CASM (or other appropriate model and index) and a 1 hour average atrazine concentration of 1500 μg/L. These thresholds should not be breached more than once every 3 years on the average (or other appropriate return frequency sufficient to allow ecosystem recovery). For saltwater, the chronic criterion is 17 μg/L (implemented as a 30 day average) and the acute criterion is 760 μg/L (1 hour average). A freshwater chronic criterion was not developed because of the uncertainty surrounding recent claims of reproductive impairment in amphibians, but will be re-examined when additional data are available that conclusively demonstrate a significant reproductive effect (or other endpoint that significantly impairs long term population viability) to aquatic species.

Atrazine has also been under review in the European Union. Syngenta issued a media release on 4 October 2003, indicating that the EU will not reregister atrazine, despite a favourable scientific review.

Risk Factors

Three key factors have been identified for assessing the vulnerability of a watershed to surface water contamination by agricultural chemicals (Blanchard and Lerch, 2000). The chemical properties of a compound determine which hydrologic pathways are available for the chemical to be lost from soil. Moderately sorbed compounds such as atrazine are more likely to be lost in surface runoff or degraded in the soil rather than leached. The hydrology of a region will determine the relative importance of runoff and leaching. Land use, including proportions and locations within a watershed that are cropped and the chemicals that are used, is the third factor. Climate is an implicit fourth factor, as streamflow represents the hydrologic response of a watershed to climate. The extent of contaminant transport is critically determined by the frequency, intensity and duration of rainfall events following application. The bulk (80-90%) of the annual atrazine transport can occur during a few post-application runoff events. Best management practices to minimise water quality problems need to be tailored to fit the hydrology of a watershed.

The authors review monitoring data from North America and elsewhere, finding that land use factors (applied mass of pesticide or row-cropping intensity) are generally the key determinant of herbicide concentrations or mass flux in streams but may not always be the dominant factor. For example, surface water draining the Deep Loess Hills of southwestern Iowa and northwestern Mississippi contained relatively low concentrations of triazine herbicides despite high cropping intensity. Contamination tends to be higher in smaller tributaries or runoff-prone basins than in catchments with higher infiltration soils. Concentrations in streams and reservoirs of the midwestern US are significantly higher than in groundwater, largely because chemicals used on summer crops are lost in surface runoff from spring rains or by degradation within the soil, before groundwater recharge occurs in autumn and winter.

Atrazine in USA Surface and Ground Water

Considerable information on the occurrence of atrazine in surface and ground water in the United States is available on the publications home page of the US Geological Survey (USGS) website (http://water.usgs.gov/pubs/). Some of these data were used in the probabilistic assessment by Solomon *et al* (1996) and the review by Blanchard and Lerch (2000).

The USGS collects data on pesticide contamination of surface and groundwaters under the National Water-Quality Assessment (NAWQA) Program. The building blocks of the NAWQA Program are Study-Unit Investigations in 60 major hydrologic basins (study units). The 60 NAWQA study units cover about one-half of the conterminous United States, encompass 60-70 percent of national water use of the population served by public water supplies and include diverse hydrologic systems that differ widely in the natural and human factors that affect water quality. This selection of study units ensures that the most important national water-quality issues can be addressed by comparative studies. The study units are divided into three groups, which are

intensively studied on a rotational schedule. The first cycle of assessment for each group of 20 study units consists of 2 years of initial planning and Retrospective Analysis of existing data, 3 years of intensive data collection and analysis, and 6 years of report preparation and low-level assessment activity before the second cycle of intensive data collection and analysis begins. One-third of the study units are in the intensive study phase at any given time, and the 10 year cycle is repeated perennially. The first complete cycle of intensive investigations of all 60 study units is scheduled to be completed in 2002 (Gilliom *et al*).

Results available at this time from the first cycle of NAWQA water-quality data collection during 1992-1996 include analyses of 76 pesticides and 7 selected pesticide degradation products in about 8,500 samples of ground water and surface water in 20 study units. The 76 herbicides, insecticides, and fungicides targeted in the study account for approximately 75 percent of agricultural pesticide use in the US and a substantial portion of urban and suburban use.

The occurrence of pesticides in streams and ground water follows broad patterns in land use and associated pesticide use. The patterns are complex, however, and differ between streams and ground water because of the wide range of use practices and processes that govern the movement of pesticides in the hydrologic environment.

Herbicides are the most common type of pesticide found in streams and ground water within agricultural areas. The most common herbicides in agricultural streams were atrazine and its breakdown product desethylatrazine (DEA), metolachlor, cyanazine, alachlor, and EPTC. All 5 of the parent compounds rank in the top 10 in national use.

Atrazine was found in about two-thirds of all samples from agricultural streams, often occurring year-round. Similar to streams, the most common compounds found in shallow ground water were atrazine and DEA, but only about one-third of the samples had detectable levels. The lower rates of atrazine and DEA detection in ground water compared to streams result from longer travel times, greater opportunity for sorption or breakdown, and greater variability of source water in wells. One of the most striking results for shallow ground water in agricultural areas, compared with streams, is the low rate of detection for several high-use herbicides other than atrazine. This is probably because these herbicides break down faster in the natural environment compared to atrazine (USGS, 1999a).

In December 2000, full reports of results were available on the USGS website as USGS circulars for sixteen study units from the first cycle, with summary reports for the remaining four. Atrazine was detected in at least 50% of surface water samples taken from nine of the sixteen study units. Three study units exceeded this frequency of detection for ground water. The metabolite DEA was found in at least half the samples of surface water taken from six study units, and in ground water from three study units. Atrazine concentrations above 1 µg/L occurred in surface water from eight and ground water from six study units. DEA only exceeded 1 µg/L in groundwater samples taken from three study units. Surface water contamination in excess of 10 µg/L atrazine (accompanied by DEA at around 1 µg/L) was recorded in three study units (Central Nebraska Basin, Potomac River Basin and Trinity River Basin). High concentrations occurred in drainage basins dominated by row crops, notably corn, during late spring and early summer, often in conjunction with storm events.

Information is also available on contamination of the Mississippi River Basin. Maximum concentrations during 1991-92 of the most extensively used herbicides such as alachlor, atrazine, cyanazine, and metolachlor ranged from 3 µg/L to about 6 µg/L in large rivers such as the Mississippi, Missouri, and Ohio, compared to 50 to more than 100 µg/L reported in previous studies of smaller tributaries. Most of the pesticides used are applied in the upper parts of the Mississippi Basin.

The maximum concentration of atrazine reached about 4 µg/L in the Mississippi during 1987-92, and about 6 µg/L in smaller rivers such as the Illinois and Missouri during 1991-92. Runoff caused by rainstorms following the application of atrazine to cornfields early in the growing season flushes a portion of the atrazine into streams that eventually flow into the Mississippi River. These high concentrations generally represent extreme conditions that do not persist past midsummer (Goolsby and Pereira, 1995).

Atrazine continued to be found in samples of water taken from the Mississippi River at Baton Rouge (Louisiana) during 1991-97. The temporal pattern of contamination was characterised by a spring peak, typically in the range 2-4 µg/L, in late May and early June. The annual average load discharged to the Gulf of Mexico was around 2% of annual use across the basin, or 3% if dealkylated metabolites are included (USGS, 1999b).

Atrazine was detected in 82.1% of samples taken from the outflow from reservoirs in the midwest of the United States during 1992-93. The median concentration was 0.43 µg/L, with a mean of 1.36 µg/L for positive samples and a peak of 12.4 µg/L. Dealkylated metabolites were also commonly detected (71.6% of samples for DEA with median of 0.17 µg/L and mean in positive samples of 0.39 µg/L, and 61.8% for DIA (desisopropylatrazine) with median of 0.08 µg/L and mean in positive samples of 0.26 µg/L). Atrazine concentrations were lowest during winter and early spring, before planting of corn, and peaked during summer. Similar but less pronounced trends were evident for DEA, with the peak concentration tending to be later in the summer. Peak concentrations tended to be higher leaving smaller impoundments but more protracted leaving larger reservoirs. The key determinant of reservoir concentrations appeared from statistical models to be the quantity of herbicide used in the drainage basin. The models also indicate that when drainage basins have steep slopes and poorly drained clay-rich soils, the receiving reservoirs tend to have higher herbicide concentrations. These findings suggest that best-management practices targeted at reducing the use of herbicides and reducing the loss of herbicides to surface- and ground-water systems will be the most successful in lowering herbicide concentrations in reservoirs (USGS, 1998).

A recent study (Scribner e*t al*, 2003) across nine midwestern States (154 samples taken from 51 streams during three runoff events) found atrazine in 93% of samples. Concentrations have been on a declining trend since 1989, during which maximum application rates have twice been reduced but overall volumes of use have increased slightly. The median concentration in pre-emergence samples, which contain the highest residues because of the use pattern, was 4.2 µg/L. This is less than half of the median concentrations recorded in 1989 and 1990.

Aquatic exposure to atrazine in the US is summarised in the recent aquatic life criteria document (US EPA, 2003). Atrazine surface water concentrations are highest in field

runoff, peaking in the low mg/L range after major storm events that occur within a few weeks of application. Concentrations in natural surface waters (streams and lakes) are much lower, typically in the 1-10 µg/L range. Maximum concentrations of 5-70 µg/L have been reported in some creeks and river from midwestern areas, as well as in some small reservoirs. Smaller streams have been shown to have higher peak concentrations, but of shorter duration, than larger streams. High concentrations have been recorded in the surface microlayer.

11.5 Timber plantation trials

In the early 1990s, Syngenta proposed to discontinue registration of atrazine for a number of uses, including forestry. Although herbicides are used less frequently in timber plantations than for annual cropping, forestry use merits particular attention. Application rates tend to be higher and timber plantations are often situated in hilly country that may be runoff prone. Aquatic contamination by atrazine tends to be highest in low-order streams. Timber plantations are often located in headwater areas. Furthermore, atrazine use in timber plantations generally occurs in late autumn or winter, while use on annual crops mostly occurs in late spring. Plantation soils in southern States are more likely to be wet at this time of the year, which increases the runoff potential, and are also cold, which slows the degradation of atrazine and prolongs the period after application when runoff is likely to be contaminated with atrazine.

The withdrawal of atrazine from forestry was opposed by both public and private forestry interests. As a compromise the APVMA introduced restrictions on the use of atrazine, requesting forest plantation users to provide objective evidence on the effects of these restrictions on water quality.The Forest Herbicide Research Management Group (FHRMG) was formed to co-ordinate a nation wide research and monitoring study.

The study was divided into two components, the first utilised large-scale study sites (catchments 8-3351 ha) to assess risk to surface waters and the second, using small-scale plots (<1 ha) to assess the risk of atrazine leaching through the soil profile to groundwater. At the surface water study sites stream monitoring stations were established in catchments with either high or low catchment area ratio (CAR - ratio of atrazine treated area to untreated area). Sampling at the high CAR catchments allowed evaluation of peak atrazine concentrations from adjacent treated areas, whereas sampling at low CAR catchments provided an opportunity to assess the level of dilution provided by streamflow from untreated areas of the catchment. The monitoring program was based around collecting routine grab samples during periods of baseflow or zero flow and intensive event sampling collected by automated samplers during flood events. Most monitoring stations were within catchments solely managed by individual stakeholders, thus minimising outside influences. Local best management practices for plantation establishment were followed with respect to site preparation techniques and herbicide application methods.

At the groundwater sites, the study design consisted of sampling the soil profile at graduated intervals of increasing length following a single application of atrazine to a number of replicate plots. At sites where shallow aquifers existed, groundwater was also sampled. The groundwater monitoring program was also repeated during a range of seasonal periods at a number of sites.

11.5.1 Experimental details

Surface water monitoring studies were conducted in a 1st rotation hoop pine (*Araucaria cunninghamii*) plantation at Imbil Qld, radiata pine plantations at Canobolas NSW (1st and 2nd rotation) and Merriang VIC (2nd rotation), and a 1st rotation *Eucalyptus nitens* plantation at Watson's TAS. Surface water monitoring data were also received from the catchment for the Warren Reservoir in the Mt Lofty ranges of SA.

Leaching studies were conducted on small plots at Imbil Qld (2nd rotation hoop pine), Toolara Qld (2nd rotation *Pinus elliottii* and *Pinus caribea* var *hondurensis*), Watson's TAS (1st rotation *Eucalyptus nitens*), Mt Gambier SA and Myalup WA (2nd rotation radiata pine).

The studies entailed sampling the soil profile to at least 90 cm at intervals (-1, 1, 7, 14, 28, 56, 112 days after treatment, and immediately after rainfall of more than 100 mm) until neither atrazine nor metabolites could be detected. Intact core samples were taken at Mt Gambier, a split tube corer was used at Watson's and Myalup, and small soil pits were excavated at Imbil and Toolara to allow sampling of the freshly cut profile. Four samples were taken, bulked and sub-sampled for each depth interval (0-10, 10-20, 20-30, 30-45, 45-60 and 60-90 cm, with additional sampling at 90-120 and 120-150 cm at Myalup and Mt Gambier). Atrazine was determined by reverse phase HPLC after wet extraction and filtration. Groundwater was sampled at one site (Toolara) with a shallow perched aquifer.

Full experimental details and raw data are contained in the FHRMG report to the APVMA (Bubb and Barnes, 2000). Key results are summarised below.

11.5.2 Surface Water Studies

Surface Water Monitoring for Atrazine in NSW Timber Plantations

Surface water monitoring in NSW was conducted at Canobolas in the central-west of the State, near the town of Orange. This is a high altitude location where rainfall is relatively evenly distributed through the year. Two moderately sloping catchments on acidic (pH 5.0-5.5) basaltic loam soils were studied, situated on the eastern and western flanks of Mount Canobolas. Soils were deep (at least 60 cm, and up to 2-3 m) and free draining with good infiltration capacity.

A first rotation site on the western flank (slope 0-12^{o}) was established on former pasture by strip cultivation, with mound ploughing along the contours and retention of pasture between. A second rotation site (slope 12-25^{o}) on the eastern flank was prepared by heaping and burning debris. The site was mound ploughed with retention of much of the smaller size debris and litter. Both sites received two applications of atrazine (4-4.5 kg/ha) in consecutive years (October 1996 and August 1997). Rainfall remained below long-term averages. Ground based spray equipment was used to apply atrazine in strips at the first rotation site, and aerial broadcast methods at the second. Liquid formulations were applied in the first year, and core-coated granules in the second.

Atrazine concentrations in water were monitored at two locations leaving the first rotation site and a third station downstream. Concentrations remained below 1 µg/L at

both upstream locations in the second year, reflecting the prolonged dry conditions. They were above the threshold in the first year for about 2 months at one station (maximum 2.9 µg/L 25 days after treatment) and about a month at the other station (maximum 20 µg/L on the morning of the fourth day after treatment, declining to 5 µg/L by the evening of the same day). The peak of 20 µg/L occurred in the first of four flood events that were sampled during the first year in the high CAR (78%) station. Peak concentrations were 1.0, 0.9 and 0.2 µg/L, respectively, in subsequent flood events at 25, 34 and 120 days after treatment. Desethylatrazine (DEA) was found at the former station at concentrations up to 0.9 µg/L, but only in the second year of the trial. Atrazine was the only analyte detected at the low CAR (8.1%) downstream station, and only in the first season. Concentrations of 2 µg/L were detected in two samples taken during the early event on the fourth day after treatment.

Atrazine undergoes moderate sorption to soil, where it degrades through dealkylation and dechlorination reactions. Dealkylated metabolites sorb less strongly than parent atrazine. The occurrence of DEA in the second year of the study probably reflects the treatment applied in the first year, part of which appears to have been dealkylated in the soil and leached to groundwater. Streamflow is a combination of baseflow, as provided by groundwater, and surface runoff. With prolonged dry conditions, streamflow would consist almost entirely of groundwater, and the DEA contaminant would receive no dilution from surface runoff. In a normal season, the DEA concentration would be expected to be considerably reduced, but accompanied by significant concentrations of atrazine transported in surface runoff.

The second rotation site was served by two monitoring stations, one at the exit to the plantation and the second 7 km downstream. Atrazine concentrations in stream water leaving the site did not exceed 1 µg/L in the first year, except for the day of treatment when 13 µg/L was recorded. In the second year, a marked spike of atrazine (61 µg/L) was detected in water at the first weir on the first day after treatment (20 August 1997). Concentrations remained elevated at this location for the next three months (26.5 µg/L on 5 September, 4.7 µg/L on 8 October, 1.2 µg/L on 7 November). Residues were diluted below 0.2 µg/L at the downstream monitoring station, except for a single sample in September 1997 containing 0.6 µg/L. Metabolites remained undetectable in the first season, but desethylatrazine reached 1 µg/L in September 1997 at the upstream station.

The spike of 61 µg/L was attributed to overflying first order gullies of intermittent streams. Drainage depressions which are not incised and only carry water from heavy rainfall events were not buffered against treatment. The main (second order) intermittent streams were adequately buffered. The granular formulation may have contributed to this incident, as surface applied granules will have a tendency to move with overland water flow. Higher contributions to streams from pelleted rather than liquid herbicides have been reported elsewhere (Michael and Neary, 1993).

Two flood events were sampled, 131 days after the first treatment and 15 days after the second. Peak concentrations at the high CAR station were 1.0 and 26.5 µg/L, respectively. The higher figure in the second year probably reflects the closer proximity to the treatment date and difficulties with aerial application rather than a general problem with runoff from the total plantation area.

Despite no further testing to confirm these hypotheses having been carried out, overall, results from this site indicate that, with good management, herbicide residues in surface water leaving treated plantation areas should not exceed 20 µg/L. Peak concentrations occur for short periods (in the order of a day) but concentrations may remain elevated (above 2 µg/L) for up to 3 months after treatment. Higher concentrations can occur if care is not taken to avoid application to ephemeral drainage lines, and this aspect requires careful management. Application should not occur to any surface channel that flows after rainfall. Atrazine concentrations further down the catchment are diluted by water from other sources, such that concentrations above 2 µg/L are unlikely to occur, even for brief periods.

Surface Water Monitoring for Atrazine in Queensland Timber Plantations

Surface water monitoring in Queensland was conducted in a hoop pine plantation at Imbil in the south-east corner of the State. The 8 ha study site was situated on silty clay soils, at an elevation of 100-300 m with slopes of 5 to 30^{o}. Atrazine was manually applied along the tree row, by knapsack at 5 kg/ha (overall rate 2.25 kg/ha) on six occasions over a 2 year period. The subtropical climate demanded more frequent treatments in order to achieve satisfactory weed control, but also leads to a shorter half-life for atrazine in soil. Summer rainfall is dominant at this subtropical location, and was near average at 1130 mm in year 1 and well above at 1703 mm in year 2, with a correspondingly high number of flood events (19 at the upstream station and 21 downstream). One large storm in February of the second year delivered 540 mm over a 3 day period and caused a major flood event.

Atrazine was applied four times in the first year, in December 1997 and April, August and November 1998. Flood events occurred 2, 34 and 53 days after the first treatment, 1 and 9 days after the second, 27 and 91 days after the third, and 2, 29 and 43 days after the fourth in the high CAR (94%). Atrazine concentrations at the upstream station remained in the low ppb range for the first and last treatments, but reached 109 µg/L for the second treatment (in the second event) and 127.7 µg/L for the third (first event). Atrazine was accompanied by significant amounts (in the order of 10%) of the dealkylated metabolites DEA and DIA, the former being predominant. Peak concentrations at the low CAR (4.4%) downstream station, after each application were 7.6, 18.2, 105.5 and 25.6 µg/L (note not always in the first flood event, and that the duration of the peaks was brief).

Previous experience with hoop pine plantation establishment had indicated that surface runoff is much more likely to be generated from point sources than from the general plantation area, the high infiltration capacity of which is enhanced by slash retention and contoured windrows which pond runoff water. Direct contamination of water courses was discounted as these were protected and chemical was applied manually. Road areas (including access tracks and snig tracks within the plantation) were suspected as the main source of contamination because of their high rainfall runoff coefficient. Drainage outlets compounded the problem as they flowed directly to watercourses via roadside drains, rather than being directed back into the general plantation area via water spreading structures.

A number of procedural changes were introduced in late 1998 to minimise the risk of contamination. Roadside transfer of herbicide mix from tanker to knapsacks was

restricted to areas where drainage was directed back into the plantation, with staff instructed to minimise the possibility of spillage during transfer and test the spray units only within the plantation area. Application to potential point sources in the general plantation area, such as access tracks and snig tracks, was to be avoided.

Treatments in 1999 occurred in February and October, with flood events at the high CAR station 11, 77, 126, 189 and 234 days after the first treatment and 1 and 13 days after the second. Atrazine concentrations, at the upstream stations, were elevated after the first treatment, reaching 41.8 µg/L in the first of two events 11 days after treatment, and 50.4 µg/L in the second. Concentrations remained in the low ppb range after the second treatment. At the downstream station, concentrations did not exceed 2.3 µg/L.

Results indicate that with improvements to application practices, runoff from hoop pine plantations should not give rise to atrazine concentrations above 2 µg/L downstream from the plantation, although much higher concentrations may occur in water leaving the plantation in flood events.

Surface Water Monitoring for Atrazine in Victorian Timber Plantations

Surface water monitoring in Victoria was conducted in a second rotation *Pinus radiata* plantation situated on clay loam soils at an elevation of 460-740 m at Merriang in the NE of the State. The site has a predominant winter rainfall pattern, with annual precipitation of 1042 mm. Rainfall was 79% of average in the first year, and 111% in the second. Atrazine was spot applied manually at 4.24 kg/ha (overall rate 0.78 kg/ha) in September 1997 and by helicopter at 5.1 kg/ha (overall rate 4.5 kg/ha) in September 1998. Although slopes were fairly steep at 16-28^{o}, surface runoff was limited by the high infiltration capacity of the soils.

A single grab sample from the high CAR station tested positive for atrazine, at 0.2 µg/L some 48 days after the initial treatment. Only one flood event was sampled in year one, 272 days after treatment, and only at the low CAR station. Two flood events in the second year, 260 and 336 days after treatment, were sampled. All tested negative for atrazine.

Surface Water Monitoring for Atrazine in Tasmanian Timber Plantations

Surface water monitoring in Tasmania was conducted in a first rotation *Eucalyptus nitens* plantation at an elevation of 410-492 m in a high winter rainfall area of Tasmania (annual precipitation 1536 mm). The plantation was situated on free draining clay loam soils (10% organic carbon in the surface 10 cm) with good infiltration capacity. Slopes ranged from 6 to 20^{o}. Atrazine was broadcast applied by tractor in November 1996 and October 1997 at 8 kg/ha (treated rate 5.4 kg/ha after discounting buffer areas). Rainfall was 85% of average in the first year and 113% in the second.

A single sampling station with a high catchment area ratio (67.5%) was used. The maximum concentration of atrazine detected in routine grab samples was 0.2 µg/L, with all but three samples remaining below 0.5 µg/L. Flood event samples were taken 5, 52 and 59 days after the first treatment and 7 and 171 days after the second. Only the initial sample, at 2 µg/L, contained more than 1 µg/L atrazine. This peak level was maintained for about 2 hours. The herbicide could not be detected in flood events that

occurred more than 2 months after treatment. The only metabolite detected was DIA, at 0.4 µg/L in the November 1997 event.

Atrazine in South Australian Water Catchments.

Surface water monitoring in South Australia did not form part of the formal forest herbicide research program, but was implemented in response to a contamination incident. Problems with persistent atrazine and hexazinone contamination in three reservoirs providing raw water to the Barossa Water Treatment Plant were brought to the attention of the APVMA by SA Water in early 1999. Monitoring data were provided for the Barossa Reservoir (near the outlet to the water treatment plant) between September 1997 and July 1999, and for the two upstream impoundments (South Para and Warren) between July 1998 and July 1999, as tabulated below (refer Table 8) for atrazine.

(Note - all of the concentrations are above the draft NHMRC guideline value). Hexazinone concentrations in the reservoirs followed similar trends. Contamination has incurred significant additional operating expenditure, including the need for activated carbon treatment.

Table 8: Monitoring data for the Barossa Reservoir and two upstream impoundments

Reservoir	**Year**	**No of samples**	**Minimum Atrazine**	**Maximum Atrazine**	**Mean**
Barossa	1997	4	2.10 µg/L	2.50 µg/L	2.20 µg/L
	1998	38	1.03 µg/L	2.26 µg/L	1.64 µg/L
	1999	25	1.3 µg/L	1.80 µg/L	1.53 µg/L
South Para	1998	19	1.40 µg/L	2.12 µg/L	1.76 µg/L
	1999	26	1.2 µg/L	1.80 µg/L	1.49 µg/L
Warren	1998	20	2.47 µg/L	5.69 µg/L	3.95 µg/L
	1999	18	1.4 µg/L	2.93 µg/L	2.02 µg/L

The Barossa Reservoir system consists of three reservoirs (see table 9) located in the northern part of the Mount Lofty Ranges. The Warren Reservoir receives water from the South Para River, Waterholes Creek, and by pipeline from the Murray River. It discharges into the South Para Reservoir, which also receives water from other tributary streams such as Victoria Creek and Malcolm Creek. Water then flows downstream to the Barossa Weir where it either discharges to the Gawler River or is diverted through a tunnel to the Barossa Reservoir. Ambient water conditions in the Barossa reservoir are strongly dependent on residual conditions in the much larger South Para reservoir upstream.

Table 9: Details of the Barossa Reservoir system

Reservoir	**Capacity**	**Catchment area**	**Surface area at full supply level**
Warren	4770 ML	11900 ha	105 ha
South Para	44770 ML	22100 ha	399 ha
Barossa	4510 ML	800 ha	62 ha

For historical and operational reasons, plantation establishment occurred only in the Warren catchment during 1996-98, rather than being spread across other catchments as in previous years. Some relatively large areas established in 1995-97 were treated twice in 2 years with Forest Mix granules (label rate 1.5 kg/ha hexazinone and 4.5 kg/ha atrazine). Shallow soils with limited moisture holding capacity favoured storm runoff, which was exacerbated by mounding perpendicular to the contour to minimise problems with waterlogging. Logging debris was pushed into windrows and burnt, rather than being retained as a mulch, as now occurs. Application by helicopter in 1998 meant that internal firebreaks and ephemeral drainage lines were treated.

Areas treated are identified on the attached map (Appendix 4). The two main plantation areas were situated on each side of a ridge, one draining east to the South Para River and the other west to Waterholes Creek.

Tributary streams in the larger South Para catchment drain an extensive area, mostly under agricultural or pasture use but with pine plantations located mainly in the Big Flat area. Soil at this location is typically a sandy duplex, with a highly permeable sand layer overlying an impervious clay that permits runoff. Previous studies in the area had indicated that atrazine contamination is likely when treatment occurs within 4-5 m of a stream or incised drain on coarse grained soils or in landscape with low relief. Three areas of 1997 plantation were treated with Forest Mix granules in May 1997 and again in May 1998, at a lower rate. The largest area (Dewells East, 31.3 ha) to be monitored was mounded perpendicular to the contour almost continuously from ridge-top to valley flat. Runoff ponded along the eastern firebreak, where a metalled road diverted water through culverts into an adjacent stream. Areas treated in 1998 are tabulated below (refer Table 10), as total area and percentage of catchment.

Table 10: Areas treated in 1998

Site	Flows to:	Treated area (cumulative)	Catchment area	Catchment area ratio
Dewells 1997 East (10)	2	31.3 ha	56.9 ha	55%
Sandy Corner (2)	3	31.3 ha	626 ha	5%
Centennial Drive Ford (3)	4	31.3 ha	7825 ha	0.4%
Rocky Ford (4)	Warren	49.0 ha	8032 ha	0.6%

Concentrations of atrazine (µg/L) detected at the four monitoring stations during the spring of 1998 are represented graphically (see Attachment B). High concentrations were detected leaving the treated plantation (Dewells 1997 East) even though 3 months elapsed between application and the first sampling. It is possible that concentrations were even higher closer to the time of application. Significant streamflow is estimated to have commenced in early July, with major streamflow and spillway flow at Warren following a major storm event on 28 July. This event is likely to have delivered the largest load of atrazine to Warren Reservoir. Some care is needed in interpreting the data, as streamflow was not measured and some relatively high concentrations were recorded when flow declined during spring, particularly in 1999.

Atrazine concentrations at sampling points further down the catchment declined rapidly through dilution, but still reached more than 3 µg/L during August at station 4 just

above the Warren reservoir. Concentrations in the following season when no atrazine was applied remained for the most part below 1 μg/L, but with occasional values above 2 μg/L at stations 10 and 2 close to the sites of treatment. Stream concentrations declined rapidly with passage of less contaminated water, but reservoir concentrations declined only slowly even when the source of contamination was removed. Note only the concentrations at site 2, close to the plantation exit, exceeded the ANZECC guideline.

Waterholes Creek is relatively small, consisting of ephemeral drainage lines that flow for short periods (a few days). Most of the catchment is forested, either with pine plantations or native forest. Second rotation sites, mounded perpendicular to the contour to minimise waterlogging, were treated with Forest Mix granules in 1997 and again at a marginally lower rate in 1998. Catchment details are tabulated below (Table 11).

Table 11: Catchment details

Site	Flows to:	Treated area (cumulative)	Catchment area	Catchment area ratio
Dewells 1997 West (11)	6	51.0 ha	157.9 ha	32.3%
Forties 1998 inlet (9)	3	0.0 ha	300.0	0.0
Forties 1998 Exit 1 (8)	6	16.0 ha	333.3 ha	4.8%
Forties 1998 Exit 2 (7)	6	6.0 ha	63.8 ha	9.4%
Road bridge (6)	23	86.2 ha	2308.1 ha	3.7%
Ford (23)	5	94.9 ha	2630.0 ha	3.6%
Yatala 1998 W Exit (12)	5	4.0 ha	9.0 ha	44.4%
Forbes Ford (5)	Warren	98.9 ha	2719.0 ha	3.6%

Atrazine concentrations found in the catchment are represented graphically in Appendix 5. Highest concentrations were again found in the upper part of the catchment as water left Dewells 1997 West. High concentrations were also found just below Forties 1998. No data were available for Yatala 1998 over this sampling period, but sub-ppb levels were detected the following winter. Atrazine contamination above 20 μg/L was detected at station 5, just above Warren Reservoir, during August 1998. This indicates significant inputs from Yatala 1998 as concentrations at station 23 just upstream remained in the low ppb range. The picture is more complicated here due to the three contributing sites, but again the ANZECC guidelines fail to be met only at sampling sites relatively close to the plantation exits.

Hexazinone concentrations followed similar trends during 1998, but tended to be higher than atrazine concentrations in the following season, with peaks after rain events.

Site preparation practices have been modified since the 1998 season. Rather than pushing into windrows and burning, logging debris on second rotation sites is now largely retained, after chopper rolling. More woody biomass is removed. Ripping and/or mound ploughing now conforms more closely to the contour. Care continues to be taken to avoid spraying in 10 m buffer strips retained along each side of stream lines, including first and second order drainage lines. Spraying occurred in these areas during 1998 because of uncertainties regarding their definition. Tracks, streams and buffer

strips occupied only 2% of the treated area, but it is possible that they contributed the bulk of the contamination to downstream reservoirs.

11.5.3 Summary of Surface Water Studies

Surface water sampling at a range of Australian timber plantation sites detected atrazine in a number of samples, with most samples collected during periods of baseflow remaining below the detection limit in low CAR stations. Some samples collected as water left plantation areas in Queensland and NSW following storm events were found to contain residues in excess of 20 µg/L. Contamination in Queensland is thought to have arisen from point sources such as road areas rather than runoff from the general plantation area. Changes to management practices were effective in reducing this contamination, although residues around 2.0 µg/L remained in low CAR stations downstream from the plantation. Also flood events for the second year were mainly months after application, not days as in the previous year. Contamination in NSW is thought to have occurred because ephemeral drainage lines were treated, although this hypothesis was not confirmed.

Stream monitoring in the Mount Lofty ranges occurred in response to low level contamination detected in downstream impoundments. Residues reached as high as 100 µg/L in water leaving treated areas, but were diluted to the low µg/L level further down the catchment. Downstream impoundments were persistently contaminated in the low µg/L range. Contamination at this site reflected its vulnerable soils, with a highly permeable sand layer overlying an impervious clay that permits runoff. Site preparation practices were also a major contributor, as mounding perpendicular to the contour facilitated runoff, which was diverted into streams after leaving the treated area.

11.5.4 Leaching Studies

Leaching Studies in Queensland Timber Plantations

Leaching studies at Imbil were conducted on a slightly acidic red podzolic loam soil with high levels (4-7%) of organic carbon in the surface 20 cm. Site preparation in the preceding winter consisted of raking large woody debris into windrows spaced at 15 m intervals across the contour. Atrazine was applied manually by knapsack at 5 kg/ha in February, April and August 1998 and February 1999, to a small cover of weeds on each occasion. Significant rainfall (65 and 44 mm respectively) was recorded in the week following the first two treatments. Conditions were dry in the week following the third treatment, and 156 mm was recorded in the 3 weeks following the fourth.

Application rates were confirmed using alfoil targets. Anomalous results were obtained after the third application when only 10% of the nominal rate was recovered. The reasons for this shortfall are unclear. Field recovery from the soil was quantitative for this treatment with around 6 mg/kg recovered from the surface 10 cm on the day after treatment, but only 4% of applied for the preceding treatment and well below expected for the remainder, suggesting interception by and dissipation from surface debris. Bromide tracer leached to the 60-90 cm sample by 14 days after treatment, but atrazine residues mostly remained confined to the surface 30 cm, with detections deeper in the soil on one occasion attributed to sample contamination. Both dealkylated metabolites were recovered at low levels, with DEA reaching 0.12 mg/kg at 60-90 cm 14 days after

the third treatment and 0.22 mg/kg at 20-30 cm 21 days after treatment, and DIA recovered from the surface 10 cm at 0.24 mg/kg on the day after the fourth treatment. The half-life was 12 days after the third treatment but could not be determined for the other three treatments because of low initial recoveries. Residue accumulation with successive treatments was neither expected nor detected. The limit of detection was 0.04 mg/kg.

The Toolara studies were conducted on an acidic gleyed podzolic sand with less than 1% organic carbon. Atrazine leaching was considered more likely to occur at Toolara than other sites because of high rainfall, low organic carbon (less than 1%) and high sand content (88%) in the soil. The site was prepared in the preceding winter with a single pass using a dozer drawn winged ripper to provide 2 m wide cultivated planting strips spaced 5 m apart, which carried a small cover of weeds. Atrazine was applied manually at 5 kg/ha in August and November 1998 and March and June 1999.

Bromide tracer was not used at this site. Atrazine residues reached a maximum of about 6 mg/kg in the surface 10 cm on the day after the third treatment and remained confined mostly to the surface 30 cm, with concentrations remaining below 0.7 mg/kg deeper in the soil. The half-life was 21 days after the initial treatment and 14 days after the later treatments. Residue accumulation with successive treatments was neither expected nor detected. Dealkylated metabolites were occasionally detected at low levels in surface samples, for example 0.16 mg/kg DEA and 0.33 mg/kg DIA in the surface 10 cm on the day after the final treatment.

A network of piezometers was installed to a depth just above the clay restriction layer (1.6-1.8 m) in order to sample shallow groundwater. Atrazine was detected at a maximum concentration of 0.6 µg/L 14 days after treatment, accompanied by 0.3 µg/L DEA. The residence time was less than 43 days, with disappearance thought to reflect degradation as the low slope and modest soil hydraulic conductivity typical of the study area would have allowed only limited lateral flow to occur.

Leaching Studies in Tasmanian Timber plantations

Tasmanian investigations were carried out on a free draining acidic ferrosol with 8% organic carbon in the surface 5 cm. Atrazine was applied by tractor to bare ground at 8 kg/ha in November 1996, immediately prior to planting of *Eucalyptus nitens*. The former pasture was sprayed out with glyphosate and the site was cultivated with a mound plough on a 3.5 m spacing. Significant rain (44 mm) fell in the week after treatment.

Bromide was only detected in the surface 5 cm, and only at the fourth analytical attempt nearly 3 years after sampling. The failure to detect the tracer can not be explained.

Atrazine recovery from alfoil plates was about half that expected from the application rate. Atrazine residues remained confined to the surface 30 cm, apart from some low level detections in the 30-60 cm segment between 7 and 28 days after treatment. Maximum residues in the surface 10 cm were about 4 mg/kg, 7 days after treatment. Results from the final (912 day) sampling are not yet available. The estimated half-life of atrazine in the soil was 140 days (this was confirmed by Environment Australia as 144 days using a pseudo-first order kinetics, $r^2 = 0.7321$). Only one of the dealkylated

metabolites (DEA) was detected, reaching significant levels near the surface (for example, 0.66 mg/kg 112 days after treatment) but remaining below 0.1 mg/kg deeper in the profile.

Leaching Studies in Western Australian Timber Plantations

The Myalup site was situated on an acidic podzolised sand with low organic carbon (less than 1%) that was cultivated in April 1996 with retention of considerable harvest debris from the previous pine crop. Atrazine was applied manually at 4.5 kg/ha to weed free ground prior to planting. Significant rain (39 mm) fell in the week after treatment.

The bromide tracer leached rapidly through the soil profile, being found in all samples to 150 cm taken at 7 days after treatment.

Recovery of atrazine from alfoil collectors was double that expected from the application rate, perhaps reflecting separation of atrazine and the bromide tracer in the spray tank. Recovery from soil was less than expected based on the application rate, perhaps reflecting interception by surface debris. Atrazine residues reached a peak of 1.6 mg/kg in the surface 10 cm on the day after treatment and were mainly retained in the surface 30 cm, with traces (0.02 mg/kg) detected to 90 cm in 7 day samples and to 60 cm in 14 and 28 day samples. Traces of DEA (0.03-0.06 mg/kg) were recovered from surface samples at 28 and 56 days after treatment. The estimated half-life for atrazine was 25 days.

Leaching Studies in South Australian Timber Plantations

The Mt Gambier site was situated on an acidic podzolised sand with high organic carbon (6.5%) in the surface 5 cm. The site was mound ploughed on a 2.5 m spacing in November 1995 after preparation with a crusher roller in February 1995. Atrazine was applied by knapsack at 4.5 kg/ha to a small cover of grass and broad leafed weeds in August 1996 and June 1997. Only the second treatment was closely followed by rain (23 mm over 7 days).

Bromide leached rapidly through the soil profile, being found in all samples to 150 cm by 14 days after the first treatment, and to 90 cm by 7 days after the second treatment.

Atrazine recovery from the soil was low after the first treatment (initial residues of 0.93 mg/kg in the surface 10 cm) but more normal after the second (initial surface residues of 4.53 mg/kg). Residues were retained in the surface 30 cm after the first treatment, but some low level detections (0.08-0.19 mg/kg) were detected to 90 cm on the day after the second treatment. The estimated half-life of atrazine at this site was 35 days, and accumulation with successive treatments was neither expected nor detected. As at Myalup, DEA was the only metabolite detected, at 0.05-0.08 mg/kg in the surface 20 cm at the 28 and 56 day samplings.

Summary of Leaching Studies

Leaching studies at timber plantation sites indicated a low likelihood of groundwater contamination. Low level groundwater contamination can occur at vulnerable sites, notably those where opportunities exist for rapid contamination via bypass flow.

The studies also provided insights regarding the effect of climate on persistence of atrazine. Short soil half-lives of 2-3 weeks in Queensland support the use of multiple treatments to control heavier weed growth in that State. Accumulation of soil residues is not expected, even with three treatments per year.

11.6 Atrazine in annual cropping areas

Atrazine is a commonly found contaminant of surface and ground waters in Australia, because of its widespread use particularly within irrigated agriculture and timber plantation areas. The NRA's existing chemicals review records that atrazine concentrations in the order of 100 µg/L have been found in irrigation drainage water from rice growing areas. It occurs commonly in natural surface waters, generally at concentrations below 10 µg/L but with occasional higher excursions, generally associated with storm events. Only limited monitoring of groundwater has been conducted, but atrazine was detected at concentrations in the order of 1 µg/L, accompanied at some sites by the metabolite DEA.

Monitoring has continued in annual cropping areas since the APVMA review in 1997. Results from Syngenta Crop Protection P/L are from projects on the Atherton Tablelands, Darling Downs, Liverpool Plains, Lachlan River near Cowra, and Western Australian canola growing areas. Further information on aquatic contamination by atrazine in Queensland and likely contributory factors is contained in a report from the Condamine Balonne Water Committee (CBWC, 2002).

Atrazine is a common contaminant of surface waters, particularly in the summer months, but occurs at relatively low levels (for the most part below 1 µg/L). The highest concentration found was 15 µg/L in a farm dam at Blackwood, indicating that runoff transports atrazine from canola growing areas. Significant metabolite concentrations also occurred, indicative of high metabolic activity. Unless a major rainfall event occurs, such dams are well suited for retaining contaminated runoff as they are kaolin lined and will not leak to groundwater.

Occasional low level detections occurred in groundwaters. More work is needed to determine their significance, but no widespread problems with groundwater are apparent from results to date.

11.6.1 Western Australian Canola

Monitoring for atrazine has been carried out in Western Australia from November 1998 to July 1999 as part of a requirement for a permit for atrazine use in TT canola (Stubbs and Eksteen 1999). Three geographically distinct areas were selected (near Geraldton, the Blackwood River catchment and Esperance) and a total of 113 samples were taken from surface (river, creek, dam or seep) and groundwaters (bores or peizometers). Seventy-four detections (65%) higher than the level of quantification *detection?* (LOD = 0.05 µg/L for atrazine and desethylatrazine) were found with the highest of 15 µg/L of atrazine from a dam (all dams are used for stock water only) in the Blackwood catchment and 12 µg/L for desethylatrazine in a dam in the Arrowsmith River catchment near Geraldton. The highest concentration from a natural waterway was 9.4

μg/L atrazine from a creek in the Blackwood catchment. Surface and groundwaters in the Esperance region experienced rare low-level spikes of atrazine only from runoff (maxima of 0.68 and 0.16 μg/L respectively). The highest concentration of desethylatrazine, which is reported to be of comparable toxicity as the parent atrazine, was 9.6 μg/L from a creek in the Blackwood catchment. Together with the atrazine concentration of 5.8 μg/L in that sample, the combined concentration of 15.4 μg/L can be taken as total atrazine. Hydroxyatrazine was never detected above its LOD of 0.05 mg/L.

The two bores in the Arrowsmith catchment registered pulses of atrazine which may require further investigation as little is known about the fate of pesticides in water-repellent sands. Streit (1999) suggests a possible point source contamination, a shallow waterbody beneath a treated sandy soil or possible extensive groundwater contamination.

Most river samples were below the LOD. There were 46 positive surface water samples for atrazine but only one sample, from a dam, exceeded the ANZECC/ARMCANZ Australian Water Quality Guideline value of 13 μg/L. The data suggests that the atrazine appears to be runoff from treated TT canola fields and is metabolised to desethylatrazine. As on-farm stock water dams in WA are lined with kaolin clay to reduce seepage into groundwater, they are well suited for retaining runoff from treated fields unless high rainfalls occur (Streit 1999). The detections in the Irwin and Arrowsmith Rivers indicate that monitoring should continue and management measures may need to be implemented if detections exceeding the guideline continue. The monitoring should be more targeted to creeks and rivers in vulnerable areas that have high triazine use, high water tables and sandy soils. Flow and rainfall data should also be included.

When monitoring of stream and groundwater in WA for the year 2000 was to commence, issues were raised on the methodology and the suitability of bores when selecting the sites (Eksteen 2000). The uncertainty delayed implementation and no data are available from the Blackwood and Irwin areas. However, as the sites were already selected in Esperance, monitoring continued from April to December 2000. Three bore sites and one river site was selected. Rainfall data and bore depth was also provided. The use of atrazine in this catchment increased substantially from 1000 L in 1998 to 7800 L in 1999. 19 samples were taken from bores and 7 samples from the river. Six samples were positive, three from the bores and three from the river. Concentrations from the bore sites varied from 0.08 to 0.57 μg/L and from the river varied from 0.30 to 0.39 μg/L. All were well below the ANZECC Guidelines for Fresh and Marine Water Quality value of 13 μg/L. However, they were well above the NH&MRC guideline value of 0.1 μg/L. The study recommended further monitoring and "that monitoring commences in the groundwater tables of regions where drinking water is derived from aquifers (eg Geraldton and Mingenew). This matter has already been taken up in a separate report for use of atrazine (and simazine) on TT canola.

11.6.2 Liverpool Plains

Syngenta has commissioned an assessment of the transport and fate of atrazine on the Liverpool Plains after studies in the late 1990s found atrazine at concentrations up to

14 µg/L in a significant proportion of groundwater samples (Peirson *et al*, 1999). Atrazine is commonly used in this area for weed control in sorghum, which tends to be grown on long rotations with wheat, with each crop followed by a long fallow period (at least 10 months) to allow soil moisture to accumulate.

Rainfall is variable but generally heaviest during the summer growing season, often occurring in heavy storms that cause flooding. Floodplain soils are prone to waterlogging after winter floods. Ephemeral streams or flood runners are present in many paddocks, facilitating the transport of contaminants in surface runoff following rain.

Atrazine is commonly detected in surface waters of the region, generally at concentrations in the order of 1 µg/L when present. Higher concentrations occur at some locations, for example 17 µg/L in the Mooki River at Ruvigne in September 1996, 25 µg/L upstream at Caroona in January 1997, and 29 µg/L higher in the catchment in Big Jacks Creek at Warrah Ridge in August 1996. The metabolite DEA was present in these samples at about 10% of the atrazine concentration. Estimated mass flux of atrazine from the Liverpool Plains in fluvial transport is about 500 kg per annum, or 0.1% of the estimated use (470 tonnes).

Peak atrazine concentrations were synchronised across the region, tending to follow periods of heavy atrazine use but not necessarily associated with periods of heavy rain. Similar patterns of contamination in Canada, independent of runoff events, have been linked to carelessness associated with operating equipment close to streams. Evidence was found for the deposition of pesticides close to or directly into stream water during the process of drawing water, mixing pesticides, spraying, or cleaning equipment, or from seepage from containers discarded in and around the spray site (Fawcett, 1998).

Groundwater beneath the Liverpool Plains is commonly contaminated by atrazine, usually in the order of 1 µg/L but reaching 14 µg/L in one sample. Higher levels generally occur close to sorghum crops. Some of the detections are thought to reflect rapid bore leakage as atrazine detections were accompanied by chemicals regarded as non-mobile, and discarded chemical drums were found around some bores. Rapid bypass flow is also possible as the reactive clay soils of the area are prone to cracking.

11.6.3 Central and North West Regions of NSW Water Quality Program

Earlier results from this long running and valuable program were described in the APVMA interim review report published in November 1997. Residues are found in the Gwydir, Namoi and Border Rivers of NSW, but have been absent from the Macquarie basin in recent years, perhaps reflecting better flows and improved farm management practices (Cooper, 1995). Atrazine can be found throughout the river basins including their headwaters, with levels approaching 10 µg/L occurring within irrigated agriculture sites. Detailed investigations at one site on the Gwydir revealed that the contamination arose via irrigation supply channels, notwithstanding that no applications of atrazine to the channels had been made for at least 12 months. It was noted that some 25% of cotton growers used atrazine for irrigation channel hygiene during the 1994-95 season, and that only 45% of growers were aware of the proposed removal of this use, to take effect by December 1995.

Detection of atrazine continued to be a common feature when monitoring in the Gwydir, Namoi and Border river basins during the 1995-96 season, but with no clear temporal pattern. The Macquarie basin, where impacts from the drought continued to be felt, was again free of this contaminant (Cooper, 1996). Detections occurred upstream of and within irrigation areas, with 19% of samples taken within irrigated areas between November and March recording more than 0.5 µg/L. Peak concentrations approached 5 µg/L in Coxs Creek and exceeded 4 µg/L in the Mooki River following storm events during January, with peak instantaneous loads of 120 and 100 kg/day, respectively. The Liverpool Plains in the Upper Namoi valley, where dryland farming of cotton, sorghum, sunflowers and maize predominates during summer, was identified as a problem area due to a limited capacity to harvest storm runoff and store the water on-farm. Atrazine loads were considerably increased from the previous season when planting was reduced by drought and peak instantaneous loads during January storms were relatively low at 29 kg/day.

All river basins showed a stable or declining trend of atrazine levels in the 1996-97 season (Muschal, 1997). Slight seasonal fluctuations were evident, from higher concentrations over summer to generally lower levels during the winter months, consistent with the use pattern. Use occurs predominantly in spring prior to planting of summer crops.

Results from the 1997-98 season (Muschal, 1998) indicated that the upper Namoi River basin had the highest frequency of atrazine detections and the highest concentrations of all catchments, reflecting the high usage of atrazine on broadacre dryland cropping in the Liverpool Plains area. Highest concentrations (6.5-7.5 µg/L) occurred in late spring and early summer, but 1-2 µg/L were recorded throughout the year.

The highest median concentration of atrazine in the 1998-99 season was 0.2 µg/L at two sites on the Darling River, at Bourke and 25 km upstream. The Namoi and Gwydir Rivers also recorded substantial levels of atrazine contamination. Broadacre farming in the upper Namoi River area was again identified as a significant contributor to the total atrazine load in this catchment. No overall rising or falling trends were evident for the catchments studied, although some fluctuations had occurred since 1991, including the slight upward trend for the Darling River this season (Muschal, 2000).

The report notes that broadacre dryland cropping adjacent to waterways and through natural drainage lines means that runoff flows through crops and directly into waterways. The major source of atrazine across the central and north west regions of NSW is believed to be dryland farms that are not designed to collect and control surface runoff arising from storms. No real increasing or decreasing trends were discernible over the last few seasons for any river basin.

11.6.4 North Queensland – Atherton Tableland Cane fields

This site was selected because it represented a newly developed cane growing area that was previously used for growing rice. The site has a significant component of surface-driven hydrology, but also a number of ground water issues. This site is also unique for the sugar industry as it drains west into the Gulf of Carpentaria, rather than into the Pacific Ocean.

Monitoring in Cattle Creek commenced in 1997 and included monitoring the concentrations of a number pesticides in surface runoff from three sites, upper catchment (mainly horticulture) sugarcane, sub-catchment (100% sugarcane) and downstream of Cattle creek (whole catchment). Monitoring in stream samples over a three year period showed virtually no atrazine in the upper catchment. The sugarcane sub-catchment showed concentrations similar to that found in the lower catchment. The lower catchment site was well below the mixing zone and concentrations of atrazine were up to 25 μg/L but mainly below 15 μg/L in the "wet" November-February. Many were above ANZECC Water Quality Guideline of 13 μg/L. In the seasonal dry periods, atrazine concentrations were usually <5 μg/L.

During early 1999, bore sampling was commenced at monthly intervals at three sites that are located near the existing surface sampling sites. Bores from the upper catchment and downstream (whole catchment) showed no detectable levels of atrazine over the sampling period indicating that atrazine was not entering these aquifers in any significant quantity. Both bores showed limited hydrological response to seasonal changes with bore height changes ranging between approximately 0.3 and 0.8 m even after heavy rainfall.

The sugarcane sub-catchment was the most hydrologically responsive with approximately 5 m recharge response between wet and dry seasons. Atrazine concentrations varied from approximately 0.12 μg/L, increasing over the monitoring period of 19 months to approximately 0.95 μg/L. Desethylatrazine concentrations varied from approximately 0.25 μg/L at the start of the monitoring period to 4 μg/L at the end of the monitoring period.

The data suggest that the main pathway for off-site movement of atrazine in this study area is via surface runoff. Data indicates that 90% of the annual loss of atrazine occurs during the wet season, even though only 35% of the total atrazine is applied during this period. If applications of atrazine were confined to the "dry" 8 months, exports would be approximately 10% of that currently occurring (Simpson 2001).

11.6.5 Southern Queensland – Condamine Balonne

Pesticide contamination is a particular issue on the upper floodplain of the Condamine River, from Warwick downstream to Chinchilla. This area encompasses the Darling Downs, a highly productive agricultural region characterised by intensive dryland and irrigated agriculture on gently sloping recent alluvial plains. Agricultural activities predominantly occur adjacent to the river and its main tributaries, taking advantage of the fertile soils (deep, self-mulching black earths) and flatter terrain. This river is ephemeral, flowing only after significant rainfall events, which mainly occur in summer with high intensity summer storms causing high runoff and erosion. Atrazine concentrations are typically low in the upper part of the floodplain but high at Chinchilla Weir (lower end of the upper floodplain) and moderate further downstream.

An exception to this typical pattern of atrazine contamination occurred when elevated concentrations of atrazine were detected in Oakey Creek at Fairview (downstream from Toowoomba, near the confluence with the Condamine River) in November 2001.

Extensive sorghum plantings, with associated atrazine applications, had occurred across the region in response to good rains (around 50 mm) that had wetted the soil profile. A further 50 mm fell in the fortnight following planting. Atrazine concentrations in samples taken from Oakey Creek at Fairview were 11.1 µg/L on 11 November and 8.0 µg/L the day after. Downstream on the Condamine, concentrations of 9.8 µg/L and 12.0 µg/L were recorded in Loudon Weir on the morning and afternoon of 12 November, together with small concentrations (0.21 and 0.55 µg/L) of desethylatrazine. Concentrations in five samples taken from Loudon Weir earlier in 2001 and two from 2000 had remained below 1 µg/L, and for the most part below 0.1 µg/L. More persistent contamination was evident further downstream at Chinchilla Weir, with concentrations in the order of 1 µg/L (together with desethylatrazine at around 10% of the atrazine concentration) in eleven samples taken between February 2000 and August 2001. The subsequent storm event does not appear to have been sampled at Chinchilla Weir. Note that the dealkylated metabolite desethylatrazine is likely to have similar aquatic toxicity to atrazine, based on findings in the 1997 interim review.

Sampling of groundwater at three randomly selected bores in 2001 found significant contamination (1.84 µg/L) at one location (St Ruth, Dalby) in April, decreasing to 0.73 µg/L by the following September. Earlier sampling at five other locations had found no contamination. The Dalby incident appeared to reflect direct contamination rather than leaching, as high concentrations (19 µg/L) of metolachlor were also found at the earlier sampling, soon after the commencement of pumping following the off season, but had declined to 0.16 µg/L in the later sample, taken after 3 weeks of constant pumping (CWBC, 2002).

The above project, which has been partly funded under the Natural Heritage Trust, also includes extension work on methods to minimise off-farm movement of pesticides, such as use of buffer strips along local waterways and conservation tillage farming practices which can reduce runoff by retarding overland flow and improving infiltration of water into the soil. The CBWC report noted that dry land cultivation adjacent to the Condamine at Chinchilla occurs well within the 60 m buffer where atrazine should not be applied. The Queensland Department of Natural Resources and Mines has recently formed a partnership with local government, community leaders and the principal atrazine registrant, Syngenta, to further investigate these problems, for example by improving chemical application methods.

The pattern of contamination seen on the upper floodplain of the Condamine is similar to that seen in the US midwest. High surface water concentrations of atrazine occur in drainage basins dominated by row crops, often in conjunction with storm events. Goundwater contamination is much less of an issue because atrazine tends to be lost in runoff or by degradation in warm and biologically active soils through the summer growing season. Higher peaks of contamination are detected leaving smaller impoundments downstream, while contamination in larger impoundments tends to occur at lower levels but is more protracted.

11.6.6 South West Victoria

A study to assess the environmental impacts of three different cropping treatments was undertaken in south-west Victoria. Surface runoff samples were collected and analysed for atrazine and simazine as well as a number of other parameters (Hollywell, 2001).

All samples were below the LOD (0.5 µg/L) for atrazine and simazine. However, south-west Victoria experienced very dry conditions for a number of years and water samples could not be collected and analysed until 17 months after applying the herbicides making it very unlikely that target chemicals would be detected.

11.6.7 Summary of Monitoring in Annual Cropping Areas

Data from studies across Australia in major atrazine use areas, indicate atrazine is a commonly detected contaminant. Generally levels are below 1 µg/L but some are found in excess of 20 µg/L. As in forestry situations, the main contributor to riverine contamination appears to be surface runoff. Dryland paddocks in the upper Namoi, for example, often contain ephemeral flood runners. Overland flow of water during flood events transports atrazine residues into waterways.

11.7 Conclusions

Available monitoring data for atrazine in Australian and US surface waters indicate broadly similar patterns of contamination. At vulnerable sites, atrazine may reach concentrations in the order of 100 µg/L in small streams leaving high CAR treated sites, if storm events occur within a short time of application. Concentrations in larger rivers and reservoirs downstream tend to be below 1 µg/L, but may exceed this threshold in areas with intensive atrazine use on soils conducive to runoff. Contamination is likely to persist year round with little variation in reservoirs with limited outflow, but declines between seasons in flowing waters. Degradation half-lives of atrazine in soil range from 12 days in Queensland to 140 days in Tasmania depending on the climate and soil temperature.

The pattern of atrazine contamination in Australian surface waters indicates that safety margins continue to be narrow in some areas, both for timber plantations and annual cropping. The key factor that determines the likelihood of aquatic contamination appears to be the vulnerability of the soil to surface runoff. Water infiltration into the soil needs to be addressed and rapid movement of surface runoff to waterways minimised. Best management practices (BMPs) have a key role to play in reducing chemical losses in runoff. BMPs can be defined as practices or combinations of practices, industrial techniques and good house keeping principles determined to be the most effective and practical known means of preventing or reducing the amount of non-point source pollution.

Storm events pose the highest risk. A major risk factor in both timber plantations and annual cropping appears to be the treatment of ephemeral drainage lines. Subsequent water flow mobilises atrazine residues. Ephemeral drainage lines should not be treated with atrazine, particularly if runoff events are likely to follow. Ideally, they would not be cultivated, nor cropped.

An associated risk factor is the treatment of runoff water. This water should be directed into permeable areas, ideally through spreading structures, rather than channelled into waterways. Retention in dams may be another option for reducing river contamination, but may conflict with State water supply requirements.

Careless handling can also be a major contributor to stream contamination, as exemplified by the high residues found in Queensland and linked to mixing and loading activities on runoff prone surfaces (forestry roads). The observation that broadscale surface water contamination in NSW cotton areas is synchronised with heavy use but not necessarily with rainfall suggests that careless handling may also be a problem in annual cropping areas. Such contamination can be reduced by avoiding handling and mixing activities in areas such as roads and river margins from which surface runoff can move easily to surface waters.

Site preparation practices are also important, particularly in timber plantations. Mounding perpendicular to the contour improves drainage but facilitates the rapid transport of atrazine residues from general cropping areas into waterways. Mounding along the contour helps retain water on site, which provides greater opportunity for residues to infliltrate the soil. Where drainage may be a problem, trees can be grown on top of these mounds.

Improving infiltration and discouraging drainage to waterways reduces risks of surface water contamination but potentially increases risks to groundwater. Moderately sorbed compounds such as atrazine are more likely to be lost in surface runoff or degraded in the soil. Although groundwater contamination appears in general to be a lesser problem than contamination of surface water for such chemicals, risks may still be significant at vulnerable sites. Groundwater is vulnerable to contamination where soils are permeable and water tables are shallow. Permeable soils are usually sandy, but rapid bypass flow in cracking clay soils or in karstic terrain can also lead to groundwater contamination. Particular care is needed when handling chemicals in such areas, with strict avoidance of such activities in the vicinity of bores or recharge areas. For example, some recorded instances of higher groundwater contamination appear to have been caused by careless handling of atrazine concentrate or working solutions near bores.

A key reason for conducting the FHRMG trials was to demonstrate that, with the label restrictions introduced in the mid 1990s, the Water Quality Guidelines could be met. The data show that this effort has largely been successful but modifications to practices already made need to be underpinned through BMPs. BMPs need to be tailored to fit the hydrology of a watershed, but the main focus should be on avoiding or minimising runoff from treated areas into waterways, particularly soon after application. Key issues are the use of chemicals near water or on steep slopes or erodible soils.

A variety of BMPs, appropriate to local conditions, have been developed for forestry activities across Australia, under the umbrella of flexible codes of practice in each State. These BMPs mainly address erosion and soil movement, and may be less effective for contaminants such as atrazine which are mobile in solution. The key to minimising off-site transport of atrazine is to avoid use and handling on hard impermeable surfaces or in areas such as ephemeral drainage lines where water may flow. Such drainage lines will show evidence of the action of periodically flowing water (for example, gravel, pebble, rock or sand bed, scour hole or nick point) and/or an incised channel at least

30 cm deep. These issues can be addressed through label instructions, as outlined below. If these label instructions are followed, atrazine concentrations in rivers should be below the relevant water quality guidelines when forestry activities are conducted according to established codes of practice.

The ANZECC Guidelines for Fresh and Marine Waters are recommended limits to acceptable changes in water quality that will continue to protect the environmental values. It should be noted that water quality should not be degraded to these levels. Whether ambient water quality is above or below the guideline values, the philosophy of continual improvement is promoted. Long-term management aims should be to improve water quality to levels that are better than those defined by the guidelines.

The NHMRC Australian Drinking Water Guidelines has the same philosophy as the ANZECC guidelines as indicated by the following. "The guidelines should never be seen as a licence to degrade the quality of a drinking water supply to the guideline level" (the guideline value for atrazine is 0.1 μg/L). Pesticides should not be found in drinking water, hence the guideline value is set at the limit of detection. This value is the level at which the pesticide can be reliably detected using practicable and readily available and validated analytical methods. If a pesticide is detected at or above this value the source should be identified and action taken to prevent further contamination. The health value is intended to be used by health authorities in managing the health risks associated with inadvertent exposure such as a spill or misuse of a pesticide.

Based on the data available, the results indicate that contamination of streams during periods of baseflow and zero flow is low when following BMP, but that ANZECC guidelines may be breached when flow is elevated soon after application of atrazine. Various contributing factors were identified: treatment of ephemeral drainage lines in NSW, mixing activities on roads in Qld, and mounding perpendicular to the contour on soils conducive to runoff in SA. Reducing atrazine contamination of runoff from storm events in particular, requires further attention to minimise contamination of streams.

Accordingly, DEH recommends that the APVMA not be satisfied that use of atrazine products in accordance with their recommendations for use (label instructions) would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment.

DEH recommends that the APVMA not be satisfied that labels for atrazine products contain adequate instuctions to ensure that the use of the products in accordance with their recommendations for use (label instructions) would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment.

DEH recommends that if labels are varied as set out below, the APVMA can be satisfied:

(i) that use of atrazine products in accordance with the recommendations for their use (new label instructions) would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment; and

(ii) that labels for atrazine products would contain adequate instructions to ensure that the use of the products in accordance with their recommendations for use (new label instructions) would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment.

It is recommended that the following instructions be added to product labels:

- DO NOT apply product to any drainage line. Drainage lines show evidence of the action of periodically flowing water (for example, gravel, pebble, rock or sand bed, scour hole or nick point) and/or an incised channel at least 30 cm deep;
- DO NOT handle, mix, apply or conduct testing operations to areas susceptible to runoff where drainage results in rapid entry into waterways. These areas include roads, access tracks, snig tracks and compacted log dumps where no specific action has been taken to prevent runoff into waterways, or areas mounded perpendicular to the contour.

The APVMA accepts the recommendations of the DEH assessment and therefore proposes the regulatory approach outlined in Section 6.

2. Use patterns may be modified

Due to the climatic conditions in Queensland forestry (and areas in northern NSW with a similar climate) up to three treatments per season may be required because the climate favours growth of weeds and atrazine has been shown to degrade relatively quickly in such a climate. The available monitoring data supports this application frequency.

Should registrants wish to incorporate these instructions on to product labels, they would still need to make application to the NRA. However the data requirements for the environmental component of this application may be reduced in light of the findings noted in this report.

11.8 References

Ball J, Donnelly L, Erlanger P, Evans R, Kollmorgen A, Neal B & Shirley M (2001) Inland Waters, Australia State of the Environment (Theme Report), CSIRO Publishing on behalf of the Department of the Environment and Heritage.

Blanchard PE & Lerch RN (2000) Watershed Vulnerability to Losses of Agricultural Chemicals: Interactions of Chemistry, Hydrology and Land-Use. Environ Sci Technol, 34: 3315-3322.

Bubb K & Barnes C (2000) Results from a Nation-wide Study on the Risk of Atrazine Contamination to Surface Water and Groundwater Resulting from Routine Application by Australian Forest Growers. Forest Herbicide Management Group. Draft report dated May 2000 to the NRA.

CBWC (2002) Fate of Nutrients and Minimising Pesticides in the Riverine Environment – Final Technical Report. Condamine Balonne Water Committee Inc, Dalby, Queensland.

CCREM (1989) Canadian Water Quality Guidelines, Appendix V. Canadian Council of Resource and Environment Ministers, September 1989.

Cooper B (1995) Central and North West Regions Water Quality Program, 1994/95 Report on Pesticides Monitoring. TS95.087 Water Quality Unit, Department of Land and Water Conservation NSW.

Cooper B (1996) Central and North West Regions Water Quality Program, 1995/96 Report on Pesticides Monitoring. TS96.048 Water Quality Services Unit, Department of Land and Water Conservation NSW.

Eksteen, D (2000) Report on Atrazine Monitoring in Western Australia. Agriculture Western Australia.

Fawcett RS (1998) The Role of Best Management Practices in Reducing Triazine Runoff. In: Ballantine LG, McFarland JE & Hackett DS (Eds) Triazine Herbicides: Risk Assessment. Americal Chemical Society, Washington DC. Oxford University Press.

Giddings JM & Hall LW (1998) The Aquatic Ecotoxicology of Triazine Herbicides. In: Ballantine LG, McFarland JE & Hackett DS (Eds) Triazine Herbicides: Risk Assessment. American Chemical Society, Washington DC. Oxford University Press.

Gilliom RJ, Alley WM & Gurtz ME, Design of the National Water-Quality

Assessment Program: Occurrence and Distribution of Water-Quality Conditions. USGS Circular 1112.

Girling AE, Tattersfield L, Mitchell GC, Crossland NO, Pascoe D, Blockwell SJ, Maund SJ, Tayloer EJ, Wenzel A, Janssen CR & Jüttner I (2000) Derivation of Predicted No-Effect Concentrations for Lindane, 3,4-Dichloroaniline, Atrazine and Copper. Ecotoxicology and Environmental Safety, 46: 148-162.

Goolsby DA & Pereira WE (1995) Pesticides in the Mississippi River. In: Meade RH (Ed) Contaminants in the Mississippi River. USGS Circular 1133, Reston, Virginia, 1995.

Gruessner, B & Watzin, MC (1996) Response of Aquatic Communities from a Vermont Stream to Environmentally Realistic Atrazine Exposure in Laboratory Microcosms. Environ Toxicol Chem, 15: 410-419.

Hollywell (2001) Department of Natural Resources and Environment, VIC, Letter to the NRA, 13 February 2001.

Huber, W (1993) Ecotoxicological Relevance of Atrazine in Aquatic Systems. Environ Toxicol Chem, 12: 1865-1881.

Michael JL & Neary DG (1993) Herbicide Dissipation Studies in Southern Forest Ecosystems. Environmental Toxicology and Chemistry, 12: 405-410.

Muschal M (1997) Central and North West Regions Water Quality Program, 1996/97 Report on Pesticides Monitoring. CNR97.063 Ecosystem Management, Department of Land and Water Conservation NSW.

Muschal M (1998) Central and North West Regions Water Quality Program, 1997/98 Report on Pesticides Monitoring. CNR98.038 Ecosystem Management, Department of Land and Water Conservation NSW.

Muschal M (2000) Central and North West Regions Water Quality Program, 1998/99 Report on Pesticides Monitoring. CNR2000.004 Ecosystem Management, Department of Land and Water Conservation NSW.

NH&MRC (1996) Australian Drinking Water Guidelines, National Health and Medical Research Council/Agricultural and Resource Management Council of Australia and New Zealand.

Peirson WL, Acworth RI, Timms W & Dorairaj S (1999) Preliminary Assessment of the Transport and Fate of Atrazine on the Liverpool Plains. WRL Technical Report 99/13 dated November 1999, University of New South Wales Water Research Laboratory.

Scribner EA, Battaglin WA, Dietze JE & Thurman EM (2003) Reconnaissance Data for Glyphosate, Other Selected Herbicides, Their Degradation Products, and Antibiotics in 51 Streams in Nine Midwestern States, 2002. Open File Report 03-217, USGS, Lawrence Kansas, 2003.

Simpson B (2001) Atrazine Monitoring Program, Atherton Tablelands, North Queensland. Department of Natural Resources, Queensland.

Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, La Point TW, Kendall RJ, Weisskopf CP, Giddings JM, Giesy JP, Hall LW Jr & Williams WM (1996) Ecological Risk Assessment of Atrazine in North American Surface Waters. Environ Toxicol Chem, 15: 31-76.

Streit L, (1999) Monitoring for Atrazine in Ground and Surface Water – Progress Report December 1999. Novartis Crop Protection Australasia P/L

Stubbs P & Eksteen D (1999) Final Preliminary Report on Atrazine Monitoring in Western Australia. Moora District Office, Agriculture Western Australia, Moora, Western Australia.

US EPA (2000) Status of Chemicals in Special Review. EPA-738-R-00-001 dated March 2000, Office of Prevention, Pesticides and Toxic Substances.

US EPA (2003) Ambient Aquatic Life Water Quality Criteria for Atrazine – Revised Draft. Document no EPA-822-R-03-023 dated October 2003. US EPA Office of Water.

USGS (1998) Herbicides in Midwestern Reservoir Outflows, 1992-93. USGS Fact Sheet 134-98, December 1998

USGS (1999a) The Quality of Our Nation's Waters - Nutrients and Pesticides. USGS Circular 1225, Reston, Virginia.

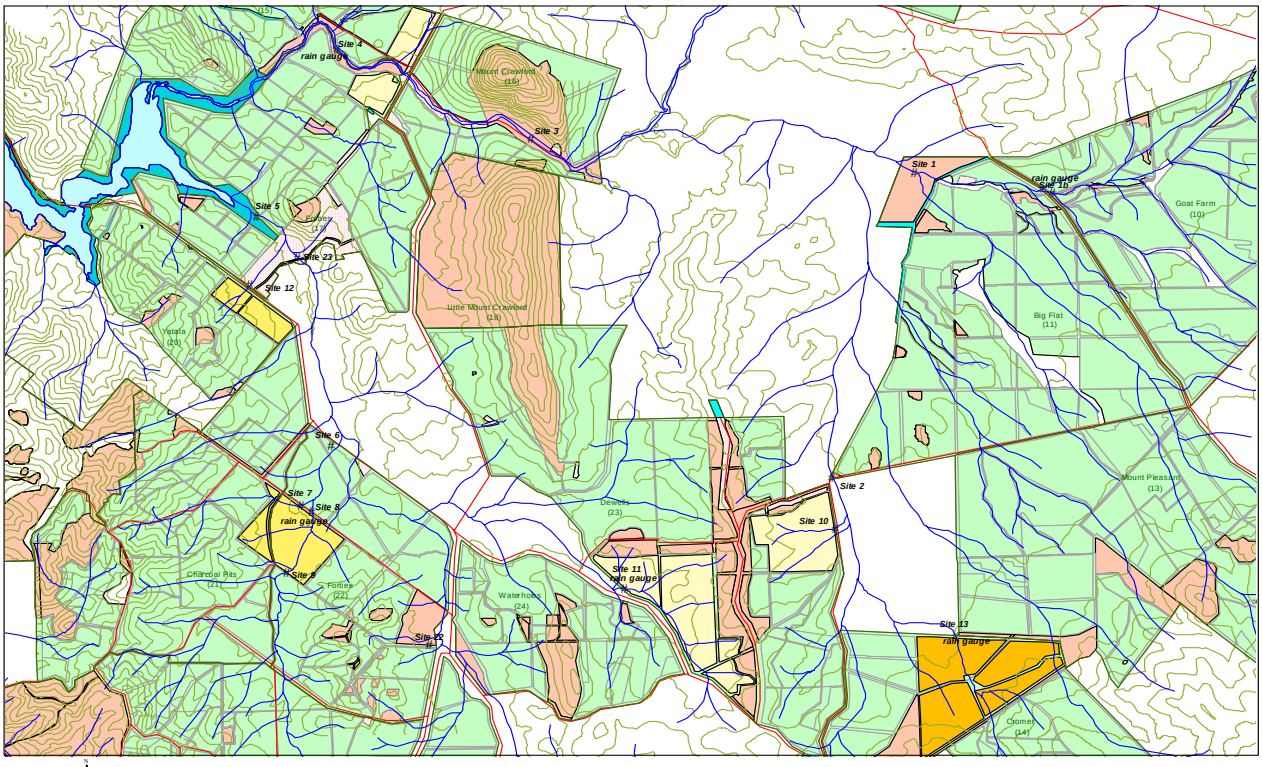
USGS (1999b) Discharge of Herbicides From the Mississippi River Basin to the Gulf of Mexico, 1991-97. USGS Fact Sheet 163-98, April 1999.

Wilkinson GR (2001) Building Partnerships – Tasmania's Approach to Sustainable Forest Management. In: International Conference on the Application of Reduced Impact Logging to Advance Sustainable Forest Management: Constraints, Challenges and Opportunities. 26 February to 1 March 2001, Kuching, Sarawak, Malaysia. Compendium of Conference Papers, pp 219-226.

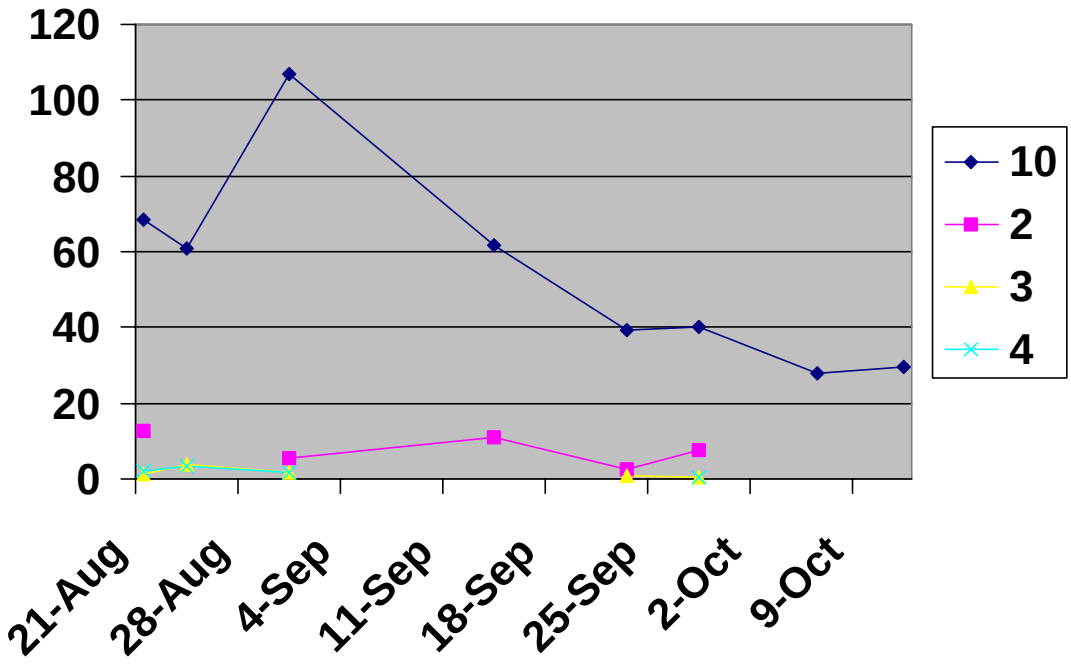
Appendix 4 – Water monitoring sites

Green areas are *Pinus radiata* plantation, with 1997 plantings represented as pale yellow and 1998 plantings as yellow. Orange areas were planted in 1999 but treated with Trounce (glyphosate/metsulfuron) rather than atrazine/hexazinone, following a directive from the SA EPA. Brown areas are native forest under ForestrySA control, while areas around reservoirs are native forest under SAWater control.

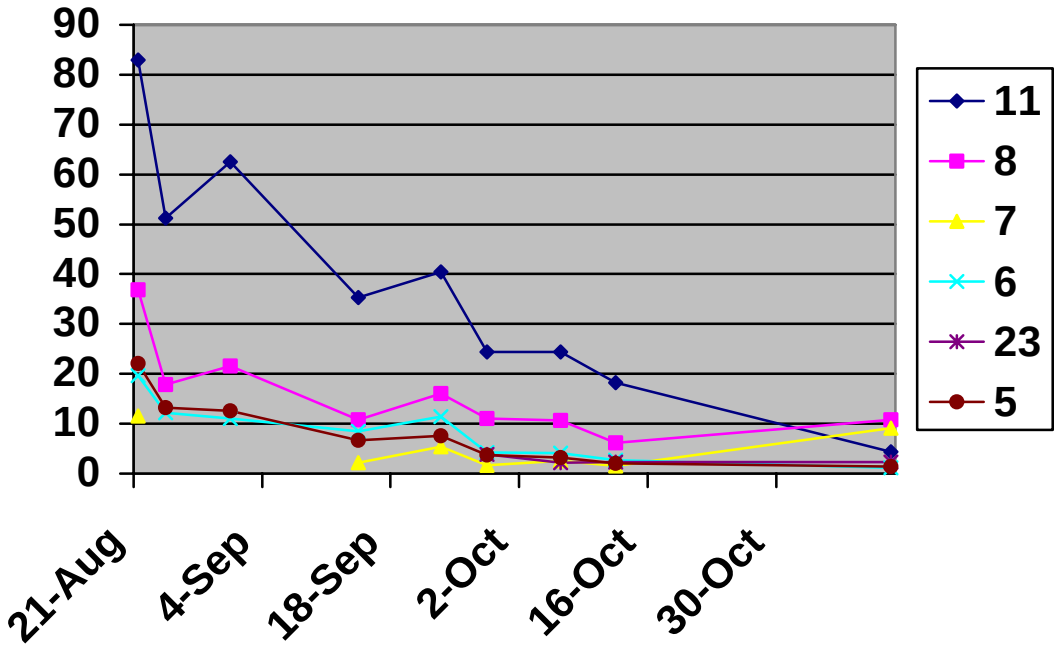
Water Monitoring Sites - Mt Crawford Forest



Appendix 5 – Atrazine Monitoring data



Atrazine concentrations in South Para catchment during spring 1998



Atrazine concentrations in Waterholes Creek catchment during spring 1998

12. ATRAZINE AND AMPHIBIANS

12.1 Introduction

The ongoing reviews of atrazine in the US and Australia have been delayed by several recent studies concerning the potential effects of atrazine on amphibian development and sexual differentiation. Some of these studies report effects at very low concentrations (as low as 0.1 µg/L).

The interim Australian review report for atrazine (APVMA, 1997) found atrazine to be slightly toxic to fish (LC50s in the 10-100 mg/L range) but with sub-lethal effects such as reduced motility and increased pigmentation evident in the low mg/L range, and minor structural alterations seen in kidneys at autopsy following exposure to 5-40 µg/L. The no observed effect level in life cycle studies on fathead minnow was 0.25 mg/L based on reduced growth of offspring.

Atrazine was found to be much more toxic to aquatic vegetation than to fish, as expected given its herbicidal activity. Laboratory EC50s for algae and aquatic macrophytes were typically in the 40-200 µg/L range following 3-14 day exposures. The interim Australian review found that aquatic ecosystems may be damaged if exposures were maintained above a threshold of 20 µg/L.

Predicted environmental concentrations in the interim Australian review did not raise significant concerns for acute toxicity to aquatic fauna, but were sufficiently high to indicate a potential risk of disruption to aquatic vegetation and consequent indirect impacts on aquatic fauna. However, these predicted concentrations were based on conservative assumptions and expected to be transient, with prolonged exposure to concentrations that may damage aquatic ecosystems not anticipated. Monitoring data for Australia indicated that most aquatic exposures to atrazine were likely to be in the low µg/L range, around two orders of magnitude below the predicted environmental concentration. Peak levels in Australian natural surface waters were generally less than 10 µg/L and declined rapidly, and revisions to use patterns such as the cessation of most non-crop use including in irrigation channels had been introduced to help reduce aquatic contamination.

12.2 Background

The toxicity of atrazine to frogs was not specifically assessed in the interim Australian review as tadpoles generally are no more sensitive than fish to the toxic effects of chemicals. However, a flow-through mesocosm study (Detenbeck *et al*, 1996) was described, in which the effects of chronic atrazine exposure were studied under simulated wetland conditions (freshwater emergent marsh). Caged leopard frog tadpoles were subjected to a stepped exposure regime (2 weeks at a nominal concentration of 15 µg/L, 2 weeks at 25 µg/L, 1 month at 50 µg/L and 2 weeks at 75 µg/L). Control mesocosms received water from the Mississippi River, containing 0.69 µg/L atrazine.

No significant effects on tadpole growth or development were detected. There appeared to be a tendency towards accelerated development in treated mesocosms, but this could not be confirmed because of variability between mesocosms. Periphyton gross productivity was reduced at concentrations as low as 15 µg/L. Reduced maximal dissolved oxygen and increased ammonium concentrations were probably related to this reduced productivity. The authors note that exposure to atrazine tends to select against filamentous green and blue-green algae and to shift community composition towards dominance by more tolerant diatoms and chrysophytes. Metaphyton (floating filamentous algae) may be especially vulnerable because of the tendency for atrazine to concentrate in the organic rich surface microlayer.

This section evaluates laboratory toxicity and microcosm studies of atrazine in amphibians that have become available since the interim Australian review, as well as the new information on developmental and other sublethal effects. Longer term toxicity testing in tadpoles presents numerous difficulties, particularly if conducted to metamorphosis, and few if any methods for assessing chronic larval toxicity in laboratory amphibians have been accepted internationally. The current state of development in this emerging field will therefore be discussed in some detail. As relatively few studies have been reported to date, this will necessarily include consideration of chemicals other than atrazine.

12.3 Acute toxicity

A revised draft (US EPA, 2002) of the ongoing US EPA review of atrazine's environmental fate and effects assesses one acute toxicity study in frog larvae (Howe *et al*, 1998). Another laboratory study of the acute toxicity of atrazine to frogs (embryos, larvae and adults) has been published more recently (Allran and Karasov, 2001). These two new laboratory studies are described below. They confirm that, as for fish, atrazine is slightly toxic to tadpoles (LC50s in the 10-100 mg/L range).

12.3.1 Leopard frogs and American toads

The toxicity of atrazine has been studied in wild-collected larvae of the northern leopard frog (*Rana pipiens*) and the American toad (*Bufo americanus*) after gradual acclimatisation in the laboratory to deionised well water over a period of a week, during which they were fed twice daily with fish food and cultured green algae. Holding tanks were cleaned daily, and water flow rates were maintained to provide a minimum of one exchange of water per hour. Early and late stage larvae [Gosner (1960) stages 29 and 40 - body weights of 0.7-0.9 g and 1.4-1.9 g for the frogs, and 0.1-0.2 and 0.4-0.5 g for the toads] were then exposed according to standard procedures (ASTM, 1985) in duplicate groups of 10 organisms at 22^{o}C in glass jars containing 15 L of oxygen saturated exposure water, using nine unspecified exposure concentrations (range finding studies were conducted in the 0.1-1000 mg/L range) and a control. The test material was a commercial formulation containing 40.8% atrazine. Deviations from nominal concentrations were confirmed by immunoassay to be within 10%. Mortality and obvious sublethal effects were recorded at 1, 3, 6, 24, 48, 72 and 96 hours, and dead animals were removed daily.

Table 12: Toxicity of atrazine to different life history stages of *Rana pipiens* and *Bufo americanus* tadpoles.

Species (stage)	**24 h LC50 (95% CI)**	**96 h LC50 (95% CI)**
R pipiens (early)	69.7 (63.1-77.2) mg/L	47.6 (41.4-54.8) mg/L
R pipiens (late)	45.3 (42.3-48.5) mg/L	14.5 (11.9-17.5) mg/L
B americanus (early)	66.4 (58.9-74.9) mg/L	26.5 (23.0-30.5) mg/L
B americanus (late)	15.8 (13.5-18.4) mg/L	10.7 (9.2-12.5) mg/L

Results are tabulated above, as nominal concentrations. Surviving larvae in the 2.8-23 mg/L range exhibited abdominal oedema after 6-24 hours exposure, which the study authors suggest was probably caused by renal disfunction. They also appeared sluggish with impaired locomotion and orientation, which may increase susceptibility to predation in natural environments. Late stage larvae were more sensitive than early stage larvae, perhaps reflecting increased stress with the onset of metamorphosis (Howe *et al*, 1998).

12.3.2 Leopard frogs, wood frogs and American toads

Embryos were collected from ponds in the US mid-west where atrazine levels were negligible (<0.1 µg/L). The anurans (seven clutches of *R pipiens* and three of *R sylvatica*) were put under treatment as early to late gastrula (Gosner stages 10-12) and the toads (four clutches of *B americanus*) as midcleavage embryos to late gastrula (Gosner stages 8-12). Groups of 50 embryos (30 for the wood frogs) from each clutch were exposed at 22^{o}C according to standard procedures (ASTM, 1996) in glass petri dishes (10 x 2 cm containing 75 mL treatment solution) to six concentrations (0-20 mg/L) of 99% pure atrazine, under static conditions with daily transfer by pipette to renewed test medium. Hatchability was determined at day 6, and exposure continued for a further 96 hours to assess mortality, deformity and swimming speed.

Hatchability of embryos and mortality and swimming speed of larvae did not differ among atrazine treatments and controls. However, there was a dose-dependent increase in deformity (blistering and oedema, tail and axial malformations) in all three species, with no difference between species. The NOEL was 2.6 mg/L, and the LOEL 4.3 mg/L.

Adult leopard frogs (7.5-9 cm in length) from a commercial supplier were tested individually in aquaria for 14 days under similar static renewal conditions, with forced cutaneous exposure between 11 am and 7 pm each day. Buccal and thoracic ventilation rates were measured each day at noon. There was a dose dependent increase in buccal ventilation up to 7.2 mg/L and a subsequent decline, with increasing thoracic ventilation beyond this point. It was suggested that this may reflect reduced availability of oxygen with higher concentrations of atrazine, increased metabolism associated with detoxification, or a direct toxic effect on the physiological systems that control ventilation. Reduced oxygen-carrying ability of the blood was thought to be unlikely as haemoglobin levels remained stable.

Adult frogs stopped eating at the highest exposure of 20 mg/L but did not lose weight, apparently because of compensatory oedema (Allran and Karasov, 2001).

12.4 Microcosm studies

As noted in the introduction, the effects of longer term exposure to atrazine on frog growth and survival have also been studied in mesocosms. Atrazine may indirectly affect the growth of larval anurans by decreasing phytoplankton and periphyton biomass, thereby reducing dissolved oxygen levels and food availability.

Another study (Diana *et al*, 2000) on the effects of atrazine on frog growth and survival in artificial pond microcosms has been published since the interim Australian review. Microcosms (1.22 m diameter plastic wading pools containing 90 L pond water) containing phytoplankton, periphyton, macrophytes and larval grey tree frogs (*Hyla versicolor*) were treated with various levels (nominally 0, 20, 200 and 2000 µg/L) of atrazine (technical grade, in acetone solution) 5 weeks after establishment (11 days after hatching, when larvae were 15 days old). Initial concentrations were confirmed by analysis, and declined by 10-20% in the three weeks after treatment. Predusk dissolved oxygen levels dropped sharply at the two higher doses before recovering after about 10 days, but again declined at 21 days after exposure. Similar declines in pH occurred. Frogs in these microcosms were 5% shorter and 10% lighter in weight at metamorphosis than those exposed to 0 or 20 µg/L atrazine, and the larval period was extended by 5% at 2000 µg/L. There were no significant treatment related effects on survival.

The effects noted were thought to reflect oxygen deficiency rather than reduced availability of food or a direct toxic effect of atrazine, although the latter was considered conceivable at the highest exposure. Resource availability was considered an unlikely cause of the developmental delays as chlorophyll *a* concentrations remained largely unaffected, although the authors conceded the possibility that atrazine resistant species prevailing under herbicide exposure could have been less palatable, less nutritious or toxigenic.

This study supports the conclusion of the interim Australian review that the no effect concentration for atrazine in aquatic ecosystems is 20 µg/L.

12.5 Longer term toxicity testing

Acute toxicity testing with larval amphibians can be conducted using procedures similar to those developed for fish. The African clawed frog (*Xenopus laevis*), sometimes referred to as the laboratory rat of the amphibian world, has a long history of use in the 96 hour whole embryo developmental toxicity test known as FETAX (Frog Embryo Teratogenesis Assay – *Xenopus*) which is a well established test for human developmental hazard identification and also applicable to ecotoxicological hazard assessment using water/soil/sediment samples (NIEHS, 2000).

Chronic testing is less straightforward, particularly if conducted to metamorphosis. As noted above, late stage larvae may be more sensitive than early stage, in contrast to fish where the smaller early life stages are generally most sensitive. Many amphibians switch from aquatic to terrestrial life forms following metamophosis, and from vegetarian to carnivorous diets, as well as undergoing stress associated with rapid physiological change at this time.

12.5.1 Test organisms

The National Research Coucil of the National Academy of Sciences reported in 1974 on the breeding, care and management of laboratory amphibians (NRC, 1974). Relatively little was known about the critical factors involved in long-term laboratory culture when the committee was convened. When the committee reported, defined laboratory amphibian lines were still under development.

The committee noted that extreme care must be exercised in providing optimal environmental conditions during two delicate stages in amphibian life cycles, namely embryonic development and metamorphosis. Rapid physiological changes occur at both periods and sudden environmental changes may prove detrimental or lethal.

The committee also reported that unusual sex ratios may be observed in laboratory-reared or laboratory bred amphibians. Some laboratories administer estradiol or testosterone to larval stages to control the numbers of males and females needed for breeding stock. Thus, animals may be phenotypically female but genetically male or vice versa. Experimental results that may vary as a consequence of disparity between genetic and phenotypic sex should be interpreted with caution, given the normal lability of sex determination in certain species of amphibians.

Use of amphibians in longer term toxicity testing has attracted interest in recent years under the US EPA's Endocrine Disruptor Screening Program (Battelle, 2002). Two anuran species (*X laevis* and *R pipiens*) are widely used in amphibian studies.

12.5.1.1 African Clawed Frogs (*Xenopus laevis*)

The NRC (1974) reported that the African clawed frog, or platanna, is widely distributed in sub-Saharan Africa, and has also established wild populations in southern California and elsewhere. It is a highly aquatic species that may migrate overland or aestivate under dry conditions. Ponds in its native habitat may dry out during long periods of low rainfall.

Larvae of *Xenopus laevis* develop rapidly, with cleavage and gastrulation taking place within a day of fertilisation at 20-22^{o}C. Hatching (Nieuwkoop-Faber stage 35, Niewkoop and Faber, 1975) occurs on the second or third day. They may be transferred to larval enclosures by siphoning or dipping (not netting) after they have fed for a few days. Larvae should be fed finely ground food when they begin to swim along the bottom. Care is needed with feeding as introduction of coarse food particles can congest filter-feeding mechanisms. Larvae should be able to clear any added food within 4-5 hours. Food slurries should be freshly prepared each day. Growth is facilitated by a second daily feeding. Larval density should not exceed 6-8 per litre. Tanks should be cleaned at 3 day intervals.

Under these conditions, metamorphosis should commence 5-6 weeks after fertilisation and be complete within 15-20 days. Care is needed to keep the water clear, particularly after forelimb eruption when larvae stop feeding until the tail is half resorbed. Animals

will then take solid food, such as finely diced meat, mosquito larvae or redworms. Juveniles may be treated as adults after a month or so.

Further information on this species is available in a recent detailed review paper (Battelle, 2002). Adults, embryos and tadpoles can be obtained from several commercial vendors who specialise in the rearing and distribution of these frogs. Adults can be maintained under static or flow-through systems, but care is needed to ensure that flow does not disturb the frogs because they live naturally in static environments.

Larval development may be divided into three phases (Nieuwkoop-Faber stages 46-53, 54-57 and 58-65). The first, premetamorphosis, is a period of embryonic and early larval development (including the development of hind-limb buds) that takes place without thyroid hormone. This is followed by prometamorphosis which is characterised by rising levels of endogenous thyroid hormone, with more specific morphogenesis such as differentiation of toes and rapid elongation of hind limbs. A surge of thyroid hormone occurs during metamorphic climax with forelimb development, tail resorption and other final processes.

Although acute toxicity testing with this species in FETAX is well established, there are no standardised approaches to testing amphibians for developmental effects in longer term exposures. Many amphibians prefer a static environment, but the difficulties associated with longer term static renewal exposure studies necessitate the use of a flow-through design. For static renewal studies, partial rather than complete replacement of culture water is recommended, particularly during metamorphosis when these animals are sensitive to sudden environmental changes. *X laevis* tadpoles should be maintained in small groups at low densities (ideally 6-8 metamorphic-age tadpoles per litre, although slightly greater densities can be used during earlier development) if they are to be successfully raised through metamorphosis in a normal period of time (2-3 months).

Adults are sexually dimorphic, with females 10-15 cm in length and males 5-10 cm. Sexually mature females possess an enlarged cloaca from which eggs emanate during breeding, while males possess thick black nuptial pads on their forearms. Metamorphic age froglets are 2-3 cm in length.

A recent US EPA White Paper (Steeger and Tietge, 2003) notes that *X laevis* larvae can be successfully raised to metamorphosis with minimal mortality in around 55 days, using a flow-through system delivering 0.025 L/minute to groups of 20 larvae. Larvae reach a maximum weight of 1.8 g, with metamorphic weight around half the maximum weight. Conditions that delay development of unexposed organisms beyond 70 days suggest problems with food or water quality and introduce uncertainties regarding the effects of overall delayed development on system specific development.

12.5.1.2 Leopard Frogs (*Rana pipiens*)

The NRC (1974) reported that the leopard frog is widely used in laboratory research. It is a highly variable species, perhaps a species complex, with wide differences in body size, colouration, and pattern. Local populations tend to differ, even between adjacent ponds. They are semiterrestrial and found in shallow water habitats throughout their

range; where protective cover occurs, they will often wander well away from water. Leopard frogs occur from southern Labrador to southern MacKenzie and eastern British Columbia southward throughout the United States and into Central America.

Embryos are held in shallow water (15-20 mm) until feeding begins a few days after hatching, as indicated by strands of faecal material appearing in the water. Dead embryos should be removed regularly, and the water clarity should be maintained by changing at least every 3 days. Maximum larval density should be 50 per litre, dropping to 6 per litre at metamorphosis. Overcrowding stunts growth. Young larvae tend to be vegetarian while older larvae are omnivorous. Postmetamorphic survival and maturation efficiency are greatly improved by adequate larval nutrition.

The detailed review paper (Battelle, 2002) notes that, in the laboratory, metamorphosis is complete within 3-4 months, and sexual maturity is reached in 1-2 years. Sexual dimorphism is not readily apparent in this species. Larvae can be raised under static or flow-through conditions although attention to water quality is required. Husbandry, breeding and rearing are more difficult than for *Xenopus*.

12.5.2 Husbandry considerations

A key consideration for chronic studies is the considerable developmental plasticity exhibited by amphibians as an adaptation to the ephemeral environments in which they often occur. Such adaptation has been demonstrated by many researchers under controlled laboratory conditions. Various factors may affect larval development, including nutrition, competition and predation pressure. The effects of resource limitation may vary depending on whether this occurs early or later in the developmental process. It is thought that amphibians need to attain a minimum size or accumulate sufficient reserves to allow them to complete metamorphosis. Resource limitation during early development tends to extend the larval period, while similar restriction during later development is likely to reduce the larval period, although metamorphs will tend to be smaller than would occur in the absence of such constraints. Species may respond differently to resource limitation during development depending on the characteristics of their natural habitat, with plasticity especially pronounced in species that breed in ephemeral and unpredictable habitats. The following selected experimental observations exemplify this behaviour.

12.5.2.1 Stocking density

The larval stage is extended and fewer animals reach metamorphosis when tadpoles of *Rana temporalis* are maintained at higher density in the laboratory. Size at metamorphosis declines significantly with increase in the density of rearing. These effects are less pronounced when siblings are raised together, while competition is greater with mixed rearing (Girish and Saidapur, 2003).

Stocking density is often progressively reduced through the larval period in order to overcome these effects in laboratory culture. For example, Gendron *et al* (2003) progressively thinned leopard frog tadpoles to a final density of 1 per litre in order to attenuate the negative impact of growth inhibitors emitted by fast-growing individuals.

12.5.2.2 Resource constraints

Studies with larvae of the desert amphibian Couch's spadefoot toad (*Scaphiophus couchii*) indicate that development rate and age at metamorphosis are primarily determined by the early food regime, while size at metamorphosis is determined by food level late in the larval period (Newman, 1998).

Laboratory studies with western spadefoot toads (*Scaphiophus hammondii*) found that early to mid prometamorphic tadpoles (Gosner stages 30-32) were capable of accelerating metamorphosis in response to declining water levels, reaching metamorphic climax in as little as 22 days, albeit with reduced body size. These effects were observed whether tadpoles were raised in groups or individually. Larval body mass was restored to control levels within 2-3 days when tanks were refilled. Early prometamorphic tadpoles (Gosner stages 26-28) did not grow or develop when challenged in this way (Denver *et al*, 1998).

12.5.2.3 Australian studies

Recent Australian studies (Doughty and Roberts, 2003) have examined the effects of resource constraints on development of the quacking frog (*Crinia georgiana*). This species occurs in SW Western Australia in wetlands on the Swan coastal plain and granite outcrops and streams in the Darling Range. In their natural habitat, quacking frogs are under strong time constraints to complete development rapidly under low nutrient conditions. Larvae hatch at a relatively advanced stage (Gosner 26-27) from a large aquatic egg and complete development rapidly compared with other species.

When reared in the laboratory under static-renewal conditions (water changes every 2 days) with daily feeding through the larval period, metamorphosis was reached in about 33 days irrespective of the food provided (boiled and frozen lettuce or two different levels of protein rich mixed chow). Tadpoles maintained on lettuce had a similar metamorphic size to those captured from the field, but those fed the high protein diet achieved much larger metamorphic size. Deteriorating conditions (reduced water levels with less or no food) reduced the larval period to 29-32 days, depending on the stage when they were imposed (more rapid development from Gosner stages 34-37 than from Gosner stage 32) and the severity of the constraints. Size at metamorphosis was reduced, particularly when more severe conditions were imposed earlier in development.

The authors note that resource constraints early in the larval period tend to delay metamorphosis in other species, but suggest that treatments were imposed too late in the development process to detect such a threshold in these studies. Alternatively, options for delaying metamorphosis when resources are low during early development may be limited in this species because of the strong time constraints and low nutrients that exist in its natural habitat. Most tadpoles in this species have sufficient reserves at hatching to complete metamorphosis because of the large egg size.

12.5.2.4 Endogenous hormones

The response of amphibian larvae to resource constraints and other stresses is hormonally mediated through the hypothalamo-pituitary-interrenal (HPI) axis. Corticotropin-releasing hormone (CRH) secreted by the hypothalamus controls the release of adrenocorticotropic hormone (ACTH) by the pituitary, which in turn stimulates glucocorticoid release from the interrenal glands (homologous to mammalian adrenal cortex). Glucocorticoids mediate the metabolic changes that are associated with the stress response and act on the hypothalamus and pituitary to decrease synthesis and release of CRH and ACTH. Corticoids mobilise stored fuels and increase metabolism under emergency situations (hence the common designation as stress hormones) at the expense of slowed tadpole growth. Basal plasma corticoid concentrations in amphibian larvae tend to increase during development. The concentration of these hormones in many species is low during premetamorphosis but increases during late prometamorphosis and peaks at metamorphic climax. Experimental studies of the role of the HPI axis in responding to stress and modulating development are described below, while interactions of this axis with thyroid and sex hormones are discussed later in this report in the context of the US EPA's endocrine disruptor screening program.

Measurement of whole body corticosterone content by radioimmunoassay in tadpoles of two anuran species (*Xenopus laevis and Rana pipiens*) commonly used in laboratory testing has confirmed the above trends, except that basal levels in *X laevis* were highest during premetamorphosis (foot paddle stages) but much lower during prometamorphosis (hind limb stages). The response of the HPI axis to an external stressor (shaking/confinement) or ACTH injection (0.04-1.36 IU/g) was most sensitive in premetamorphs, which may increase the ability of these vulnerable earlier developmental stages to cope with competition or predation (Glennemeier and Denver, 2002a).

Chronic exposure to moderate doses of exogenous corticosterone, sufficient to elevate whole body content by 50%, was associated with slowed growth and retarded hindlimb development in premetamorphic *R pipiens*, while reduction of whole body content by 50% using the corticoid synthesis inhibitor metapyrone (MTP) was associated with increased size at metamorphosis but no change in metamorphic timing (Glennemeier and Denver, 2002b).

Elevation of whole-body corticosterone content in premetamorphic *R pipiens* by short term (4 day) exposure to environmental stress (increased conspecific density or limited resource levels) was associated with reduced tadpole growth and slowed development. The corticosterone elevation appeared to be responsible for the growth effects of crowding, but not the developmental, as blocking corticosteroid biosynthesis with MTP reversed the growth suppression caused by high tadpole density but not the developmental effects. The authors suggest that the developmental effects may be due to some other physiological factor such as thyroid activity, noting that hormones interact in complex ways to orchestrate tadpole growth and development (for example, corticoids synergise with thyroid hormone during prometamorphosis to accelerate metamorphic changes). MTP did not reverse the developmental and growth effects caused by limited resources, suggesting that these probably reflect metabolic constraints involving insufficient nutrients rather than modulation by corticosteroids (Glennemeier and Denver, 2002c).

Sex steroids may also vary in response to stress. For example, large declines in testosterone that have been measured in adult male and female water frogs (*Rana esculanta*) following capture from the field have been attributed to short term captivity stress (Gobbetti and Zerani, 1996). The decrease in testosterone was shown to be accompanied by increased aromatase activity and 17β-estradiol levels.

12.5.2.5 Water quality

Poor water quality (for example low dissolved oxygen and high ammonia) is unfavorable to optimum survival, growth and development of laboratory raised amphibian larvae. Recently reported research indicates that these effects of poor water quality may involve endocrine mechanisms.

Hypoxia has recently been identified as an endocrine disruptor in immature adult carp. Levels of testosterone, 17β-estradiol and triiodothyronine were significantly reduced in male carp after 4 weeks exposure to hypoxia and in female carp after 8 weeks. Levels of 17β-estradiol were significantly increased in male carp after 8 weeks. Gonadal development was retarded in males and females. There were adverse effects on reproductive performance (reduced spawning success, sperm motility, fertilisation success, hatching rate and larval survival) after 12 weeks of exposure to hypoxia (Wu *et al*, 2003). An accompanying press release from the American Chemical Society notes that carp are naturally resistant to hypoxia, and that hypoxia may cause endocrine disruption in other fish and amphibian species.

A recent news report (Pelley, 2003) indicates that nitrate pollution is also a suspected endocrine disruptor, although more work needs to be done to demonstrate cause and effect. According to the report, a developmental endocrinologist (Guillette) from the University of Florida has found an association between low testosterone levels in alligators and high nitrogen concentrations in Florida lakes (these findings were announced at a water quality conference in Iowa in February 2002). The report also notes that a steroid endocrinologist (Panesar) from the Chinese University of Hong Kong has shown that nitrate suppresses testosterone production in cells from mouse testes. It is suggested that the effect is due to reduction in the mitochondria of nitrate to nitric oxide, which depresses testosterone synthesis.

The alligator research was also presented at the International Symposium on Environmental Endocrine Disruptors, held in Tsukuba, Japan in December 2001. Apart from the inhibition of steroidogenesis by nitric oxide, it was also reported that exposure of male yearlings to moderate levels (100 mg/L) of nitrate gave rise to aromatase induction.

These research findings indicate that endocrine function in laboratory amphibians may be disrupted by poor water quality. When conducting static testing with atrazine, hypoxia and excessive nitrate may be more likely in exposed replicates than in controls because of the detrimental effects of atrazine on algae, which take up nitrogen from the water and release oxygen during photosynthesis. Atrazine has been shown to reduce dissolved oxygen and increase ammonia concentrations in microcosms, and to shift algal community composition. Such effects are likely to be less pronounced in single species bioassays, but algal colonisation of test solutions is likely to be retarded, with possible adverse consequences for oxygen levels and tadpole nutrition.

Sexual differentiation in anurans is directed by steroid hormones, particularly androgens and oestrogens, during sensitive developmental periods. Given that testosterone and estradiol levels in fish and reptiles have been shown or are suspected to vary in response to changes in oxygen availability and nitrate exposure, sexual differentiation in anurans and the incidence of intersex individuals may be influenced by these water quality parameters. Furthermore, since the timing of onset of sexual differentiation depends on growth and development, this may be affected by various abiotic factors such as temperature, water quality, food availability and space, and may vary between different strains and laboratories. As noted by Mackenzie *et al* (2003) in their model studies with steroid hormones (see below) an increased incidence of intersex may simply reflect delayed development rather than hormonal responses to chemical exposure. In the absence of any dose-response relationship, husbandry considerations offer a more likely explanation for such phenomena than chemical exposures.

12.5.3 Protocol development

The US EPA and the OECD are developing protocols for longer term toxicity testing with amphibians. The current focus is on screening assays for detecting substances that interfere with the thyroid axis. Thyroid hormone controls amphibian metamorphosis. Test protocols for substances that interfere with reproduction are scheduled for investigation after the initial thyroid screening assays.

12.5.3.1 US EPA Endocrine Disruptor Screening Program

The US EPA's now disbanded Endocrine Disruptors Screening and Testing Advisory Committee recommended a battery of screening and testing assays in its 1998 final report. A frog metamorphosis assay was recommended for inclusion in the tier 1 screening battery, and an amphibian development and reproduction assay for inclusion in the tier 2 test battery. Tier 1 screens were recommended for use to make initial judgements about areas of concern in order to direct the focus of tier 2 tests. An Endocrine Disruptor Methods Validation Subcommittee (EDMVS) has been established by the US EPA to provide advice and counsel on scientific issues associated with the conduct of studies necessary for validation of these assays.

A draft detailed review paper (Battelle, 2002) is available for the proposed tier 1 amphibian metamorphosis assay. Amphibian metamorphosis is under hormonal control with thyroid hormone the causative agent. As in most other developmental processes other hormones also control metamophosis, including those from the pituitary and adrenal glands. Metamorphosis can be influenced by a variety of abiotic factors, such as temperature, water availability, crowding, light, diet, and environmental iodine levels, as well as by chemical stressors. Recent investigations have shown that thyroid hormone also plays a role in sex differentiation. Synthesis of thyroid hormone in the thyroid is under complex neuroendocrine control, with complicated feedback loops at the central nervous system, hypothalamus, and pituitary levels. These interactions form the hypothalamus-pituitary-thyroid axis. Interactions also occur between thyroid hormones, sex steroids and glucocorticoids (stress hormones). Overall, it must be understood that the link between the thyroid axis and metamorphosis can be influenced by several different forms of extraneous factors as occurs in many other developmental processes.

The amphibian metamorphosis assay was considered by the EDMVS on 24 July 2002 at its fourth plenary meeting. The US EPA reported that more studies would need to be done before the assay could be considered ready. One source of uncertainty was the interactions of the thyroid with other endocrine systems. A different species (*X tropicalis*) was under consideration for the tier 2 developmental assay because it matures more quickly, but the husbandry had not yet been sufficiently determined for it to be used in the tier 1 metamorphosis assay, although it would likely be used once those issues had been resolved. Only one member of the EDMVS responded to the question of whether prevalidation efforts should be phased in for the metamorphosis assay, noting that the program seemed to be a long way from prevalidation and was rather in the stage of test development.

12.5.3.2 OECD Endocrine Disrupters Testing and Assessment

The OECD has established an *ad hoc* expert group on amphibian testing to discuss and agree on the preferred approach for a *Xenopus* metamorphosis assay as an *in vivo* screening assay to identify chemicals that interfere with the thyroid axis. The assay would serve as a model for thyroid disruption in vertebrates. An advantage of testing in this species is its well characterised development, based on the staging criteria of Nieuwkoop and Faber.

The expert group met for the first time in Duluth (US) on 26-27 June 2003. Based on the outcome of that meeting, the OECD has circulated a proposal for phase 1 of the validation of the assay (Kloas *et al*, 2003). Under this proposal, exposure is initiated at stage 51 (premetorphosis) or 54 (early prometamorphosis) and maintained to stage 58-59 (early metamorphic climax) which requires 21 or 14 days, respectively. The optimum duration is to be determined during validation studies. Larval density is 20 tadpoles per 10 L tank, and tadpoles are fed daily (sera micron at 300 mg/day/tank). The main endpoints are developmental stage, hind limb length and whole body length, measured on a weekly basis. Mortality is checked daily, and should remain below 5% in controls. Thyroid histology is conducted at termination. Larvae are exposed under static renewal conditions with continuous aeration, but flow-through conditions are also to be investigated.

Note that this test uses different endpoints compared with its US counterpart, which measures tail resorption at the end of metamorphic climax. Endogenous thyroid hormones are high during this stage of development, reducing the sensitivity of this endpoint for detecting substances that interfere with the thyroid axis.

12.5.4 Model studies of sexual differentiation

Several recent studies have reported that sexual differentiation in amphibians can be altered by exposure to low doses of atrazine, and have invoked hormonal pathways to explain these observations. The default (homogametic) sex in most anurans is female. Females develop normally in the absence of gonadal steroids, but gonadal steroids are needed for appropriate sexual differentiation of males.

There are no established protocols for such testing. Model compounds with known or suspected hormonal activity have also been tested recently, as outlined below.

12.5.4.1 Steroids and nonylphenol

Amphibian development studies with model compounds (17β-estradiol, 17α-ethinylestradiol, nonylphenol and two aromatase inhibitors) have recently been reported (Mackenzie *et al*, 2003). Only the results with the first three compounds will be discussed here, as the concerns that have been raised in relation to atrazine are said to involve induction of aromatase rather than inhibition.

Leopard frog tadpoles, hatched in the laboratory from wild-harvested eggs, were exposed under static renewal conditions (water change every 48-72 hours) from Gosner stage 25 (start of independent feeding) through metamorphosis, a period of about 124 days (but with some tadpoles yet to reach metamorphosis after 162 days). Tadpole density was maintained at 1 g/5 L. Intersex animals were defined as containing both ovarian and testicular gonadal tissue and germ cells, as determined by histological analysis of 40-50 sections through the dissected gonads of metamorphs.

Analysis of water samples found mean measured concentrations to be 16.3% of nominal for estradiol (1, 10, 50 or 100 µg/L), 41.4% for ethinylestradiol (1 or 10 µg/L) and 9.1% for nonylphenol (10 or 100 µg/L). Concentrations declined between renewals, with the presence of metabolites such as estriol from estradiol indicating a metabolic contribution. Mortality was relatively high (40-58%) in all groups but did not appear to be treatment related. Cannibalism could have played a role, and may help explain differences in development rate (cannibalistic tadpoles would metamorphose faster than noncannibalistic conspecifics). Time to metamorphosis (124±18 days for most control animals) and metamorphic weight and length were not significantly affected by any treatment.

Gonads in control animals were clearly differentiated, with a single intersex individual from 20 examined animals and equal split between males and females. Treated metamorphs had a female-biased sex ratio and there was an increased incidence of intersex gonads in some treatment groups, albeit of variable appearance (isolated oocytes within predominantly testicular tissue, unilateral intersex with regions of testicular tissue within an ovotestis characterised by ovarian tissue, bilateral intersex with two ovotestes, and a single lateral intersex with one testis and one ovary in the same ethinylestradiol exposed individual). The female bias was complete, with no intersex animals, at the two higher doses of estradiol. The authors assume that the intersex animals were genetic males with gonads undergoing transformation to female morphology.

Estrogenic activity was also indicated by a significant increase in the proportion of ovaries containing early vitellogenic oocytes in frogs exposed to ethinylestradiol or the lower dose of nonylphenol, compared with controls and intersex animals where oocytes remained previtellogenic. The rate of atresia (oocyte degeneration) did not appear to be affected by treatment.

The presence of intersex gonads in some control animals indicates that the leopard frogs sampled likely belong to a semi-differentiated race of amphibians, in which developing

tadpoles go through a stage of natural juvenile hermaphroditism that in some cases persists beyond metamorphosis. Previous reports of intersex gonads in the control groups of frog species thought to be gonochoristic (separate sexes, with male and female reproductive organs in different individuals) are cited. The authors conclude that sexual differentiation is a complicated process in some frog populations, based on their observation of natural juvenile hermaphroditism in some broods but not others during similar studies with thousands of leopard frogs sampled from the same pond over several years. The increased incidence of intersex may reflect delayed development, but other mechanisms need to be explored. Further study is also needed to determine whether these alterations in sex differentiation persist into adulthood and influence fitness or reproductive success.

Similar but less pronounced responses were seen in wood frogs (*Rana sylvatica*) which develop more rapidly than leopard frogs (all control animals reached metamorphosis in 47±9 days in this study).

12.5.4.2 Bisphenol A

Considerable detail is reported in a recently published chronic toxicity study in *Xenopus laevis* (Pickford *et al*, 2003) conducted under flow-through conditions. The authors of this study conclude that the method used has considerable potential for adoption as an amphibian chronic larval development test, in support of environmental risk assessment.

The test was initiated with 4 day old larvae obtained by induced spawning of 6 male/female pairs of captive-bred adult *Xenopus laevis*. Test vessels were rectangular glass tanks with a working volume of 9.5 L and water depth of approximately 150 mm. Larvae were exposed in carbon filtered, dechlorinated and UV-sterilised tap water, delivered to the tanks at a nominal flow rate of 400 mL/minute (approximately 6 tank volumes per day) via glass mixing chambers. Stock solutions of bisphenol A and the positive control (17β-estradiol) in basified dechlorinated water were delivered simultaneously to the mixing chambers to provide nominal exposure concentrations of 1, 2.3, 10, 23, 100 and 500 µg/L (2.3 µg/L for the positive control).

Dissolved oxygen (7-9 mg/L), pH (7.2-7.8) and temperature (21.2-22.4^{o}C) were measured in all test vessels, and alkalinity (15-23 mg/L $CaCO_3$), total hardness 42-53 mg/L $CaCO_3$), conductivity (212-237 µS/cm) and salinity (0.5-1.5 ppt) in one test and one control replicate at weekly intervals. A photoperiod of 12:12 light:dark with 20 minute dawn/dusk transitions was used.

Four replicates of 40 larvae (nominal static loading of 4.2 larvae/L) were used for each test concentration and control. Larvae were fed a proprietary fry food, twice per day for 6 days and three times (twice on weekends) thereafter in increasing amounts according to requirements, supplemented at day 40 with a proprietary juvenile pellet food in order to avoid cannibalism of smaller larvae by faster developing froglets.

Larvae were observed daily for mortality, behaviour and appearance, and any dead larvae were removed. All larvae from one replicate were removed and lightly anaesthetised on days 32 and 62 for measurement of total and snout-vent length. From day 41 onwards, individual larvae reaching stage 66 (completion of metamorphosis) were removed and killed by terminal anaesthesia followed by destruction of the brain

stem. Wet weight and total and snout-vent lengths were recorded, and froglets were dissected to assess gross gonadal morphology (male, female or intersex, based on presence of testes, ovaries or mixed tssue types). This assessment was performed before and after fixation of the gonadal-adrenal-mesonephros with Bouin's fixative.

The test was terminated on day 90, and surviving larvae were terminally anaesthetised. Those that had yet to reach development stage 58 were weighed, measured and fixed in formalin, while those between stages 58-66 inclusive also underwent dissection and gonadal assessment as for the froglets.

Time-weighted mean measured concentrations were 83-100% of nominal with the greatest shortfall at 1 µg/L due to more rapid degradation in the stock concentrate for this test concentration. This concentrate was therefore replenished more frequently. Significant shortfalls (29-59% of nominal) also occurred initially in the positive control, but this was corrected by altering the method for preparing the stock concentrate. The time-weighted mean measured concentration of 17β-estradiol over the 90 day test was 2.0 µg/L (75% of nominal). Note that *X laevis* larvae are feminised by exogenous oestrogens during premetamophosis but not during metamorphic climax.

Larval survival was 67.5-97.5% in individual test vessels, and 81.4-90.7 (mean 86.6%) for each test concentration. Mortalities were recorded throughout the exposure period but were most frequent soon after test initiation. It appeared that some larval mortalities went unrecorded, probably reflecting decomposition or concealment in faecal debris of early stage larvae or cannibalism by faster developing froglets, but these unrecorded individuals were nevertheless utilised for statistical analysis of survival. There were no significant differences in mean survival for any test concentration compared with the dilution water control, but survival in one of the highest concentration replicates was significantly lower than in two of the control replicates, and mean survival in the high dose group was significantly lower than in the low dose (1 µg/L) group.

A spread of development stages was evident in the replicates used for measuring tadpole growth at days 32 and 62, but development stage distributions in the bisphenol A exposed groups and positive control did not differ from the dilution water control. Time to metamorphosis remained unaffected except for a significant increase (about 10 days for males and 4 days for females on average) in the positive control. Total length of stage 66 metamorphs also remained unaffected, except for a significant increase in the positive control. Snout-vent length was also significantly increased in males from the positive control. Wet weights remained unaffected in all groups.

Development through metamorphosis reached 77.8% (89.8% of survivors) by the end of the 90 day test. There were only 15 cases among the 996 metamorphosed froglets examined where assessment of gonadal sex differed pre- and post-fixation, and a further 4 cases among the 75 where animals had not reached metamorphosis, with no gender bias in post-fixation reassignment. Only 4 cases had indeterminate structure pre-fixation, and only two of these were diagnosed after fixation as intersex, occurring at 23 µg/L bisphenol A and in the positive control. The percentage of males in test and control replicates varied from 32.0 to 74.2% but remained in the 40-60% range apart from these two outliers. Males represented 47.7% in the pooled controls and 45.2% in the pooled test replicates. A female bias was evident in the positive controls (24.2-37.5, mean 30.0% male) indicating a feminising effects of 17β-estradiol.

The pooled incidence of testicular abnormalities was markedly greater in the positive control (34 animals) than in the dilution water control (8 animals) or bisphenol A exposed groups (5-12, mean 9 animals). Ovarian abnormalities showed a similar pattern (14 in the positive control, 8 in the dilution water control, and 2-8 in bisphenol A exposed groups).

The absence of significant effects of bisphenol A exposure on survival, growth, development and sexual differentiation contrasts with an earlier report (Kloas *et al*, 1999) that exposure of *Xenopus* larvae to 27 µg/L bisphenol A resulted in a female biased sex ratio, this being interpreted as a feminising effect on gonadal differentiation. This more recent study incorporated a number of improvements over the earlier, including more test concentrations, greater replication, improved statistical power, analytical confirmation of test concentrations and use of flow-through conditions. This study achieved a control survival of 87.1% over 90 days, which compares favourably with the earlier study. The feminising effect of the positive control was similar in both studies with around 30% males recorded.

The results of this study confirm previous findings that estrogens can influence gonadal differentiation in anurans, but the response does not appear to be particularly sensitive. The study also confirms previous findings that exposure to estrogens influences development and metamorphosis in anurans, presumably through interaction with the hypothalamus-pituitary-thyroid axis (Pickford *et al*, 2003).

12.6 Studies with atrazine

A number of recent chronic exposure studies conducted under static-renewal conditions have reported effects of atrazine on sexual differentiation in anuran larvae. Gonadal abnormalities and laryngeal effects have been reported at concentrations typical of those that occur in the environment, leading to speculation that atrazine may impact on amphibian populations. Effects on metamorphosis and immune function have also been reported. These studies are described below.

12.6.1 Polygonadism and laryngeal effects in *Xenopus*

Low µg/L doses of atrazine have been reported to disrupt the sexual development of the African clawed frog under laboratory conditions, with gonadal alterations observed at concentrations as low as 0.1 µg/L. These findings were presented at a meeting of the Society for Environmental Toxicology and Chemistry, held in Baltimore in November 2001, and at the International Symposium on Environmental Endocrine Disruptors, held in Tsukuba, Japan in December 2001. The published paper (Hayes *et al*, 2002a) appeared on 16 April 2002 and was widely reported in the media on the same date, which coincided with release of the US EPA's revised risk assessment for atrazine.

In this study, eggs were manually stripped from female frogs after treatment with human choriogonadatropin and fertilised *in vitro* with sperm obtained from the dissected testes of male frogs. After hatching, larvae were netted into tanks where they were reared under crowded conditions (triplicate groups of 30 in 4 L aerated 10% Holtfreter's solution) and fed daily with a "solution" of ground Purina rabbit chow, in increasing amounts as the animals grew. Note that netting is not recommended, particularly for young larvae, as the delicate egg yolk is easily detached, compromising

early nutrition. Larvae were exposed from hatching (stage 48) throughout the entire larval period until complete tail reabsorption (stage 66) under static renewal conditions with change of test medium every 3 days. Larvae were exposed to nominal concentrations of 0.01, 0.1, 1, 10 and 25 µg/L atrazine in one study using animals from a long-term captive colony, and 0.1, 0.4, 0.8, 1.0, 25 and 200 µg/L atrazine in a second study using frogs obtained from the commercial supplier Nasco. Atrazine was dispensed from ethanol stock solution and controls were treated with ethanol such that all tanks contained 0.004%. No effects were reportedly seen on mortality, time to metamorphosis, length, or weight at metamorphosis, although the data are not presented.

Males and females were differentiated at metamorphosis based on gross gonadal morphology, with phenotypic sex confirmed by histology for 10 males and 10 females from each replicate. All animals where sex was ambiguous were subjected to histological examination. Gonadal abnormalities were observed at all doses except 0.01 µg/L, with the incidence of single sex polygonadism or hermaphroditism (intersex animals with multiple testes and ovaries) at 16-20%. It appears that the incidence of gonadal abnormalities was the same in both studies and did not vary with dose. Raw data are not included and no information is provided in the published paper regarding any dose-response relationship, except for the observation that these abnormalities were never seen in controls, either in this study or in many others conducted over the preceding 6 years.

In his Japanese conference presentation, the lead author clarified that affected males were described as having multiple testes rather than a fragmented testis because no connections or ducts were present between the testes. He also stated that exposure of stage 52-54 larvae to low doses (< 1 ng/mL) of estradiol does not give rise to complete feminisation but produces "these exact same types of abnormalities". Other researchers (Villalpando and Merchant-Larios, 1990) have reported the formation of ambiguous gonads in *X laevis* tadpoles exposed to estradiol benzoate from stages 51 to 54. Earlier administration of the hormone led to complete sex reversal in presumptive genetic males, while testes differentiated normally when exposure was initiated later in the development process. Disruption of gonadal differentiation in this species, including the formation of ovotestes, has also been reported (Qin *et al*, 2003) following exposure to PCBs throughout the larval stage. Ovotestes were characterised by morphological ovaries in the cranial and caudal parts and morphological testes in the middle part. Morphologically normal and abnormal testes from a few frogs were also interspersed with oocytes in histological sections.

Effects were also reported on laryngeal muscle size, as determined by measuring the area of a transverse section taken from roughly one-third through the larynx. Laryngeal size was reduced in exposed males, with a threshold of 1 µg/L but no obvious dose-response relationship. Within treatment groups, mean laryngeal size was around 30-40% larger in males than in females, but laryngeal size was 20-30% larger in males and females from the commercially derived animals compared with the internal laboratory population.

The authors hypothesise that the effects observed reflect disruption of steroidogenesis, occurring as a result of an induction of the enzyme aromatase which converts androgens to oestrogens. Reduced androgen levels may favour loss of masculine features, such as

the reduction in laryngeal muscle size, while increased oestrogen levels may induce the development of ovaries in male larvae. Separate experiments which exposed adult frogs to 25 µg/L atrazine for 46 days found that sexually mature males suffered a 10-fold decrease in plasma testosterone relative to controls (larvae were too small to allow these measurements). Aromatase itself was not measured, and nor was estradiol. The authors note that exposure to exogenous estrogen in this species is known to result in 100% females, whereas androgens increase laryngeal growth but do not affect gonadal differentiation.

12.6.2 Gonadal dysgenesis and testicular oocytes in male leopard frogs

Leopard frog larvae hatched in the laboratory from eggs collected in Wisconsin have been tested in much the same way as the African clawed frogs in the above study, using three exposure concentrations (0, 0.1 and 25 µg/L) said to have been confirmed by chemical analysis (Hayes *et al*, 2002b, 2003). It is unclear whether test vessels were aerated. Exposure occurred from 2 days post-hatch until complete tail reabsorption. No effects were observed in females. Microscopic histological analysis of gonads revealed gonadal dysgenesis (under-developed testes with poorly structured, closed or absent lobules and low to absent germ cells) and testicular oogenesis (presence of oocytes, but no ovarian tissue, within testicular lobules, particularly in later metamorphs) in male frogs. Oocytes were vitellogenic and protruded through the testicular lobules making them observable by gross morphology in two exposed males. Testicular oocytes were not seen in the control animals, although two control males contained degenerating extragonadal oocytes and a single male showed gonadal dysgenesis. The effects (gonadal dysgenesis and the presence of testicular oocytes) in this species appear to differ from the polygonadism (single sex and hermaphroditic) reported in *Xenopus*. The authors claim that these kinds of abnormalities have never been seen in more than 7000 animals reared in the laboratory from four different populations, unless they were exposed to atrazine. These abnormalities occurred more frequently at low exposure (36% gonadal dysgenesis and 29% testicular oocytes) than at higher exposure (12 and 8%). The authors argue that this kind of inverted dose-response is common with chemicals that affect the endocrine system, and that atrazine induces the enzyme aromatase which converts androgens to oestrogens.

Similar abnormalities were observed in specimens caught from the field, with an emphasis on smaller animals that were considered likely to have metamorphosed recently. Sites were selected based on atrazine sales. Testicular oocytes were seen at seven of eight sites studied, the remaining site being the only one where atrazine was present at less than 0.2 µg/L when specimens were taken. Gonadal dysgenesis was reported from only one site. The highest incidence of testicular oocytes (92%) was a site in Wyoming where many animals showed advanced stages of complete sex-reversal. Histological analysis of the gonads was conducted on 20 males from the 100 animals collected at each site, which is hard to reconcile with the reported observation of testicular oocytes in 92% of the males examined. As significant atrazine usage does not occur in this area, the authors speculate that contamination at this site reflects riverine transport from significant atrazine use areas in Colorado.

The authors conclude that, given the widespread use and ubiquitous contamination by atrazine, its pattern of use, and its potency as an endocrine disruptor, atrazine likely has a significant impact on amphibian populations. This conclusion seems at odds with the

discovery of apparently healthy juvenile leopard frog populations at sites with significant atrazine contamination, but the authors suggest that effects are reversible, that some percentage of the population does not show this response, that these developmental abnormalities do not impair reproductive function at sexual maturity, and/or that continuously exposed populations have evolved resistance to atrazine. The possibility that the observed juvenile hermaphroditism is a natural phenomenon is suggested, but the natural frequency of hermaphroditism is said to be difficult to determine. As noted above, the authors claim never to have seen testicular oogenesis or hermaphroditism in more than 7000 laboratory raised animals from four populations, unless they had been exposed to atrazine.

12.6.3 Gonadal dysgenesis in male and female *Xenopus*

A static exposure study has been conducted in *Xenopus laevis* tadpoles during gonadal differentiation (early metamorphosis). Test organisms were obtained commercially just prior to sexual differentiation and acclimated to the laboratory for a week before exposure at Nieuwkoop-Faber stage 56. A single test concentration (21 µg/L nominal, 18 µg/L measured) was used, with no carrier solvent, and animals were not fed during the 48 hour exposure period. Two replicates of 16 frogs each in 15 L test solution were used for exposure and controls, with half being male. After exposure, the gonad-kidney complex and the brain with attached pituitary gland were dissected out, fixed and examined microscopically. Three indicators of reproductive impairment were evaluated, being an index for total testicular volume, estimated numbers of spermatogonial cell nests and integrity of nurse cells (analogous to Sertoli cells in mammals).

The paper reports results but no raw data. Total testicular volume was 57% lower in exposed male frogs than in controls, number of spermatogonial cell nests 70% lower, and number of nurse cells 74% lower. Testicular resorption was observed in 70% of exposed male frogs, and aplasia (failure of full development) of the testis in 10%. The authors suggest that these results may reflect induction of the enzyme aromatase. Histological sections of the pituitary gland revealed only undifferentiated chromophobes, indicating that the pituitary was not actively secreting hormones (Tavera-Mendoza *et al*, 2002a).

Two indicators of reproductive impairment were evaluated in the female frogs from this study, being the frequency of occurrence of primary and secondary oogonia, and of atresia (resorption of developing eggs in the ovary) among primary and secondary oogonia. There was no evidence of gonadal resorption or reduced ovarian volume in females, but enhanced levels of oogonial development and increased levels of atresia were observed. The frequency of occurrence of primary oogonia was lower in exposed groups than in controls. Conversely, the frequency of occurrence of secondary oogonia was higher, but 20% of these underwent resorption (atresia) before progressing to primary oocytes. The authors suggest that the increased atresia could reduce the reproductive capacity of the exposed frogs. Induction of aromatase was again suggested as the causative mechanism (Tavera-Mendoza *et al*, 2002b).

12.6.4 Intersex and discontinuous gonads in *Xenopus*

Naturally fertilised eggs for this study (Carr *et al*, 2003) were obtained from breeding pairs imported from South Africa, after laboratory acclimation for 7 days and artificial induction of spawning by injection of human chorionic gonadatropin. Groups of 60-65 larvae were exposed in FETAX medium to atrazine (nominal concentrations of 0, 1, 10 and 25 µg/L) from 48 hours to 80 days post-hatch, under continuously aerated, semi-static conditions (50% water change every 72 hours). Solvent controls (0.0025% ethanol) and positive controls (dihydrotestosterone or estradiol, nominally 100 µg/L, with 0.0025% ethanol) were also studied. Each atrazine exposure was replicated eleven times, and the controls six times. The initial very high density of 60-65 per 100 mL was reduced by transfer to 1 L FETAX medium on the 5th day post-hatch, when feeding (0.4 g powdered frog brittle in 2 mL FETAX solution) commenced, and again on the 19th day by transfer to 4 L FETAX medium. Feeding and 50% water exchange continued on a 72 hour schedule. Dead or moribund animals were removed daily, and any unusual observations such as bent or asymmetric tails, oedema and abnormal swimming were recorded, together with forelimb emergence and tail resorption. Additional food was provided as needed, based on daily observations of food depletion.

Dissolved oxygen was monitored every 2 days and found to remain in the 3.9-9.3 mg/L range, within the ranges previously reported for aerated FETAX medium. Ammonia was monitored every 7 days, but the results are not reported.

Animals were removed from tanks at metamorphosis, euthanased, rinsed and weighed before fixing and storage. Snout-vent lengths and sex (based on gonadal morphology) were recorded on preserved specimens. Any tadpoles that did not complete metamorphosis (stage 66) were processed similarly at the termination of the 78 day exposure period, but only data from stage 66 animals were used.

Laryngeal size in males and females was compared based on the largest cross-sectional area, determined by digital photography of every 20th 8 µm section. Gonadal morphology was examined under a binocular dissecting microscope, with females identified by the presence of ovaries (long and lobular, with small areas of dark pigmentation) and males by testes (shorter and lacking lobes or pigmentation). Animals were identified as intersex if gonads contained separate testicular and ovarian characteristics or if testicular and ovarian tissues were mixed. Discontinuous gonads exhibited abnormal segmentation of the gonad along the rostral-caudal axis. Gonadal morphology was also examined microscopically after sectioning at 10 µm and staining with hematoxylin and eosin.

Atrazine did not affect hatching success (at least 90% in all treatments), post-hatch mortality (10-14% in all groups at 80 days post-hatch) or larval growth, although time to complete metamorphosis varied inversely with body weight and snout-vent length. This would appear to reflect competitive effects at the relatively high densities used (15 larvae/L approaching metamorphosis) with larger larvae developing more rapidly at the expense of their smaller cohorts. It would appear that the crowded culture conditions impacted adversely on development as less than half the animals completed metamorphosis in the atrazine treated and steroid/solvent controls. Only 49-60% reached forelimb emergence, and complete tail resorption was only achieved in 37-52% of the test animals.

There were no statistically significant departures from a 50:50 sex ratio except for the estradiol treatment where males were reduced to 26%, females increased to 67%, and 7% of animals were classified as intersex based on gross gonadal morphology. Concentrations of estradiol in the exposure tanks were a lot lower than they should have been. Histological analysis revealed ambiguous gonadal materials in some of these affected animals. The incidence of intersex in atrazine exposed animals as determined by gross morphology increased with increasing atrazine concentration with a corresponding decrease in males, but only at 25 µg/L (14/296, compared with 2/334 in FETAX medium controls, 3/309 at 1 µg/L, and 1/276 at 10 µg/L). At this concentration, the incidence of discontinuous gonads (8%) also exceeded that in controls (4%) or at lower exposures (4 and 7%). Histological analysis found obvious testicular or ovarian morphology in the atrazine exposed intersex animals, although testes were a little small and appeared flat at times.

The laryngeal dilator muscle was 30-40% larger in stage 66 males than in females in all treatments except the steroid controls. This finding, based on careful histological observation, conflicts with earlier reports (Sassoon and Kelley, 1986) that sexually dimorphic features do not appear in the larynx of this species until after metamorphosis. However, the developing larynx is clearly capable of responding to androgens before the completion of metamorphosis as muscle cross-sectional area was doubled in males and females exposed to dihydrotestosterone. There was no indication that atrazine had any effect on laryngeal dilator muscle size. Estradiol treatment appeared to slightly increase this parameter in females, such that no sex differences were apparent, but the increase was not statistically significant.

12.6.5 Delayed metamorphosis in *Xenopus*

Tadpoles in these studies (Brown Sullivan and Spence, 2003) were bred from three distinct natural pairs and maintained in moderately hard water, with feeding commenced (commercial tadpole mash) when larvae reached the free swimming stage at around 5 days old. In range finding studies, groups of six tadpoles, randomly chosen from full siblings, were exposed to atrazine (nominal concentrations of 0-320 µg/L, dispensed from 10 g/L acetone stock solution) from 11 days after hatching (NF stage 46-48) through till metamorphosis. Previous work had shown that acetone at these low concentrations (up to 0.032 mL/L) would not affect these tadpoles. Tanks were cleaned and test solutions completely changed on an alternating 3 and 4 day schedule, and volumes were increased as the tadpoles grew, from 500 mL initially to 1.5 L at 33 days old and 6 L at 50 days old. It appears that tanks were not aerated. Tadpoles were removed at metamorphosis (NF stage 66) or when found dead during daily checks.

The mean time to metamorphosis increased dose responsively from 60 to 64 days under atrazine exposure, with concomitant decreases in mean weight from 0.63 g in controls to 0.52 g at 20 µg/L and 0.45 g at 320 µg/L atrazine. In contrast to the dose response observed between treatments, there was little correlation in general between time to metamorphosis and weight at metamorphosis within treatments. Separate studies found that nitrate concentrations to 400 mg/L did not affect time to metamorphosis but that weight at metamorphosis increased with increasing nitrate exposure.

Further studies were conducted at 0, 40 or 320 µg/L atrazine in combination with nitrate (0, 37 or 292 mg/L) but under different husbandry conditions (2.5 L test solution at

10 days, increasing to 6 L at 24 days and reduced back to 3 L when only half the original tadpoles remained). Under these conditions, the mean time to metamorphosis increased from 65.6-74.3 days in controls through 83.9-91.1 days at 40 µg/L to 90.0-108.2 days at 320 µg/L, compared with 60-64 days in the earlier experiments. The authors note that the longer time to metamophosis is likely to reflect intraspecific variation as different clutches of tadpoles were used. Similar dose related delays were seen in other developmental stages such as limb emergence and head elongation. Mean weight at metamorphosis (0.92-1.00 g in controls, 0.76-0.93 g at 40 µg/L, 0.67-0.72 g at 320 µg/L) also increased under these less crowded conditions but was again lower with increasing exposure to atrazine. The presence of nitrate appeared to result in earlier metamorphosis at 40 µg/L atrazine but delayed metamorphosis at 320 µg/L atrazine.

The authors note that the effects of atrazine on tadpole development, namely inhibited growth and delayed metamorphosis, differ from the usual effect of environmental stressors such as overcrowding. They argue that such stress tends to result in an earlier transition to metamorphosis and smaller size at metamorphosis, and that such outcomes have reportedly been seen in *Xenopus laevis* tadpoles raised under high density laboratory conditions. However, this oversimplifies the situation, as a number of studies have shown that increased competition for resources early in the larval period tends to decrease larval growth rate, survivorship, and size at metamorphosis and to increase the length of the larval period. The authors argue that the response seen with atrazine would be maladaptive as tadpoles would be smaller and more vulnerable to predation for a longer period.

The mechanism through which atrazine might slow larval development and influence metamorphosis was not determined, but the authors suggest a direct or indirect effect on hormonal mechanisms associated with metamorphosis, while discounting differences in resource availability caused by the herbicide on the basis that food availability was constant and unlimited among treatments.

Although discounted by the authors, an alternative explanation for the slower larval development and smaller size at metamorphosis with increased exposure to atrazine may well be resource availability. Algal growth under static-renewal conditions may help to sustain larvae, particularly while they are small which is when resource restriction is known to extend the larval period. Increasing concentrations of atrazine would likely be associated with reduced algal production. This effect could be offset by increased nutrients – note that size at metamorphosis responded positively to nitrate in the absence of atrazine. The competing effects of atrazine and nitrate on algal growth could explain why metamorphosis occurred more rapidly in the presence of nitrate under moderate atrazine exposure (40 µg/L) but more slowly under higher atrazine exposure (320 µg/L).

Earlier static-renewal studies (Allran and Karasov, 2000) with leopard frogs found no effects on survival, development or metamorphosis when larvae were exposed for 138 days to atrazine (0, 20 or 200 µg/L). Exposure to nitrate (5 or 30 mg/L) slightly decreased larval length.

12.6.6 Trematode infection in wood frogs

A field study (Kiesecker, 2002) has examined whether variations in the level of deformity at natural breeding sites could be related to exposure to agricultural runoff. Laboratory studies were also conducted, in which tadpoles were subjected to infection pressure after exposure to a number of pesticides including atrazine.

The field component used 6 ponds, situated within 10 km of each other and all populated by snails infected with a trematode (*Ribeiroia* sp) known to be capable of inducing limb deformities. Three were in close proximity to agricultural fields and had evidence of exposure to agricultural runoff. Wood frog (*Rana sylvatica*) tadpoles were raised in enclosures at each site. Three enclosures per pond were coarsely screened (500 µm nitex) to allow access by trematode larvae (cercariae), and three were finely screened (75 µm) to exclude the parasites.

Pond water samples were tested externally for the presence of pesticide contamination, using EPA standard methods 8081 (organochlorine pesticides) and 8141a (organophosphorous pesticides). The author states that "in three ponds, detectable levels of both organochlorine pesticides and organophosphorous compounds (eg atrazine and malathion) were detected". This statement is misleading. Atrazine is not an organophosphorous compound, and is not generally regarded as an organochlorine compound, although it does contain chlorine. Importantly, the cited analytical method states that simazine and atrazine give poor responses on the ECD detector, and should be analysed using a different method. There is no evidence to support the claim that ponds were contaminated by atrazine.

Limb deformities developed in 28.6% of the exposed tadpoles in the ponds contaminated by agricultural runoff, but only in 4% of those from the uncontaminated ponds. Metacercariae (encysted larvae) were consistently found in proximity to the site of deformity. Trematode infection appeared to retard growth, with exposed frogs 37% smaller in the contaminated ponds and 22% smaller in the uncontaminated ponds.

The laboratory component examined susceptibility to trematode infection and immune response of larval wood frogs after exposure to atrazine (3 or 30 µg/L). Individual tadpoles (12 per exposure) were exposed for 4 weeks in 3.5 L dechlorinated tap water, with renewal of the test medium every other day, and then transferred to 75 mL containers containing 50 cercariae (*Ribeiroia* sp or *Telorchis* sp) harvested from snails. All cercariae penetrated the tadpoles within 4 hours. Tadpoles were then returned to their original containers for a week before being sampled for blood, and then killed and preserved for enumeration of metacercarial cysts.

Analysis of blood samples showed an alteration in immune response following pesticide exposure, as indicated by a decrease in the number of eosinophils (a type of white blood cell). The proportion of cercariae that successfully encysted increased following atrazine exposure, from 20-40% in controls to 80-90% in exposed tadpoles.

The author concludes that stress in the form of atrazine exposure decreased the ability of tadpoles to resist infection, resulting in higher parasite loads and increased incidence of deformity.

Limb deformities caused by *Ribeiroia* infection have been identified recently (Johnson *et al*, 2003) as an emerging infectious disease. Although such malformations have occurred since at least the 1940s, they appear to have become more prevalent in recent years. Resurvey of six historical malformation sites found that three continued to support severe limb malformations. No pesticides were detected (atrazine and its two monodealkylated metabolites were < 0.5 µg/L) but amphibians at all sites carried *Ribeiroia* infections. It is suggested that the increased abundance in the landscape of eutrophic artificial wetlands, which provide habitat for the aquatic snails that harbour the parasite, is the main factor behind these infections. Additional insults such as certain pesticides (as identified by Kiesecker, 2002) may further increase the incidence of malformation by reducing resistance to infection, although they were not detected at the sites studied.

12.6.7 Nematode infection in leopard frogs

A more recent study (Christin *et al*, 2003) with juvenile leopard frogs (*Rana pipiens*) has investigated whether exposure to a mixture of pesticides (atrazine, metribuzin, aldicarb, endosulfan, lindane and dieldrin) has immunotoxic effects, as determined by challenging the exposed animals with larvae of the skin penetrating parasite *Rhabdias ranae*, a nematode commonly found in semiterrestrial ranid frogs.

Post-metamorphic juvenile frogs were exposed for 21 days in groups of 100 to the pesticide mixtures, using DMSO as solvent vehicle. Half of the frogs were then exposed for 24 hours to the parasites on moistened filter paper within individual containers. The degree of infection was determined after a further 21 days in clean aquaria.

Lymphocyte proliferation was significantly reduced in exposed frogs, but recovered within 3 weeks of exposure and was stimulated in frogs challenged with parasites, except for those previously exposed to the highest concentration (210 µg/L atrazine, well above concentrations that would be expected to occur in the environment). Phagocytosis and splenocyte numbers were unaffected by pesticide exposure itself but were diminished at the end of the 21 day recovery period in the most highly exposed group. The prevalence of lung infection tended to be higher in this group. The authors conclude that these results suggest that pesticides can alter the immune response of frogs and affect their ability to deal with parasitic infection.

The authors note that the solvent vehicle DMSO appears to modulate immune function, but that other carriers exert more important effects. For example, when methanol was used to dissolve some of the pesticides in early experiments, sex reversal occurred in developing frogs and survival was negatively affected.

12.6.8 Corticosterone production *in vitro*

In vitro bioassays have been conducted with adrenal cell suspensions (>95% viability) from *X laevis* (10^7/mL) and *R catesbieiana* (10^5/mL). The former were prepared from the whole kidney, and the latter from adrenal tissue. When stimulated with optimal concentrations of ACTH (2 IU/mL) and dibutyryl cyclic adenosine monophosphate (10 mM) these cells secreted corticosterone, a hormone that helps maintain blood glucose levels and liver glycogen reserves and controls osmoionic balance.

Corticosteroid hormones are important for the maintenance of homeostasis and normal development in amphibians.

Acute (60 mins) exposure to atrazine (10^{-4}-10^{-8} M) had no effects on *X laevis* cells and no effects on the viability of *R catesbeiana* cells. However, the secretory capacity of the bullfrog cells was inhibited (EC50 1.1 x 10^{-5} M, or approximately 2 mg/L). The authors suggest that the bullfrog adrenal system might be extremely vulnerable to atrazine, and that the results obtained *in vitro* are evidence of the high endocrine toxicity of atrazine, but acknowledge that impairment of the neuroendocrine stress response by atrazine has yet to be demonstrated *in vivo* (Goulet and Hontela, 2003).

12.7 Critical evaluation of atrazine studies

The recently reported studies linking atrazine exposure with alterations in sexual differentiation have prompted detailed investigation by the principal registrant (Syngenta) and the US EPA.

Syngenta expert panel

Syngenta Crop Protection Inc has convened an expert panel to examine endocrine system responses to atrazine exposure in amphibians and other aquatic fauna. An unpublished report by the panel (Solomon *et al*, 2002) includes progress reports on studies conducted with the intention of clarifying suggestions that low-dose exposure atrazine influences sexual differentiation in the African clawed frog, including the recently published study by Carr *et al* (2003) that is described earlier in this report.

A progress report of a second study conducted under similar conditions by one of the authors (Giesy) is also included. Time to initiate and complete metamorphosis remained unaffected by atrazine exposure (0.1-25 µg/L), and length and weight at metamorphosis did not differ significantly between treatments. Gonadal abnormalities such as mixed sex, discontinuous gonads, and irregular size and shape were seen in stage 66 tadpoles, but these features occurred independently of dose, including in controls. The incidence of mixed sex animals remained below 5%. Sex ratios were skewed in the positive control (100 µg/L oestradiol) with 77% of metamorphosed froglets being phenotypically female. Measurements of laryngeal dilator muscle area were ongoing at the time of reporting.

Groups of adult frogs were exposed under static renewal conditions to 25 µg/L atrazine for 47 days. None of the testicular tissue samples from atrazine exposed or control male frogs had significantly different aromatase activity from that of the bovine serum albumin blank. Generally low to non-detectable levels of aromatase activity were observed for male frogs in previous experiments. Mean aromatase activity of brain tissue in male frogs was less than in controls, but the difference was not significant. No effects were seen in gonadal or brain samples from atrazine exposed female frogs.

The panel report notes that gonadal abnormalities were seen in control animals in these sponsored studies, in contrast to the original study by Hayes *et al* (2002a) which claims that such abnormalities have never been seen in control animals.

The expert panel report includes preliminary details of field studies from corn growing areas of South Africa, where African clawed frogs occur in natural or constructed farm ponds that receive atrazine residues in runoff. Adults and juveniles were collected in 2001-2 and processed for gross morphological, histological and biochemical assessment of possible effects on the larynx, gonads, biochemical and hormonal status. Gross morphological examinations have been completed, and show low rates (<5%) of gonadal abnormality (single testis, discontinuous testes, and unusually large size differences between testes) in adult males from exposed and reference sites. Females showed no abnormality, while a small proportion (2.5%) of metamorphs showed abnormalities, but only at reference sites.

Updated reports of this research have not been presented to Australian authorities, but more recent results have been evaluated by the US EPA (see below). It is considered that the US EPA evaluations contain sufficient detail for the purposes of this review.

US EPA White Paper

The US EPA (Steeger and Tietge, 2003) has reviewed recent studies on developmental effects in amphibians. The review includes evaluation of further laboratory work by the Syngenta expert panel, examining larval development and adult responses to atrazine in *X laevis* and green frogs (*R clamitans*). The larval studies were conducted under overcrowded conditions, which delayed metamorphosis and contributed to high levels of mortality, particularly for green frogs which suffered an average 77% mortality during the 273 day exposure period. No effects were seen on sexual differentiation in green frogs. Gross morphology indicated the presence of discontinuous gonads in *X laevis* but with no dose response between 0.1 and 25 µg/L. Preliminary histology revealed a higher incidence of mixed sex/intersex characteristics than indicated by gross morphology. The studies with adults found no significant effects on aromatase activity following exposure to 25 µg/L atrazine, but there was considerable variability in gonadal aromatase activity and plasma steroid concentrations within treatment groups.

The principal difficulty with the interpretation of the laboratory studies is that there are no consistent and reproducible effects. Laryngeal effects have not been replicated, while polygonadism has been reported from different laboratories but at different threshold concentrations (0.1 and 25 µg/L). It is hard to reconcile the different experimental designs, but all have shortcomings, particularly in relation to husbandry. Excessive loadings created unfavourable environmental conditions, which in turn impacted adversely on the condition of animals as indicated by delayed metamorphosis. Measured hormone levels were highly variable, reflecting handling stresses and different sampling times (hormone levels in amphibians fluctuate with the time of day and with their level of sexual maturity, among other factors). The response to steroid controls was limited in some studies. The suggested mechanism of aromatase induction during a sensitive premetamorphic period of gonadal development remains hypothetical as this was not demonstrated in any laboratory anuran.

The White Paper also reviews a number of field studies. A microcosm study with *X laevis* (groups of 800 larvae in 1100 L water) suffered from delayed metamorphosis, which the authors ascribe to low water temperature. Gonadal deformities (discontinuous testes only) in recently metamorphosed froglets were reported at low levels (< 5%) in control and exposed (1, 10 and 25 µg/L atrazine) animals based on

gross morphology, with histology yet to be performed. Frogs grew slightly better in controls, which could reflect the effect of atrazine on algae. A South African representative of this research group (Du Preez) reported orally to the Scientific Advisory Panel on 17 June 2003 on the histological examinations, noting that testicular oocytes were seen in 56% of animals from reference ponds and 38-58% from exposed ponds when these were looked for carefully (examination of every 6 µm slide through the testis). The average incidence was about 10 oocytes per specimen, but this dropped markedly in more mature animals.

A low incidence (3% at reference sites and 2% in exposed animal, but some atrazine was present at all sites) of gonadal abnormalities (testicular oocytes) was also reported from field studies in South Africa with *X laevis*. Males at exposure sites had slightly larger testes. Plasma steroid levels and aromatase activity were highly variable in the frogs sampled, confounding analysis of these parameters. The sexual response in this species may have been affected by trapping, which held males and females in close proximity, and by exogenous steroids from the beef liver used as bait. Trap success was low at some sites because of the introduction of predatory catfish.

A preliminary report of north American field studies using three reference and six exposed sites found a low incidence (< 1%) of mixed or unknown sex animals among more than 600 green frogs. Plasma steroid measurements were very highly variable.

A reconnaissance study of cane toads (*Bufo marinus*) in Florida found an increased incidence of intersex (39 and 29%) in cane toads with testes at two agricultural (sugarcane dominated) sites, compared with the University of Miami where no such abnormalities were seen. The toads at agricultural sites were exposed to a complex mixture of agrochemicals, including fertilisers, while exposures at the university site are unclear as no analyses were conducted. Intersex animals were found to have ovarian tissue associated with Bidder's organ (characterised as a nonfunctional, rudimentary ovary) rather than with the testes as reported in frogs. Many of these intersex animals (100 and 55%) exhibited female colouration. At one site only, intersex toads (71%) had developed nuptial pads. Similar intersex characteristics were noted in Bidder's organs of southern toads (*B terrestris*) at agricultural and nonagricultural sites.

A progress report of a field study in southern Iowa (3 reference and 11 exposed ponds) indicates that no gross gonadal abnormalities were found in bull frogs (*R catesbiena*). The snout-vent length was lower in juveniles from reference sites.

Most of the field studies reviewed did not provide sufficient information to characterise study sites or provide sufficient rationale for site selection and variability. Atrazine exposure occurred in reference/control sites, with likely exposure to a much wider array of chemicals. Potential confounding effects of other stressors such as habitat condition, prey availability and nutrient loading, were not described or evaluated. The field studies do not clarify the situation because of likely interference by additional stressors which varied widely from site to site.

The US EPA also questioned the ecological relevance of the reported laryngeal and gonadal effects, noting that apparently healthy leopard frogs were easily obtained from environments contaminated by atrazine.

Given these uncertainties, the US EPA concluded that laboratory studies should be designed and conducted to test the working hypothesis that atrazine exposure causes gonadal developmental effects in amphibians. Further phases would examine sex steroid levels, aromatase activity and ecological relevance, assuming that gonadal effects could be demonstrated. This proposal was discussed in an open meeting on 17-20 June 2003 by the US EPA's Scientific Advisory Panel.

US EPA Scientific Advisory Panel

The US EPA Scientific Advisory Panel reported in early August on the outcome of its deliberations on the scientific issues associated with the potential developmental effects of atrazine in amphibians (Lewis and Roberts, 2003).

The Panel agreed that further studies are warranted, given the inconsistencies between laboratory studies that have reported developmental abnormalities in atrazine exposed amphibians, and the design or methodological flaws that limit the usefulness of associated field studies. It was agreed that sufficient data were available to establish the hypothesis that atrazine interferes with normal gonadal development in anurans, with a threshold concentration between 0.1 and 25 μg/L, but that more data are necessary to properly test the hypothesis.

The Panel noted that stress associated with sampling can have profound effects on plasma sex steroid concentrations, and that these also vary according to distinct circadian rhythms. Other physiological parameters such as body weight and reproductive status may also account for variability in plasma sex steroid concentrations.

The additional data should be generated under standardised conditions and must be subject to independent verification. ASTM water quality guidelines should be followed. Flow-through conditions should be preferred, but static-renewal studies would be acceptable provided that ASTM guidelines for loading are followed and that water quality is maintained within ASTM guidelines and assessed daily. A positive estrogen control (17β-estradiol) should be used but there is no need for an androgen control. A clear set of definitions concerning the terminology for classifying gonadal deformities should be developed.

The ideal study would use sufficient individuals to allow for its continuation, in order to assess the consequences of any gonadal abnormalities for fertility and reproduction. Initial studies should be conducted with *X laevis*, with confirmatory studies in a North American *Rana* species. Experimental field-based studies in small temporary wetlands with established seasonal breeding populations of amphibians should also be considered at an early stage.

12.8 Summary of atrazine studies

Atrazine does not exert acutely toxic effects on amphibians at typical exposure levels. There may be some indirect effects of atrazine exposure on the growth and development of tadpoles because of reduced food resources associated with herbicidal activity, but this is only likely at concentrations above the previously established threshold of 20 μg/L.

Longer term laboratory toxicity tests with amphibians are complicated by numerous factors. Test protocols remain under development and have yet to be agreed internationally, but there is a general consensus that flow-though conditions are desirable in order to avoid complications associated with declining water quality under static conditions. Most of the longer term toxicity studies in amphibians that have been reported to date used static-renewal conditions. A flow-through method has been reported very recently and tested with bisphenol-A. No adverse effects were reported in tadpoles exposed from early post-hatch through metamorphosis, in contrast to earlier findings of a feminising effect of this substance on gonadal differentiation under less controlled, static-renewal conditions.

A number of recent studies conducted under static or static-renewal conditions have reported effects of atrazine on sexual differentiation, based on dissection and microscopic examination of tadpoles or newly metamorphosed froglets. Polygonadism (single sex and intersex) has been reported in *X laevis* with a threshold of 0.1 µg/L, and reduced laryngeal size has been reported in males with a threshold of 1 µg/L. Effects on gonadal differentiation were also reported in *R pipiens*, again with a threshold of 0.1 µg/L, but the effects (gonadal dysgenesis and the presence of testicular oocytes) appear to differ from the polygonadism reported in *X laevis*. Similar abnormalities (primarily testicular oocytes) were also reported in field caught specimens. Both studies reported that these effects were never seen in unexposed controls. Another group of researchers reported effects on gonadal development (reduced testicular volume and reduced spertagonial cell nests and nurse cells in males, and faster oogonial development and increased resorption in females) following acute exposure of *X laevis* tadpoles at the prometamorphic stage when gonads are in the early stages of differentiation. The authors of all these studies suggest that induction of the enzyme aromatase by atrazine is the cause of these feminising effects, but the hypothesis remains unproven.

Attempts to reproduce these findings have been largely unsuccessful. Low incidences of gonadal abnormalities have been reported in various frog species, but independent of atrazine exposure. The incidence of these abnormalities was only increased above background in one study with *X laevis*, where an increased incidence of discontinuous gonads was reported at a nominal concentration of 25 µg/L atrazine. Animals in this study, and in all the other static-renewal studies that have been reported, were developmentally challenged by maintenance at high population density. The laryngeal effects have not been reproduced.

Recently reported model studies with steroid hormones have found that juvenile hermaphroditism is a natural phenomenon in some anuran broods, including leopard frogs. This finding, and the observation of intersex animals in control populations by other researchers, contradict claims by one research group that this phenomenon has never been seen unless tadpoles had been exposed to atrazine, notwithstanding the testing of thousands of individuals from different populations.

The occurrence or higher incidence of abnormalities in atrazine exposed replicates compared with unexposed controls should not be disregarded simply because of difficulties in reproducing the results. However, the suggestion that gonadal abnormalities are a direct effect of atrazine exposure needs to be considered cautiously

as a mechanism for these effects and a dose-response relationship have yet to be demonstrated.

It is possible that the effects reported by some research groups are caused indirectly by atrazine. Hypoxia and nitrate pollution have both been identified recently as potential endocrine disruptors in aquatic organisms, and both would be more likely to occur under atrazine exposure because of the herbicidal effects of atrazine on algae, which take up nitrogen from the water and release oxygen during photosynthesis. Indirect effects from elevated nitrate concentrations (dissolved oxygen remained within an acceptable range) could therefore account for the abnormalities seen at 25 µg/L in the study by Carr *et al* (2003) as algae are likely to be sensitive to such concentrations. Nutrition may also be compromised, particularly while tadpoles are young, if algal colonisation of test vessels is retarded by atrazine. Effects reported at lower concentrations are unlikely to be an indirect consequence of atrazine exposure, as atrazine concentrations of 1 µg/L or less would not be expected to affect algae. Hypoxia and nitrate pollution arising from high tadpole density and limited water exchange may be responsible for these reported effects, and would explain the lack of any dose response relationship (although not the negative response in controls). In the absence of any dose-response relationship, it is more likely that the effects reported at low exposure concentrations are associated with delayed development under less than optimal conditions, rather than a yet to be confirmed hormonal response to chemical exposure.

One recent laboratory study has reported that chronic atrazine exposure in *X laevis* delays metamorphosis and reduces metamorphic size. Hormonal mechanisms have also been invoked in this instance, but without supporting evidence. The effects reported appear more likely to have been an indirect effect associated with the herbicidal activity of atrazine, as recorded in mesocosm and field studies.

Effects on immune function have also been reported, with atrazine exposed animals more vulnerable to trematode infection. However, the main factor behind an apparent increase in trematode infection in recent years appears to be eutrophication, which favours the snails that harbour the parasite. Atrazine has not been detected in water samples collected from infection sites. Increased vulnerability to nematode infection has also been reported in tadpoles exposed in the laboratory to a mixture of pesticides including atrazine, but at elevated concentrations that would not be encountered in the field.

Atrazine has also been shown to inhibit the neuroendocrine stress response in suspensions of bullfrog adrenal cells, but a similar response in the whole animal has yet to be investigated.

The US EPA has concluded that sufficient data are available to establish the hypothesis that atrazine interferes with normal gonadal development in anurans, with a threshold concentration between 0.1 and 25 µg/L, but that more data are necessary to properly test the hypothesis.

12.9 Conclusion

The requirement for continued registration in Australia is that the APVMA be satisfied that use of atrazine products in accordance with their recommendations for use would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment.

Currently available data are inadequate to support a conclusive risk assessment of the effects of atrazine in amphibians. There is disagreement as to whether atrazine impacts on amphibian development and on the levels of exposure where such effects may occur. Further research may clarify these issues and allow more confident conclusion. Shortcomings in the available data mean that there is some uncertainty associated with the conclusions that may be drawn at this time.

The main unintended effect identified in this report is disruption of sexual differentiation. Some studies have reported such effects at low exposure levels typical of those that may occur in the Australian environment, but under artificial laboratory conditions where other stressors such as poor water quality and high population densities are likely to impact adversely on amphibian development. A dose response relationship between exposure to atrazine and alterations to sexual differentiation has not been established. The raw data for these studies are unavailable, and there are inconsistencies in the results reported (for example, the incidence of abnormalities in a sample of 20 frogs is reported as 92%). It has not been possible to independently reproduce these effects at the same low exposure levels, although they have been replicated in the laboratory at higher exposure (25 µg/L) that potentially could give rise to indirect effects through reduced primary productivity. In the absence of any dose-response relationship, it would appear more likely that the reported effects reflect impaired development under less than optimal rearing conditions, rather than chemical exposure. The likelihood that atrazine is disrupting sexual differentiation in Australian frogs at current exposure levels (peak concentrations typically 1-10 µg/L in Australian surface waters) does not therefore appear high, based on currently available evidence.

Delayed metamorphosis and reduced metamorphic size have been identified as potential unintended adverse effects of atrazine exposure (40 and 320 µg/L) under laboratory conditions in one study. An earlier study found no such effects at concentrations to 200 µg/L. Given that atrazine levels in the Australian environment are unlikely to exceed 1 µg/L over extended periods, the likelihood that atrazine is delaying metamorphosis or reducing metamorphic size in Australian frog populations is considered low.

Reduced immune function has also been reported in atrazine exposed (3 and 30 µg/L) laboratory amphibians. Similar effects have been reported in the field, but with no evidence of atrazine exposure, and in another laboratory study where tadpoles were exposed to a mixture of pesticides including atrazine, at concentrations well above those that would be expected to occur in the environment. The likelihood that atrazine is reducing immune function in Australian frogs does not appear to be high, based on this limited evidence.

Continued registration depends not only on whether unintended effects are likely to occur, but also on whether these unintended effects will have adverse consequences for

populations. Extrapolation from laboratory effects to population impacts in the field can be difficult. The researchers that report disruption of gonadal differentiation in laboratory amphibians at very low atrazine exposure levels (0.1 µg/L) have claimed that atrazine likely has a significant impact on amphibian populations and could be a contributing factor in amphibian declines. However, these same researchers have reported that frogs were easily sampled from apparently healthy populations at sites said to be contaminated by atrazine.

Inconsistencies between studies, the difficulty in independently replicating the low dose effects of atrazine in amphibians and the likely influence of other stressors, together with the occurrence of healthy amphibian populations at sites where atrazine is present, indicate that it is unlikely that atrazine is impacting adversely on populations of Australian amphibians at current levels of exposure.

The US EPA has conducted a detailed ecological risk assessment of atrazine, which concluded that atrazine is likely to result in community and population level risk at 10-20 µg/L. Detailed consideration of recent amphibian findings has not altered this conclusion. The US EPA has noted the inconsistency and lack of reproducibility across studies and an absence of a dose-response relationship, and will seek additional data to reduce any uncertainty regarding the potential risk of atrazine to amphibians. The issue of atrazine and amphibians should be revisited if these additional data demonstrate that atrazine may be likely to impact on frog populations at realistic levels of exposure, but such outcomes are not considered likely.

12.10 References

Allran JW & Karasov WH (2000) Effects of Atrazine and Nitrate on Northern Leopard Frog (*Rana pipiens*) Larvae Exposed in the Laboratory from Posthatch Through Metamorphosis. Environ Toxicol Chem, 19: 2850-2855.

Allran JW & Karasov WH (2001) Effects of Atrazine on Embryos, Larvae and Adults of Anuran Amphibians. Environ Toxicol Chem, 20: 769-775.

ASTM (1985) Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. E-729-80. In *Annual Book of ASTM Standards*, Vol 11.4. Philadelphia, PA, pp 272-296.

ASTM (1996) Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians. E-729-96. In *Annual Book of ASTM Standards*, Vol 11.05. Philadelphia, PA, pp 1-19.

Battelle (2002) Revised Draft Detailed Review Paper for Amphibian Metamorphosis Assay. US EPA Contract No 68-W-01-023. Document dated 3 July 2002. Battelle, Columbus, Ohio.

Brown Sullivan K & Spence KB (2003) Effects of Sublethal Concentrations of Atrazine and Nitrate on Metamorphosis of the African Clawed Frog. Environ Toxicol Chem, 22: 627-635.

Carr JA, Gentles A, Smith EE, Goleman WL, Urquidi LJ, Thuett K, Kendall RJ, Giesy JP, Gross TJ, Solomon KR & Van Der Kraak G (2003) Response of Larval *Xenopus laevis* to Atrazine: Assessment of Growth, Metamophosis, and Gonadal and Laryngeal Morphology. Environ Toxicol Chem, 22: 396-405.

Christin M-S, Gendron AD, Brousseau P, Ménard L, Marcogliese DJ, Cyr D, Ruby S & Fournier M (2003) Effects of Agricultural Pesticides on the Immune System of *Rana pipiens* and on its Resistance to Parasitic Infection. Environ Toxicol Chem, 22: 1127-1133.

Denver RJ, Mirhadi N & Phillips M (1998) Adaptive Plasticity in Amphibian Metamorphosis: Response of *Scaphiophus hammondii* Tadpoles to Habitat Dessication. Ecology, 79: 1859-1872.

Detenbeck NE, Hermanutz R, Allen K & Swift MC (1996) Fate and Effects of the Herbicide Atrazine in Flow-Through Wetland Mesocosms. Environ Toxicol Chem, 15: 937-946.

Diana SG, Resetarits WJ, Schaeffer DJ, Beckman KB & Beasley VR (2000) Effects of Atrazine on Amphibian Growth and Survival in Artificial Aquatic Communities. Environ Toxicol Chem, 19: 2961-2967.

Doughty P & Roberts JD (2003) Plasticity in Age and Size at Metamorphosis of *Crinia georgiana* Tadpoles: Responses to Variation in Food Levels and Deteriorating Conditions During Development. Australian Journal of Zoology, 51: 271-284.

Gendron AD, Marcogliese DJ, Barbeau S, Christin M-S, Brousseau P, Ruby S, Cyr D & Fournier M (2003) Exposure of Leopard Frogs to a Pesticide Mixture Affects Life History Characteristics of the Lungworm *Rhabdias ranae*. Oecologia, 135: 469-476.

Girish S & Saidapur SK (2003) Density-dependent Growth and Metamorphosis in the Larval Bronze Frog *Rana temporalis* is Influenced by Genetic Relatedness of the Cohort. J Biosci, 28: 489-497.

Glennemeier KA & Denver RJ (2002a) Developmental Changes in Interrenal Responsiveness in Anuran Amphibians. Integrative and Comparative Biology, 42: 565-573.

Glennemeier KA & Denver RJ (2002b) Small Changes in Whole-Body Corticosterone Content Affect Larval *Rana pipiens* Fitness Components. General and Comparative Endocrinology, 127: 16-25.

Glennemeier KA & Denver RJ (2002c) Role for Corticoids in Mediating the Response of *Rana pipiens* Tadpoles to Intraspecific Competition. Journal of Experimental Zoology, 292: 32-40.

Gobbetti A & Zerani M (1996) Possible Mechanism for the First Response to Short Term Captivity Stress in the Water Frog, *Rana esculanta*. Journal of Endocrinology, 148: 233-239.

Gosner KL (1960) A Simplified Table for Staging Anuran Embryos and Larvae. Herpetologica, 16: 183-190.

Goulet BN & Hontela A (2003) Toxicity of Cadmium, Endosulfan and Atrazine in Adrenal Steroidogenic Cells of Two Amphibian Species, *Xenopus laevis* and *Rana catesbeiana*. Environ Toxicol Chem, 22: 2106-2113.

Hayes TB, Collins A, Lee M, Mendoza M, Noriega N, Stuart AA & Vonk A (2002a) Hermaphroditic, Demasculinized Frogs after Exposure to the Herbicide Atrazine at Low Ecologically Relevant Doses. Proceedings of the National Academy of Sciences, 99: 5476-5480.

Hayes TB, Haston K, Tsui M, Hoang A, Haeffele C & Vonk A (2002b) Feminisation of Male Frogs in the Wild. Nature, 419: 895-6 (31 October 2002).

Hayes TB, Haston K, Tsui M, Hoang A, Haeffele C & Vonk A (2003) Atrazine-Induced Hermaphroditism in American Leopard Frogs (*Rana pipiens*): Laboratory and Field Evidence. Environmental Health Perspectives, 111: 568-575.

Howe GE, Gillis R & Mowbray RC (1998) Effect of Chemical Synergy and Larval Stage on the Toxicity of Atrazine and Alachlor to Amphibian Larvae. Environ Toxicol Chem, 17: 519-525.

Johnson PTJ, Lunde KB, Zelmer DA & Werner JK (2003) Limb Deformities as an Emerging Infectious Disease in Amphibians: Evidence from Museum Specimens and Resurvey Data. Conservation Biology, 2003, 17: 1724-1737.

Kiesecker JM (2002) Synergism Between Trematode Infection and Pesticide Exposure: A Link to Amphibian Limb Deformities in Nature. Proceedings of the National Academy of Sciences, 99: 9900-9904.

Kloas W, Lutz I & Einspanier R (1999) Amphibians as a Model to Study Endocrine Disrupters: II. Estrogenic Activity of Environmental Chemicals *In Vitro* and *In Vivo*. Sci Total Environ, 225: 59-68.

Kloas W, Opitz R & Lutz I (2003) Standard Operating Procedure for the Conduct of the Xenopus Metamorphosis Assay (XEMA) (Comparison Study 2003). Document dated July 2003. Department of Inland Fisheries, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin.

Lewis P & Roberts S (2003) Report: FIFRA Scientific Advisory Panel Meeting, June 17-20 2003, held at the Crowne Plaza Hotel, Arlington, Virginia. A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding: Potential Developmental Effects of Atrazine on Amphibians. SAP Report No 2003-01 dated 4 August 2003.

Mackenzie CA, Berrill M, Metcalfe C & Pauli BD (2003) Gonadal Differentiation in Frogs Exposed to Estrogenic and Antiestrogenic Compounds. Environ Toxicol Chem, 22: 2466-2475.

Newman RA (1998) Ecological Constraints on Amphibian Metamorphosis: Interactions of Temperature and Larval Density with Responses to Changing Food Level. Oecologia, 115: 9-16.

NIEHS (2000) Background Review Document. Frog Embryo Teratogenesis Assay – *Xenopus*. National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, National Institute of Environmental and Health Sciences. 10 March 2000.

Niewkoop PD & Faber J (1975) Normal Table of *Xenopus laevis*, Second Edition: Amsterdam, The Netherlands, North Holland Publishing.

APVMA (1997) The NRA Review of Atrazine. Interim Report dated November 1997.

NRC (1974) Amphibians: Guidelines for the Breeding, Care, and Management of Laboratory Animals. Subcommittee on Amphibian Standards, Institute of Laboratory Animal Resources, National Research Council. National Academy of Sciences, Washington DC, 1974.

Pelley J (2003) Nitrate Eyed as Endocrine Disrupter. Environ Sci Technol, May 2003, p 162A.

Pickford DB, Hetheridge MJ, Caunter JE, Hall AT & Hutchinson TH (2003) Assessing Chronic Toxicity of Bisphenol A to Larvae of the African Clawed Frog (*Xenopus laevis*) in a Flow-through Exposure System. Chemosphere, 53: 223-235.

Qin Z-F, Zhou J-M, Chu S-G & Xu X-B (2003) Effects of Chinese Domestic Polychlorinated Biphenyls (PCBs) on Gonadal Differentiation in *Xenopus laevis*. Environmental Health Perspectives, 111: 553-556.

Sassoon D & Kelley DB (1986) The Sexually Dimorphic Larynx of *Xenopus laevis*: Development and Androgen Regulation. American Journal of Anatomy, 177: 457-472.

Solomon KR, Carr JA, Du Preez LH, Giesy JP, Gross TS, Kendall RJ, Smith EE & Van Der Kraak G (2002) Endocrine System Responses in Fish, Amphibians and Reptiles to Atrazine: Assessment of an Expert Panel. Ecorisk Inc. Syngenta Study no 1725-02 dated 1 July 2002. Unpublished.

Steeger T & Tietge J (2003) White Paper on Potential Developmental Effects of Atrazine on Amphibians. Document dated 17 May 2003. Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, Environmental Fate and Effects Division, United States Environmental Protection Agency, Washington DC.

Tavera-Mendoza L, Ruby S, Brousseau P, Fournier M, Cyr D & Marcogliese D (2002a) Response of the Amphibian Tadpole (*Xenopus laevis*) to Atrazine during Sexual Differentiation of the Testis. Environ Toxicol Chem, 21: 527-531.

Tavera-Mendoza L, Ruby S, Brousseau P, Fournier M, Cyr D & Marcogliese D (2002b) Response of the Amphibian Tadpole *Xenopus laevis* to Atrazine during Sexual Differentiation of the Ovary. Environ Toxicol Chem, 21: 1264-1267.

US EPA (2002) Reregistration Eligibility Science Chapter for Atrazine, Environmental Fate and Effects Chapter. Document dated 22 April 2002.

Villalpando I & Merchant-Larios H (1990) Determination of the Sensitive Stages for Gonadal Sex-reversal in *Xenopus laevis* Tadpoles. International Journal of Developmental Biology, 34: 281-285.

Wu RSS, Zhou BS, Randall DJ, Woo NYS & Lam PKS (2003) Aquatic Hypoxia is an Endocrine Disruptor and Impairs Fish Reproduction. Environ Sci Technol, 37: 1137-41.

13. ADDITIONAL TOXICOLOGICAL ASSESSMENT

In 1996, the Office of Chemical Safety (OCS) completed a comprehensive evaluation of the mammalian toxicology and metabolism/toxicokinetics of atrazine as part of the Australian Pesticides and Veterinary Medicines Authority's (APVMA) Chemical Review Program. The current review was undertaken to consider whether recent published reports on carcinogenicity, amphibian development and endocrine-disruptor potential of atrazine would change the recommendations of the 1996 review.

The published reports were epidemiological studies, which considered a possible link between atrazine exposure and human cancer, and environmental studies, which investigated possible effects on frog development. These environmental studies were included because of possible links to the endocrine disrupting potential of atrazine. The published epidemiological data provided support for the absence of a carcinogenicity potential for atrazine. The environmental studies are considered unlikely to have a direct relevance to human health. The 1996 review identified that atrazine caused neuroendocrine disruption in Sprague-Dawley (SD) rats, but did not bind to the oestrogen receptor or have any oestrogenic activity. Therefore it is unlikely to be an endocrine disruptor in humans based on the known mechanism of action in SD rats. No changes to the existing health standards for atrazine are recommended.

13.1 Introduction

The APVMA has requested that the OCS, within the Therapeutic Goods Administration (TGA) group of regulators, assess any relevant current information on atrazine and identify and evaluate any variations from its earlier conclusions on toxicology matters published in the APVMA's November 1997 *Atrazine Review Report*. Specific issues for consideration included atrazine's carcinogenicity, endocrine-disruptor potential and effects on vertebrate development, particularly amphibian development and reproduction.

In conducting this review, the conclusions of the OCS's 1996 report with respect to the chronic, developmental and reproductive studies have been reconsidered along with the relevant findings of the Interim Re-registration Eligibility Decision (IRED)[1] document of the US EPA. In addition, relevant recent published material has been considered.

13.2 Background

Atrazine is a systemic triazine herbicide used pre-emergence and early post-emergence for selective control of broad-leaf and grassy weeds in various food crops (such as corn, sorghum, sugar cane), forestry plantations and in non-crop situations. Atrazine has been used in Australia for more than 25 years. At present, atrazine is in Poisons Schedule 5 (S5) of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP). It has

[1] A copy of the US EPA's Interim Reregistration Eligibility Decision (IRED) document can be found at http://www.epa.gov/oppsrrd1/REDs/atrazine_ired.pdf

an acceptable daily intake (ADI) of 0.005 mg/kg bw/d, but due to its low acute toxicity, an acute reference dose (ARfD) for atrazine has not been set in Australia.

Several major evaluations of the toxicology of atrazine have been conducted in Australia:

In December 1985, the Commonwealth Department of Health evaluated a large toxicology data submission from Ciba-Geigy. The Pesticide and Agricultural Chemicals Standing Committee (PACSC) tentatively set an ADI of 0.0003 mg/kg bw/d, based on a NOEL of 0.6 mg/kg bw/d in a 2-year rat study and using a 2000-fold safety factor. Since this study reported a high incidence of mammary tumours in controls, with a dose-related trend for increased incidence in atrazine-treated rats, it was necessary to await the submission of a replacement chronic mouse study. The PACSC concluded that atrazine was of low oncogenic potential but requested the final report of ongoing chronic studies in rodents.

In November 1990, a review of newly submitted data concluded that rodent studies showed no evidence of carcinogenic potential and available epidemiological data showed no association between atrazine exposure and cancer. An ADI of 0.005 mg/kg bw/d was set, based on the NOEL of 0.5 mg/kg bw/d in a 2-year rat study and using a 100-fold safety factor. Atrazine remained exempt from the poisons schedule.

In January 1994, the Advisory Committee on Pesticides and Health (ACPH) considered atrazine use and water contamination issues, noting public concern raised in Tasmania following atrazine use in the establishment of eucalypt plantations and the contamination of stream water. The ACPH recommended the development of forestry guidelines to reduce the possibility of water contamination with pesticides and agreed to review the ADI and the drinking water Health Guideline Value. In May 1994, the ACPH reviewed the toxicology database and concluded that rat mammary tumours were not relevant to the human risk assessment of atrazine. The ACPH confirmed the NOEL of 0.5 mg/kg bw/d, the ADI of 0.005 mg/kg bw/d and the water quality Health Guideline Value of 0.02 mg/L. In November 1994, an extensive package of supplementary toxicology studies of atrazine was evaluated. There was no change to the NOEL or ADI, but atrazine was rescheduled from exempt to S5.

The OCS completed a *Review of the Mammalian Toxicology and Metabolism-Toxicokinetics of Atrazine* in December 1996. The OCS evaluated a number of new data submissions on the toxicology of atrazine in addition to all previously submitted data. The review recommended that:

- Apart from significantly stricter controls over uses in riparian zones there were no objections to the continued approval of atrazine.
- No change to the current NOEL for atrazine was warranted. The NOEL of 0.5 mg/kg bw /d (10 ppm) was established in a 2-year SD rat study, with a LOEL of 70 ppm (2.8-4.5 mg/kg bw/d) based on a statistically-significant increase in mammary tumour incidence at this dose. Whilst the mammary tumours were not considered to be relevant to human health, the response was considered to reflect a hormonal interaction and an appropriately conservative endpoint for setting the ADI.

- The current ADI for atrazine of 0.005 mg/kg bw/d (based on the NOEL of 0.5 mg/kg bw/d and using a safety factor of 100) was confirmed.
- The Health Guideline Value for atrazine and its metabolites of 0.02 mg/mL should be reconsidered by the National Health and Medical Research Council (NHMRC). This value was subsequently amended to 0.04 mg/mL in 2001.
- No change to the poisons schedule (S5 of the SUSDP) was warranted.

13.3 Carcinogenicity

The potential carcinogenicity of atrazine was considered by the OCS as part of the 1997 APVMA review. The OCS evaluated a series of chronic feeding studies in SD and Fischer-344 rats, which were performed to determine the possible carcinogenic action of technical grade atrazine on the pituitary and mammary glands and its effect on hormone levels and oestrous cycle in females.

In SD rats, no increase in the overall incidence of pituitary or mammary tumours was seen but there was a somewhat earlier onset of mammary fibroadenoma/carcinoma at 20 mg/kg bw/d. Similarly, there appeared to be an earlier onset of pituitary tumours. An increased number of days in oestrus or under oestrogen dominance were observed, which suggested that the earlier onset of mammary tumours could relate to an accelerated ageing of the neuroendocrine system. In contrast, Fischer 344 rats did not exhibit any treatment-related effects on the length of the oestrous cycle, oestradiol or progesterone. The results of the lifetime studies in female Fischer-344 rats indicated that the only toxicological effect was reduced bodyweight gain (NOEL of approximately 3.5 mg/kg bw/d). There was no evidence of a carcinogenic effect of atrazine. It was noted that the proportion of time Fischer 344 rats spent in oestrus tended to decline with age, in contrast to SD rats.

The mammary tumour response observed in various female SD rat studies was inconsistent. For example, the study of Spindler & Sumner (1981) revealed a non dose-related increase in the incidence of fibroadenomas. In contrast, the study of Mayhew (1986) revealed a dose-related increase in adenocarcinomas, but no effect on fibroadenomas. In a subsequent study, there was no increase in mammary tumours (Rudzki et al 1991). Studies provided with the most recent submission from Ciba-Geigy showed an earlier onset of mammary tumours, without an increase in total tumour incidence (Wetzel et al 1994). Considering the large variation noted in the spontaneous occurrence of mammary tumours in SD rats, the inconsistent response in the various rat studies is not surprising (Haseman et al 1986). Collectively, the findings have led to the hypothesis that certain triazines can produce an endocrine-mediated imbalance, which results in precocious reproductive ageing in SD rats, with the possible earlier onset or increased incidence of mammary tumours.

In a published study using Fischer 344/LATI rats, there was an increase in benign mammary tumours in high-dose males (with a small increase in latency cf. tumours in control animals) but not in females (Pinter et al 1990). The increase in male mammary tumours may be attributable, at least in part, to the significantly longer lifespan of 750 ppm males than controls. However, in this study neither tumour to age adjustment nor comparison to background control data of the laboratory, were performed. In a

subsequent Hazleton study (Thakur 1992), no increase in mammary tumours was noted in male Fischer 344 rats at the highest dose of 400 ppm; the increase in this study was only seen at 750 ppm, not at 375 ppm. An increase in malignant uterine tumours in females and an increase in haematopoietic system tumours also was noted. It is possible that atrazine treatment may have affected hormonal balance since the mammary gland and uterine tumours may be hormone-dependent tumours; however, the lack of any increase in mammary tumours in females argues against a direct oestrogenic action of atrazine.

On the basis of these data the OCS reached the following conclusions:

- The earlier onset in mammary tumours was not seen in male SD rats, in female Fischer 344 rats, or male or female CD-1 mice;
- It was likely that the response observed in SD female rats only occurs above a certain threshold;
- The background incidence of mammary tumours was significantly higher in female SD than in female Fischer 344 rat. For example, NCI data (1980) indicated a 36.4% historical control incidence for mammary tumours in SD rats and a 17.9% incidence in Fischer rats;
- The available evidence indicates that neither atrazine nor its metabolites are genotoxic in animal cells;
- In humans, menopausal women develop episodes of declining oestrogen secretion and longer periods of low oestrogen levels, in contrast to the situation in ageing SD rats. Therefore, it would appear that the atrazine response in SD rats is not an appropriate surrogate for the assessment of human risk for mammary tumour development.

These data were also considered by the Advisory Committee on Pesticides and Health (ACPH) at its 12th meeting (5th February 1997). The Committee considered that the benign mammary tumours observed in SD rats were not relevant for a human health risk assessment.

The US EPA has also downgraded the carcinogenicity potential of atrazine. In 1988, the US EPA had classified atrazine as a "possible human carcinogen" under its then cancer assessment guidelines. In 1994, a special review of atrazine's potential to cause human cancer through dietary or occupational exposure was initiated. The outcome of this review was the tentative conclusion that atrazine should be reclassified as a "likely human carcinogen" based on more contemporary cancer guidelines. However, this hazard classification was reviewed by the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Science Advisory Panel (SAP) and the Cancer Assessment Review Committee (CARC). They concluded that the mechanism of mammary tumour formation in SD rats is not relevant to humans and therefore atrazine should be reclassified as "not a likely human carcinogen". The US EPA has accepted this cancer classification.

After a review of a similar database for atrazine, the International Agency for Research on Cancer (IARC) concluded in 1999 that the mode of action of atrazine is species specific and thus not relevant to humans and downgraded the classification from Group 2B "possible human carcinogen" to Group 3 "not classifiable".

13.4 Epidemiological data

Sathiakumar and Delzell (1997) conducted a review of epidemiological data from 10 case-control studies and one follow-up study on the association of atrazine exposure and human cancer. Collectively, this epidemiological data revealed no link with the occurrence of non-Hodgkin's lymphoma, Hodgkin's disease, leukemia, multiple myeloma, soft tissue sarcoma, colon cancer and ovarian cancer. However, the authors reported that there were limitations to the majority of studies including the relatively small numbers of subjects and the absence of detailed quantitative exposure data. A recent study on the cancer incidence among triazine herbicide manufacturing workers concluded that there was no data that supports a causal link between atrazine exposure and prostate cancer (MacLennan et al 2002). A follow-up study provided no evidence that triazine manufacturing workers have an increased mortality rate (MacLennan et al 2003).

It is concluded that these recently published epidemiological data provide support for the absence of any carcinogenicity potential of atrazine.

13.5 Developmental Effects on vertebrates

The possible effect of atrazine on the development of vertebrates, particularly of amphibians, has recently been raised in the light of studies reporting effects on frog development (Hayes et al 2002a & b). The so-called "Hayes studies" reported hermaphrodism in two species of frogs at relatively low concentrations.

In the first of these studies (Hayes et al 2002a), the effect of 0.01-200 ppb atrazine on sexual development in African clawed frogs (*Xenopus laevis*) was examined in 2 separate experiments. Atrazine caused hermaphroditism ($\geq$0.1 ppb), demasculinized larynges ($\geq$1.0 ppb) and decreased testosterone (25 ppb) in males. In the second study (Hayes et al 2002b), which tested only 2 concentrations of atrazine (0.1 and 25 ppb), retarded gonadal development (gonadal dysgenesis) and testicular oogenesis (hermaphroditism) was induced by atrazine at 0.1 ppb in American leopard frogs (*Rana pipiens*). However, the occurrence of these abnormalities at 25 ppb was approximately 3-fold lower than at 0.1 ppb (ie. there was no dose-response). These abnormalities were also observed in animals collected from atrazine-contaminated sites across the USA. However, the possibility that other contaminants caused the effects in frogs can not be ruled out because the chemical profiles of these sites were not determined.

A number of criticisms have been levelled against the Hayes studies. There is no validated test method for determining the endocrine effects of atrazine; it is unclear whether these findings are reproducible. Publicly available communications from Syngenta contend that these studies have been unable to be repeated by two members of a panel originally set up by Syngenta to investigate the possible effects of atrazine on amphibian development. This panel also included Hayes, who subsequently left the panel allegedly due to questions over the validity of preliminary findings.

In their most recent environmental risk assessment, the US EPA concluded that based on the existing uncertainties in the available database, atrazine should be subjected to

more definitive testing once the appropriate testing protocols have been established. The FIFRA SAP is apparently reviewing new and supplementary studies relating to the potential effects of atrazine on amphibian endocrinology and reproductive and developmental responses. The outcome of this review will feed in to the existing environmental assessment and it is anticipated that an amended IRED will be published at a later date.

During the APVMA's 1997 review of atrazine, the OCS evaluated a range of studies conducted in mice, rats and rabbits, which examined the ability of atrazine to perturb normal reproduction and development. These studies indicated that atrazine is not a reproductive or developmental toxicant. The utility of these and other laboratory animal species (eg. guinea pigs, dogs and non-human primates) stems not only from their physiological similarities to humans but also because validated test methods exist which substantiate their relevance and reliability in chemicals risk assessment.

The Organisation for Economic Co-operation and Development (OECD) publishes a series of test guidelines, which are a recognised international standard for chemical testing. The methods described in these guidelines cover tests for physical and chemical properties, effects on human health and wildlife, and accumulation and degradation in the environment. There is currently no validated test method for the use of amphibians (or reptiles) in assessing the hazard to human health from chemical exposure. Therefore, the OCS considers that while the reports of Hayes et al (2002a & b) may impact on the environmental assessment of atrazine, they are unlikely to have any direct relevance to human health.

13.6 Endocrine-disrupting potential

The OCS, along with other Australian Government agencies, considers that endocrine disruption is but one part of a spectrum of effects that chemicals can cause if animals and humans are exposed to levels that overwhelm normal inactivation processes such as metabolism and excretion. That is, endocrine disruption is not considered to be an adverse end-point per se, but rather is a mode or mechanism of action potentially leading to other toxicological or eco-toxicological outcomes eg. reproductive, developmental, carcinogenic or ecological effects.

Studies evaluated by the OCS as part of the 1997 APVMA review suggested that atrazine perturbs the neuroendocrine system of female SD rats, which leads to precocious reproductive ageing and the possible earlier onset or increased incidence of mammary tumours. As such it can be considered as an endocrine disruptor in this particular rat strain. However, as discussed above, this mechanism of mammary tumour formation in SD rats is not relevant to humans and therefore it is unlikely that atrazine is an endocrine disruptor in humans. Other studies evaluated by the OCS indicated that atrazine does not bind to the oestrogen receptor and has no intrinsic oestrogenic activity. These findings were also made by the US EPA in their latest IRED and by IARC in their 1999 evaluation of atrazine.

As part of their environmental risk assessment, the US EPA was uncertain as to whether atrazine causes endocrine effects on the environment. Based on the existing uncertainties in the database, the US EPA stated that atrazine should be subjected to more definitive testing once appropriate test protocols have been established. As

mentioned above, the agency is awaiting the outcome of the review of new and supplementary data on the potential effects of atrazine on amphibian endocrinology and reproductive and developmental responses by their SAP.

13.7 Conclusions

1. Published epidemiological data provides support for the absence of carcinogenicity potential for atrazine.
2. Effects on frog development should be considered as equivocal until such times as validated test methods can reliably reproduce recent findings. While these findings may impact on the environmental assessment of atrazine, any findings are unlikely to have a direct relevance to human health.
3. Atrazine causes neuroendocrine disruption in SD rats, but does not bind to the estrogen receptor or have any estrogenic activity. It is unlikely that atrazine is an endocrine disruptor in humans based on the known mechanism of action in SD rats.

13.8 Recommendation

No changes to the existing health standards for atrazine are proposed.

13.9 References

Haseman JK, Winbush JS and O'Donnell MR (1986) Use of Dual Control Groups to Estimate False Positive Rates in Laboratory Animal Carcinogenicity Studies. Fund. Appl. Toxicol. **7**: 573-584

Hayes TB, Collins A, Lee M, Mendoza M, Noriega N, Stuart AA & Vonk A (2002a) Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proceedings of the National Academy of Sciences USA. **99**(8)5476-5480.

Hayes TB, Haston K, Tsui M, Hoang A, Haeffele & Vonk A (2002b) Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (*Rana pipiens*): Laboratory and field evidence. Environmental Health Perspectives. **111**(4): 568-575

MacLennan PA, Delzell E, Sathiakumar N, Myers SL, Cheng H, Grizzle W, Chen VW & Wu XC (2002) Cancer incidence among triazine herbicide manufacturing workers. Journal of Occupational & Environmental Medicine **44**(1):1048-58.

MacLennan PA, Delzell E, Sathiakumar N & Myers SL (2003) Mortality among triazine herbizide manufacturing workers. Journal of Toxicology and Environmental Health, Part A **66**: 501-517.

Mayhew DA (1986) Twenty Four Month Combined Chronic Oral Toxicity and Oncogenicity Study in Rats utilizing Atrazine Technical. Ciba-Geigy Corp., Greensboro, NC. Lab: American Biogenics Corporation, Decatur, IL. (called ToxiGenics Inc. prior to 12 Feb. 1985). Study No. 410-1102. EPA MRID no. 00141874. Report date 29 April 1986 [Boxes DP and AQ; R1226]

Pinter A, Torok G, Borzsonyi M, Surjan A, Csik M, Kelecsenyi Z and Kocsis Z (1990) Long-Term Carcinogenicity Bioassay of the Herbicide Atrazine in F344 Rats. National Institute of Hygiene, Dept of Morphology, Budapest, Hungary. Neoplasma 37: 533-544

[A3162/17 B3: R10561; data submission date 13 May 1994]

Rudzki MW, McCormick GC & Arthur AT (1991) Chronic Toxicity Study in Rats. Ciba-Geigy Corporation, Summit, New Jersey, USA. Division of Toxicology/Pathology, Safety Evaluation Facility, Lab. Study no. 852214. Study completion date 28 January 1991 [A3162/20 B5]

Sathiakumar N & Delzell E (1997) A review of epidemiological studies of triazine herbicides and cancer. Critical Reviews in Toxicology **27**(6):599-613.

Spindler M & Sumner DD (1981) Two-Year Chronic Oral Toxicity Study with Technical Atrazine in Albino Rats.Ciba-Geigy Corp. Lab: Industrial Biotest Labs Inc., Northbrook, Illinois; reported by Ciba-Geigy, NC. Study no. 622-06769, EPA MRID no. 00089151 [A3162/2 B27; no submission no.]

Thakur AJ (1992) Two-Year Dietary Oncogenicity Study in Fischer-344 Rats with Atrazine Technical. Ciba-Geigy Corp., Greensboro, NC. Lab: Hazleton Washington Inc., Vienna, Virginia, USA. Study no. HWA-483-277. Report date 18 Feb 1992. [A3162/25 B8 & B9: R9657]

Wetzel LT, Luempert LG, Breckenridge CB, Tisdel MO, Stevens JT, Thakur AK, Extrom PC & Eldridge JC (1994) Chronic Effects of Atrazine on Estrus and Mammary Tumour Formation in Female Sprague-Dawley and Fischer 344 Rats. Dept Toxicology, Ciba-Geigy, Greensboro, NC and Dept Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC. J Toxicol Env Hlth **43**: 169-182