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Australian Pesticides and
Veterinary Medicines Authority

TECHNICAL REPORT
ATRAZINE

The reconsideration of the active constituent, registration of products containing Atrazine and approvals of their associated labels.

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ACRONYMS AND ABBREVIATIONS

ACPH	Advisory Committee on Pesticides and Health (superseded by the Advisory Group on Chemical Safety)
ADI	Acceptable daily intake
ANZECC	Australian and New Zealand Environment and Conservation Council
ARfD	Acute reference dose
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
ATSDR	Agency for Toxic Substances and Disease Registry
bw	body weight
CCC	Community Consultative Committee
d	Per day
DEWHA	Department of the Environment, Water, Heritage and the Arts
DoHA	Department of Health and Ageing
FAO	Food and Agriculture Organization of the United Nations
IARC	International Agency for Research on Cancer
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LOEL	Lowest observable effect level
µg	microgram
mg	milligram
MOA	Mode of action
MRL	Maximum residue limit
NHMRC	National Health and Medical Research Council
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NWQMS	National Water Quality Management Strategy
OCS	Office of Chemical Safety
ppb	Parts per billion
ppm	Parts per million
SD rats	Sprague-Dawley rats
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

CONTENTS

1. UPDATED TOXICOLOGY ASSESSMENT OF 2004, TAKING INTO ACCOUNT PUBLIC COMMENTS AND OVERSEAS REPORTS (OCS, APRIL 2007)

In 1996, the Office of Chemical Safety (OCS) completed a comprehensive assessment of the mammalian toxicology and metabolism/toxicokinetics of atrazine as part of the Australian Pesticides & Veterinary Medicines Authority's (APVMA) Chemical Review Program. The APVMA published the assessment in its 1997 atrazine review report. The current assessment (first published in 2004 and updated in April 2007 following consideration of public comments) was undertaken to consider whether recent published reports on carcinogenicity, amphibian development and the endocrine-disruptor potential of atrazine would change the recommendations of the 1996 assessment, as discussed in the APVMA's 2004 report.

The current level of exposure to mixtures of triazine compounds via food and drinking water was also considered and whether a cumulative risk assessment is warranted, in response to public comments and further information that became available after publication of the 2004 report. (A separate supplementary report is at section 2. It was prepared immediately before the June 2007 atrazine forum requested by the APVMA's Community Consultative Committee.)

The published studies considered by the OCS in the 2004 report 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 no support for any carcinogenicity potential of atrazine. The environmental studies are considered unlikely to have a direct relevance to human health. The 1996 assessment 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. The current level of exposure to mixtures of triazine compounds via food and drinking water is not of concern, although cumulative risk assessment would be a consideration if the level of exposure was significant. No changes to the existing health standards for atrazine are recommended[1].

1.1 INTRODUCTION

Prior to publication of the 2002 report the APVMA requested the OCS to assess any relevant current information on atrazine and whether this changed its conclusions published in the APVMA's November 1997 report. Specific issues for consideration included atrazine's carcinogenicity, endocrine-disruptor potential and effects on vertebrate development, particularly amphibian development and reproduction.

[1] APVMA note: Changes to existing health standards are undertaken by the DoHA as a parallel action to the implementation of review outcomes when warranted.

In conducting this review, the OCS considered:

- its 1996 assessment with respect to chronic, developmental and reproductive studies;
- relevant findings of the US EPA's Interim Re-registration Eligibility Decision (IRED)[2]; and
- the US EPA's relevant recent published material.

1.2 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 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 Pesticides 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 two-year rat study and using a 2,000-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 two-year rat study and using a 100-fold safety factor. Atrazine remained exempt from poisons scheduling.

- 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

[2] See http://www.epa.gov/oppsrrd1/REDs/atrazine_ired.pdf.

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 Schedule 5.

- The OCS completed a Review of the Mammalian Toxicology and Metabolism-Toxicokinetics of Atrazine in December 1996[3]. 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 two-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[4] to 0.04 mg/mL in 2001.
  - No change to the poisons schedule (S5 of the SUSDP) was warranted.

1.3 CARCINOGENICITY

The OCS considered atrazine's potential carcinogenicity, and the resulting report was published as part of the APVMA's 1997 atrazine report. 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 cycles 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

[3] APVMA note: The assessment from the OCS was included in the APVMA's November 1997 report.
[4] APVMA note: Amendments to drinking water standards are determined by the NHMRC.

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 the laboratory's background control data, 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 per cent historical control incidence for mammary tumours in SD rats and a 17.9 per cent 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 12^{th} meeting (5 February 1997). The ACPH considered that the benign mammary tumours observed in SD rats were not relevant for a human health risk assessment.

In relation to mammary tumours associated with atrazine exposure in female SD rats, Meek *et al.* (2003) postulated that atrazine suppresses the release of luteinizing hormone (LH) from the pituitary gland such that there is an insufficient level of LH to trigger ovulation. When ovulation fails, follicles within the ovum continue to produce oestrogen. Repetitive failure of ovulation leads to prolonged exposure to endogenous oestrogen and/or prolactin creating a state of persistent oestrus. Over a sufficiently long period of time, this would translate into a hastened ageing process and the development of mammary fibroadenoma/carcinoma. Thus, in SD female rats, reproductive ageing is characterised by persistent hyperestrogenemia and hyperprolactinemia with low levels of LH and follicle-stimulating hormone (FSH). In contrast, reproductive ageing in women is characterised by exhaustion of ovarian follicles resulting in low levels of oestrogen and prolactin. LH and FSH levels in postmenopausal women remain high. Meek *et al*. concluded that on the basis of differences in reproductive physiology, it would appear that the atrazine response in SD rats is not relevant to humans.

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 Science Advisory Panel and the Cancer Assessment Review Committee. 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 atrazine's MOA is species specific and thus not relevant to humans and downgraded its classification from Group 2B 'possible human carcinogen' to Group 3 'not classifiable' (IARC, 1999).

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 2002 study on the cancer incidence among triazine herbicide manufacturing workers concluded that there were no data that support 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).

The OCS concludes that these recently published epidemiological data provide no support for the carcinogenicity potential of atrazine.

1.4 DEVELOPMENTAL EFFECTS ON VERTEBRATES

Atrazine's possible effect 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 two separate experiments. Atrazine caused hermaphrodism ($\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 two 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 three-fold lower than at 0.1 ppb (i.e. there was no dose-response relationship). 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 cannot 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 atrazine's endocrine effects; it is unclear whether these findings are reproducible. Publicly available communications from Syngenta contend that two members of a panel originally set up by Syngenta to investigate the possible effects of atrazine on amphibian development have been unable to repeated the results. This panel also included Hayes, who subsequently left the panel allegedly due to questions over the validity of preliminary findings.

In June 2003, after evaluating the available literature on the potential effects of atrazine on amphibian gonadal development, the US EPA concluded that while there was sufficient information to formulate an hypothesis that atrazine exposure can affect amphibian gonadal development, there was insufficient information to refute or confirm that hypothesis, mainly because of the limitations of the study designs and uncertainties in the data. The US EPA's 2003 white paper critically evaluated the data from 17 laboratory and field studies, discussed remaining uncertainties in evaluating the potential effects of atrazine on amphibian development, and outlined future studies that could address these uncertainties (US EPA 2003a). The Scientific Advisory Panel reviewed the white paper and concluded that the US EPA's review was thorough, the conclusions were valid, and the approaches and criteria for new studies were appropriate. The Scientific Advisory Panel also agreed that additional studies were warranted and that a tiered testing approach was appropriate.

In response to a November 2004 Data Call-In Notice from US EPA, Syngenta, the principal atrazine registrant, developed a testing protocol for determining the effects of atrazine alone on amphibian gonadal development, and conducted two studies consistent with the first tier of testing described in the 2003 white paper (US EPA 2003). In June 2007, Syngenta submitted to the US EPA its final report on the potential effects of atrazine on gonadal development of amphibians.

As of September 2007, the US EPA had reviewed 19 laboratory and field studies, including registrant-submitted studies and studies available in the public literature. Only the two Data Call-In studies submitted by the registrant incorporated all of the design elements recommended by the US EPA and the Scientific Advisory Panel to address uncertainties identified in the 2003 white paper. These studies were conducted separately in two independent laboratories. Although both studies have limitations, the overall design and conduct of the studies reflect a high degree of quality control that allows them to be used to test the hypothesis that atrazine exposure affects amphibian gonadal development. Other laboratory and field studies reviewed by the US EPA did not fully account for experimental and environmental conditions that could influence relevant endpoints.

Based on a thorough examination of these studies and their results, US EPA finds that atrazine does not adversely affect amphibian gonadal development, and believes there is no compelling reason to pursue additional testing of atrazine for amphibian gonadal effects.

As part of 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 (e.g. 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.

1.5 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 which overwhelm normal inactivation processes such as metabolism and excretion. That is, endocrine disruption is not considered to be an adverse endpoint *per se*, but rather is an MOA potentially leading to other toxicological or eco-toxicological outcomes e.g. 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. The relevance of induced aromatase activity in *in vitro* systems to altered oestrogenic signalling *in vivo* is at present unclear. On the weight of evidence from the *in vitro* and *in vivo* studies, atrazine is unlikely to be an oestrogenic compound. These findings were also made by the US EPA in their latest IRED[5] and by IARC in their 1999 evaluation of atrazine.

In relation to the affects of atrazine on sperm count, mixed results have been observed in animal studies. No testicular toxicity, as assessed by sperm morphology, sperm counts and testicular weights, was seen in mice treated daily for five days with atrazine intraperitoneally at doses of 38–600 mg/kg bw/d (Osterloh *et al.* 1983). In contrast, decreases in sperm numbers and motility were observed in Fischer rats treated with atrazine intraperitoneally at doses of 30–120 mg/kg bw twice a week over 60 days (Kniewald *et al.* 2000). However, given that the likely human exposure to atrazine would be via the oral and dermal routes, no occupational or public exposure will ever be likely to achieve these high doses. It should also be noted that atrazine, when administered orally to both male and female rats at 30 mg/kg bw/d, did not cause any impairment in the reproductive performance. Therefore, the animal data suggest that effects of atrazine on sperm counts in men exposed to atrazine at environmental levels typical of those seen in human populations are unlikely.[6]

1.6 CUMULATIVE RISK ASSESSMENT

In establishing whether a cumulative risk assessment is warranted for a group of

[5] APVMA note: Information on the US EPA regulatory position on atrazine is compiled in the Decision Documents for Atrazine at http://www.epa.gov/oppsrrd1/REDs/atrazine_combined_docs.pdf, comprising:

- Atrazine IRED [Interim Reregistration Eligibility Decision] (January 2003);
- Revised Atrazine IRED (31 October 2003); and
- Finalisation of Atrazine IRED, and Completion of Tolerance Reassessment and Reregistration Eligibility Process (6 April 2006).

[6] APVMA note: The APVMA has initiated a project to re-examine the possibility that the triazines (atrazine and related chemicals with a similar mechanism of action) may have unintended harmful effects on humans, taking into account ongoing research into a newly hypothesised endocrine MOA. This project will take account of international reports.

similarly acting compounds, the OCS should be satisfied that exposure is likely to occur. The OCS noted in the December 1996 assessment that atrazine was used in high volumes, predominantly as a herbicide in preparation for plantings with coarse grains and sugarcane, with minor uses in forestry and legumes.

The 1992 Australian Market Basket Survey (AMBS) (National Food Authority, Australian Government Publishing Service) conducted assays for atrazine and simazine in meat and cereal foods. Because of their use pattern (just before or after crop emergence) it was considered unlikely that residues would be present in food. In fact, no residues of either herbicide were detected. This finding was in agreement with US data; in over 30 years of use, atrazine had not been detected in edible portions of plants or livestock in the US nor had it been detected in market basket surveys. Thus the OCS concluded that exposure of the population to atrazine in food is very unlikely.

However, the fact that atrazine is both mobile in the soil and reasonably stable in the environment indicates that non-occupational exposure to atrazine, if it occurs, is likely to occur through contamination of drinking water. Indeed the OCS noted in its 1996 assessment that consideration should be given to amending the Australian Drinking Water Guidelines to include the four metabolites with parent atrazine in the definition of atrazine; this action would have the equivalent effect of lowering the guideline value (0.0005 mg/L) for atrazine alone since, in water samples in which atrazine is detected, one or more metabolites are commonly detected but that had been disregarded in the existing Standard.

The OCS also referred this issue to the Advisory Committee on Pesticides and Health (ACPH). Recognising the need to take into account toxicologically significant metabolites from an exposure risk assessment perspective, the ACPH supported the modification of the atrazine guideline value in the Australian Drinking Water Guidelines (1996). The ACPH proposed that, rather than including all four metabolites (desethylatrazine, desisopropylatrazine, diaminochlorotriazine and hydroxyatrazine) as outlined above, only the atrazine-specific metabolites, desethylatrazine and hydroxyatrazine, be included with atrazine in the definition for the guideline value. It was therefore recommended by ACPH that it was appropriate that the issue of drinking water guidelines for atrazine be referred to the NMHRC and ARMCANZ for consideration by the joint committee responsible for updating the Australian Drinking Water Guidelines. In response to this recommendation, a health based guideline of 0.04 mg/L was established. However it was noted that if atrazine is detected in the drinking water, then remedial action should be taken to stop the contamination; the practical limit of determination is 0.0001 mg/L. It was also recognised that the metabolites of atrazine (desethylatrazine, desisopropylatrazine, diaminochlorotriazine and hydroxyatrazine) may constitute approximately 50 per cent of the total atrazine-derived triazine compounds in some ground and surface water samples, and this was accounted for in the guideline value by the addition of an extra two-fold safety factor. Similarly, for simazine, a health based guideline value of 0.05 mg/L was established, with a guideline value of 0.0005 mg/L, above which remedial action should be taken to remove the source of contamination. It should be noted that there is regular monitoring in Tasmania at 54 sites for atrazine and simazine (and 16 other chemicals), with results available at www.dpiw.tas.gov.au.

It may be noted that atrazine has rarely been found in Australian reticulated water supplies. In groundwater it has been reported at concentrations of up to 0.002 mg/L in an area where atrazine was used to suppress weed growth in irrigation channels for 10 years (NHMRC 1996). The OCS furthermore concluded that all uses that contributed to the total environmental load of atrazine such as atrazine products applied to lawns, golf courses, irrigation channels, drains, roadsides, industrial premises and non-agricultural areas could not be maintained.

Therefore, while acknowledging there is a basis for consideration of a risk assessment of atrazine as part of a group of triazine pesticides based on a common MOA, current exposure to atrazine and simazine in food is very unlikely, while available data suggest that exposure to atrazine, simazine and propazine in reticulated drinking water is likely to be negligible. Considering the negligible exposure to the major triazine compounds available in Australia, a cumulative risk assessment of these compounds on public health grounds is not warranted at present. Nevertheless, the utility of both aggregate and cumulative risk assessment methodology for the assessment of risks posed by agricultural and veterinary chemicals to public health is under consideration by the OCS.

1.7 CONCLUSIONS

1 Published epidemiological data provide no support for any carcinogenicity potential of atrazine.[7]

2 Effects on frog development should be considered as equivocal until such time as validated test methods can reliably reproduce recent findings. While these findings may impact on the environmental assessment of atrazine, they are unlikely to have a direct relevance to human health.

3 Atrazine causes neuroendocrine disruption in SD rats, but does not bind to the oestrogen receptor or have any oestrogenic activity. It is unlikely that atrazine is an endocrine disruptor in humans based on the known MOA in SD rats.

4 Considering the negligible exposure to the major triazine compounds available in Australia, a cumulative risk assessment of these compounds on public health grounds is not warranted at present. However, a cumulative risk assessment would be a consideration if the level of exposure became significant.

1.8 RECOMMENDATION

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

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[7] APVMA note: The JMPR report published in September 2007 concludes in relation to atrazine '*A range of epidemiological studies (including cohort studies, case-control studies, and ecological or correlational studies) assessed possible relationships between atrazine or other triazine herbicides and cancer in humans. For some cancer types, such as prostate or ovarian cancer and non-Hodgkin lymphoma, the increased risks reported in single studies could either be explained by the methodology used or had not been confirmed in more reliable studies. Thus, the weight-of-evidence from the epidemiological studies did not support a causal association between exposure to atrazine and the occurrence of cancer in humans. The Meeting concluded that the existing database on atrazine is adequate to characterize the potential hazards to foetuses, infants and children.*'

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2. SUPPLEMENTARY TOXICOLOGICAL ASSESSMENT (OCS, JUNE 2007)

The following is the report from the OCS to the APVMA dated June 2007, titled *Commentary to APVMA on additional atrazine information provided by the CCC for the atrazine forum*.

2.1 INTRODUCTION

As requested by the APVMA's Community Consultative Committee, this report addresses the issues related to atrazine that will be discussed at the June 2007 forum:

- Endocrine-disrupting potential
- Developmental effects
- Carcinogenic potential
- Water contamination issues
- Cumulative assessment of mixtures
- Links between atrazine and myalgic encephalomyopathy
- Effects on children's health.

2.2 ENDOCRINE-DISRUPTING POTENTIAL

The OCS re-affirms that it considers endocrine disruption to be a part of a spectrum of effects that chemicals can cause if animals and humans are exposed to levels of a chemical which overwhelm normal inactivation processes, such as metabolism and excretion. Thus, endocrine disruption is not considered to be an adverse endpoint *per se*, but rather an MOA potentially leading to other toxicological outcomes e.g. reproductive, developmental, carcinogenic.

Previous 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 in section 13.3 of the 2004 atrazine report[8] and in section 1, Volume 2 of this report, 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 via this MOA.

More recently, other studies evaluated by the OCS indicate that atrazine does not bind to the oestrogen receptor. Radiobinding studies showed that triazines, including atrazine, did not interact with rat uterine oestrogen receptors at concentrations up to 100 mM. The triazines were approximately 100,000 times less potent than oestradiol itself in causing a 50 per cent reduction in labelled oestradiol binding to the rat oestrogen receptors (at 25°C).

[8] APVMA note: See http://www.apvma.gov.au/chemrev/downloads/atrazine_draftfinal2.pdf.

2.3 INDUCTION OF AROMATASE

Fan et al. (2007) Atrazine induced aromatase expression is SF-1 dependent: Implications for endocrine disruption in wildlife and reproductive cancers in humans (National Institute of Environmental Health Sciences)

Since atrazine does not bind to the oestrogen receptor, other mechanisms of affecting oestrogen production have been sought. Recent evidence suggests that atrazine may increase oestrogen production by increasing levels of aromatase, an enzyme of the cytochrome P450 family, which is important in sexual development. It is involved in the production of oestrogen by catalysing the conversion of testosterone (i.e. to aromatise androgens) to oestradiol (an oestrogen). Aromatase is located in oestrogen-producing cells in the adrenal glands, ovaries, placenta, testicles, adipose (fat) tissue, and brain. Atrazine has been shown to increase aromatase levels in mammalian cells *in vitro* by binding to and inhibiting phosphodiesterase, which results in increased levels of cyclic AMP (cAMP) leading to increased transcription of the aromatase gene, CYP19, that increases aromatase activity.

A series of *in vitro* experiments were conducted in order to test the hypothesis that the transcription factor, steroidogenic factor-1 (SF-1) is required for atrazine-induced aromatase expression (Fan *et al*. 2007).

The authors compared SF-1 expression in atrazine responsive human H295R adrenocortical carcinoma cells and non-responsive human ovarian granulosa-like tumour cell line (KGN). Although both cell lines expressed SF-1 mRNA, polymerase chain reaction revealed that copy numbers of SF-1 mRNA were 54 times lower in the non-responsive KGN cell lines compared to the atrazine-responsive H295R cells. Western blotting confirmed that the responsive cell line (H295R) contained higher SF-1 protein levels than the non-responsive KGN cell line.

Of the six tissue and cell-specific aromatase promoters that are known in humans, atrazine was shown in the study to only affect aromatase expression in cell and tissue types that use the SF-1 dependent aromatase promoter II (ArPII), utilised during gonadal differentiation and development. This was shown by induction of ArPII luciferase reporter activity by atrazine in the atrazine-responsive H295R cells without co-transfection of Adeno-SF-1. Atrazine did not affect the ArPII in the absence of SF-1 co-expression in NIH/3T3 fibroblast cells, which lack endogenous SF-1 expression. Transfection of the fibroblast cells with SF-1, however, resulted in a 4.2-fold increase in basal luciferase activity and increased responsiveness to atrazine. These experiments confirmed that atrazine only affects aromatase expression in cells using the SF-1 dependent ArPII and that cells that do not use this promoter or do not express SF-1 are not responsive to atrazine. When the atrazine-non-responsive KGN ovarian granulosa cells were transfected with adeno-SF-1, the exogenous SF-1 conferred approximately a four-fold increase in aromatase responsiveness to atrazine. In addition, the transfected granulosa cells showed a significant increase in aromatase activity as determined by a tritium release assay.

Chromatin-immunoprecipitation assays showed that ArPII DNA sequences in the immunoprecipitates were increased by 100 mM atrazine, showing that the pesticide did enhance binding of SF-1 to the promoter. No binding occurred when the binding site (AGGTCA) was mutated to AttTCA, showing that the responsiveness to atrazine was eliminated.

The authors of the study conclude that atrazine is a potent endocrine disruptor that increases aromatase expression in select human cancer cell lines. The mechanism involves the inhibition of phosphodiesterase and subsequent elevation of cAMP. The effects of atrazine on aromatase were shown to vary between cell lines, with the data suggesting a role for SF-1 in atrazine-induced aromatase expression as the effects were observed only in cells that use the SF-1-dependent ArPII promoter. Non-responding cells lacked expression of SF-1.

The atrazine-responsive ArPII does play a vital role in the reproductive system and has been shown to be involved in oestradiol production which may be involved with certain breast cancer oncogenesis. Aromatase expression and oestrogen production is regulated by ArPII and is SF-1 dependent. In human medicine, aromatase inhibitors have been shown to be effective at treating breast cancer induced by adipose aromatase.

OCS conclusions

A brief commentary on the study provided by the CCC regarding the endocrine-disrupting potential of atrazine on human-derived cell types *in vitro* (Fan *et al*. 2007) has been made.This study has merits in that it provides further evidence of endocrine disrupting potential of atrazine; indicates potential class effects; and comes forward with a proposed MOA.

The relevance of induced aromatase activity in *in vitro* systems to altered oestrogenic signalling *in vivo* is, at present, unclear. The Fan *et al.* (2007) study findings contrast with a recent *in vivo* study that reported a decrease in aromatase expression in the mammary glands of rat pups exposed to atrazine (Rayner *et al*. 2004). Since increased oestrogen levels occur in atrazine-treated female SD rats (due to anovulation) but not in the female Fisher 344 rats, the role of atrazine in aromatase induction *in vivo* remains questionable. With regard to androgen effects, there is no consistent experimental evidence to support the claim that atrazine suppresses androgen production and/or interferes with androgen-receptor complex formation. More research is needed to confirm the role of aromatase in potential adverse effects. It is not known whether aromatase levels are increased in humans exposed to atrazine.

The OCS would like to reaffirm the finding reported in 1997, reaffirmed in the OCS June 2006 report (resubmitted in April 2007, entitled *A review of supplementary data relating to the carcinogenicity, developmental effects and endocrine-disrupting potential of atrazine*), that atrazine causes neuroendocrine disruption in SD rats. Based on the existing evidence at that time, the relevance of this finding to an MOA of atrazine in humans was not known. There is no doubt that there are new postulated MOAs for atrazine. More importantly, in light of the new proposed MOA for atrazine, the existing toxicological data sets could be re-examined against newly postulated MOAs.[9]

[9] APVMA note: The molecular biological investigations (possibly related to a previously unreported endocrine MOA for atrazine) are also questionable when viewed in the context of the negative findings in apical studies

2.4 DEVELOPMENTAL EFFECTS

Souder (2006) It's not easy being green: Are weed killers turning frogs into hermaphrodites?

The author describes the experiments carried out by Tyrone Hayes from 1997 to 2002, which have been considered by OCS in previous evaluations. The following is a summary extracted from the supplementary OCS report of June 2006 (finalised June 2006 and resubmitted to APVMA in April 2007). The possible effect of atrazine on the development of vertebrates, particularly of amphibians, was 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 two separate experiments. Atrazine caused hermaphroditism (>0.1 ppb), demasculinized larynges (>1.0 ppb) and decreased testosterone (25 ppb) in males. In the second study (Hayes *et al*. 2002b), which tested only two 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 three-fold lower than at 0.1 ppb (i.e. there was no dose-response relationship). 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 cannot be ruled out because the chemical profiles of these sites were not determined.

According to Souder (2006), in his published articles Hayes argued that atrazine activates the aromatase gene to produce aromatase, which then converts testosterone to estradiol. It was proposed that elevated levels of aromatase could explain the males' stunted voice boxes and the hermaphroditism observed in the male frogs. To Hayes, it was not surprising that low doses of atrazine were associated with the abnormalities in the frogs, since it is known that hormones such as testosterone and estradiol function at very low concentrations. Souder noted that the studies conducted by Hayes were not acceptable to the manufacturer, Syngenta, who described Hayes' findings as 'inconclusive'. Furthermore, two teams of scientists have tried to replicate Hayes' results but have been unable to do so. However, according to Souder, the experiments conducted by these teams deviated from Hayes' methods, particularly in the rearing of the frogs, which could render the results inconclusive. It is noted that there is a body of studies outside the Hayes studies that report similar findings in frogs. This was revised at the APVMA's Atrazine Forum and remains a topic of evaluation and advice for the environmental assessment area of DEWHA.

OCS conclusions

OCS concluded in its report of June 2006 (resubmitted in April 2007) that the

(i.e. *in vivo* studies in whole animals, looking at effects on the whole organism). Nonetheless, the APVMA has tasked the OCS to re-examine this concern.

following confounded these findings:

- It is possible that other contaminants caused the effects in frogs, as chemical profiles of these sites were not determined.
- There is no validated test method for determining the endocrine effects of atrazine in non-mammalian species.

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*. (2002 a & b) may impact on the environmental assessment of atrazine, they are unlikely to have any direct relevance to human health.

In support of these conclusions the OCS notes:

- No impairment was found in the reproductive performance in either male or female rats (administered atrazine orally up to 30 mg/kg bw/d);
- There is no direct evidence to support the assertion that triazine herbicides cause developmental effects in humans.
- Furthermore, animal studies investigating the developmental effects of atrazine in drinking water at doses up to 0.05 mg/L, in combination with other pesticides, revealed no signs of developmental toxicity (as described in the OCS December 1996 assessment).

OCS conclusions were based on the weight of evidence on the relevance of frog data to mammalian toxicology and human health; issues in regard to effects on frog populations should be addressed by DEWHA.

2.5 CARCINOGENIC POTENTIAL

Rodents: mammary and pituitary tumours in rats

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. No new data were submitted for this supplementary assessment. The following is a summary of previous considerations in order to address the concerns raised by the CCC for discussion at the forum.

The carcinogenic potential of atrazine has been investigated in a number of studies on different strains of rats and mice. The only tumourigenic response is the formation of mammary tumours, limited to female Sprague-Dawley (SD) rats. Several mechanistic studies indicate that the MOA is unique to the female SD rat. Therefore, occurrence of mammary tumours in SD rats is not relevant to humans as the MOA is different. Differences in susceptibility to chemical induced alteration in hormonal balance may account for the presence or absence of tumours in certain strains. Atrazine when administered at doses at or above the maximum tolerated dose (MTD) significantly increased the incidence and onset of mammary tumours in SD rats but not in F-344 rats. The observed increase in atrazine induced mammary tumourigenesis at the MTD in SD rats was associated with a lengthening of the estradiol cycle and an elevation in endogenous plasma prolactin and estradiol levels correlated with the early appearance of mammary neoplasms in this strain. This did not occur in F-344 rats.

Disruption of cycle with prolonged periods of proestrus/oestrus may be an indication of future mammary gland stimulation and mammary tumour development caused by an imbalance of endogenous reproductive hormones. The important question arising from differences in strain responsiveness to chemical induced mammary carcinoma in particular if the neoplasm is hormone related, is which observation is reflective of changes in humans. It is to be noted that the incidence of mammary tumours with ageing is common in SD and F-344 rats but the type of neoplasm is different. In the case of the SD rats the tumour incidence was approximately 40 per cent with 22 fibroadenomas and 21 adenocarcinomas. The incidence of mammary tumours in F-344 rats was predominately fibroadenomas and overall rate also was lower (23 per cent). Physiologically the endocrine changes in ageing SD and F-344 rats are markedly different and may account for the enhanced susceptibility to mammary carcinogenesis in SD rats. The physiological alterations noted in SD rats such as low progesterone level resulting in unopposed oestrogens are not similar to those in humans. Potential risk to humans is significant when mammary carcinoma occurs in a strain with a spontaneous low incidence of these tumours (F-344 strain).

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 per cent historical control incidence for mammary tumours in SD rats and a 17.9 per cent 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 (5 February 1997). The ACPH considered that the benign mammary tumours observed in SD rats were not relevant for a human health risk assessment.

In relation to mammary tumours associated with atrazine exposure in female SD rats, Meek *et al*. (2003) postulated that atrazine suppresses the release of luteinizing hormone (LH) from the pituitary gland such that there is insufficient level of LH to trigger ovulation. When ovulation fails, follicles within the ovum continue to produce oestrogen. Repetitive failure of ovulation leads to prolonged exposure to endogenous oestrogen and/or prolactin creating a state of persistent oestrus. Over a sufficiently long period of time, this would translate into a hastened aging process and the development of mammary fibroadenoma/carcinoma. Thus, in SD female rats, reproductive aging is characterised by persistent hyperoestrogenemia and hyperprolactinemia with low levels of LH and follicle-stimulating hormone (FSH). In contrast, reproductive aging in women is characterised by exhaustion of ovarian follicles resulting in low levels of oestrogen and prolactin. LH and FSH levels in postmenopausal women remain high. The article concluded that on the basis differences in reproductive physiology, it would appear that the atrazine response in SD rats is not relevant to humans.

The US EPA noted in their report on cumulative risk assessment of triazines that, even though the evidence showed that a common mechanism could be used to group the triazines for the development of mammary tumours, it was recommended that this endpoint was not relevant to humans (cited in US EPA 2006). This was based on similar considerations as those outlined above. Though hypothalamic disruption of pituitary function (attenuation of LH surge) and resulting oestrus cycle disruption may occur in humans following exposure to atrazine, the hormonal environment would be different from that observed in rats. The prolonged/increased exposure to oestrogen and prolactin observed in the rat would not be expected to occur in humans. It is the prolonged/increased exposure to oestrogen and prolactin in the rat that is the basis of early-onset and increased mammary tumours in susceptible strains of rats. In addition, atrazine has been shown not to be mutagenic. Therefore, atrazine is 'not likely to be carcinogenic in humans' as previously stated.

Epidemiological studies in humans.

The National Toxics Network Inc. claimed that 'there was an association between atrazine exposure and increased incidence of various cancers'. This assertion was based on four published studies (Mill 1998; van Leeuwen 1999; Scammell 2005, MacLennon *et. al.* 2002). In general, these studies did not support a causal link between atrazine and cancer due to the lack of exposure data. The International Agency for Research on Cancer (IARC) and the US EPA have assessed the epidemiology data and concluded that the human epidemiology database did not provide sufficient evidence to associate atrazine with cancer of any human tissue. Consequently, the IARC downgraded the classification from 'possibly causing cancer in humans' (Group 2B) to 'unlikely to cause cancer in humans' (Group 3) in 1999. The APVMA review of all available atrazine data initially published in the 1997 review report and updated in the 2002 and 2004 reports, and the US EPA review in 2000 independently arrive at the same conclusion as IARC's 1999 classification.

A similar conclusion was reached by the Canadian regulatory agency in 2004. The Pest Management Regulatory Agency noted that data from animal cancer studies in conjunction with atrazine's MOA are not consistent with those cancers that are purported by some epidemiological studies to be associated with atrazine. The Canadian agency concluded, 'in the absence of any dose-response evidence for atrazine-related carcinogenicity in humans, there is little evidence to support atrazine-induced carcinogenicity in humans' (PMRA 2004).

OCS conclusions

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. The US EPA and the OCS each independently concluded that the proposed mechanism of mammary tumour formation in SD rats, although likely to be associated with hormonal (endocrine) perturbation, is not relevant to humans. This was supported by lack of effects in F344 rats, which exhibit a similar oestrous cycle to humans.

The US EPA and IARC have assessed the human epidemiology database (including Mill 1998; Van Leewen 1999; Syngenta/NRDC 2003) and concluded that 'in general, these studies did not support a causal link between atrazine and cancer at any tissue site. Further epidemiology studies by Dr M Scammell, February 2005 (in relation to the alleged increase in cancer of reproductive organs in St Helens in Tasmania) and MacLennan *et al*. 2002 (prostate cancer incidence among triazine herbicide manufacturing workers), were assessed by the OCS in the June 2006 review (resubmitted in April 2007, entitled *A review of supplementary data relating to the carcinogenicity, developmental effects and endocrine-disrupting potential of atrazine*), as providing insufficient evidence of a causal relationship with atrazine exposure. Thus, the published epidemiological data provide no support for any carcinogenicity potential of atrazine.

It is noted however, that a number of new data studies provided and others in the literature raise issues of multiple MOAs for atrazine and linkage to endocrine effects including cancer end points. The OCS June 2006 advice has fully examined the cancer MOA for the rat mammary tumours via changes in luteinizing hormone and the relevance of these findings to humans. However, in light of the more recent data on atrazine-induced changes to aromatase expression the OCS has been tasked by the APVMA to undertake a focused re-examination of the cancer MOA using the newly developed World Health Organisation/International Program on Chemical Safety (WHO/IPCS) Conceptual Framework for MOA and Human Relevance (Boobis *et. al.* 2006)[10].

2.6 WATER CONTAMINATION ISSUES

Levels in water

Atrazine can be extracted from water using a non-polar solvent or by solid phase extraction and analysed with gas chromatography using nitrogen-phosphorus detection. The limit of detection is approximately 0.0001 mg/L.

If present in drinking water, atrazine would not be a health concern unless the concentration exceeded 0.04 mg/L (level revised from 0.02 mg/L, a safety factor of two was taken into consideration for the likely presence of metabolites of atrazine which have a similar toxicity profile to parent atrazine). However, the

[10] APVMA note: The full JMPR (2007) monograph (expected to be published in 2008) will contain an analysis of atrazine's cancer MOA using the WHO/IPCS framework.

WHO guideline value is 0.002 mg/L determined using an additional safety factor of 10 for potential oncogenicity. This should be considered in Australia also. It should be noted that the permissible levels of atrazine in water are 60 times lower (3 mg/L) than in Australia and in the EU the level is 0.1 mg/L (0.0001 mg/L).

Bleaney A (2007) Upper catchment issues in Tasmania

This article reports on findings of recent investigations into pesticide usage conducted by a community group in Tasmania, the Break O'Day Catchment Risk Group, formed in 2004 to 'bring local knowledge and expertise into the assessment and management of risks in the main catchments of the Break O'Day municipality' (Bleaney 2007). This was in response to a helicopter crash 250 metres uphill from the South George River in December 2003. The helicopter was carrying the insecticide alpha-cypermethrin, a synthetic pyrethroid insecticide used to control beetles and other insects in plantation forests. Four weeks after the crash, in January 2004, there was a major flood in the area.

The article states that 16 weeks after the crash and 11 weeks after the flood, an investigation conducted by the Department of Primary Industries, Water and Environment (DPIWE) found other pesticides, such as atrazine, simazine, terbacil and chlorothalonil, in significant amounts at the crash site. It was noted that there was a 90 per cent mortality of intertidal oysters and other species approximately five kilometres downstream from the St Helens drinking water intake. Among the many postulated causes, the high rate of oyster mortality was also attributed to pesticides 'washed down the George River by the flood'. The group postulated that pesticides 'recently sprayed in the catchment and those that had been deposited on the ground at the time of the crash may have washed with topsoil into the river' and into the oyster farming operations. It was hypothesised that 'oysters, being filter feeders, ingested pesticide, which were adsorbed onto suspended soil particles and other detritus'.

In carrying out its investigations, the Catchment Risk Group obtained information by data gathering and analysis from senior government officials (ministers and bureaucrats) and local government officials (presented in Part 1 of the article); from historical information relating to water issues and pesticide usage (Part 2); and from community-based testing as well as findings from commissioned reports, together with data from the literature (Part 3).

This assessment will concentrate on Part 3 (community-based testing and literature review), particularly the toxicological considerations of pesticides. One of the main concerns of this article relates to the perceived inability of testing regimes (analytical and toxicological) to deal with the complexity of chemical interactions and changes occurring in aquatic systems. It was felt that there is a lack of understanding on the part of authorities in Tasmania as to the toxic impact of many of the pesticides and chemicals used in catchments.

The article briefly dealt with a possible mechanism of pesticide transfer through riverine systems; mainly pyrethroid interactions with aquatic sediments resulting in long half-lives of up to 163 days. As a result, detecting pyrethroids in waters may be of limited value, as the chemical would be adsorbed into the sediments, suggesting that searching for the pesticide in the waters inhabited by the oysters (which are filter feeders) would be fruitless. It was suggested that sediment analysis should have been conducted to identify the 'toxins' contributing to oyster deaths.

A brief summary of risks posed by pesticides concentrated on the potential for immunotoxicity, endocrine disruption and epigenetic changes.

Immunotoxicity

It was stated that pesticides, such as pyrethroids and atrazine, can cause immune system dysfunction, prompting the suggestion that immunosuppression could play a role on the effects of pesticides on native Australian wildlife, for example fungal infection affecting the platypus population and the aggressive facial tumours affecting the Tasmanian devil population.

No specific details on any of the cited literature were given, making it difficult to assess the studies.

Endocrine disruption

It was acknowledge that interest in the role of pesticides in endocrine disruption continues to be an area of active research. Reference was made to the paper by Hayes *et al*. (2002), describing the potential for atrazine to castrate and feminise male amphibians at extremely low (0.1 to 1ppb) levels (see above). No assessment of the paper was conducted. In addition, the group highlighted the potential link of atrazine to prostate and breast cancers, which has been assessed above (Section 1 of Volume 2).

Epigenetic changes

The article also briefly touched on the issue of pesticides possessing the ability to alter gene expressions, including many genes shown to be involved in causing human disease. Again, there was no analysis of any of the studies cited in the references; but the article acknowledged that evidence has only been shown in experimental animals and no definitive proof exists that 'pesticides are the ultimate cause of disease in humans'.

Other issues discussed

The article made mention of the fact that effects of chemical mixtures are not studied in great detail, notwithstanding that chemicals are nearly always 'encountered in real-life as mixtures'. In particular, concerns were raised about the potential for synergistic effects of chemical combinations on the oysters, which was not considered in any investigations on the causes of oyster mortality.

In addition, the article expressed concern that there is a lack of evaluation of the toxicity of pesticide products (pesticide plus additives and contaminants), 'as the toxicity of the tested active pesticide is often less and very different from that of the retail product'. The role of degradation products and/or metabolites in relation to the oyster deaths was not investigated by the DPIWE.

OCS conclusions

In general, the article served to highlight concerns raised about the causes of the high rate of oyster mortality in oyster farming areas adjacent to the George River in the Break O'Day municipality of Tasmania. No specific data were submitted for a definitive conclusion as to whether atrazine was responsible for the deaths observed. Therefore, the OCS cannot comment.

With respect to the perceived deficiencies of regulatory agencies in assessing or evaluating the public health aspects of pesticides, the OCS reaffirms that the regulatory process is robust and rigorous and does take into account effects of degradation products and/or metabolites, before recommending approval of any chemical or pesticide.

Noting that this paper raises three potential MOAs for atrazine to have adverse effects on human health, the OCS has been tasked by the APVMA to re-examine each of these MOAs against the existing database and available information. Specifically, the potential for immunotoxicity, endocrine disruption and epigenetic changes will be examined using the new WHO/IPCS Conceptual Framework for MOA and Human Relevance. In doing so, the OCS can provide additional information on the weight of evidence considerations for the postulated MOAs and scientific judgements involved in human health risk assessment. Further, this approach will identify any data gaps, uncertainties and inconsistencies in establishing MOA.

2.7 CUMULATIVE ASSESSMENT OF MIXTURES

Cumulative risk assessment of the triazine group of chemicals

With respect to assessing chemical mixtures, the OCS is currently (in 2008) participating in an international harmonisation project to develop methodologies for the cumulative and aggregate risk assessment of chemical mixtures. The OCS is therefore cognizant of the worldwide interest in this area and acknowledges that effects of chemical mixtures are being included in the overall risk assessment methodologies.

In establishing whether a cumulative risk assessment is warranted for a group of similarly acting compounds, the OCS should be satisfied that exposure is likely to occur. It was noted in the December 1996 OCS assessment that atrazine was used in high volumes, predominantly as a herbicide in preparation for plantings of coarse grains and sugarcane, with minor uses in forestry and legumes.

The 1992 Australian Market Basket Survey (AMBS) (National Food Authority, Australian Government Publishing Service) conducted assays for atrazine and simazine in meat and cereal foods. Because of their use pattern (just before or after crop emergence) it was considered unlikely that residues would be present in food. In addition, no residues of either herbicide were detected. This finding was in agreement with US data; in over 30 years of use, atrazine had not been detected in edible portions of plants or livestock nor had it been detected in market basket surveys. Thus it was concluded that exposure of the population to atrazine in food is very unlikely.

However, the fact that atrazine is both mobile in the soil and reasonably stable in the environment indicates that non-occupational exposure to atrazine, if it occurs, is likely to occur through contamination of drinking water. Indeed it was noted in the 1997 report that consideration should be given to amending the Australian Drinking Water Guidelines to include the four metabolites with parent atrazine in the definition of atrazine; this action would have the equivalent effect of lowering the guideline value (0.0005 mg/L) for atrazine alone since, in water samples in which atrazine is detected, one or more metabolites are commonly detected, but that had been disregarded in the existing Standard. This issue was also referred to the Advisory Committee on Pesticides and Health (ACPH). Recognising the need to take into account toxicologically significant metabolites from an exposure risk assessment perspective, the ACPH supported the modification of the atrazine guideline value in the Australian Drinking Water Guidelines (1996). They proposed that, rather than including all four metabolites (desethylatrazine, desisopropylatrazine, diaminochlorotriazine and hydroxyatrazine) as outlined above, only the atrazine-specific metabolites, desethylatrazine and hydroxyatrazine be included with atrazine in the definition for the guideline value. It was therefore recommended that it was appropriate that the issue of drinking water guidelines for atrazine be referred to the NMHRC and ARMCANZ for consideration by the joint committee responsible for updating the Australian Drinking Water Guidelines. A health based guideline of 0.04 mg/L was established. However it was noted that if atrazine is detected in the drinking water, then remedial action should be taken to stop the contamination; the practical limit of determination is 0.0001 mg/L. It was also recognised that the metabolites of atrazine (desethylatrazine, desisopropylatrazine, diaminochlorotriazine and hydroxyatrazine) may constitute approximately 50 per cent of the total atrazine-derived triazine compounds in some ground and surface water samples, and this was accounted for when the guideline value was derived, by the addition of an extra two-fold safety factor. Similarly, for simazine, a health based guideline value of 0.05 mg/L was established, with a guideline value of 0.0005 mg/L, above which remedial action should be taken to remove the source of contamination.

It may be further noted that atrazine has rarely been found in Australian reticulated water supplies. In groundwater it has been reported at concentrations of up to 0.002 mg/L in an area where atrazine was used to suppress weed growth in irrigation channels for 10 years (NHMRC 1996). It was furthermore concluded that all uses that contributed to the total environmental load of atrazine such as atrazine products applied to lawn, golf courses, irrigation channels, drains, roadsides, industrial premises and non-agricultural areas could not be maintained.

ATSDR interaction profile of triazine mixtures

A media report from Pesticides & Toxic Chemical News by McMenamin (2006) made reference to a recently finalised review of an interaction profile of triazine mixtures with nitrates and diazinon conducted by the Agency for Toxic Substances and Disease Registry (ATSDR 2006). This review is not specific to atrazine; however, atrazine and its degradation product, desethylatrazine, were included in the triazine mixtures. Cancer risk due to the combinations of nitrates and triazines (including atrazine) and the possible potentiation of diazinon’s neurological effects by triazines were the main concerns of the review.

ATSDR reported that no toxicological data or physiologically based pharmacokinetic models were available for the complete mixture. Therefore, the document is an exposure-based screening assessment of potential health hazards for the mixture, which depended on an evaluation of the health effects and mechanistic data for the individual components and on the joint toxic action (additivity and interaction) and mechanistic data for the various combinations of the components. The profile assesses the evidence for joint toxic action among atrazine, its degradation product, simazine, diazinon and nitrate. The report also discussed how public health assessments could incorporate concerns about interactions, additivity and potential human exposure to mixtures of these chemicals (ATSDR 2006).

The toxic endpoints of concern for the mixture are reproductive, neurological, haematological and carcinogenic. With respect to atrazine's presence in the mixture, effects of concern included reproductive effects. Although atrazine is not known to be a carcinogen, the ATSDR interaction profile states that it can react with nitrite (nitrate metabolite) in the environment and *in vivo* to form N-nitrosoatrazine, which on structure-activity considerations raises concerns for potential carcinogenicity. This has been disputed, on the grounds that the formation of N-nitrosoatrazine in the environment is highly unlikely and does not occur at neutral pH found in soil and water (McMenamin 2006; Syngenta 2005). In addition, N-nitrosoatrazine possesses a short photolytic half-life precluding any interaction with target organisms, should the compound form. The company states that no N-nitrosoatrazine 'has been confirmed as present in environmental samples over a 50-year triazine use'. The formation of N-nitrosoatrazine in the stomach has also not been demonstrated, even at high doses of nitrate and atrazine. However, the document concluded that the potential risk in humans remains unresolved, requiring further research (ATSDR 2006).

The model used to assess the mixture of the triazines, diazinon and nitrates for potential human hazards was the hazard index, where hazard quotients (ratios of exposures to health guidance values) were estimated for the individual components. To screen the mixtures for potential reproductive health hazard, an endpoint specific hazard index for reproductive effects needs to be estimated for the triazines present in the mixture. Additivity assumptions are the basis for the hazard index.

The triazines present in the interaction profile mixture (atrazine, simazine and desethylatrazine) are considered by the US EPA as belonging to the common mechanism group, based on their common mechanism of toxicity (US EPA 2002). They suppress/attenuate the luteinizing hormone ovulatory surge and the resulting effects on reproductive function and reproductive development. They are not oestrogenic. Their mechanism of reproductive toxicity involves neuroendocrine disruption of hypothalamic-pituitary gonadal function in rats. In March 2006, the US EPA released a report on the cumulative risk from triazine pesticides (US EPA 2006).

The authors concluded, with a high degree of confidence, that the triazines have additive effects on reproduction via their suppression of the ovulatory surge of luteinizing hormone. They were not as confident that triazines potentiate the neurological effects of diazinon. Atrazine levels in the studies were 40 to 200 parts per billion (ppb) although the maximum contaminant level is 3 ppb.

The purpose of the US EPA review was to guide environmental health scientists addressing concerns about potential hazards due to additivity and interactions among contaminants at hazardous waste sites or in other situations. The extent to which nitrates and triazines interact to form more genotoxic compounds and their effects were considered. The mixtures were chosen because the pesticides and nitrates are the most commonly found chemicals in untreated groundwater samples taken during the National Water Quality Assessment Program of the US Geological Survey. Concerns were expressed that millions of Americans in the Corn Belt could be exposed to the endocrine effects of atrazine in drinking water. Critics of the release of the profiles by ATSDR have questioned its motives when the US EPA is close to finalising its 10-year review of atrazine.

Mixtures of atrazine and diazinon were found at seven sites while mixtures of atrazine and nitrate were found at 14 sites. In three instances, exposure involved two or more components of the mixture addressed in the ASTDR report (McMenamin 2006). Concerns have been raised about the ATSDR report (McMenamin 2006; Syngenta 2005) and a request has been made by Syngenta to withdraw the interaction profile for atrazine, desethylatrazine, diazinon, nitrate and simazine from ATSDR's chemical interaction register.

The paper by McMenamin (2006) on the ATSDR review indicates that in addition to 'additive effects' of triazine compounds, certain compounds may potentiate certain effects of pesticides, which include:

- neurological effects of the organophosphate diazinon; and
- genotoxicity of nitrate and triazine interactions (formation of nitroso compounds).

Although the OCS has not reviewed the ATSDR report, it is noted that certain criticisms of the review have been made by US EPA Office of Pesticide Programs (US EPA 2006), in particular that certain 'interaction profiles' were based on:

- *in vitro* studies;
- non-mammalian *in vivo* toxicity tests; and
- studies at concentrations which were considerably higher than encountered in environmental media (i.e. drinking water).

With respect to the potentiating effect of atrazine on the toxicity of diazinon, the US EPA noted that none of the cited studies were based on mammalian studies. The studies were conducted using midge larvae, the common housefly and a small shrimp-like amphipod at concentrations 13 to 67 times those relevant for drinking water sources. Thus, the potentiating effects of atrazine on the toxicity of diazinon were not demonstrated at concentrations relevant to humans (US EPA 2006a). Similarly, the potential for formation of N-nitrosoatrazine following chemical interactions between atrazine and nitrite was not convincing in the ATSDR profile (ATSDR 2006). Again, with the *in vivo* gavage studies in mice, the doses of atrazine used were high, exceeding the doses expected in humans through drinking water. In addition, none of the studies investigating the joint action of atrazine and nitrate on northern leopard frog and newt larvae are relevant to humans.

The US EPA (2006) critique cited a carcinogenicity study performed by Weisenburger (1988) in both mice and rats that may be more relevant than the studies cited in the ATSDR report. That study concluded that atrazine and N-nitrosoatrazine were not carcinogenic in either rodent species. The OCS believes this data to be more relevant to human exposure than the studies cited in the ATSDR report (although this study was only published as an abstract).

It appears that the US EPA is currently developing a cumulative risk assessment for triazine herbicides, to account for additive effects (from other triazines) on reproductive function and development (US EPA 2006).

OCS conclusions:

The OCS acknowledged (in its 2007 response to public submissions, see section 1, volume 2) that there is a theoretical basis for consideration of a risk assessment of atrazine as part of a group of triazine pesticides based on a common MOA (suppression of luteinizing hormone) and recommended that a factor for 'additive effects' be incorporated into the drinking water guidelines.[11]

The OCS would need to comprehensively review the ATSDR report to determine whether there are any data which could be used to refine the cumulative risk assessment of atrazine.

Therefore, while acknowledging there is a basis for consideration of a risk assessment of atrazine as part of a group of triazine pesticides based on a common MOA, current exposure to atrazine and simazine in food is very unlikely, while available data suggest that exposure to atrazine, simazine and propazine in reticulated drinking water is likely to be negligible. Considering the negligible exposure to the major triazine compounds available in Australia, a cumulative risk assessment of these compounds on public health grounds is not warranted at present. Nevertheless, the utility of both aggregate and cumulative risk assessment methodology for the assessment of risks posed by agricultural and veterinary chemicals to public health is under consideration and could be undertaken in conjunction with an OCS assessment of MOA issues.

2.8 POTENTIAL LINK BETWEEN MYALGIC ENCEPHALOMYOPATHY AND ATRAZINE EXPOSURE

Downs (2006) Crop spraying: Could pesticide exposure have caused your M.E?

Note: ME refers to myalgic encephalomyopathy, also known as chronic fatigue syndrome (CFS).

This refers to a medical feature article that appeared in the June 2006 edition of a British journal *InterAction*, written by Georgina Downs who runs the UK Pesticides Campaign to highlight adverse health and environmental effects of pesticides. Ms Downs apparently lived for 22 years adjacent to fields that were sprayed with

[11] APVMA note: A factor has been included in the current Health drinking water guideline value to take into account the contribution of atrazine's main metabolites.

pesticides and has long-standing health problems. Her campaign aims to show the inadequacies in the risk assessment of pesticides by UK agencies, particularly with respect to rural areas. She has presented material to the UK Advisory Committee on Pesticides (ACP).

Ms Downs' activities resulted in the Royal Commission on Environmental Pollution carrying out a study to re-examine the evidence regarding the risks to people from pesticides in 2004. According to the article, the Royal Commission concluded 'that crop spraying is a potential health risk and that chronic illnesses and diseases reported by people in rural areas, including cancer, Parkinson's, ME and Multiple Chemical Sensitivity (MCS) could be associated with pesticide exposure'. The Royal Commission's report was apparently also critical of the UK ACP and the pesticide regulator, the Pesticides Safety Directorate. It recommended a need for a 'no spray zone' of five metres width between sprayed fields and residential property. Ms Downs was critical of this distance, suggesting it should have been wider as pesticides can travel over vast distances. The author claims that the ACP 'hit back' at the criticisms of the Royal Commission. The OCS has not seen this report and, therefore, cannot comment on the report.

The main thrust of the article, however, relates to a potential link between ME and exposure to pesticides. Ms Downs states that the UK ACP has concluded that chemical toxicity does not play a role in ME but instead could 'represent a psychologically mediated response to a triggering event or exposure (e.g. a viral infection that causes acute fatigue, or perceived exposure to an environmental hazard) that is conditioned by cultural influences as well as by individual beliefs and mental health'.

The author reviewed three studies linking pesticides to ME (Fernandez-Soto *et al.* 2005; Kerr *et al.* 2005; Racciatti *et al.* 2001). Three case studies linking exposure to pesticides and ME were also reported in the article. The subjects were apparently diagnosed with ME after exposure to pesticides. However, Ms Downs does acknowledge that there is uncertainty over the exact nature and importance of the link between ME and pesticides, even though there is a growing body of evidence linking pesticides to various neurological and neuro-degenerative diseases, as many pesticides are toxic to the nervous system.

The author is also critical of the use of a predictive 'bystander' model to assess the risk to public health from crop spraying. It is pointed out that the model assumes there will only be occasional, short-term exposure from spray drift at the time of application of the pesticide. As such, it was stated that this model is inadequate to address long-term exposure, particularly to mixtures of chemicals over long periods of time. The issue of assessment of exposure to chemical mixtures is discussed in section 2.7.

It is rightly pointed out that exposure to pesticides will involve transmission by air, water, contaminated surfaces and food, amongst other ways. The routes of exposure involve inhalation (through the lungs), dermal absorption (through the skin), orally (through ingestion) and through the eyes. Once exposure occurs, the chemicals can potentially enter the bloodstream and be carried throughout the body.

2.9 EFFECT ON CHILDREN'S HEALTH

McMenamin (2007) Toxicity & exposure assessment for children's health

A recent media communication by McMenamin (2007) alerted scientists to the latest update on exposure assessment of the effects of atrazine on children by the US EPA (US EPA 2007). The latter report summarises the implications of possible endocrine disruption for children's health, which may be related to effects during pregnancy and sexual development, increased risks for preterm delivery and intrauterine growth retardation associated with atrazine exposure. Exposure of children can occur from ingestion of contaminated drinking water, from dermal contact or ingestion following agricultural and lawn applications.

The US EPA report (2007) is a chemical summary of atrazine, which draws on information derived from the US EPA and ATSDR resources as well as the Technology Education and Copyright Harmonization (TEACH) database. The database contains summaries of research studies relating to developmental exposure and/or health effects for atrazine and other chemicals. None of the studies listed in this database have been evaluated by TEACH. The following is therefore a modified extract of the unevaluated summaries in the TEACH database that were incorporated in the US EPA report (US EPA 2007):

Most of the studies cited originated in the USA, except for a report from New Zealand stating that atrazine was not detected in a New Zealand survey of infant formula and infant weaning foods, in contrast to a US study which detected very low levels of atrazine in a variety of foods. Little information is available regarding the effects of atrazine in children. Five studies were summarised. Maternal exposure to atrazine in drinking water was associated with low foetal weight and with heart, urinary, and limb defects in humans. Atrazine has been shown to slow down the development of foetuses in animals, and exposure to high levels of atrazine during pregnancy caused reduced survival of foetuses. It is unclear whether or at what level of exposure this might occur in humans. It is not known whether atrazine or its metabolites can be transferred from a pregnant mother to a developing foetus through the placenta or from a nursing mother to her offspring through breast milk.

2.10 OTHER ISSUES

Kilburn KH (2006) Why is chemical brain injury ignored? Pondering causes and risks

This is a general article not specific to atrazine. The article talks about various chemicals and possible brain injury such as disturbances of brain function by mercury in mirror silverers, and palsy and psychosis caused by lead.

Weiner et al. (2007) Atrazine-induced species-specific alterations in the subcellular content of microalgal cells

This publication was highlighted by the CCC and was included in the material sent to the OCS for information only. The paper dealt with the effect of atrazine on inorganic carbon incorporation into different macromolecules, such as proteins, polysaccharides and lipids, following biochemical fractionation of the end products of photosynthesis. Five microalgal test species were chosen for toxicity testing of atrazine. This study was not relevant to the OCS but applicable to environmental testing. The contents of the publication were noted but not evaluated.

2.11 NOEL FOR ADI

The OCS completed its Review of the Mammalian Toxicology and Metabolism-Toxicokinetics of Atrazine in December 1996 (included in the APVMA's 1997 atrazine report). In its report the OCS evaluated a number of new data submissions on the toxicology of atrazine in addition to all previously submitted data. No change to the NOEL for atrazine was warranted. The NOEL of 0.5 mg/kg bw/d (10 ppm) was established by the OCS in November 1990, derived from a two-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 neuroendocrine effect. This was considered to be an appropriately conservative endpoint for establishing the ADI, at 0.005 mg/kg bw/d and using a safety factor of 100. Following the December 1996 review and the 2004 review, the NOEL for the ADI remained unchanged.

2.12 INTERNATIONAL ACTIVITIES

The US EPA concluded in 2000 that atrazine is an endocrine disruptor. In a December 2007 status update, the US EPA concluded that atrazine does not adversely affect amphibian gonadal development based on a thorough review of 19 laboratory and field studies, including studies submitted by the principal registrant (Syngenta) and other studies in the public literature. Except for the studies conducted by Hayes (2002a&b), the OCS has not cited or reviewed the other studies. To ensure the quality and transparency of its assessment of atrazine's potential to affect amphibian gonadal development, the US EPA sought advice for the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) at a public peer review meeting on October 9–11 2007. The SAP's final report and recommendations were expected to be made publicly available in January 2008. The US EPA believes that there is no compelling reason to pursue additional testing of atrazine for amphibian gonadal effects.

Based on available scientific studies, the US EPA believes that atrazine is not likely to cause cancer in humans. According to a December 2007 status update, the US EPA is currently reviewing three epidemiological cancer studies for atrazine. The US EPA expects to receive two additional epidemiological studies and analyses concerning atrazine and cancer from the National Cancer Institute's (NCI's) Agricultural Health Study during 2008. After receiving and reviewing the two pending studies, the US EPA plans to convene another FIFRA SAP meeting on atrazine and its possible association with carcinogenic effects. The US EPA will present its assessment of all available data on the potential carcinogenicity of atrazine, both epidemiological studies and laboratory animal studies, and will reconsider whether the determination that atrazine is not likely to cause cancer in humans should be revisited. The US EPA's January 2003 Atrazine IRED did not include a quantitative risk assessment for cancer due to a determination that it is unlikely that atrazine's cancer MOA in the rat is operative in humans. Results of recent epidemiological studies regarding atrazine's potential link to prostate cancer taken to the SAP in July 2003 did not alter that conclusion. Accordingly the US EPA concluded in its October 2003 revised IRED that atrazine is not likely to be a human carcinogen.

In 2002, atrazine was included in the global program Regionally Based Assessment of Persistent Toxic Substances as atrazine residues were detected in many sectors of the environment with the potential for regional transport (United Nations Environment Programme Chemicals 2002).

2.13 OCS CONCLUSIONS

The OCS has provided commentary regarding the potential impact on human health outlined in the studies discussed above. The OCS notes that label changes proposed will provide further protection to human health, in addition to existing health standards for atrazine; therefore implementation of the 2004 review outcomes is supported. Nevertheless, there is no doubt that since the toxicological review has been completed there are new postulated MOAs for atrazine that warrant investigation.

Therefore, the APVMA has initiated a project to re-examine the possibility that the triazines (atrazine and related chemicals with a similar MOA) may have unintended harmful effects on humans, taking into account ongoing research into a newly hypothesised endocrine MOA. This project will take into account international reports, such as the work of the Joint Meeting on Pesticide Residues (JMPR).

The APVMA will consider initiating a new review if further data provide credible evidence of a previously uncharacterised risk.

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3. ADDITIONAL ENVIRONMENTAL ASSESSMENT (DEWHA, FEBRUARY 2008)

The following DEWHA report was originally provided to the APVMA in mid 2006. It was prepared in response to public comments on the 2004 atrazine report. The DEWHA updated the report in February 2008 following consideration of a further submission from Southern Farming Systems in relation to the use of atrazine post-emergence on TT canola grown on raised beds.

3.1 TANK MIXTURES

One submission from an Honorary Research Associate at the University of Sydney raised a concern regarding tank mixes of simazine and atrazine. The submission details concerns that these herbicides have similar MOAs, and therefore their combined effects would be additive. If additive, effective concentrations in the environment may be the combined amount of the two herbicides, which has not been evaluated in the review.

The DEWHA is aware of this issue. The US EPA is currently evaluating the human health issue of common MOAs for all the triazine herbicides ('The grouping of a series of triazine pesticides based on a common mechanism of toxicity'). However, due to the extremely complex and intensive nature of such an assessment, results are not expected for several years to come. While both the DEWHA and the APVMA acknowledge that the effects of simazine and atrazine are likely to be additive, consideration of such effects is not within the scope of this particular review, which is restricted to atrazine in isolation.

A joint submission received from the Allergy, Sensitivity and Environmental Health Association Qld Inc (ASEHA Qld) and the Australian Chemical Trauma Alliance (ACTA) highlighted a study by Porter et al. (1999) which investigated the effects of aldicarb, atrazine and nitrate on mice. It appears from the submission that ASEHA/ACTA are concerned about harmful effects of chemical mixtures.

The DEWHA has acknowledged that combinations of chemicals can be sprayed. However, as discussed above, such issues are not within the scope of the review of atrazine and may instead be considered in another forum.

3.2 LABEL RESTRAINTS – FORESTRY

Forests NSW commented that roads and tracks are currently oversprayed when atrazine is applied aerially, and therefore the new label instruction requiring that the product must not be applied to roads will greatly increase the costs of plantation establishment. Forests NSW is of the view that roads are drained adequately, although it is not possible to ensure no runoff. Forests NSW has suggested that the restriction may be more relevant to wetter states such as Queensland, and suggested alternative wording for the label, to:

> *Do NOT handle, mix or conduct testing operations to areas susceptible to run-off where drainage results in rapid entry into waterways, unless specific action has been taken to prevent run-off into waterways. These*

> *areas may include roads, access tracks, snig tracks and compacted log dumps.*
>
> *Do not apply to areas mounded perpendicular to the contour unless specific action has been taken to prevent runoff into waterways.*

A submission from Nufarm made essentially the same comment and suggestion in regard to mounding perpendicular to the contour. In addition, the submission from Tree Plantations Australia requested that this label instruction be amended similarly (although they retained the word 'apply').

The DEWHA has assessed these comments and confirms that the intent of the statement was to minimise runoff of atrazine into waterways. However, DEWHA is concerned that although Forests NSW are of the view that roads are drained adequately, the crucial point at issue is, 'Where are the roads drained to?' In addition, monitoring results from the Barossa indicate that draining perpendicular to the contour greatly increases runoff. If effective measures can be taken to minimise runoff of atrazine then the DEWHA is agreeable to modifying the statement along the following lines:

> *Do NOT handle, mix, apply or conduct testing operations in areas susceptible to run-off where drainage results in rapid entry into waterways, particularly where no specific and effective action has been taken to prevent run-off into waterways. These areas may include areas mounded perpendicular to the contour, roads, access tracks, snig tracks, and compacted log dumps.*

3.3 TRIGGER VALUES

A submission from Syngenta requested clarification on several points in the environmental assessment, including whether there are freshwater trigger values set for discharge points or mixing zones, whether these values are for a specific duration, and whether these values include both the parent atrazine and its degradates.

The Australian and New Zealand Guidelines for Fresh and Marine Water Quality are prepared under 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)[12]. These guidelines are for ambient water that will protect their designated values, and are of relevance when considering aquatic ecosystems. The freshwater moderate reliability trigger value for atrazine of 13 mg/L was derived using the statistical distribution method with 95 per cent protection and an acute to chronic ratio (ACR) of 20.2. Moderate reliability trigger values are derived from acute toxicity data. The guidelines state that 95 per cent protection level trigger values apply to ecosystems classified as slightly to moderately disturbed (noting that a higher protection level could be applied to slightly disturbed ecosystems where the goal is no change in biodiversity). ACR is

[12] APVMA note: The Natural Resource Management Ministerial Council (NRMMC) replaced ANZECC and ARMCANZ after 2001. The NRMMC and the Environment Protection and Heritage Council now jointly manage the NWQMS.

the species mean acute value divided by the chronic value for the same species.

The Australian Drinking Water Guidelines are developed and set by the National Health and Medical Research Council (NHMRC) in collaboration with the Natural Resource Management Ministerial Council (NRMMC). These guidelines are set in relation to human health. The APVMA is not involved in setting values for either these or the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. According to the 2004 Australian Drinking Water Guidelines, atrazine would not be a health concern unless the concentration exceeded 0.04 mg/L. This value takes into consideration atrazine metabolites, and includes an additional safety factor for this reason. The DEWHA also notes that the reasons for any differences between Australian and WHO guideline values are also discussed in the Australian Drinking Water Guidelines (see http://www7.health.gov.au/nhmrc/publications/synopses/eh19syn.htm).

The Guidelines prepared under the NWQMS advocate a risk-based approach to water quality management. In the absence of sufficient local data, default trigger values for ambient water quality are provided as a general guide to the water quality that is needed to protect the environment as well as human uses for waterways. Where default trigger values are exceeded, further investigation should be undertaken to determine environmental impacts and mitigation strategies, if needed. Ambient waters are specifically defined as excluding discharge points and mixing zones. In addition, the DEWHA highlights that it considers effects at the scale of populations and ecosystems. While populations of aquatic species are likely to recover from infrequent contamination at concentrations up to the trigger value over a day or two, any contamination at higher levels or for extended periods is likely to lead to significant environmental impacts.

3.4 RUNOFF

The Victorian Department of Primary Industries requested clarification on what would constitute an appropriate specific action to prevent runoff into waterways.

The DEWHA does not believe it is possible or appropriate to list all possible permutations of actions for all forestry, cropping and other situations on a label. The purpose of the label is to specify the outcome that must or must not occur. In this case, runoff of atrazine into waterways at unacceptable levels must not occur. In some situations, diversion of runoff into onsite dams may be effective. However, it may not be possible to hold water on a property, due to state government or other requirements. This may not necessarily lead to atrazine in runoff if sufficient time has elapsed since treatment for the atrazine to infiltrate and/or sorb to soil before any runoff occurs. However, if the user cannot prevent unacceptable runoff, then atrazine should not be used.

A submission from the National Toxics Network (NTN) listed several concerns. The NTN felt that narrow safety margins are not acceptable, given that increasing areas in Tasmania are under hardwood plantation, with commensurate increases in atrazine use. It was also remarked that there will be runoff from mild slopes and logging tracks after heavy rain that will not be prevented by proposed label restrictions.

Runoff is very dependent on soil types. For example, there is little runoff where soil has good infiltration capacity, while runoff may be higher where soils have a high clay content. Indeed, results indicate that there was relatively little runoff of water during Tasmanian monitoring. If atrazine has had sufficient time to infiltrate or sorb to soils, its presence in runoff will be reduced. The issue is whether atrazine in runoff causes effects on aquatic populations or ecosystems at the levels that are likely to occur. If concentrations do not exceed the freshwater trigger value, then the aquatic environment should be able to recover from brief exposure to low concentrations of atrazine.

3.5 RAISED BED CROPPING OF CANOLA

Respondents to the 2004 atrazine report raised concerns that the furrows between raised beds may be classified as a channel or a drain, and thus be subject to label restrictions. It was also noted that raised beds are normally mounded perpendicular to the contour (which the APVMA atrazine report of 2004 proposed to preclude on product labels as a review outcome).

Spraying atrazine across the furrows between raised beds within a paddock, which by their very nature are designed to move water, could lead to an increased load of atrazine reaching drains and waterways.

Raised bed cropping is used to overcome waterlogging and poor soil structure by mounding earth into raised beds interspersed with filter furrows. The beds run perpendicular to the contour usually on the steepest slope in a paddock and generally for no more than 400 metres. Raised bed cropping is now not recommended for slopes more than 1.5 per cent. Beds can range from around 80 cm to 1.5 m in width, but are more usually on the lower side of this range. Crop is sown in the beds and in the furrows across approximately 100,000 ha in southeastern Australia. The furrows usually end in a head or collector drain, which runs at right angles to the beds and which may also be sown. Ideally, these drains are engineered to move water away in order to prevent bogging of vehicles. Engineering controls include underground slotted pipes to the fence line, and filling with crushed rock to remove surface water.

The environmental assessment in the 1997 atrazine report discussed a study in which atrazine runoff after application to maize in Australia was modelled using GLEAMS (Groundwater Loading Effects from Agricultural Management Systems). This study simulated an application rate of 1.2 kg atrazine/ha (reflecting use of 3.0 kg/ha of the product Primextra which contains both atrazine and metolachlor). Almost all the atrazine leaving the field was dissolved in surface runoff. The maximum concentration leaving the field, based on an overland flow model, was 183 µg/L. Runoff losses were also increased by 180 per cent when atrazine was surface applied rather than incorporated into the top 5 cm of soil.

It is noted that atrazine is generally only used post-emergence on canola and therefore is surface applied. The DEWHA modelled the flow through irrigation furrows in comparison to conventional flat cropping and predicted increased sediment losses of 260 per cent and increased atrazine losses via sediment of 130 per cent. A 30 mm rainfall event on the day following application increased runoff losses by 340 per cent and caused 7.5 per cent of the applied atrazine to be lost from the field. After rain, a maximum concentration of 2,296 µg/L was predicted in runoff water after surface application of atrazine. The DEWHA concluded that this finding highlighted the importance of spraying only when heavy rainfall is not forecast within 48 hours.

A field study in the USA which measured atrazine runoff from a tile-drained farm (tile farming involves placement of pipes in the soil or subsoil to enhance or accelerate drainage of water from the soil profile) found a direct correlation between increased runoff flow rates and increased atrazine concentration off-field (Rothstein *et al*. 1996). In this experiment, 1.4 kg of atrazine was applied to 1 ha of a tile-drained field. A 20 mm rainfall event occurred six days after application, leading to atrazine concentrations of 34.5 µg/L at a tile line outlet feeding a stream. In this respect, tile-drained farms are not unlike the head drains in raised bed cropping systems. This study indicated that measures to transport water off-field may also increase the levels of atrazine moving off-field.

In other field studies conducted in the USA, measured loss of atrazine from corn fields following 30.5 mm rain falling five days after application ranged from 1.2 to 7.2 per cent (Sadeghi & Isensee 2001). In the following year, 18.5 mm of rain falling 25 days after atrazine application led to losses of 0.01 to 0.08 per cent of applied atrazine (Sadeghi & Isensee 2001). The application rate was 1.7 kg/ha. The same authors also conducted a laboratory study that simulated a higher rainfall event (48.6 mm over two hours) at five to six days after application, with measured atrazine losses in runoff of 6.7 to 22.7 per cent. The first 500 mL of runoff contained the highest atrazine concentration (Sadeghi & Isensee 2001), indicating that even if smaller quantities of water were to move off-field, they may still carry the bulk of the available residues of atrazine. Consequently, based on available measured data, and given that raised beds are designed to move water off-field, it may not be unreasonable to expect 5 per cent of applied atrazine to move off-field following rain within five days of application.

Although it has previously been recognised that irrigation channels or drains have the capacity to carry more water than the furrows between raised beds, it is nevertheless apparent that furrows that are designed to move water are likely to also increase losses of atrazine substantially, particularly when furrows are directly sprayed (they are directly sprayed because they are sown with crop[13]). It is not possible to directly model the expected environmental concentrations in catchments in Victoria or elsewhere because the variables of slope, proportion planted, width of furrows, effectiveness of any retaining structures, variability in soil types, distance to streams and drainage lines, and so on are unknown and too numerous to realistically determine. As well, runoff also depends on intensity of rainfall. Indeed, a study in Switzerland found that different field properties caused the relative losses of herbicides from fields in two subcatchments to vary by up to a factor of 56. This variability was predominantly caused by factors that affect the fraction of rain lost to surface water by fast transport mechanisms (Leu *et al*. 2004a). A companion field study in the same catchment determined that major losses of herbicides occurred during the first two rain events following application,

[13] APVMA note: Furrows are sprayed and sown with crop to help prevent the development of resistant weeds. TT canola grown in combination with atrazine used post-emergence is an important weed resistance management rotation crop.

with applied atrazine mobilised to runoff water (Leu *et al*. 2004b).

Nevertheless, a standard initial-level model can be calculated. For broad-acre crops such as canola, an initial-level model involves calculating runoff from a 10 ha field to a pond that is 15 cm deep and 1 ha in size (volume of pond = 1,500 m^3). If the maximum label application rates for TT canola of 4 L/ha pre-emergence, and 2 L/ha post-emergence are used, and a not-unrealistic rainfall event of 50 mm is also used, then the following calculations apply: 2 L/ha of product, containing 500 g/L atrazine, is applied to 10 ha of land, meaning that 10,000 g atrazine is applied in total over the 10 ha; 50 per cent of rainfall flows off the field into the pond carrying with it 5 per cent of the applied atrazine. The pond then contains a volume of 4,000 m^3 of water, and the concentration of atrazine in the pond would be 125 µg/L. Given that the first 500 mL of runoff contained the highest atrazine concentration in the study described above, if rainfall were less (say 30 mm instead of 50 mm), then the concentration may be even higher as it would be diluted in less water.

This estimated concentration of 125 µg/L in a pond receiving runoff water is the result of an initial-level model and because it is almost 10 times the freshwater 'moderate reliability' trigger value[14] for atrazine of 13 µg/L, it indicates that there may be cause for concern, particularly where heavy rain falls soon after application across runoff prone catchments that are widely planted to TT canola in raised beds.

In order to validate model estimates, actual data are required. Australian data on this issue are very scarce; however, the Victorian Department of Primary Industries conducted a pilot study to investigate the impact of raised bed cropping on runoff volumes and nutrient flows and provided these data to the APVMA in late 2005 to be assessed by the DEWHA. It is emphasized that the following data are from a small pilot study conducted during years of below average rainfalls. The study was designed to investigate nutrient movement and should not be regarded as a comprehensive scientific experiment to evaluate atrazine in runoff from raised beds. Analysis for atrazine was conducted opportunistically on the first samples collected from runoff events. The concentrations detected in these early samples are likely to contain higher residues than would prevail throughout the event. They cannot be used to determine average concentrations during a runoff event, or total loadings.

The studies investigated runoff from conventional flat cropping with a shallow (50 mm) cultivation, conventional flat cropping with a deep (250–300 mm) cultivation, and raised bed cropping with a deep (250–300 mm) cultivation. The soil at the study site was a brown Sodosol, typical of the cropping soils in south-west Victoria, and characterised by low infiltration capacity. These soils commonly generate runoff when rainfall exceeds 5 mm/hour. Growers in the region on different soil types such as black Vertosols have reported very low amounts of runoff during the same period because of much higher infiltration capacity and water holding capacity. Treatments were replicated three times, sites were 0.2 ha and no sites were irrigated. As well, samples from a weir installed to measure flow from a 26 ha

[14] The freshwater moderate reliability trigger value is set in the *Australian and New Zealand Guidelines for Fresh and Marine Water Quality.* 'Moderate reliability' trigger values apply to ecosystems classified as slightly to moderately disturbed.

raised bed crop paddock catchment were also analysed. Overall, the results indicate that there are higher volumes of flow from the raised beds than from flat bed cropping.

In 2002, atrazine was applied to TT canola post-emergence, at a rate of 2 L/ha along with approximately 100 L water/ha. A rainfall event 22 days after application led to atrazine concentrations off field ranging from 160–230 µg/L, while the weir recorded a concentration in one sample of 500 µg/L. Another rainfall event 48 days after application led to concentrations between 65–120 µg/L, with the lower concentration coming off raised beds. As flow was 23,000 L/ha from shallow flat cropping and 42,000 L/ha from the raised bed treatment, the lower concentration was assumed to result from dilution because of higher volumes of water flow. The sample collected at the weir after the same runoff event recorded a concentration of 94 µg/L and a flow of 39,000 L/ha.

Mean annual surface runoff volume measured from 1999–2004 ranged from 0.01 ML/ha/yr to 1.4 ML/ha/yr in the study. Again, higher runoff was generally observed for raised bed treatments. However, water runoff from raised beds was slightly lower than conventional flat shallow cropping treatments in 2004 when soil was more waterlogged and there had been no runoff events (although it was still higher than from conventional flat deep cropping treatments). In one case, drains to the weir overflowed during a storm event and consequently runoff volumes could not be accurately measured. The total number of overland flow events ranged between 0–7 in a given year.

It was also noted that peak initial flows from raised beds are typically more rapid than from conventional flat cropping systems, indicating a greater potential for flash flooding downstream unless buffer dams or other interceptors were to be in place. It was noted that less than 2 per cent of growers have installed such buffer dams. As the concentration of atrazine is expected to be highest in the first samples of water running off soil, this initial flow from raised beds may lead to higher concentrations of atrazine reaching ambient waters.

It is difficult to determine from these data whether the runoff is predominantly from the beds themselves or from the furrows in-between beds. However, because 20–30 per cent of the surface area of a paddock is composed of compacted furrows, it may be anticipated that furrows are a large contributor to the slightly increased runoff volumes observed in otherwise dry conditions.

The concentrations reported in this pilot study are well above the ANZECC trigger value of 13 µg/L. Note that the atrazine concentrations detected in the pilot study are not unusual for edge-of-field runoff. Concentrations above 100 µg/L were recorded leaving a grain paddock on the Liverpool Plains, some three months after application (Sun and Cornish 2003). A concentration of 85 µg/L (flow-weighted average) was detected 57 days after application to sorghum on a black Vertosol soil (which has higher infiltration capacity) on the Darling Downs (Rattray *et al*. 2007). Concentrations above 1 mg/L have been recorded in effluent from sugarcane fields in Louisiana (Selim 2003).

Because safety margins are already narrow under conventional cropping practices, and because data indicate that atrazine may continue to run off treated fields up to 48 days after it was applied, there are significant concerns that application of atrazine to raised beds may increase the load of atrazine reaching natural waterways. Given that the data above indicate that runoff events may occur up to seven times a year even in years of below average rainfall, and also that raised beds may have a greater potential for flash flooding downstream unless interceptors are in place, there are significant concerns that raised beds may contribute to increased spikes in atrazine concentrations following storm runoff events.

In January 2008, the DEWHA received a submission from Southern Farming Systems that suggested changes to the worst case screening model by considering a more realistic level of agricultural activity and a different runoff scenario (SFS 2007). According to their submission some 33 per cent of cropped land will typically be under canola, as standard practice in south-west Victoria is to grow canola in a three-course rotation with cereals. Not all the canola grown is TT. The current estimate is 80 per cent, and is expected to decline within a few seasons following the lifting of the GM moratorium. Crops are typically grown across 50-60 per cent of a farm. Therefore, TT canola would currently be expected to represent up to 26 per cent of the cropping land area or occupy 13–16 per cent of a typical farm, with this estimate expected to decline as growers adopt newer varieties.

The model was compiled on an Excel spreadsheet to predict atrazine concentrations in a 1.5 ML receiving water body on a notional farm area of 100 ha of which 50–60 per cent was cropped at any time. It was assumed 26 per cent of this was cropped with TT canola, 1 kg of atrazine was applied with a mean half-life of 42 days in soil, and 20–30 cm rain fell two days after application (in line with the label restraint), of which 20 or 30 per cent respectively containing 0.4 per cent of the applied atrazine ran off into the pond, the final volume of which was 5.5 or 10.5 ML. This results in an atrazine concentration range in the receiving water body of between 5-11 µg/L, i.e. just below the ANZECC trigger level of 13 µg/L.

However, two important assumptions have been made which affect this result. Only 1 kg of atrazine was applied, which is at the bottom end of the current post sowing pre-emergence rate. Crucially only 0.4 per cent of atrazine was assumed to run off under these conditions. This was used as it was a 'median value' based on the 0.4 per cent obtained from Rattray *et al*. (2007), and the range of 0.04–4 per cent said to be found by Sadeghi & Isensee (2001). Since the recommended slopes for raised beds are 0.5–1.5 per cent for south-west Victoria, literature reports on slopes >2 per cent were not considered relevant, as were the results for tile-drained fields [e.g. Rothstein (1996) and Leu *et al*. (2004)] noted above. While the rate is equivalent to the maximum now proposed, it cannot be guaranteed that a maximum of 0.4 per cent runs off, noting that if 1 per cent runs off all concentrations would be equal to or exceed the ANZECC trigger level of 13 µg/L. Note that observed seasonal runoff losses are commonly 2–3 per cent of applied, based on a review of numerous studies (Leonard 1988).

Based on their revised modelling above, Southern Farming Systems also proposed three label restrictions to assist in reducing atrazine at source. These would prohibit use of atrazine on TT canola on raised beds:

- where slopes exceed 1.5 per cent;

- on more than 20 per cent of a property; and
- more than once per season at a maximum of 1 kg ac/ha, EITHER post sowing pre-emergence (previously up to 2 kg ac/ha) OR post-emergence.

Importantly the post sowing pre-emergence treatment is currently allowed at up to 2 kg ac/ha, and the current label does not specifically rule out a second post-emergence treatment at up to 1 kg ac/ha).

In 2006, the DEWHA suggested that options to reduce atrazine export from farms should be investigated, such as band spraying and buffer dams. Southern Farming Systems has declared an intention to review the efficacy of such options. Research work on atrazine degrading enzymes is proposed for 2008. Growers may move to newer varieties that do not rely on atrazine, as seed stocks become available.

In conclusion, based on the current information it cannot be guaranteed that the amount of atrazine runoff will be sufficiently low to result in ambient levels which are below the ANZECC Water Quality Guidelines trigger value of 13 µg/L. In addition, the DEWHA has concerns that the argument provided in January 2008 by SFS to refine the screening level model used an unrealistically low value for the amount of atrazine running off (0.4 per cent). Therefore the revised modelling and the proposed label amendments resulting from this provide insufficient confidence for the DEWHA to be able to recommend that the APVMA be satisfied that the continued use of atrazine on TT canola grown on raised beds would not be likely to have an unintended effect that is harmful to animals, plants or things, or to the environment.

If the APVMA were to seek additional actual data there must be agreement on the data collection protocols and data should be collected over a period of not more than two growing seasons.

3.6 WATER MONITORING

The Victorian Department of Primary Industries asked about the criterion for more targeted water monitoring, and for a definition of a 'vulnerable area'.

In regard to the water monitoring referred to in the report, the DEWHA noted an exceedence of the guideline value in WA, and water monitoring in this state formed part of the conditions of registration for label extensions to use on TT canola. Therefore, this criterion has been met as part of the registration for label extension and is not considered further in the review. The DEWHA definition of a vulnerable area is one that experiences high rainfall, where conditions are conducive to runoff or leaching (soil, topography) and where there is extensive use of atrazine within a catchment.

A submission was received from Tasmanian marine farmers, who felt that the water monitoring data provided by industry were not objective and were liable to be biased.

In accordance with international pesticide regulation schemes, all data are assessed by the DEWHA for good practice and confidence in the results. If datasets are flawed then more conservative models and assumptions (that use larger safety margins) are used by the DEWHA in their assessments. It is also worth noting that the DEWHA was represented on the Forest Herbicide Research Management Group to assist with sampling design and guard against potential bias.

The Tasmanian marine farmers also claimed that tests taken by the local council of St Helens in Tasmania in 1993–1994 showed low levels of atrazine. Apparently, no further testing was conducted after that date. The marine farmers claim that testing was stopped because atrazine was detected.

The DEWHA provides objective science-based assessment based on data, and therefore cannot comment on council decisions to conduct or not conduct water testing. The DEWHA can, however, comment on the water test results provided. From the information provided to the APVMA and thence to the DEWHA by the marine farmers, it appears that atrazine was detected in two samples in July 1994, at levels of 0.09 and 0.3 μg/L. It is not clear where the samples were taken from, as they were marked only 'S. George Forest Lodge' and 'Millybrook off U. Esk Rd' (i.e. were these man-made streams, rivers, farm dams, drainage ditches off a property, etc.), and no map marking these places was provided. Atrazine was not detected in five other samples taken at the same time. It was also not detected in any of 13 samples taken from various sites in Ringarooma, Mt Victoria and Pyengana in September 1994. These levels of atrazine are well below the ANZECC freshwater trigger values of 13 μg/L, and therefore the DEWHA would consider that such concentrations are unlikely to cause long-term harm to the aquatic ecosystem.

A joint submission was received from the Allergy, Sensitivity and Environmental Health Association Qld Inc (ASEHA Qld) and the Australian Chemical Trauma Alliance (ACTA). Only one of the recommendations appeared to pertain directly to environmental risks, which was that 'environmental studies must be undertaken in an effort to ascertain an accurate level of atrazine contamination in the Australian environment'.

While additional monitoring data are always useful, there has already been extensive water monitoring conducted by the Forest Herbicide Research Management Group. These data are sufficient for the DEWHA to conduct a risk assessment, which was outlined in the 2004 report.

A submission from the NSW Department of Environment and Conservation also put forward the view that the water monitoring studies were inadequate, and concluded that general and widespread use of atrazine is not acceptable given that water quality trigger values may be commonly exceeded after storm events. Alternative mitigation strategies such as eliminating use of granules and setting a maximum catchment application ratio were suggested.

The water monitoring studies were very extensive and represent almost a unique trial in Australia. Sampling practices were necessarily different across the many widely differing land types and practices across each state and territory in Australia. As well, any monitoring study will necessarily experience natural variability in weather conditions. The DEWHA is of the view that the results of the studies were adequate to conduct a risk assessment. Such results indicate that runoff events can occur after storms, but whether such events result in movement of atrazine into waterways is very dependent on soil types and land use practices.

For example, in the case of forestry, the higher concentrations of atrazine in runoff events near the Barossa likely reflected practices such as mounding perpendicular to the contour, removal of debris, and aerial spraying across buffer strips. Contamination in Queensland was thought to result from runoff from road areas and snig tracks, where it was directly diverted into streams. Such high-risk practices are the reason that the label restraint has been recommended. If this label restraint is followed, it is anticipated that runoff of atrazine will be greatly reduced.

In the case of cropping, water monitoring results indicate that concentrations of atrazine in waterways are generally lower than for forestry, which may reflect the lower rates of application, smaller slopes, differences in application practices such as no aerial spraying, or the like. The only concentrations that exceeded the water quality guideline in WA were in farm dams, where the water would largely be contained. In contrast, some samples of surface water in the Liverpool Plains did contained atrazine in concentrations that exceeded the guideline value on several occasions. This was thought to reflect careless handling practices near stream water because peak concentrations were found after heavy atrazine use but not necessarily after heavy rain that would cause runoff.

Relatively high levels of atrazine found in water samples from the Condamine Balonne in southern Queensland were thought to predominantly result from poor practices such as land cultivation within the 60 m buffer and inadequate measures to reduce runoff such as buffer strips and conservation tillage practices. The Queensland Government formed partnerships with local governments, and community leaders and industry to address these issues.

In contrast to these patterns, the Atherton Tableland Cane Fields did experience spikes in atrazine in surface runoff from sugercane fields. The bulk of the movement of atrazine occurs in the wet season. Restricting of atrazine application to the eight 'dry' months of the year would substantially decrease the amount lost to runoff.

It is acknowledged that runoff of atrazine is undesirable and, in sufficient quantities, is likely to have an unintended effect that is harmful to animals, plants, things and the environment. However, whether the concentrations in runoff that would occur if label directions were followed would cause adverse consequences on aquatic populations is more difficult to determine. Existing data indicate that aquatic plant populations can recover from brief fluctuations in atrazine up to the water quality guideline. Available information indicates that if best management practices and label restrictions are followed then atrazine concentrations in runoff should be minimised. If landholders wish to retain the right to use atrazine then they must ensure that atrazine does not run off their properties at unacceptable concentrations, which is to say, at concentrations above the water quality criterion.

With regard to catchment application ratios, the DEWHA considers that the potential runoff in one catchment may be very different to the potential runoff in another, depending on slopes, soil types, crop rotations, etc. Therefore, the DEWHA recommends that one ratio will not be relevant for every catchment. The DEWHA is of the view that the state governments, who regulate use, are in a better position to understand the catchments in their jurisdictions. State governments are also better placed to ascertain land use and pesticide use within a catchment. For this reason, it would be more appropriate for state governments to establish the catchment application ratios that are appropriate for their own catchments.

The DEWHA has no data to indicate that granules may pose a greater or lesser risk to the aquatic environment than do liquid formulations. Granules may move off-site more easily or be taken up by birds; however, granules have certain other environmental advantages in that they are less likely to drift after aerial application.

3.7 RAINWATER

The ASEHA/ACTA submission pointed to a brief article in New Scientist *and made the case that atrazine can be found in rainwater, making it 'unsafe for drinking'. Although this appears to be a health concern, residues in rainwater can also affect the environment.*

The DEWHA discussed the potential for atrazine to be present in rain and fog in the 1997 atrazine report. The report concluded that quantities of atrazine found in rainwater in some agricultural areas in the US (approximately 1.5 µg/L) are many times smaller than the environmental guidelines. The DEWHA has also sourced the original article referred to in *New Scientist*, by Bucheli *et al*. (1998). The article documented rainwater on roofs in Switzerland near agricultural areas. A maximum concentration of 903 ng/L was measured (1 ng is 1/1000th of a µg, which itself is 1/1000th of a mg). It is not clear if the atrazine was washed off the roofs or was actually in the rainwater. In any case, the maximum concentration was 0.9 µg/L, which is well below the freshwater trigger value set by the NWQMS.

3.8 POTENTIAL EFFECTS ON AMPHIBIANS

The National Toxics Network (NTN) submission argued that the studies on the potential for adverse impacts of atrazine at low concentration on amphibians outweigh both the ANZECC guidelines for freshwater quality and the DEWHA conclusions that permanent damage is unlikely at concentrations below 13 µg/L. The NTN contends that studies with negative findings (meaning no effects found) regarding low use of atrazine are unusable.

The DEWHA discussed the amphibian studies at length in the 2004 report. Unfortunately the data on impacts on amphibians are equivocal and it is for this reason that the US EPA has required substantial further testing. In its assessment, the DEWHA considered the weight of evidence and has concluded that there are significant methodological flaws and confounding factors with many of the papers on this subject.

The DEWHA has also considered more recent studies such as that by Hayes (2004), Roberge *et al*. (2004), Freeman and Rayburn (2005) and Coady *et al*. (2005). Hayes (2004) made the case that studies postulating no effects of atrazine also have flawed methodologies or inappropriate endpoints. For this reason, the additional studies required by the US EPA, which has also specified the necessary measurement protocols and endpoints to be met, should assist in resolving the debate. However, it is also noted that several laboratories have had difficulties in repeating the effects noted by Hayes, and that healthy metamorphling frogs were collected at allegedly contaminated locations sampled by Hayes (2004). Due to their age the metamorphs were likely to have developed in the ponds rather than migrating from elsewhere as suggested by NTN.

Freeman and Rayburn (2005) found that atrazine, when applied to *Xenopus laevis* tadpoles at life history stages earlier than 56, over a period of five weeks, caused developmental delays at concentrations of 100 µg/L. This concentration is higher than the freshwater trigger value of 13 µg/L, and also reflects constant exposure over five weeks. Another recent article found that mortality, growth, gonad development, laryngeal muscle size and aromatase activity in juvenile X. laevis was not significantly affected by concentrations of atrazine between 0.1 and 25 µg/L (Coady *et al*. 2005). However, this research was also overseen by the Ecorisk Inc panel, which is currently at the heart of the current controversy regarding atrazine studies because it is sponsored by a company that sells atrazine. Roberge *et al*. (2004) found that atrazine did not affect the oestrogen receptor at concentrations up to 100 µM (= 20 mg/L) in a competitive binding assay. However, it did inhibit phosphodiesterase, derived from bovine heart, at concentrations of 5 nM atrazine and above (where 0.2 µg/L = 0.9 nM). Reduced levels of phosphodiesterase may lead to increased levels of cAMP, which functions in transcription factors and binding proteins, and may also increase expression of aromatase. However, at least 11 different families of PDE are known, which may respond differently to atrazine. Moreover, the link from inhibition of bovine heart PDE in the lab to actual fitness effects on aquatic organisms is not clear. Therefore, DEWHA has no compelling evidence at this time to alter its previous conclusions.

The NSW Department of Environment and Climate Change felt that the emphasis in the summary on the shortcomings of the amphibian studies was stronger than the data warranted and suggested redrafting.

The changes recommended by the NSW Department of Environment and Climate Change have been incorporated into this report.

3.9 BUFFER ZONES AND NO-TILL SYSTEMS

The submission from Tasmanian marine farmers commented that some label restrictions are impractical, such as 10 m buffer zones in the case of helicopters.

The DEWHA notes that there are no 10 m buffers listed on labels. However, the label specifies 20 m buffers around wells, sink holes and streams, when mixing or applying. The label also specifies that the product is not to be applied within 60 m of downwind lakes and dams. All label directions must be followed. The DEWHA recommends that if a helicopter cannot target a load with sufficient accuracy to spray up to the 20 m buffer, then either it should cease spraying further out so that spray does not fall within the buffer, or other application methods that enable greater accuracy must be used.

The Victorian Department of Primary Industries' submission requested clarification on whether a 20 m buffer was to apply to drainage lines.

As specified in the 2004 report, the DEWHA recommended that atrazine not be applied to drainage lines. However, an additional buffer is not regarded as necessary in this instance.

The NSW Department of Environment and Climate Change suggested that buffer zones to intercept and absorb surface runoff should be considered, pointing to research that indicates that some buffer zones can intercept suspended solids in runoff.

While this is a good suggestion, the DEWHA assessment indicates that the predominant medium by which atrazine reaches waterways is being dissolved in water, not via adsorption to suspended solids. While some spreading buffer zones that slow water flow can allow atrazine to infiltrate soil, buffer zones on a slight slope do not give the product sufficient time to infiltrate sediment and thus may not reduce the amount flowing in to waterways. For this reason it is difficult to set label directions for spreading buffer zones that both the DEWHA and the APVMA can have confidence will be effective. Instead, it is more crucial that product is allowed sufficient time to infiltrate and sorb to soil, which means that it is not applied to waterlogged soil, nor when rain is in any way likely within 48 hours, nor when soil is irrigated to the point of runoff. Nevertheless, because there may be spray drift and because there can also be some movement of atrazine that is sorbed to soil particles, buffer zones around waterways should remain on labels and must be observed.

Similarly, a submission from an agronomist suggested that a move towards no-till systems would increase water infiltration and therefore alleviate the problems of runoff.

The DEWHA has considered this suggestion, which at first sight has merit. Again, the available data show that results may be variable across different soil types, conditions and crops. As well, atrazine is often incorporated into the top 4 cm of the soil layer when used pre-emergence, which is not compatible with no-till systems. As discussed earlier, losses of atrazine in runoff are generally higher with surface application than when atrazine is incorporated into soil. While no-till systems appear to be very good in reducing sediment runoff (e.g. Owens *et al*. 2002), they may often, but not always, reduce surface water runoff. Increases in soil organic matter under a no-till system can lead to greater soil porosity and thus reduce runoff (e.g. Rhoton *et al*. 2002). Conversely, in some cases light tillage may break up a surface crust, allowing greater water penetration. Mickelson *et al*. (2001) found that atrazine concentrations were higher in runoff water under a no-till system (33–140 µg/L) than under three other tillage systems (concentrations of 15–18 µg/L, 19-36 µg/L and 10–17 µg/L) in a two-year field study on corn. It is assumed that this may be related to the surface application needed with the no-till system, and the reduced levels of actual runoff water (consequently leading to higher concentrations in the water that does run off). The DEWHA recommends that the onus is on users to prevent runoff of atrazine. No-till systems, while not appropriate for all situations and crops, may be an effective means to do so in some cases.

3.10 CONCLUSIONS

In conclusion, the DEWHA does not have any compelling evidence to alter its conclusions regarding potential endocrine disruption in frogs. However, the data are not convincing either way and therefore the DEWHA recommends that this aspect be re-visited at a future point should the outcome of the testing ordered by the US EPA indicate adverse effects at concentrations in the same order of magnitude as the trigger value of 13 µg/L.

The DEWHA also has significant concerns in relation to raised bed cropping. Because safety margins are already narrow under conventional cropping practices, and because data indicate that atrazine may continue to run off treated fields up to 48 days after it is applied, the DEWHA does have concerns that application of atrazine to raised beds may increase the load of atrazine reaching natural waterways.

In mid 2006 the DEWHA recommended to the APVMA the following options to address these concerns:

> Option 1: Atrazine is no longer used on TT canola grown on raised beds (Example proposed label statement: *'TT canola: Do NOT use or apply atrazine on raised beds or where furrows have been created in soil for the purposes of holding or channelling water'*.)
>
> Option 2: Buffer dams or other effective interceptors become mandatory where raised bed cropping is used. (Example proposed label statement: '*TT canola: Do NOT use or apply atrazine on raised beds or where furrows have been created in soil for the purposes of holding or channelling water UNLESS that channelled water is effectively directed to buffer dams or other effective interceptors*').
>
> Option 3: Further investigation be undertaken to determine whether spray application equipment, such as band spraying, which only applies pesticide to the tops of beds (and not across beds and furrows together, as currently practised), may effectively reduce the presence of atrazine in runoff. However, it is noted that weeds may then occur in the furrows. If this would lead to weeds being present in the crops planted in the furrows, then another alternative is that the furrows should not be sown. This option, to only apply atrazine to the tops of beds, should only be considered if it will be possible and enforceable to ensure that furrows are not sprayed. (Example proposed label statement: '*TT canola: Do NOT apply atrazine to the furrows in-between raised beds, or to channels between beds that are intended to hold or move water*').

In late 2007 and early 2008 the APVMA and DEWHA discussed the revised finding with affected registrants, state officials and TT canola grower groups. No consensus was reached on the issue.

In January 2008, the DEWHA received a submission from Southern Farming Systems that suggested changes to the worst case screening model, as discussed in section

3.5 above. Additional actual data are required to refine the risk assessment.

At this stage the APVMA cannot conclude that the use of atrazine on TT canola, when applied post emergence to raised beds, would not be likely to have an unintended harmful effect on the environment. However, evidence to date is very limited to address this concern. Therefore the APVMA has determined that affected registrants will be provided with an opportunity to demonstrate that this potential problem is either non-existent or can be mitigated with enforceable amended label instructions. It is anticipated that it will require a further two cropping seasons for additional data to be generated and another 12 months after that for the results to be evaluated and a regulatory position adopted.

In the interim the APVMA has concluded that the review of atrazine should not be further delayed while more investigation of alternative methods and/or containment controls is undertaken. The APVMA will therefore apply the label amendments detailed in the 2004 report. In addition, any registrant who does not elect to provide a commitment to generate the required data will be required to amend their label to exclude the post-emergent use of atrazine on TT canola grown on raised beds.

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4. SUPPLEMENTARY ENVIRONMENTAL ASSESSMENT BEFORE PUBLIC FORUM 22 JUNE 2007 (DEWHA, JUNE 2007)

The Community Consultative Committee (CCC) requested further public consultation to address particular topics of concern. This took the form of a forum held on 22 June 2007. As preparation for the forum, the APVMA referred three items that were highlighted by the CCC to the DEWHA for agency information and attention, as outlined below. Two additional items were also referred, as well as a case study on the issues surrounding use of agricultural chemicals in north-east Tasmania.

1. An email dated 12 March from a member of the CCC to the APVMA identifies six issues for discussion, of which three are relevant to environmental assessment.

Questions how frog studies, including those looking at gonadal differentiation, were considered in the APVMA risk assessment process: In brief, the many available studies of the effects of atrazine in frogs were evaluated for their reliability. Part of this evaluation involved comparing studies for mutual consistency. Only reliable studies can be used for regulatory decision-making. The environmental assessment then evaluated whether harmful effects in Australian frog populations would be likely at current levels of exposure to atrazine in Australian surface waters, based on the weight-of-evidence from reliable studies and field observations.

The issue of atrazine impacts on other wildlife: The environmental assessment considered toxicity data for a wide range of vertebrate and invertebrate organisms. In general, the toxicity of atrazine to these organisms was found to be moderate (endpoints for aquatic fauna in the 1–10 ppm range). Atrazine impacts on aquatic fauna were considered more likely to arise through indirect rather than direct mechanisms, as atrazine is very highly toxic to algae and aquatic plants that form the basis of aquatic food chains. This toxicity formed the basis for determining levels of atrazine that would pose a risk in the environment.

The issue of ground and surface water contamination and the APVMA risk assessment process: Aquatic contamination is assessed using very conservative models in the first instance. Where such initial screening assessments indicate a possible risk, the assessment is refined using more realistic approaches. In the case of atrazine, monitoring data were available as the basis for a refined assessment. The risk assessment is based on protecting the most sensitive organism from harmful population effects.

2. Meeting notes prepared by a member of the CCC and sent to APVMA expand on the above issues.

Groundwater is again raised, but from a human health angle: The low-level contamination of Australian groundwater by atrazine appears to mainly reflect seepage through soils from diffuse rather than point sources, although there are notable exceptions such as the incident reported in Perth where a pest control operator had washed equipment into a soakaway, leading to high levels of atrazine contamination in the local aquifer.

The notes also refer to difficulties caused to desalination plants by the presence of atrazine. The relevance of this observation is unclear, given that atrazine is a contaminant of fresh rather than marine waters. As noted in the APVMA's 1997 report, atrazine breaks down relatively quickly under estuarine conditions.

A number of questions are raised in relation to the APVMA conclusion that 'the likelihood that atrazine is disrupting sexual differentiation in Australian frogs at current exposure levels … does not appear to be high, based on currently available evidence': The evidence on which this conclusion is based is detailed in the APVMA's 2004 report. The shortcomings of the available studies in this emerging area of research are also noted. Some of the studies are published and have therefore undergone peer review, but many of the unpublished studies have also undergone thorough peer review through the US EPA's Scientific Advisory Panel. The evidence for the Australian evaluation was primarily taken from overseas sources. A weight-of-evidence approach was adopted for data evaluation, which naturally excluded outlying and irreproducible data points from the risk assessment.

There has been some monitoring of Australian frog populations at sites where atrazine is likely to be present. For example, Wassens *et al*. (2005) surveyed frogs in various habitats in the Murrumbidgee Irrigation Area, finding a relatively high number of species compared with other irrigation areas. Frog diversity was highest in rain-fed depressions and dams, and lowest in irrigation canals and rice bays. No chemical monitoring was conducted, but these researchers attributed the variation in diversity to habitat factors such as aquatic and fringing vegetation and the local diversity of habitat types. Hyne *et al*. (2007) surveyed tadpoles and adults of four frog species in rice bays, finding a significantly lower occurrence of one species, the endangered southern bell frog *(Litoria raniformis)*, in northern compared to southern regions of the Coleambally Irrigation Area in NSW. Concentrations of two herbicides (thiobencarb and diuron) were higher in the northern regions, but other herbicides including atrazine were similar across all study sites.

The claim that the use of atrazine in TT canola is being phased out in Canada because registrants did not want to generate data to support it: The Proposed Acceptability for Continuing Registration (PACR) document that provides a summary of the environmental data and information reviewed as well as the rationale for the proposed regulatory decision for atrazine in Canada was released for public comment on 22 May 2007. The Canadian assessment found the highest risks to be to aquatic plants and communities exposed to atrazine through surface runoff. Precautionary measures were proposed to mitigate this. The Canadian authorities have undertaken to conduct a risk assessment of chronic amphibian effects when new data on reproductive and developmental effects are received.

The Canadian assessment considers current registrations for use on corn. It is unclear why triazine-tolerant canola is not included in the PACR document, but the availability to Canadian farmers of genetically modified varieties may be pertinent.

As stated in the APVMA's 2004 report, the use of atrazine in canola raises particular concerns where raised beds are used to improve drainage in duplex soils prone to waterlogging, because atrazine residues are likely to leave the treated area as residues in runoff. It may be noted that one technical option would be to plant different canola varieties, as occurs in Canada.

3. Two recent publications were also highlighted.

The first (Fan *et al*. 2007) is an *in vitro* study of aromatase expression in human cell lines. The focus of this study is on human health issues. Its relevance to environmental assessment is limited because comparable cell lines and molecular tools for wildlife species are not available, as noted by the authors. Concerns are expressed for endocrine-mediated effects in wildlife, particularly amphibians, citing numerous publications by one of the authors (Hayes) but not presenting any new science.

Note that the hypothesis that atrazine exposure induces aromatase in tadpoles and that this induction gives rise to effects on gonadal differentiation was examined by the US EPA's Scientific Advisory Panel (Lewis and Roberts 2003). The panel agreed with the US EPA that aromatase induction by atrazine had yet to be demonstrated by controlled laboratory studies. Recent field studies (Murphy *et al*. 2006) in which measured atrazine concentrations were compared with the gonadosomatic index, plasma steroid levels and aromatase activity in green frogs failed to discover any correlations. These authors conclude that atrazine does not up-regulate aromatase in green frogs in the wild, and does not appear to affect plasma steroid concentrations.

The second study (Weiner *et al*. 2007) examined carbon allocation into macromolecular pools in five microalgal species exposed to atrazine at the EC50, and at half and double this level. A decrease in algal proteins was found in three species, similar to effects seen under conditions of nutrient deficiency. Other effects on carbon uptake into cells and allocation into macromolecular pools were noted, together with the implications of such alterations for nutrition in algal consumers, particularly in their early stages of development. These alterations were observed at atrazine concentrations as low as 25 ppb.

This study is interesting, but does not alter regulatory outcomes for atrazine which are already based on the high sensitivity of algae to atrazine and the recognition that microalgae are at the base of aquatic food chains. The possibility that exposure to such concentrations of atrazine may indirectly affect amphibian development by reducing algal food resources is noted in the APVMA's 2004 report.

4. The APVMA also referred an article (Souder 2006) that had been brought to its attention. The article describes the research of Dr Tyrone Hayes and his relationship and disagreements with Syngenta, the principal registrant of atrazine.

This polemical article does not contain any new science, and has a journalistic rather than scientific focus, consistent with its origins. It has no implications for the atrazine review.

5. A recent article on the toxicology of triazine mixtures with nitrates and diazinon was referred by the APVMA on the basis that it may contain or reference additional relevant material. The article has a human health focus, with the mixtures for

study selected on the basis of their occurrence in groundwater rather than surface water.

The effects on frogs of mixtures of atrazine and nitrate are described in the APVMA's 2004 report. Atrazine and nitrate both appear to exert indirect effects on amphibian development at elevated concentrations. There is now new research (Orton *et al*. 2006) on the effects of this combination of chemicals on development and sexual differentiation in leopard frogs. Testicular oocytes were seen in control groups and in frogs exposed to the individual chemicals, but not the combination. These authors suggest that premature maturation of the gonad may occur as a result of such exposures during larval development, based on histological evidence.

The environmental effects of mixtures can be more complex than the effects on human health because of the variety of organisms involved and the opportunity that this presents for indirect effects. In the same way that atrazine can indirectly harm some organisms by reducing algal food resources, insecticides such as diazinon may indirectly benefit the same organisms by harming other algal consumers. Recent studies (Boone and Bridges-Britton 2006) with mixtures of atrazine, carbaryl and nitrate found that tadpoles of the gray treefrog did not appear to be more susceptible to single versus multiple contaminants, possibly reflecting positive changes in food resources. Previous findings that exposure to the insecticide carbaryl produces positive outcomes for anuran metamorphosis were confirmed.

The effects of mixtures of aquatic contaminants (herbicides) on leopard frog development have been explored by Hayes (2006). This work has produced some interesting results, but to this time has not been replicated by other research groups, and suffers from similar shortcomings as this researcher's work on the low-dose effects of atrazine on the development and sexual differentiation of frogs. For example, the published paper does not include the raw data, and does not describe the quality of the water or of the food that was provided. It would not be appropriate to use these unreplicated findings as the basis for regulation of atrazine, particularly given the implications for regulation of other herbicides. These authors conclude that reliance on studies that examine relatively high exposures to individual chemicals may grossly underestimate risks as mixtures of chemicals at low concentrations had much greater effects on the parameters measured in this study. Interestingly, these parameters related to the duration of larval development and size at metamorphosis rather than sexual differentiation. Effects of atrazine on the gonads were not detectable in the population studied because the frogs did not complete sexual differentiation of the gonads before metamorphosis.

The authors of this study suggest that exposure to the pesticide mixture rendered the frogs more vulnerable to disease as a result of immunosuppression. Increased rates of flaviobacterial infection in leopard frogs exposed to low-dose pesticide mixtures led the authors to conduct histological analyses of the thymus, which revealed damage in the form of thymic plaques. Parallel studies in African clawed frogs suggested that these adverse effects may be due to an increase in plasma levels of the stress hormone corticosterone, but this remains hypothetical, as the collection of blood samples by cardiac puncture is likely to have elevated the parameter being measured.

These authors cite the work of Carr *et al*. (2003) as support for their findings of smaller metamorphic size following an extended larval period under low-dose atrazine exposure. This is misleading, as the effects observed by Carr *et al*. (2003) reflect inadequate husbandry (starvation and poor environmental conditions) rather than any direct effect of atrazine exposure. Reduced metamorphic size after an extended larval period is known to result from increased competition for resources early in the larval period, as documented in the APVMA's second draft final review report.

The immunosuppressive effects of atrazine on frogs are receiving increasing attention, as is warranted given that pathogens are recognised as a major stress on frog populations. As with the reported effects on gonadal differentiation, findings in this area are too preliminary and inconclusive to be used for regulatory decision making, but merit close attention. Some of the evidence in this area is described in the APVMA's second draft final review report. More recent work (Brodkin *et al*. 2007) extends the hypothesis that atrazine may harm amphibian populations by increasing susceptibility to disease into the same low-dose exposure range where effects on gonadal differentiation have been reported. The study was conducted in adult leopard frogs, which were held individually in plastic containers filled with aged tap water or atrazine supplemented water and stimulated with the inflammatory mediator thioglycollate. The inflammatory response and the phagocytic activity of white blood cells were measured as indicators of the innate immune response.

Nominal exposure concentrations of 0.01, 0.1, 1 and 10 ppb were used for a dose-response study, conducted over eight days with daily renewal of the medium. Two control groups were used, one stimulated by intraperitoneal injection with thioglycollate and the other injected with Ringer solution. Stimulation occurred on the seventh day of exposure, prior to euthanasia on the eighth day and peritoneal lavage with physiological Ringer solution.

The number of white blood cells in peritoneal exudates was suppressed relative to the stimulated control group at all atrazine exposure levels. At the highest exposure of 10 ppb, the number of peritoneal white blood cells from frogs stimulated by thioglycollate was similar to the number from nonstimulated resident peritoneal cells. A similar response was seen in the percentages of highly phagocytic cells and nonphagocytic cells.

The extent to which adult leopard frogs would show increased susceptibility to infection under atrazine exposure is not known, but the authors conclude based on these indicators of the innate immune response that immunosuppression may be caused by exposure to very low doses of atrazine alone, as well as by exposure to ecologically relevant mixtures of pesticides and herbicides as reported by others, and that such immunosuppression may play a role in the global amphibian decline.

This research merits close attention, given the potential implications for amphibian populations. However, at this stage the significance to individual or population health of the effects seen on the activity of white blood cells taken from leopard frogs after atrazine exposure is uncertain, and the reproducibility of the effect remains untested. Therefore, these preliminary findings would not be a sound basis for regulating the use of atrazine.

6. The case study (Bleaney 2007) describes the results of a two-year community based audit conducted in north-east Tasmania. It does not appear to have been peer-reviewed.

The report documents ongoing concerns that low level exposure to pesticides, particularly as mixtures, could be interfering with endocrine and immune systems in wildlife and humans. It is further alleged that the immunosuppressive effects may be behind the current afflictions in Tasmanian devils and in platypus in Tasmania. DEWHA commented on this hypothesis in December 2004 in relation to the AERP report (E-04-9228-A) regarding pesticide use in Tasmanian forests. In short, DEWHA did not believe that the APVMA could, as requested by the authors of that report, give a guarantee that adverse effects from low level exposures to combinations of chemicals will not occur, but believed that the APVMA could be satisfied that such outcomes are very unlikely. Those beliefs remain unchanged. According to the present report, Dr Bleaney was one of the authors of the AERP report.

David Parsley's report dated 21 May 2004 on the helicopter crash that formed part of the rationale for this AERP report was provided by the Break O'Day Council to Dr Bleaney for information and not public distribution, but has been reproduced in her publicly available report. David Parsley concluded that the incident was of minor environmental significance.

Much of Dr Bleaney's report is occupied by claims of incompetence or negligence on the part of state and local government. At the Australian Government level, the APVMA and its advisory agencies are criticised for not comprehensively testing for immunotoxicity or endocrine disruption. This criticism would appear misplaced as there are no internationally agreed guidelines for such testing, while considerable controversy surrounds the claims that have been made in the open literature regarding these phenomena. The DEWHA is closely following overseas regulatory developments in these emerging areas. Atrazine is included on the initial draft list for the US EPA's Endocrine Disruptor Screening Program (news release dated 11 June 2007, see http://www.epa.gov/endo/pubs/prioritysetting/listfacts.htm).

The particular issues surrounding atrazine and amphibians are scheduled to again be discussed by the US EPA's Scientific Advisory Panel on 9–12 October 2007. DEWHA will be following the progress of this meeting with great interest, and will review its previous advice to the APVMA appropriately should the US EPA determine that harmful effects are likely at very low concentrations (i.e. below the ANZECC guideline of 13 ppb).

Dr Bleaney's report claims on page 50 that the current system for evaluating the safety of pesticides is clearly dysfunctional. Her view appears to be that mainstream science should be replaced by more innovative approaches, such as an approach known as post-normal science. Such major policy changes are likely to fall outside the scope of the National Registration Scheme. DEWHA has confidence in the current approach of evaluating risk according to established and internationally accepted scientific procedures, with targeted monitoring and scientific investigation of significant detections of pesticide residues in water as occurs for example in Tasmania, and doubts that the broader community would be receptive to such innovation. As noted in relation to the Tasmanian pesticide monitoring program by the Minister for Primary Industries and Water, David Llewellyn MHA, in his letter of 7 November 2006 to Dr Bleaney (page 87 of her report) 'The broader community can and does have confidence in the transparency of the program and its role in further understanding the nature and extent of the impact of chemical use in primary industries on Tasmanian water quality'.

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