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Proposed Special Review Decision PSRD2023-01

Proposed Special Review Decision of Atrazine and Its Associated End-use Products

Consultation Document

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Proposed special review decision for atrazine and associated end-use products

Under the authority of the *Pest Control Products Act*, pesticides are regulated by Health Canada's Pest Management Regulatory Agency (PMRA) on behalf of the Minister of Health. The *Pest Control Products Act* prescribes both the pre-market and post-market assessment (re-evaluations and special reviews) of pesticides to determine the acceptability or continued acceptability of human health and environmental risks, and, acceptable value of a pesticide in Canada. Unlike a re-evaluation, a special review is triggered only under certain circumstances, as described in section 17 of the *Pest Control Products Act*, and the intent of a special review is to address specifically the identified aspect(s) of concern. The special review approach is described in the PMRA Guidance Document: *Approach to Special Reviews of Pesticides*. More details on the legislative framework are provided under the section of Legislative Framework of this document.

Health Canada evaluates the aspect(s) of concern that prompted the special review in accordance with subsection 18(4) of the *Pest Control Products Act*. The internationally accepted science-based approach is used for the assessment of the aspect(s) of concern, similar to all other scientific assessments (for example, new product registrations, re-evaluations). This step includes both risk (or value, if applicable) assessment and risk management to address the concerns identified. Health Canada's approach to risk and value assessment as well as risk management is outlined in the Framework for Risk Assessment and Risk Management of Pest Control Products.[1]

Pursuant to subsection 17(1) of the *Pest Control Products Act*, Health Canada conducted a special review of all registered pest control products containing atrazine which was initiated in 2017 (REV2017-10). Health Canada became aware of additional information on atrazine and a preliminary analysis of this information indicated that the criteria listed in subsection 17(1) of the *Pest Control Products Act* were met, and a special review was warranted. The identified aspects of concern are:

- Potential changes to toxicology endpoint(s) used for human health and environmental risk assessment, and impact thereof, including potential human health (drinking water) and environmental risk from atrazine in surface water.

Pursuant to subsection 18(4) of the *Pest Control Products Act,* Health Canada has evaluated the aspects of concern that prompted the special review of pest control products containing atrazine, which are relevant to both human health and the environment.

Atrazine is a herbicide and is a member of the chlorotriazine group of chemicals. It is registered to control broadleaf weeds in/around corn (field, grain, silage, seed, and sweet), sorghum, and switchgrass when crops are in the very early stages of development (pre- or post-emergence). Atrazine can also be mixed with liquid fertilizers or incorporated onto granular fertilizers

[1] PMRA Guidance Document, A Framework for Risk Assessment and Risk Management of Pest Control Products (https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/policies-guidelines/risk-management-pest-control-products.html)

(fertilizer impregnation) in commercial fertilizer facilities. There are no registered domestic-class products. All currently registered products containing atrazine have been considered in this special review. Currently registered pest control products containing atrazine are listed in Appendix I.

This proposed special review decision is a consultation document.[2] Health Canada will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (please see contact information on the cover page of this document).

Proposed special review decision for atrazine

Under the authority of the *Pest Control Products Act* and based on an evaluation of available relevant scientific information related to the aspects of concern for human health and the environment, Health Canada is proposing that continued registration of most uses of atrazine are acceptable with additional risk mitigation measures.

The assessment of the aspects of concern from this special review indicate that risks to human health and the environment for the following uses of atrazine are considered to be acceptable provided that proposed label amendments are implemented:

- Foliar uses on corn (field, seed, sweet), sorghum and switchgrass; and
- Liquid fertilizer as a carrier for use on corn (field, seed, sweet).

The assessment of the aspects of concern from this special review indicates that based on the available information, the risk to the environment from the use of impregnated granular fertilizers is found to be acceptable. However, the risk to human health from this use for the following use of atrazine is not shown to be acceptable. Therefore, the impregnated granular fertilizer use of atrazine is proposed to be cancelled.

The proposed additional mitigation measures are summarized below, and details are outlined in Appendix II and III.

Proposed risk mitigation measures

Human health

Evaluation of available relevant scientific information related to the aspects of concern indicated that most uses of atrazine showed acceptable risk to human health with implementation of the proposed mitigation outlined below and in Appendix II.

---

2 “Consultation statement” as required by subsection 28(2) of the *Pest Control Products Act.*

To protect mixer/loaders and applicators:

- Require closed mixing/loading when handling more than 85 kg a.i. of atrazine in a day;
- Require a closed cab during application when handling more than 133 kg a.i. of atrazine in a day; and
- Cancel the impregnation of granular fertilizer uses, as feasible mitigation measures were not identified for treating granular fertilizer in commercial facilities.

Environment

Evaluation of available relevant scientific information related to the aspects of concern indicated that uses of atrazine showed acceptable risk to the environment with implementation of the proposed mitigation outlined below and in Appendix III:

- Updated standard environmental precaution statements to inform users of the potential toxicity to terrestrial and aquatic organisms;
- Updated spray buffer zones for non-target terrestrial and aquatic habitats;
- Updated standard precautionary runoff statements to reduce the potential for runoff of atrazine to adjacent aquatic habitats; and
- Updated precautionary statement to indicate that leaching to groundwater is possible.

Next steps

Before making a special review decision on atrazine, Health Canada will consider all comments received from the public in response to this consultation document. A science-based approach will be applied in making a final decision on atrazine. Health Canada will then publish a special review decision document, which will include the decision, the reasons for it, a summary of the comments received on the proposed decision, and Health Canada's response to these comments.

The following additional data may be submitted during the consultation for consideration during the final decision phase:

Human health

No additional scientific data are being requested. However, during the consultation period, the registrants and other stakeholders may submit the following information that could help address uncertainties in the available information for atrazine and support revised assessments of exposure and risk.

- **Occupational exposure:**
    - Additional information on granular fertilizer impregnation in a commercial treatment facility. This could include:
        - Current Canadian information on the types of tasks/activities typically conducted in a commercial fertilizer treatment facility;

        - Current information on the time spent on each task/activity in a commercial fertilizer facility and potential for exposure to atrazine or atrazine-treated fertilizer;
        - Clarification on whether the treated fertilizer is bagged (if so, how large the bags are, and if manually or automatically bagged);
        - Clarification on whether the equipment is manually or automatically cleaned and if the equipment is cleaned after each batch or at the end of the day; and
        - Information on the typical duration and thoroughness of the equipment cleaning (for example, whether workers physically enter the mixing/blending equipment).
    - Additional information and data on the potential exposure sources during loading of impregnated granular fertilizer in Canada. For example, whether the granules are loaded into the application equipment directly at the fertilizer facility or whether they are loaded into bags or a truck for transport to the field. If the latter, then information on how the granules are transferred from bags or trucks into the application equipment (for example, is the transfer done manually or automatically and whether the loading equipment is fully enclosed) should be provided.

Environment

As part of efforts to provide continual oversight for registered pesticides in Canada, additional data will be considered as they become available. Health Canada encourages registrants, stakeholders, and partners to provide, during the consultation period, additional available water monitoring data for shallow waterbodies in corn growing regions in Canada.

Other information

The relevant confidential test data on which the proposed decision is based (see References section of this document) are available for public inspection, upon application, in the PMRA's Reading Room. For more information, please contact the PMRA's Pest Management Information Service.

Legislative framework

The Minister of Health's primary objective under the *Pest Control Products Act* subsection 4(1) is to prevent unacceptable risks to individuals and the environment from the use of pest control products.

As noted in the preamble of the Act, it is in the national interest that the attainment of the objectives of the federal regulatory system continue to be pursued through a scientifically-based national registration system that addresses risks to human health, the environment and value both before and after registration and applies to the regulation of pest control products throughout Canada; and that pest control products with acceptable risk and value be registered for use only if it is shown that their use would be efficacious and if conditions of registration can be established to prevent unacceptable risks to human health and the environment.

For the purposes of the Act, the health or environmental risks of a pest control product are acceptable if there is reasonable certainty that no harm to human health, future generations or the environment will result from exposure to or use of the product, taking into account its conditions of registration as per subsection 2(2) of the *Pest Control Products Act*.

Risk for human health and the environment, and value are defined under the Act in subsection 2(1) as follows:

> **Health risk**, in respect of a pest control product, means the possibility of harm to human health resulting from exposure to or use of the product, taking into account its conditions or proposed conditions of registration.
>
> **Environmental risk**, in respect of a pest control product, means the possibility of harm to the environment, including its biological diversity, resulting from exposure to or use of the product, taking into account its conditions or proposed conditions of registration.
>
> **Value**, in respect of a pest control product, means the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (a) efficacy; (b) effect on host organisms in connection with which it is intended to be used; and (c) health, safety and environmental benefits and social and economic impact.

When evaluating the health and environmental risks of a pesticide and determining whether those risks are acceptable, subsection 19(2) of the *Pest Control Products Act* requires Health Canada to apply a scientifically-based approach. The science-based approach to assessing pesticides considers both the toxicity and the level of exposure of a pesticide in order to fully characterize risk.

Health Canada's approach to risk and value assessment is outlined in A Framework for Risk Assessment and Risk Management of Pest Control Products.[3]

For this special review on atrazine, the aspects of concern are related to human health and the environment.

[3] PMRA Guidance Document, A Framework for Risk Assessment and Risk Management of Pest Control Products

Evaluation of the aspects of concern that prompted the special review

Following initiation of the special review, Health Canada requested information under section 19 of the *Pest control Products Act* that were related to the aspects of concern.

To assess the aspects of concern, Health Canada considered the information that prompted the special review and other information currently available relevant to the aspects of concern, including relevant published scientific literature on potential health and environmental effects of atrazine, information considered for the previous re-evaluation of atrazine (RRD2004-12, RVD2007-05), information from the consultation of the previous special review for atrazine (REV2017-09), information from the United States Environmental Protection Agency (USEPA) preliminary ecological risk assessment for atrazine (USEPA, 2016), information gathered from other federal/provincial government departments and agencies, and any relevant information obtained since then (for example, information from the Canadian incident report database; water monitoring data).

1.0 Potential change(s) to toxicology endpoint(s) previously used for human health risk assessment

As part of the special review, Health Canada assessed new information to determine whether toxicology reference values previously used for the 2003 human health risk assessment warranted revision. Information submitted as part of the special review under section 19 of the *Pest Control Products Act,* as well as relevant information from scientific literature published subsequent to the release of PACR2003-13 was examined. Information provided in comments submitted in response to the previous special review of atrazine (REV2015-11, REV2017-09) was also considered.

Health Canada previously determined that atrazine and its chlorotriazine and hydroxylated metabolites and transformation products (TPs) are relevant to human exposure (PACR2003-13). Atrazine and its chlorotriazine metabolites are considered as major metabolites in plant and animal systems; as well as major TPs in drinking water sources, which is a result of degradation of atrazine in the environment. Hydroxylated metabolites of atrazine are major metabolites in plants, but not in livestock animals, and they are also identified as major TPs in drinking water. Chemical names of these metabolites/TPs can be found in Appendix IV, Tables 1A and 1B.

The toxicology database on atrazine is robust and extensive, consisting of a comprehensive set of studies on atrazine, its key metabolites and/or transformation products, and includes a large number of published toxicity studies as well as unpublished guideline toxicity studies. The majority of these studies were comprehensively evaluated by the USEPA and other international regulatory authorities, as well as independent scientists, including the USEPA Scientific Advisory Panel (SAP) that convened and evaluated all atrazine data and its key aspects for risk

assessment on 12 separate occasions since 2000.[4] In addition to conducting an independent evaluation of available studies, Health Canada also considered a number of recent evaluations of the same data by other national and international authorities and organizations, such as the USEPA and the Australian Pesticides and Veterinary Medicines Authority (APVMA). The majority of the guideline studies complied with good laboratory practice (GLP) standards. A search of the published scientific literature was conducted according to the principles of systematic review in order to select the relevant published information for use in the hazard assessment. Studies that were deemed relevant in addressing the aspects of concern were included in the review. Studies were classified as acceptable, acceptable with limitations, supplemental, or unacceptable, as outlined in Health Canada's Information Note: Determining Study Acceptability for use in Pesticide Risk Assessments. Overall, the scientific quality of the data is acceptable and the database is considered adequate to characterize the potential health hazards associated with atrazine.

Toxicokinetic studies

Metabolism studies available in the toxicity database included studies that were conducted with triazine-radiolabelled atrazine, as well as studies in which tissue levels of non-radiolabelled atrazine and its major metabolites were quantified by analytically sensitive and specific methods, such as high-performance liquid chromatography (HPLC) or tandem mass spectrometry (MS/MS).In single or repeat-dose oral gavage radiolabel metabolism studies in rats, oral absorption was extensive, relatively rapid and dose-dependent. Peak plasma levels were reached within 2 hours and 24 hours for low and high doses, respectively. Distribution was extensive, dose-dependent and greatest in highly perfused tissue sites. The highest tissue residue levels were in red blood cells, followed by the liver, kidneys, ovaries, pituitary, brain and mammary glands. The distribution pattern of the radiolabelled administered dose (AD) did not vary across dose levels, but the amount distributed varied in a dose-dependent manner. The elimination half-lives for tissue radioactivity levels were estimated to be 30 days in red blood cells, 10 days for brain, and approximately 4 days for all other tissues or organs.

Atrazine was extensively metabolized, with more than 25 metabolites detected in rats. The major metabolic pathway was identified as the stepwise dealkylation via either desethyl-atrazine (DEA) or desisopropyl-atrazine (DIA) to the terminal chlorotriazine metabolite, namely diaminochlorotriazine (DACT). The chlorotriazine metabolites may also undergo glutathione conjugation followed by further metabolism to mercapturic acid derivatives. The hydroxylation or dechlorination of the triazine ring was a very minor metabolic pathway, as detection of these metabolites in rat metabolism studies was essentially negligible.

4 FIFRA Scientific Advisory Panel Meetings on Atrazine. Available online from https://www.epa.gov/ingredients-used-pesticide-products/atrazine#fifra-sap [last accessed November, 2021]

Atrazine and its chlorotriazine metabolites were rapidly cleared, with over 50% of the AD excreted within 24 hours of dosing. Within a week of dosing, excretion was largely complete, accounting for more than 90% of the AD. The primary routes of excretion were the urine (70–76% of the AD), the feces (13–15% of the AD) and to a lesser extent the bile (7% of the AD). The excretion profile of atrazine did not vary across dose levels. Overall, there were no sex-related differences in the toxicokinetic profile of atrazine. The plasma elimination half-life of radiolabelled residues was estimated at approximately 40 hours.

Based on plasma and tissue elimination half-lives, the atrazine toxicokinetic profile does not appear to fit a one compartment toxicokinetic model. Rather, the toxicokinetic profile may be better described by a multiple-compartment model where the levels of atrazine and its metabolites may differ among various organs and tissues.

Several non-guideline single or repeat dose oral gavage toxicokinetic studies in different strains of rats and other species were available that measured the levels of atrazine and its metabolites in tissue and excreta samples. The majority of these studies in rats assessed distribution of atrazine and chlorotriazine metabolites in the body following gestation only, or gestational and lactational treatment paradigms. The results indicate that atrazine and chlorotriazine metabolites can distribute widely in the body, with highest levels detected in the plasma, brain, adrenal and mammary glands. They further demonstrated that atrazine and chlorotriazine metabolites can cross the placenta, be transferred via milk to the young, cross the blood-brain barrier (BBB), and reach the gonads. The major chlorotriazine metabolite recovered in the plasma and tissue samples of the dams, fetuses, and pups was the terminal chlorotriazine metabolite, DACT. DEA, DIA, and unchanged atrazine were also detected at lower levels, although the hydroxymetabolites of atrazine, DEA, DIA and DACT were not reliably quantifiable in tissue and plasma samples. These studies also confirmed the results of the radiolabelled toxicokinetic studies with atrazine, including the stepwise dealkylation metabolic pathway of atrazine to DACT. Single or repeat dose oral gavage toxicokinetic studies in rats conducted with DIA and DACT also indicated that DIA is metabolized to DACT and the major chlorotriazine metabolite detected in plasma was unchanged DACT.

Two single oral or intravenous (IV) dose non-guideline toxicokinetic studies in monkeys were available to supplement the findings of the main toxicokinetic studies in rats. The results of these studies suggested a similar toxicokinetic profile to that noted in rats. Atrazine was relatively rapidly absorbed following oral administration of single doses ranging from 1 to 100 mg/ kg bw. Peak blood concentrations were reached after 2, 8, and 24 hours at the lowest, intermediate, and highest dose levels, respectively. The bioavailability was estimated to be 75–92% based on the area under the curve data. DACT was identified as the terminal chlorotriazine metabolite in plasma and urine samples, and accounted for the highest portion of the AD. The predominant route of excretion was via the urine, accounting for up to 90% of the AD, while elimination via the feces accounted for the remaining portion. The renal clearance half-life was approximately 21 hours. The terminal half-lives for elimination of radiolabelled test material from plasma were 32, 22, and 20 hours for the lowest, intermediate and highest dose levels, respectively. The toxicokinetic profile, including the metabolites identified, was comparable between the oral gavage and IV dose studies in the monkey.

A non-guideline single low oral dose study of kinetics and metabolism in six adult human male volunteers was also available. This study was subjected to a human research ethics review by the USEPA, which found that the study met all USEPA human study requirements and thus, the study could be considered in their hazard assessment. Atrazine and chlorotriazine metabolites were quantified in the urine samples collected from all individuals, as well as in the blood samples collected from one individual at various intervals until the end of the seven-day study period. The peak plasma levels of DEA were detected within two hours and rapidly declined thereafter, with a half-life of 2.8 hours. Decreased levels of DEA corresponded with increased plasma levels of DACT, which peaked at five hours after dosing. The elimination half-life of DACT was approximately 18 hours. These results suggested a step-wise dealkylation of atrazine to DEA and then to DACT. In urine, DEA, DIA, and DACT accounted for 5.4%, 1.4% and 7.7% of the AD, respectively. However, a significant portion (85%) of the AD was not recovered in blood or urine during the study period and remained unaccounted for. Taking into account the limitations of this study, the kinetics and metabolism profile of atrazine in these human volunteers was similar to that observed in rats and monkeys.

The USEPA and the registrant have developed physiological-based pharmacokinetic (PBPK) models for atrazine and chlorotriazines. PBPK models typically consist of a series of mathematical representations of biological and physiological processes in the body that simulate the toxicokinetic and toxicodynamic properties of a substance that enters an organism. PBPK models may be used to refine the interspecies and intraspecies extrapolation factors used in developing reference values for risk assessment, in characterizing route-to-route extrapolation, and to assist with interpretation of other similar types of toxicokinetic data. Given that a Special Review is intended to focus on the aspect(s) of concern, the available information related to further refinement of toxicology reference values using PBPK models was not required, and therefore, was not included in the scope of this assessment.

Acute toxicity profile

Based on existing reviews, atrazine was determined to be of low to slight acute oral toxicity in rats and mice. It was of low acute dermal and inhalation toxicity in rats. It was non- to slightly-irritating to rabbit skin, and non- to mildly-irritating to the rabbit eye, and caused skin sensitization in one of the two available maximization tests in guinea pigs.

Short-term toxicity studies

In short-term guideline toxicity studies in rats, dogs and rabbits, common effects indicative of systemic toxicity across species were reduced body weight and food consumption. In the rat oral dietary studies, changes in several organ weights such as decreased liver and kidney weights and an increased incidence of splenic hemosiderin deposits were also observed, while in dogs there was evidence of marked cardiac toxicity.

In the 12-month dietary toxicity study in dogs, toxicity was restricted to the highest dose tested. Clinical signs of toxicity observed throughout the study were consistent with results of the electrocardiographic assessment as well as gross and histopathological findings in the hearts of these animals. These treatment-related findings included ascites, dyspnea, and laboured and shallow breathing. Observed electrocardiographic changes included a moderate increase in heart

rate as well as moderate decreases in the height of the P-wave and in the PR and QT values. The gross pathological lesions consisted of moderate to severe dilatation of right and/or left atria, and in some dogs, a fluid-filled pericardium and enlarged and soft hearts. Histopathological findings included myolysis and focal atrophy of myocardial fibres, and oedema of the heart. Cardiac toxicity was also observed in the long-term dietary oncogenicity study in mice as a dose-related increase in the incidence of cardiac thrombosis in both sexes, beginning at the mid-dose.

Short-term dermal administration of atrazine to rabbits, at the limit dose of testing, produced dermal irritation at the test site. Increased spleen weight was also observed with changes in some of the associated hematological and clinical chemistry parameters. Other signs of systemic toxicity, such as decreased body weight and body weight loss, were also noted.

Studies investigating preovulatory luteinizing hormone (LH) surge and estrous cyclicity

Perturbation of the neuroendocrine system, particularly, the hypothalamus-pituitary-gonadal (HPG) axis, as evidenced by the attenuation of the pulsatile LH secretion and the preovulatory LH surge, is the hallmark of atrazine toxicity in rats and provides the most sensitive endpoint and point of departure (POD) for risk assessment. Suppression of the pulsatile LH levels is the key event of the cascade of changes leading to adverse reproductive and developmental effects. The most sensitive apical endpoint associated with the attenuation of the LH surge is the disruption of the estrous cycle in regularly cycling female rats. Effects of atrazine on the suppression of LH levels and/or estrous cyclicity have been extensively investigated. Available repeat-dose studies cover a wide dose range, and include dietary or gavage exposures, guideline and non-guideline formats, and different strains of rats. Overall, there was evidence of durational effects, as characterized by attenuation of the LH surge and the subsequent disrupted estrous cyclicity occurring at doses of greater than 100-fold lower in long-term studies compared to the studies of shorter duration in Sprague-Dawley (SD) rats. Based on studies of similar duration across several strains of rats, Long-Evans (LE) rats were considered the strain most sensitive to an attenuation of the LH surge. However, there was a lack of robust studies of longer duration in LE rats examining this effect and/or alterations of estrous cyclicity. The uncertainty resulting from the lack of a long-term study investigating these endpoints in the most sensitive species is addressed through the application of a database uncertainty factor (refer to Section 1.2).

As noted above, the mechanism of LH suppression has been extensively investigated in several strains of rats, using various study designs and dosing strategies. Many of these non-guideline short-term oral mechanistic studies included female rats that received standard bilateral ovariectomies along with a constant subcutaneous dose of estradiol to generate less variable and more reliable hormone data from synchronized cohorts of female animals. In the intact female rat exhibiting a normal 4-day estrous cycle, estradiol begins to rise on diestrus day 2, reaching its apex on the day of proestrus. This helps promote the secretion of gonadotropin-releasing hormone (GnRH) and the subsequent surge of LH on the afternoon of proestrus. Thus, studies that included sampling of LH levels at 2-hour intervals during the afternoon of the proestrus stage produced more reliable LH data. Several studies have shown that the brain is the site of the molecular initiating event (MIE) for atrazine to suppress LH levels (PMRA# 2945607, USEPA 2018; see subsequent sections below for detailed discussions of the atrazine neuroendocrine mode of action (MOA)). For studies of shorter duration, the timing and method of dosing were critical in the design of high quality mechanistic studies because of reliance on peak plasma

concentration levels of atrazine and chlorotriazine metabolites to produce an effect on LH levels. Overall, given the hormonal profile of the estrous cycle and toxicokinetic and toxicodynamic properties of atrazine, the timing and the method of dosing as well as post-surge hormonal sample collection required optimization to produce reliable LH data.

Therefore, studies that ensured that atrazine and/or chlorotriazine metabolites would be available at the target site of action at the time of the critical period for the neural trigger of the LH surge, thus allowing for the mechanism of LH suppression to be elucidated following oral gavage dose administration of atrazine, were considered of higher quality for the hazard characterization.

Of all the available short-term toxicity studies, a non-guideline 4-day oral gavage toxicity study (PMRA# 2945603, 2945604 and 2945570, Cooper et al., 2007 and 2010[5]) in intact regularly cycling female LE rats generated the most robust LH data. The robust design of this study was in part due to the inclusion of a high number of doses, particularly in the low dose range, and a narrow gap between the dose groups. In addition, this study included the following key elements in its design in order to generate the most robust and reliable LH surge data:

1) The strain of rat most sensitive to the attenuation of LH surge elicited by atrazine was selected, namely LE rats (further discussed below).
2) During the pre-treatment period: the ovarian cycle of each female rat was monitored for a period of two weeks by daily vaginal lavages. Only female rats displaying regular, 4-day estrous cycles during the two week pre-treatment period were included in the experiments.
3) During the treatment period: cohorts of LE female rats were dosed on each day of the estrus cycle with the last dose given on proestrous day. The afternoon spontaneous LH surge was evaluated in large cohorts of animals from each dose group via blood sample collections at 12:00, 14:00, 16:00, 18:00 or 20:00 hours.
4) Animals were required to meet the following three criteria for inclusion in the results: a) a proestrous vaginal smear, b) an increase in uterine weight (>500 mg), and c) elevated levels of progesterone.

In this study, attenuation of the LH surge was observed beginning at the second lowest dose following the four day dosing period. This effect was characterized by a statistically significant and dose-related reduction in measured LH levels at the 18:00 hour time point on the day of proestrous. This study achieved the lowest NOAEL for the attenuation of the LH surge across the database, which was considered the most appropriate POD for establishing the toxicology reference values for atrazine.

A supplemental oral gavage mechanistic study (PMRA# 2816728 and 2016034, Coder, 2011a) also examined the attenuation of LH surge over a single estrous cycle in intact female LE rats using several study design elements that were the same as those of the 4-day oral toxicity study (PMRA# 2945603, 2945604 and 2945570, Cooper et al., 2007 and 2010) above. While

[5] This study was conducted by the Reproductive Toxicology Division of National Health and Environmental Effects Research Laboratory of USEPA. This study was published in 2007 in the Birth Defects Research journal while additional details for this study were available in unpublished USEPA reports submitted to USEPA SAP meetings for consideration and inclusion in the atrazine human health risk assessment.

reductions in LH levels were observed at all doses within this study, they did not show a dose-related pattern, and statistically significant attenuation of the LH surge was restricted to the high dose in this study. However, there was uncertainty in whether the LH data included measurements from the females with confirmed vaginal proestrous smears. This uncertainty, combined with the variability in the results, constituted a major limitation of this study. High variability in the data also precluded treatment-related determinations for the other measured hormones in this study. Several other supplemental studies (PMRA# 2816746 and 2816011, Coder, 2010a; PMRA# 2816736 and 2816024, Coder et al., 2011b; PMRA# 2816739 and 2816026, Coder et al., 2011c), performed at the same laboratory with similar study designs, had the above-noted as well as other significant limitations, such as animals dying as a result of complications from ovariectomies, and missing sections of some study reports.

In a supplemental single oral gavage dose study (PMRA# 3292815, Goldman et al., 2011) in ovariectomized (OVX) female SD rats, in which atrazine treatment was on the day of presumed vaginal proestrous, an increased LH surge was observed compared to that of the control group. The increase in LH surge was characterized by examining the peak amplitude and the amount released. The study authors and the USEPA SAP members attributed this effect to the role of atrazine-induced increased levels of progesterone. (For more details, see section on the hypothalamus-pituitary-adrenal (HPA) axis hormone studies). An earlier single or repeat dose oral gavage study (PMRA# 2945601, Cooper et al., 2000) was conducted by the same laboratory with the primary objective of characterizing differences between rat strains and the effect of duration of dosing on the response to atrazine effects on LH surge and ovarian function. In the first set of experiments, attenuation of the LH surge was observed in high-dose OVX female LE rats, while no effect was observed in OVX female SD rats at the same dose. Following a 3-day dosing period where OVX was performed on day 0, suppression of LH and prolactin surges were observed at all doses and at the second highest dose in the LE rats and SD rats, respectively. In the LE rats, increased pituitary prolactin levels were also observed that were consistent with decreased serum prolactin levels across all doses. Following a 21-day dosing period, LH suppression was observed at all doses in LE rats and beginning at the mid-dose in SD rats, respectively. In all of these studies, suppression of the LH surge was characterized by a statistically significant reduction in serum LH levels in treated animals compared to the control animals. These studies demonstrated that female LE rats were more sensitive to the LH and prolactin suppressive effects of atrazine than female SD rats. These studies also demonstrated evidence for durational effects as characterized by the attenuated LH levels noted at lower doses in experiments with longer duration of dosing compared to those with shorter duration. There was no treatment-related effect on the number of oocytes with atrazine up to 300 mg/kg bw and pseudopregnancy was observed following high dose treatment over three days.

A number of supplemental oral gavage mechanistic studies (PMRA# 2815995 and 2816757, Foradori et al., 2009a; PMRA# 2815998 and 2816756, Foradori et al., 2009b) examined the role of GnRH neurons in the atrazine-attenuated LH surge over the span of one estrous cycle in OVX Wistar rats. GnRH neuronal activation was examined using immediate early gene product FOS (cFOS) as a marker. A statistically significant reduction in activated GnRH neuronal activity was observed starting at the mid-dose following four days of dosing. The mechanism of atrazine GnRH disruption was examined as part of another mechanistic oral gavage study (PMRA# 2945603, 2945604 and 2945570; Cooper et al., 2007 and 2010) in intact LE rats. Altered GnRH regulation, as evidenced by statistically significant increased GnRH levels in the median

eminence region of the hypothalamus, was observed beginning at the second highest dose. Increased GnRH levels remaining in the hypothalamus is consistent with the lower levels reaching the pituitary to produce an optimal LH surge. Thus, this finding was supportive of the atrazine neuroendocrine MOA. In summary, these studies demonstrated that atrazine interference with central mechanisms controlling GnRH neuronal activation in the hypothalamus is the potential site of the MIE for its neuroendocrine MOA.

Several oral gavage or dietary mechanistic studies examined ovarian function and estrous cyclicity following various dosing periods. In a 21-day oral gavage mechanistic study (PMRA# 2945602, Cooper et al., 1996), estrous cyclicity alterations were observed at all doses in intact SD and LE female rats, with LE rats displaying altered cyclicity more frequently compared to SD female rats. Decreased body weight was also observed in the LE rats at the same doses that produced altered cyclicity. At higher doses, an increased incidence of repetitive pseudopregnancies was observed in both strains. However, ovarian regression and anestrus were observed only in the LE rats. This study further affirmed that LE female rats are more sensitive than the SD strain to the attenuation of the LH surge elicited by atrazine. In a different oral gavage mechanistic study (PMRA# 3292817, Shibayama et al., 2009) in SD rats, alterations of estrous cyclicity and histopathological lesions in the reproductive organs, as characterized by lobular hyperplasia in the mammary gland, were observed at lower doses following a 4-week compared to a 2-week dosing period. In another 4-week oral gavage mechanistic study (PMRA# 1167781, 1167779, 1167780 and 1180052, Morseth 1996a) in intact female SD rats, decreased body weight gain, increased estrous cycle alterations and attenuation of the LH surge were observed beginning at the mid-doses. A subsequent oral dietary mechanistic study (PMRA# 1180044, Morseth 1996b) in intact female SD rats, conducted by the same investigators and laboratory as the study above, examined estrous cyclicity parameters over a 6-month dosing period. Since the female rats were about two months old before they were included in this study, a 6-month dosing period was selected to avoid confounding effects on cyclicity parameters due to the normal reproductive senescence exhibited in female SD rats around 9 months of age (PMRA# 2945614, USEPA, 2010). An attenuated LH surge and altered estrous cyclicity were observed starting at the mid-dose. In the last several weeks of the study, the estrous cycle alterations were more pronounced. At the high-dose, decreased body weight and body weight gain as well as increased incidences of enlarged pituitary and thickened mammary glands were observed. Both of these studies clearly demonstrated the potential for atrazine to produce LH suppressive effects at lower doses following extended dosing periods in SD rats. The second study also covered the estimated time period needed for atrazine and its metabolites to reach plasma-to-brain steady state. However, high variability of the LH data as well as uncertainty as to whether LH data were obtained from females with confirmed vaginal proestrus were limitations in both of these studies.

Genotoxicity

Atrazine was assessed for genotoxicity through an extensive battery of in vitro and in vivo tests. There were two in vitro Comet assays available, one was considered acceptable and the other supplemental. Although there was some indication of increased DNA damage in the supplemental study, the DNA damage was observed only at cytotoxic doses. Similarly, an in vivo Comet assay in mice showed DNA damage at doses considered to be overtly toxic to the animals. Multiple in vitro sister chromatid exchange (SCE) and unscheduled DNA synthesis

(UDS) assays showed a lack of genotoxicity. A series of bacterial reverse gene mutation assays indicated that atrazine was not mutagenic. Increased micronucleus frequency was limited to a single, supplemental in vivo study in the presence of overt toxicity and mortality in mice. No increase in micronucleus frequency was observed in other in vitro and in vivo studies. Acceptable in vitro chromosomal aberration assays did not show any changes in the frequency of chromosomal aberrations. Male germ cell genotoxicity assays were also available for consideration. Two chromosomal aberration studies in spermatocytes did not demonstrate evidence of genotoxicity. Atrazine was also negative for male germ cell genotoxicity in dominant lethal assays. Overall, the weight of evidence based on the review of a wide array of genotoxicity assays indicated that atrazine was not genotoxic.

Chronic toxicity/Carcinogenicity studies

In a guideline 18-month mouse dietary oncogenicity study (PMRA# 1234783, 1233356 and 1233357, Hazelette and Green 1987), administration of atrazine resulted in a dose-related increase in the incidence of cardiac thrombosis in both sexes, beginning at the second highest dose. Changes in hematological parameters in both sexes, a marked decrease in the body weight and increased mortality in females were observed at the highest dose. There was no evidence of tumourigenicity in mice.

In a guideline 24-month dietary chronic toxicity/carcinogenicity study conducted with SD rats (PMRA# 1203786, 1203787, 1203788, 1203789, 1203790, 1203791 and 1204001 Mayhew et al., 1986), systemic toxicity, consisting of dose-related decreases in body weight and body weight gain in both sexes, was observed beginning at the second highest dose. The magnitude of this effect early in the study was slight, but progressively worsened over the course of the study, resulting in marked reductions in the final values. There were also increased incidences of retinal degeneration in both sexes, as well as increased incidences of myeloid hyperplasia in the bone marrow of femur and sternum, and splenic extra-medullary hematopoiesis in female rats at the same dose. Additional findings at the highest dose included an increased incidence of degeneration of rectus-femoris muscles in both sexes, increased incidences of epithelial hyperplasia in the prostate, acinar hyperplasia of mammary glands, and calculi in the renal pelvis in males, and decreased survival and lower red blood cell indices, increased incidences of transitional epithelial hyperplasia in the bladder and kidney, and an increased incidence of centrilobular liver necrosis in females.

Evidence of carcinogenicity in female SD rats consisted of treatment-related increased incidences of mammary gland adenocarcinomas and fibroadenomas, which were observed starting at the lower mid dose. These findings were further supported by evidence from numerous mechanistic carcinogenicity studies where dose-related increased incidences of mammary tumours were observed in female SD rats. Subsequent to the conduct of this study, extensive research has been conducted to explore the underlying mechanism(s) responsible for the formation of these tumours. The research to date has established that certain other members of the triazine chemical class also have the potential to produce mammary gland tumours in female SD rats following long-term repeat dosing.

Briefly, the mechanism(s) of the mammary gland tumour formation in female SD rats was explored in four acceptable non-guideline dietary chronic toxicity studies (PMRA# 1167680, 1167765 and 1167774, Pettersen et al., 1995; PMRA# 1135430, 1135427, 1159810 and 1167679, Thakur 1991a; PMRA# 2815961 and 2816711 Thakur 1992a; PMRA# 1078579 and 1078580, Morseth 1998), with the primary objective of investigating the mechanism(s) of the mammary gland tumour formation in female SD rats. The potential for atrazine to produce tumourigenic and non-tumourigenic effects in various other endocrine-related organs were also assessed in these studies. Increased incidences of mammary gland tumours characterized by adenomas, carcinomas, and/or fibroadenomas with or without an earlier onset (relative to controls) were observed in two of these four studies (Pettersen et al., 1995 and Morseth 1998). In the two other studies (Thakur 1991a and Thakur 1992a), there was an earlier onset of mammary tumours relative to controls without any increase in their overall lifetime incidence. One of the studies above (Morseth 1998) also included an assessment of the tumourigenic potential of atrazine in an OVX cohort of female rats. Neither increases in the incidences of the mammary-gland proliferative changes nor mammary tumour formation were observed, suggesting that the mode of action for mammary gland tumours caused by atrazine in SD rats is related to ovarian function.

Two further acceptable non-guideline dietary long-term studies (PMRA# 1115083, 1115084, 1115085, 1135415, 1159809 and 1167679, Thakur 1991b; PMRA# 1123336, 1123316, 1123317 and 1150103, Thakur 1992b) investigating the tumourigenic potential of atrazine in female F344 rats produced no evidence of proliferative changes or tumours in the mammary gland.

In a 127-week non-guideline dietary mechanistic carcinogenicity study (PMRA# 3292818, Pinter et al., 1990) conducted in F344 rats and supported by the International Agency for Research on Cancer (IARC), decreased body weight was observed in both sexes. The study authors reported statistically significant increases in survival and increased incidences of benign mammary gland tumours in high dose males. In the high dose females, the study authors reported statistically significant increases in uterine adenocarcinomas and combined leukemia and lymphomas. However, Health Canada could not conclude that there was a carcinogenic response in this study due to significant flaws in the study design, and inadequate histopathological examination of animals across all doses, as well as reporting deficiencies and errors in data summaries. Overall, this study was considered unacceptable. Other international regulatory agencies as well as independent scientists at the USEPA SAP meetings reached the same conclusion for this study.

Assessment of the MOA for the formation of mammary gland tumours

As demonstrated in the mechanistic short-term and carcinogenicity studies discussed above, atrazine has the potential to produce mammary gland tumours in intact female SD rats, but not in F344 rats, OVX female SD rats or mice. As noted above, the underlying MOA responsible for the formation of mammary gland tumours in female SD rats has been the subject of intensive research by the international scientific community and in-depth scrutiny by multiple regulatory authorities as well as the USEPA SAP meeting proceedings. This work led to the extensive evidence that atrazine causes mammary gland tumours in the female SD rat via a neuroendocrine MOA involving premature (accelerated) reproductive senescence.

The key events (KEs) of this MOA as are as follows:

> KE1: hypothalamic effects resulting in changes in catecholamine function and regulation of the pulsatile release of GnRH;
> KE2: attenuation of LH surge (released from the pituitary gland); the attenuated LH surge is of insufficient amplitude or duration to trigger ovulation;
> KE3: repetitive failure to ovulate results in persistent secretion of estrogen/estradiol from the ovarian follicles;
> KE4: increased prolactin release by the pituitary as a secondary consequence resulting from the elevated estrogen level;
> KE5: prolactin and estrogen-induced proliferative processes in the mammary gland leading to tumour formation.

Health Canada also conducted an independent evaluation of all sources of information and data related to the MOA for atrazine in the formation of mammary gland tumours as part of PACR2003-13 and also considered the more recent information. A summary of this evaluation is briefly discussed herein. Altered GnRH regulation (Cooper et al., 2007; Foradori et al., 2009b), as well as attenuation of the LH surge, observed across several mechanistic studies (Cooper et al., 1996-2010; Morseth 1996b), supported that the initial KEs of this MOA occur in female SD rats. Evidence of altered estrous cyclicity was consistently noted across many repeat-dose toxicity studies in female SD rats. This finding supported KE2. Increased estradiol and prolactin levels, and corresponding stimulation of mammary gland tissue, as evidenced by increased incidences of mammary galactoceles, secretory activity and lobular development were observed at the 9-month time point in a 24-month serial necropsy study (Thakur 1991a). These findings were supportive of KEs 3–5. Long-term studies of atrazine's carcinogenic potential in female SDs rats demonstrated an increased incidence of mammary gland tumours starting at the 9 month time-point (Thakur 1991a; Pettersen et al., 1995; Thakur 1992a; Mayhew et al., 1986; Morseth 1998), which was supportive of the final KE5 of this MOA. The strength, consistency and biological plausibility of the MOA were demonstrated by the evidence of each KE being observed in a dose- and temporal-concordant manner across the database. The available data also demonstrated that when this MOA and associated endocrine physiology were not operative, as is the case in mice, F344 rats and male or OVX female SD rats, treatment-related mammary gland tumours were not observed.

There were some minor inconsistencies in the available MOA data. The exact MIE has not been adequately characterized. Therefore, the dose concordance relationship between the MIE and the subsequent suppression of GnRH secretion during the estrous cycle could not be clearly established. GnRH neuronal activity was also altered at higher doses than those that suppressed LH. However, all downstream KEs from the point of decreased hypothalamic secretion of GnRH were clearly defined and supported by available data. Robust data on key hormone levels to support and strengthen the evidence for the middle KEs were also not available. For example, increased estradiol and prolactin levels were observed only in a single study at the 9-month sample collection time point.

However, indirect examination of these KEs occurred via histopathological correlations, such as the observation of increased incidences of mammary gland secretory activity and galactoceles, which provided indirect evidence for prolonged exposure to prolactin levels. Finally, it is uncertain whether other peripheral factors, such as a disruption of the hypothalamus-pituitary-adrenal (HPA) axis, could play a facilitatory role within this MOA.

The weight of evidence assessment of the data did not support alternative MOAs. There was no evidence of a direct estrogenic response and no evidence of genotoxicity. While the available data do reflect varying levels of response in estrogen-related endpoints across species and studies, the potential for interaction with the estrogenic pathways is supported by later KEs in the neuroendocrine MOA discussed above. Numerous in vitro studies also showed evidence of increased aromatase (CYP19) mRNA expression/enzyme activity. However, the weight of evidence from robust in vivo data did not support an MOA involving aromatase upregulation.

In summary, despite some minor inconsistencies, robust data from reliable, well-conducted guideline and non-guideline studies support the biological plausibility of the MOA as well as the weight of evidence linking the KEs with the formation of mammary gland tumours in female SD rats specifically.

The USEPA (PMRA# 2945607), APVMA (PMRA# 2815962), the Joint Meeting on Pesticide Residues (JMPR) (PMRA# 2815961) and the USEPA SAP meetings (PMRA# 2945614) as well as other members of the scientific community (PMRA# 3304257, Meek et al., 2003; Cooper et al., 2007) have extensively assessed all available atrazine toxicity data in accordance with the IPCS framework for evaluating a carcinogenic MOA (PMRA# 3304258, Sonich-Mullin et al., 2001) and established that the available data supported the above MOA for the formation of mammary gland tumours.

The KEs of the neuroendocrine MOA listed above also describe the normal physiological process and cause of reproductive aging in female SD rats, which is accelerated by exposure to atrazine. However, reproductive aging in humans is mediated via different physiological processes. Within this context, the basic physiological differences in reproductive cycles as well as in reproductive aging in female SD rats, compared to humans, inform the relevance of the mammary gland tumours produced by atrazine for the human health risk assessment. The key aspects of the physiological differences include, but are not limited to, the fact that the main driver for LH surge in female SD rats is the brain regulation of GnRH, while in women, it is ovarian estrogen. Reproductive aging is also characterized by depletion of oocytes in women, in contrast to LH failure in female SD rats. Therefore, the mammary gland tumours, when occurring via the MOA involving acceleration of reproductive senescence in female SD rats, are not considered relevant for the human health risk assessment. However, LH has many vital functions in humans and thus the disruption of early KEs, such as attenuation of the LH surge, may lead to adverse effects on HPG function through other endocrine pathways, which are considered relevant to humans. The scientific community, as noted above, has also assessed the atrazine data in accordance with the IPCS human relevance framework (PMRA# 3304256, Boobis et al., 2006) and reached the same conclusions.

Reproductive and developmental toxicity studies

Numerous guideline and non-guideline developmental and reproductive toxicity studies were available. None of these studies evaluated key endpoints such as sperm morphology, prostate histopathology and ovarian follicle count parameters, following extended dosing during pre-and postnatal periods as well as during adulthood. However, the evidence of pre- and postnatal toxicity noted across this group of studies was consistent with the downstream effects of the overarching atrazine MOA of HPG/LH suppression in rats.

In the guideline dietary 2-generation reproductive toxicity study (Unpublished study: PMRA# 1233367 and 1233368, Mainiero et al., 1987; Published study: PMRA# 2816056 and 2816783 DeSesso et al., 2014) in SD rats, parental and offspring toxicity consisted of decreased body weight and body weight gain, which were observed at the highest dose tested. There was no evidence of sensitivity of the young in this study. No treatment-related effects were observed on the reproductive parameters that were assessed. However, the data for several of these parameters were highly variable, thus hampering the identification of treatment-related effects. Furthermore, several parameters required in the current Organisation for Economic Co-operation and Development (OECD) reproductive toxicity test guideline, such as ovarian follicle counts, estrous cycle length and periodicity, sperm parameters (motility and morphology), and onsets of puberty, were not assessed, as this study predated the current OECD guideline. However, several of the parameters not assessed in this study were investigated in additional mechanistic studies (described below).

Two guideline rat gavage developmental toxicity studies were available for atrazine, both of which were conducted with SD rats. In both studies, maternal toxicity consisted of decreased body weight, body weight gain, and food consumption, as well as increased incidences of clinical signs of toxicity, such as salivation, at the highest dose. In the first study (PMRA# 1137002, Infurna 1984), increased mortality and post-implantation loss were also observed at the highest dose, a dose that exceeded the maximum tolerated dose and was close to the limit dose of testing. In fetuses, increased incidences of skeletal variations, such as incomplete ossification of the skull bones, were observed at the mid-dose. Due to the significant number of mortalities in dams and the marked reduction in fetal weight, limited fetal evaluations were conducted at the high dose. Fetal effects were observed at a dose that did not cause maternal toxicity in this study, which suggests sensitivity of the young. However, the large dose spacing used in the study may have contributed to this observation. A narrow and a more optimal dose range was selected in the second study (PMRA# 1233374, Giknis 1989). In the fetuses, there was an increased incidence of incomplete ossification of various skull bones at the high dose, which was also toxic to the maternal animals. There was no evidence of treatment-related malformations or sensitivity of the young in this study. In a gavage rabbit developmental toxicity study, significant maternal toxicity in the form of reduced body weight gain and food consumption was observed at highest dose. Body weight loss during the first few days of treatment and increased post-implantation loss and resorptions were also observed at this dose. Two dams were necropsied due to signs of impending abortion at the same dose.

Fetal effects in this study included increased incidences of incomplete ossification of appendicular elements, as well as decreases in the number of live fetuses and reduced fetal body weight. Taken together, these studies showed similar patterns of maternal and developmental toxicity, with no evidence of treatment-related malformations or sensitivity of the young.

Several oral gavage mechanistic developmental toxicity studies in rats were available that examined the effects of atrazine during critical periods of gestation or lactation. This was considered an important area of atrazine research given its neuroendocrine MOA, the vital roles of prolactin in pregnancy initiation early in gestation, and the role of LH in the maintenance of pregnancy in mid-gestation in rats. In one of the first such studies[6] (PMRA# 3292819, Cummings et al., 2000), pregnant female cohorts of four different strains of rats (LE, SD, F344 and Holtzman (HLZ)) were treated during gestation days (GD) 1-8. Increased pre-implantation loss in F344 rats and post-implantation loss in HLZ rats were observed at the high dose. Decreased body weight in all strains, decreased serum progesterone in HLZ rats, and decreased LH levels in HLZ and LE rats were also observed in maternal animals. In a follow-up study (PMRA# 2945579, Narotsky et al., 2001) conducted by the same laboratory, dams from three different strains of rat, F344, LE, and SD, were dosed from GD 6-10 to examine the effects of atrazine on pregnancy maintenance. Full litter resorptions were observed starting at the mid-dose in F344 rats and at the high dose in the other two strains. Body weight loss was noted starting at the low-dose in F344 and SD dams and at the high dose in LE rats. Delayed parturition was also observed in the high-dose SD and F344 dams.

In another mechanistic study examining effects of early postnatal exposure to atrazine (PMRA# 2945583, Stoker et al., 1999), maternal Wistar rats were dosed from lactation days (LD) 1 to 4. Starting at the intermediate-low dose, suckling-induced prolactin release (serum levels) was inhibited in dams, and an increased incidence of prostatitis was observed in the male offspring of these dams, when assessed on postnatal days (PND) 70 and 170.

A modified oral gavage 1-generation reproductive toxicity study in SD rats (PMRA# 2816744, 2816014, 2816022, and 2816741, Coder 2011d) examined the effects of atrazine treatment on female pubertal development. Parameters assessed included LH surge and estrous cyclicity changes using multiple cohorts subjected to dosing during gestation, lactation, and/or peripubertal periods. When dams were treated from GD 0 to LD 20, there was an increased incidence of pups with no milk in the stomach as well as decreased pup survival at the high dose. Delayed vaginal opening (VO) was observed in the female offspring when treated in utero, via milk and peripubertally at the same dose. Several limitations in reporting and design of this study were noted. For example, the study authors stated that the timing of the blood sample collections were not ideal for detecting hormonal effects. In addition, estrous cyclicity data were not organized in tables with summaries of means and standard deviations across doses to facilitate interpretation. Furthermore, SD rats are not the most sensitive strain to the effects of atrazine on pregnancy and onsets of puberty.

In a 4-day oral gavage mechanistic study (PMRA# 2816740 and 2816023, Coder et al., 2011e), cohorts of SD or LE female rats were dosed with atrazine over the duration of one estrous cycle immediately before mating to assess fertility and reproductive performance. An additional cohort

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[6] Conducted by the USEPA Office of Research and Development (ORD) laboratories.

of LE female rats received atrazine in the diet for four days. Decreased ova and corpora lutea counts were observed in the SD females starting at the lowest dose tested. The same effect was noted at the highest dose tested in LE females, whether treatment was via either gavage or diet. Increased total resorptions and/or post-implantation loss were also observed in SD females at the highest dose. This last finding was consistent with the overarching atrazine MOA of HPG/LH suppression, suggesting that LH and prolactin surges did not completely rebound to sustain pregnancy following cessation of dosing at mating. This is further supported by the available toxicokinetic data, which indicate that atrazine and its chlorotriazine metabolites can cross the BBB and have an extended retention in brain tissue (with an estimated half-life of elimination of 10 days). Several limitations in the design and reporting of this study prevented the determination of whether the rest of endpoints measured in the study were related to treatment. For example, estrous cyclicity data were not reported in a manner that could inform and facilitate analysis of LH surges. Since female rats are considered as induced and spontaneous ovulators, the act of mating included as part of the study design further confounded the interpretation of the fertility and reproductive performance data.

In two oral gavage mechanistic developmental studies (PMRA# 2816730 and 2815991) in Wistar rats, dams were treated with atrazine during gestation or lactation to examine effects on the reproductive system of their male offspring, which were assessed on either PND 70 or PND 170. In the cohort treated during gestation, decreased body weight gain and food consumption, an increase in total litter losses, and decreased litter size and viability as well as decreased weaning indices were observed in the dams. In the F1 male pups, increased mortality and decreased weight were observed at the high dose. The effects on the F1 male reproductive system included dose-related decreased weights of several organs. At higher doses, increased percent abnormal sperm and decreased sperm and spermatid numbers were observed. Due to excess pre- and post-natal mortality, and a low number of remaining male animals, reproductive endpoints were only evaluated at the high dose on PND 70. In the cohort treated during the lactation period, no effect on pup viability or litter size was observed. The other effects observed in dams and offspring were similar to those observed in the cohort that were dosed during the gestation period. However, the effects were less severe and observed at higher doses. In a mechanistic developmental gavage study (PMRA# 2816792, Rayner et al., 2007) in LE rats, a cross-fostering design was used to examine effects on male reproductive endpoints with a primary focus on the prostate gland and onset of puberty. The F1 cohorts receiving post- or peri-natal treatment had more significant prostate effects when examined on PND 120 or PND 220 than the cohort receiving treatment in utero only. These effects were characterized as increased incidence and severity of inflammation in the lateral prostate and decreased myeloperoxidase (MPO) activity levels. The results of this study were consistent with those of another similar study (Stoker et al., 1999).

Overall, the series of non-guideline studies discussed above provided robust mechanistic information, including examination of currently required endpoints that were not examined in the older guideline 2-generation reproductive toxicity studies or developmental toxicity studies. These studies included examination of the young animal following dosing with atrazine over the critical period of development in utero as well as the reproductive parameters such as ovarian follicle counts, estrous cycle length and periodicity, and sperm parameters (motility and morphology), following short-term dosing during vulnerable windows of susceptibility. Although a multi-generational study examining the above-noted endpoints following longer

durations of dosing was not available, taking the results of this group of studies together, the pattern of effects observed was consistent with the downstream effects of the overarching neuroendocrine MOA of atrazine. There was also no evidence of increased sensitivity of the young compared to the parental animals across this group of studies. The lowest NOAELs across this group of studies were several fold higher than the NOAELs that were based on LH suppression in key studies, including the non-guideline 4-day oral gavage toxicity study (PMRA# 2945603, 2945604, and 2945570, Cooper et al., 2007 and 2010) in intact regularly cycling female LE rats, the latter of which is the basis of the revised toxicology reference doses provided in Section 1.2.

Studies on mammary gland development

Several unpublished and published oral gavage mechanistic developmental toxicity studies designed to evaluate the effects of atrazine on the development of the mammary gland were available for review. These studies used different types of study designs, including cross-fostering modules, and dosing of maternal animals during critical windows of in utero development of the young. In two of the first such published studies (PMRA# 2816791 and 2816793, Rayner et al., 2004 and 2005) in LE rats, conducted by the USEPA ORD, pregnant dams were dosed during late gestation to examine the potential for any treatment-related effects on the development of the mammary glands in their female offspring. Stunted epithelial development in the mammary glands of offspring from treated dams were observed at the only dose tested in both studies. This was characterized by a decreased area of the glands, as well as fewer terminal end buds (TEB), and lower density of epithelial branches compared to control females when examined at various postnatal time points. The effects on mammary glands of F1 females were observed when assessed on PND 67 and in F2 females when assessed on PND 4. Decreased body weight gain was observed in maternal animals in both of these studies. In a subsequent unpublished study (PMRA# 2816001, Coder, 2010b), pregnant LE rats were dosed late in gestation to assess treatment-related effects on the development of mammary glands in the offspring across multiple doses. Decreased body weight and body weight gain were observed at the mid-dose in the maternal animals. Structural changes, such as increased ductal length and number of TEBs, in the mammary gland of the female offspring were observed starting at the mid-dose when examined at various postnatal time points. The cross-fostering and pair-feeding modules of this study, which used the animals from the high dose group, were terminated on PND 2 due to several reported issues, including maternal aggression, poor nesting behaviour, and alienation and cannibalization of the pups.

In an attempt to repeat the above study (PMRA# 2816726, Hovey et al., 2011), a similar design without a cross-fostering module was used across multiple doses to better characterize effects on mammary gland development. A blinded and quantitative method for the assessment of key morphological features and structures of mammary glands using a whole mount technique was used. Specifically, ductal elongation, ductal network area, epithelial area, TEB incidence, and epithelial density as well as epithelial proliferation within different parenchymal structures were assessed on PNDs 1, 21, 33, day of VO, and as adults. Decreased ductal network area and lower epithelial density was noted, starting at the mid-dose, across the various postnatal time points that were assessed. However, the developmental ontology and pattern of these effects did not show consistency across all the different time points assessed. This inconsistency, combined with the lack of clearly dose-related changes in some of these measurements as well as the presence of

systemic toxicity in the dams and in the offspring at the same dose levels, confounded the toxicological interpretation of these findings. In the only study (PMRA# 2816025 and 2816805, Davis et al., 2011a) conducted in SD rats among this group of studies, pregnant dams were grouped in two different types of dosing regimens that consisted of either once daily or twice daily gavage dosing, the latter resulting in the same overall dose levels as single daily doses. The goal of the twice daily dosing was to study the effects of maintaining a longer steady-state level of atrazine in its unmetabolized form. Mammary glands were assessed using the whole mount technique on PND 45, which was shortly after attainment of puberty. No treatment-related effects were observed on mammary gland development; however, the study lacked detailed reporting of the data. Other signs of toxicity included slightly increased post-implantation loss and an increased rate of pup mortality shortly after birth at the high-dose, regardless of the type of dosing regimen used. Signs of offspring toxicity included decreased body weight and a statistically significant delay in the time to reach VO at the same dose in both types of dosing regimens. The twice daily dosing regimen produced slightly more toxicity in the same parameters, especially in the rate of pup mortality shortly after birth, compared to the once daily dosing. This pattern of toxicity, specifically the higher rate of pup mortality following the twice daily dosing regimen, was also observed in a second study conducted by the same laboratory (PMRA# 3292813, Fraites et al., 2011b). Other notable findings relating to additional developmental parameters that were assessed in this study are discussed in the following sections.

Overall, this group of studies examined mammary gland development over a critical period of development and following short-term dosing with atrazine. The effects on mammary gland development were observed at doses that were toxic to maternal animals. Although there were some inconsistencies in the pattern of effects observed on mammary gland development across the various studies, all of the above studies demonstrated an effect of atrazine treatment on mammary gland development in the young animal, which is consistent with the downstream effects of the overarching atrazine MOA of HPG/LH suppression. However, the lowest NOAELs across this group of studies were several-fold higher than the NOAELs that were based on LH suppression in key studies, including the 4-day oral gavage toxicity study (PMRA# 2945603, 2945604, and 2945570, Cooper et al., 2007 and 2010) in intact regularly cycling female LE rats, the latter of which is the basis of the revised toxicology reference doses provided in Section 1.2.

Studies on onset of puberty

A number of published and unpublished oral gavage mechanistic or developmental toxicity studies designed to assess the potential for atrazine and its chlorotriazine metabolites to alter the onset of puberty were available. Several of these studies, which were compliant with their respective USEPA Endocrine Disrupter Screening Program (EDSP) test guidelines, included additional measurements to better elucidate the underlying mechanism of changes in the attainment of puberty. In an initial female pubertal development and thyroid function assay (PMRA# 2945573, Laws et al., 2000) in juvenile Wistar rats, delayed VO and altered estrous cyclicity were observed at the top three doses tested. No treatment-related effects on thyroid function were noted. The inclusion of a pair-fed control group showing a lack of delayed VO indicated that this effect was not secondary to reduced body weight. In a second pubertal development assay (PMRA# 1078516, Ashby et al., 2002) using several strains of female rats, delayed VO was observed at mid- and high-doses in SD and Wistar rats, respectively. Other

notable findings were decreased uterine weights beginning at the mid-dose in Wistar rats and at the high-dose in SD rats. A subsequent mechanistic study (PMRA# 2816806 and 2815972, Breckenridge et al., 2015) examined the effects of atrazine exposure on the onset of puberty and the LH surge in different cohorts of animals following various dosing periods covering critical windows of development, including in utero and postnatal periods, as well as through puberty and/or adulthood. A statistically significant delay in VO was observed starting at the mid-dose in cohorts that were subjected to the most extended period of dosing, which included perinatal and peripubertal periods. The cohort that was retained in the study through adulthood also displayed attenuation of the LH surge and episodes of prolonged diestrus at the same dose. During the early postnatal period, decreased birth and survival indices were noted, which is a typical finding for atrazine when dams are dosed at the levels used in this study.

In a pubertal assay (PMRA# 2945586, Stoker et al., 2000) conducted in male Wistar rats, decreased LH levels and a treatment-related delay in preputial separation (PPS) were observed starting at the second lowest dose. At higher dose, decreases in various reproductive organ weights as well as changes in other hormone levels were observed. No treatment-related effects on serum thyroid hormone levels were noted. In another mechanistic study (PMRA# 2945587, Trentacoste et al., 2001) in which male SD rats dosed during the peripubertal period to examine effects on reproductive function, decreased weights in several reproductive organs were observed at the top two doses, along with delayed PPS and decreased LH and intratesticular testosterone levels. In a supplemental Hershberger assay (PMRA# 2815982 and 2816747), treatment-related changes in reproductive organs were noted beginning at the second lowest dose. However, this analysis was limited due to the lack of detailed data summaries and individual animal data from the study report. In a different mechanistic study (PMRA# 2945581, Rosenberg et al., 2008), development of the male reproductive system following in utero treatment was examined in SD rats. Maternal toxicity consisted of decreased body weight, while offspring toxicity was observed as decreased body weight and increased mortality during PNDs 0-2. A delay in PPS followed by a subsequent decrease in testosterone levels on PND 60 were observed in cohorts of the in utero-treated male rats that were maintained on a control diet during the postnatal period. At higher doses, decreased anogenital index and intratesticular testosterone levels were observed. In a second study (PMRA# 3292813, Fraites et al., 2011b) of similar design in SD rats, delayed PPS was observed at the high dose. This study also included an assessment of rough-and-tumble play behaviour, which was discussed as an indirect measurement of testosterone levels during the perinatal period. However, the study author did not report any treatment-related effects on this parameter and data was not provided to validate this conclusion.

In summary, this group of studies provided robust mechanistic information on development and attainment of puberty following dosing with atrazine over critical periods of development and short-term durations. A multi-generation study assessing the effects of atrazine on the onset of puberty following extended dosing was not available. However, delayed puberty was consistently observed across this group of studies and was consistent with the downstream effects of the overarching atrazine MOA of HPG/LH suppression. In studies in which animals were exposed in utero, the effects on onsets of puberty were also observed in the presence of maternal toxicity.

The lowest NOAELs across this group of studies were several fold higher than the NOAELs that were based on LH suppression in key studies, including the 4-day oral gavage toxicity study (PMRA# 2945603, 2945604, and 2945570, Cooper et al., 2007 and 2010) in intact regularly cycling female LE rats, the latter of which is the basis of the revised toxicology reference doses provided in Section 1.2.

Studies on potential effects on the HPA axis

A multitude of single and repeat-dose oral gavage mechanistic studies in rats were conducted to explore the potential effects of atrazine and/or its chlorotriazine metabolites on the HPA axis. These studies sought to characterize the dose-response and time-course effects of treatment on the secretion of HPA hormones in rats. A number of these studies were conducted at excessively high doses, and were not summarized in Appendix IV, Table 2. Among the group of studies using more appropriate doses, two high-quality studies (PMRA# 2945575, Laws et al., 2009; PMRA# 3292812, Fraites et al., 2009b), one in male Wistar rats and the other in female LE rats, demonstrated clear treatment-related marked increases in the adrenocorticotropic hormone (ACTH) and adrenal steroid hormones, namely, progesterone and corticosterone, immediately post-dosing. The effects were similar among animals dosed with atrazine or one of its chlorotriazine metabolites, with the exception of DACT. Dosing with DACT resulted in a less significant effect on the HPA activity in male Wistar rats than atrazine and the other chlorotriazine metabolites, and no effects on the HPA activity in female LE rats. More importantly, the data demonstrated that all HPA effects characterized by marked increases in secretion of HPA hormone levels declined and returned to control levels within one hour of dosing. In the four-day repeat-dose experiments conducted as part of the second study, the treatment-related increases in the HPA hormone levels were not of the same magnitude as those observed following single oral dose experiments. Both sets of experiments tested the same dose range and assessed hormone levels at the same time points. In a subsequent supplemental study in female Wistar rats (PMRA# 2816814 and 2816028, Foradori et al., 2011), adrenalectomies and ovariectomies were performed to further elucidate how activation of this endocrine axis by chlorotriazines could contribute to induced changes in female reproductive function. Several experiments were conducted as part of this study. In two experiments, animals were dosed at the same time periods of a single estrus cycle. In one of these two studies, the preovulatory LH surge was assessed post-dosing in adrenalectomized (ADX) and sham-treated animals. In the other study, the pulsatility profile of LH secretion was assessed. The limited data from this published study indicated that a treatment-related attenuation of the LH surge occurred in both ADX and sham-treated animals. However, the pulsatile secretion of LH was only altered in the sham-treated animals. The study authors concluded that atrazine treatment-related changes in the hormones of the HPA axis did not play a role in suppression of the LH surge, since a supressed LH surge was observed in ADX animals.

Overall, in the context of this group of studies, the underlying mechanism for atrazine and its chlorotriazine metabolites to activate the HPA axis was not elucidated. However, the results indicated that the changes to the HPA axis by atrazine and its chlorotriazine metabolites likely do not induce effects on the LH surge. Treatment-related changes in the hormones of the HPA axis also occurred at a lower magnitude following repeated dosing compared to single dose administrations and at doses higher than those resulting in attenuation of the LH surge, the latter of which is the basis of the revised toxicology reference doses provided in Section 1.2.

Studies on immune and nervous systems

Several non-guideline toxicity studies were available that explored the potential effects of atrazine and its chlorotriazine metabolites on the immune and nervous systems using a variety of in vitro and in vivo test systems and designs. The studies considered relevant to the scope of this assessment were reviewed and summarized in Appendix IV, Table 2. Discussed herein are findings only from the higher quality studies, albeit all these studies were considered supplemental.

In oral gavage or dietary mechanistic studies (PMRA# 3292827, Foradori et al., 2017; PMRA# 2816013) of immune function, assessments of adrenal hormonal levels, as well as antibody forming cell (AFC) and natural killer cell (NKC) assays were performed. As observed in the studies discussed in the preceding section, acute and transient increases in adrenal hormonal activity were observed following gavage dosing only. At higher doses, decreased body weight and increased NKC activity (effector to target ratios) were observed. In a mechanistic developmental immunotoxicity study (PMRA# 2945593, Rooney et al., 2003) in SD rats, a single group of dams received oral gavage doses from mid-gestation to the end of lactation. Treatment-related immunotoxic responses were observed, which were characterized by a decreased immunoglobin M response to sheep red blood cells and a delayed-type hypersensitivity to bovine serum albumin in male offspring only. In mechanistic studies (PMRA# 3292825, Filipov et al., 2005; PMRA# 3292826, Karrow et al., 2005; PMRA# 3292830, Zhao et al., 2013; PMRA# 2816042, Chen et al. 2013; PMRA# 2945594, Rowe et al. 2006) in mice, additional evidence of effects on the immune system were observed. These included altered thymic and splenic T-cell populations and splenic cellularity as well as reduced spleen and thymus weights. Although some of these alterations were observed at all doses in males on the first day post-dosing, the rapid recovery in these parameters at a later time point in the study lessened the toxicological concern for these findings. Overall, treatment-related effects on the immune system were observed at doses greater than those eliciting attenuation of LH levels and disruption of estrous cyclicity in female rats.

In oral gavage or dietary mechanistic studies examining potential effects on the nervous system, behavioural assessments and evaluations of some neurotransmitters, such as dopamine, were performed. In mouse studies, short-term treatment resulted in alterations in avoidance of a novel object and an increase in water maze swim time as well as other behavioural abnormalities (PMRA# 2815986, Lin et al., 2013). In rat studies (PMRA# 3292833, Li et al., 2019; PMRA# 3292829, Bardullas et al., 2011), decreased platform crossing times as well as alterations in other parameters tested in the Morris water maze were observed. Histopathology changes in the hippocampus were also noted. In developmental toxicity studies in rats (PMRA# 2945592, Li et al., 2014a; PMRA# 2945591, Sun et al., 2014; PMRA# 2945589, Li et al., 2014b), gestational or lactational treatment as well as treatment during puberty resulted in reduced dopamine concentrations in the striatum of the offspring. These studies had significant limitations and deficiencies in reporting that interfered with toxicological interpretation of the results. However, any reported effects suggestive of impaired nervous system functioning were only observed at doses greater than those resulting in attenuation of LH levels and disruption of estrous cyclicity in female rats.

Chlorotriazine metabolites of atrazine

In a variety of organisms, such as in animals, plants and bacteria, atrazine can be dealkylated at the 4th and 6th position of its ring structure to form either of the mono-dealkylated metabolites (desethyl-atrazine, desisopropyl-atrazine) during metabolism, which, in turn, can be further dealkylated to DACT. Several key studies, conducted according to OECD and other internationally accepted test guidelines, were available for each of the chlorotriazine metabolites. In PACR2003-13, the toxicity profiles of these metabolites were considered similar to that of unchanged atrazine with regard to their potential to attenuate LH levels and result in downstream reproductive and developmental effects. The toxicology reference values for atrazine were considered applicable to its chlorotriazine metabolites for inclusion in the residue definition for the purposes of the risk assessment. After a detailed review of the available toxicology database for each of these metabolites, a change to the conclusions regarding the comparative toxicity of atrazine and its chlorotriazine metabolites reached in PACR2003-13 was not considered necessary.

Diaminochlorotriazine (DACT)

DACT was of low acute oral toxicity in rats. In the short-term oral gavage and dietary toxicity studies in rats, reduced body weight and food consumption were observed. In addition, indications of disrupted estrous cyclicity, such as an increased number of females with shortened or prolonged estrous cycles and with persistent estrus or diestrus, were observed. In the short-term dietary toxicity study in dogs, cardiac toxicity, characterized by increased incidences of pathological findings in the heart, as well as liver toxicity were observed at the high-dose. A non-guideline long-term dietary toxicity study in intact female SD rats with the objective of evaluating effects of DACT on organs and systems associated with estrous cyclicity was available. At the high-dose, decreased body weight, altered LH surge, and an increased incidence of mammary gland tumours were observed. DACT was not genotoxic in a battery of tests, which included in vitro assays for gene mutation in bacteria and DNA repair in mammalian cells as well as an in vivo clastogenicity test. In an oral gavage developmental toxicity study in SD rats, decreased body weight was observed in the maternal animals at the intermediate-high dose. Developmental toxicity consisted of increased incidences of incomplete ossification of several sites in the skull bones, which were observed starting at the intermediate-low dose and in the absence of maternal toxicity. At the intermediate-high dose, decreased fetal body weight as well as increased incidences of incomplete ossification of several other sites, such as the hind- and forepaw, were noted in the presence of maternal toxicity. At the high dose, noteworthy effects included increased resorptions and post-implantation loss.

There was evidence of sensitivity of the young in this study, but no evidence of treatment-related malformations. In a pubertal assay with DACT in Wistar rats, delayed PPS and VO were observed in males and females, respectively, beginning at the mid-dose.

Desisopropyl-atrazine (DIA)

DIA was of moderate acute toxicity in rats. In the short-term dietary toxicity study in rats, reduced body weight and body weight gain and histopathological changes in the pars distalis of the pituitary gland were observed in males starting at the mid-dose. Increased incidences of

histopathological changes in the adrenal cortex and thyroid were observed in the high-dose males. In high-dose females, decreased body weight and increased incidences of extramedullary hematopoiesis in the liver and spleen were observed. In the short-term dietary toxicity study in dogs, reduced body weight, body weight gain and food consumption as well as reduced red blood cell (RBC) parameters were observed in both sexes starting at the mid-dose. Decreased heart, prostate, and testes weights were observed starting at the mid-dose in males. DIA was not genotoxic in a battery of tests including assays for point mutation and DNA repair in vitro. In the guideline oral gavage developmental toxicity study in rats, maternal toxicity included decreased body weight, body weight gain, and food consumption as well as body weight loss of up to 7 g following the first day of dosing at the high-dose. Developmental toxicity was comprised of increased incidences of fused sternebrae starting at the mid-dose. At the high-dose, increased incidences of absent/incomplete ossification of the proximal phalanx of several posterior digits as well as metatarsal 1 were observed. There were no treatment-related malformations, but evidence of sensitivity of the young was observed. In a pubertal assay in male Wistar rats with DIA, delayed PPS was observed starting at the second lowest dose. At higher doses, decreased body weight as well as reductions in serum testosterone levels and prostate and seminal vesicle weights were observed.

Desethyl-atrazine (DEA)

DEA was of moderate acute oral toxicity in rats. In a guideline short-term dietary toxicity study in rats, reduced body weight in females and food efficiency in both sexes were observed at the high-dose. In the short-term dietary toxicity study in dogs, reduced body weight and food consumption, decreased RBCs, and increased incidences of renal tubular epithelial hyperplasia/basophilia were observed at the high-dose. Increased incidences of histopathological changes as well as decreased heart weights were also observed at the high-dose. DEA was not genotoxic in a battery of tests including assays for point mutations and DNA repair in vitro and clastogenicity in vivo. In an oral gavage developmental toxicity study in rats, maternal toxicity consisted reduced body weight, body weight gain, and food consumption as well as body weight loss during the first day of dosing at the high-dose. Increased post-implantation loss was also noted at this dose. Developmental toxicity was characterized by increased incidences of fused or incompletely ossified sternebrae, and incomplete ossification of the proximal phalanges at the high-dose. There were no treatment-related malformations or evidence of sensitivity of the young. In a pubertal assay in male Wistar rats with DEA, delayed PPS was observed starting at the second lowest dose. At higher doses, decreased body weight as well as reductions in serum testosterone and prostate, epididymides and seminal vesicle weights were observed.

Hydroxylated transformation products of atrazine

Four hydroxylated TPs of atrazine were considered relevant for the risk assessment in PACR2003-13 and for the current review. These include hydroxyatrazine (HA, G 34048), desethylhydroxyatrazine (DEHA GS-17794), desisopropylhydroxyatrazine (DIHA, GS-17792) and ammeline (GS-17791). Toxicology studies were available for HA only, and included several key studies conducted according to OECD and other internationally accepted test guidelines. In PACR2003-13, the other hydroxylated TPs of atrazine were assumed to be of equivalent toxicity to HA. Therefore, the toxicology reference value established for HA, was considered applicable to other hydroxylated TPs for inclusion in the residue definition for the risk assessment.

After a detailed review of the toxicology database for HA coupled with the lack of any new reliable toxicity data available for other hydroxylated TPs, a change to this conclusion was not considered necessary.

HA was of low acute oral toxicity in rats. In the guideline short-term dietary toxicity studies in rats and dogs, effects included reduced body weight and body weight gain at the top two doses. Evidence of kidney toxicity, observed at the same doses, included changes in relevant clinical chemistry and urine analysis parameters as well as gross and histopathological lesions. The histopathological lesions consisted of crystal formation, dilatation, and basophilia in the renal tubules.

In the guideline dietary chronic toxicity and carcinogenicity study in SD rats, kidney toxicity, including changes in hematological, clinical chemistry and urinalysis parameters as well as macroscopic and microscopic kidney lesions, were observed at the two highest doses. Microscopic lesions consisted of increased incidences of papillary interstitial fibrosis and kidney dilatations with crystal deposits. Clinical signs of toxicity such as tremors, reduced body weight, and increased mortality, as well as histopathological lesions in several other tissues were noted at the high-dose. There was no evidence of carcinogenicity.

HA was not genotoxic in a battery of in vitro and in vivo tests, including bacterial reverse mutation assays and a DNA repair assay in vitro, as well as in the micronucleus test in mice.

In the oral gavage developmental toxicity study in SD rats, reduced food consumption and body weight gain as well as enlarged mottled kidneys in dams were observed at the high-dose. Developmental toxicity included decreased fetal weight, increased incidences of incompletely ossified hyoid and interparietal bones and unossified forepaw metacarpals, which was also observed at the high-dose. Single incidences of gastroschisis and umbilical hernia, noted at this dose in different fetuses, were considered incidental. There was no evidence of sensitivity of the young or treatment-related malformations.

Two oral gavage pubertal studies in Wistar rats were available for HA. In the first study, a slight treatment-related delay in VO was observed at the highest dose. The second study included an assessment of pubertal development in both sexes as well as an assessment of kidneys and other key target organs.

In this study, renal toxicity in the form of hydronephrosis, renal tubule dilatation and ascending pyelonephritis was observed starting at the lowest dose tested for both sexes. No treatment-related effects on any relevant pubertal endpoints, including the onsets of VO or PPS, were observed.

In summary, HA has a different toxicological profile compared to unchanged atrazine and its chlorotriazine metabolites. The most sensitive effect of HA was kidney toxicity, which is presumed to be due to its low solubility in water, characterized by crystal formation and a consequent inflammatory response. There was limited evidence of HA resulting in the perturbation of the HPG axis.

The identities of major atrazine metabolites and TPs are presented in Appendix IV, Table 1A and 1B. Results of the relevant toxicology studies conducted on laboratory animals with atrazine and the noted metabolites and TPs are summarized in Appendix IV, Tables 2, 3, and 4. The toxicology reference values for use in the human health risk assessment are summarized in Appendix IV, Tables 5 and 6.

Epidemiology

Numerous studies were identified in the published scientific literature that explored the potential health effects of atrazine in human populations. The majority of studies identified in the literature, which included ecological, cross-sectional and case-control studies, lacked adequate characterization of atrazine exposure and were not considered further in the hazard assessment. Instead, the epidemiological component for this special review was primarily focused on prospective cohort studies, many of which used data from the Agricultural Health Study (AHS). The AHS follows a cohort of approximately 53 000 licensed pesticide applicators and their spouses in Iowa and North Carolina. More specifically, the focus of the epidemiological component for this special review was on studies that either, 1) assessed human health outcomes that were also identified as key endpoints in animal studies, or 2) identified a positive association between atrazine use and adverse health outcomes. Therefore, human health outcomes that were examined further included female reproductive and developmental effects, and cancers of the prostate and breast.

Female reproductive effects

A hybrid retrospective and prospective cohort study was conducted among a population of 102 pre-menopausal women aged 18 to 40, residing in agricultural communities in Illinois in 2003, that were reported by the authors as areas with high atrazine use. The reference group were women from Vermont, which was selected due to the low amounts of atrazine used in that area. The aim of the study was to determine the association between atrazine exposure and changes to the menstrual cycle. Exposure to atrazine was estimated through quantification of atrazine and metabolite levels in both tap water and urine samples, and through information obtained from questionnaire data. Menstrual cycle irregularities were determined retrospectively using questionnaire data, and menstrual cycle length was investigated in more detail using data from prospective cycle diaries. To better characterize menstrual cycle status, a subset of participants submitted urine samples to be analysed for the metabolites estrone 3-glucuronide, a measure of follicular growth, and pregnanediol 3-glucuronide, levels of which can be used to provide evidence of ovulation. In a few cases, LH levels were quantified to better understand the pre-ovulatory LH surge. Analyses of menstrual cycles showed that Illinois women were more likely to have reported cycle length irregularity (odds ratio (OR) = 4.69; 95% confidence interval (CI): 1.58, 13.95), and a cycle duration of longer than 6 weeks between periods (OR = 6.16; 95% CI: 1.29, 29.38) as compared to Vermont women. Another analysis showed that consumption of more than 2 cups of unfiltered Illinois water daily was also associated with an increased risk of irregular periods (OR = 5.73; 95% CI: 1.58, 20.77). None of the atrazine level indices were significantly associated with LH levels. Although the above CIs did not contain the null value of 1.0, the range of values was large, which may have been due to low participation rates and reduced statistical power. Also, cases and controls were defined based on state of residence, meaning that there may have been additional confounders that were not accounted for. The data

were further limited by the low proportion of tap water samples (43%) that had detectable levels of atrazine or its metabolites. Overall, there was insufficient evidence to conclude that there was an association between atrazine exposure and disruption of the menstrual cycle. A similar conclusion was reached in the 2010 USEPA SAP meetings.

There were three AHS studies available that examined female reproductive outcomes; however, there were several limitations with these studies, including low participation rates and the fact that the AHS was not originally designed to specifically address questions related to reproductive outcomes. These studies did not provide any additional information regarding potential associations between atrazine use and changes to the menstrual cycle.

Breast cancer

Two prospective cohort AHS studies were conducted to investigate an association between atrazine use and breast cancer. The first study showed an adjusted OR of 0.7 (95% CI = 0.4, 1.2) for breast cancer among wives of farmers who had reported that they had previously used atrazine themselves. In the second study, the observed relative risk (RR) was 1.14 (95% CI = 0.47, 2.50) for breast cancer among female pesticide applicators who had ever reported using atrazine. The CIs for both of these studies contained the null value of 1.0, suggesting no association between atrazine use and breast cancer.

Other studies were available that investigated an association between atrazine use and breast cancer. The CIs for each risk estimate either contained, or closely approximated the null value of 1.0, suggesting no association between atrazine and breast cancer. However, it is important to note that these studies were largely ecological or case-control by design, which limited their overall utility in informing the weight of evidence review.

Overall, the weight of evidence review did not demonstrate a clear association between atrazine use and breast cancer outcomes; therefore these studies were not considered further in the hazard assessment.

Developmental effects

A nested case-cohort study was conducted on a sub-cohort of 579 woman and child pairs enrolled in the PELAGIE (Perturbateurs endocriniens: Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance) cohort from the Brittany region of France from 2002 through 2006 to explore associations between atrazine exposure and various adverse developmental outcomes. Cases and controls were defined as having a urinary level of atrazine or metabolites above and below the limit of detection (LOD), respectively. The developmental outcomes of interest included congenital anomalies, fetal growth restriction (FGR), and small head circumference. After adjustment for maternal age, education level, smoking status, alcohol consumption and blood pressure, individuals with detectable levels of atrazine or its metabolites had a slight, but statistically significant increased odds ratio for FGR (OR = 1.50; 95% CI = 1, 2.20) and small head circumference (OR = 1.70; 95% CI = 1, 2.70). However, both sets of CIs included the null value of 1.0, suggesting a lack of association between urinary atrazine levels and adverse developmental outcomes. The data were limited by reliance on a single urinary sample to reflect chronic exposure. Also, cases and controls were assigned based on whether

they had an atrazine or metabolite level above the LOD, meaning that case ascertainment was dependent on the sensitivity of the analytical method used. Overall, there is no clear evidence to support an association between atrazine and developmental effects.

Prostate cancer

An epidemiological study was conducted on workers employed at a plant that manufactured atrazine to explore the association between occupational exposure to atrazine and prostate cancer. Data was collected during a follow-up period from 1985 to 1997, and included 2045 workers who were offered prostate-specific antigen (PSA) screening beginning in 1989. Incident prostate cancer cases were identified through a combination of state tumour registry records, plant records, or death certificates. Incident cancer cases were compared to the number expected from the general population of the surrounding area to determine the excess number of cancer cases that could potentially be attributed to working at the plant. For all workers, 11 prostate cancers in total were observed, with only 6.3 cancers expected based on general population rates (standardized incidence rate (SIR) = 175; 95% CI = 87, 312). The authors calculated these rates for different subgroups, including based on race (white and non-white), years worked, years since hire, and employee group (company, contract workers, and maintenance). Company employees, who, on average, were employed at the plant for longer than the other employee types, had the highest SIR for prostate cancer of 217 (95% CI = 94, 428). Both SIR values were associated with wide CIs that contained the null value 100, suggesting that the excess prostate cancer risk may not be overly different between workers and the general population. Furthermore, the study was limited by a lack of confounder data, including smoking status, previous employment history, or potential exposure to other occupational factors. There were no biomonitoring data to definitively demonstrate that these workers were indeed exposed to atrazine. A nested case-control study was conducted using the same worker population to determine whether the elevated prostate cancer incidence was a result of increased PSA screening. It was found that workers who had prostate cancer had more PSA screening tests performed as compared to workers without prostate cancer (OR = 8.54; 95% CI = 1.69, 82.20). The data were limited by the low number of prostate cancer cases out of the total number of participants (11 out of 2045), resulting in low statistical power and a wide confidence interval. Overall, there was no clear evidence to support an association between atrazine exposure and prostate cancer in workers involved in the manufacturing of atrazine. Two prospective cohort studies using AHS data were also available, which supported a lack of an association between atrazine exposure and prostate cancer.

Overall, majority of the available epidemiological studies were not designed to collect data on adverse health outcomes that could be used quantitatively or qualitatively in regulatory decision-making, and were more exploratory in nature. The evidence from the available epidemiological studies does not support a cause-and-effect relationship between atrazine use and any adverse health outcome. The majority of these studies have also been comprehensively evaluated by the USEPA and expert panels, such as the USEPA Scientific Advisory Panel (SAP). The overall conclusion from these assessments was that the evidence was insufficient to conclude a clear association between atrazine and any adverse health outcomes in humans. While epidemiological data have inherent limitations, reported findings have the advantage of being directly based on human exposures and population responses.

As a result of this advantage, epidemiological studies may provide valuable insights in the adverse outcome pathway framework. Health Canada continues to support the conduct of well-designed epidemiological studies where exposure conditions are well characterized.

1.1 Pest Control Products Act hazard characterization

For assessing risks from potential residues in food or from products used in or around homes or schools, the *Pest Control Products Act* requires the application of an additional 10-fold factor to threshold effects to take into account completeness of the data with respect to the exposure of, and toxicity to, infants and children, and potential prenatal and postnatal toxicity. A different factor may be determined to be appropriate on the basis of reliable scientific data.[7]

With respect to the completeness of the database in characterizing the toxicity to infants and children, the toxicology database for atrazine contains the full complement of required studies including oral gavage developmental toxicity studies in rats and rabbits, and a dietary 2-generation reproductive toxicity study in rats. For the chlorotriazine metabolites and hydroxyatrazine, oral gavage developmental toxicity studies in rats and rabbits were available. In addition, a large number of non-guideline studies investigating the mechanism of toxicity relating to the developmental, reproductive, and endocrine effects of atrazine, chlorotriazine metabolites and hydroxyatrazine were also available. Many of these studies utilized robust study designs to examine effects in rats via a variety of testing strategies, including dosing during critical windows of prenatal and postnatal development, dosing at the expected peak time of hormonal surges, cross-fostering designs and peripubertal dosing regimens.

With respect to potential prenatal and postnatal toxicity, there was no clear indication of increased sensitivity of fetuses or offspring compared to parental animals across the extensive toxicity database for atrazine, the chlorotriazine metabolites and hydroxyatrazine.

For atrazine, decreased pup weight and body weight gains were observed at the maternally toxic dose in the dietary 2-generation reproductive toxicity study in rats. Increases in the mean number of resorptions and post-implantation loss were observed in the rat and rabbit guideline gavage developmental toxicity studies at doses causing moderate to significant maternal toxicity. At the same doses, increased incidences of incomplete ossification of various skeletal sites were observed in the fetuses in both test species. Two abortions as well as reduced fetal weights and decreased number of live fetuses were also noted in the rabbit at the high dose. Although serious effects in the form of resorptions, post-implantation loss and abortions were observed in the rat and rabbit developmental toxicity studies, concern for these findings was tempered by the presence of maternal toxicity.

In the various non-guideline mechanistic studies in rats examining effects on pregnancy outcomes and development of the young, no increased sensitivity of the young was observed compared to parental animals. In the first two studies of this group, pre- and post-implantation loss, full litter resorptions, and decreased live litters were noted with differential sensitivity of the strains of rats tested (PMRA# 3292819, Cummings et al., 2000; PMRA# 2945579, Narotsky et

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[7] SPN2008-01. The Application of Uncertainty Factors and the Pest Control Products Act Factor in the Human Health Risk Assessment of Pesticides.

al., 2001). In the study (PMRA# 2816740, Coder et al., 2011e) in which animals were dosed over the span of one estrous cycle immediately before mating, a slight increase in post-implantation loss was observed in one of the cohorts in the presence of maternal toxicity (decreased ova and corpora lutea counts). In a different study (PMRA# 2816744, 2816014, 2816022, and 2816741, Coder, 2011d) by the same laboratory, increased incidence of pups with no milk in the stomach as well as decreased pup survival were observed when dams were treated during gestation and lactation periods. Other studies (PMRA# 2816806 and 2815972, Breckenridge et al., 2015) have also demonstrated decreased birth and pup survival indices. However, all these effects in the young noted across this group of studies were observed at doses that were associated with toxicity (decreased body weight) to the parental animal, and doses were several fold higher than those eliciting attenuation of the LH surge in the adult female animals.

No increased sensitivity of the young was observed compared to parental animals in another group of mechanistic studies (PMRA# 2816791 and 2816793, Rayner et al., 2004 and 2005; PMRA# 2816001, Coder 2010b; PMRA# 2816726, Hovey et al., 2011; PMRA# 2816025 and 2816805, Davis et al., 2011a) that assessed effects on the mammary glands during critical windows of development. Structural changes in the development of the mammary gland, such as lower density of the epithelial branches, fewer TEBs, and altered ductal networks were observed. However, these effects were observed in the presence of maternal toxicity and at higher doses than those producing LH suppression.

No increased sensitivity of the young was observed compared to parental animals across the group of studies that examined effects on development of male or female reproductive systems and function following dosing during critical windows of development. In the series of studies that focused on the development of the female reproductive system and function, delayed VO was observed following perinatal and/or peripubertal dosing strategies (PMRA# 2816025 and 2816805, Davis et al., 2011a; PMRA# 2816744, 2816014, 2816022 and 2816741, Coder, 2011d; PMRA# 2816806 and 2815972, Breckenridge et al., 2015).

In one of these studies (Breckenridge et al., 2015) in which the cohort of animals was dosed perinatally and/or peripubertally, as well as through early adulthood, attenuation of the LH surge and episodes of prolonged diestrus were observed in the treated animals.

No increased sensitivity of the young was observed compared to parental animals across the series of studies that focused on development and function of the male reproductive system. Treatment of dams during early lactation resulted in an increased incidence of prostatitis in their male offspring when assessed in adulthood. This effect was observed in the presence of maternal toxicity (reduced prolactin levels) (PMRA# 2945583, Stoker et al., 1999). Inflammation of the prostate gland in F1 male offspring that received treatment perinatally or postnatally when assessed as adults was also observed in a study of cross-fostering design (PMRA# 2816792, Rayner et al., 2007). In the study (PMRA# 2816730 and 2815991) where perinatal dosing was used to assess the developmental effects on the reproductive system in male progeny, decreased litter size and viability as well as increased total litter loss and pup mortality were observed at maternally toxic doses.

F1 male rats assessed later in their adulthood demonstrated decreased weights in some of the reproductive system organs as well as decreased sperm counts and increased percent abnormal sperm levels. In a subsequent study (PMRA# 2816731) by the same group, male offspring treated via maternal milk demonstrated the same effects noted in the study above, when assessed later in their adult life, but at higher doses.

In a series of studies in which peripubertal dosing strategies were used in juvenile rats, delayed VO and altered estrous cyclicity were noted in females (PMRA# 2945573, Laws et al., 2000; PMRA# 1078516, Unpublished study Ashby et al., 2002; PMRA# 2816806 and 2815972 Breckenridge et al., 2015). In males, delayed PPS, and decreased serum LH and testosterone levels in males as well decreased weight in several reproductive organs were observed (PMRA# 2945586, Stoker et al., 2000; PMRA# 2945587, Trentacoste et al. 2001). All the effects noted in this group of studies occurred at doses several-fold higher than those resulting in the attenuation of LH surge in adult female rats.

No increased sensitivity of the young was noted in the series of non-guideline studies exploring effects on the developing nervous and immune systems. Since these studies were considered supplemental, clear NOAELs or LOAELs could not be established for them. However, any effects suggestive of impaired development of the nervous or immune systems were observed only at doses much greater than those resulting in attenuation of LH levels and disruption of estrous cyclicity in female rats.

For the chlorotriazine metabolites, increased incidences of incomplete ossification of various skeletal sites, such as the appendicular elements, were observed at maternally toxic doses in the rat oral gavage developmental toxicity studies. Increased incidences of fused sternebrae were also observed starting at mid- and high-dose for DIA and DEA, respectively. For DEA, increased post-implantation loss was observed at the same dose as the increased incidences of fused sternebrae, both of which were observed in the presence of maternal toxicity. Some fetal findings, including increased incidences of fused sternebrae and incomplete ossification of several skeletal sites for DIA and DACT, respectively, were observed in the absence of apparent maternal toxicity, suggesting sensitivity of the young. In pubertal assays, the chlorotriazine metabolites also resulted in delays in the onset of puberty, namely, time to VO and PPS. Overall, although there was some indication of sensitivity of the young in the rat developmental toxicity studies conducted with DIA and DACT, all of the effects in the young resulting from exposure to the chlorotriazine metabolites were observed at doses several-fold higher than those of atrazine that resulted in the attenuation of the LH surge. Since the toxicology reference values of atrazine are based on the attenuation of the LH surge, and were deemed applicable to these metabolites, the degree of concern was low for the findings in the young attributed to the chlorotriazine metabolites when considering the margins of protection afforded by the atrazine toxicology reference values.

For HA, increased incidences of incomplete ossification of several appendicular elements and skull bones were observed at maternally toxic doses in the rat oral developmental toxicity study. In a pubertal assay with HA, there were no treatment-related effects observed on any relevant pubertal endpoints.

Overall, the database is adequate for determining the sensitivity of the young. Some of the developmental effects, such as post-implantation loss, total litter loss, and decreased pup survival, are considered serious in nature. However, the concern for these effects was tempered by the presence of maternal toxicity at the same doses. Furthermore, the weight of evidence supported the linkage between the observed effects in the young to the overarching atrazine MOA of HPG/LH suppression. Given that the attenuation of the LH surge in adults regularly cycling female rats was observed at much lower doses than the effects observed in the young, and formed the most sensitive point of departure across the database and for the risk assessment, the level of concern was low for all of the observed effects in the young. Therefore, the PCPA factor was reduced to onefold for atrazine, and its chlorotriazine metabolites for exposure scenarios in which the attenuation of LH surge was used to establish the point of departure. For HA, the endpoints in the young were well-characterized and not considered serious in nature, and there was no evidence of sensitivity of the young. On the basis of this information, the PCPA factor for HA was reduced to onefold.

1.2 Toxicology reference values for atrazine and its chlorotriazine metabolites

Acute reference dose (ARfD)

To estimate acute dietary risk, the 4-day oral gavage toxicity study with atrazine in LE rats with a NOAEL of 1.6 mg/kg bw/day was selected for risk assessment. Attenuation of the LH surge was observed at the LOAEL of 3.12 mg/kg bw/day. The possibility that this effect was a result of an acute exposure during the peak LH surge period could not be ruled out, and therefore, this endpoint was considered relevant to the acute risk assessment. Standard uncertainty factors of 10-fold for interspecies extrapolation and 10-fold for intraspecies variability have been applied. As discussed in the *Pest Control Products Act* hazard characterization section, the PCPA factor was reduced to onefold. Thus, the composite assessment factor (CAF) is thus 100.

The ARfD is calculated according to the following formula:

$$\text{ARfD} = \frac{\text{NOAEL}}{\text{CAF}} = \frac{1.6 \text{ mg/kg bw/day}}{100} = 0.02 \text{ mg/kg bw of atrazine}$$

Acceptable daily intake (ADI)

To estimate risk following repeated dietary exposure, the NOAEL of 1.6 mg/kg bw/day from the 4-day oral gavage toxicity study with atrazine in LE rats was selected. At the LOAEL of 3.12 mg/kg bw/day, attenuation of the LH surge was observed. Standard uncertainty factors of 10-fold for interspecies extrapolation and 10-fold for intraspecies variability have been applied. The application of an additional threefold database uncertainty factor (UF_{DB}) was also considered appropriate, since studies with a robust study design for assessing effects on the LH surge over a longer duration were not available in LE rats, the strain of rats considered the most sensitive to LH suppression following treatment to atrazine. This was further supported by evidence of durational effects, as characterized by attenuation of the LH surge and the subsequent disrupted estrous cyclicity, observed at lower doses in repeat-dose toxicity studies of longer duration compared to shorter duration in SD rats. As discussed in the *Pest Control Products Act* hazard characterization section, the PCPA factor was reduced to onefold. The CAF is thus 300.

The ADI is calculated according to the following formula:

$$ADI = \frac{NOAEL}{CAF} = \frac{1.6 \text{ mg/kg bw/day}}{300} = 0.005 \text{ mg/kg bw/day of atrazine}$$

The NOAELs of a few guideline short-term dietary toxicity studies in rats were lower than the NOAEL of 1.6 mg/kg bw/day selected for risk assessment. However, the weight of evidence did not support using any of these NOAELs for reference dose selection. The factors that contributed to this weight of evidence included less optimal dose spacing, the low magnitude of the adversity of the effects noted at higher dose levels in these studies, as well as higher NOAELs established for the same endpoints in the long-term dietary toxicity studies in rats.

Short- and intermediate-term dermal and inhalation

For short- and intermediate-term occupational exposures via the dermal and inhalation routes, the NOAEL of 1.6 mg/kg bw/day from the 4-day oral gavage toxicity study in the LE rats was selected for risk assessment. At the LOAEL of 3.12 mg/kg bw/day, attenuation of the LH surge was observed. The available route-specific 25-day dermal toxicity study with atrazine in rabbits did not assess the endpoint of concern (LH surge and/or estrous cyclicity), and a short-term inhalation toxicity study was not available. Thus, it was necessary to use an oral study for short-term dermal and inhalation risk assessment. Standard uncertainty factors of 10-fold for interspecies extrapolation and 10-fold for intraspecies variability were applied. An additional threefold UF_{DB} was also applied, since studies with a robust study design for assessing effects on the LH surge over a longer duration were not available in LE rats, the strain of rats considered most sensitive to LH suppression following exposure to atrazine. This was further supported by evidence of durational effects, as characterized by attenuation of the LH surge and the subsequent disrupted estrous cyclicity, observed at lower doses in repeat dose toxicity studies of longer duration compared to shorter duration in the SD rats.

Therefore, the target margin of exposure (MOE) for these scenarios is 300. The selection of this study and target MOE is considered to be protective of all populations, including nursing infants and the unborn children of exposed female workers.

Cancer assessment

A treatment-related increased incidence and early onset of mammary gland tumours was observed in female SD rats following chronic dosing with atrazine. No evidence of carcinogenicity was observed in mice, F344 rats, or OVX female SD rats following chronic dosing. An MOA for tumour induction has been well characterized for atrazine in female SD rats, is considered unique to certain strains of rats, and is not relevant to humans. Thus, the mammary gland tumours, occurring in the female SD rats via this MOA, are not considered relevant to the human health risk assessment, as further described earlier in this document.

1.3 Toxicology reference values for hydroxyatrazine and hydroxylated transformation products

Acute reference dose (ARfD)

Establishment of an acute reference dose was not required, as an endpoint of concern attributable to a single exposure was not identified in the oral toxicity studies with hydroxyatrazine.

Acceptable daily intake (ADI)

To estimate risk resulting following repeated dietary exposure, the 2-year combined chronic toxicity and carcinogenicity study conducted with hydroxyatrazine in rats with a NOAEL of 1 mg/kg bw/day was selected for risk assessment. At the LOAEL of 8 mg/kg bw/day, kidney toxicity as characterized by an increased incidence of crystal formation and a subsequent inflammatory response, was observed. This study provided the lowest NOAEL in the database for these metabolites. Standard uncertainty factors of 10-fold for interspecies extrapolation and 10-fold for intraspecies variability have been applied. As discussed in the *Pest Control Products Act* hazard characterization section, the PCPA factor was reduced to onefold. The CAF is thus 100.

The ADI is calculated according to the following formula:

$$\text{ADI} = \frac{\text{NOAEL}}{\text{CAF}} = \frac{1 \text{ mg/kg bw/day}}{100} = 0.01 \text{ mg/kg bw/day of hydroxyatrazine}$$

Cancer assessment

There was no evidence of tumourigenicity in the available data for hydroxyatrazine. Therefore, a cancer risk assessment was not necessary for hydroxyatrazine and the other hydroxylated transformation products.

2.0 Dietary exposure and risk assessment

Health Canada assessed potential acute and chronic dietary (food and drinking water) risks from exposure to residues of atrazine and its metabolites/transformation products for this special review. Atrazine and its chlorotriazine metabolites/transformation products produce different toxic effects compared to its hydroxylated metabolites/transformation products; attenuation of LH surge and renal effects, respectively. Therefore, atrazine and its chlorometabolites do not share a common mechanism of toxicology with the hydroxylated metabolites, and risks are quantified separately using their respective toxicology reference values (sections 1.2 and 1.3). Since separate toxicology reference values were established for a) atrazine and the chlorotriazine metabolites/transformation products, and b) hydroxyatrazine and other hydroxylated metabolites/transformation products, two different residue definitions were also specified for risk assessment purposes: 1) atrazine and its chlorotriazine metabolites/transformation products (DEA, DIA, DACT) for drinking water, plant and animal commodities; and 2) hydroxyatrazine (HA) and other hydroxylated metabolites (DEHA, DIHA, and ammeline) for drinking water and plant commodities only. Therefore, two separate dietary exposure and risk assessments were

conducted based on the relevant toxicology reference values and residue definitions noted above. The reference values used in the dietary exposure are summarized in sections 1.2 and 1.3, and listed in Appendix IV, Tables 5 and 6. The dietary risk assessment results are summarized in Appendix V.

Acute and chronic dietary (food plus drinking water) exposure and risk assessments were conducted using the Dietary Exposure Evaluation Model - Food Commodity Intake Database™ (DEEM-FCID™, Version 4.02, 05-10-c) program, which incorporates food consumption data from the National Health and Nutrition Examination Survey/What We Eat in America for the years 2005-2010 available through the Centers for Disease Control and Prevention's National Center for Health Statistics. Dietary exposure assessments are age-specific and incorporate the different eating habits of the population at various stages of life (infants, children, adolescents, adults and seniors). For example, the assessments take into account differences in children's eating patterns, such as food preferences and the greater consumption of food relative to their body weight when compared to adults.

The atrazine dietary exposure assessments considered all foods that may potentially be treated with atrazine, including imported foods that may be treated outside of Canada. The dietary assessments were conducted using Canadian maximum residue limits (MRLs) or American tolerances for most commodities; however, for a few commodities with no anticipated residues from field trials or MRL/tolerance levels, residues obtained from metabolism studies were used. Percent crop treated (PCT) was not available and all commodities were assumed to be 100% treated. These inputs result in an exposure estimate that is considered to be conservative. In addition, default and experimental food processing factors were applied for relevant processed commodities.

Residues of atrazine and its chlorotriazine transformation products in drinking water were estimated based on water monitoring data, and residues of hydroxylated transformation products in drinking water were estimated using modelling as discussed in Section 3.

For atrazine and its chlorotriazine metabolites/transformation products, dietary exposure and risks are shown to be acceptable (< 32% of the ARfD and < 72% of the ADI). Milk (Canadian MRL as residue input) is the major risk contributor, accounting for about 45% of the total acute exposure and 47% of the total chronic exposure for children 1–2 years old (the highest exposed population subgroup). Drinking water accounted for about 5% of the total acute exposure and 12% of the total chronic exposure for children 1–2 years old.

For hydroxylated metabolites/transformation products, dietary exposure and risks are shown to be acceptable (< 72% of the ADI). Drinking water (modelling EEC as residue input) is the major risk contributor, about 99.5% of the total exposure chronic exposure for all infants (<1 year old) (the highest exposed population subgroup). An acute dietary risk assessment is not required for hydroxylated metabolites/transformation products as there was no acute reference value established for this group of metabolites/transformation products. Dietary exposure and risks from atrazine and its chlorometabolites and from hydroxylated metabolites are not combined because they produce different toxic effects and do not share a common mechanism of toxicity.

Maximum residue limits (MRLs) for pesticides in/on food are specified by Health Canada under the authority of the *Pest Control Products Act*. Canadian MRLs for atrazine are currently specified for corn at 0.2 ppm and animal commodities at 0.04 ppm. The residue definition for enforcement purposes in plant and animal commodities was previously established by Health Canada as atrazine and its chlorotriazine metabolites (DEA, DIA and DACT).

Hydroxylated metabolites of atrazine were not included in the residue definition for enforcement, as they are of lower toxicity and residues of atrazine and the chlorotriazine metabolites are sufficient biomarkers for monitoring purposes. A complete list of Canadian MRLs can be found in Health Canada's MRL Database, an online query application that allows users to search for specified MRLs, regulated under the *Pest Control Products Act*, for both pesticides and food commodities.

As a result of this special review of atrazine, dietary risks were shown to be acceptable from exposure to atrazine and its metabolites/transformation products through food and drinking water. Therefore, no amendments to the currently established MRLs are being proposed as part of the special review decision and the current Canadian MRLs for atrazine will be maintained.

3.0 Exposure from drinking water

3.1 Concentrations in drinking water

Estimated environmental concentrations (EECs) of atrazine and its transformation products in potential drinking water sources (groundwater and surface water) are presented in Tables 1 and 2 below.

For atrazine and its chlorotriazine transformation products (DEA, DIA and DACT), Canadian drinking water monitoring data (2005–2020) were considered. The sum of maximum concentrations of atrazine + chlorotriazine transformation products (DEA, DIA and DACT) in surface water (15.38 µg/L, sum of surface water maxima in Table 2) was used in the acute and chronic drinking water risk assessments.

Canadian water monitoring data are not available for the hydroxylated transformation products (HA, DEHA, DIHA, ammeline). Concentrations determined through water modelling were used in the drinking water assessment. For the chronic drinking water risk assessment, the combined residue of the hydroxylated transformation products of 94 µg/L was used.

Table 1 Level 1 (Modelling) EECs in potential sources of drinking water for combined residues of (1) atrazine and its chlorotriazine transformation products and (2) hydroxylated transformation products of atrazine

Combined residue	Groundwater (µg a.i./L)		Surface water (µg a.i./L)		
	Daily[1]	Yearly[2]	Daily[3]	Yearly[4]	Overall[5]
Atrazine and its chlorotriazine transformation products (DEA, DIA, DACT) [EECs as parent equivalent]	1496	1495	107	21	10
Hydroxyatrazine (HA) and other hydroxylated transformation products (DEHA, DIHA, ammeline) [EECs as HA equivalent]	94	94	19	8.1	3.8

1. 90th percentile of daily concentrations
2. 90th percentile of 365-day moving average concentrations
3. 90th percentile of the highest 1-day average concentration from each year
4. 90th percentile of yearly average concentrations
5. Average of all yearly average concentrations

Table 2 Summary of Canadian drinking water monitoring data from 2005 to 2020

Compound	Groundwater		Surface Water		Treated Water	
	Detections	Max	Detections	Max	Detections	Max
Atrazine	141 of 1902 samples (7.4%)	2.32 µg/L	3608 of 11319 samples (31%)	13 µg/L	176 of 10103 samples (1.7%)	5.7 µg/L
DEA	28 of 851 samples (3.3%)	0.14 µg/L	1906 of 10016 samples (19%)	1.5 µg/L	54 of 1240 samples (1.5%)	0.35 µg/L
DIA	0 of 511 samples (0%)	ND[1]	579 of 6061 samples (9.6%)	0.76 µg/L	3 of 207 samples (1.5%)	0.075 µg/L
DACT	14 of 52 samples (27%)	0.44 µg/L	103 of 155 samples (66%)	0.12 µg/L	92 of 207 samples (44%)	0.38 µg/L

1. ND = Not Detected

3.2 Drinking water exposure and risk assessment

Exposure from drinking water and food sources were combined to determine the total dietary exposure and risk. Refer to Section 2 for the results of the acute and chronic dietary exposure and risk assessments.

4.0 Occupational and non-occupational exposure and risk assessment

Occupational and non-occupational (for example, residential) risk is estimated by comparing potential exposures with the most relevant endpoint from toxicology studies to calculate a margin of exposure (MOE). This is compared to a target MOE incorporating uncertainty factors protective of the most sensitive subpopulation. If the calculated MOE is less than the target MOE, it does not necessarily mean that exposure will result in adverse effects, but mitigation measures to reduce risk would be required.

The toxicology reference values for non-occupational and occupational exposures are summarized in Section 1.2 and shown in in Appendix IV, Table 5.

Although the chlorotriazine and hydroxylated metabolites/transformation products may be found in plants, occupational and non-occupational exposure is not expected since these metabolites are a product of plant metabolism and are unlikely to be present on plant surfaces.

Dermal absorption

A dermal absorption value of 6% based on a human in vivo study was previously used for the re-evaluation of atrazine (PACR2003-13, RRD2004-12) and was revisited for the special review in light of current policies. Based on a review of the available dermal absorption studies, the 6% value from the human in vivo study was found to be acceptable, appropriate, and consistent with current policies and has been maintained in the current assessment.

4.1 Non-occupational exposure and risk assessment

Non-occupational (for example, residential) risk assessment involves estimating risks to the general population, including youth and children, during or after pesticide application.

4.1.1 Residential exposure and risk assessment

Since there are no registered domestic-class products containing atrazine, residential handler (mixer/loader and applicator) exposure is not anticipated. Postapplication exposure to residents is also not expected because, based on the use pattern, commercial application of commercial-class products to residential areas is not anticipated.

4.1.2 Bystander exposure and risk assessment

To minimize spray drift and potential bystander exposure from agricultural uses, spray drift statements are currently on all registered product labels. However, updates to the spray drift statements are being proposed to meet current labelling standards (Appendix II).

Concentrations of atrazine were measured in the air in Canadian agricultural regions during the spray season. A bystander inhalation assessment was conducted using the maximum measured air concentration from the available data and assumed bystanders would be exposed for short- and intermediate-term durations. The assessment is considered conservative because it uses an upper bound estimate of exposure. Calculated MOEs were greater than the target MOE and, therefore, risks were shown to be acceptable. Results are summarized in Appendix VI, Table 5. Since bystander inhalation exposure was minimal compared to other routes of exposure (such as dietary and drinking water), it was considered qualitatively in the aggregate risk assessment.

4.2 Occupational exposure and risk assessment

4.2.1 Mixer/loader and applicator exposure and risk assessment

Workers can be exposed through mixing, loading, and applying atrazine. Based on the registered atrazine use pattern, mixer/loader and applicator exposure is expected to occur for short- and intermediate-term durations via the dermal and inhalation routes.

4.2.1.1 Spray uses

For the spray uses of atrazine (that is, broadcast application of liquid atrazine to agricultural areas), exposures were estimated using unit exposure values from the Pesticide Handler Exposure Database (PHED) and/or Agricultural Handler Exposure Task Force (AHETF) data, and a dermal absorption factor for route-to-route extrapolation. Exposures from mixing atrazine with liquid fertilizer are considered to be covered by the spray use scenarios, as exposures would be similar. Inputs for the exposure assessment included: standard and refined area treated per day (ATPD) values, and unit exposure values for workers wearing current labelled personal protective equipment (PPE) (a single layer of clothing, chemical resistant (CR) gloves, and coveralls). No chemical-specific exposure studies (passive dosimetry, biomonitoring) were submitted to Health Canada; however, many are available in the published literature. Due to study design and/or reporting, these studies were unable to be used quantitatively in the exposure assessment.

The occupational mixer/loader and applicator exposure and risk assessment associated with the spray uses of atrazine is presented in Appendix VI, Table 1. MOEs were greater than the target MOE and, therefore, risks were shown to be acceptable for farmer application to most crops with a closed mix/load system. In addition, an enclosed cab is required for custom applicators or when very large areas of crops/fields are to be treated. As such, proposed mitigation will be based on the amount of active ingredient handled, as specified in Appendix II.

4.2.1.2 Impregnation and application of granular fertilizer

Atrazine can also be applied to granular fertilizer (impregnation) in commercial fertilizer facilities, and the impregnated granular fertilizer can be applied to corn fields by farmers and custom applicators.

For the impregnation of granular fertilizer in commercial facilities, no appropriate chemical-specific handler exposure data were available. Therefore, to address all possible activities and exposure scenarios in commercial fertilizer facilities (for example, treaters, cleaners), worker exposure was estimated using unit exposure values derived from seed treatment exposure studies. Seed treatment studies representative of the current label mitigation (closed mix/load system, PPE), or with additional PPE, were used. Cleaner unit exposures were determined by normalizing by the time spent cleaning in the study (~8 hours), rather than the study application rate, which is specific to seed treatment. A dermal absorption factor was used for route-to-route extrapolation. Other exposure assessment inputs included the maximum amount of atrazine impregnated onto granular fertilizer per day currently stated on labels (1500 kg/day) and an 8 hour workday.

For workers loading and applying the impregnated granular fertilizer to corn fields, exposure was estimated using unit exposures from PHED and a dermal absorption factor for route-to-route extrapolation. Inputs included standard area treated per day values, and unit exposure values for workers wearing current label PPE.

The occupational handler exposure and risk assessment for the impregnated granular fertilizer uses of atrazine in commercial facilities is presented in Appendix VI, Table 2. For impregnation of granular fertilizer in commercial facilities, MOEs were less than the target MOE for all activities with the PPE specified on the atrazine labels (a single layer of clothing, CR gloves, and coveralls). Potential risks were not shown to be acceptable with additional PPE. Potential mitigation by further limiting the amount handled per day was not considered to be practical, as only a small amount of fertilizer could be treated per day.

The occupational handler exposure and risk assessment for loading and applying impregnated granular fertilizer is presented in Appendix VI, Table 3. For loading and applying impregnated granular fertilizer, MOEs were less than the target MOE based on current label-specific conditions (open loading, open cab application) and PPE. Risks could be mitigated with additional PPE or using a closed transfer system. However, because feasible mitigation measures were not identified for treating granular fertilizer in commercial facilities, this use is proposed for cancellation.

4.2.1.3 Mixer/loader and applicator exposure and risk assessment conclusion

For farmer application to switchgrass, occupational mixer/loader and applicator MOEs were greater than the target MOE and risks were shown to be acceptable with the PPE specified on the labels (a single layer of clothing, CR gloves, and coveralls). However for farmer application to corn and sorghum, risks were not shown to be acceptable and a closed mixing/loading system is required for MOEs to be greater than the target MOE.

For custom application (or when very large areas of crops/fields are to be treated) to corn, sorghum and switchgrass, occupational mixer/loader and applicator MOEs were less than the target MOE, and risks were not shown to be acceptable with the PPE specified on the labels. A closed mixing/loading system and an enclosed cab are required for custom applicators in order for MOEs to be greater than the target MOE.

For all activities associated with impregnation of granular fertilizer in commercial facilities, MOEs were less than the target MOE and risks were not shown to be acceptable with the PPE specified on the atrazine labels (a single layer of clothing, CR gloves, and coveralls). Potential risks were not shown to be acceptable with additional PPEEven with additional PPE, risks could not be mitigated. Potential mitigation by further limiting the amount handled per day was not considered to be agronomically practical, as only a small amount of fertilizer could be treated per dayfeasible.

For loading and applying impregnated granular fertilizer, all MOEs were less than the target MOE based on current-label specific conditions and PPE, and risks were not shown to be acceptable. Risks could be mitigated with additional PPE or using a closed transfer system. However, because feasible mitigation measures were not identified for treating granular fertilizer in commercial facilities, this use is proposed for cancellation.

- Additional information on granular fertilizer impregnation in a commercial treatment facility could help address uncertainties in the available information for atrazine and support revised assessments of exposure and risk. This information could include:

- Current Canadian information on the types of tasks/activities typically conducted in a commercial fertilizer treatment facility;
  - Current information on the time spent on each task/activity in a commercial fertilizer facility and potential for exposure to atrazine or atrazine-treated fertilizer;
  - Clarification on whether the treated fertilizer is bagged (if so, how large the bags are, and if manually or automatically bagged);
  - Clarification on whether the equipment is manually or automatically cleaned and if the equipment is cleaned after each batch or at the end of the day; and
  - Information on the typical duration and thoroughness of the equipment cleaning (for example, whether workers physically enter the mixing/blending equipment).

- Additional information and data on the potential exposure sources during loading of impregnated granular fertilizer in Canada. For example, whether the granules are loaded into the application equipment directly at the fertilizer facility or whether they are loaded into bags or a truck for transport to the field. If the latter, then information on how the granules are transferred from bags or trucks into the application equipment (for example, is the transfer done manually or automatically and whether the loading equipment is fully enclosed) should be provided.

4.2.2 Occupational postapplication exposure

Workers can be exposed to atrazine when entering a treated site to conduct activities, such as scouting and/or handling of treated crops.

Based on the registered use pattern, postapplication exposure of workers entering treated fields is expected to be short-term and via the dermal route. Inhalation exposure is expected to be minimal when entering after the shortest standard restricted entry interval of 12 hours. This would account for potential inhalation of fine spray droplets and any volatilization of atrazine.

For workers entering a treated site, restricted-entry intervals (REIs) are calculated to determine the minimum length of time required before workers can enter after application to perform tasks involving hand labour. The REI is the duration of time that must elapse in order to allow residues to decline to a level where risks are considered to be acceptable for postapplication worker activities.

Exposure of workers entering treated sites was estimated using activity-specific transfer coefficients (TCs), a dermal absorption factor for route-to-route extrapolation, and peak dislodgeable foliar residue (DFR) and dissipation rate values from a chemical-specific DFR study on corn. Additional inputs included an 8-hour workday for all activities and an average worker body weight.

Atrazine is currently registered for pre-emergent application (for example, pre-plant incorporated and before crop emergence) to all crops (corn, sorghum, switchgrass) and for post-emergent application to corn and sorghum. For workers entering treated sites (agricultural fields) following post-emergent applications, the MOEs are above the target MOE and, therefore, risks were shown to be acceptable at the standard 12 hour REI, as shown in Appendix VI, Table 4.

Postapplication dermal exposure following pre-emergent applications is expected to be minimal and is considered to be addressed by the quantitative post-emergent postapplication assessment and the minimum 12 hour REI. The 12 hour REI was also considered to address potential postapplication activities following application of atrazine impregnated granular fertilizer and liquid fertilizer mixed with atrazine. On this basis, the potential risks for postapplication workers entering treated sites (agricultural fields) are considered to be acceptable with a 12 hour REI. No additional mitigation measures are proposed. However, not all registered atrazine labels contain an REI statement; therefore, the standard REI statement is proposed to be added to the PRECAUTIONS section, when applicable (see Appendix II).

4.3 Aggregate exposure and risk assessment

Aggregate exposure is the total exposure to a single pesticide that may occur from food, drinking water, residential, and other non-occupational sources, and from all known or plausible exposure routes (oral, dermal and inhalation).

For atrazine, the aggregate assessment consisted of combining food and water exposure along with inhalation exposure to bystanders from spray drift, since residential exposure is not expected (see section 4.1.1). Chronic dietary (food + drinking water) exposure and was shown to be acceptable (see section 2), and, as noted in section 4.1.2, since bystander inhalation exposure was minimal compared to other routes of exposure (such as dietary and drinking water), the aggregate risk for bystanders was also considered to be acceptable.

In addition, human biological monitoring data for the Canadian population, including children above 3 years of age, from the Canadian Health Measures Survey (CHMS, Cycle 2; 2009-2011; Health Canada, 2013) are available for atrazine metabolites (atrazine mercapturate, and DACT, DEA). All monitored atrazine metabolites in this survey were below the limit of detection in all samples. Population-based biological monitoring surveys represent aggregate exposure from all routes and sources and further support the acceptability of the assessment.

5.0 Environmental risk assessment

5.1 Fate and behaviour in the environment

Physical and chemical properties of atrazine that are relevant to the environment are summarized in Appendix VII, Table 1. A summary of fate information for atrazine is presented in Appendix VII, Table 2. The summary consists of data reported in previous Health Canada environmental assessments (PACR 2007-05), as well as new fate information, primarily from open literature sources, reported by the USEPA (USEPA, 2016). Available water monitoring data from Canada and the United States were considered, which is presented in Appendix VIII, Table 10).

Laboratory studies show that atrazine is non-persistent to persistent in soil under aerobic and anaerobic conditions. While atrazine can break down by phototransformation, this process is not likely to have a significant impact on the dissipation in the terrestrial environment given that the laboratory half-life is 12 days under natural light. Atrazine has medium to very high mobility in soil depending on the soil type.

Under field conditions, atrazine has been reported to be slightly persistent to persistent. Residues as high as 41% remained in the soil during the subsequent growing season; therefore, a potential for carryover was identified. Under field conditions, most of the applied material dissipates from the root zone, but traces of atrazine residues can leach into soil depths greater than one meter approximately one year after application.

Transformation products identified in various laboratory soil studies included hydroxyatrazine (HA), desethylatrazine (DEA), desisopropylatrazine (DIA) and diaminochlorotriazine (DACT). Of these DEA, DIA and DACT were identified as mobile in soil, whereas HA is immobile to moderately mobile in soil. Under field conditions, the transformation products were first detected in soil at 450 days after the application of atrazine and were detected up to 938 days.

Atrazine is subject to transport from treated fields through surface runoff. Dissolved atrazine accounts for greater runoff losses than atrazine bound to eroded soil. Results of field studies have shown that surface runoff from corn fields is expected to be ≤ 2% of applied atrazine.

Atrazine is moderately persistent in aquatic systems under aerobic conditions and persistent under anaerobic conditions. Hydrolysis and phototransformation are not important routes of transformation.

Atrazine was shown to be moderately persistent to persistent in lake water. Results from artificial stream experiments show no significant accumulation of atrazine in sediments. In marine/estuarine systems, atrazine has been shown to be non-persistent to moderately persistent.

Atrazine is detected in the atmosphere, in areas removed from agricultural sites. Deposition of atrazine into surface waters occurs through gas exchange, particulate deposition and precipitation. The highest concentrations of atrazine in air (vapour + particulate) are expected to coincide with the application period. Atrazine is not expected to bioaccumulate.

5.2 Environmental toxicity

Atrazine toxicity data available for terrestrial and aquatic organisms were assessed primarily from information that was considered in the Health Canada 2007 environmental review of atrazine (PACR2007-05) and the more recent 2016 USEPA Refined Environmental Risk Assessment for Atrazine (USEPA, 2016). Additional toxicity data reported in the recent 2020 USEPA Biological Evaluation (USEPA, 2020) was also considered.

The 2016 USEPA refined ecological risk assessment (USEPA, 2016) is based on the results of hundreds of toxicity studies that look at the effects of atrazine on terrestrial and aquatic organisms, higher-tiered aquatic community level studies, over 20 years of surface water monitoring data and higher tiered exposure aquatic modelling. The USEPA classified studies as qualitative (or supplemental;, useful information but not directly used in the risk assessment) and quantitative (provided a valid endpoint that could be used directly in the risk assessment).

Although some studies were not conducted to guideline requirements, valuable information was obtained. If the studies were not of high enough quality to derive precise/accurate endpoints, qualitative conclusions were drawn. In some cases, studies may be deficient in some information and may restrict how a study should be interpreted. In these cases, uncertainties are considered

when summarising all the data. Despite such limitations, these studies may be deemed acceptable for consideration in the risk assessment if the methods/study design are considered reasonably scientifically sound, the study design/results are relevant within the Canadian context (for example, environmental conditions) and the results are consistent with results of other studies that were fully reviewed by Health Canada and found acceptable.

Studies classified as acceptable by the USEPA (or other OECD foreign review agencies) are considered acceptable by Health Canada. OECD member regulatory agencies evaluate the quality of toxicity data following standards and guidelines that are deemed acceptable to Health Canada. If Health Canada determines that a foreign review of an environmental fate or toxicity study is inaccurate or has reason to believe that the study may be unacceptable, Health Canada will conduct a review of the original study.

Summary tables of toxicity data are provided in Appendix IX (Tables 1 to 27). Studies considered acceptable, either from a quantitative or qualitative standpoint only, are listed in the toxicity tables for each taxon.

5.3 Environmental risk characterization

The environmental risk assessment integrates the environmental exposure and ecotoxicology information to estimate the potential for adverse effects on non-target species. This integration is achieved by comparing exposure concentrations with concentrations at which adverse effects occur. Estimated environmental concentrations (EECs) are concentrations of pesticide in various environmental media, such as food, water, soil and air.

The EECs (presented in Appendix X) are estimated using standard models which take into consideration the application rate(s), chemical properties and environmental fate properties, including the dissipation of the pesticide between applications.

Ecotoxicology information includes acute and chronic toxicity data for organisms (invertebrates, vertebrates and plants) from both terrestrial and aquatic habitats. For the risk assessment, effects metrics are established for all organism groups. These metrics can include unaltered laboratory or higher-tiered endpoints, laboratory or higher-tiered endpoints to which an uncertainty factor is applied, and geomeans of laboratory or higher-tiered endpoints. The effects metric is considered representative of the estimated exposure level at which adverse effects could result. A summary of effects metrics used in the risk assessment is presented in Table 3.

Where possible, the analysis of toxicity data may include the determination of the hazardous concentration to five percent of species (HC_5) from species sensitivity distributions (SSDs). The HC_5 is the concentration that is assumed to be protective for 95% of species of the assessed taxonomic group or assemblage as related to the assessment endpoint and ecological protection goal. At an EEC equal to the HC_5, 95% of all species (within each taxonomic group) are not expected to be exposed to concentrations exceeding their threshold toxicity value (for example, LC_{50}, NOEC). The HC_5 is calculated for acute and chronic data sets using the LC_{50}/EC_{50} values and NOEC values, as appropriate (an HC_{10}, hazardous concentration to 10% of species, may also be considered using EC_{25} values for terrestrial plants when no other data is available).

Initially, a screening level risk assessment is performed to identify pesticides and/or specific uses that do not pose a risk to non-target organisms, and to identify those groups of organisms for which there may be a potential risk. The screening level risk assessment uses simple methods, conservative exposure scenarios (for example, direct application at a maximum cumulative application rate) and sensitive toxicity endpoints. For characterizing acute risk, acute toxicity values (EC_{50}, LC_{50}, and LD_{50}) from the relevant toxicity studies are divided by an uncertainty factor. The uncertainty factor is used to account for differences in inter- and intra-species sensitivity. Thus, the magnitude of the uncertainty factor depends on the group of organisms that are being evaluated (10 for fish, 2 for aquatic invertebrates). The EC_{50} is the effective concentration estimated to cause an effect to 50 percent of the test population. Similarly, the LC_{50} or LD_{50} is the lethal concentration or lethal dose estimated to cause mortality to 50% of the test population. When assessing chronic risk, the no-observed effect concentration or level (NOEC or NOEL) is used and an uncertainty factor is not applied.

Integration of the environmental exposure and ecotoxicology is achieved by comparing exposure concentrations with concentrations at which adverse effects occur to derive a risk quotient. A risk quotient (RQ) is calculated by dividing the EEC by the effects metric, and the risk quotient is then compared to the level of concern (LOC). The LOC = 1 for all organisms with the exception of honeybees (acute LOC = 0.4) and beneficial terrestrial arthropods (LOC = 2). If the screening level risk quotient is below the level of concern, the risk is considered acceptable and no further risk characterization is necessary. If the screening level risk quotient is equal to or greater than the level of concern, then a refined risk assessment is performed to further characterize the risk. Refined assessments take into consideration more realistic exposure scenarios (such as drift to non-target habitats) and may consider different toxicity endpoints. Refinements may include further characterization of risk based on exposure modelling, monitoring data, results from field or mesocosm studies, and probabilistic risk assessment methods. Refinements to the risk assessment may continue until the risk is adequately characterized or no further refinements are possible.

Atrazine is shown to transform into various products through multiple pathways. Environmental fate data show that hydroxyatrazine (HA), desethylatrazine (DEA) and diaminochlorotriazine (DACT) are major transformation products of atrazine (> 10% of applied a.i.). Toxicity information for the transformation products of atrazine is available for birds, mammals, aquatic invertebrates, fish and algae and indicates transformation products have similar or lower toxicity compared to parent atrazine. Conducting the risk assessment based solely on toxicity and exposure to atrazine, is therefore considered sufficiently protective.

Table 3 Summary of toxicity effects metrics for the atrazine risk assessment

Taxa	Effects metrics (uncertainty factor)	Endpoint for risk assessment with uncertainty factor	Comments
Terrestrial organisms			
Soil dwelling invertebrates	Acute most sensitive sp. (14-day LC_{50}/2)	90.2 mg a.i./kg soil	Earthworm (*Eisenia fetida*); 14-d LC_{50} = 180.4 mg a.i./kg soil.

Taxa	Effects metrics (uncertainty factor)	Endpoint for risk assessment with uncertainty factor	Comments
	Chronic most sensitive sp. (30-day $LC_{50}/2$)	8.5 mg a.i./kg soil	Collembola (*O. Apuanicus*); 30-d LC_{50} = 17.2 mg a.i./kg soil.
Pollinators (Honeybee)	Adult acute most sensitive sp. (contact LD_{50})	> 97 µg/bee	Honeybee (*Apis mellifera*)
	Larva acute oral (72-h LD_{50})	33 µg a.i./larva	
	Larva chronic oral (22-d NOED)	6 µg a.i./larva	
Beneficial arthropods	Acute most sensitive sp. (NOEC)	2.24 kg a.i./ha	Laboratory test data for atrazine with standard test species, the predatory mite – *T. pyri* (DACO 9.2.5) and the parasitic wasp – *A. rhopalosiphi* (DACO 9.2.6), not available. NOEC representative of lowest definitive endpoint reported for 5 species of carabid beetles.
Birds	Acute most sensitive sp. ($LD_{50}/10$)	78.3 mg a.i./kg bw	Northern bobwhite quail (*Colinus virginianus*); LD_{50} = 783 mg a.i./kg bw
	Chronic most sensitive sp. (20-week NOEL)	7.9 mg a.i./kg bw/day	Mallard duck (*Anas platyrhynchos*). NOEL based on reduced number of eggs laid per pen.
Mammals	Acute most sensitive sp. ($LD_{50}/10$)	133.2 mg a.i./kg bw	Mouse; LD_{50} > 1332 mg a.i./kg bw
	Chronic most sensitive sp. (NOEL)	4.0 mg/kg bw/day	Two-generation reproductive toxicity study (Sprague-Dawley rat). NOEL based on offspring toxicity (reduced body weight gain, body weight).
Terrestrial Plants	Seedling emergence (14-day ER_{25})	2.8 g a.i./ha	Seedling emergence endpoint based on reduced dry weight for lettuce.
	Vegetative vigour (HC_{10} from an SSD of 21-28-d ER_{25} values)	22.4 g a.i./ha	Calculated by Health Canada (n = 33 species); details provided in Appendix XI.
Freshwater aquatic organisms			
Freshwater invertebrates	Acute 48-h $LD_{50}/2$	360 µg a.i./L	Midge (*Chironomus tentans*); 48h LC_{50} = 720 µg a.i./L.
	Chronic 30-day NOEC	60 µg a.i./L	Scud (*Gammarus fasciatus*). NOEC based on 25% reduction in development to seventh instar (LOEC = 140 µg a.i./L).

Taxa	Effects metrics (uncertainty factor)	Endpoint for risk assessment with uncertainty factor	Comments
Freshwater fish	Acute most sensitive sp. (96-h LC_{50}/10)	35 µg a.i./L	African Catfish fingerlings, (*Clarias gariepinus*); 96h LC_{50} = 350 µg a.i./L
	Chronic full life cycle – 44-week NOEC	65 µg a.i./L	Brook trout (*Salvelinus frontinalis*). NOEC based on reduced growth.
Freshwater algae	Acute most sensitive sp. (48-hour EC_{50}/2)	2.2 µg a.i./L	Chlorophycean green algae (*Chlorella vulgaris*); 4-day EC_{50} = 4.3 µg a.i./L atrazine (based on reduced abundance).
Freshwater vascular plants	Screening level: Most sensitive sp. (14-day EC_{50}/2)	2.3 µg a.i./L	Waterweed (*Elodea Canadensis*); 14-day EC_{50} of 4.6 based on reduced biomass.
	Refined risk assessment: Acute HC_5 value (SSD of EC_{50} values)	18.7 µg a.i./L	Calculated by Health Canada (n = 8 species); details provided in Appendix XI.
	Screening and drift assessment: Surrogate terrestrial plant: Vegetative vigour (HC_{10} from an SSD of 21-28-d ER_{25} values)	22.4 g a.i./ha	Freshwater vascular plants may be exposed to atrazine in water and from drift of spray. There are no toxicity endpoints for emerged freshwater species representative of exposure from drift of spray onto emerged plants. The vegetative vigour HC_{10} value determined for terrestrial plants was used as a surrogate endpoint to estimate risk to emerged freshwater vascular plants for the screening and drift assessment.
Amphibians	Acute most sensitive sp. (96-h LC_{50}/10)	41 µg a.i./L	American bullfrog (*Rana catesbaeiana*); 4-day LC_{50} of 410 µg a.i./L.
	Chronic 20–25-day NOEC	8.0 µg a.i./L	Black-spotted frog tadpoles *(Pelophylax nigromaculatus)*. Stage G26 exposed for 20 and 25 days; NOEC based on reduced growth.
Aquatic higher tier (freshwater)	NOEC	20 µg a.i./L	Generally, community-level effects were observed at atrazine concentrations of ≥10 µg a.i./L, but more often at 20 µg a.i./L.
Marine/estuarine organisms			
Marine invertebrates	Acute most sensitive sp. (48-h LC_{50}/2)	24 µg a.i./L	Opossum shrimp (*Neomysis integer*); 48-hour LC_{50} = 48 µg a.i./L.
	Chronic most sensitive sp. (41-day NOEC)	< 3.5 µg a.i./L	Copepod (*Amphiascus tenuiremis*). NOEC based on decrease in viable offspring production per female (F1).
Marine fish	Acute most sensitive sp.	200 µg a.i./L	Sheepshead Minnow (*Cyprinodon variegatus*); 96-hour LC_{50} = 2000 µg a.i./L.

Taxa	Effects metrics (uncertainty factor)	Endpoint for risk assessment with uncertainty factor	Comments
	($96\text{-h } LC_{50}/10$)		
	Chronic NOEC	8.5 µg a.i./L	Salinity challenge test - Atlantic salmon (*Salmo salar*): 21-day exposure in freshwater followed by 24-hour transition to seawater and 3-month rearing in seawater. NOEC is based on significant reduction in growth rate observed during the first month in seawater.
Marine algae	Acute most sensitive (96-h $EC_{50}/2$)	5.9 µg a.i./L	Chlorophycean green algae (*Ankistrodesmus* sp.); 4-day EC_{50} = 11.9 µg a.i./L atrazine (based on reduced chlorophyll a concentration
Marine vascular plants	Acute most sensitive (96-h $EC_{50}/2$)	15 µg a.i./L	Pondweed (*Potamogeton perfoliatus*); 28-day biomass EC_{50} = 30 µg a.i./ L
	Refined risk assessment: Acute HC_5 value (SSD of EC_{50} values)	16.5 µg a.i./L	Calculated by Health Canada (n = 23 species); details provided in Appendix XI.
	Screening and drift assessment: Surrogate terrestrial plant: Vegetative vigour (HC_{10} from an SSD of 21-28-d ER_{25} values)	22.4 g a.i./ha	Marine vascular plants may be exposed to atrazine in water and from spray drift. There are no toxicity endpoints for emerged freshwater species representative of exposure from drift of spray onto emerged plants. The vegetative vigour HC_{10} value determined for terrestrial plants was used as a surrogate endpoint to estimate risk to emerged marine vascular plants for the screening and drift assessment.

5.3.1 Terrestrial organisms

Earthworms and soil-dwelling organisms

At the screening level, RQ values did not exceed the level of concern (Appendix VIII, Table 1). The risks associated with the use of atrazine are acceptable for earthworms and soil-dwelling organisms.

Pollinators

The pollinator risk assessment was conducted according to the *2014 Guidance for Asssessing Risk to Bees*. The risk assessment uses a tiered approach in which Tier I considers the most conservative exposure to individual bees, whereas Tiers II and III progressively take into consideration more realistic exposure scenarios to bee colonies under semi-field and field conditions.

Pollinators can be exposed to atrazine from contact and/or feeding on contaminated parts of plants (for example, pollen and nectar). In-hive bees, including immature bees, can be exposed via contaminated plant materials brought back by foraging bees. For the Tier I assessment for foliar application, the highest single spray application rate for corn and switchgrass (1500 g a.i./ha), and sorghum (1000 g a.i./ha) was used to estimate the EEC. The most sensitive endpoints for acute contact toxicity tests on adult bees were used in the risk assessment as well as the larval oral acute and chronic toxicity tests. Acute oral and chronic oral toxicity tests on adult bees were not available. The Tier 1 risk assessment for bees is summarized in Appendix VIII, Table 2.

There is uncertainty with respect to potential risks to pollinators because a full Tier 1 suite of pollinator toxicity studies is not available. Nor is additional Tier 2 or 3 test data available to further refine the pollinator risk assessment. Based on the results of the Tier 1 screening level risk assessment, the use of atrazine may pose a risk to bee larvae. This potential risk, however, is expected to be low based on the following lines of evidence and reasoning:

1) The acute risk quotient for bee larvae only marginally exceeds the LOC for application to corn. For the Tier I assessment, dietary exposure is estimated based on upper-bound food consumption rates of pollen and nectar for honeybees from laboratory studies conducted under controlled conditions.
2) Corn, sorghum or switchgrass do not produce nectar, and pollen is produced in late summer. Tier 1 residue estimates do not consider dissipation or transformation of atrazine following application. Atrazine residue data for total foliar, nectar or pollen in plants is not available; however, dissipation data for dislodgeable residues of atrazine on field corn (half-life = 1.2–1.5 days; PMRA# 3263195) show that atrazine residues on corn do not persist long after application. Furthermore, given that atrazine is applied pre- or post-emergence in early spring, atrazine residues in nectar or pollen are likely significantly lower than the Tier 1 exposure estimates. Chronic exposure of bee larva to atrazine from nectar and pollen residues, therefore, is not anticipated.
3) Incident report reviews (see details in Section 6.2) indicate that atrazine was not the causal agent in any Canadian pollinator incidents.

The risks associated with the use of atrazine are acceptable for pollinators.

Beneficial arthropods

Laboratory test data for atrazine with standard test species for beneficial arthropods [the predatory mite - *Typhlodromus pyri* (DACO 9.2.5) and the parasitic wasp - *Aphidius rhopalosiphi* (DACO 9.2.6)], were not available for the review. The risk to beneficial arthropods from exposure to direct application of atrazine was determined based on a definitive acute no observed adverse effect concentration (NOAEC) value of 2.24 kg a.i./ha reported for five species of carabid beetles. At the maximum single field sprayer application rate for corn and switchgrass (1500 g a.i./ha), the risk quotient does not exceed the level of concern (LOC =1) for beneficial arthropods (RQ = EEC/NOAEC = 0.67). In higher-tier studies (field), no effects on arthropod populations (abundance) were observed up to 2 kg a.i./ha atrazine, which is higher than the maximum Canadian registered application rate for corn (1.5 kg a.i./ha). The risks associated with the use of atrazine are acceptable for beneficial arthropods.

Terrestrial plants

Non-target plants may be exposed to atrazine by overspray and spray drift. Sufficient laboratory data for seedling emergence and vegetative vigour are available to determine species sensitivity distributions for terrestrial plants.

The most sensitive terrestrial plant endpoint based on seedling emergence is a 14-day ER_{25} (the 25% effect rate) of 2.8 g a.i./ha for lettuce (based on reduced dry weight). Although a sufficient number of species endpoints were available for seedling emergence to estimate a hazardous concentration to ten percent of species (HC_{10} value) from a species sensitivity distribution (SSD), the model provided a poor fit (by visual inspection and rejection of the null hypothesis at alpha = 0.05 for the Anderson-Darling goodness-of-fit test).

The HR_{10} for vegetative vigour is 22.4 g a.i./ha (based on 21 to 28-day ER_{25} values, n = 33 species). Details regarding the calculation of the HR_{10} value (estimation method, endpoint selection criteria and specific data included) are provided in Appendix XI.

The results of the risk assessment are presented in Appendix VIII, Table 3. The risk quotients for terrestrial plants (seedling emergence and vegetative vigour) exceed the level of concern (LOC = 1) for all registered crops (corn, sorghum and switchgrass), indicating that terrestrial plants are at risk from direct overspray and from drift of spray. Information from environmental incidents (summarized in Section 6.2) is consistent with the known toxicity hazard of atrazine to terrestrial plants.

Spray buffer zones are required to mitigate risks to non-target terrestrial plants.

Birds and mammals

For the bird and mammal risk assessment, the ingestion of food items contaminated by spray droplets is considered to be the main route of exposure. The risk assessment is thus based on the estimated daily exposure, which takes into account the expected concentration of atrazine on various food items immediately after the last application and the food ingestion rate of different sizes of birds and mammals. At the screening level, only the most conservative exposure estimates are used, that is, the maximum single application rate for agricultural uses that results in the highest estimated daily exposures (corn and switchgrass – 1500 g a.i./ha).

Screening level risk quotients (RQ) are shown in Appendix VIII, Table 4. The level of concern (LOC = 1) is exceeded for certain birds and mammal sizes and feeding guilds at the screening level for acute and reproductive effects (RQ values up to 34) with the exception of large birds and mammals, and small mammals for acute effects.

To further characterize the risk to birds and mammals, the assessment was expanded to include a range of atrazine residue concentrations on all relevant food items at the single ground application for sorghum (1000 g a.i./ha) and the single ground application rate for corn and switchgrass (1500 g a.i./ha). The risk associated with the consumption of food items contaminated from spray drift off the treated field was assessed. The risk to birds and mammals based on maximum and mean residue values on terrestrial food sources is characterized in Appendix VIII, Tables 5 and 6, respectively.

The refined risk analysis shows that acute on-field risk to birds and mammals is not expected at the sorghum application rate, except for small insectivorous birds (based on maximum residue values only – RQ = 1.04). Atrazine is not expected to pose an acute risk off field to birds or mammals at the sorghum application rate due to drift. Atrazine may pose a risk to birds and mammals where it is applied to corn and switchgrass. Despite the risks identified, acute lethality to birds and mammals resulting from feeding on field is considered unlikely for the following reasons:

1) Risk is identified for birds and mammals feeding on-field only. No risk is identified for off-field feeding for birds and mammals. The RQ values that exceed the acute LOC for on-field feeding are low for birds and mammals.
2) For birds, the effect metric is based on a single dose oral gavage with technical atrazine (LD_{50} = 783 mg a.i./kg/day, Northern bobwhite quail). Lower acute sensitivity is demonstrated in other species based on end-use products up to the highest test concentration (LD_{50} > 2000 mg a.i./kg, Mallard and Ring-necked pheasant). Short-term acute dietary tests conducted with technical atrazine demonstrate relatively little to no toxicity to birds up to the highest test concentration (LD_{50} > 5000 to > 10000 mg a.i./diet; Northern bobwhite quail, Mallard, Ring-necked pheasant and Japanese quail).
3) For mammals, the endpoint selected for the risk assessment is conservative (the LD_{50} is a greater than value, >1332 mg a.i./kg in mice). Definitive acute mammalian endpoints show lower acute sensitivity (for example, LD_{50} = 1869 mg a.i./kg bw – rat, LD_{50} = 3992 mg a.i./kg bw –mouse).
4) The risk assessment is conservative in that it assumes dietary intake by birds and mammals comprises 100% of each type of food item (insect, grain, seed, fruit or plant). In some cases, although an acute exposure risk is identified, the risk is unlikely to manifest in birds and mammals as they would need to consume an unrealistically large proportion of a single food item (for example, 83% diet of insects for medium-sized birds feeding on fields treated at the highest single application rate for corn and switchgrass, based on maximum residue values).
5) Dissipation data for dislodgeable residues of atrazine on corn show that residues do not persist long after application (half-life = 1.2–1.5 days, PMRA# 3263195). Residues remaining on food items above the acute LOC, therefore, are expected to be short-lived.
6) The major use of atrazine in Canada is on field corn. Up to two pre-plant or pre-emergence applications may be made to field corn, but only one post-emergence application may be made; the yearly total application cannot exceed 1500 g a.i./ha. Atrazine residues on food items resulting from split application to corn (pre-plant or post-emergent) are not expected to exceed the acute LOC for birds and mammals.
7) The potential exposure to atrazine residues in food items resulting from application to switchgrass is limited relative to atrazine use on corn. Application to switchgrass is permitted once in the year of establishment only (pre-plant or pre-emergent).
8) There are no incident report reviews showing a causal link with atrazine.

The refined reproductive risk assessment shows that in most cases the risk quotients exceed the reproductive LOC for birds and mammals feeding on field based on both maximum and mean residue values. Overall, the refined risk assessment shows that reproductive effects from atrazine may pose a low risk to birds and mammals due to the following reasons:

1) The risk assessment is conservative in that it assumes that the dietary intake of birds and mammals comprises 100% of each type of food item (insect, grain, seed, fruit or plant). In the field, birds and mammals would not be expected to consume all of their diet from one single food source. However, for reproductive risk, in many cases, the proportion of a single food item required to reach the LOC is potentially very low (for example, as low as 8% and 6% for reproductive effects in medium-sized birds and mammals feeding on small insects at the highest application rate for corn and switchgrass, respectively).
2) The potential period of exposure is anticipated to be short. Dissipation data for dislodgeable residues of atrazine on corn show that residues do not persist long after application (half-life = 1.2–1.5 days, PMRA# 3263195). Residues remaining on food items, therefore, are expected to be short-lived. Although rapid dissipation would act to shorten the window of opportunity to which birds and mammals may be exposed to residue concentrations capable of eliciting reproductive effects, the potential for exposure during a critical reproductive period cannot be ruled out.
3) There is some uncertainty regarding the chronic effect metrics used for the bird and mammal risk assessment. For birds, the chronic effect metric is based on reduced number of eggs laid per pen for Mallard duck (the lowest test dose group - 75 mg a.i./kg diet, equivalent to 7.9 mg a.i./kg bw/day - NOEL). A slight increase in the number of eggs laid per pen was observed at the NOEL (% effect = -1.91%) whereas a significant decrease was observed at 22.5 mg a.i./kg bw/day (LOEL) and at the highest dose group, 65.6 mg a.i./kg bw/day (% effect = 21.3 and 49.0%, respectively). No significant effects, however, were observed at the 7.9 or 22.5 mg a.i./kg/day dose level for any of the other offspring parameters measured (live embryos per eggs set, live embryos per viable embryos, hatchlings per eggs set, hatchling survival). For adults, a statistically significant reduction in food consumption was also observed at 22.5 mg a.i./kg bw/day, albeit the effect is very small (% effect = 7.62). Considerable data overlap is present across control and treatment groups for both parameters (eggs laid per hen and adult food consumption). Based on the aggregate of these results, there is some uncertainty as to whether the extent of these effects is biologically-relevant or treatment-related. The chronic effect chosen for the avian risk assessment, therefore, is potentially overly conservative.
4) For mammals, no treatment-related mortalities or clinical observations were observed in Norway rats (*Rattus norvegicus*) after dietary exposures up to the highest test dose concentration (500 mg a.i./diet) for two consecutive generations. The chronic effect metric is based on the NOEC of 50 mg a.i./kg diet (NOEL - 4.0 mg a.i./kg bw/day) reported for offspring effects: a slight decrease in mean pup body weight gain and body weight was observed at the highest test concentration (500 mg a.i./kg diet) in F1 and F2 males on postnatal day 21 and in F1 female pups. A slight but statistically significant increase in relative testes weight was also reported in both generations at the highest dose, however, atrazine did not significantly impair reproductive performance at any test dose concentration. Based on the overall results for effects to the F1 and F2 generation, the chronic effect metric (based on reduced pup weight) is, therefore, potentially overly conservative.

There are no incident reports involving birds and mammals from the use of atrazine, albeit none would be expected from adverse chronic exposure; chronic problems affecting wildlife from the use of atrazine may be largely unnoticed in the field.

The risks associated with the use of atrazine are acceptable for birds and mammals. Although the reproductive risk to birds and mammals is considered low, a label statement is required to inform the user of the potential hazard.

5.3.2 Aquatic organisms

Screening level risk assessment for aquatic organisms

For the screening level risk assessment, expected environmental concentrations (EECs) of atrazine in water were calculated based on the maximum single foliar application rate for corn and switchgrass (1500 g a.i./ha), and direct application to water bodies with a depth of 15 cm (seasonal water body for amphibian endpoints) and 80 cm (permanent water body for remaining endpoints).

For the assessment of risk, toxicity endpoints chosen from the most sensitive species tested were used as surrogates for the wide range of species that can be potentially exposed following treatment with atrazine. The acute endpoints were derived by dividing the EC_{50} or LC_{50} from the appropriate laboratory study by a factor of two (2) for algae and aquatic plants, and by a factor of 10 for fish and amphibians to account for potential differences in species sensitivity as well as varying protection goals (protection at the community, population, or individual level).

The screening level risk assessment for aquatic organisms is summarized in Appendix VIII, Table 7. Risk quotients exceed the level of concern (LOC = 1) for all aquatic organisms with the exception of freshwater invertebrates and marine fish for acute effects.

Refined risk assessment for aquatic organisms

Sufficient laboratory toxicity data were available for freshwater algae, vascular plants, and fish, and marine/estuarine algae and vascular plants to determine acute HC_5 values (the 5th percentile of the species sensitivity distribution (SSD) for the LC_{50}/EC_{50} at 50% confidence intervals). For freshwater vascular plants and marine/estuarine algae, HC_5 values of 18.72 and 16.5 µg a.i./L, respectively, were considered for the refined risk assessment. For freshwater fish, although a sufficient number of species endpoints were available to estimate an HC_5 value from a species sensitivity distribution (SSD), the model provided a poor fit (by visual inspection and rejection of the null hypothesis at alpha = 0.05 for the Anderson-Darling goodness-of-fit test). For marine/estuarine vascular plants, it was not possible to create an SSD for atrazine because the exposure duration for available EC_{50} values is too variable. Details regarding the calculation of the acute HC_5 value (in other words, estimation method and data handling, study endpoints and study references) are provided in Appendix XI.

Spray drift

The risk to aquatic invertebrates was further characterized by taking into consideration the concentrations of atrazine that could be deposited through spray drift in off-field aquatic habitats that are downwind and directly adjacent to the treated field. The maximum amount of spray that is expected to drift 1-m downwind from the application site during spraying using field sprayer based on a medium spray droplet size is 6%. The potential risk from drift was assessed for the single ground application for sorghum (1000 g a.i./ha), corn and switchgrass (1500 g a.i./ha).

In marine/estuarine habitats, chronic exposure resulting from spray drift is not expected given the high rates of water replacement due to tidal flushing. For this reason, risk from spray drift for marine organisms is determined based on the acute effects metrics, with the exception of marine fish. The chronic endpoint for Atlantic salmon (*Salmo salar*) remains relevant for the drift risk assessment because fish were exposed to atrazine for 21 days in freshwater followed by a 24-hour transition to seawater.

The risk to aquatic organisms resulting from spray drift are presented in Appendix VIII, Table 8. The risk to aquatic organisms resulting from spray drift is summarized in Appendix VIII, Table 7. The acute risk quotients indicate that the LOC is exceeded for amphibians (RQ = 1.5 based on application to corn only) and freshwater algae (RQ = 3.4–5.1). On a chronic basis, the LOC is exceeded for amphibians (RQ = 5.0–7.5), marine fish (RQ = 1.3 based on application to corn only) and marine algae (RQ = 1.3–1.9).

There are no toxicity endpoints for emerged freshwater or marine vascular plant species representative of direct application exposure onto emerged plants. The vegetative vigour HC_{10} value determined for terrestrial plants (22.4 g a.i./ha) was used as a surrogate endpoint to estimate risk to emerged freshwater and marine vascular plants. The associated risk quotient (EEC/(HC_{10})) for spray drift from application for sorghum (1000 g a.i./ha), and corn and switchgrass (1500 g a.i./ha) is 2.7 and 4.0, respectively. Spray buffer zones will be required to mitigate risks to aquatic organisms from spray drift.

Runoff

Water modelling risk assessment

Aquatic organisms can also be exposed to atrazine as a result of runoff into a body of water. The Pesticide in Water Calculator (PWC) model (v.1.52) was used to predict estimated environmental concentrations (EECs) resulting from runoff of atrazine following field sprayer application. The models were run at foliar application rates representative for sorghum, corn and switchgrass. The Level 1 atrazine EECs in a 1-ha receiving water body (80- and 15-cm deep) predicted by PWC for these crop applications are presented in Appendix VIII, Tables 3 and 4. The values reported by PWC are 90th percentile concentrations of the concentrations determined at a number of timeframes including the yearly peak, 96-hour, 21-day, 60-day, 90-day and yearly average.

Acute and chronic RQ values were calculated using an EEC for the timeframe which most closely matched the exposure time used to generate the endpoint (for example, a 96-hour LC_{50} would use the 96-hour value generated by the model; a 21-day NOEC would use the 21-day EEC value).

The acute and chronic RQ values for aquatic organisms determined for runoff modelling of ground field sprayer applications that exceed the LOC for each crop and region are summarized in Appendix VIII, Table 9. Acute RQ values are as high as 61 and chronic RQ values are as high as 74. However, the PMRA considered water monitoring information as outlined below to further characterize the risk.

Water monitoring risk assessment

Canadian water monitoring data were available for consideration in the aquatic risk assessment. Data from the year 2006 onward (the last 15 years) were considered relevant for the assessment; older data were deemed unlikely to represent current Canadian use conditions and were not included. Atrazine concentrations were measured in 17 527 surface water samples collected from Canada's ten provinces (only two samples from Newfoundland) (Appendix VIII, Table 10). Examples of waterbodies sampled included rivers, lakes, creeks, small streams, ponds, sloughs, and a few wetlands. Waterbodies sampled were typically located in agricultural areas. A total of 6750 samples were from Ontario and Quebec, where most of the corn is grown in Canada. The number of samples, the sampling frequency and the number of years of monitoring varied among programs. In Ontario, several rivers and creeks located in corn-growing areas were sampled at weekly to monthly intervals. In Quebec, sampling was more frequent, with sampling from every two or three days, to weekly. While some sites have only one year of monitoring, at least 20 rivers and creeks in agricultural areas of Ontario and Quebec had more than seven years of monitoring (some up to 13 years).

From the 17 527 Canadian surface water samples, the highest concentration of atrazine detected was 37 µg/L in a river from Quebec sampled in 2013. The second highest detected atrazine concentration was 18 µg/L, in two Ontario waterbodies (one in 2015, one in 2019). Out of the 17,527 samples collected in Canada (6750 in Ontario and Quebec), none of them had atrazine concentrations exceeding the acute or chronic effects metrics for freshwater invertebrates or the acute effects metrics for amphibians. Only one sample exceeded the acute effects metric for freshwater fish and the effects metrics for aquatic vascular plants and algae (Appendix VIII, Table 11). A total of 18 samples (0.1% of total samples) from 12 sites in Ontario and Quebec exceeded the chronic effects metric for amphibians, a 25-day NOEC of 8 µg/L.

Looking at longer-term average concentrations at the twelve sites showing atrazine concentrations exceeding the chronic effects metric for amphibians, only three of them had maximum 25-day (approximate) rolling average concentrations exceeding 8 µg/L (Appendix VIII, Table 12). The average in two of the three cases was associated with a higher degree of uncertainty; only two values were used to calculate the average at these sites, and one of the values was a non-detect. The sampling frequency at two of the sites did not allow for the calculation of an average over the span of approximately 25 days. The shortest time interval would have been 49 days, which is almost twice as long as the exposure period in the amphibian study. The risk quotients associated with the longer-term average concentrations at these twelve sites ranged from 0.1 to 1.6. For the two sites where the sampling frequency did not allow for the calculation of a 25-day average using the observed data, the highest concentration detected (1.25 and 1.63 µg/L) was used as a conservative estimate of exposure in the risk quotient calculation.

The available Canadian water monitoring data are considered appropriate for risk assessment purposes for freshwater invertebrates, fish, aquatic vascular plants and algae; however, they are less representative of the most vulnerable habitat for amphibians. The waterbodies sampled in Ontario and Quebec where most of the corn is grown in Canada were mainly flowing rivers and creeks (no wetlands).

While rivers and creeks are considered potential habitat for amphibians, they may not represent shallow seasonal waterbodies, which are considered the most vulnerable exposure scenario for amphibians. The dataset for Canada included ten samples from wetlands in British Columbia, where the highest atrazine concentration measured was 0.03 µg/L.

Two studies published in the literature reported concentrations of atrazine in wetlands in the United States. One of the studies was conducted in Iowa where corn may have been grown (PMRA# 2526244), while the other study was not conducted in areas where corn was grown (PMRA# 2988073). In the Iowa study, six wetlands were sampled two to three times between April and July in 2012 and 2013. Concentrations in the wetlands ranged from 0.07 to 19 µg/L and were below the acute effects metric for amphibians. The second highest detection was 0.9 µg/L. An estimate of a longer-term exposure concentration in the wetland showing the detection of 19 µg/L could not be calculated because no samples were collected following that detection.

Some Canadian water monitoring data from more than 15 years ago is available from Environment and Climate Change Canada's Pesticide Science Fund. The data had been excluded because they are older, but data collected specifically from small stream tributary sites and amphibian breeding habitat (farm ponds and streams) are considered below.

Agricultural sites based in two areas of the Thames River watershed, north of London, ON and surrounding Chatham, ON, were sampled in PMRA# 1311111. Both are areas of intensive row crop agriculture; wetlands are primarily agricultural drains with few natural farm ponds. In 2004 and 2005, monthly sampling for atrazine was conducted as part of this project at wetland sites. The highest level recorded was 4.74 µg/L in 2004 from a site in Chatham. Atrazine concentrations followed a seasonal pattern peaking at the beginning of July and then tapering off in the fall. There were no significant differences in atrazine concentrations between Chatham and London sites. Concentrations of atrazine were significantly higher in drains as compared to ponds. Results for 2005 were not presented in the original report.

Results of 229 water samples were analyzed from 18 small stream tributary sites and 10 amphibian breeding sites (Ontario farm ponds and streams) are reported in PMRA# 1403269. Atrazine was detected in 90.1% of samples, indicating that the sites were in areas of use. Concentrations ranged from 0.00643 to 14.9 µg/L (median of 0.098 µg/L), which are below the acute effects metric for amphibians. No details were provided that could help determine amphibian chronic exposure.

The availability of a large database of highly relevant Canadian water monitoring data allowed for further refinement of the aquatic risk assessment. This water monitoring data indicates risks to aquatic organisms are acceptable. Chronic risk to amphibians is identified as the key driver of the aquatic risk assessment. While rivers and creeks are considered potential habitat for amphibians, these habitats may not be representative of the most vulnerable exposure scenario for amphibians (in other words, shallow seasonal waterbodies with no flow, such as, ponds and wetlands).

Available water monitoring data from shallow farm ponds and wetlands in Canadian corn-growing areas are consistent with the larger database of water monitoring data for flowing water bodies which supports the conclusion that chronic risks to amphibians are acceptable. As part of efforts to provide continual oversight for registered pesticides in Canada, additional data will be considered as they become available.

Precautionary label statements are currently on all atrazine end-use product labels to reduce the potential for runoff to adjacent aquatic habitats. Updated label statements to minimize the potential for runoff are proposed in Appendix III.

5.4 Environmental risk conclusions

Terrestrial organisms

Risks are acceptable for soil-dwelling invertebrates, pollinators, and beneficial arthropods.

The refined risk assessment indicates atrazine may pose a low reproductive risk to birds and mammals. Although there are no incident reports involving birds and mammals, chronic problems affecting wildlife from the use of atrazine may not be easily noticed in the field. Considering all the available information, including an understanding that the period of exposure to residues above a level of concern is expected to be short, the weight of evidence indicates that risks to birds and mammals are acceptable. A label statement is required to inform the user of the potential hazard to birds and mammals.

Potential risks were identified for plants. These risks can be mitigated with spray buffer zones of 10 to 15 metres for sensitive terrestrial habitats (Appendix III). With spray buffer zones in place, risks are acceptable.

Overall. risks to terrestrial organisms are acceptable when products are used according to label directions.

Aquatic organisms

Spray drift poses potential risks to amphibians, freshwater algae, marine fish and marine/estuarine vascular plants. These risks can be mitigated with spray buffer zones of 2–5 metres for freshwater habitats and 2–3 metres for marine habitats (Appendix III). With spray buffer zones in place, risks associated with spray drift are acceptable.

Atrazine is subject to transport in surface runoff from treated fields, can leach through the soil and has been detected in groundwater. Precautionary label statements are currently on all atrazine end-use product labels to reduce the potential for runoff to adjacent aquatic habitats and leaching to groundwater.

Health Canada’s aquatic risk conclusions are based on the weight-of-evidence from an extensive amount of effects and exposure data including chronic toxicity data, surface water modelling and recent Canadian environmental monitoring data.

Atrazine surface water modelling estimates exceedances of the level of concern for acute and chronic effects to aquatic organisms in most regions. Water modelling inputs and assumptions are conservative, and the EECs generated are likely to be higher than actual concentrations present in waterbodies.

The availability of a large database of highly relevant Canadian water monitoring data allowed for further refinement of the aquatic risk assessment. This water monitoring data indicates risks to aquatic organisms are acceptable. Chronic risk to amphibians is identified as the key driver of the aquatic risk assessment. While rivers and creeks are considered potential habitat for amphibians, these habitats may not be representative of the most vulnerable exposure scenario for amphibians (shallow seasonal waterbodies with no flow – for example, ponds, wetlands). Available water monitoring data from shallow farm ponds and wetlands in Canadian corn-growing areas are consistent with the larger database of water monitoring data for flowing water bodies which supports the conclusion that chronic risks to amphibians are acceptable. As part of efforts to provide continual oversight for registered pesticides in Canada, additional data will be considered as they become available.

Updates to the label statements (for example, toxicity hazard, runoff and groundwater leaching statements, use directions) are required. Risks to aquatic organisms are acceptable when products are used according to label directions.

6.0 Incident reports

6.1 Health incident reports

As of 29 December 2021, nine human incidents (4 American and 5 Canadian incidents) and nine domestic animal incidents involving atrazine were submitted to Health Canada through the Incident Reporting Program.

In the four American human major incidents, the details surrounding the reported circumstances of atrazine exposure (for example, eating atrazine contaminated food) were either lacking or the reported effects (for example, cancer, lymphoma) were unclassifiable due to the role of other unknown confounding factors (for example, biological or environmental). In the Canadian human incidents that were considered to be related to the reported product (3 reports), the exposure scenarios involved contact with product residues when handling an atrazine product or inhaling fumes from a fire lit in a barrel containing atrazine residues. The reported symptoms were mainly minor and included effects such as eye and skin irritation or chest pain.

In the domestic animal incidents considered to be possibly related to the reported product (5 out of 9 incidents), animals were exposed to atrazine either as a result of suspected contact with treated areas or by ingesting a bag containing atrazine. The symptoms reported in animals included lethargy, anorexia, ataxia or death.

No consistent exposure or adverse effects patterns were noted following the review of atrazine incidents. As such, no health issues relating to the aspects of concern were identified. Therefore, no additional mitigation measures are being proposed based on the incident report review.

6.2 Environment incident reports

As of 29 December 2021, 13 environment incidents, relating to effects on herbaceous plants, trees and shrubs, have been reported to Health Canada through the Incident Reporting Program. Five incidents were reported in Canada for injuries sustained to herbaceous plants that involved atrazine only. It is probable that these incidents were a direct result of the application of atrazine. Eight of the incidents to herbaceous plants, trees and shrubs, involved multiple herbicidal active ingredients; therefore, it is impossible to determine if they were a result of atrazine alone.

Between 2012 and 2016, a large number of pollinator incidents in the corn- and soybean-growing region of Ontario and Quebec were reported to the PMRA as potentially related to a number of pesticides that are highly toxic to pollinators. Atrazine was not suspected as contributing to the incidents by the individuals who reported the incidents. During the analysis of the bee samples, atrazine was detected at low quantities in a small number of incidents in Quebec. The analytical methods used for the incidents in Ontario did not include atrazine. Of the incidents where atrazine was detected, one occurred in 2011, five in 2012, four in 2013 and two in 2014. The effects observed in these incidents (trembling, aggressive behaviour, confusion, disorientation) are not expected for pollinators exposed to atrazine and are known to be associated with exposure to pesticides that are highly toxic to bees or bee pathogens. Based on the information available for these incidents, it is unlikely that atrazine contributed to the effects observed and reported during these pollinator incidents.

The United States Ecological Incident Information System (EIIS) was also searched for environment incidents involving atrazine. As of October 2015 (the last update available to Health Canada), 666 incidents involving atrazine were reported to the EIIS database. The assigned certainty index was as follows: 605 were possible or higher, 60 were unlikely and one was unrelated.

Most incidents (584 reports, 87%) involved plants. Soybeans (202 reports) and corn (294 reports) were the most commonly reported species in incidents. Exposure scenarios noted in incidents were mainly carryover (147 reports) followed by direct application to plants or drift from an application site. Carryover as high as 41% was observed in terrestrial field dissipation studies; Canadian labels have a statement advising that atrazine is persistent and may carryover. Effects reported in plants were mainly non-specific plant damage.

Aquatic species were reported in 19 unique incidents in the United States. No incident reports occurred after 2005. Reported species affected include bass, bluegill, channel catfish, bullhead, bream and others. Run-off from a registered application site was the most common exposure scenario that resulted in fish mortality. The USEPA (2016) reports:

> *"The presence of atrazine at levels thought to be sufficient to cause either direct or indirect effects was confirmed in 3 aquatic incidents evaluated. Atrazine use was also correlated with 14 incidents where its presence in the affected water was not confirmed, but the timing of atrazine application was correlated with the incident.*

The remaining incidents were likely caused by some factor other than atrazine. Other causes primarily include the presence of other pesticides at levels known to be toxic to affected animals."

In seven incident reports, the affected organism was noted as "terrestrial". Reported species were honeybees (two), cows (two) and birds (three). Reported exposure scenarios were mainly drift from an agricultural area under conditions of misuse (unspecified).

List of abbreviations

<	less than
>	greater than
≤	less than or equal to
≥	greater than or equal to
↑	Increased
↓	Decreased
µg	microgram(s)
µM	Micromolar
♀	Female
♂	Male
^{14}C	carbon-14 or radiocarbon
5-HIAA	5-hydroxy-indoleacetic acid
A/G	albumin/globulin
abs	Absolute
ACTH	adrenocorticotropic hormone
AD	administered dose
ADI	acceptable daily intake
ADX	Adrenalectomized
AFC	antibody forming cell
AGD	anogenital distance
AHETF	Agricultural Handler Exposure Task Force
AHS	agricultural health study
a.i.	active ingredient
ALT	alanine transaminase
APVMA	Australian Pesticides and Veterinary Medicines Authority
ARfD	acute reference dose
ARTF	Agricultural Re-entry Task Force
ASAE	American Society of Agricultural and Biological Engineers
AST	aspartate transaminase
atm	atmosphere(s)
ATPD	area treated per day
ATR	Atrazine
AUC	area under the curve
b.i.d	bis in die
BBB	blood brain barrier
BDNF	brain-derived neurotropic factor
BE	Biological evaluation
BrdU	Bromodeoxyurdine
BSA	bovine serum albumin
BSS	bagger, sewer, stacker
BUN	blood urea nitrogen
bw	body weight
BWG	bodyweight gain

C	Control
CA1	cornu ammonis 1
CAF	composite assessment factor
CD	classification determinant or cluster of differentiation
CEC	cation exchange capacity
CF	correction factor
cFOS	immediate early gene product FOS
CG	crop group
CHMS	Canadian Health Measures Survey
CI	confidence interval
cm	centimeter(s)
C_{max}	maximum serum concentration
CNS	central nervous system
COMT	catechol-O-methyl transferase
Con A	concanavalin A
CR	chemical resistant
CREB	cAMP response element-binding protein
CYP	cytochrome P
DA	Dopamine
DACT	Diaminochlorotriazine
D-Ala-6 GnRH	GnRH analog
DAT	dopamine transporter
DEA	desethyl-atrazine
DEEM-FCID™	Dietary Exposure Evaluation Model - Food Commodity Intake Database™
DEHA	Desethylhydroxyatrazine
DER	Data evaluation record
DFR	dislodgeable foliar residue
DHT	Dihydrotestosterone
DIA	desisopropyl-atrazine
DIHA	Desisopropylhydroxyatrazine
DNA	deoxyribonucleic acid
DOPAC	3,4-dihydroxyphenylacetic acid
DPR	Department of Pesticide Regulation
DT_{50}	dissipation time 50% (the time required to observe a 50% decline in concentration)
DT_{90}	dissipation time 90% (the time required to observe a 90% decline in concentration)
DTH	delayed hypersensitivity
dw	dry weight
EC_{10}	effective concentration on 10% of the population
EC_{20}	effective concentration on 20% of the population
ECCC	Environment and Climate Change Canada
EDSP	Endocrine Disruptor Screening Program
EEC	estimated environmental concentration
ELISA	enzyme-linked immunosorbent assay
ER	estrogen receptor
ER_{25}	effective rate on 25% of the population
ERK	extracellular signal-regulated kinase

ET	exposure time
F1	first filial generation
F2	second filial generation
FA	fraction of species affected
FC	food consumption
FGR	fetal growth restriction
FSH	follicle-simulating hormone
FST	forced swim test
g	gram(s)
GD	gestation day
GLP	good laboratory practice
hr(s)	hour(s)
HA	hydroxyatrazine
ha	hectare(s)
HC_5	hazardous concentration estimate that is assumed to be protective of 95% of species in a species sensitivity distribution
HCT	Hematocrit
HDL	high density lipoprotein
HGB	Hemoglobin
HLZ	Holtzman
HPA	hypothalamus-pituitary-adrenal
HPG	hypothalamus-pituitary-gonadal
HPLC	high-performance liquid chromatography
HPLC	high performance liquid chromatography
HVA	homovanillic acid
i.p.	intraperitoneal
IARC	International Agency for Research on Cancer
IC_{50}	inhibition concentration on 50% of the population
IFN-γ	interferon gamma
IgM	immunoglobin M
IL-2	interleukin 2
IL-4	interleukin 4
IORE	Indeterminate Order Rate Equation Model
IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
IV	Intravenous
JMPR	Joint Meeting on Pesticide Residues
Kd	soil-water partition coefficient
Kfoc	organic-carbon normalized Freundlich distribution coefficient
kg	kilogram(s)
K_{oc}	organic-carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	litre(s)
LA/BC	levator ani with bulbocavernosus muscles
LC	liquid chromatography
LC_{50}	concentration estimated to be lethal to 50% of the test population

LD	lactation day
LD_{50}	dose estimated to be lethal to 50% of the test population
L-DA	levodopa (DA precursor)
LDL	low density lipoprotein
LE	Long-Evans
LH	luteinizing hormone
LOAEL	lowest observed adverse effect level
LOC	level of concern
LOD	limit of detection
LOEC	lowest observed effect concentration
LPS	lipopolysaccharide
m	metre(s)
meq	millequivalents
M/L	mixer/loader
M/L/A	mixer/loader/applicator
m^3	cubic meter
MAO	monoamine oxidase
Max	maximum
MCF-7 cell line	Michigan cancer foundation-7 cell line
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MEK	mitogen-activated protein kinase
mesDA	mesencephalic dopamine neuron
mg	milligram(s)
MHC	major histocompatibility complex
MHPG	3-methyoxy-4-hydroxyphenylglycol
MI	mitotic index
MIE	molecular initiating event
min	minute(s)
mL	millilitre(s)
MLR	mixed leukocyte proliferative response
mm	millimetre(s)
MOA	Mode of Action
MOE	margin of exposure
MPO	myleoperoxidase
MPS	mononuclear phagocytic system
MRID	master record identification
MRL	maximum residue limit
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MTT	3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenylte-trazolium bromide
MWM	Morris water maze
N	sample size
N/A	not applicable

N/R	not required
NC	not calculated
ND	not detected
NE	norepinephrine
ng	nanogram(s)
NHEERL	National Health and Environmental Effects Research Laboratory
NKC	natural killer cells
NMRI	Naval Medicine Research Institute
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
NOR	novel object recognition
NPI	novelty preference index
NR	not reported
Nurr1	nuclear receptor related 1 protein
NZW	New Zealand white
OC	organic carbon content
OECD	Organisation for Economic Cooperation and Development
OM	organic matter content
OR	odds ratio
ORD	Office of Research and Development
OVX	ovariectomized/ovariectomies
P	parental generation
PACR	Proposed Acceptability for Continuing Registration
PBPK	physiological-based pharmacokinetic
PCE	polychromatic erythrocytes
PCPA	*Pest Control Product Act*
p-CREB	phosphorylated CREB
PCT	percent crop treated
PELAGIE	Perturbateurs endocriniens: Etude Longitudinale sur les Anomalies de la Grossesse, l'lnfertilite et l'Enfance
p-ERK	phosphorylated ERK
pg	picogram
PHED	Pesticide Handler Exposure Database
pk	peak
pKa	dissociation constant
p-MEK	phosphorylated MEK
PMRA	Pest Management Regulatory Agency
PND	postnatal day
POD	point of departure
ppb	parts per billion
PPE	personal protective equipment
ppm	parts per million
PSA	prostate-specific antigen
PW	porewater
RBC	red blood cells

REI	restricted-entry interval
rel	relative
REV	Re-evaluation Note
RIA	radioimmunoassay
RQ	risk quotient
RR	relative risk
RRD	Re-evaluation Decision Document
RT-PCR	reverse transcription-polymerase chain reaction
s.i.d	semel in die
SAP	scientific advisory panel
SCE	sister chromatic exchange
SD	Sprague-Dawley
SFO	single first order
SIR	standardized incidence rate
SNpc	substantia nigra pars
SOP	standard operating procedures
sp.	species (singular)
spp.	species (plural)
SRBC	sheep red blood cells
SSD	Species Sensitivity Distribution
$t_{1/2}$	half-life
$T_{1/2}$	elimination half-life
TC	transfer coefficient
TCT	total chlorotriazines
TEB	terminal end buds
TH	tyrosine hydroxylase
TK	toxicokinetic
T_{max}	time to reach C_{max}
TNF-α	tumour necrosis factor alpha
TP	transformation products
TRR	total radioactive residue
UDS	unscheduled DNA synthesis
UF_{DB}	database uncertainty factor
USEPA	United States Environmental Protection Agency
UV	ultraviolet
VMAT2	vesicular monoamine transporter 2
VO	vaginal opening
vs	versus
VTA	ventral tegmental area
WHO	World Health Organisation
wt(s)	weight(s)
Zif268	zinc finger protein 225

Appendix I Registered products containing atrazine as of 1 September 2022

Registration number	Marketing class	Registrant	Product name	Guarantee
16641	C	BASF Canada Inc.	LADDOK HERBICIDE	200 g/L
18438	T	Syngenta Canada Inc.	ATRAZINE TECHNICAL	96 %
18450	C	Syngenta Canada Inc.	AATREX LIQUID 480 HERBICIDE	480 g/L
19349	C	BASF Canada Inc.	MARKSMAN HERBICIDE	261 g/L
25730	C	Syngenta Canada Inc.	PRIMEXTRA II MAGNUM HERBICIDE	320 g/L
26277	C	Bayer CropScience Inc.	CONVERGE 480 HERBICIDE	480 g/L

Registration number	Marketing class	Registrant	Product name	Guarantee
29358	C	Syngenta Canada Inc.	PRIMEXTRA II MAGNUM AGRICULTURAL HERBICIDE	320 g/L
30373	M	Syngenta Canada Inc.	ATRAZINE BASE MIX MANUFACTURING CONCENTRATE	57.8%
30519	C	BASF Canada Inc.	FRONTIER MAX PLUS	396 g/L
30726	M	BASF Canada Inc.	MARKSMAN BULK HERBICIDE	261 g/L
30864	C	Syngenta Canada Inc.	LUMAX EZ HERBICIDE	112 g/L
31846	C	Syngenta Canada Inc.	ACURON HERBICIDE	120 g/L

Registration number	Marketing class	Registrant	Product name	Guarantee
34235	C	Syngenta Canada Inc.	A22668 HERBICIDE	105 g a.e./L

Appendix II Proposed label amendments for end-use products containing atrazine, for the protection of human health

The proposed label amendments presented below do not include all label requirements for individual end-use products, such as first aid statements, disposal statements, precautionary statements and supplementary protective equipment. Information on labels of currently registered products should not be removed unless it contradicts the label statements below.

Commercial class products

Uses proposed for cancellation: All label language related to the impregnated granular fertilizer use must be removed from all applicable labels.

1.1 General label improvements

The aerial application language in the **PRECAUTIONS** and **DIRECTIONS FOR USE** sections is to be modified as follows:

- Replace "**DO NOT** apply using aerial application equipment" with "**DO NOT** apply by air"

In order to promote best practices, and to minimize human exposure from spray drift or from spray residues resulting from drift due to the use of atrazine, the following label statement is proposed to be added to labels under **PRECAUTIONS**. If a similar statement is already present, it should be replaced with the following statement:

- "Apply only to agricultural crops when the potential for drift to areas of human habitation and human activity, such as houses, cottages, schools, and recreational areas, is minimal. Take into consideration wind speed, wind direction, temperature inversions, application equipment, and sprayer settings."

1.2 Precautions

Under the **PRECAUTIONS** section (Personal Protective Equipment; PPE) add the following, unless the current mitigation is more restrictive. Should the PPE on the label be more restrictive (for example, respirator requirement), then those PPE should be incorporated into the applicable statement(s) below.

- "If mixing/loading more than [85 kg a.i. of atrazine to be reported in product equivalent value] per person per day, a closed mixing/loading system must be used." As indicated by square brackets, the amount of atrazine in the statement (85 kg a.i) is to be converted into the corresponding amount of product by the registrant for each product label.
- "A closed system means removing a pesticide from its original container, rinsing, mixing, diluting, and transferring the pesticide through connecting hoses and couplings that are sufficiently tight to prevent exposure to the pesticide."
- "If applying more than [133 kg a.i. of atrazine to be reported in product equivalent value] per person per day a closed-cab tractor is required during application. This restriction is

required to minimize exposure to the worker." As indicated by square brackets, the amount of atrazine in the statement (133 kg a.i) is to be converted into the corresponding amount of product by the registrant for each product label.

- "Wear coveralls over a long-sleeved shirt, long pants, chemical-resistant gloves, socks and shoes during mixing, loading, application, clean-up and repair. In addition, wear protective eyewear (goggles or face shield) during mixing, loading, clean-up and repair. Gloves are not required during application within a closed cab."

The following Restricted-Entry Interval (REI) label statement is to be added under **PRECAUTIONS:**

- "**DO NOT** enter or allow worker entry into treated areas during the Restricted Entry Interval (REI) of 12 hours."

For labels that currently include directions for early re-entry in the **PRECAUTIONS** section, the language is to be modified as follows:

- **Replace:** "If required, individuals may re-enter treated areas within 12 hours of treatment for short term tasks not involving hand labor if at least 4 hours have passed since application and long-sleeved shirt, long pants and chemical resistant gloves are worn" **with:**
  - "If required, certified applicators may enter treated areas within 12 hours for short-term tasks not involving hand labour if at least 4 hours have passed since application and long-sleeved shirt, long pants, chemical-resistant coveralls, chemical-resistant footwear, socks, goggles, chemical-resistant gloves, hat and a respirator with a NIOSH-approved organic-vapour-removing cartridge with a prefilter approved for pesticides OR a NIOSH-approved canister approved for pesticides is worn. Time spent in the treated area cannot exceed 1 hour in a 12-hour period."

Appendix III Proposed label amendments for technical and end-use products containing atrazine, for the protection of the environment

Information on approved labels of currently registered products should not be removed unless it contradicts the label statements provided below.

1.0 Labels of technical products

1.1 Under the **ENVIRONMENTAL PRECAUTIONS** section:

Add the following statements:

TOXIC to aquatic organisms.
DO NOT discharge effluent containing this product into sewer systems, lakes, streams, ponds, estuaries, oceans or other waters.

1.2 Under the **DISPOSAL** section:

Add the following statement:

Canadian manufacturers should dispose of unwanted active ingredients and containers in accordance with municipal and provincial regulations. For additional details and cleanup of spills, contact the manufacturer and the provincial regulatory agency.

2.0 Labels for commercial class products

2.2 Under the **ENVIRONMENTAL PRECAUTIONS** section:

Add the following statements**:**

- TOXIC to aquatic organisms and non-target terrestrial plants. Observe spray buffer zones specified under DIRECTIONS FOR USE.
- TOXIC to birds and small wild mammals.
- To reduce runoff from treated areas into aquatic habitats avoid application to areas with a moderate to steep slope, compacted soil, or clay.
- Avoid application when heavy rain is forecast.
- Contamination of aquatic areas as a result of runoff may be reduced by including a vegetative filter strip between the treated area and the edge of the water body.
- This product demonstrates the properties and characteristics associated with chemicals detected in groundwater. The use of this product in areas where soils are permeable, particularly where the water table is shallow, may result in groundwater contamination.
- Atrazine is persistent and may carry over. It is recommended that this product not be used in areas treated with any products containing atrazine during the previous season.

2.3 Under the **GENERAL DIRECTIONS FOR USE** section (after the Mixing Instructions):

Add the following statements:

- As this product is not registered for the control of pests in aquatic systems, DO NOT use to control aquatic pests.
- DO NOT contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes.
- DO NOT apply by air.

2.4 Under the **DIRECTIONS FOR USE** section

Field sprayer application: DO NOT apply during periods of dead calm. Avoid application of this product when winds are gusty. DO NOT apply with spray droplets smaller than the American Society of Agricultural Engineers (ASAE S572.1) medium classification. Boom height must be 60 cm or less above the crop or ground.

Spray buffer zones

A spray buffer zone is NOT required for uses with hand-held application equipment permitted on this label.

The spray buffer zones specified in the table below are required between the point of direct application and the closest downwind edge of sensitive terrestrial habitats (such as grasslands, forested areas, shelter belts, woodlots, hedgerows, riparian areas and shrublands), sensitive freshwater habitats (such as lakes, rivers, sloughs, ponds, prairie potholes, creeks, marshes, streams, reservoirs and wetlands) and estuarine/marine habitats.

Method of application	Crop	Spray buffer zones (metres) required for the protection of:				
		Freshwater habitat of depths:		Estuarine/Marine habitat of depths:		Terrestrial habitat:
		Less than 1 m	Greater than 1 m	Less than 1 m	Greater than 1 m	
Field sprayer	Sorghum	3	2	2	2	10
	Corn (field, sweet, seed), switchgrass	5	3	3	3	15

When tank mixes are permitted, consult the labels of the tank-mix partners and observe the largest (most restrictive) spray buffer zone of the products involved in the tank mixture and apply using the coarsest spray (ASAE) category indicated on the labels for those tank mix partners.

The spray buffer zones for this product can be modified based on weather conditions and spray equipment configuration by accessing the Spray Buffer Zone Calculator on the Pesticides portion of the Canada.ca website.

2.5 Under the **DISPOSAL** section

Add the following statement:

- For information on disposal of unused, unwanted product, contact the manufacturer or the provincial regulatory agency. Contact the manufacturer and the provincial regulatory agency in case of a spill and for clean-up of spills.

The following statement would apply to plastic or metal containers that contain agricultural and non-crop land uses (for example, forestry) pesticide products, and that are designed to contain 23 L or less of product.

Disposal of container:

DO NOT reuse this container for any purpose. This is a recyclable container and is to be disposed of at a container collection site. Contact your local distributor/dealer or municipality for the location of the nearest collection site. Before taking the container to the collection site:

1. Triple- or pressure-rinse the empty container. Add the rinsings to the spray mixture in the tank.

2. Make the empty, rinsed container unsuitable for further use.

If there is no container collection site in your area, dispose of the container in accordance with provincial requirements.

2.6 Under the **STORAGE** section

Add the following statement:

- Store this product away from food or feed.

Appendix IV Toxicity tables and figures

Table 1A Identity of select atrazine chlorotriazine metabolites/Transformation products

Common name (Other names)	Chemical name (IUPAC)
Desethyl-atrazine (DEA, G-30033)	2-amino-4-chloro-6-ethylamino-s-triazine
Desisopropyl-atrazine (DIA, G-28279)	2-amino-4-chloro-6-isoproylamino-s-triazine
Diaminochlorotriazine (DACT, G-28273)	2-amino-4,6-diamino-s-triazine

Table 1B Identity of select atrazine hydroxylated metabolites/Transformation products

Common name (Other names)	Chemical name (IUPAC)
Hydroxyatrazine (HA, G-34048)	2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine
Desethylhydroxyatrazine (DEHA, GS-17794)	2-amino-4-hydroxy-6-ethylamino-s-triazine
Desisopropylhydroxyatrazine (DIHA, GS-17792)	2-amino-4-hydroxy-6-isoproplyl-s-triazine
Diaminohydroxyatrazine (Ammeline, GS-17791)	2,4,-diamino-6-hydroxy-s-triazine

Table 2 Toxicity profile of technical atrazine

Effects observed in both sexes are presented first followed by sex-specific effects in males, then females, each separated by semi-colons. Organ weight effects reflect both absolute organ weights and relative organ to bodyweights unless otherwise noted in Table 2.

Study type/ Animal/PMRA#	Study results
Toxicokinetics studies	
Toxicokinetics – oral (gavage) repeated dose studies ♂ SD rats PMRA# 2945557	Studies of blood kinetics and urine/feces/tissue residue levels were conducted with ^{14}C-triazine-labelled atrazine. Animals received doses of 0.4 or 4 mg/kg bw/day for up to 7 days. Blood and tissue samples were collected for analysis from three animals per dose at 4, 6, 8, 10, 14, and 18 days following the commencement of dosing. Feces and urine samples were collected daily for analysis of radioactivity. **Rate and extent of absorption and excretion**: Oral absorption was extensive and dose-dependent. For the majority of tissues (plasma, RBC, muscle, kidney, fat and liver) sampled, the radioactivity levels peaked at day 8 for both low and high doses. Recovery of radioactivity, as percent of AD, was approximately 74% in urine, 28% in feces, and 3% in the tissue samples. **Elimination**: The estimated half-lives for elimination was 4 days for most tissues, 10 days for brain, and 25–30 days for the RBC.
Toxicokinetics – oral (gavage) single dose, and repeated dose studies	Studies of blood kinetics, bile/urine/feces/tissue residue levels, enterohepatic recirculation, and metabolite identification and isolation were conducted with ^{14}C-triazine-labelled atrazine. Single low and high dose experiments included doses of 1 and 100 mg/kg bw, respectively. The repeated dose experiment included 15 days of 1 mg/kg bw/day. The radioactivity was measured in the bile as well as in the plasma, tissue, urine

Study type/ Animal/PMRA#	Study results
SD rats PMRA# 2945558, 2945565, 2945563, 2945567	and feces samples. Depending on the experiment, 3 to 12 animals per sex and group were used. **Rate and extent of absorption and excretion**: Oral absorption was extensive, relatively rapid and dose-dependent. Peak plasma levels were reached at 2 hr and 24 hr (T_{max}) for the low and high doses, respectively. Urinary excretion accounted for 65% and 66% of the AD in the low and high dose, respectively over a 2-day period. Excretion via the fecal route accounted for 11% and 20% of the AD in the low and high dose, respectively. Over a 48 hr period, biliary excretion was 7% of the AD in the low dose. Approximately 95% of the AD was excreted within 7 days of dosing. The urinary route accounted for about 75% of the excretion while feces accounted for 20% over the 7 day period. Route of excretion did not seem to vary between sexes or with dose. **Distribution and target organ(s)**: Distribution was extensive and dose-dependent (radioactivity levels were greatest in erythrocytes and highly perfused organs and tissues, such as the liver and kidneys). Elimination from tissues appeared to follow first order kinetics and the half-life of elimination in the tissues was 31 hr and 7-days in low and high doses, respectively. Except for RBC, whole blood, skeletal muscles, the tissue burden for any specific tissue or organ was < 1% of total AD by 14 day post-dosing. **Metabolism**: Atrazine was nearly completely metabolized. Metabolites indicate that dechlorination of the triazine ring and N-dealkylation are the metabolite pathways. Dechlorination and hydroxylation of the triazine ring was a minor metabolic pathway. The major metabolic pathway was a stepwise, N-dealkylation via desisopropylatrazine (DIA) and deethylatrazine (DEA) to the major urinary metabolite, diaminochlorotriazine (DACT). These metabolites are also known as chlorotriazines. Urinary, biliary and fecal metabolites detected revealed minimal dose-related differences in metabolite profile. In urine, 26 metabolites were detected, but only two accounted for greater than 5% of the AD. Nine biliary metabolites were detected, but were less than 1.6% of AD. Three biliary metabolites were also identified as urinary and fecal metabolites. No major sex-related differences were evident in the metabolic profile. The major fecal metabolite was DACT, which accounted for 40% of the total fecal radioactivity. Twelve other metabolites were also identified in the feces of high dose rats, and 9 in the feces of low dose rats, but were < 2.42% of the AD radioactivity levels
Toxicokinetics – oral (gavage) repeated dose studies ♀ SD rats PMRA# 2945559, 2945560	Studies of blood kinetics and urine/feces/tissue residue levels were conducted with ^{14}C-triazine-labelled atrazine. Doses of 0, 1, 3, 7, 10, 50, or 100 mg/kg bw/day were administered to the animals (2♀/dose) for 10 days. Blood samples were collected daily and. tissue samples were collected at necropsy for the measurement of radioactivity. **Rate and extent of absorption and excretion**: Proportions excreted in the urine (70–76% of the AD) and feces (13–15% of the AD) did not vary across doses. Half-lives for elimination from plasma and RBC were 39 hr and 8 days, respectively. **Distribution and target organ(s)**: Distribution was highest in red blood cells, followed by liver, kidneys, ovaries, pituitary, brain and mammary tissue samples. The distribution pattern of the radiolabel did not vary across doses, but the amount distributed varied in a dose-dependent manner.
Toxicokinetics – Distribution of ^{14}C-atrazine following acute lactational treatment oral (gavage)	Studies of tissue residue levels were conducted with ^{14}C-triazine-labelled atrazine. Single doses of 2 or 4 mg/kg bw were administered to nursing dams (2 per dose) on LD 3. Two and half hours after dosing the dams, the pups (20 per dose) were allowed to nurse for 30 min. At the end of the nursing period, radiolabelled residues of ^{14}C-atrazine were measured in the organs and tissues of the perfused dam and in the stomachs and brain of the pups **Dams**

Study type/ Animal/PMRA#	Study results
Wistar rats Published USEPA NHEERL/ORD study PMRA# 2945582 Stoker et al., 2007 Non-guideline	The liver, kidney, lung, heart, spleen, mammary glands and uterus had the highest levels of the radioactivity, with percent total per organ ranging from 0.05 to 2.5% of the AD. The anterior pituitary, ovaries, adrenals and brain (hypothalamus, caudate, cortex) had less than 0.01% of the AD. A dose-dependent increase in TRR levels was noted, with an approximately two-fold increase in all tissues, except the heart, lung and mammary gland – the tissues with the highest levels. **Pups** The amount of radioactivity detected in the stomach accounted for 0.0074% and 0.0069% of the AD in the low-and high-doses, respectively. The amount of radioactivity detected in the brain accounted for 0.00022% and 0.00018% of the AD in the low- and high-dose, respectively. Thus, a dose-dependent increase was not evident. The results of this study demonstrated that atrazine and metabolites were present in small quantities in the brain and tissues of the dams (adult ♀) and provided evidence that atrazine or its metabolites can cross the BBB. The results also provide information for postnatal distribution into the suckling neonate during early lactation. The study author concluded that the amount reaching the neonate following nursing is minimal and does not dispute the previous finding that the effects of atrazine on prolactin production in the dam was the primary MOA for the development of prostatitis in the ♂ offspring.
Toxicokinetics – Distribution of atrazine and metabolites following gestational /lactational treatment and potential to cross blood-brain barrier – oral (gavage) Wistar rats Unpublished USEPA NHEERL/ORD internal report PMRA# 2945584 Non-guideline	Studies of blood kinetics, determination of the tissue residue levels, and metabolite identification and isolation were conducted. Pregnant dams received doses of 0, 5 or 25 mg/kg bw/day from GD 18-20, 14-20, or GD 14 - LD 10 (3♀/dose). Transfer of maternally administered radioactivity and metabolites into plasma, milk and tissues (brain, adrenal, mammary glands, and gonads) were assessed by collecting appropriate samples within one or two hours of the last dose. Atrazine or metabolites were determined in the milk, plasma and tissue samples using LC/MS/MS methods. The plasma concentrations were determined on a per volume basis and the tissue, whole fetus and milk samples were determined on a per gram basis. In the GD 14-LD 10 treatment groups, the neonates were removed from the dam for 3 hr and then allowed to nurse for 30 min just prior to necropsy. Following this nursing period, one ♂ and one ♀ PND 10 pup were perfused and plasma, milk from the stomachs, adrenal gland and the gonads (testes and ovaries) from each pup were collected and weighed. The major metabolite detected in dams, fetuses, and pups was DACT although atrazine, DEA, and DIA were also detected at lower levels. The hydroxymetabolites of DIA and DEA (DIHA and DEHA) were not reliably quantifiable. **Gestational treatment (GD18-20 or GD14-20)** **Dam** Greater than 99% of total residues was chlorotriazines (TCT) (atrazine plus all chlorotriazine metabolites). DACT accounted for 74% and 82% of the TCT detected in plasma. DACT, DIA and DEA accounted for 85%, 10% and 2% of the TCT in brain tissue, respectively. Atrazine accounted for less than 1% of the TCT in the brain, but accounted for 13% and 5% in the adrenal and mammary gland tissues. DACT, DIA, and DEA accounted for 65%, 19% and 7% of the TCT in the adrenal and 65%, 22% and 6% of the TCT in the mammary gland, respectively. The amounts of hydroxylated metabolites (DIHA and DEHA, and HA) in the tissues and plasma were negligible

Study type/ Animal/PMRA#	Study results
	(<0.1% or undetectable). The amount of TCT was lower in the adrenals after a 7-day treatment compared to a 3-day treatment. **Fetus** Greater than 99% of total residues was TCT. DACT accounted for approximately 78% of the TCT in the fetus, with atrazine, DIA and DEA accounting for less than 1%, 17% and 3% of the TCT, respectively. The amount of chlorotriazines found in the whole fetus was similar whether the dams were treated for 3 or 7 days. The amounts of hydroxylated metabolites in the tissues and plasma were negligible (<0.1% or undetectable). **Gestational/Lactational treatment (GD14 – LD10)** **Dam** Greater than 99% of total residues was TCT. DACT accounted for ~ 90, 95, 75, and 90% of TCTs detected in plasma, brain, adrenal, and mammary glands, respectively. DIA, the second most prevalent metabolite detected in all tissues (except the adrenals), accounted for 3-8% of the TCTs detected. In the adrenal glands, unchanged atrazine accounted for ~ 11% of the TCTs (and thus was the second most detected chlorotriazine) while in other tissues it accounted for < 1% of the TCT. HA accounted for 0.7% of the total residues in the adrenals and 0.5% in the mammary tissue. The amounts of other two hydroxylated metabolites in the tissues and plasma were negligible (<0.1% or undetectable). **Pup** Greater than 99% of total residues was TCT. DACT was the major metabolite detected accounting for 99%, 91%, and 96% of the total residues in plasma, stomach content (milk), and brain, respectively. The adrenal glands only contained DACT. Small amounts of atrazine (2.5%), DIA (0.4%) and DEA (0.1%) and HA (1%) of total residues were detected in the brain. Only DACT was detected in the gonads (ovaries or testes) in the neonate. DACT was only detected in the ovaries of the of high-dose animals while it was detected in the testes at both doses. The amounts of other hydroxylated metabolites in the tissues and plasma were negligible (<0.1% or undetectable). It is noteworthy that for all tissues, except the brain, the content of atrazine and/or its metabolites was markedly higher in dams than in their pups (80-97% higher). In the brain, however, the content of atrazine in the pups was comparable or ~ fivefold higher than in the dams while DACT and DIA content in the pup brain was 3–19% of that detected in dam brain.
Toxicokinetics – Single or repeated-dose, oral (gavage) Plasma concentrations of atrazine and primary metabolites LE rats Published USEPA NHEERL/ORD study	Studies of blood kinetics were conducted with atrazine or equimolar levels of chlorotriazines. Doses of 0, 12.5, 75 mg/kg bw/day (atrazine); 10, 60.2 mg/kg bw/day (DIA); or 8.4, 50.6 mg/kg bw/day (DACT) were used in single dose (4–9♀/dose) or repeated dose experiments (9-14♀/dose). The levels of atrazine and chlorotriazines were determined using mass spectrometry (MS). **Plasma levels of atrazine and metabolites following single or 4-day repeated dose treatment** Dose-related levels of atrazine and the chlorotriazine metabolites were detected in plasma 15 min after single and 4-day repeat dose treatments. Low, but detectable, plasma levels of atrazine were observed following both single and repeat dose treatment paradigms. DACT (5-31 µM) was the most abundant plasma metabolite following any dose of atrazine; followed by DIA (5 µM) and DEA (0.5 µM), respectively. Single oral doses of DIA or DACT yielded ~ 10× and ~14× higher plasma levels of these metabolites,

Study type/ Animal/PMRA#	Study results
PMRA# 3292812 Fraites et al., 2009a Non-guideline	respectively, compared to levels of these metabolites generated from the equimolar atrazine oral doses.
Toxicokinetics – Distribution of atrazine and its metabolites in maternal, fetal, and neonatal fluid and tissue samples following gestational / lactational treatment SD rats Published USEPA NHEERL/ORD study PMRA# 3292813 Fraites et al., 2011a Non-guideline	Studies of blood kinetics, determination of the tissue residue levels, and metabolite identification and isolation were conducted. Pregnant dams received dose levels of 0, 5 or 25 mg/kg bw/day from GD 18-20, GD 14-20, or GD 14 - LD 10 (3♀/dose level). 1-2 ♂ and ♀ fetuses per group were selected for analysis upon necropsy. Atrazine or metabolite levels were quantified in the milk, plasma and tissue samples using MS/MS methods. **Atrazine and metabolite levels following gestational treatment:** **Dams** A dose-dependent increase in levels of atrazine and chlorinated metabolites was consistent in the plasma and all tissues of the dams and was observed following both gestational treatment durations. The primary chlorotriazine metabolite detected in dams following both gestational treatment durations was DACT, which accounted for the largest percent of total chlorotriazines detected in plasma (~ 86%) or tissues (55–92%). In contrast, atrazine levels represented less than 1% of the total chlorotriazines in the plasma and brain tissue following both treatment periods. Higher levels of atrazine were present in the adrenal (7-14%) and mammary tissue (3–7%). Of the two intermediate metabolites, DIA was present at higher levels than DEA, and both were generally detected at higher concentrations than atrazine. The amount of HA in the tissues and plasma was negligible (<0.1% or undetectable). Reduced tissue concentrations of the chlorotriazines were noted after longer duration of treatment (7 days vs 3 days). **Fetuses** DACT accounted for ~ 60–90% of the total residues in the fetal tissue. Dose-dependent increases in atrazine, DIA, and DEA were also observed in the fetus and these levels were similar to those measured in the maternal tissues and plasma. The levels of HA were negligible (<0.1% or undetectable). As with maternal tissues, fetal tissue from 7-day high-dose treated dams had 50–75% less atrazine, DIA or DEA compared to fetal tissues from 3-day high-dose treated dams. **Atrazine and metabolite levels following gestational and lactational treatment:** **Dams** The concentrations and distribution of the chlorotriazine metabolites in the plasma, brain, adrenal, and mammary gland of dams treated from GD 14 to LD 10 were similar to those observed in dams treated from GD 14-20. DACT accounted for the majority of the chlorotriazines in the tissues and plasma collected. Atrazine accounted for only 0.4–0.6% of the total chlorotriazines in mammary tissue. The amount of HA was about 0.5% of the total chlorotriazines in mammary tissue **Neonates** On PND 10, offspring plasma, milk, and tissues contained primarily DACT. The milk obtained from the neonatal stomachs following the 30 min nursing period contained 91-93% DACT. DACT also accounted for 99% of the total chlorotriazines detected in neonatal plasma. Atrazine was found at higher, but variable, concentrations in the neonatal brain (2–5% of the total chlorotriazine) compared to other tissues or fluids. HA levels in the tissues and plasma were negligible. The gonads of the neonates were

Study type/ Animal/PMRA#	Study results
	analyzed for all metabolites, but only DACT was detected. Note: although DEHA and DIHA were measured, it was not indicated in the report whether they were detected in the tissue samples.
Toxicokinetics – Single dose oral (gavage) or IV Cynomolgus monkeys PMRA# 2945595, 2549387 2014 Non-guideline	Studies of blood kinetics, determination of the tissue residue levels, and metabolite identification and isolation were conducted. Atrazine was administered to the same 6 ♀ monkeys in 6 different phases. Each phase was separated by a washout period of 34 to 42 days. The experiments covered four oral phases including doses of 0.25 to 2.5 mg/kg bw and two IV phases including a dose of 0.125 mg/kg bw. One of the IV phases was conducted with ^{14}C-triazine-labelled atrazine. Blood, urine and feces samples were collected frequently starting immediately post-dosing until 7-days post-dosing to characterize the metabolic profile and determine the internal dose metrics. HPLC-tandem MS/MS was used to analyze samples from phases 1–5. Samples from phase 6 were analyzed for total radioactivity. Atrazine was rapidly and extensively absorbed (T_{max} = 1 hour), metabolized to DEA and DIA, and cleared from plasma with a $T_{1/2}$ of 4.0 hours. DEA and DIA appeared rapidly in plasma with a similar pharmacokinetic profile to atrazine. DACT took slightly longer to reach maximum plasma concentration (T_{max} = 1.8 hr) and cleared with a longer half-life ($T_{1/2}$ = 10.3 hr). Internal dose metrics (C_{max} and AUC) revealed that DACT was the major metabolite, which was approximately 10-fold more than DEA and DIA and 200-fold more than atrazine. Internal dose metrics for the chlorotriazines scaled linearly with the AD indicating that absorption and metabolic processes did no saturate over the 20-fold tested dose range. Ninety percent of the chlorotriazines identified were found in urine and 10% in feces. The TK profile of plasma metabolites following IV injection was comparable to those seen following oral administration indicating that atrazine is rapidly and completely absorbed.
Toxicokinetics – Single dose oral (gavage) or IV Rhesus monkeys Published study PMRA# 3292814 Hui et al., 2011 Non-guideline	Studies of blood kinetics, determination of the urine/feces tissue residue levels were conducted with ^{14}C-triazine-labelled atrazine. Animals (4♀/dose) received oral doses of 0, 10, or 100 mg/kg bw or IV dose of 0.25 mg/kg bw. Plasma, urine, and feces samples were collected at various times up to 7 days post-dosing. **Oral administration**: Atrazine was rapidly absorbed [kinetic parameters such as AUC and Cmax were linearly correlated with doses] and cleared from plasma with a $T_{1/2}$ of 5.5 hr. Bioavailability was determined to be 60% of the AD. At the end of dosing period, urinary and fecal excretion reached 91–95% of the AD. **IV administration**: At the end of dosing period, urinary (85% of the AD) and fecal excretion (12%) reached 99% (combined) of the AD.
Pharmacokinetic – Single dose oral Humans PMRA# 2945568	A pharmacokinetic study was conducted in 6 adult ♂s using a single oral dose of unlabelled atrazine at 0.1 mg/kg bw. Urine samples were collected for 168 hr (fecal excretion not measured). Blood samples were obtained from one individual at 0, 2, 3, 4, 5, 6, 8, 24, 32, 72, and 168 hr after oral ingestion of the dose. Using gas chromatography methods, blood and urine samples were analyzed for atrazine and its major chlorotriazine metabolites

Study type/ Animal/PMRA#	Study results
1988 Non-guideline	DEA and DACT were detected in blood for up to 24 hr. Atrazine and DIA were detected in blood at very low levels. The detection of DEA reached a peak plasma level within 2 hr and declined rapidly thereafter with a half-life of 2.8 hr. This decline in DEA levels corresponded in an increase in DACT plasma levels. The rate of appearance of DACT in blood peaked at 5 hr and was eliminated with a half-life of 17.8 hr. This suggests a step-wise dealkylation of atrazine to DEA and then to DACT. Atrazine was not detected in urine, while DEA, DIA and DACT accounted for 5.4%, 1.4% and 7.7% of the dose, respectively. As significant amounts of atrazine were not found in blood or urine and only 15% of the dose could be accounted for in urine, 85% of the dose was unaccounted for. The urinary kinetics of DACT indicated an elimination half-life of 11.5 hr. Urinary monitoring of DACT was considered to be the best indicator of human atrazine exposure. In rats, the three chlorotriazine metabolites represent about 60% of radioactivity in the urine. Thus, it is possible that atrazine was incompletely absorbed in humans (extent of absorption in rodents was > 70%) or underwent complete ring cleavage and metabolized to CO_2 and N_2 (but rodent studies showed that the triazine ring is biologically stable). Other possibilities include extensive biliary excretion or there are many metabolites excreted in the urine which were not extracted and identified.
Acute Toxicity Studies	
Acute oral toxicity (gavage) Tif.RAI rats	LD_{50} = 1869 (1405–2487) mg/kg bw Clinical signs of toxicity: sedation, dyspnoea, exophthalmos, curved body posture **Slight acute toxicity**
Acute oral toxicity (gavage) SD rats	LD_{50} = 3520 mg/kg bw Clinical signs of toxicity: piloerection, reduced activity and salivation **Low acute toxicity**
Acute oral toxicity (gavage) SD rats	LD_{50} > 3100 mg/kg bw **Low acute toxicity**
Acute oral toxicity (gavage) SD rats	LD_{50} = 2850 mg/kg bw (both sexes combined) **Low acute toxicity**
Acute oral toxicity (gavage) Tif:MAG Mice	LD_{50} = 3992 (3557–4479) mg/kg bw (both sexes combined) Clinical signs of toxicity: sedation, ataxia, diarrhoea, polyuria, ptosis, salivation, dyspnoea, curved body posture, ruffled hair **Low acute toxicity**
Acute oral toxicity (gavage) HSD: ICR Mice	LD_{50} > 1332 mg/kg bw Clinical signs of toxicity: sedation, ataxia, body tremors, polyuria, ptosis, sensitivity to touch **Slight acute toxicity**

Study type/ Animal/PMRA#	Study results
Acute inhalation toxicity SD Rats	LC_{50} > 5.82 mg/L **Low acute toxicity**
Acute dermal toxicity Tif:RAIf rats	LD_{50} > 3100 mg/kg bw **Low acute toxicity**
Acute dermal toxicity SD rats	LD_{50} > 2000 mg/kg bw **Low acute toxicity**
Acute dermal toxicity Rabbits	LD_{50} = 7550 mg/kg (both sexes combined) **Low acute toxicity**
Eye irritation Himalayan rabbits	**Non-irritant**
Eye irritation NZW rabbits	**Mild irritant**
Skin irritation Himalayan rabbits	**Mild irritant**
Skin irritation NZW rabbits	**Non-irritant**
Skin irritation Rats	**Non-irritant**
Dermal sensitization Guinea pigs	**Negative**
Dermal sensitization (Maximization test) Guinea pigs	**Potential skin sensitizer**
Short-term toxicity studies	
14-day oral toxicity (gavage) Juvenile Albino rats PMRA# 1234778 Non-guideline (dose-range finding)	Supplemental The study investigators specified that the purpose of the study was not to derive a NOAEL, but to examine the effects of treatment on the endocrine and the immune systems of juvenile rats as well as to determine a dose range for testing in definitive studies. Rats were 23 day old on the first day of dosing. Five rats per sex and dose were maintained on control diet for an additional two weeks to assess reversibility of the findings. **≥ 25 mg/kg bw/day:** ↓ thymus wt (♂/♀); ↑ ALT, ↓ spleen wt (♂); ↓ ovary wt, ↑ anovulation (no corpora lutea – indicative of lack of ovulation) (♀)

Study type/ Animal/PMRA#	Study results
	≥ 100 mg/kg bw/day: ↓ BW (did not show complete recovery), ↓ FC (some recovery was observed) (♂/♀); ↓ brain wt, ↓ liver wt, ↓ adrenals wt, ↓ spleen wt, ↑ ALT (♀) **400 mg/kg bw/day:** mortality, fatty atrophy of bone marrow (♂/♀); ↓ brain wt, ↓ liver wt, ↓ testes wt, ↓ adrenal wt (♂); ↑AST, ↑ moderate necrosis of the thymic cortex (♀) Note: Anovulation or evidence of any other treatment-related histopathological findings was not observed in the animals assigned to the recovery groups. Histopathology was conducted on spleen, mesenteric and other lymph nodes, sternum with bone marrow, bone marrow of sternum and femur, liver, large intestine, testis, ovary, thymus, brain and the adrenals.
90-day oral toxicity (diet) with a 4-week recovery period Albino rats PMRA# 2945548	NOAEL = 0.6/3.4 mg/kg bw/day (♂/♀) **≥ 3.3/3.4 mg/kg bw/day:** ↑ splenic hemosiderin pigments (and was still present in ♀ after recovery period) (♂/♀); ↓ BW, ↓ liver wt, ↓ kidney wt (changes in organ wts did not completely recover at the end of recovery period) (♂) **34/35 mg/kg bw/day:** ↓ FC (♂/♀); ↓ BWG (9%), ↑ water intake (♀)
12-month oral toxicity (diet) Beagle dogs PMRA# 1233358, 1233359, 1233361	NOAEL = 5 mg/kg bw/day **34 mg/kg bw/day:** ↓ FC, ↓ BWG, ↓ platelet counts, ↑ cardiac toxicity [tachycardia and ↑ heart rate, ↓ height of the P-wave amplitude, ↓ PR and QT values, ↑ moderate to severe atrial dilation in 4/5 ♂ and all ♀, enlarged and soft hearts and fluid in pericardium, ↑ cardiac myolysis in 3/6 ♂ and 6/6 ♀ (not seen in controls), ↑ focal atrophy of myocardial fibers in 2/6 ♂ and 5/6 ♀ (not seen in controls), ↑ edema of the heart, ↑ effects secondary to cardiac dysfunction (ascites, dyspnea, liver fibrosis/atrophy), cardiac dysfunction (myocardial lesions) noted in two high-dose decedents (one ♂ and one ♀ found in moribund conditions and subsequently killed, atrial premature complexes and atrial fibrillation found in one ♀)(♂/♀); ↑ rel. liver wt (♂); ↓ abs. heart wt (♀) Note: Electrocardiography was performed at three month intervals throughout the study.
25-day dermal toxicity NZW rabbits PMRA# 2815961, 2816711	NOAEL = 100 mg/kg bw/day **1000 mg/kg bw/day:** ↓ FC, ↓ BW, BW loss, ↓ BWG, ↓ RBC, ↓ HGB, ↑ % reticulocytes, ↓ total serum albumin, ↓ chloride, ↑ spleen wt (♂/♀); ↑ minimal to severe acanthosis, focal subacute lymphocytic inflammation in treated skin (♀)
Chronic toxicity/Oncogenicity studies	
18-month oncogenicity (diet) CD-1 mice PMRA# 1234783, 1233356, 1233357 Hazelette and Green, 1987	NOAEL = 38/43 mg/kg bw/day (♂/♀) **≥ 194/247 mg/kg bw/day:** ↓ BWG, ↑ cardiac thrombi **386/483 mg/kg bw/day:** ↓ FC, ↓ RBC, ↓ HGB, ↓ HCT (♂/♀); ↓ BW, ↑ mortality, ↓ % neutrophils and lymphocytes (♀) **No evidence of carcinogenicity** Mammary glands were examined histopathologically.
24-month chronic toxicity/oncogenicity (diet)	NOAEL = 2.6/3.5 mg/kg bw/day (♂/♀) **≥ 3.5 mg/kg bw/day:** ↑ mammary gland tumours including adenocarcinomas and fibroadenomas (♀)

Study type/ Animal/PMRA#	Study results
SD rats PMRA# 1203786, 1203787, 1203788, 1203789, 1203790, 1203791, 1204001, Mayhew et al., 1986	**≥ 20/30 mg/kg bw/day:** ↓ BWG, ↓ BW, ↑ retinal degeneration (♂/♀); ↑ myeloid hyperplasia in bone marrow of femur and sternum and splenic extra-medullary hematopoiesis (♀) **42/65 mg/kg bw/day:** ↑ degeneration of rectus-femoris muscles (♂/♀); ↓ serum triglyceride levels, ↑ prostate epithelial hyperplasia, ↑ acinar hyperplasia of mammary gland, ↑ calculi in renal pelvis (♂); ↓ survival, ↓ HGB, ↓ HCT, ↓ RBC, ↑ transitional epithelial hyperplasia in bladder and kidney, ↑ centrilobular liver necrosis, ↑ mammary gland adenocarcinomas at interim necropsy and in early deaths (♀) **Evidence of carcinogenicity in ♀ SD rats** Mammary gland tumour incidences in terminal sacrifice ♀ at 0, 0.5, 3.5, 30, and 65 mg/kg bw/day: Adenocarcinomas: 15/66, 15/64, 26*/68, 27*/65, 35**/64 Carcinosarcomas: 0/66, 0/64, 0/68, 0/65, 2/64 Fibroadenomas: 29/66, 29/64, 35/68, 38/65, 42**/64 Mammary gland tumour incidences in all ♀ combined (interim sacrifice, early deaths, and terminal sacrifice) at 0, 0.5, 3.5, 30, and 65 mg/kg bw/day: Benign: 29/88, 29/69, 36/69, 39/70, 46**/89 Malignant: 15/88, 16/69, 27*/69, 27**/70, 45**/89 Combined: 35/88, 40/69, 48/69, 48**/70, 65**/89 * statistically significant at p <0.05 ** statistically significant at p<0.01
Developmental/Reproductive toxicity studies	
2-generation reproductive toxicity (diet) SD rats Unpublished study: PMRA# 1233367, 1233368 Mainiero et al., 1987 Published study PMRA# 2816056, 2816783 DeSesso et al., 2014	**Parental toxicity** NOAEL = 3.6/4.0 mg/kg bw/day (♂/♀) **36/41 mg/kg bw/day**: ↓ FC, ↓ BWG (in P and F1), ↓ BW (in P and F1 – started within the 1st week of pre-mating and persisted throughout the study) **Offspring Toxicity** NOAEL = 4.0 mg/kg bw/day (♂/♀) **41 mg/kg bw/day**: ↓ BWG, ↓ BW (F1 and F2 ♂ on PND 21 and F1 ♀) **Reproductive Toxicity** NOAEL = 36/41 mg/kg bw/day (♂/♀) The reproductive indices data were variable (the fertility index in F_1 control was 86%) Sperm parameters (counts, motility and morphology), estrous cycle length and periodicity, and ovarian follicle were not examined. Onsets of puberty were not examined. Only testes and ovaries were weighed. Mammary gland was not examined histopathologically except for one P animal in the 4 mg/kg bw/day dose that showed palpable mass at gross necropsy which was subsequently confirmed as adenocarcinoma histopathologically Histopathology was limited to evaluation of the pituitary gland testes, epididymides, seminal vesicles, prostate, coagulating glands, ovaries, uterus, cervix, and vagina in control and high dose groups for P and F1 animals and a few F2 offspring (4–5) animals.

Study type/ Animal/PMRA#	Study results
	Limited details on microscopic evaluation methods (for example, sectioning of the tissues) as well as limited details regarding which parts of the examined organs were histologically evaluated (for example, no information re: which section of rat prostate was examined. Note that the rat's dorsal and lateral prostates are known to be the most homologous to humans and the lateral prostate is most sensitive to prolactin effects) **No evidence of sensitivity of the young**
Developmental toxicity (gavage) SD rats PMRA# 1137002, 1167663, 1233370, 1144845, 1233371 Infurna 1984	**Maternal Toxicity** NOAEL = 70 mg/kg bw/day ≥ **70 mg/kg bw/day**: ↓ BWG, ↓ FC (GD 6-7) (non-adverse) **700 mg/kg bw/day**: ↓ BW, ↓ BWG, ↓ FC (throughout treatment period and including necropsy), ↓ liver wt, ↑ clinical signs of toxicity (salivation, oral/nasal discharge, ptosis, bloody vulvas, swollen abdomen, enlarged stomach and adrenals, and discoloured lungs) ↑ mortality, ↑ post-implantation loss **Developmental Toxicity** NOAEL = 10 mg/kg bw/day ≥ **70 mg/kg bw/day**: ↑ incomplete ossification of skull, teeth, hyoid, forepaw metacarpals and hindpaws distal phalanx, ↑ incidence of rudimentary and wavy ribs **700 mg/kg bw/day**: ↓ fetal wt, ↑ post-implantation loss (skeletal examination was not performed at this dose due to high maternal mortality and extremely reduced fetal wts) **Evidence of sensitivity of the young** **No evidence of treatment-related malformations**
Developmental toxicity (gavage) SD rats PMRA# 1233374 Giknis 1989	**Maternal Toxicity** NOAEL = 25 mg/kg bw/day **100 mg/kg bw/day**: ↓ BW (started immediately post-dosing and persisted throughout the treatment period), ↓ BWG, ↓ FC (GD 6-12), ↑ clinical signs of toxicity (salivation, alopecia), one dead on GD20 **Developmental Toxicity** NOAEL = 25 mg/kg bw/day **100 mg/kg bw/day**: ↑ incomplete ossification of various skull bones (hyoid, interparietal, occipital, and parietal bones) **No evidence sensitivity of the young or treatment-related malformations**
Developmental toxicity (gavage) NZW rabbits PMRA# 1137003, 1167663, 1137876, 1144767, Arthur 1984	**Maternal Toxicity** NOAEL = 5 mg/kg bw/day ≥ **5 mg/kg bw/day**: ↓ FC, ↓ BWG (non-adverse) **75 mg/kg bw/day**: ↓ BW, BW loss, ↑ clinical signs of toxicity (bloody vulvae, little or no stool), two dams killed due to signs of impending abortion, ↑ mean resorptions, ↑ post-implantation loss **Developmental toxicity** NOAEL = 5 mg/kg bw/day

Study type/ Animal/PMRA#	Study results
	75 mg/kg bw/day: ↑ resorptions, ↑ post-implantation loss, ↓ number of live fetuses/litter, ↓ fetal BW, ↑ incomplete ossification of appendicular elements (forepaws and hindpaws) **No evidence sensitivity of the young or treatment-related malformations**
Genotoxicity studies	
Bacterial Reverse Mutation Assay S. typhimurium (TA98, TA100, TA1535, TA1537) Unpublished study PMRA# 1234587	Negative ± metabolic activation Tested up to a limit concentration.
Bacterial Reverse Mutation Assay / Rec-assay S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538), E. coli (WP2), B. subtilis (H17, M45) Unpublished study PMRA# 1234637	Negative ± metabolic activation and negative in the rec-assay Tested up to a limit concentration. Precipitation formed in the rec assay between 500–10 000 µg/plate. Precipitation formed in the E. coli mutagenicity assay between 1000–5000 µg/plate. Precipitation formed in the S. typhimurium mutagenicity assay between 1000–10 000 µg/plate.
Unscheduled DNA synthesis ♂ rat primary hepatocytes Unpublished study PMRA# 1234573	Negative Precipitation occurred at 100 and 150 µg/mL
Unscheduled DNA synthesis CRL 1121 human fibroblasts Unpublished study PMRA# 1234574	Negative without metabolic activation Metabolic activation was not performed. Precipitation occurred at 100 and 150 µg/mL.
Chromosomal aberration / micronucleus assay Human lymphocytes	Negative ± metabolic activation

Study type/ Animal/PMRA#	Study results
Published study PMRA# 3292853 Ribas et al., 1998 Non-guideline	
Sister chromatid exchange test / chromosomal aberration Human lymphocytes Published study PMRA# 3292846 Kligerman et al., 2000a Non-guideline	Negative without metabolic activation Metabolic activation was not performed.
Micronucleus assay / excision repair Human lymphocytes Published study Surralles et al., 1995 PMRA# 2815961 Non-guideline	Negative without metabolic activation Metabolic activation was not performed.
Sister chromatid exchange assay Human lymphocytes Published study PMRA# 3292844 Dunkelberg et al., 1994 Non-guideline	Negative with metabolic activation There was no suitable positive control for the experiments without metabolic activation.
Comet assay (Single-cell gel electrophoresis) Human lymphocytes Published study	Negative ± metabolic activation

Study type/ Animal/PMRA#	Study results
PMRA# 3292855 Zeljezic et al., 2006 Non-guideline	
Bone marrow chromosome aberration test (in vivo) Mice (strain unknown) Unpublished study PMRA #1234575	Negative One ♀ in the mid-dose group died within the treatment period of 24 h. There was no increase in the number of micronucleated PCEs at any dose at 24 h as compared to control.
Bone marrow chromosome aberration test (in vivo) Chinese hamsters Unpublished study PMRA # 1234638	Negative There was no increase in the number of micronucleated PCEs at any dose at 24 h as compared to control. The purity was not given but was derived from the Dominant lethal assay which used the same batch of technical active and was performed by the same laboratory.
Bone marrow chromosome aberration test (in vivo) ♀ C57B1/6 mice Published study PMRA# 3292847 Kligerman et al., 2000b Non-guideline	Negative
Dominant lethal assay (in vivo) ♂ mice (strain unknown) Unpublished study PMRA # 2945569	Negative Common clinical signs were piloerection and reduced locomotor activity.
Dominant lethal assay (in vivo) ♂ NMRI-derived mice	Negative There was no change in mating ratio, number of implantations or resorptions in ♀s after mating with treated ♂s.

Study type/ Animal/PMRA#	Study results
Unpublished study PMRA # 1234572	
Sperm head morphology (in vivo) ♂ C57BL/6 mice Published study PMRA# 3292851 Osterloh et al., 1983 Non-guideline	Negative Repeated dose (i.p.) of seven concentrations ranging from 38 to 600 mg/kg bw for 5 days Mice were sacrificed 35 days after first injection
DNA damage in blood leukocytes (single cell electrophoresis assay) (in vivo) ♀ C57B1/6 mice Published study PMRA# 3292854 Tennant et al., 2001 Non-guideline	Negative Positive in the presence of excessive cytotoxicity
Bacterial Reverse Mutation Assay S. typhimurium (TA98, TA100, TA1535, TA1537) Unpublished study PMRA #: 1234615	Supplemental This study was completed in 1978 prior to introduction of OECD guidelines. Atrazine was found to be non-genotoxic in all three S. typhimurium strains tested. The following details were not provided: chemical purity, phase of growth (late exponential, or early stationary phase) and whether there was testing up to a precipitating or cytotoxic concentration. As a result of these deficiencies, this study is considered supplemental.
Bacterial Reverse Mutation Assay S. typhimurium (TA97, TA98, TA100) Published study PMRA # 1234590 (with other metabolites)	Supplemental Four concentrations ranging from 2.16–2157 µg/plate without metabolic activation Atrazine was found to be non-genotoxic in all three S. typhimurium strains tested. In addition to lack of tests with S9 activation, the following items were not provided: phase of growth (late exponential, or early stationary phase) and whether there was testing up to a precipitating or cytotoxic concentration. As a result, this study is considered supplemental.

Study type/ Animal/PMRA#	Study results
Butler et al., 1989 Non-guideline	
Bacterial Reverse Mutation Assay / Host Mediated Assay S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538) / ♂ Swiss Webster mice Unpublished study PMRA # 1234593 Non-guideline	Supplemental This study was completed in 1977 prior to introduction of OECD guidelines. The compound was administered by oral intubation; The bacteria were administered by i.p. injection. In vivo exposure was either acute or sub-acute (5 days). Neither showed an increased revertant count.
Unscheduled DNA synthesis (in vitro) Rat primary hepatocytes Unpublished study PMRA# 2815961, 2816711 Hertner et al., 1992	Supplemental Negative according to the JMPR Study limitation: limited reporting of study results
Chromosomal aberration assay Human lymphocytes Published study PMRA# 3292850 Meisner et al., 1992	Supplemental Culture and exposure conditions were adequately described. No metabolic activation was performed and no rationale was given for its exclusion. It is recommended by guideline studies that the concentration of the highest test substance suppress mitotic activity by 50%. However, the authors did not provide the MI because there was no consistent difference between treated and control cultures, and the MI did not follow any significant dose response. Nonetheless, the authors showed a dose dependent increase in chromosomal damage in the absence of MI changes following atrazine treatment. It was also not stated whether the slides were blindly coded. Historical control data was not given. The purity was not given.
Comet assay (Single-cell gel electrophoresis) Human lymphocytes Published study	Supplemental Metabolic activation was performed. Positive at cytotoxic doses

Study type/ Animal/PMRA#	Study results
PMRA# 3292852 Ribas et al., 1995 Non-guideline	
Bone marrow chromosome aberration test (in vivo) B6C3F1 mice Published study PMRA# 3292850 Meisner et al., 1992 Non-guideline	Supplemental No treatment-related indication of chromosomal aberrations Study limitation: limited reporting of study results
Bone marrow micronucleus test (in vivo) NMRI mice Published study PMRA# 3292845 Gebel et al., 1997 Non-guideline	Supplemental (positive in the presence of excessive toxicity) **1750 mg/kg bw/day:** ↑ mortality Study limitation: inadequate number of animals per group and limited reporting of study results
Spermatocyte chromosomal aberration test (in vivo) NMRI mice Unpublished study PMRA # 1234571	Supplemental Only the summary and procedure were available. Result values were not provided. The purity was not given but was derived from the Dominant lethal assay which used the same batch of technical active and was performed by the same laboratory. The study authors found that in the high-dose group, one aberration in the form of a fragment was noted in one spermatocyte metaphase (1 of 800) from one animal.
Spermatogonia chromosomal aberration test (in vivo) NMRI mice Unpublished study PMRA # 1234639	Supplemental Only the summary and procedure were available. Result values were not provided. The purity was not given but was derived from the Dominant lethal assay which used the same batch of technical active and was performed by the same laboratory. One chromosome-type aberration per 800 cells was observed in the low-dose group. This was considered spontaneous in origin by the authors and within historical control range.

Study type/ Animal/PMRA#	Study results
Special studies (non-guideline)	
a) Studies on preovulatory LH surge and estrous cyclicity	
1-, 2-, and 4-day oral (gavage) LH surge OVX ♀ SD rats Unpublished USEPA NHEERL ORD internal report PMRA# 3292815 (Goldman et al, 2011)	Supplemental The purpose of this study was to investigate the impact of 1, 2, or 4-day(s) of treatment with 100 mg/kg bw/day atrazine on the LH surge and circulating adrenal progesterone (one of the hormones involved in the regulation of the LH surge) levels. Blood was collected at 1400, 1600, and 1800 hr from the lateral tail vein. At 2000 hr, the animals were necropsied and trunk blood was collected. OVX animals were implanted with an estradiol benzoate capsule three days before dosing. Circulating LH was determined using RIA and progesterone was determined using a commercial kit. **Single dose test** **100 mg/kg bw**: ↑ LH surge (manifested as an ↑ in both peak amplitude and AUC), ↑ progesterone levels within 1 hr of dosing, however, levels returned to background (control) levels by 3 hr **2- or 4-day test** No statistically significant change was observed in progesterone levels [progesterone data not provided] **2-day test** **100 mg/kg bw/day**: ↓ LH (↓ AUC but not in peak amplitude] **4-day test** **100 mg/kg bw/day**: ↓ LH surge (↓ AUC and peak amplitude) Study limitation: Summary data tables with means and standard deviations were not available for some datasets in the study report.
4-day oral (gavage) LH surge Intact ♀ LE rats Unpublished USEPA NHEERL ORD internal report (Cooper et al., 2010) Published USEPA NHEERL ORD study (Cooper et al., 2007) PMRA# 2945603, 2945604, 2945570	NOAEL = 1.56 mg/kg bw/day The purpose of this study was to generate a NOAEL for the pre-ovulatory surge of LH (an endocrine event necessary for normal ovulation) in LE rats considering that a NOAEL was not identified for LE rats in the Cooper et al. 2000 study. Three blocks were included in the study. For all blocks, treatment started on the day of vaginal estrous until the day after proestrous (4 consecutive days). The doses included were: 0, 1.6, 3.12, 6.25, 12.5, 25, and 75 mg/kg bw/day. Each block included a concurrent control along with the lowest dose from the previous block. The number of animals in each group included in the data analysis had to meet three criteria at the time of necropsy: 1) a proestrous vaginal smear, 2) increased uterine weight (500 mg) and 3) elevated concentration of progesterone at later time points (1600 hr to 1800 hr). A large proportion of animals that did not meet the criteria were excluded. Uterine weights, progesterone levels, and the onset of the LH surge (timing) were unaffected by atrazine treatment in ♀s reaching proestrous **≥ 3.12 mg/kg bw/day**: ↓ magnitude of LH surge (1800 hr) **≥ 25 mg/kg bw/day**: ↑ altered GnRH regulation (block 1: ↑ GnRH content in the median

Study type/ Animal/PMRA#	Study results
	eminence of hypothalamic region)
1-, 3- or 21- day oral (gavage) Disruption hypothalamic control of pituitary-ovarian function OVX ♀ SD or LE rats Published USEPA NHEERL/ORD study PMRA# 2945601 Cooper et al., 2000	Experiment 1**:** Effect of atrazine on LH surge in SD and LE rats. Animals (60 days old) received OVX and estrogen implant on day 0. 40 ♀ per dose and strain were used. 10 ♀ per dose were necropsied at 1200 hr, 1300 hr, 1500 hr, and 1800 hr. **Single dose test** Doses of 0, 50, 100, 200 or 300 mg/kg bw were used. NOAEL = 200 mg/kg bw (LE) NOAEL ≥ 300 mg/kg bw (SD) **300 mg/kg bw**: ↓ LH and prolactin surges in LE rats, but not SD rats **3-day test** Doses of 0, 50, 100, 200 or 300 mg/kg bw were used. LOAEL = 50 mg/kg bw/day (LE) NOAEL = 200 mg/kg bw/day (SD) ≥ **50 mg/kg bw/day**: ↓ LH and prolactin surges in LE rats, ↑ pituitary prolactin levels in LE rats **300 mg/kg bw/day**: ↓ prolactin surges in SD rats **21-day test** Doses of 0, 75, 150, or 300 mg/kg bw were used. Animals received OVX on day 0 and estrogen implant on day 21 LOAEL = 75 mg/kg bw/day (LE) NOAEL = 75 mg/kg bw/day (SD) ≥ **75 mg/kg bw/day**: ↓ LH surge in LE rats, ↑ pituitary prolactin levels in both strains ≥ **150 mg/kg bw/day**: ↓ prolactin surges in LE and SD rats, ↓ LH surge in SD rats The LE ♀ rats appear to be more sensitive to the hormone suppressive effects of atrazine than ♀ SD rats. **Experiment 2** (intact LE rats only): effect of atrazine on ovulation. Vaginal cytology recorded for 3 weeks and oocytes were counted. **Single dose test** Doses (0, 75, 150, or 300 mg/kg bw) administered at 1200 hr on the day of vaginal proestrus NOAEL = 300 mg/kg bw No treatment-related effect **3-day test** Three daily doses of 30 mg/kg bw/day. Last dose administered at 1200 hr on the day of vaginal proestrus

Study type/ Animal/PMRA#	Study results
	LOAEL = 300 mg/kg bw/day **300 mg/kg bw/day:** ↑ pseudopregnancy*, ↑ no eggs on estrus (subsequent proestrus and ovulation blocked) *defined as displaying a diestrous vaginal smear for approximately 12 days or more and having elevated serum progesterone **Experiment 3** (limited reporting): To determine atrazine target sites, 3 special studies were performed: (1) Hypophysectomized ♀s with pituitary auto transplants (ectopic pituitaries) and serum prolactin levels were measured hourly; (2) synthetic GnRH to induce LH surge in 3-day atrazine-treated ♀s; (3) atrazine (in vivo or in vitro) to suppress LH and prolactin secretion from pituitaries by perfusion procedure i) This study indicated that the secretion of prolactin by the pituitary was not altered by atrazine if the gland was removed from the influence of the CNS factors. ii) serum LH concentration levels in the atrazine + GnRH ♀s were comparable to the estrogen-induced LH surge observed in control ♀s iii) Study author conclusion (data not provided): No differences in either LH or prolactin levels were noted from the pituitaries of untreated ♀s exposed to atrazine in vitro. Similarly, no changes in basal or GnRH-stimulated LH release or thyroid releasing hormone-stimulated prolactin release were noted in the pituitaries obtained from ♀s dosed with atrazine (0, 100 or 200 mg/kg bw) by gavage for days. Overall, study authors concluded that experiment 3 revealed that the target site was the hypothalamus (alteration of hypothalamic control of LH and prolactin pituitary secretion by atrazine).
4-day oral (gavage) LH surge OVX ♀ Wistar Rats Published study PMRA# 2815995, 2816757 Foradori et al., 2009a	Supplemental **Experiment 1** (atrazine effect on LH secretory pattern): To determine if atrazine alters LH secretory patterns: animals given doses of 0, 50, 100, or 200 mg/kg bw/day for 4 days. On the second day of treatment, intra-atrial cannulae were implanted. Three to 4 hr after the final dose, blood was withdrawn at 5 min intervals for 3 hr for analysis of LH levels using RIA **200 mg/kg bw/day:** ↓ LH pulse frequency and concomitant ↑ in LH pulse amplitude **Experiment 2 and 3**: 2. Validation of protocol for GnRH immunoneutralization and pituitary response: Animals fitted with intra-atrial cannulae and administered GnRH antiserum. Two days later, blood samples were obtained at 10 min intervals for 100 min period. After 3rd sample was taken, animals were treated with one of four doses of the GnRH agonist. Blood samples were taken and plasma was analyzed for LH levels by radioimmunoassay. 3. Effect of atrazine on pituitary response to GnRH: Animals dosed with doses above. On the second day, intra-atrial cannulae were implanted and animals were treated with anti-GnRH serum. On the afternoon of the final day of atrazine treatment, blood was sampled at 10 min intervals. After 3 samples were taken, a single bolus dose of D-Ala-6 GnRH was administered. Sampling continued for 90 min. Plasma samples were analyzed for LH levels by radioimmunoassay.

Study type/ Animal/PMRA#	Study results
	Atrazine-treated animals that had been immunoneutralized with the anti-GnRH serum showed an LH response comparable to that observed in the control group. Administration of GnRH agonist caused an increase in the circulating concentration of LH. However, there was no significant change in the levels of LH following atrazine treatment. The authors concluded that although the LH pulse period and amplitude were increased in atrazine-treated animals, the anterior pituitary function does not seem to be altered by atrazine, as LH levels were not reduced in atrazine-treated animals in response to GnRH agonist after GnRH immunoneutralization. Additionally, the inhibition of LH pulses and disruption of cyclicity in ♀ rats treated with atrazine are likely to be mediated by interference with central mechanisms controlling GnRH release from the hypothalamus. The brain appears to be the primary target mediating the effect of atrazine on LH release in the rat. This conclusion is consistent with the overall neuroendocrine MOA of atrazine. Study limitation: Summary data tables with means and standard deviations were not available for some datasets in the study report
4-day oral (gavage) Role of GnRH neurons OVX ♀ Wistar rats Published study PMRA# 2815998, 2816756 Foradori et al., 2009b	Supplemental **Experiment 1**: Effect of atrazine on hormone-induced LH and FSH surges: Animals received doses of 0, 50, 100 or 200 mg/kg bw/day. Animals were given estrogen and progesterone on appropriate days to induce pre-ovulatory surge of LH. Blood samples were taken on the afternoon of the 4th day of treatment at 1hr intervals via intra-atrial cannulated-implants (rats were freely moving during blood sampling). Plasma LH and FSH were determined using radioimmunoassay **Experiment 2**: Effects of atrazine on GnRH neuronal activation: Same doses and treatment methods were used as those used in experiment 1. Near the peak of the predicted LH surge (1700 hr in this colony), animals were necropsied and sections of their brains were examined for GnRH neuron activation using immuno-histochemistry, To determine if atrazine alone would have a stimulatory effect on GnRH neuronal activation, a second cohort was given 50 mg/kg bw/day of atrazine for 4 days as described above without hormone induction. Brains were processed as described above **Experiment 3:** Effects of atrazine withdrawal on hormone-induced LH surge and GnRH activation: Two treatment groups were used. LH was induced 2 days or 4 days after cessation of atrazine treatment. On final day of hormone induction, blood samples were collected for analysis. A second cohort of animals was prepared as described in this experiments above, but without the intra-atrial cannula implants. Near the peak of the LH surge, animals were necropsied and brains were examined histologically. **≥ 50 mg/kg bw/day**: statistically significant ↓ LH levels and AUC (in Experiment 1) **≥ 100 mg/kg bw/day**: statistically significant ↓ activated GnRH (in Experiment 2) **200 mg/kg bw/day**: statistically significant ↓ FSH AUC (in Experiment 1), Results of experiment 3 indicated that LH levels and AUC were comparable to those of control level 4 days after cessation of treatment.

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	GnRH neuronal activation was examined using the immediate early gene product FOS (cFOS) in the GnRH neurons – as a measure of cellular activity. Study authors concluded: atrazine treatment inhibited the hormone-induced LH surge and GnRH neuronal activation, an effect that was transient and no longer present 4 days after withdrawal. Thus, the inhibition of the HPG axis and the disruption of cyclicity in ♀ rats treated with atrazine are likely to be mediated by interference with central mechanisms controlling GnRH activation in the preoptic area and hypothalamus. Conclusion: Although the study authors conclusions are consistent with well-established neuroendocrine MOA of atrazine, the dose concordance of LH inhibition and GnRH neuronal activation did not match. LH levels were reduced at 50 mg/kg bw/day while GnRH neuron activation were lowered starting at 100 mg/kg bw/d. The number of activated GnRH neuronal cells at 50 mg/kg bw/day were similar to the control levels. Study limitations: Summary data tables with means and standard deviations were not always provided. Purity level was not provided.
4-day oral (gavage) LH surge Intact ♀ LE rats Unpublished study PMRA# 2816728 2816034 Coder P, 2011a	Supplemental Animals (11–12♀/dose) received treatment (0, 1.5, 3.0, 6.0, 12, or 50 mg/kg bw/day) over a 4-day cycle. Corticosterone, progesterone, prolactin, and LH levels in intact ♀ LE rats were determined at 1100 hr (Cohort A), and 1300 hr (Cohort B) at the presumed proestrous stage. Vaginal lavages were performed daily for 14 days before treatment began and ♀s exhibiting 4-day cycles were intended for LH analysis. **50 mg/kg bw/day:** ↓ LH surge peak by 50% and AUC in Cohort B, ↓ BWG, ↓ FC The LH levels were lower by ~ 25% at lower doses, but they did not attain statistical significance nor did they show dose-related patterns Study limitations: Estrous cyclicity data were not used to inform LH surge analysis. High variability in the corticosterone, progesterone and prolactin data.
4-day oral (Gavage) LH surge and Corticosterone levels in OVX, estrogen-treated animals ♀ SD rats Unpublished study PMRA# 2816746, 2816011 Coder P, 2010a	Supplemental Animals (20♀/dose) received treatment (0, 100 mg/kg bw/day) at approximately the same time of day (1200 to 1500 hr). On the fourth day of treatment, blood samples were collected at 1300, 1600, 1800, 2000, 2300, and 0100 (study day 4) hr to analyze for LH and corticosterone blood levels. OVX surgery and estrogen implant via subcutaneous injection were conducted on Day 0 **100 mg/kg bw/day**: ↑ wet yellow material in the anogenital area and right hindlimb, ↓ BW, ↓ FC, ↓ LH surge/circulating levels (at 1300, 1600, 1800, and 2000 hr), ↓ LH peak and AUC, ↑ corticosterone levels (at 1300 and 2000 hr), ↓ corticosterone levels (at other time points), ↑ corticosterone peak Study limitations (issues performing ovariectomies): five and 2 rats died prior to the first administration of atrazine in the 0 and 100 mg/kg bw/day group, respectively. One ♀ died in the 100 mg/kg bw/day group after the first atrazine administration, but necropsy indicated that this death was also due to post-operative complications. Due to these mortalities after the surgical procedure (typically before they were treated with atrazine), animals were reassigned to ensure that at least 16 rats/group were available for hormone assessments

Phase I: 4 or 5-day oral (gavage or diet) LH surge Phase II: 28- to 36-day oral (gavage or diet) Immunotoxicity potential ♀ SD rats Unpublished study PMRA# 2816736, 2816024 Coder et al., 2011b	Supplemental **Phase I** (LH surge in intact ♀ SD rats): Animal (21 ♀/dose level) received treatment via diet (0, 3, 9, or 40 mg/kg bw/day) or oral gavage (0, 0.75, 1.5, 3, 6, 10, or 50 mg/kg bw/day). Vaginal lavages were performed daily for determination of estrous cycles beginning at least 14 days prior to the start of the study. The start of dosing for each ♀ was reportedly (but not confirmed with data) based on the stage of the estrous cycle. On last day of treatment, blood samples were collected regularly for LH analysis. Dietary administration No effects on LH concentration levels measured at various time of the day, AUC, or peak levels; however, it was not possible to determine whether the animals that were not in proestrus stage by necropsy were included to inform LH analysis. **40 mg/kg bw/day**: ↓ BW (by day 2 of dosing and persisted until the end of the 4–5 day treatment period), ↓ androstenedione levels Gavage administration **50 mg/kg bw/day**: ↓ BW (by the end of the 4–5 day treatment period), ↓ LH levels (at 1300 hr post lights on), ↓ LH peak and AUC **Phase II** (immunotoxicity assessments): 20♀/dose received treatment similar to phase I above. AFC (10♀/dose) and NKC (10♀/dose) activity assays were conducted. No treatment-related effects on hematology parameters. High variability in the data from the AFC assay. No treatment-related effect was noted in the effector: target ratios determined in the NKC assay. Blood samples from the animals designated to the NKC assay were collected regularly for hematology and hormone analysis (corticosterone determined via radioimmunoassay; ACTH and prolactin determined via ELISA; aldosterone, androstenedione, estrone, estradiol, estriol, progesterone, testosterone, and dihydrotestosterone determined via LC/MS/MS) Spleen and thymus wts were measured for animals designated in the NKC assay Dietary administration **51 mg/kg bw/day**: ↓ BW (AFC and NKC assays), ↓ abs. thymus wt, ↓ abs. spleen wt, Gavage administration **≥ 3 mg/kg bw/day**: ↓ abs. thymus wt (non-adverse) **50 mg/kg bw/day:** ↑ salivation, ↓ BW (AFC and NKC assays), ↓ androstenedione, ↓ aldosterone, ↓ estradiol Study limitations (both phases): The hormone data for plasma prolactin, progesterone, testosterone, dihydrotestosterone, and estrone, and urinary and plasma corticosterone concentrations were considered inadequate for assessment due to a high level of individual animal data variability. The variability include inter and intra-group variability, and levels below the LOD, in addition to some data, for example for dihydrotestosterone were not reported. Study report sections on study design discussed collection of blood samples from animals that were not in estrous stage of cycle by the next morning. This was in direct contradiction of the objectives of the study.

Study type/ Animal/PMRA#	Study results
	Study limitation: Histopathology was not conducted in any group or phase of the study.
4, 8 or 14 day Oral (gavage) LH surge OVX ♀ SD rats Unpublished Study PMRA# 2816739, 2816026 Coder et al., 2011c Published study: PMRA# 3304255 Zimmerman et al., 2018	Supplemental Doses (0. 6.5, 50, or 100 mg/kg bw/day) were administered in 25♀/dose for 4- (Cohort A), 8- (Cohort B), or 14- (Cohort C) days. OVX surgery performed 3 days prior to scheduled blood collection for LH assessments. Animals received estradiol benzoate at the time of surgery via a femoral vein catheter implant. Vaginal lavages were performed daily and slides were evaluated microscopically to determine the stage of the estrous cycle beginning 10-days prior to the initiation of treatment and continued until the day of OVX surgery. Blood samples were taken for plasma analysis of atrazine on the day following LH blood collection. **Cohort A:** **≥ 6.5 mg/kg bw/day**: ↑ clinical signs of toxicity (wet yellow material in the urogenital area), ↓ LH surge **100 mg/kg bw/day**: ↓ BW, ↓ BWG **Cohort B:** **≥ 50 mg/kg bw/day**: ↓ BW, ↓ BWG **100 mg/kg bw/day**: ↓ LH surge **Cohort C:** **≥ 6.5 mg/kg bw/day**: ↑ clinical signs of toxicity (wet yellow material in the urogenital area, dried red material around nose and left eye) **≥ 50 mg/kg bw/day**: ↓ BW, ↓ BWG, ↓ LH surge, ↑ length of estrous cycle Study limitations (for all Cohorts): A number of animals died or were killed which appeared to be related to errors during OVX surgery. Stages of estrous cycle were not used to inform LH analysis. Note plasma analysis of atrazine was not included in the study report.
A 21-day oral (gavage) Ovarian function ♀ SD or LE rats Published USEPA NHEERL ORD study PMRA# 2945602 Cooper et al., 1996	LOAEL = 75 mg/kg bw/day The purpose of this study was to assess the effect of treatment on the ovarian function. 11–12 ♀/dose/strain received treatment at doses of 0, 75, 150 or 300 mg/kg bw/day. Cycles were determined by vaginal smears. Blood samples were collected at necropsy to determine hormone levels. Progesterone and estradiol were measured. Ovariectomies were performed according to the following criteria: If the ♀ demonstrated a regular or irregular pattern of cyclicity during the 21-day treatment period, she was OVX on the first subsequent day of vaginal estrus (1400 hr) and oviducts were flushed to verify that ovulation had occurred and to determine the number of oocytes. Alternatively, in those rats displaying a predominantly leukocytic vaginal smear pattern throughout the treatment period, ovariectomies were performed either 10 days after the last observed estrous smear or on day 21. The ovaries were examined immediately microscopically. By using the vaginal smear pattern to select the day of OVX, it was possible to determine if the animal was pseudopregnant as confirmed by presence of corpora lutea, or anestrous as indicated by ovarian atrophy. **≥ 75 mg/kg bw/day**: ↑ estrous cycle alterations in both strains, ↓ BW in LE rats

Study type/ Animal/PMRA#	Study results
	≥ **150 mg/kg bw/day**: ↑ repetitive pseudopregnancy in both strains, ↑ percent of days in diestrus, ↓ percent of days in estrus, ↑ progesterone levels in both strains, **300 mg/kg bw/day**: ↓ mean number of eggs in both strains, regression of ovaries and anestrous (confirmed by ovarian atrophy) in LE animals only
2- or 4-week (gavage) Ovarian toxicity and fertility ♀ SD rats Published study PMRA# 3292817 Shibayama et al., 2009	Supplemental **Main study** Animals (10♀/dose) received treatment at 0, 3, 30 or 300 mg/kg bw/day for 2-or 4-week period. Clinical signs were observed twice daily, BW and FC were measured twice weekly. Vaginal smears taken every day and mean estrous cycle length was calculated. Ovaries, uterus, and pituitary gland were removed and weighed. These organs as well as vagina and mammary glands were examined histopathologically. Serial ovarian sections were subjected to proliferating cell nuclear antigen analysis **≥ 30 mg/kg bw/day:** ↑ lacrimation (no incidences given; seen in both 2- and 4-week studies), ↑ irregular estrous cycles, ↑ mean estrous cycle length (slight), ↑ lobular hyperplasia of mammary gland **300 mg/kg bw/day:** ↑ clinical signs of toxicity (salivation, soiled perineal region in both 2- and 4-week studies; however no incidences given; decreased stool was noted in the 4-week study only), ↓ BW (in the 2- and 4-week dosing periods), ↓ BWG, ↓ abs. ovaries wt (in the 2- and 4-week dosing periods), ↓ abs. uterus (in the 4-week study only), ↑ histopath findings in reproductive organs in either or both of the studies (ovaries: loss of currently formed corpora lutea, ↓ in number of previously formed corpora lutea, ↑ in large-sized atretic follicles, swelling of previously formed luteal cells, uterus: atrophy, mammary gland: ↑ lactation) **Fertility Study** Animals (10♀/dose) received treatment at 0, 3, 30 or 100 mg/kg bw/day. Dosing was performed two weeks before mating to GD 7 for a total of 5-week period. Clinical signs were observed twice daily, BW measured twice a week during premating and mating periods, and on Days 0 to 8, 11, and 14 of gestation. FC measured twice a week during pre-mating and mating periods and on GD 1, 4, 8, 10, and 13. Vaginal smears taken every day until the day of successful copulation and mean estrous cycle length was calculated. Necropsy on GD 14: The numbers of corpora lutea, implantations, live embryos, and dead embryos were counted and the copulation rate and pregnancy rate calculated **≥ 30 mg/kg bw/day:** ↑ salivation **100 mg/kg bw/day:** ↑ lacrimation and ↓ stools, copulation failure caused by prolongation of diestrus was seen in 1 animal (thought to be due to the anovulatory effect of the treatment) Study limitation: Reproductive indices data (copulation index, fertility index, number of corpora lutea, number of implantation sites, numbers of live and dead embryos, and percentage of preimplantation and post-implantation loss) were highly variable.

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2-week oral (gavage) The effect of atrazine on serum hormone levels ♀ SD rats PMRA# 1234780 Morseth 1990	Supplemental The purpose of this study was to examine the effect of treatment (atrazine and DACT) on serum hormone levels. 15♀/dose received treatment at 0, 100, 200 or 400 mg/kg bw/day (atrazine or DACT). A positive control (vehicle + metoclopramide) was included but the results were not reported. The high dose was lowered to 300 mg/kg bw/day from day 4 due to mortality). When animals reached diestrus, serum was collected for analysis of prolactin, LH, FSH, progesterone, and estrogen. Key organs were weighed. **Atrazine** ≥ **100 mg/kg bw/day:** ↓ thymus wt, ↓ BWG, ↓ mammary gland wt ≥ **200 mg/kg bw/day:** ↑ clinical signs of toxicity (thin and hunched appearance, rough haircoat and the presence of few or no feces, alopecia), ↓ BW, ↓ uterus wt, ↓ estrogen levels **300/400 mg/kg bw/day:** ↑ mortality (2 animals died), ↓ LH level **DACT** ≥ **100 mg/kg bw/day:** ↑ clinical signs of toxicity (thin and hunched appearance, rough haircoat and the presence of few or no feces, alopecia), ↓ BW, ↓ BWG, ↓ thymus wt, ↓ mammary gland wt, ↓ uterus wt, ↓ LH levels ≥ **200 mg/kg bw/day:** ↑ mortality (1 and 8 at this dose and high-dose levels, respectively), ↓ estrogen levels, ↓ progesterone levels **300/400 mg/kg bw/day:** ↓ ovaries wt, ↓ spleen wt
4-week oral (gavage) Estrous cycle alteration and pre-ovulatory LH surge ♀ SD rats PMRA# 1167781, 1167779, 1167780, 1180052, Morseth 1996a	NOAEL = 5 mg/kg bw/day The purpose of the study was to assess the effect of treatment on estrous cyclicity and the LH surge in 90 ♀ per dose. Animals received treatment at 0, 2.5, 5, 40 or 200 mg/kg bw/day. Ten days before necropsy, animals received OVX surgery, followed by capsules releasing estradiol (implanted subcutaneously) 3 days before necropsy. Histopathology was not conducted. Vaginal cytology and serum LH, Prolactin, and Estrogen were determined at 1100, 1400, 1600, 1800, 2000, and 2300 hr from two cohorts of animals – one set from non-repeat bled animals and another from repeat bled animals. ≥ **40 mg/kg bw/day:** ↓ BWG, ↑ estrous cycle alterations, ↓ LH surge levels (more prominent in the repeat bled cohort) **200 mg/kg bw/day:** ↓ BW Study limitation: Vaginal cytology was not used to support LH surge levels
6-month oral (diet) Estrous cycle alteration and LH surge ♀ SD rats PMRA# 1180044	NOAEL = 1.8 mg/kg bw/day The purpose of the study was to assess the effect of treatment on estrous cyclicity and LH surge in 90 ♀ per dose. Animals received treatment at 0, 1.8, 3.6 or 29 mg/kg bw/day. Ten days before necropsy, animals received OVX surgery, followed by capsules releasing estradiol (implanted subcutaneously) 3 days before necropsy. Histopathology was not conducted. Vaginal cytology and serum LH, prolactin, and estrogen were determined at 1100, 1400, 1600, 1800, 2000, and 2300 hr from two cohorts of animals – one set from non-repeat bled animals and another from repeat bled animals.

Study type/ Animal/PMRA#	Study results
Morseth 1996b	**≥ 3.6 mg/kg bw/day:** ↓ (presumed) LH surge, ↑ estrous cycle alterations (consistent with apparent pseudopregnancy and accelerated reproductive senescence, namely demonstration of estrous cycle changes identical to those observed in aging control ♀ SD rats, except they occurred at a younger age) **29 mg/kg bw/day:** ↓ BWG, ↓ BW, ↑ enlarged pituitary, ↑ thickened mammary glands Study limitation: Vaginal cytology was not used to support LH surge levels
30-day oral (gavage) LH surge and other related hormone measurements OVX ♀ Rhesus Monkeys PMRA# 2815980, 2815981, 2816762, 2816761 Unpublished study 2004	Supplemental The purpose of this study was to assess the effect of treatment on LH surge and other related hormone levels in 6 ♀ per dose. Animals received treatment at 0 or 25 mg/kg bw/day. The 30-day treatment was followed by a 60-day recovery period. Animals were acclimated to handling and dosing procedures by receiving vehicle once daily via gavage for 7 days, followed by a 7-day recovery period prior to treatment. Estradiol benzoate was administered on day 5 of pre-treatment, and at 5 and 26 days after initiation of treatment followed by blood collection for a period of 72 hours post-treatment with estradiol benzoate. After a 26-day recovery period, the animals were re-challenged with estradiol benzoate followed by blood collection. LH, FSH, prolactin, progesterone, estradiol, and cortisol were assessed by radioimmunoassay **25 mg/kg bw/day:** ↓ BW (mostly contributed by one animal BW loss, which started prior to treatment and continued during treatment), ↓ FC The study is considered inconclusive in determining treatment-related effects on hormones due to highly variable hormone responses Authors/Expert Panel conclusion: This study demonstrated the limitation of using the OVX, estrogen-primed, ♀ rhesus monkey as a model for evaluating effect of chemicals on estrogen-induced LH surge. Limitations include a high degree of intra-subject and inter-subject variability in the LH response upon repeated testing, and the need to optimize the test-retest interval at 28 days, the average duration of the menstrual cycle in the rhesus monkeys. Experimental design modifications could be employed that may overcome some of these issues; however, it is expected that a number of animals would have to be evaluated over a long duration of time to achieve the statistical robustness needed to reliably determine if atrazine has an effect on the LH surge in the monkey. Such an experiment would be prohibitive from an operational, cost and animal welfare point of view.
b) Studies on mammary gland tumour formation	
12-month oral (diet) Effects of atrazine on the mammary and pituitary glands, the estrous cycle, and plasma hormone levels ♀ SD rats PMRA# 1167680, 1167765, 1167774 Pettersen et al., 1995	NOAEL= 2.8 mg/kg bw/day The purpose of this study was to assess the effect of treatment on the mammary gland and pituitary glands, the estrous cyclicity, and select plasma hormone levels in 55 ♀ per dose. Animal received treatment at 0, 0.8, 1.7, 2.8, 4.1 or 24 mg/kg bw/day. Serial sacrifices were conducted in 10 ♀ per dose at 3, 6, 9 months to determine estrous cyclicity, estradiol, LH, progesterone, and prolactin levels. Histopathology of the mammary and pituitary glands was conducted. Brain, ovaries, pituitary and uterus wts were examined. **≥ 4.1 mg/kg bw/day:** ↓ BW (with similar magnitude throughout the study), ↓ BWG, ↑ ovaries wt at terminal necropsy **24 mg/kg bw/day:** ↑ pituitary wt, ↑ uterine wt, ↑ mammary gland hypertrophy, ↑ mammary gland tumours, ↑ enlarged pituitary

Study type/ Animal/PMRA#	Study results
	Mammary gland tumour incidences in ♀ at 0, 0.8, 1.7, 2.8, 4.1 and 24 mg/kg bw/day: Adenocarcinoma: 1/55, 2/55, 0/55, 1/55, 1/55, 6/24 Adenoma: 0/55, 0/55, 1/55, 0/55, 1/55, 1/55 Fibroadenoma: 2/55, 2/55, 2/55, 1/55, 4/55, 4/55 Total: 3/55, 4/55, 3/55, 2/55, 6/55, 10*/55 * statistically significant at $p < 0.05$ Study limitations: The results of plasma hormone analyses were not reported. Summary means and standard deviation data for estrous cyclicity were not provided. Individual animal vaginal smears data with stage of estrous cycle were provided.

Study type/ Animal/PMRA#	Study results
24-month oncogenicity with serial necropsy (diet) ♀ SD rats PMRA# 1135430, 1135427, 1159810, 1167679 Thakur 1991a	NOAEL = Not determined LOAEL = 4.2 mg/kg bw/day The purpose of this study was to assess the effect of treatment on key endocrine tissues and endpoints with the primary focus on the oncogenic potential of the treatment on the mammary glands through a serial necropsy study design in 70 ♀ per dose. Animals received treatment at 0, 4.2 or 26 mg/kg bw/day. Serial sacrifices were conducted in 10 ♀ per dose at 1, 3, 6, 9, 12, 15, 18, and 24 months. The pituitary, mammary glands, uterus and ovaries from all animals were examined histopathologically. Estrous cycles were assessed by vaginal cytology, and selected serum hormone concentrations (prolactin, estradiol, progesterone, and corticosterone) were determined at all time-points. Serum hormone levels were measured using RIA technique. The ovaries, uterus, vagina, mammary gland and pituitary were re-evaluated histologically for specific indications of reproductive senescence, which might relate to the onset-time of hormonally-mediated mammary tumours. ≥ **4.2 mg/kg bw/day:** ↑ clinical signs of toxicity (alopecia, rough haircoat, swollen body areas), ↑ serum estradiol/prolactin at 9 months, ↑ estrous cycle alterations, ↑ animals with “old corpora lutea” (non-cycling) at 3 months, ↑ number of animals with reduced number of corpora lutea, ↑ antral follicles (anovulation) at 3 and 9 months (at 12 months, control animals were the same as treated animals in terms of parameters representing anovulation), ↑ histopath findings in the ovaries (cysts or medullary tubule hyperplasia at different time points), ↑ histopathological findings in the mammary glands (acinar/lobular development at ≥ 3 months, secretory activity at 3 and 9 months, galactoceles at ≥ 3 months, except at 24 months) **26 mg/kg bw/day:** ↓ BW, ↓ BWG, ↑ mortality (↓ survival), ↑ clinical signs of toxicity (hunched posture, small moveable tissue, pale body, large moveable tissue mass), ↑ histopathological lesions in the mammary glands (chronic inflammation at 9 and 12 months), ↑ mammary gland tumours (consistent with changes in estradiol and prolactin levels observed at 9 months) Mammary gland tumour incidences in ♀ at 0, 4.2 and 26 mg/kg bw/day: 9-month: Fibroadenoma: 0/10, 0/10, 2/10 Carcinoma: 0/10, 0/10, 3/10 12-month: Fibroadenoma: 1/10, 0/10, 0/10 Carcinoma: 0/10, 1/10, 1/10 18-month: Fibroadenoma: 2/10, 4/10, 4/10 Carcinoma: 3/10, 2/10, 4/10 No statistical analysis was performed
24-month oncogenicity (diet) Oncogenic potential in the ovaries, pituitary, uterus and mammary gland ♀ SD rats	Supplemental The purpose of this study was to assess the effect of treatment on the key endocrine tissues and endpoints with the primary focus on the oncogenic potential of the treatment in the mammary gland tumour formation in 60 ♀ per dose following 24 months of dosing. Animals received treatment at doses of 0, 3.5, or 20 mg/kg bw/day. ≥ **20 mg/kg bw/day:** ↓ BWG (12%), ↑ palpable masses in the mammary region (confirmed histologically as mammary gland fibroadenomas and/or carcinomas at 12-month time point), ↑ mortality

Study type/ Animal/PMRA#	Study results
PMRA# 2815961, 2816711 Thakur 1992a	Mammary gland tumour incidences in ♀ at 0, 3.5 and 20 mg/kg bw/day: Fibroadenoma and/or carcinoma at 12-month: 2/10, 3/10, 9*/10 Fibroadenoma and/or carcinoma at 24-month: 46/60, 34/59, 49/60 * statistically significant at p <0.05 Study limitations: detailed study reports were not available
24-month oncogenicity with serial necropsy (diet) ♀ F344 Rats PMRA# 1115083, 1115084, 1115085, 1135415, 1159809, 1167679 Thakur 1991b	NOAEL = 4.8 mg/kg bw/day The purpose of this study was to assess the effect of treatment on key endocrine tissues and endpoints with the primary focus on the oncogenic potential of the treatment on the mammary glands through a serial necropsy study design in 70 ♀ per dose. Animals received treatment at doses of 0, 0.7, 4.8, 14 or 33 mg/kg bw/day. Serial sacrifices were conducted in 10 ♀ per dose at 1, 3, 9, 12, 15, 18, and 24 months. The pituitary, mammary glands, uterus and ovaries from all animals were examined histopathologically. Estrous cycles, vaginal cytology, and selected serum hormone concentrations (prolactin, estradiol, progesterone, and corticosterone) were collected at all time-points. Serum hormone levels were measured using RIA technique. The ovaries, uterus, vagina, mammary gland and pituitary were re-evaluated histologically for specific indications of reproductive senescence, which might relate to the onset-time of hormonally-mediated mammary tumours. Results were evaluated as a function of treatment and as a function of treatment over time. **≥ 14 mg/kg bw/day:** ↓ BW, ↓ BWG The hormone data were quite variable, and the estrous cyclicity data were unreliable. The ovarian histomorphology data were reportedly used to confirm the estrous cycle data and to ensure that the hormone samples for each animal was collected in the same stage of estrous cycle across all animals of a dose group. However, the validity of this statement could not be confirmed based on the data included in the study reports. No treatment-related histopathological effects in the uterus, ovary, and pituitary and mammary glands were observed. Rats in all groups displayed histomorphological alterations in mammary gland that would be expected in normally aging F344 ♀ rats. This included some evidence of lobular/acinar development with secretory activity and occasional galactoceles in all groups at 15, 18, and 24 months. There was not an increased incidence of any tumour type, nor an early onset of mammary tumours.
24-month oncogenicity study (diet) F344 rats PMRA# 1123336, 1123316, 1123317, 1150103, Thakur 1992b	NOAEL = 3.4/4.4 mg/kg bw/day (♂/♀) The purpose of this study was to assess the effect of treatment on the key endocrine tissues and endpoints with the primary focus on the oncogenic potential of the treatment in the mammary gland tumour formation in 60 animals per sex and dose following 24 months of dosing. Animals received treatment at doses of 0, 0.5/0.6, 3.4/4.4, 10/13, or 20/26 mg/kg bw/day in ♂/♀. Hematology and clinical chemistry assessments were not performed. **≥ 10/13 mg/kg bw/day:** ↓ BW, ↓ BWG, ↓ FC
24-month oncogenicity (diet) Tumour incidence in OVX vs intact animals	NOAEL = 3.1 mg/kg bw/day The purpose of this study was to assess the effect of treatment on the key endocrine tissues and endpoints with the primary focus on the oncogenic potential of the treatment in the mammary gland tumour formation in 80 ♀ per dose in two cohorts – OVX and intact following 24 months of dosing. For each cohort, 20 ♀ per dose were

Study type/ Animal/PMRA#	Study results
♀ SD Rats PMRA# 1078579, 1078580, Morseth 1998	allocated for interim necropsy at 12-month time point. OVX ♀ received treatment at doses of 0, 1.2, 2.5, 3.5, or 20.9 mg/kg bw/day. Intact ♀ received treatment at doses of 0, 1.5, 3.1, 4.2, or 24.4 mg/kg bw/day. Hematology, clinical chemistry and urinalysis assessments were not performed **Intact** ♀: ≥ **3.1 mg/kg bw/day:** ↑ incidence of mammary tumours ≥ **4.2 mg/kg bw/day:** ↑ ovarian histopathology (cysts and bursa), ↑ mammary gland secretory activity **24 mg/kg bw/day:** ↑ mortality, ↓ BW, ↑ mammary gland chronic inflammation Mammary gland tumour incidences in ♀ at 0, 1.5, 3.1, 4.1 and 24 mg/kg bw/day at 12 months: Carcinoma: 2/22, 2/22, 0/23, 2/23, 6/25 Fibroadenoma and/or carcinoma: 2/22, 3/22, 2/23, 4/23, 6/25 No statistical analysis performed Mammary gland tumour incidences in ♀ at 0, 1.5, 3.1, 4.1 and 24 mg/kg bw/day at 24 months: Fibroadenoma: 16/80, 25/80, 33**/78, 29*/80, 25*/80 Adenomas: 0/80, 0/80, 1/78, 0/80, 0/80 Carcinomas: 12/80, 18/80, 20/78, 14/80, 27**/80 Total: 24/80, 34/80, 44**/78, 38*/80, 43**/80 * statistically significant at p ≤0.05, ** p ≤0.01 **OVX** ♀: **21 mg/kg bw/day:** ↓ BW, ↑ palpable masses Total incidence of mammary neoplasia in ♀ at 0, 1.5, 3.1, 4.1 and 24 mg/kg bw/day at 24 months: 0/64, 0/66, 0/70, 0/71, 0/72
127-week carcinogenicity (diet) F344 rats Published study PMRA# 3292818 Pinter et al., 1990	**Unacceptable** due to significant limitations and flaws in the study design, conduct and reporting**.** This study was conducted under auspices of IARC. The purpose of this study was to assess the oncogenic potential of the treatment in 50–56 animals per sex and dose following 127 weeks of dosing (0, 19, or 38 mg/kg bw/day) in diet. A statistically significant increase in the total number of benign mammary gland tumours in ♂ as well as in two other tumour types in ♀ (combined incidences of leukemia and lymphoma and uterine adenocarcinomas) were reported in the article. Increased survival in ♂ was also reported in the article. Due to significant limitations and serious flaws in the study design and its conduct as well as missing details in the published article, this study was considered unacceptable. ≥ **19 mg/kg bw/day:** ↓ BW (based on growth curves, data not provided) Doses were lowered following 8 weeks of treatment due to signs of toxicity. The USEPA, JMPR, California DPR and IARC monograph also considered this study unacceptable due to significant limitations and study design flaws.
c) Studies on reproductive and developmental effects	

Study type/ Animal/PMRA#	Study results
Developmental (gavage) Implantation and embryo viability Four strains of rats – HLZ, LE, SD or F344 Published USEPA NHEERL ORD study PMRA# 3292819 Cummings et al., 2000	The purpose of this study was to assess the effect of treatment on implantation and embryo viability in 9–15 ♀ per dose and strain in two different cohorts. These two cohorts were defined as receiving doses from GD 1-8 at specific times of the day, namely either diurnally or nocturnally, during the expected peak prolactin surge times. Animals received treatment at doses of 0, 50, 100, or 200 mg/kg bw/day. Necropsies were conducted on GD 8-9. **Maternal toxicity** NOAEL = 50 mg/kg bw/day (F344, LE and HLZ) NOAEL = 100 mg/kg bw/day (SD) **≥ 50 mg/kg bw/day:** ↓ BWG in all strains (mean BW data were not provided) (non-adverse) **≥ 100 mg/kg bw/day:** ↓ serum progesterone in HLZ rats, ↓ serum LH in HLZ and LE rats, ↑ pre-implantation loss in F344 (seen during nocturnal dosing), ↑ post-implantation loss in HLZ (seen during both nocturnal and diurnal dosing) **200 mg/kg bw/day:** ↓ BW in all strains, BW loss in F344 and HLZ, ↓ serum LH in F344 **Developmental Toxicity** NOAEL = 50 mg/kg bw/day (F344 and HLZ) NOAEL ≥ 200 mg/kg bw/day (LE and SD) **≥ 100 mg/kg bw/day:** ↑ pre-implantation loss in F344 (seen during nocturnal dosing), ↑ post-implantation loss in HLZ (seen during both nocturnal and diurnal dosing) Study author conclusion: F344 rats are most susceptible to preimplantation effects and HLT rats appear most sensitive to the post-implantation effects of atrazine. LE and SD are least sensitive. Study limitation: Summary data tables including means and standard deviations were not available for a number of the measured parameters in the study article.
Developmental (gavage) Pregnancy loss F344, LE or SD rats Published USEPA NHEERL ORD study PMRA# 2945579 Narotsky et al., 2001	The purpose of this study was to assess the effect of treatment on pregnancy loss in 9–15 ♀ per dose and strain of rats through four experiments. Dosing was performed at different intervals of mid-gestation. Dams were allowed to deliver and litters were examined postnatally in all experiments. **Maternal Toxicity** LOAEL = 25 mg/kg bw/day (F344, SD) NOAEL = 50 mg/kg bw/day (LE) **≥ 25 mg/kg bw/day:** BW loss within the first day of dosing (F344 and SD) (not clearly dose-responsive at this dose and BW data for subsequent days of dosing was not provided to assess the extent of the effect), ↓ BWG (F344: GD 6-20) (non-adverse) **≥ 50 mg/kg bw/day:** ↑ full-litter resorption/↓ live litters (F344), ↓ BWG (SD: GD 6-20) **≥ 100 mg/kg bw/day:** BW loss (LE rats, in the first day of treatment) ↓ BWG (GD 6-20 in LE rats), delayed parturition (SD and F344), ↑ prenatal loss in surviving litters (F344 rats) **200 mg/kg bw/day:** Two F344 dams had delayed parturition with no surviving pups, ↑ mortality (F344), ↑ full-litter resorption/↓ live litters (SD and LE),

Study type/ Animal/PMRA#	Study results
	Developmental Toxicity NOAEL= 25 mg/kg bw/day (F344) NOAEL =100 mg/kg bw/day (SD and LE) ≥ **50 mg/kg bw/day:** ↑ full-litter resorption/↓ # of live litters (F344) ≥ **100 mg/kg bw/day:** ↑ percent of prenatal loss in surviving litters (F344 rats) **200 mg/kg bw/day:** ↑ full-litter resorption/↓ live litters (SD and LE) F-344 was the most sensitive to atrazine effects on pregnancy (showing full litter resorptions at > 50 mg/kg bw/day). Study limitation: Summary data tables including means and standard deviations were not available for a few of the measured parameters in the study article.
4-day oral (gavage) Suckling-induced prolactin release in nursing rat dams causes prostatitis in ♂ offspring Wistar rats PMRA# 2945583 Published study Stoker et al., 1999	The purpose of this study was to assess the effect of treatment on the suckling-induced prolactin release in the nursing dams and the subsequent effect on prostate in the ♂ offspring. Dosing was performed in 5–7 nursing dams per dose on LD 1-4. Bromocriptine was used in separate group as a positive control. The day of delivery was designated PND 0. On PND 1, the pups were culled to 10 per litter. Each treatment group consisted of ♂ from at least 10 different litters (n = 13–64 of offspring ♂ – varied depending on the endpoint measured). Serum prolactin concentrations were measured on PND 3 using a serial sampling technique and in-dwelling cardiac catheters. ♂ offspring were examined on PND 90 and 120. Myeloperoxidase (MPO) assay and histology were used to assess prostate inflammation. **Maternal toxicity** NOAEL = 12.5 mg/kg bw/day ≥ **25 mg/kg bw/day:** ↓ suckling-induced prolactin release (serum levels) **Offspring toxicity** NOAEL 12.5 mg/kg bw/day ≥ **25 mg/kg bw/day:** ↑ incidence rate of lateral prostatitis (in 120-day old ♂ offspring, but not in 90-day ♂, based on MPO assay results – defined as > 0.042 MPO/mg in the lateral prostates of the ♂) ≥ **50 mg/kg bw/day:** ↑ incidence rate and severity of lateral prostatitis (in 120-day old ♂ ♂ based on histology and MPO assay, but not in 90-day old ♂) Study article also discussed the results of the experiments conducted with combined treatment of ovine prolactin and atrazine at 50 or 100 mg/kg bw/day on PND 1-4 which reduced the incidence of inflammation observed at 120 days (data not reported). Additional dams were also given atrazine at 50 and 100 mg/kg bw/day on PND 6-9 and PND 11-14. Inflammation was increased in offspring from dams treated on PND 6-9 although not statistically significant. Dosing on PND 11-14 did not produce any inflammation (data not reported).
Oral (Gavage) ♀ pubertal development, LH surge and estrous cyclicity	The purpose of this study was to assess the effect of treatment on ♀ pubertal development endpoints, LH surge and estrous cyclicity parameters via various dosing schedules during gestation and lactation in parental animals (Cohort 1) as well as various intervals around puberty and early adulthood in F1 (Cohort II). Clinical observations, BW, FC, and reproductive indices were recorded at appropriate intervals. Estrous cyclicity was determined for some F1 subsets. Hormone analysis (LH and corticosterone) was done for all subsets. Plasma atrazine and its metabolites were assessed for all subsets.

Study type/ Animal/PMRA#	Study results
SD rats Unpublished study PMRA# 2816744, 2816014, 2816022, 2816741, Coder P 2011d	**Parental toxicity** NOAEL = 25 mg/kg bw/day No treatment-related effects on BW during gestation and lactation in Cohort 1 dams **≥ 25 mg/kg bw/day:** ↓ BWG **50 mg/kg bw/day:** One total litter loss in Cohort 1, ↑ milk not present in pup stomach, ↓ BW (F1 Cohort 1) **Offspring Toxicity** NOAEL = 25 mg/kg bw/day **≥ 25 mg/kg bw/day:** ↑ salivation (non-adverse) **50 mg/kg bw/day:** ↓ BW and BWG (in F1 pups Cohort I all Subsets), ↓ pup survival (PND 1-4 in Cohort 1 all subsets), ↑ pups found dead or partially cannibalized, ↑ milk not present in pup stomach (Cohort 1 subset A and B), delayed VO (in Cohort 1 all subsets) **Reproductive toxicity** NOAEL = 25 mg/kg bw/day **50 mg/kg bw/day:** ↓ birth wt (PND 1), ↓ pup survival (PND 0-1), Concentrations of atrazine (and metabolites in plasma): Plasma concentrations of atrazine and its chlorotriazine metabolites 10.5 to 12 hours after dose administration indicated that little or no atrazine remained in the plasma. The plasma chlorotriazine metabolites (DIA, DEA, and DACT) were detectable at all doses. The concentrations of DACT were greater than DEA and DIA. Study limitations: The study authors did state that the timing of the blood sample collections were not ideal for observing the hormonal effects. Estrous cycle data were not summarized in means and standard deviations to facilitate data interpretation.
4-day (or 5-days) oral (gavage or diet) Fertility and reproductive performance in intact ♀ rats LE and SD rats Unpublished study PMRA# 2816740, 2816023 Coder et al., 2011e Published study PMRA# 2816046, 2816808	Experiment 1: The purpose of this study was to assess the effect of treatment on fertility and reproductive performance in 25 intact ♀ per dose, strain and cohort. For cohorts A (SD) and B (LE), the vehicle and atrazine were administered via gavage beginning at the time the lights were turned on (0500 hr) on the 1st day of the estrous cycle (day of estrous; study day 0) and continuing over one complete 4- or 5-day estrous cycle. Cohort C (LE) ♀s were given the control or test diets ad libitum beginning on the 4th day of the previous estrous cycle (study day -1) and continuing over the next complete 4- or 5-day estrous cycle. Vaginal lavages were performed daily during the pre-treatment and treatment periods for the determination of estrous cycles. On the last day of the treatment period for each ♀ (4th or 5th day of the cycle for ♀s exhibiting 4- or 5-day estrous cycles, respectively), ♀s were paired with untreated ♂s followed by measurements of reproductive performance and fertility parameters at necropsy at the end of gestation. Experiment 2 (Published article): A different cohort of animals were dosed in the same manner as in experiment one and LH levels, estrous cyclicity and number of CL and ova were determined. During necropsy, the ampulla of each oviduct was removed, placed on a clean glass slide, and opened, allowing the eggs within to spill out into saline. The number of ova on the slide was counted. Ovaries were grossly examined and number of corpora lutea was determined. **Parental toxicity**

Study type/ Animal/PMRA#	Study results
Foradori et al., 2014	**≥ 12 mg/kg bw/day:** ↓ ova and CL (cohort A) **≥ 50 mg/kg bw/day:** ↓ BW (cohort C on day 4), ↓ conception index in cohort C (number with confirmed pregnancy/no with evidence of mating) **100 mg/kg bw/day:** ↓ BW (cohort A on day 4), ↑ incidence of decreased defecation (cohort C), slight ↑ total resorptions/post-implantation loss (cohort A), ↓ ova and CL (cohort B and C) **Developmental toxicity** **100 mg/kg bw/day**: ↑ total resorptions/post-implantation loss (Cohort A), Study limitations: Several limitations in reporting, such as estrous cyclicity data not summarized in a manner to facilitate data interpretation. Study design problems were noted. For example, LH surge in rats could be induced by the act of mating and that there are several other factors like steroid hormones and other compensatory mechanisms that tightly regulate LH surge, which can make up for atrazine-induced LH suppression.
Oral (gavage) Reproductive Development of ♂ rats after in utero treatment Wistar rats Unpublished study PMRA# 2816730, 2815991 Published study PMRA# 2816056, 2816783 DeSesso et al., 2014 In-life dates: 1999 Report Issue data: 2008 and amended in 2012	The purpose of this study was to assess the effect of treatment on development of the ♂ reproductive system following in utero exposure. 25 pregnant ♀ per dose received treatment from GD 6-21. Dams were allowed to litter and rear their offspring to weaning. Parental animals, ♀ offspring, and one ♂ pup per litter were euthanized at weaning and necropsied. After weaning, the remaining pups (25 ♂/group) were kept in the study on control diet until the scheduled necropsies on PND 70 or PND 170. At necropsy, adrenal glands, epididymides, pituitary, prostate, seminal vesicles, and testes were weighed. Plasma testosterone was measured. Assessment of the sperm and spermatid numbers, and sperm morphology were performed on PND 70 and 170. **Maternal toxicity** NOAEL = 5 mg/kg bw/day **≥ 25 mg/kg bw/day:** ↓ BWG, ↓ FC, ↑ total in utero litter loss, **125 mg/kg bw/day:** ↓ BW (during treatment/gestation period only. Full recovery noted by the end of lactation – the high dose animals gained weight almost twice as much as their control counterparts during LD 1-21), ↓ litter size, ↓ viability index (LD 0-4), ↓ weaning index (LD 4-12) **Developmental toxicity** NOAEL = 5 mg/kg bw/day Due to high variability in plasma testosterone levels on PND 70 or PND 170, the data were deemed inadequate for the assessment of a treatment-related effect. **≥ 1 mg/kg bw/day:** ↓ abs. prostate wt (non-adverse) **≥ 5 mg/kg bw/day:** ↓ abs. seminal vesicles wt (PND 70) (non-adverse) **≥ 25 mg/kg bw/day:** ↓ prostate wt (PND 170), ↑ percent of abnormal sperm (PND 70 and 170) **125 mg/kg bw/day:** ↓ abs. pituitary wt, ↑ pup mortality, ↓ spermatid numbers, ↓ sperm numbers, ↓ pup BW (PND 1-21, note: by PND 40, the group mean BWs in the surviving animals were comparable to the control values)

Study type/ Animal/PMRA#	Study results
	Due to excess pre- and postnatal mortality at 125 mg/kg bw/day, there were too few ♂ to evaluate reproductive endpoints on PND170, so ♂ in this dose group were only evaluated on PND 70
Oral (gavage) Reproductive development of ♂ rats after postnatal treatment (treatment during lactation/via milk) Wistar rats Unpublished Study PMRA# 2816731, 2815992 Published study PMRA# 2816056, 2816783 DeSesso et al., 2014 In-life dates: 1999	The purpose of this study was to assess the effect of treatment on development of the ♂ reproductive system following exposure via milk. 25–31 dams per dose received treatment from LD 2-21. Parental animals, ♀ offspring, and one ♂ pup per litter were euthanized at weaning and necropsied. After weaning, the remaining pups (25 ♂/group) were kept in the study in control diet until scheduled necropsies on PND 70 or PND 170. Adrenal glands, epididymides, pituitary, prostate, seminal vesicles, and testes were weighed. Plasma testosterone was measured. Assessment of the sperm and spermatid numbers, and sperm morphology was performed on PND 70 and 170. **Maternal toxicity** NOAEL = 25 mg/kg bw/day **≥ 25 mg/kg bw/day:** ↓ BWG (LD 21) (non-adverse) **125 mg/kg bw/day:** ↓ BW (started as early as LD 4), ↓ FC **Developmental toxicity** NOAEL = 25 mg/kg bw/day Due to high variability in plasma testosterone levels on PND 70 or PND 170, the data were deemed inadequate for the assessment of a treatment-related effect. **≥ 25 mg/kg bw/day:** ↓ spermatid numbers (PND 70 and PND 170) (non-adverse at this dose) **125 mg/kg bw/day:** ↓ BW (PND 4-21), ↓ BWG, testes wt (PND 70 and PND 170), ↓ epididymis wt (PND 70), ↓ prostate wt (PND 170), ↓ sperm numbers (PND 70), ↑ percent abnormal sperm (PND 70 and PND 170) Histopathology assessment was not conducted. Thus, the endpoint prostatitis observed by Stoker et al., (1999) was not investigated in this study.
Developmental (gavage) – Cross-foster design to determine effects on puberty and reproductive tissues in ♂s LE rats Published USEPA NHEERL ORD study PMRA# 2815987, 2816792 Rayner et al., 2007	Supplemental The purpose of this study was to assess the effect of treatment on puberty and reproductive tissues of ♂ offspring via a cross-foster design. 20 dams per dose received treatment from GD 15-19. Only a single dose was included in this study. On PND 1, half of the litters were cross-fostered to create the following four groups: gestational treatment dams-control pups (Atrazine-C); milk source or lactational treatment (C-Atrazine), pre- and postnatal treatment (Atrazine-Atrazine), or control (C-C). At weaning, pups were weighted and two ♂s from each cross-fostered litter in each group were selected (total n >18 per group) for continued evaluation of puberty and necropsy on PND120 (n = 9–10) and PND 220 (n = 9–10). Measurements: PPS beginning on PND 37, pituitary gland, testes, lateral and ventral prostates, and seminal vesicles of each animal were weighed. Prolactin, T, and androstenedione levels were measured on PND 120. Lateral and ventral prostate were subjected to histology. **Maternal toxicity**

Study type/ Animal/PMRA#	Study results
	100 mg/kg bw/day: ↓ BW, ↓ BWG **Developmental toxicity** **100 mg/kg bw/day:** ↓ BW (atrazine-atrazine group on PND 4), ↓ BW (C-atrazine, atrazine-C, and atrazine-atrazine groups on PND 120), delay in PPS (atrazine-atrazine and C-atrazine). ↓ BW at PPS in atrazine-atrazine group, ↑ pituitary wt (atrazine-atrazine), ↑ lateral prostate wt (atrazine-atrazine on PND 120, in all atrazine groups on PND 220), ↑ inflammation in lateral prostate (C-atrazine and atrazine-atrazine), ↑ distribution (% of gland affected) and severity score of inflammation of prostate (C-atrazine and atrazine-atrazine groups on PND 120), ↓ MPO levels (C-atrazine and atrazine-atrazine), ↓ prolactin level in all atrazine groups on PND 220
d) Studies on the mammary gland development	
Developmental (gavage) Cross-fostering, pair-feeding and quantitative evaluation of the mammary glands LE rats Unpublished study PMRA# 2816001, 2816759 Coder, P. 2010b	The purpose of this study was to assess the effect of treatment on the development of the mammary gland via cross-fostering study design. 18–42 dams per dose received treatment from GD 13-19, considered the presumptive critical period for the development of mammary glands. The control and high-dose groups contained 42 dams each while the low- and mid-dose groups contained 18 dams in order to accommodate the need for rats used in the cross-fostering portion of the study. In addition, a group of 18 dams was used for the pair-feeding component of the study and their feed was restricted to match the food consumption of the 100 mg/kg bw/day rats on GD 13 through LD 21. Clinical observations, BW, FC, and reproductive indices were recorded at appropriate intervals. Necropsies were performed on F1 ♀ pup/litter on the following time points: PND 1, PND 21, PND 33, day of VO, and the first day of diestrus following the first observed day of estrus after PND 58. At necropsy, mammary glands were obtained from each ♀ for morphometric and microscopic evaluations. Additional F1 ♀ pups were maintained in control diet to produce F2 generation. Mammary gland assessments: Mammary gland assessments were conducted on the 4th Mammary gland collected on PND 1, 21, 33, day of VO, and first diestrus after PND 58. The 4th left mammary gland was used for whole mount and subjected to morphometric measurements of the ductal length, ductal network area, epithelia area, and number of end buds. The 4th right mammary gland was used for cell proliferation analysis using BrdU immunoassay. **Maternal toxicity** NOAEL = 6.5 mg/kg bw/day No treatment-related effects on F2 or F1 post-PND 70 **≥ 50 mg/kg bw/day:** ↓ BW (during treatment period in P generation which persisted throughout the lactation), ↓ BWG **100 mg/kg bw/day:** BW loss, ↓ BW (after the first dose), ↓ BW (in F1 generation on PND 28-70), ↓ BWG, ↑ total litter loss (6 total litter losses in P generation, 5 of which were observed on LD 0 or 1 compared to none in other groups during this period - attributed to diminished nursing behaviour (no milk band on pups), poor nesting behaviour, scattered nest, alienation of individual pups, and/or aggression towards pups), ↓ litter size, ↓ pup survival between PND 0-1, ↓ implantations sites, ↓ number of pups born **Offspring Toxicity** NOAEL = 6.5 mg/kg bw/day

Study type/ Animal/PMRA#	Study results
	No treatment-related effect in F2. No treatment-related effect on VO in F1 or F2. **≥ 50 mg/kg bw/day:** ↑ mammary gland ductal length on PND 1, ↑ # of terminal end buds of the mammary gland on day of VO **100 mg/kg bw/day:** ↓ litter wt (PND 7-21), ↓ pup BWG (PND1-21), ↓ proliferation of epithelial cells of the mammary gland on PND 1, ↑ # of end buds of the mammary gland on PND 21, ↑ pups found dead Study limitations: Due to maternal aggression and cannibalization of pups (resulting in total litter loss), the cross-fostering module of the study was terminated on PND 2 after only 8 litters were produced for both groups included in the cross-fostering module. No further attempts were made to cross-foster and all remaining animals previously assigned to these groups were necropsied on PND 2. Data collected for these animals were not available via the study report.
Developmental (gavage) Effects on mammary gland development and puberty LE rats Published USEPA NHEERL/ORD study PMRA# 2816793, Rayner et al., 2004	Supplemental The purpose of this study was to assess the effect of treatment on the development of the mammary gland via cross-fostering study design. 14–16 dams per dose received treatment from GD 15-19. On PND 1, half of the litters were cross-fostered to create the following four groups: gestational treated dams-control pups (atrazine-C); milk source or lactational treatment (C-atrazine), pre-and postnatal treatment (atrazine-atrazine), or control (C-C). **Maternal toxicity** **100 mg/kg bw/day:** ↓ BWG (in all periods), BW data was not provided, **Developmental toxicity** **100 mg/kg bw/day:** ↑ delay in VO, ↑ BW at VO (in ♀ offspring of the treated dam groups), ↑ stunted epithelial development in MGs of all treated groups PND 4 through PND 40 with least developed MGs in the atrazine-atrazine groups
Developmental (gavage) Critical period of MG development LE rats Published USEPA NHEERL ORD study PMRA# 2816791, 2815984 Rayner et al., 2005	Supplemental The purpose of this study was to assess the effect of treatment on the development of the MG during critical period of development. 8 dams per group received treatment twice daily (half the dose each time) on various intervals of late gestation. Control dams received vehicle on GD 13-19. Treated dams were dosed on GD 13-15, GD 15-17, GD 17-19, or GD 13-19. ♀ pups (n = 8/group) were necropsied on PND 4, 22, 25, 33, 46, and 67. Beginning on PND 29, ♀ offspring (more than 24 ♀s/group) were evaluated for VO. Estrous cyclicity patterns were observed from PND 37 to PND 67. The number of consecutive normal cycles (4–5 days) were determined and analyzed according to the treatment for all animals. MG Development: MGs were removed from all ♀ offspring on PND 4, 22, 25, 33, 46, and 67 and examined to determine if epithelial development of mammary gland was affected. Stained epithelia were measured (area and length) to observe outgrowth into the fat pad. Mammary gland development was scored through whole mount analysis. **Maternal toxicity** **100 mg/kg bw/day:** ↓ BWG (in all periods), BW data was not provided, **Developmental toxicity**

Study type/ Animal/PMRA#	Study results
	100 mg/kg bw/day: ↓ BW (on PND 67 in the GD 15-17 and GD 13-19 groups only), delay in MG development [↓ area of the MG in all treated groups (on PND 4, mammary glands from the GD13-19 measured less than half the size of controls on PND 4, PND22, PND25), ↓ area of the mammary gland in GD13-19 group (on PND 33), ↓ mammary gland developmental scores in the GD 13-19 and GD 17-19 groups (mammary glands displayed fewer terminal end buds, and less dense lateral epithelial branches, and not migrated as far through the fat pad as the control on PND 22, 33, and 46, the developmental scores of mammary glands in all treatment periods were still different than control on PND 67 when all ♀s were sexually mature [mammary gland s contained many large lobular units, with only moderate epithelial branching, the mammary gland s from 3-day treatment period (GD17-19) closely resembled the mammary glands from 7-day treatment period (GD13-19)], ↑ ovaries wt (in all treated groups), ↑ uterine wt (in all treated groups) Estrous cyclicity patterns: the study authors concluded that the majority of animals in each group had three to four consecutive normal cycles, and fewer than two animals per group displayed persistent estrus. No significant differences due to treatment were found in the number of consecutive normal cycles or irregular cycles among the treatment groups. No data or individual animal values were provided to substantiate these statements. Consequence of the brief prenatal treatment on second generation: The offspring of control and treated dams were bred to control LE ♂s starting on PND 68. **100 mg/kg bw/day**: ↓ fetal BW (PND 4), ↓ mammary gland developmental scores in GD 13-19 and GD 17-19 ♀ pups on PND 4 (fewer ductal buds from lateral epithelial branches and undersized compared to other groups) Study limitations: limited data reported for some measured endpoints.
Developmental (gavage) mammary gland development LE rats Published study PMRA# 2816726, 2816019 Hovey et al., 2011	The purpose of this study was to assess the effect of treatment on the development of the MG during critical period of development in utero. 18-42 dams per dose received treatment from GD 13-19. 42 dams were assigned to control and high-dose groups, while 18 dams were assigned to the low- and mid-doses. A separate group (n =18) was pair fed the level of food consumed each day by dams in the high-dose group between GD 13 through PND 21. A blinded, quantitative analysis of key morphological features in mammary gland whole mounts (ductal elongation, ductal network area, epithelial area, TEB incidence, and epithelial density) as well as epithelial proliferation within different parenchymal structures was conducted on PND 1, PND 21, PND 33, day of VO (regardless of age), and as adults. **Maternal Toxicity** NOAEL = 6.5 mg/kg bw/day **≥ 50 mg/kg bw/day**: ↓ BWG, ↓ BW on GD 20 and PND 21 **Developmental Toxicity** NOAEL = 6.5 mg/kg bw/day **≥ 50 mg/kg bw/day:** ↓ BW at VO, ↑ ductal length (PND 1), ↓ epithelial area (at first diestrus after PND 58), ↓ epithelial density (PND 1), ↓ ductal network area (after PND 58)

Study type/ Animal/PMRA#	Study results
	100 mg/kg bw/day: ↓ ductal network area (PND 21), ↑ TEB, ↓ total proliferative epithelial cells in MGs (incidence of BrdU-labelled cells) (PND 1 and age at VO), ↑ total proliferative epithelial cells in mammary glands (PND 1)
Developmental (gavage) Mammary gland development Published USEPA NHEERL/ORD study SD rats PMRA# 2816025, 2816805 Davis et al., 2011	The purpose of this study was to assess the effect of treatment on the development of the mammary gland during critical period of development in utero. 12 dams per dose received treatment from GD 14-21. Two studies were conducted with either once daily doing (s.i.d) or twice daily dosing (b.i.d) paradigms. The b.i.d study used half the volume and dose of vehicle or treatment each time for total daily doses that matched those of the first study. The b.i.d. dosing regimen was implemented to maintain a longer steady-state of the parent compound in the serum and tissues given the rapid metabolism of atrazine to chlorotriazine metabolites. A cage control including 6 dams/group were included. These animals did not receive oral gavage, but were weighed daily. Mammary gland development, estrous cyclicity and VO were assessed. **Maternal toxicity** NOAEL = 20 mg/kg bw/day Maternal BW data/results were not provided although the individual animal BW was measured daily. **100 mg/kg bw/day:** ↑ % post-implantation loss (in both studies) **Developmental toxicity** NOAEL = 20 mg/kg bw/day Mammary gland development assessed on PND 45 revealing no significant effects based on the level of data provided in the study article. **100 mg/kg bw/day:** ↓ BW (PND 1-21, comparable to control by PND 33), ↑ pup death (PND 0-4 in both studies), ↑ delay in VO in both studies Estrous cyclicity data were not provided in the study article, but the study author stated that estrous cyclicity was followed until PND 272 to determine whether a premature emergence of reproductive senescence could have been attributable to the gestational atrazine treatment. Several rats in the 5–20 mg/kg bw/day dose groups reportedly stopped cycling and entered persistent estrus between PND 119 and 132 in the s.i.d. study. All of high-dose animals were reportedly cycling. There were fewer non-cycling individuals in the b.i.d study. The authors concluded that there were no significant differences in cyclicity between studies or dose groups The study also confirmed that the delay in VO was not due to lower BW. The high dose BW was comparable to control values on PND 33. However, control animals reached VO on PND 33 on average while high-dose animals reached VO by PND 35.
e) Studies on female pubertal development	
19-day oral (gavage) ♀ pubertal development and thyroid function Wistar rats Published USEPA NHEERL ORD	The purpose of this study was to assess the effect of treatment on ♀ pubertal and thyroid function. 15 ♀ per dose received treatment from PND 22 to 41. A group of high-dose pair-fed controls was included whose daily food intake was dependent upon the amount consumed by their counterpart in the high-dose group. The purpose of this group was to examine whether delay in VO is secondary to reduced BW or the primary effect of treatment. The majority of the endpoints were assessed according to the USEPA EDSP test guideline for a pubertal assay. NOAEL = 25 mg/kg bw/day

Study type/ Animal/PMRA#	Study results
study PMRA# 2945573, 3292820 Laws et al., 2000	No treatment-related effect on thyroid function (hormone levels or histology) was noted. No delayed VO was observed in the pair-fed control group. **≥ 50 mg/kg bw/day:** ↑ delayed VO (not observed in pair-fed controls), ↑ estrous cycle alteration (prolonged diestrus) following VO **≥ 100 mg/kg bw/day:** ↑ number of animals not cycling **200 mg/kg bw/day:** ↓ BW, ↓ BWG, no VO occurred in a few ♀s at terminal necropsy (PND 41), ↓ adrenal wt, ↓ kidney wt, ↓ pituitary wt, ↓ ovary wt, ↓ uterine wt (with and without fluid), ↓ corpora lutea development
22/25-day oral (gavage) ♀ pubertal development Wistar and SD rats Unpublished study PMRA# 1078516 Ashby et al., 2002	The purpose of this study was to assess the effect of treatment on the onset of puberty in ♀ Wistar or SD rats. Various numbers of ♀ per dose received treatment from PND 21 to 43 (Wistar) or PND 21 to 46 (SD). Necropsies were conducted at various intervals starting on PND 30 using (8-10♀ per dose) until PND 46. BW, uterine wt and VO were assessed. A separate group of Wistar rats was given the GnRH agonist, antarelix, which is known to block the release of LH from the pituitary and delay VO. NOAEL = 10 mg/kg bw/day (SD rats) NOAEL = 30 mg/kg bw/day (Wistar rats) **≥ 30 mg/kg bw/day:** delayed VO (SD rats), ↓ abs. uterine wt (PND 30, PND 33, PND 43 in Wistar rats) **100 mg/kg bw/day:** ↓ BW (PND 30, 33, 43 in Wistar rats), ↓ uterine wt (SD rats), delayed VO (Wistar rats) Antarelix completely prevented VO and uterine growth
Oral (gavage) developmental / postnatal Effects on sexual maturation parameters and LH surge after in utero and/or postnatal treatment through puberty/adulthood SD rats Published study: PMRA# 2816806, 2815972 Breckenridge et al., 2015	The purpose of this study was to assess the effect of treatment on the onset of puberty, LH surge, and estrous cyclicity via dosing periods during development. Animals were assigned to four different cohorts. Cohort 1: Dams treated during gestation and lactation, ♀ pups administered vehicle or atrazine by gavage from PND 21 until 5 days after they attained sexual maturation (2-3 weeks). Cohort 2: Dams treated during gestation and lactation, ♀ pups administered vehicle or atrazine by gavage from PND 21 until PND 133. Cohort 3: Untreated dams, ♀ pups administered vehicle or atrazine by gavage from PND 21 until 5 days after they attained sexual maturation (2–3 weeks). Cohort 4: Untreated dams, ♀ pups administered vehicle or atrazine by gavage from PND 120 to PND 133. Each dose included 25 dams and/or 30–50 ♀ offspring per group. Note: In all cohorts, pups did not receive treatment directly during lactation. **Parental toxicity** NOAEL = 6.5 mg/kg bw/day No treatment-related effects on BW and BWG. **≥ 25 mg/kg bw/day**: ↓ LH (at 0900 hr in cohort 2), ↑ percentage of animals that displayed at least one episode of prolonged diestrus (cohort 2) **50 mg/kg bw/day**: ↓ FC (cohort 1 and 2 at the end of treatment), ↓ gestation index (number of ♀s with live born/number of sperm positive ♀s in cohort 1 [note only data from cohort 1 was reported; however, study author indicated that similar effects were noted in the cohort 2]), ↓ LH (0900 hr in cohort 1 and 4), ↑ percentage of animals that displayed at least one episode of prolonged diestrus (cohort 4), ↓ live birth index (mean percentage of pups per litter alive on PND 0 in cohort 1 [note only data from cohort 1 was reported]),

<table>
<tr><th>Study type/
Animal/PMRA#</th><th>Study results</th></tr>
<tr><td></td><td>Offspring toxicity
NOAEL = 6.5 mg/kg bw/day

≥ 25 mg/kg bw/day: delayed VO (cohort 1 and 2)

50 mg/kg bw/day: ↓ BW (cohort 1 and 2 on PND 21 and in cohort 2 from PND 95-133), delayed VO (cohort 3), ↓ pup survival index (mean percentage of pups surviving PND 1-21 in cohort 1 [note only data from cohort 1 was reported, however, study author indicated that similar effects were noted in the cohort 2])

Study limitation: The LH surge data were not matched with stages of estrous cyclicity.</td></tr>
<tr><td colspan="2">f) Studies on male pubertal developmental and reproductive function</td></tr>
<tr><td>Pubertal assay

Wistar rats

Published USEPA NHEERL ORD study

PMRA# 2945586

Stoker et al., 2000</td><td>NOAEL = 6.25 mg/kg bw/day

The purpose of this study was to assess the effect of treatment on the onset of puberty in ♂ Wistar rats. 6–20 ♂ per dose received treatment from PND 23-53. The majority of the endpoints were assessed according to the USEPA EDSP test guideline for a pubertal assay. A PF group was also included. Beginning on the first day of dosing (PND 23), the daily FC of 10 ♂s at the high-dose was monitored. Then on the following day, each pair-fed ♂ was fed the same amount of food as the corresponding dosed animal. This feeding regimen was continued until the ♂s were killed on PND 53. PPS was monitored beginning on PND 33, until all ♂s showed separation. Reproductive organs and blood samples were collected following necropsy. LH, TSH, and T4 were measured using RIA. In addition, testicular and serum testosterone were assessed, while the former was only measured for control and high-dose animals. Two additional experiments were also conducted. Experiment 1: ♂s were killed on PND 45 (n = 6 for controls and high-dose) to evaluate LH receptor number in the testes and serum and testicular testosterone levels. Experiment 2: ♂s were killed at 120 days of age (n = 8 per group) to evaluate recovery of reproductive tract weights and hormone levels.

≥ 12.5 mg/kg bw/day: ↓ serum LH, delay in PPS (also delayed in PF group)

≥ 50 mg/kg bw/day: ↓ abs. ventral prostate wt on PND 53

≥ 100 mg/kg bw/day: ↓ BW (PND 53)

≥ 150 mg/kg bw/day: ↓ serum prolactin

200 mg/kg bw/day: ↓ abs. seminal vesicle on PND 53, ↓ abs. epididymis wt on PND 53, ↓ abs. pituitary wt, ↓ intra-testicular testosterone at PND 45, ↑ serum estradiol, ↑ estrone, ↑ T3

There were no treatment-related effects on serum TSH or T4 concentrations, or pituitary prolactin or LH concentrations. The study authors concluded that atrazine delayed PPS at doses below which it affects BW.

Although testicular LH receptor analysis is not part of the ♂ pubertal protocol, it was included in this study to determine whether decreases in prolactin may have resulted in a decrease in normal upregulation of receptors during puberty. No treatment-related effect was observed between the number of LH receptors in the control and 200 mg/kg bw/day groups on PND 45 or 53. Complete data were not available for this experiment.</td></tr>
</table>

Study type/ Animal/PMRA#	Study results
	Although serum testosterone levels (measured in all groups) were lower in all doses than controls, there was no pattern or statistical significance identified likely due to variability in the data provided. Study authors also acknowledged the variability of serum testosterone levels and added that testosterone is highly variable at this age. They further indicated that testosterone levels rise gradually from PND 20 to 40, and abruptly double by PND 50. For this reason, they proposed a more complete evaluation would include a time-point analysis, with testosterone measurements conducted at 5-day intervals (PND 28, 33, 38, 43, 48) following the first doses of atrazine. By PND 120, all BW and reproductive tract organ wt values had returned to control values in all treatment groups, except for the ventral prostate, which was still lower than that of controls values.
Oral (gavage) Hershberger Assay Castrated rat assay for anti-androgens 6-wk old Alpk: AFfSD (AP) Rats Unpublished Study PMRA# 2815982, 2816747	Supplemental The purpose of this study was to assess the effect of treatment in a Hershberger assay using three different cohorts of rats. **≥ 25 mg/kg bw/day**: ↓ LA/BC wt (cohort 1), **≥ 50 mg/kg bw/day**: ↓ seminal vesicles wt, ↓ LA/BC wt (cohort 2), ↓ prostate wt (cohort 2) **100 mg/kg bw/day**: ↓ BW (cohort 1, 2 and 3), ↑ adrenals wt (cohort 1 and 2), ↓ Cowper's wt (cohort 2 and 3), ↓ LA/BC wt (cohort 3), ↓ seminal vesicle wt (cohort 3), ↓ prostate wt (cohort 3), ↑ glans penis wt (cohort 3) Study limitations: Inadequate details on study design, materials and methods, and results provided in the study article including lack of individual animal data
Developmental (gavage) Effects of gestational treatment on postnatal development in ♂s SD rats Published study PMRA# 2945581 Rosenberg et al., 2008	The purpose of this study was to assess the effect of treatment on the development of ♂ reproductive system following in utero exposure during critical period of development. 14-16 dams per dose received treatment from GD 14-21. On PND 0, testicular testosterone levels were measured in ♂ pups from 7-8 dams. With remaining 7 to 8 dams per doses, the number of pups were culled to 8 on PND 2, and the following was assessed: BW and AGD measured on PND 21, and PPS was assessed daily beginning on PND 37. Terminal necropsy was performed on PND 60. Testes and other androgen dependent organs were weighed and testosterone levels were measured. **Maternal Toxicity** NOAEL = 10 mg/kg bw/day **≥ 50 mg/kg bw/day:** ↓ FC, ↓ BW **Developmental Toxicity** NOAEL = 10 mg/kg bw/day **≥ 50 mg/kg bw/day:** ↑ pup death (between PND 0-2), ↓ pup BW on PND 2, delay in PPS, ↓ serum testosterone levels on PND 60 **≥ 75 mg/kg bw/day:** ↓ AGD index, ↓ intratesticular testosterone levels on PND 60, ↓ testes wt, ↓ BW on PND 60 Study limitation: Summary data tables with means and standard deviations were not available for some measured parameters in the study article.

Study type/ Animal/PMRA#	Study results
Developmental (gavage) Effects of gestational treatment on reproductive development in ♂ offspring SD rats Published USEPA NHEERL ORD study PMRA# 3292813 Fraites et al., 2011b	The purpose of this study was to assess the effect of gestational treatment on the development of the ♂ reproductive system and characteristics. 12 dams per dose received treatment from GD 14-21. As was performed in the study by Davis et al., 2011 above, two studies using s.i.d and b.i.d dosing regimen was used. Note the b.i.d. dosing regimen was implemented to maintain a longer steady-state of unchanged atrazine in the serum and tissues given the rapid metabolism of atrazine. A group of cage-controls was also included in each study which served as controls for behavioral studies. These animals were weighed but they did not undergo oral gavage dosing. Litters were examined at birth and on PND 4 and 7. PPS assessment began on PND 38 and continued until PPS was observed. Necropsy was done on PND 59 to assess AGD, serum testosterone levels, and androgen-sensitive organ wts. Ex vivo testosterone production was performed at birth and on PND 59. Pup weight was measured on PND 4, 7, and 21. On PND 30-33, cage-mates (littermates; one pair per litter) were assessed for rough-and-tumble play behaviour. **Maternal toxicity** NOAEL = 20 mg/kg bw/day **100 mg/kg bw/day:** ↓ BW (dams at this dose showed marked BW loss after the first day of dosing) **Developmental toxicity** NOAEL = 20 mg/kg bw/day **100 mg/kg bw/day:** ↓ BW (some recovery was shown in subsequent measurements on PND 4, 21, 46, and 59. The BW of ♂s on PND 59 at this dose was comparable to control values in the b.i.d study. In the s.i.d study, the BW on PND 59 was lower compared to control values), ↓ pup viability (both studies on PND 1-4), delay in PPS (both studies) No trend or pattern could be identified for the serum or interstitial fluid testosterone data, although the variability in the data was acceptable within each study; the variability between the studies was quite large. No treatment-related effects were noted on unstimulated or LH-stimulated testosterone production by the testes (either at birth or on PND 59), or on androgen-dependent organ weights. Study author’s notes/conclusion: Rough-and-tumble play behaviour is strongly influenced by the amount of testosterone during the perinatal period. Thus, rough-and-tumble play behaviour was assessed as an additional measure of potential atrazine-induced changes in testicular hormone secretion. The frequency and duration of play and interactions were assessed. The duration of play for ♀s was significantly shorter than that of ♂s as expected. Analysis of rough-and-tumble play behaviour demonstrated no atrazine-induced effect. Overall, no alterations of reproductive endpoints were observed in treated offspring unless the animals received the highest dose employed in this study. Study limitations: Summary data tables with means and standard deviations were not available for some measured parameters in the study article.
Pubertal development and reproductive function SD rats PMRA# 2945587 Trentacoste et al.,	The purpose of this study was to assess the effect of treatment on the onset of puberty and some reproductive system endpoints in ♂s. Groups of 9–10 ♂ per dose received treatment from PND 22-47. A food restriction was conducted with rats pair fed to the second high-dose group. BW, PPS, testosterone (serum and interstitial fluid) and serum LH concentrations were measured. **≥ 100 mg/kg bw/day:** ↓ BW, ↓ seminal vesical wt, ↓ ventral prostate wt, ↓ serum testosterone, ↓ intratesticular fluid testosterone, ↓ LH, delayed PPS Note: the pair fed group only had ↓ testosterone and LH on PND 47 that was similar to

Study type/ Animal/PMRA#	Study results
2001	100 mg/kg bw/day. Study limitations: Summary data tables with means and standard deviations were not available for some measured parameters in the study article.
g) Studies on hypothalamic-pituitary-adrenal axis (HPA)	
Single dose, oral (gavage) Characterization of dose response and time course for effects of atrazine and its primary metabolites on pituitary and adrenal hormone secretion Wistar rats Published USEPA NHEERL ORD study PMRA# 2945575 Laws et al., 2009	The purpose of this study was to characterize dose response and time course for effects of treatment (atrazine and chlorotriazine metabolites DIA, DEA, and DACT) on pituitary and adrenal hormone secretion following a single oral dose in 9–10 ♂ per group. All chlorotriazine doses were selected as the molar equivalent of atrazine to facilitate the comparison of the potency of each test chemical with that of atrazine. The ♂s were dosed at 0900 hr and necropsied at various intervals post-dosing to collect blood samples for hormone analysis. BW was recorded. ACTH, corticosterone, progesterone and prolactin were assessed using RIA. In addition, extraction and analysis of rat plasma samples for atrazine and metabolites were performed. Animals were acclimated (given vehicle doses) for a week prior to test substance administration to assure reliable measures of pituitary-adrenal hormones and avoid gavage-dosing-related stress. Dosing procedure at 0900 hr followed because previous laboratory experience showed that ACTH and corticosterone concentration levels are more consistent during the morning hours when circadian fluctuations of these hormones are the lowest. **Atrazine** **≥ 50 mg/kg bw**: ↑ ACTH (maximum levels reached by 15 min post-dosing and returned to control values by 180 min after treatment), ↑ corticosterone and progesterone levels (by 30 min and remained elevated by 180 min post-dosing). **DIA** **≥ 10 mg/kg bw:** ↑ corticosterone and ↑ progesterone (maximum ↑ at 30 min post-dosing) **≥ 40 mg/kg bw:** ↑ ACTH (maximum ↑ by 30 min post-dosing) **DEA** **173 mg/kg bw:** ↑ ACTH, corticosterone, and progesterone all within 15 min of treatment. **DACT** **33.7 mg/kg bw:** ↑ ACTH (minimally at 30 min post-dosing), ↑ corticosterone and progesterone (minimally at 30 min post-dosing), There were no treatment-related effects on prolactin levels. Plasma concentration of test chemicals and metabolites confirmed that the major metabolite of atrazine is DACT. This study also examined simazine and propazine; however, only the results pertaining to atrazine and common triazine metabolites were summarized.
Single or repeated-dose oral (gavage) Characterization of dose response and time course for effects of atrazine and its primary	The purpose of this study was to characterize dose response and time course for effects of treatment (atrazine and chlorotriazine metabolites DIA, DEA, and DACT) on pituitary and adrenal hormone secretion following single or repeated dosing in 4–14 ♀ per group at 0900 h. All chlorotriazine doses were selected as the molar equivalent of atrazine to facilitate the comparison of the potency of each test chemical with that of atrazine. The repeat-dose study was conducted over one estrous cycle. Necropsy was conducted 15 min post-gavage (following the last dose administration), and trunk blood was collected for hormone measurements. Plasma ACTH and serum corticosterone and progesterone were

Study type/ Animal/PMRA#	Study results
metabolites on pituitary and adrenal hormone secretion Plasma concentrations of ATR and primary metabolites LE rats Published USEPA NHEERL ORD study PMRA# 3292812 Fraites et al., 2009b	determined via RIA. Single dose experiment: **Atrazine** **75 mg/kg bw:** ↑ plasma ACTH, ↑ serum corticosterone, ↑ progesterone **DIA** **60.2 mg/kg bw:** ↑ ACTH, ↑ serum corticosterone, ↑ progesterone **DACT** No treatment-related effect on any hormone measurement. 4-day repeated dose experiment: **Atrazine** **≥ 12.5 mg/kg bw/day:** ↑ serum corticosterone, ↑ progesterone **≥ 75 mg/kg bw/day:** ↑ plasma ACTH **DIA** **≥ 10 mg/kg bw/day:** ↑ ACTH, ↑ serum corticosterone, ↑ progesterone **DACT** No treatment-related effect on any hormone measurement. Study investigators also compared the atrazine/metabolite-induced HPA axis response to a well-known stressor. The single dose and repeat dose experiments were repeated (n = 14/group) with rats undergoing a 5-min restraint stress test instead of oral gavage doses of chlorotriazines. The animals were restrained in cylindrical plastic restrainers for 5 min at 0900 hr before returning them to their home cage. The study authors indicated that both of the single and multiple acute restraint stress tests produced hormonal responses of a similar magnitude to those induced by atrazine and DIA (detailed data not provided). The study authors indicated that similar hormonal responses were also observed in experiments in which rats received an oral dose of atrazine following bilateral subdiaphramatic vagotomy and following IV administration of DIA in jugular vein-catheterized animals. These additional studies were conducted to show that the activation of HPA axis by oral administration of atrazine and DIA were not simply due to the stimulation of gastrointestinal afferents. Detailed data for these experiments were not part of the study article.
Single or repeated-dose oral (gavage) Effects of atrazine on LH surge or pulses in ADX animals	Supplemental The purpose of this study was to determine if 1. increases in corticosterone levels could contribute to the attenuation of LH release following ATR treatment given that corticosterone can inhibit LH secretion 2. adrenal activation plays a role in atrazine suppression of the LH surge

Study type/ Animal/PMRA#	Study results
OVX ♀ Wistar rats Published study PMRA# 2816814, 2816028 Foradori et al., 2011	Three experiments were conducted as part of this study. In the first experiment, the effect of treatment on corticosterone levels in 3–5 per ♀ per dose and time point was assessed following single oral gavage doses. No ADX or OVX surgeries were performed. In the second experiment, the effect of treatment on LH surge was assessed. In the third experiment, the effect of treatment on the pulsatile release of LH was assessed. In both of these experiments, dosing was done over 4 days in 7–13 ♀ per dose. Ten days after OVX surgery, half of the animals were ADX and the other half were subjected to sham surgery before they were assigned to the study. **≥ 10 mg/kg bw/day:** dose-related ↓ LH peak and AUC (in ADX and sham animals in Experiment 2) **≥ 50 mg/kg bw/day:** ↑ corticosterone levels in Experiment1 (at 20 min post gavage time-point at 50 mg/kg bw/day and at all time-points measured at 200 mg/kg bw/day), **200 mg/kg bw/day:** altered pulsatile release of LH in Experiment 3 (↑ pulse amplitude and pulse period) in vehicle treated sham animals, but not in the ADX animals. Study authors concluded that the adrenal hormones do not play a role in the preovulatory surge of LH, but play a role in the basal pulsatile LH secretions affected by treatment. Study limitations: Summary data tables with means and standard deviations were not available for some measured parameters in the study article. Purity level was not provided. Estrous cyclicity data was not used to inform the inclusion of animals for LH analysis.
h) Studies on the immunotoxic potential	
Oral immunotoxicity study and hormone evaluation, 1, 7, 14, or 28 days exposure (gavage or diet) SD rats Published study PMRA# 3292828 Foradori et al., 2017	NOAEL = 25 mg/kg bw/day The purpose of this study was to assess the immunotoxic potential of treatment via gavage dosing of various durations and following 28 days dosing via diet in 10 animals/sex/dose. A high-dose pair-fed control group as well positive control groups were included as appropriate. AFC and NKC assays were conducted. Blood was collected for pituitary (ACTH, prolactin), adrenal (corticosterone, progesterone, aldosterone) and gonadal (androgens, estrogens) hormone assessment (30 minutes after the first dose and after 1, 7, and 28 days of dosing). BW and FC were recorded daily, and spleen wt, spleen cell numbers, and thymus wt were measured. Gavage dosing - ♂: **≥ 6.5 mg/kg bw/day**: ↑ plasma corticosterone (day 1 only) (♂) **≥ 25 mg/kg bw/day**: ↑ plasma ACTH (day 1 only), ↑ plasma progesterone (day 1 only), ↓ plasma aldosterone (day 28) (♂) **100 mg/kg bw/day:** ↓ BW (beginning on day 3, through to day 28), ↓ BWG, ↓ FC, ↑ plasma aldosterone (day 1), ↓ thymus wt (days 7 to 28), ↑ in NKC activity (effector; target ratios of 200:1 and 100:1) (♂) Gavage dosing - ♀: **≥ 6 mg/kg bw/day:** ↓ BWG (intermittent throughout 28-day treatment period) **50 mg/kg bw/day:** ↓ BW (study termination), ↓ plasma aldosterone, ↓ urinary corticosterone (all time points), ↓ thymus wt (♀)

Study type/ Animal/PMRA#	Study results
	Dietary dosing - ♀: **≥ 3 mg/kg bw/day**: ↓ plasma progesterone (♀) **51 mg/kg bw/day**: ↓ bw (study termination), ↓ plasma aldosterone (♀)
Oral immunotoxicity study and hormone evaluation; acute, 6-, 13-, or 28-day exposure (gavage) ♂ SD rats PMRA# 2816013/ 2816743	NOAEL = 25 mg/kg bw/day The purpose of this study was to assess the immunotoxic potential of treatment via gavage dosing of various durations in 10♂ per dose. The following subsets were used: A: dosed on study day 0 (euthanized approximately 30 minutes after dose administration); B: dosed on study days 0–6; C: dosed on study days 0–13; D and E: dosed on study days 0–27 or 28; each dosing regimen included a positive-control group and a pair-fed control group. The following assessments were performed: Clinical observations: all subsets; BW: recorded daily for all subsets until necropsy; FC: subsets B, C, D, E; Hematology: subsets C and D; Hormone analysis, urine: subsets C and D; Hormone analysis, blood: subsets A, B, C, D; Tissue collection: subsets A, B, C and D; Organ wt (liver, spleen, adrenal glands, thymus): subsets A, B, C and D (Subset E, spleen wt only); Microscopic evaluation: subsets C and D; Bone marrow smear cytology: subset C; NKC function: subset D; AFC assay: subset E Hormonal analysis included the following: 1. Stress hormones: corticosterone, ACTH, progesterone, and prolactin; 2. ♀ sex hormones: estradiol, estrone, estriol; 3. ♂ sex hormones: androstenedione, testosterone, and DHT; and 4. Mineralocorticoids: aldosterone. Bone marrow smears performed on day 13. NKC assay was performed on day 27. Splenic AFC assay and spleen wt were assayed on day 28 following immunization with SRBC on day 24. Subset A: No treatment-related clinical signs of toxicity or effects on BW. **≥ 6.5 mg/kg bw/day:** ↑ plasma corticosterone levels, ↑ plasma progesterone levels **≥ 25 mg/kg bw/day**: ↓ thymus wt Subset B: **≥ 25 mg/kg bw/day:** ↓ BWG (days 0–6), ↓ FC, ↓ thymus wt **100 mg/kg bw/day**: BW loss (days 0–2), ↓ BW, ↑ plasma corticosterone levels Subset C: **≥ 6.5 mg/kg bw/day**: ↓ thymus wt **≥ 25 mg/kg bw/day:** ↑ MCHC **100 mg/kg bw/day**: BW loss (days 0–1), ↓ BW (days 8–13), ↓ BWG (days 0–13), ↓ FC,

Study type/ Animal/PMRA#	Study results
	↑ neutrophil counts (abs. and rel.), ↑ rel. liver wt, ↑ incidence of periportal glycogen content in the liver, ↑ plasma corticosterone levels Subset D: **≥ 25 mg/kg bw/day:** ↓ BW (days 11–27), ↓ BWG (days 0–27), ↓ plasma aldosterone levels. **100 mg/kg bw/day**: salivation, BW loss (days 0–1), ↓ BWG (days 0–27), ↓ FC, ↓ HGB, ↓ HCT, ↑ MCHC, ↑ reticulocyte counts (abs. and rel.), ↑ red cell distribution width, ↑ hemoglobin distribution width, ↓ thymus wt, ↑ rel. liver wt, ↑ incidence and severity of periportal glycogen content in the liver, enhanced NKC activity (at 2 highest effector: target ratios) Subset E: **≥ 6.5 mg/kg bw/day**: ↓ BW (days 0–28), ↓ BWG (days 0–28) **100 mg/kg bw/day**: salivation, BW loss (days 0–1), ↓ FC
Oral immunotoxicity study Prenatal and early postnatal exposure (gavage) SD rats PMRA# 2945593	Supplemental The purpose of this study was to assess the immunotoxic potential following gestational and lactational treatment. Dams received treatment from GD 10 to PND 23. The following assessments were performed: PND 2: pups removed from dams, weighed, pooled and reassigned to 5/sex/litter. PND 7: pups were weighed and ear-punched for identification and tracked individually for the remainder of the experiment. PND 14: one pup/sex was euthanized, spleen and thymus were removed and weighed. PND 49+: the following immune functions in the offspring were evaluated: NKC function, DTH responses to BSA, phagocytic activity of peritoneal macrophages, and antibody response to SRBC. Spleen and thymus wt were measured. Total T4 and T3 were measured in PND 14 offspring. Dams: BW was measured daily during gestation. **Maternal toxicity:** **35 mg/kg bw/day**: ↓ pup viability (fetal and litter basis) (PND 2-14) (♂/♀) **Developmental toxicity:** **35 mg/kg bw/day**: ↑ pup mortality (fetal and litter basis) (♂/♀); ↓ BW (PND 7), ↓ primary antibody response (IgM response to SRBC, 8 weeks), ↓ DTH response (8 and 12 weeks) (♂). Numerous study limitations including the purity level of test substance was not provided
14-day oral immunotoxicity study; assessment of short- and long-term effects (gavage) ♂ C57BL/6 mice Published study PMRA# 3292825	Supplemental The purpose of this study was to assess the immunotoxic potential of treatment. Three independent identical experiments were conducted, each with 10 one-month-old ♂ per dose. Samples were collected 1 day, 7 days, or 7 weeks after the final of 14 daily doses (3–4 ♂/ dose/time point). Parameters evaluated: kidney, liver, thymus and spleen wt and cellularity; lymphocyte subpopulations in the spleen; lymphocyte subpopulations in the thymus; peripheral blood mononuclear cells

Study type/ Animal/PMRA#	Study results
Filipov et al., 2005	BW recorded daily at the time of dosing and at time of necropsy. **≥ 5 mg/kg bw/day:** ↓ thymic T-cell population (1 day post-exposure, CD4+/CD3+, CD8+/CD3+, CD4-/CD8+, CD4-/CD8-) (non-adverse) **≥ 25 mg/kg bw/day:** ↓ spleen cellularity (7 weeks post-exposure), ↓ thymic T-cell population (1 day post-exposure, CD4+/CD8-), ↓ splenic cell population (1 day post-exposure; MHC II, CD19+, CD4+/CD 44^{low}, CD8+/CD44^{low} and CD8+/ CD44-), ↑ splenic cell population (1 day post-exposure ; CD8+, CD8+/CD44^{high}), ↓ splenic cell population (7 days post-exposure, CD4+/CD 44^{low}), ↑ splenic cell population (7 days postexposure, CD4+/CD44^{med}). **≥ 125 mg/kg bw/day:** ↓ thymus wt and cellularity (1 and 7 days post-exposure), ↓ spleen cellularity (1 and 7 days post-exposure), ↓ thymic T-cell population (1 day post-exposure, CD4+/CD8+), ↓ splenic cell population (1 and 7 days post-exposure; CD11^{high}), ↓ peripheral blood mononuclear cell subpopulations (7 days post-exposure, CD4+/44-). **250 mg/kg bw/day:** ↓ spleen wt (1 day post-exposure), ↑ CD3+ splenic cell population (1 day post-exposure), rebounding effect in splenic cells 7 weeks post-exposure (↑ CD4+/CD44^{low} and CD4+/CD44-, and ↓ CD4+/CD 44 ^{med}), ↑ peripheral blood mononuclear cell subpopulations (1 day post-exposure; CD8+/44^{high} and CD4+/CD44^{high}), ↓ peripheral blood mononuclear cell subpopulations (1 and 7 days post-exposure, CD4+), ↑ peripheral blood mononuclear cell subpopulations (7 days post-exposure, CD+4/44^{high}, NKC), ↓ peripheral blood mononuclear cell subpopulations (7 days post-exposure, CD4+/44 ^{low}, MHCII+ cells). JMPR/USEPA Conclusion: Although T cell subpopulations were ↓ at all doses on day 1 post-exposure, the fact that they quickly recovered diminishes the toxicological significance of this finding and its ability to predict impaired function. Study limitations: Summary data tables with means and standard deviations were not available for some measured parameters in the study article.
14-day oral immunotoxicity study; effects in adult ♀ mice (gavage) ♀ B6C3F1 mice Published study PMRA# 3292826 Karrow et al., 2005	Supplemental The purpose of this study was to assess the immunotoxic effects of treatment following 14 days of oral (gavage) dosing in 8 ♀ per dose. The following immune assays and toxicity parameters were assessed: BW (days 8 and 15); organ wt (liver, spleen, thymus and kidney); hematology; differential cell counts; serum IgM titers; splenic NKC activity; splenic MLR; splenic IgM AFC response; splenic lymphocyte proliferation to mitogens Con A (Concanavalin A) and LPS (Salmonella typhi lipopolysaccharide); splenic B and T cell enumeration; splenic cytotoxic T lymphocyte response to mitomycin C-treated P815 mastocytoma cells; evaluation of mononuclear phagocytic system (MPS; % uptake of SRBCs into the spleen); host resistance to L. monocytogenes challenge (three challenge levels); host resistance to B16F10 tumor challenge (two doses). **≥ 25 mg/kg bw/day:** ↓ BWG, ↓ spleen wt, ↓ RBC (non-adverse) ≥ **250 mg/kg bw/day:** ↓ thymus wt, ↓ host resistance to B16F10 tumour challenge (↑ number of nodules in lungs and (counts per minute) /lung in B16F10 tumour challenge at 3×10^5 cells/ mouse challenge level). **500 mg/kg bw/day:** BW loss, ↓ abs. kidney wt, ↓ HGB and HCT, ↓ leukocytes, ↓ lymphocytes, ↓ neutrophils ↑ percentage of T cells and CD4−CD8+ splenic T cells, ↓ abs. number of splenic B cells, ↓ abs. number of CD4+CD8+ splenic T cells, stimulated MLR.

Study type/ Animal/PMRA#	Study results
	Study limitations: Summary data tables with means and standard deviations were not available for some measured parameters in the study article.
28-day oral immunotoxicity study (gavage) ♀ C57B1/b mice Published study PMRA# 3292830 Zhao et al., 2013	Supplemental The purpose of this study was to assess the immunotoxic potential of treatment following 28 days of oral (gavage) dosing in 10♀ per dose. Spleen and thymus were collected 24 hr after final dose of atrazine and weighed. BW was measured every 7 days; spleens were examined histopathologically. Tests/assays conducted: lymphocyte transformation test; NKC toxicity assay; flow cytometry (to detect surface markers of lymphocytes); ELISA for IL-2, IL-4, IFN-γ, and TNF-α. MTT assay [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenylte-trazolium bromide] was used to assess splenic lymphocyte proliferation and NKC function via cytotoxicity. **≥ 5 mg/kg bw/day:** ↓ spleen wt, ↓ splenic lymphocyte proliferation, ↓ percentage of CD3+ splenic lymphocytes (non-adverse) **≥ 25 mg/kg bw/day**: ↓ thymus wt, histopathological changes in the spleen (atrophy, effacement of germinal centres, ↓ white pulp, congestion of red pulp), ↓ CD4+ splenic lymphocytes, ↓ splenic CD4+/CD8+ ratio, ↓ serum IL-4 **125 mg/kg bw/day**: ↓ NKC cytotoxic activity Study limitations: Summary data tables with means and standard deviations were not available for some measured parameters in the study article.
28-day oral immunotoxicity (gavage) ♂ Balb/c mice PMRA# 2816042, 2816779	Supplemental The purpose of this study was to assess the immunotoxic potential of treatment following 28 days of oral (gavage) dosing in 10♂ per dose. The following assessments were performed: BW, spleen and thymus wt were measured at study termination. 1) Apoptosis/necrosis of splenocytes and thymocytes was assessed by flow cytometry. 2) Animals were immunized by i.p. injection of SRBCs 5 days prior to sacrifice. a) antibody aggregation of the serum hemolysin was determined; b) one-day prior to sacrifice, animals were sensitized with hypodermic injection of 20 µL of 20% (v/v) SRBCs in saline in the left hind footpad. The left footpad volume was measured 24 hr post-sensitization and incrassation of the left foot pad was calculated (DTH); c) Peritoneal cells were isolated from the abdominal cavity of mice sacrificed on day 28: d) Cells were cultured for 4 hr. The mononuclear phagocytic system was evaluated by measuring the clearance zone of neutral red and nitrogen oxide release. 3) Splenocytes were isolated and co-incubated with Concanavalin A (ConA): a) after co-incubation for 72 hr, the proliferation response of splenocytes was measured using the MTT assay; b) after co-incubation for 48 hr, the supernatant was harvested and IFN-γ, IL-4 and serum lysozyme levels were measured via ELISA. 4) Isolated splenocytes were co-cultured for 4 hr with YAC-1 cells and then the NKC activity was assessed by MTT assay. **≥ 44 mg/kg bw/day:** ↓ thymus wt, ↓ percentage of normal thymocytes, ↑ late apoptosis/necrosis of splenocytes, ↓ proliferation index of splenocytes in response to Con A, ↓ serum lysozyme. **≥ 88 mg/kg bw/day:** ↓ BW, ↓ spleen wt, ↑ early apoptosis of thymocytes, ↓ splenetic

Study type/ Animal/PMRA#	Study results
	IFN-γ **175 mg/kg bw/day:** ↓ NKC activity, ↓ incrassation of left footpad volume, ↓ clearance of neutral red (slight), ↓ nitrogen oxide release, ↓ antibody aggregation of the serum hemolysin, ↓ IFN-γ /IL-4 ratio. Study limitations: Summary data tables with means and standard deviations were not available for some measured parameters in the study article.
Immunotoxicity study; effects of maternal atrazine 21-day exposure (subcutaneous pellets) Balb/c mice PMRA# 2945594	Supplemental The purpose of this study was to assess the immunotoxic potential during critical windows of development. Dams received treatment via subcutaneous time release pellets starting between GD 10-12 for 21 days. Immune status was evaluated in offspring at approximately 3 months of age. The following measurements were performed: 1) In vitro T cell proliferation and cytolytic activity after allogeneic stimulation assays: a) Splenocytes from offspring were isolated and stimulated for 96 hr using irradiated splenocytes from ♀ C57B1/6 mice. Proliferation was measured using ^{3}H-thymidine. b) Cytotoxic T lymphocyte assay: T cell ability to lyse alloreactive target cells was assessed using EL-4 lymphoma cells as targets. 2) Humoral immune response was assessed with heat killed *Streptococcus pneumoniae* by ELISpot assay for pneumococcal surface protein A and phosphorylcholine 14 days post-immunization. Serum antibody titer to pneumococcal surface protein A and phosphorylchlorine were determined by ELISA, 7 and 14 days post-immunization. 3) Spleen cell phenotype (CD4+, CD8+, and B220+) was determined by flow cytometry in unimmunized and heat killed *Streptococcus pneumoniae* immunized offspring. **23-35 mg/kg bw/day:** 1a) ↑ T-cell (mixed lymphocyte) proliferation (♂), b) ↑ Cytotoxic T lymphocyte activity (♂), 2) ↑ IgM B secreting cells (♂), 3) ↑ CD8+ cells (unimmunized ♀)
i) Studies on the neurotoxic potential	
10-day oral neurotoxicity study (gavage) ♂ C57BL/6 mice Published study PMRA# 3292831 Lin et al., 2013	Supplemental The purpose of this study was to assess the potential neurotoxic effect of treatment following 10 days of dosing in 5 ♂ per dose. Open field observations and pole grip tests were performed on day 4 in all groups. NOR test and FST were conducted in all dose groups, except at the lowest dose. Spleen, thymus, liver and brain wt and BW were measured. **≥ 25 mg/kg bw/day:** dose-dependent ↓ NPI in NOR test (non-adverse) **≥ 125 mg/kg bw/day:** ↓ mean distance traveled, mean number of crossings and number of rearings in the first 5 min in open field test; approached familiar objects more than novel objects and spent more time with familiar objects in NOR test; ↑ time spent swimming and ↓ time spent immobile in FST; ↑ striatal DA, HVA and 5-HIAA; ↑ HVA, DOPAC, 5-HIAA and NE in the prefrontal cortex; trend towards ↓ TH mRNA in the substantia nigra. **250 mg/kg bw/day:** trend towards ↓ BW, trend towards ↑ rel. brain wt, ↑ MHPG in the hippocampus.

Study type/ Animal/PMRA#	Study results
	Spleen, thymus and liver wt were not affected. No statistically significant differences noted between treated and control animals for pole grip test. No significant differences due to atrazine exposure were found in the striatal protein expression of TH, DAT, VMAT-2, Drd2, or α-synuclein. Dose-dependent ↓ NPI may reflect ↑ avoidance of the novel object, indicative of ↑ anxiety. ↑ swim time in FST may also be indicative of anxiety. Study limitation: Summary data tables with means and standard deviations were not available for some measured parameters in the study article.
14-day oral neurotoxicity study (gavage) ♂ C57BL/6 mice PMRA# 2815986 / 2816781	Supplemental The purpose of this study was to assess the neurotoxic effect of treatment following 14 days of oral (gavage) dosing in 14–16 ♂ per dose. Necropsies were conducted at 15, 22, or 64 days after termination of dosing. The following parameters were assessed: BW measured daily. Striatal dopamine (DA), DOPAC (a metabolite of dopamine) and HVA (metabolite of dopamine) measured by HPLC; TH (tyrosine hydroxylase) measured by Western blot analysis; fixed brain tissue was analyzed by immuno-histochemistry / stereology. **≥ 5 mg/kg bw/day**: ↓ # TH (+) neurons in ventral tegmental area (VTA) on day 64 (non-adverse) **≥ 25 mg/kg bw/day**: ↓ # TH (+) neurons in substantia nigra pars compacta (SNpc) (days 22 and 64). **≥ 125 mg/kg bw/day**: ↓ DA, DOPAC and HVA levels (day 15), slight ↓ in DOPAC and HVA (day 22), ↓ # TH (+) neurons in VTA (day 22) **250 mg/kg bw/day**: ↓ # TH (+) neurons in VTA and SNpc (day 15). Numerous study limitations
30-day oral neurotoxicity study (gavage) ♂ SD rats Published study PMRA# 3292833 Li et al., 2019	Supplemental The purpose of this study was to assess the potential neurotoxic effects of treatment following 30 days of oral (gavage) dosing in 20 ♂ per dose starting when animals were 35 days of age. BW was measured once a week. On PND 90, 8/group were randomly chosen for behavioural tests (Morris water maze, MWM); 6/group were selected for electron microscopy of hippocampal sections, and the remaining rats were euthanized and had their hippocampus isolated and snap-frozen for subsequent experiments. MWM: **≥ 10 mg/kg bw/day**: ↓ platform crossing times (statistically significant but not dose dependent), ↓ percentage of time spent in the target quadrant and time spent in annulus (predefined area around the target) compared to controls, ↓ time spent in target annulus. No effect was noted on escape latency as ↓ was observed in all groups, indicating spatial learning/acquisition. ↓ percent time spent in the target quadrant and time spent in annulus indicate ↓ spatial memory ability. Electron microscopy: hippocampal neuron ultrastructure in dentate gyrus and cornu ammonis 1 was impaired in atrazine-treated groups. Downregulation of mRNA and protein expression levels of MEK/ERK/CREB pathway and downstream factors in hippocampal tissue

Study type/ Animal/PMRA#	Study results
	≥ 10 mg/kg bw/day: blurred and shriveled karyolemma (nuclear membrane), mitochondrial swelling, reduced cristae, and vacuolar degeneration in the dentate gyrus, evidence of degeneration of karyolemma and mitochondria and undefined synaptic clefts noted in the CA1 sub-region, ↓ CREB and BDNF mRNA expression in the hippocampus; ↓ MEK 1/2, p-MEK 1/2, p-ERK 1/2, CREB, p-CREB and Zif268 protein expression in the hippocampus **≥ 100 mg/kg bw/day**: ↑ lysosomes in the CA1 sub-region, ↓ MEK1, ERK1, ERK 2 mRNA expression in the hippocampus, ↓ BDNF protein expression in the hippocampus MWM Study limitations: - results only given in graphical format; actual numerical values not provided; - platform was submerged (2 cm) but unclear if it was visible to animals; - time intervals between trials not specified; - animals left on platform for only 10 seconds as opposed to recommended 15–20 seconds; - swim speed (to distinguish deficit in motor function versus learning), visual performance (cued learning in which cues are placed inside the tank), path length to locate hidden platform, number of passes over platform were not measured
Oral neurotoxicity study – 12 months (diet) ♂ SD rats Published study PMRA# 3292829 Bardullas et al., 2011	Supplemental The purpose of this study was to assess the potential neurotoxic effects of treatment following 12 months of dosing via diet in 10 ♂ per dose starting when animals were 21 days of age. Animals were maintained at 300 g through caloric restriction. Levels of DA and serotonin (5-HT), and their metabolites (DOPAC, 5-hydroxyindole acetic acid), were analyzed (by HPLC) in various regions of the brain, and levels of tyrosine hydroxylate (TH) were evaluated in the striatum and nucleus accumbens. Locomotor activity (collected over a 25 hr period, n = 7–9/group): evaluated monthly (months 1–6) and bimonthly thereafter. Motor coordination assessment took place following 10 months of treatment. Learning tasks including spontaneous alternation in a plus-maze, delayed alternation, and eight-arm radial maze (win-shift and non-delayed random foraging paradigm), were performed at various time points following 6–12 months of atrazine exposure. Striatum, nucleus accumbens, prefrontal cortex and hypothalamus were collected and protein levels were quantified using the Bradford technique. Protein levels from the striatal and nucleus accumbens were also analyzed using a protein assay. **10 mg/kg bw/day:** ↓ spontaneous locomotor activity following 8 months of treatment (during the first hour: horizontal and vertical activity, and stereotypic counts; 24-hr period: ↓ number and time of stereotypies), ↑ spontaneous locomotor activity (following 8 months of treatment: horizontal activity and stereotypic counts during the light cycle; 24-hr period: significant hyperactivity in a number of parameters during dark and light cycles, total distance and horizontal activity), effect in motor coordination following 10 months of treatment (latency to fall off the rod was unchanged whereas control group showed ↑ latency), ↑ number of errors during spontaneous alternation (session 3), ↑ number of re-entry errors in baited arms during non-delayed random foraging task, ↓ DA in the striatum (35%, statistically significant), ↑ DA in the hypothalamus (132%, not statistically significant) Numerous study limitations including the purity level of the test article was not provided.
Neurotoxic effects of in utero exposure on rats, 6 months or 1 year post-exposure (gavage)	Supplemental The purpose of this study was to assess the potential neurotoxic effects of treatment following in utero exposure in 5 dams per dose from GD 0-PND 1. 20 ♀ offspring per dose were assessed at 6 months and 12 months of age following necropsy. mRNA and

Study type/ Animal/PMRA#	Study results
♀ SD rats PMRA# 2945592 Li et al., 2014a	protein expression of the following were analyzed in the midbrain (substantia nigra) by RT-PCR and Western blot: Nurr1, TH, VMAT2, DAT, MAO, and COMT. Levels of the following were analyzed in the striatum using HPLC with a fluorescence detector: L-DA, DA, HVA, DOPAC, mesDA **≥ 25 mg/kg bw/day**: ↓ DA at 1 year, ↓ L-DA, DOPAC and HVA at 6 months and 1 year, ↓ Nurr1 mRNA at 6 months and 1 year, ↓ TH mRNA at 6 months and 1 year, ↓ VMAT2 mRNA at 6 months, ↓ DAT mRNA at 6 months, ↑ COMT mRNA at 6 months and 1 year (not dose-dependent at 6 months), ↓ Nurr1 protein at 6 months (dose-dependent), ↓ TH protein at 6 months, ↓ VMAT and DAT protein at 6 months. **50 mg/kg bw/day**: ↓ [DA] at 6 months, ↓ VMAT2 and DAT mRNA at 1 year, ↑ MAO mRNA at 6 months and 1 year, ↓ VMAT and DAT protein at 1 year No effects on BW or FC (data not provided). Numerous study limitations including: Positive-control results not shown or discussed. All data were presented as bar graphs. No rationale as to why parameters were measured in ♀ offspring only.
Neurotoxic effects of in utero and lactational exposure on SD rats, 1-year post-exposure (gavage) SD rats PMRA# 2945591	Supplemental The purpose of this study was to assess the potential neurotoxic effects of treatment following in utero and lactational exposure in 5 dams per dose from GD 5-PND 22. 20 offspring animals were assessed at 12 months by examining the following parameters from their brains: DA content examined by HPLC-FL. mRNA expression of TH, Nurr1, DAT and VMAT2 in the ventral midbrain were examined by fluorescence PCR. TH, DAT, VMAT2 and Nurr1 levels in the ventral midbrain were assessed by Western Blot analysis. **≥ 25 mg/kg bw/day**: ↓ DA in the striatum and Nurr1 mRNA expression in the ventral midbrain, ↓ Nurr1 and VMAT2 protein expression in the midbrain (♂/♀) **50 mg/kg bw/day**: ↓ VMAT2 mRNA expression in the ventral midbrain, ↑ DAT protein expression in the midbrain Numerous study limitations including: no positive-control included in the study. All data were presented as bar graphs.
Neurotoxic effects of exposure during pubertal development of SD rats, 1-year post-exposure (gavage) PMRA# 2945589 Li et al., 2014b	Supplemental The purpose of this study was to assess the potential neurotoxic effects of treatment following exposure in 6–10 animals per sex and per dose from PND 22 – PND 62. Then, the animals were maintained in control diet and the following parameters were assessed at the 12-month necropsy: DA of striatum examined by HPLC-FL. mRNA and protein expression of TH, Nurr1, DAT and VMAT2 were examined in samples of ventral midbrain via fluorescence PCR and Western blot analysis. **≥ 25 mg/kg bw/day**: ↓ DA in the striatum (not dose-dependent), ↓ Nurrl and TH mRNA expression (not statistically significant at LD) (♂); ↓ Nurrl, TH, VMAT2 and DAT mRNA and protein expression in the ventral midbrain (not dose-dependent for DAT protein expression) (♀). **50 mg/kg bw/day**: ↓ VMAT2 and DAT mRNA expression in the ventral midbrain, ↓ Nurrl, TH, VMAT2 and DAT protein expression (♂); ↓ [DA] in the striatum (♀). No effects on BW and FC (data not shown)

Study type/ Animal/PMRA#	Study results
	Numerous study limitations, including: positive-control group results were not included in the study article. All data were presented as bar graphs.
j) Studies on estrogenic/anti-estrogenic potential, aromatase activity, or gene expression	
In vitro aromatase induction PMRA# 2816755 2816004	Supplemental The purpose of this study was to assess the potential effect of treatment (atrazine, DACT, DEA, DIA, ammeline and hydroxyatrazine) on the aromatase of steroidogenic cell lines (H295R) In H295R, 10 µM of atrazine induced an ↑ in aromatase by 2 hr of treatment and persisted through 72 hr. 10 µM of DEA induced aromatase by 2 hr and its effect persisted up to 24 hr. 10 µM of DIA induced aromatase by 4 hours and its effect persisted up to 48 hr 10 µM of hydroxyatrazine induced aromatase by 72 hr (last measured time-point) Study limitations: Data were only reported in figures. Summaries of means, standard deviations, and individual concentration data were not provided.
Assays of direct estrogenic activity Published & unpublished studies PMRA# 1167674, 1167675, 1167676, 2815963, 2816709, 2816710, 1993-1995	The purpose of these assays were to assess direct estrogenic effect of treatment. The study reports described more than one assay. The doses varied depending on the assay. The study reports of all these experiments lacked sufficient details, such as, the summary data tables with mean and standard deviations, raw data, statistical analysis, detailed information regarding the study design and materials and methods. However, key information as summarized below was noted from these studies and was confirmed via credible international scientific review documents. PMRA# 1167674: A trio of assays was performed: uterotrophic response assay; progesterone receptor competitive binding assay; and a uterine thymidine incorporation assay. PMRA# 1167675: This study describes a series of estrogen receptor competitive binding assays both in vitro and in vivo. Overall, the results indicate that atrazine (DACT and simazine were also tested) exhibit some competitive binding with estradiol but only under conditions which favour binding at extremely high concentrations) PMRA# 1167676: This study described four separate assays: competitive binding assay with hepatocyte Ah receptor; MCF-7 cell proliferation; gel electrophoresis mobility shift assay using the progesterone receptor; and luciferase reporter gene assay in MCF-7 cells. Neither atrazine nor simazine displayed estrogenic activity or interacted with the Ah receptor in the set of experiments described in this paper. Numerous other in vitro published studies indicate that atrazine tested negative or weakly positive at very high concentrations in estrogen receptor (ER) binding and ER transactivation assays. Mixed results were obtained for the aromatase studies. In the in vivo studies, atrazine was negative for estrogenic activity in uterotrophic assays.

Study type/ Animal/PMRA#	Study results
	Although the available data indicate varying responses in estrogen-related endpoints across species and studies, the potential for atrazine to result in effects on estrogen-dependent tissues is supported by their overall well-known MOA that they function through a neuroendocrine MOA which suppresses the hypothalamic release of GnRH and therefore LH, which will then result in downstream effects on estrogen and androgen signaling pathways. Overall, the atrazine database does not support a potential for direct estrogenic activity.

Table 3 Summary of toxicology studies for chlorotriazine metabolites of Atrazine

Effects observed in both sexes are presented first followed by sex-specific effects in males, then females, each separated by semi-colons. Organ weight effects reflect both absolute organ weights and relative organ to bodyweights unless otherwise noted.

Study type/ Animal/PMRA#	Study results
Diaminochlorotriazine (2,4-diamino-6-chloro-s-triazine) [a terminal rat metabolite] **(DACT) (G-28273)**	
Acute toxicity studies	
Acute oral toxicity (gavage) SD rat	LD_{50} > 5050 mg/kg bw (♂) LD_{50} > 5550 mg/kg bw (♀) Deaths were observed up to 12 days post dosing. Clinical signs of toxicity included: piloerection, reduced activity and salivation, up to days 15 post-dosing **Low toxicity**
Short-term toxicity studies	
14-day oral toxicity (gavage) SD rats PMRA# 1234780 Non-guideline	**≥ 100 mg/kg bw/day:** hunched appearance, rough coat, no/little stool, ↓ BW, ↓ thymus wt, ↑ rel. spleen wt, ↓ LH **≥ 200 mg/kg bw/day:** one animal died, ↓ estrogen, ↓ progesterone, ↓ prolactin **300/400 mg/kg bw/day:** 7 animals died after dose reduced to 300 mg/kg bw/day, ↓ abs. spleen wt Note the 400 mg/kg bw/day dose was lowered to 300 mg/kg bw/day after deaths were observed within 2–4 days of dosing
90-day oral toxicity (diet) SD rats PMRA# 1123345, 1150097, 1150098, 1150099	NOAEL = 17/0.7 mg/kg bw/day (♂/♀) **≥ 7.6 mg/kg bw/day:** prolonged estrus cycle, ↑ incidence of rat with persistent estrus and/or diestrus (♀) **≥ 20 mg/kg bw/day:** ↓ BW, ↓ BWG (♀) **34/40 mg/kg bw/day:** ↓ BW, ↓ BWG (♂)
13- or 52-week oral toxicity (diet) Beagle dogs	NOAEL = 3.6/3.4 mg/kg bw/day (♂/♀) **24/33 mg/kg bw/day:** ↑ incidence of cardiac toxicity/moribundity, ↑ pathological cardiac findings (heart enlargement, softness, thickened vulva lesions, distension, thrombosis, myocarditis, necrosis, inflammation, hemorrhage, hemosiderosis), ↑ pathological effects in

Study type/ Animal/PMRA#	Study results
PMRA# 2815961, 2816711	the liver (enlargement, congestion, centrilobular fibrosis/atrophy, bile stasis, necrosis, hemosiderosis, adhesion, mottling and high texture), ↑ fluid in pericardial, thoracic and abdominal cavities, ↓ BWG, ↑ spleen wt, ↑ liver wt, ↑ kidney wt, ↑ anaemia with reticulocytosis, ↓ albumin, calcium and total cholesterol levels, ↑ platelet levels (♂/♀); ↑ testicular effects (hypospermatogenesis, hypospermia), ↑ thymus atrophy, ↑ bone marrow hyperplasia (♂) The high dose of 1500 ppm dose was decreased to 750 ppm from week 7 to the end of study period due to severe toxicity
Developmental/Reproductive toxicity studies	
Developmental toxicity (gavage) SD rats PMRA# 1233376, 1234570	**Maternal toxicity** NOAEL = 25 mg/kg bw/day ≥ **25 mg/kg bw/day:** ↓ BWG (during GD 6-8 only) (non-adverse) ≥ **75 mg/kg bw/day:** ↓ BW, BW loss during first few days of dosing, ↓ FC, ↓ BWG **150 mg/kg bw/day:** ↑ resorptions, ↑ post-implantation loss **Developmental toxicity** NOAEL = 2.5 mg/kg bw/day ≥ **25 mg/kg bw/day:** ↑ incomplete ossification of several sites in the skull (hyoid, interparietal, occipitals, parietals, teeth) ≥ **75 mg/kg bw/day:** ↓ fetal BW, ↑ incomplete ossification of several sites in hindpaw and forepaw (distal phalanges, metacarpal and metatarsus), ↑ rudimentary 14th ribs, ↑ wavy rib, ↑ incomplete ossification of sternebrae, ↑ incomplete ossification of sites in the skull (nasal, presphenoid) **150 mg/kg bw/day:** ↑ resorptions, ↑ post-implantation loss, ↑ of renal papilla absent, ↑ incidence of pitted kidneys, ↑ incomplete ossification of sites in the skull (frontal, basisphenoid) **Evidence of sensitivity of the young. No evidence of treatment-related malformations**
Genotoxicity studies	
Bacterial Reverse Mutation Assay S. typhimurium (TA98, TA100, TA1535, TA1537) Unpublished study PMRA# 1234577	**Negative ± metabolic activation** Tested up to a limit concentration Test substance precipitated at 5000 µg/plate
Bacterial Reverse Mutation Assay S. typhimurium (TA98, TA100, TA1535, TA1537)	**Negative ± metabolic activation** Tested up to a limit concentration Test substance precipitated at 5000 µg/plate

Study type/ Animal/PMRA#	Study results
Unpublished study PMRA# 1234577	
Bacterial Reverse Mutation Assay S. typhimurium (TA97, TA98, TA100, Published study Part of PMRA# 1234590 Butler et al., 1989	**Negative in the absence of metabolic activation** This was a non-guideline study. It is not known what guideline was followed. The study authors did not use metabolic activation due to previous studies showing negative results using this system. They also suggested that since mammalian metabolism produces metabolites identical to those produced in plants that there is no major biohazard, and using the S9 system was unnecessary. In addition to no S9 activation, the following items were not mentioned: phase of growth (late exponential, or early stationary phase) and whether there was testing up to a precipitating or cytotoxic concentration. As a result, this study is considered supplemental. TA1535, TA1537, TA1538 were also negative (data were not provided in the study report)
Unscheduled DNA synthesis CRL 1521 Human Fibroblasts Unpublished study PMRA# 1234576	**Negative** Tested up to a limit concentration. Test substance precipitated between 400-1000 µg/mL in cytotoxicity test.
Unscheduled DNA synthesis ♂ rat primary hepatocytes Unpublished study PMRA# 1234586	**Negative** Tested up to a limit concentration.
In vivo mammalian cytogenetics (Micronucleus Assay) NMRI-derived mice Unpublished study PMRA# 1234585	**Negative** There was an increase in the number of polychromatic erythrocytes observed at 48 hr in the first mutagenicity assay at 5000 mg/kg bw, and the authors attributed this to an increased value in two ♀ animals. To determine whether this was due to chance, the authors repeated the experiment, using additional doses (1250, and 2500 mg/kg bw/day). There was no observed increase in the number of PCEs at any dose or at any time point. The reviewer agrees that the observed increase in PCE in the first mutagenicity test in two ♀s is likely secondary to overt toxicity since these effects were not repeated in a second test with additional dosing.
Special studies	
Pubertal Assay (gavage) ♂ Wistar rat Published USEPA	NOAEL = 4.4 mg/kg bw/day (atrazine equimolar dose of 6.25 mg/kg bw/day) (♂) The purpose of this study was to assess the effect of treatment on onsets of puberty in 8–13 ♂ per dose (38 ♂ in control group). The animals received treatment from PND 23-53. ≥ **8.4 mg/kg bw/day:** delay in PPS

Study type/ Animal/PMRA#	Study results
NHEERL ORD study PMRA# 2945585 Stoker et al., 2002	≥ **84 mg/kg bw/day:** ↓ BW at PND 53, ↓ epididymis wt, ↓seminal vesicle wt **135 mg/kg bw/day:** ↓ ventral prostate wt, ↓ seminal vesicle wt, ↓ serum estrone
Pubertal Assay (gavage) ♀ Wistar Rats Published USEPA NHEERL ORD study PMRA# 2945574 Laws et al., 2003	NOAEL = 16.7 mg/kg bw/day (atrazine equimolar dose of 25 mg/kg bw/day) (♀) The purpose of this study was to assess the effect of treatment on onsets of puberty in 15 ♀ per dose. The animals received treatment from PND 22-41. BW was recorded daily, VO was monitored daily and the age at complete VO was recorded. Beginning on the day of VO, daily vaginal smears were collected to monitor the estrous cycle until necropsy. Histological evaluation of thyroid, uterus, and ovaries was conducted. T3, T4 and TSH serum levels were also determined. **≥ 33.8 mg/kg bw/day:** delayed VO, ↑ BW at VO, ↓ abs. pituitary wt **135 mg/kg bw/day:** ↓ BW, ↑ animals did not attain VO and were not cycling prior to necropsy (an absence of corpora lutea was noted in these animals), ↓ abs. kidneys wt, ↓ abs. adrenals wt, ↓ abs. ovaries wt, ↓ abs. uterus wt For those animals, where VO failed to occur prior to necropsy, the age of VO was recorded as the day after necropsy to determine a mean for each group. No treatment-related effect was seen for serum T3, T4 or TSH levels. No other treatment-related histopathological change was observed in the uterine, ovarian, or thyroid tissues. The study author also concluded that the LOAEL for the delay in VO for DACT was equimolar to that reported for atrazine.
29/52-week short- and long-term toxicity study (diet) SD rats PMRA# 1078581	NOAEL = 3.4 mg/kg bw/day (♀) The purpose of the study was to assess the effect of treatment following 29 weeks of dosing on the estrous cycle and LH surge, and following 52 week of dosing on the organ/systems associated with the estrous cycle in 16–50 ♀ per dose per time point. The animals designated for plasma LH analysis were ovariectomized on week 30/31 and were given estradiol via subcutaneous implants. Following 3 days of estradiol intake, serial blood samples were collected for hormone analysis. **≥ 20 mg/kg bw/day:** ↓ BWG, ↓ BW, ↓ LH surge, ↑ mammary tumours (fibroadenoma [6% (2/36) vs 2% (2/82) in control; carcinomas [6/36 (17%) vs 4/82 (5%) in control]; combined [23% vs 7% in control]) Estrous cyclicity data were not summarized in tables of means and std. deviations (only individual animal data were provided).
Study Type/ Animal/ PMRA #	**Study results**
Desisopropylazine (2-amino-4-chloro-6-ethylamino-s-triazine) [an intermediate rat metabolite] **(DIA (G-28279)**	
Acute toxicity studies	
Acute oral toxicity SD rats	LD_{50} = 2290 mg/kg bw (♂) LD_{50} = 810 mg/kg bw (♀) Clinical signs of toxicity: piloerection, reduced activity and salivation. **Moderate toxicity**
Short-term toxicity studies	

Study type/ Animal/PMRA#	Study results
90-day oral toxicity (diet) Tif: RAIf rats PMRA# 2945549, 2945550	NOAEL = 0.6/3.3 mg/kg bw/day (♂/♀) **≥ 3.2 mg/kg bw/day:** ↓ BW, ↓ BWG, ↑ changes in pars distalis of the pituitary gland (♂) **35/38 mg/kg bw/day:** ↑ rel. kidney wt, ↓ abs. heart wt, ↑ rel. testes wt, ↑ rel. kidney wt, ↑ fatty changes in adrenal cortex, ↑ hypertrophy of thyroid follicular epithelium (♂); ↓ BW, ↑ rel. liver wt, ↑ extramedullary hematopoiesis of spleen (♀)
14-week oral toxicity (diet) Beagle dogs PMRA# 2815961, 2816711	NOAEL = 3.8 mg/kg bw/day (♂/♀) **≥ 18 mg/kg bw/day:** ↓ BW, ↓ BWG, ↓ FC, ↓ RBC parameters (♂/♀); ↓ heart wt, ↓ prostate wt, ↓ testes wt (♂)
Developmental/Reproductive toxicity studies	
Developmental toxicity (gavage) Tif: RAIf rats PMRA# 2945552, 2945553	**Maternal toxicity** NOAEL = 25 mg/kg bw/day **25 mg/kg bw/day:** ↓ BWG and ↓ FC (during the first few days of dosing only) (non-adverse) **100 mg/kg bw/day:** BW loss (~ 7g following the first day of dosing), ↓ BW (at the end of gestation), ↓ BWG and ↓ FC **Developmental toxicity** NOAEL = 5 mg/kg bw/day **≥ 25 mg/kg bw/day:** ↑ incidence of fused sternebrae 1 and 2 **100 mg/kg bw/day:** ↑ absent/incomplete ossification of proximal phalanx of posterior digit 2, 3, 4 and 5, and metatarsal 1 **Evidence of sensitivity of the young. No evidence of treatment-related malformations.**
Genotoxicity studies	
Bacterial Reverse Mutation Assay S. typhimurium (TA98, TA100, TA1535, TA1537), E. coli (WP2uvrA) Unpublished study PMRA# 1234588	**Negative ± metabolic activation** Tested up to a limit concentration
Bacterial Reverse Mutation Assay S. typhimurium (TA97, TA98, TA100)	Supplemental Negative based on information available in the study report Toxicity data was not provided. Study limitations: lack of sufficient detail in study report

Study type/ Animal/PMRA#	Study results
Published study Part of PMRA# 1234590 Butler et al., 1989 Non-guideline	
Unscheduled DNA synthesis ♀ rats primary hepatocytes Unpublished study PMRA# 2815961, 2816711	Supplemental Negative based on the JMPR, WHO reports
Special studies	
Pubertal Assay (gavage) ♂ Wistar rats Published USEPA NHEERL ORD study PMRA# 2945585 Stoker et al., 2002	NOAEL = 10.4 mg/kg bw/day (atrazine equimolar dose of 12.5 mg/kg bw/day) (♂) The purpose of this study was to assess the effect of treatment on the onset of puberty in 8–13 ♂ per dose (38 ♂ in control group). The animals received treatment from PND 23-53. **≥ 21 mg/kg bw/day:** delay in PPS **≥ 40 mg/kg bw/day:** ↓ ventral prostate wt **≥ 80 mg/kg bw/day:** ↓ BW at PND 53, ↓ seminal vesicle wt, ↓ serum testosterone **161 mg/kg bw/day:** ↓ epididymis, ↓ lateral prostate wt
Study Type/ Animal/ PMRA #	**Study results**
Deethylatrazine (2-amino-4-chloro-6-isopropylamino-s-triazine) [an intermediate rat metabolite] **(DEA) (G-30033)**	
Acute toxicity studies	
Acute oral toxicity (gavage) SD rats	LD_{50} = 1890 mg/kg bw (♂) LD_{50} = 600 mg/kg (♀) Clinical signs of toxicity: piloerection, reduced activity and salivation. Survivors recovered by day 8 post-dosing **Moderate toxicity**
Short-term toxicity studies	
90-day oral toxicity (diet) Tif:RAIf rats PMRA# 2815961, 2816711	NOAEL = 3.2/3.35 mg/kg bw/day (♂/♀) **≥ 35/39 mg/kg bw/day:** ↓ FC, BWG, ↓ BW (♂/♀)
90-day oral toxicity (diet)	NOAEL = 3.7 mg/kg bw/day (♂/♀) **≥ 29/32 mg/kg bw/day:** ↓ BW, ↓ FC, ↑ renal tubular hyperplasia/basophilia, ↓ RBC (♂/♀),

Study type/ Animal/PMRA#	Study results
Beagle dogs PMRA# 2815961, 2816711	↓ heart wt, ↑ paroxysmal atrial fibrillation (♂), ↑ right atrial wall hemorrhagic inflammation with angiomatous hyperplasia, ↓ uterus wt, ↓ thymus wt (♀)
Developmental/Reproductive toxicity studies	
Developmental toxicity (gavage) Albino rats PMRA# 2945554, 2945555	**Maternal toxicity** NOAEL = 25 mg/kg bw/day **100 mg/kg bw/day:** ↓ BWG (at the end of gestation period), ↓ FC, ↓ BW loss (~ 7 g following the first day of dosing), ↓ BW (at the end of treatment period), ↓ FC, ↑ post-implantation loss **Developmental toxicity** NOAEL = 25 mg/kg bw/day **100 mg/kg bw/day:** ↑ incidence of fused sternebrae 1 and 2, ↑ incidence of asymmetrically shaped/bipartite ossification sternebrae 5, ↑ incidence of bipartite ossification sternebrae 5, ↑ incomplete ossification of proximal phalanges, ↑ post-implantation loss **No evidence of sensitivity of the young or treatment-related malformations**
Genotoxicity studies	
Bacterial Reverse Mutation Assay S. typhimurium (TA98, TA100, TA1535, TA1537), E. coli (WP2uvrA) Unpublished study PMRA# 1234589	**Negative ± metabolic activation** Tested up to a limit concentration
Bacterial Reverse Mutation Assay S. typhimurium (TA97, TA98, TA100) Published study Part of PMRA# 1234590 Butler et al., 1989	Supplemental Negative based on the level of information provided. Study limitations: lack of sufficient detail in study report
Unscheduled DNA synthesis Rat primary hepatocytes Unpublished study PMRA# 2815961,	Supplemental The following study is not available in the PMRA files. Negative based on JMPR, WHO reports

Study type/ Animal/PMRA#	Study results
2816711 Non-guideline	
Bone marrow micronucleus test (in vivo) Tif:MAGf mice PMRA# 2815961, 2816711	Supplemental This study is not available in the PMRA files. Negative based on USEPA, JMPR and WHO reports
Unscheduled DNA synthesis Tif:RAIF rat primary hepatocytes PMRA# 2815961, 2816711	Supplemental This study is not available in the PMRA files. Negative based on USEPA, JMPR and WHO reports
Special studies	
Pubertal Assay ♂ Wistar rats Published USEPA NHEERL ORD study PMRA# 2945585 Stoker et al., 2002	NOAEL = 10.8 mg/kg bw/day (atrazine equimolar dose of 12.5 mg/kg bw/day) (♂) The purpose of this study was to assess the effect of treatment on onsets of puberty in 8-13 ♂ per dose (38 ♂ in control group). The animals received treatment from PND 23-53. **≥ 22 mg/kg bw/day:** delay in PPS, ↓ seminal vesicle wt **≥ 87 mg/kg bw/day:** ↓ BW at PND 53, ↓ seminal vesicle wt, ↓ serum testosterone **≥ 174 mg/kg bw/day:** ↓ epididymis and ↓ lateral prostate wt

Table 4 Summary of toxicology studies for hydroxyatrazine

Effects observed in both sexes are presented first followed by sex-specific effects in males, then females, each separated by semi-colons. Organ weight effects reflect both absolute organ weights and relative organ to bodyweights unless otherwise noted.

Study type/Animal/PMRA#	Study results
Acute toxicity studies	
Acute oral toxicity (gavage) SD rats	LD_{50} > 5050 mg/kg bw (♂/♀) **Low toxicity**
Short-term toxicity studies	
90-day oral toxicity (diet) SD rats	NOAEL = 6.3/7.4 mg/kg bw/day (♂/♀) **≥19/23 mg/kg bw/day**: ↑ pitted or rough kidney, ↑ renal tubular dilatation and basophilia, ↑ interstitial inflammation of kidney (♂/♀); ↑ urine volume (♂); ↑ chloride

Study type/Animal/PMRA#	Study results
PMRA # 1234775	(♀) **37/46 mg/kg bw/day**: ↓ BW, ↓ BWG, ↑water consumption, ↓ RBC, ↓ HCT, ↓ HGB, ↑ leukocytes, ↑ neutrophils, ↑ BUN, ↑ creatinine, ↑ electrolytes (Cl, Na), ↑ urine volume, ↑ kidney wt, ↑ cellular casts and anisotropic crystals in renal papillary tubules (♂/♀); ↓ FC (♂); ↑ platelets, ↑ potassium, ↓ urine specific gravity (♀) LDL, HDL not assessed. Serum thyroid hormone measurements not taken. Detailed clinical observations were limited and conducted at pre-dose and termination only; motor activity/grip strength not assessed. Reticulocytes reported only for high dose ♀s
90-day oral toxicity (diet) Beagle dogs PMRA # 1234776	NOAEL = 5.8/6.2 mg/kg bw/day (♂/♀) **6.2 mg/kg bw day**: ↓ FC day 0 (♀) (non-adverse) **≥ 60/64 mg/kg bw/day**: ↓ BW, ↓ overall BWG, ↓ FC days 7 and 14, ↑ pitted or rough kidneys, ↑ chronic nephropathy (consisting of renal tubal dilatation, tubular atrophy, tubular basophilia and chronic interstitial fibrosis), ↑ intratubular crystalline casts, ↓ urine specific gravity, ↑ dilute urine excretion (♂/♀); ↓ FC day 0 (♂); ↓ RBC, ↓ HGB, ↓ HCT (♀) **248/222 mg/kg bw/day**: ↓ FC days 21–70 (♂/♀); ↓ HGB (♂); emaciation/few feces, BW loss (day 7), ↑ BUN, ↑ creatinine (♀)
Chronic toxicity/Oncogenicity studies	
24-Month Chronic Toxicity/Oncogenicity (diet) SD rats PMRA# 2945551	NOAEL = 0.96/1.2 mg/kg bw/day (♂/♀) **1.2 mg/kg bw/day**: ↑ accumulated interstitial matrix in renal papillae without alteration in kidney function (non-adverse at this dose) (♀) **≥ 7.6/9.5 mg/kg bw/day**: ↑ water consumption (week 28), ↑ renal tubules devoid of epithelium, malformed/misshapen kidneys, ↑ papillary interstitial fibrosis of the kidney, ↑ kidney dilatation with crystal deposits (♂/♀); ↑ rough pitted surface of the kidney, ↑ acute inflammation of the kidney, ↑ incidence and severity of progressive nephropathy (♀) **17/22 mg/kg bw/day**: ↑ mortality, ↑ emaciation, ↑ pallor, ↑ tremors, ↓ BW, ↓ BWG, ↓ FC, ↑ water consumption (weeks 7–52), ↓ RBC, ↓ HGB, ↓ HCT, ↓ MCHC, ↑ leukocytes, ↑ platelets, ↑ abs. segmented neutrophils, ↑ BUN, ↑creatinine, ↑ calcium, ↑ phosphorus, ↓ glucose, ↓ total protein, ↓ albumin, ↑ urine volume, ↓ specific gravity, ↓ urine colour intensity, ↓ urinary pH, ↓ urine osmolality, crystalline urinary sediments, ↑ kidney wt, ↑ kidney discoloration, ↑ calculi and cysts in the kidney, ↑ enlarged and discoloured renal lymph nodes, ↑ enlarged vessel in the cardiovascular system, ↑ enlarged parathyroid gland, ↑ kidney transitional cell erosion and hyperplasia, ↑ dilatation and crystal deposits in urinary system (ureters, bladder, or prostatic ureter) accompanied by inflammatory infiltrate, ↑ pigmented macrophage accumulation of the renal lymph nodes, ↑ congestion and sinusoidal ectasia of the renal lymph nodes, ↑ mineralization of several tissues, ↑ fibrous osteodystrophy, ↑ polyarteritis nodusa, ↑ arterial fibromuscular proliferation, parathyroid hyperplasia, (♂/♀); piloerection, stained and wet coat at abdominal areas, increased anuria, dehydration, diarrhea, ↓ activity, ↑ MCV, ↓ creatinine kinase activity, ↓ globulin, calculi and thickened wall of the bladder, ↑ rough pitted surface of the kidneys, ↑ enlarged kidneys, ↑ acute inflammation in the kidneys, ↑ severity of progressive nephropathy, ↑ testes flaccid, ↑ testicular degeneration and atrophy, ↑ oligospermia, ↑ spermatidic giant cells in the epididymides (♂); ↓ MCH, ↓ A/G ratio, ↑ potassium, ↑ cholesterol, ↑ protein and occult blood in urine, ↑ erythrocytes in urine, ↑ small kidneys, ↑ incidence and severity of progressive

Study type/Animal/PMRA#	Study results
	cardiomyopathy (♀) **No evidence of carcinogenicity** Heart, spleen, thyroid, epididymal, and uterine weights were not taken. Due to high mortality in the high dose group, surviving animals were sacrificed at 18 months. Microscopic examinations took place for controls and the top two doses.
Developmental toxicity studies	
Developmental toxicity (gavage) SD rats PMRA# 1233375	**Maternal** NOAEL = 25 mg/kg bw/day **125 mg/kg bw/day**: ↓ BWG (GD8-12), ↓ FC (GD 8-12), ↑ enlarged mottled kidneys **Developmental** NOAEL = 25 mg/kg bw/day **125 mg/kg bw/day**: ↓ fetal wt, ↑ incomplete ossification of hyoid bone, ↑ incomplete ossification of interparietal bone, ↑ non-ossified forepaw metacarpals **No evidence of sensitivity of the young or treatment-related malformations** Contents of thoracic and abdominal cavities were examined, including a slice through the kidney. Uteri and their contents were weighed. No other histopathological assessment or organ weights were taken for any maternal tissue including thyroid glands; thyroid hormones were likewise not assessed.
Genotoxicity studies	
Bacterial Reverse Mutation Assay S. typhimurium (TA98, TA100, TA1535, TA1537) PMRA# 1234583	Negative ± metabolic activation Tested up to a limit concentration Only four bacterial strains assessed (no assessment of AT base pair reversions)
Bacterial Reverse Mutation Assay S. typhimurium (TA98, TA100, TA1535, TA1537) PMRA# 2815961	Negative ± metabolic activation The number of concentrations assessed was not available
Unscheduled DNA synthesis in vitro Primary rat hepatocytes PMRA# 1234580	Negative in absence of metabolic activation Tested up to a precipitating concentration

Study type/Animal/PMRA#	Study results
In vivo micronucleus assay NMRI mouse PMRA# 1234584	Negative No mortalities occurred; clinical signs of toxicity not assessed
Special Studies	
Pubertal Assay (gavage) Wistar rats Published USEPA NHEERL ORD study PMRA# 2945574 Laws et al., 2003	NOAEL = 183 mg/kg bw/day The purpose of this study was to assess the effect of treatment on the onset of puberty in 15 ♀ per dose. The animals received treatment from PND 22-41. BW was recorded daily, VO was monitored daily and the age at complete VO was recorded. Beginning on the day of VO, daily vaginal smears were collected to monitor the estrous cycle until necropsy. Liver, kidney, adrenals, ovaries, uterus, and pituitary were weighed. Histological evaluation of thyroid, uterus, and ovaries was conducted. Serum was frozen at -80°C following euthanasia for T3, T4 and TSH analysis. All doses were selected as the molar equivalent of atrazine to facilitate the comparison of the potency of the test chemical with that of atrazine. No treatment-related effects were seen in any animal with respect to organ weights, histopathology or serum T3, T4 or TSH levels. **183 mg/kg bw/day:** slight delay in VO (1.3 days in one of two experiments only) (non-adverse) Note: In a pilot study, VO was statistically significantly delayed by 2.2 days
♂ and ♀ Pubertal Protocols (gavage) Wistar rats Published USEPA NHEERL ORD study PMRA# 3292827 Stoker et al., 2013	NOAEL = Not determined LOAEL = 11.4 mg/kg bw/day (♂) LOAEL = 45.75 mg/kg bw/day (♀) The purpose of this study was to assess the effect of treatment on the onset of puberty in 10 ♂ and 12 ♀ per dose. The animals received treatment from PND 22-41/42 (♀), PND 23-53 (♂). BW was recorded daily, VO and PPS were monitored daily and the age at completion was recorded, daily vaginal smears were collected to monitor the estrous cycle until necropsy. Liver, kidney, adrenal, pituitary, testis, epididymis, prostate, seminal vesicles, ovary, and uterus were weighed. Histological evaluation of testes, epididymis, thyroids (♂ only), and kidney. Following euthanasia, blood was allowed to clot then stored at -80°C for analysis of T3, T4 and TSH (♂/♀); testosterone, LH, and prolactin (♂ only) serum levels were also determined. ≥**11.4 mg/kg bw/day**: ↑ hydronephrosis, ↑ renal tubule dilatation, ↑ ascending pyelonephritis (♂) ≥**22.8 mg/kg bw/day**: ↑ kidney wt (♂) ≥**45.75 mg/kg bw/day**: ↑ renal tubule dilatation, ↑ ascending pyelonephrosis, ↑ renal tubule concretions (mineralized material) with associated inflammation (♀) **≥91.5 mg/kg bw/day**: ↑ pale kidney (data not shown) (♂/♀); ↑ renal tubule concretions (mineralized material) with associated inflammation, ↑ renal pelvic hyperplasia (♂); ↑ kidney wt (♀) **183.4 mg/kg bw/day**: ↓ BW (♂); hydronephrosis (♀)

Study type/Animal/PMRA#	Study results
	No treatment-related effect was seen on the onset of puberty, namely VO (♀) and PPS (♂). There were no differences in estrous cyclicity between treated and control groups (data not provided in the study report). There were no changes in the mean serum concentration of T4, T3, or TSH in ♂ or ♀, or of mean serum testosterone, LH, or prolactin levels in ♂.

Table 5 Toxicology reference values for use in health risk assessment for atrazine and chlorotriazine metabolites/Transformation products

Exposure scenario	Study	Point of departure and endpoint	CAF or target MOE[1]
Acute dietary (all populations)	4-day oral (gavage) toxicity study in Long-Evans rats	NOAEL = 1.6 mg/kg bw/day Attenuation of the LH surge	100 PCPA factor = onefold
	ARfD (all populations) = 0.02 mg/kg bw		
Repeated Dietary (all populations)	4-day oral (gavage) toxicity study in Long-Evans rats	NOAEL = 1.6 mg/kg bw/day Attenuation of the LH surge	300 UF_{DB} = threefold PCPA factor = onefold
	ADI = 0.005 mg/kg bw/day		
Short- and intermediate-term dermal[2] and inhalation[3]	4-day oral (gavage) toxicity study in Long-Evans rats	NOAEL = 1.6 mg/kg bw/day Attenuation of the LH surge	300 UF_{DB} = threefold
Cancer	Mammary gland tumours in female SD rats are not considered relevant to human health risk assessment		

UF_{DB} = Database uncertainty factor

[1]CAF (composite assessment factor) refers to a total of uncertainty and PCPA factors for dietary assessments; MOE refers to a target MOE for occupational assessments.

[2] Since an oral NOAEL was selected, a dermal absorption factor of 6% (PACR2003-13) was used in a route-to-route extrapolation.

[3] Since an oral NOAEL was selected, an inhalation absorption factor of 100% (default value) was used in route-to-route extrapolation.

Table 6 Toxicology reference values for hydroxylated metabolites/Transformation products of atrazine

Exposure scenario	Study	Point of departure and endpoint	CAF[1]
Acute dietary (all populations)	There was no toxicology endpoint attributable to a single exposure for the general population		
Repeated dietary (all populations)	2-year carcinogenicity study in rats	NOAEL = 1.0 mg/kg bw/day Kidney effects (increased incidence of crystal formation and a subsequent inflammatory response)	100 PCPA factor = onefold
	ADI = 0.01 mg/kg bw/day		
Cancer	No evidence of oncogenicity relevant to humans in available data		

[1]CAF (composite assessment factor) refers to a total of uncertainty and PCPA factors for dietary assessments

Appendix V Dietary exposure and risk estimates

Table 1 Acute and chronic dietary exposure and risk analyses for atrazine and its chlorotriazine metabolites/Transformation products

Subpopulation	Acute (%ARfD)[1]			Chronic (%ADI)[2]		
	Food alone	Drinking water alone[3]	Food and drinking water[3]	Food alone	Drinking water alone[3]	Food and drinking water[3]
General Population	12.8	4.1	15.1	18.5	6.2	24.7
All Infants (<1 year old)	20.3	14.0	29.1	27.1	23.2	50.3
Children 1–2 years old	28.7	5.9	31.2	63.0	8.5	71.5
Children 3–5 years old	21.3	4.7	23.3	48.0	7.0	54.9
Children 6–12 years old	15.5	3.7	17.2	31.5	5.2	36.7
Youth 13–19 years old	9.7	3.4	11.2	18.5	4.4	22.9
Adults 20–49 years old	7.6	4.0	9.8	14.3	6.2	20.5
Adults 50+ years old	6.1	3.5	8.4	11.8	6.0	17.8
Females 13–49 years old	7.6	4.0	9.6	14.2	6.1	20.2

1. Acute Reference Dose (ARfD) of 0.02 mg/kg bw; A deterministic acute risk assessment is conducted and exposure is reported at the 95th percentile.
2. Acceptable daily intake (ADI) of 0.005 mg/kg bw/day.
3. For drinking water exposure to atrazine and its chlorotriazine transformation products, a monitoring EEC of 15.38 µg/L was used for both acute and chronic dietary risk assessments.

Table 2 Chronic dietary exposure and risk analyses for hydroxylated metabolites/Transformation products of atrazine

Subpopulation	Acute (%ARfD)[1]			Chronic (%ADI)[2]		
	Food alone	Drinking water alone[3]	Food and drinking water[3]	Food alone	Drinking water alone[3]	Food and drinking water[3]
General Population	Not required			0.9	19.0	19.9
All Infants (<1 year old)				0.4	70.9	71.3
Children 1–2 years old				1.0	26.1	27.1
Children 3–5 years old				1.1	21.3	22.4
Children 6–12 years old				0.9	15.8	16.7
Youth 13–19 years old				0.8	13.4	14.2
Adults 20–49 years old				1.0	18.9	19.8
Adults 50+ years old				1.0	18.4	19.3
Females 13–49 years old				1.0	18.5	19.5

1. An acute dietary risk assessment is not required, as an acute toxicology reference value was not

established.

2. Acceptable daily intake (ADI) of 0.01 mg/kg bw/day.
3. The modelled Level 2 EEC of 94 µg/L (yearly) was used in the chronic dietary risk assessment.

Appendix VI Occupational and non-occupational exposure and risk assessment

Table 1 Short-term M/L/A occupational exposure and risk assessment for spray uses of atrazine (all products are liquids/suspensions)

Use	Application method	Max rate	ATPD[1]	Exposure (µg/kg bw/day)[2]		MOE[3] (Target MOE = 300)		
		(kg a.i./ha)	(ha)	Dermal	Inhalation	Dermal	Inhalation	Combined
Minimum label specified PPE[4] [Single Layer + Coveralls, CR Gloves (MLA)] + Open M/L + Open Cab (A)								
Corn	Groundboom [Custom]	1.5	140	7.17	6.06	**220**	**260**	**120**
	Groundboom [Farmer]	1.5	80	4.10	3.47	390	460	**210**
Sorghum	Groundboom [Custom]	1.0	360	12.3	10.4	**130**	**150**	**71**
	Groundboom [Farmer]		107	3.65	3.09	440	520	**240**
Switchgrass	Groundboom [Custom]	1.5	150	7.68	6.50	**210**	**250**	**110**
	Groundboom [Farmer]		20	1.02	0.866	1600	1900	850
Minimum label specified PPE[4] + Closed M/L + Open Cab (A).								
Corn	Groundboom [Farmer]	1.5	80	2.14	2.66	750	600	330
Sorghum	Groundboom [Farmer]	1.0	107	1.91	2.21	840	670	370
Minimum label specified PPE[4] + Closed M/L + Closed Cab (A).								
Corn	Groundboom [Custom]	1.5	140	2.21	0.446	720	3,600	600
Sorghum	Groundboom [Custom]	1.0	360	3.79	0.765	420	2100	350
Switchgrass	Groundboom [Custom]	1.5	150	2.37	0.478	680	3400	560

PPE = personal protective equipment; Single layer = long-sleeved shirt, long pants; Form = formulation; ATPD = area treated per day; Max = maximum; MOE = Margin of Exposure; MLA = Mixer/Loader/Applicator; ML = mixer/loader; A = Applicator; CR = chemical resistant; kg = kilogram; a.i. = active ingredient; ha = hectare; bw = body weight; µg = microgram; CF = correction factor.

1. Standard ATPD day values were used for corn and sorghum. Crop-specific ATPD values were used for switchgrass based on information supplied by growers.
2. Exposure (µg/kg bw/day) = [unit exposure (µg/kg ai) × application rate (kg a.i./ha) × ATPD (ha/day) × dermal absorption of 6% (for dermal exposure route)] ÷ body weight (80 kg). Unit exposure values are from PHED and AHETF.
3. Calculated using a NOAEL of 1.6 mg/kg bw/day from a 4-day gavage study in the rat and target MOE of 300. MOE = NOAEL (mg/kg bw/day) ÷ [Exposure (µg/kg bw/day) × CF (1 mg ÷ 1000 µg)]. MOEs less than the target MOE are in **bold** text.
4. Minimum level of PPE currently required on product labels [Coveralls over single layer (long-sleeved shirt, long pants), CR Gloves (MLA)].

Table 2 Short-and intermediate-term exposure and risk assessment for tasks conducted in commercial granular fertilizer impregnation facilities for atrazine

Activity	Amount handled (kg a.i./day)[2]	Unit exposures (µg/kg ai)		MOE (Target = 300)[1,3]		
		Dermal	Inhalation	Dermal	Inhalation	Combined
Using PMRA# 2313618 – Minimum label specified PPE (Single Layer + CR Gloves + Coveralls)[4,5]						
Treater (closed mix/load)	1500	53.5	1.12	**27**	**76**	**20**
BSS	1500	7.33	1.5	**190**	**57**	**44**
Using PMRA# 2313617 – Minimum label specified PPE + CR Coveralls[4]						
Treater (closed mix/load)	1500	7.36	0.27	**190**	320	**120**
	600[6]			480	790	300
BSS	1500	0.9	0.25	1600	340	**280**
	1400[6]			1700	370	300
Forklift operator	1500	0.72	0.105	2000	810	580
Activity	**Time spent cleaning**	**Unit exposures (µg/hr)**		**MOE (Target = 300)**		
		Dermal	**Inhalation**	**Dermal**	**Inhalation**	**Combined**
Using PMRA# 2313618 – Minimum label specified PPE (Single Layer + CR Gloves + Coveralls)[5]						
Cleaning	8 hr	1204[7]	211[7]	**220**	**76**	**56**
Using PMRA# 2313617 – Minimum label specified PPE + CR Coveralls						
Cleaning	8 hr	241[7]	67[7]	1100	960	510

PPE = personal protective equipment; Single layer = long-sleeved shirt, long pants; CR = chemical resistant; MOE = margin of exposure; kg = kilogram; a.i. = active ingredient; M/L = mixer/loader; BSS = bagger, sewer, stacker; hr = hour; CF = correction factor.

1. MOEs less than the target MOE are in **bold** text.
2. The maximum amount active ingredient incorporated per day per facility as specified on atrazine labels (1500 kg a.i./day). However, there are MOEs less than the target MOE assuming the maximum amount incorporated per day, so the amount handled per day where target MOEs are met are also shown (footnote 6), when applicable. For cleaners, as information regarding the time workers spend cleaning fertilizer equipment was not available, a standard 8 hour workday was assumed.
3. Calculated using a NOAEL of 1.6 mg/kg bw/day from a 4-day gavage study in the rat and target MOE of 300. MOE = NOAEL (mg/kg bw/day) ÷ Exposure (µg/kg bw/day) × CF (1 mg ÷ 1000 µg). Exposure (µg/kg bw/day) = [unit exposure (µg/kg ai or µg/hr) × Amount Handled per Day (kg a.i./day) or Time Spent Cleaning (hr) × dermal absorption of 6% (for dermal exposure route) × CF (1 mg/1000 µg)] ÷ body weight (80 kg).
4. The seed treatment exposure studies were conducted in facilities with a closed mix/load transfer system on canola seeds; therefore, these MOEs are representative of a closed mix/load transfer system in a commercial fertilizer facility currently required on registered product labels.
5. Minimum level of PPE currently required on product labels [Coveralls over single layer (long-sleeved shirt, long pants), CR Gloves (MLA)].
6. In order to meet the target MOEs the amount of atrazine treated per day in a facility needs to be reduced to this amount. However, these amounts were not considered practical, as only a small amount of fertilizer could be treated per day.
7. Cleaner unit exposures were determined by normalizing by time spent cleaning in the seed treatment studies (~8 hours per day) rather than the study application rate, which is specific to seed treatment.

Table 3 Short-Term Exposure and Risk Assessment for Loading and Application of Granular Fertilizer Impregnated with Atrazine Using PHED

Loader/Applicator	Application rate (kg a.i./ha)[3]	Area treated/day (ha)[4]	Unit exposures (µg/kg ai)		MOE (Target = 300)[1,2]		
			Dermal	Inhalation	Dermal	Inhalation	Combined
Open loading + Open cab solid broadcast spreader (Minimum label specified PPE: Single layer + CR gloves + Coveralls)[5]							
Farmers	1.5	65	12.76	3.8	1700	350	**290**
Custom		130			860	170	**140**
Open loading + Open cab solid broadcast spreader (Minimum label specified PPE + CR coveralls)[5]							
Farmers	1.5	65	9.92	3.8	2200	350	300
Custom		130			1100	170	**150**
Open loading + Closed cab solid broadcast spreader (Minimum label specified PPE)[5]							
Custom	1.5	130	7.98	2.5	1400	260	**220**
Open loading + Closed cab solid broadcast spreader (Minimum label specified PPE + CR Coveralls)[5]							
Custom	1.5	130	4.73	2.5	2300	260	**240**
Closed loading + Open cab solid broadcast spreader scenario (Minimum label specified PPE)[5]							
Custom	1.5	130	6.58	2.42	1700	270	**230**
Closed loading + Open cab solid broadcast spreader scenario (Minimum label specified PPE + CR coveralls)[5]							
Custom	1.5	130	4.3	1.82	2500	361	320
Closed loading + Closed cab solid broadcast spreader scenario (Minimum label specified PPE)[5]							
Custom	1.5	130	1.8	0.52	6100	1300	1050

PPE = personal protective equipment; CR = chemical resistant; MOE = margin of exposure; kg = kilogram; ai = active ingredient; ha = hectares.

1. MOEs less than the target MOE arein **bold** text.
2. MOE = NOAEL ÷ [exposure (µg/kg bw/day) × CF (1 mg/1000 µg)]. NOAEL of 1.6 mg/kg bw/day from a 4-day gavage study in the rat with a target MOE of 300. Exposure (µg/kg bw/day) = [unit exposure (µg/kg a.i.) × application rate (kg a.i./ha) × Area Treated per Day (ATPD) (ha) × dermal absorption of 6% (for dermal exposure route)] ÷ body weight (80 kg). Unit exposures are from PHED.
3. 1.5 kg a.i./ha is the maximum registered application rate for corn. Atrazine impregnated granular fertilizer is registered for use on corn only.
4. Values typically used by the PMRA for loading/application of impregnated granular fertilizer.
5. Minimum level of PPE currently required on product labels [Coveralls over single layer (long-sleeved shirt, long pants), CR Gloves (MLA)].

Table 4 Postapplication dermal exposure and risk assessment for atrazine

Crop	Activity	Transfer coefficient (cm^2/hr)[1]	Rate (kg a.i./ha)	Day 0 DFR[2] (µg/cm^2)	MOE[3] (Day 0) Target: 300	REI [4]
Pre-emergent applications (including pre-plant)						
Corn (Field, Seed, and Sweet)[5], Sorghum, Switchgrass	Minimal dermal exposure as there is no treated crop foliage available for contact.					12 hours
Post-emergent applications						
Corn (Field, Seed and Sweet)[5]	Scouting	210	1.5	1.80	710	12 hours
Sorghum	Scouting	210	1.0	1.20	1100	12 hours

MOE = margin of exposure; kg = kilogram; ai = active ingredient; ha = hectare; hr = hour; N/A = not applicable; CF = correction factor; DFR = dislodgeable foliar residues

1. Transfer coefficients are standard PMRA Agricultural values and are based on ARTF studies. Activities that have minimal postapplication exposure to treated foliage and do not have a transfer coefficient are not included in this table.
2. Day 0 DFR on Day 0 after one application (as per current labels). DFR values were calculated using the peak DFR of 12% of the application rate and 36% dissipation per day based on chemical-specific data on corn.
3. Dermal MOE = NOAEL ÷ [exposure (µg/kg bw/day) × CF (1 mg/1000 µg)]. Exposure (µg/kg bw/day) = [$DFR_{Day\ 0}$ × Transfer Coefficient × 8 hr] ÷ 80 kg. NOAEL of 1.6 mg/kg bw/day from a 4-day gavage study in the rat with a target MOE of 300.
4. Point in time when the dermal exposure results in an MOE greater than or within range of the target MOE (300) and when inhalation risks are expected to be acceptable.
5. "Field corn" includes corn used for grain, silage, and seed.

Table 5 Atrazine bystander inhalation exposure and risk assessment

Lifestage	Maximum air concentration (pg/m^3)[1]	Inhalation rate (m^3/ hr)	ET (hr/day)	Inhalation exposure (mg/kg bw/day)[2]	Inhalation MOE[3] (Target = 300)
Adults	7120	0.64	1.5	8.54×10^{-8}	19 000 000
Youth (11 to < 16 years old)		0.63	1.7	1.34×10^{-7}	12 000 000
Children (1 to < 2 years old)		0.33	3.0	4.91×10^{-7}	3 300 000
Children (6 to <12 months)		0.23	2.3	3.93×10^{-9}	410 000 000

pg = picogram; ET = exposure time; hr = hour; kg = kilogram; MOE = margin of exposure; bw = body weight.

1. Maximum value from all literature studies monitoring air in Canadian agricultural regions since the year 2000. The maximum value was found in Yao et al., 2008.

2. Inhalation exposure (mg/kg bw/day) = [maximum air concentration (pg/m^3) × conversion factor (pg/1 × 10^9 mg) × inhalation rate (m^3/hr) x exposure time (hr/day)] ÷ body weight (kg). Body weights are 80, 57, 11, and 9 kg, respectively, for adults, youth, children (1 < 2 yrs) and children (6 < 12 months) from the USEPA Residential SOPs (2012).
3. MOE = NOAEL ÷ exposure. NOAEL of 1.6 mg/kg bw/day from a 4-day gavage study in the rat and a target MOE of 300.

Appendix VII Environmental fate

Table 1 Physical and chemical properties of atrazine

Property	Value	Interpretation
Solubility in water	33 mg/L (20°C)	Soluble in water
Vapour pressure	0.04 mPa (20°C)	Low volatility
Henry's law Constant	2.61×10^{-4} Pa·m^3/mole (20°C)	Low volatility from moist soil and water
Octanol-water partition coefficient (log K_{ow})	2.7	Low potential for bioaccumulation
Dissociation constant (pK_a)	1.7	Dissociates at environmentally relevant pH Potentially mobile at environmentally relevant pH

Table 2 Summary of fate processes for atrazine in the terrestrial and aquatic Environment (as reported in PACR2007-05, open literature and the USEPA (PMRA# 2741498)).

Process	$T_{1/2}$ or DT_{50}	DT_{90}	Kinetics (T_R or $T_{1/2slow}$)	Comments
	(days – d, hours – h)			
Abiotic transformation				
Hydrolysis Non sterile, buffer solutions, 25°C	pH 2: 20 d pH 12: 20 d pH 4: 200 d pH 11: 200 d pH 6: >1000 d pH 10: >1000 d pH 3.9: 209 d	nr	SFO	Resistant to hydrolysis at environmental pH. (Armstrong et al., 1967)
Hydrolysis Sterile, soil and water, 25°C	pH:3.9: 22 d	nr	SFO	(Armstrong et al., 1968)
Hydrolysis Non sterile, buffer solution, 20 and 30°C	pH 5 (20°C): 84 d pH 5 (30°C): 42 d	nr	SFO	(Burkhard and Guth, 1981)
Hydrolysis Non sterile, buffer solution with fulvic acid, 25°C	pH 2.9: 35 d pH 7: 742 d	nr	SFO	(Khan, 1978)
Hydrolysis Non sterile: Deionized water (65 mg/L DOC, 4 and 30°C) Well water (6 mg/L DOC, 4 and 30°C)	Deionized water – 65 mg/L DOC pH 7.7 (4°C): 1565 d pH 7.7 (30°C): 2022 d Well water – 6 mg/L DOC	nr	SFO	(Widmer et al., 1993)

	pH 7.8 (4°C): 1565 d pH 7.8 (30°C): 1311 d			
Hydrolysis Non sterile, redistilled water, 25°C	pH 2: 2.48 d pH 5: 1732.87 d pH 7: 173286.80 d	nr	See comment	Half-lives estimated from equation: $t_{1/2}$ = 0.01356*(0.0245 + 10 - pH) / 10 – pH years (Gamble et al., 1983)
Hydrolysis Non sterile, groundwater – 0.05 mg/L DOC, 20°C	pH 6.66: 283 d	nr	SFO	(Navarro et al., 2004)
Photolysis on soil (natural sunlight)	7 - 12 d	nr	SFO	Not an important route of transformation in soil.
Photolysis on soil (natural sunlight)	45 d		SFO	Transformation products include DEA, DACT and DIA. (Das, Y 1989 - MRID 42089905)
Photolysis in water (natural sunlight)	pH 7: 168 d	nr	SFO	Transformation products include DEA, DACT, DIA DIHA and DHEA. (MRID 42089904; 45545301)
Photolysis in water (natural sunlight)	pH 7: 335 d	nr	SFO	Transformation products include DEA, DIA and DACT
Aerobic soil biotransformation				
Sandy loam, 4% OM, pH 4.9, 22°C	115 d	nr	nr	Application rate 5–48 mg/kg. (Armstrong et al., 1967)
Silt loam, 13% OM, pH 6.9, 22°C	220 d	nr	nr	
Clay, 2% OM, pH 7.3, 22°C	1000-1800 d	nr	nr	
Loam, 2.5% OM, pH 8, 25°C	41 d	nr	nr	17% soil water. (Walker and Zimdahl 1981)
Silt loam, 1.1% OM, pH 7.3, 25°C	28 d	nr	nr	
Sandy loam, 2.6% OM, pH 6.4, 25°C	47 d	nr	nr	
Loam, 2.5% OM, pH 8, 5°C	181 d	nr	nr	
Silt loam, 1.1% OM, pH 7.3, 5°C	133 d	nr	nr	
Sandy loam, 2.6% OM, pH 6.4, 5°C	179 d	nr	nr	
Loam, 2.5% OM, pH 8, 5°C	103 d	nr	nr	5.1% soil water. (Walker and Zimdahl 1981)
Silt loam, 1.1% OM, pH 7.3, 5°C	55 d	nr	nr	
Loam, 2.6% OM, pH 6.4, 5°C	94 d	nr	nr	
Loam, 0.55% OM, pH 5.4, 12–36°C	16 d	nr	nr	Sediment (Jones et al., 1982)
Sandy loam, 0.85% OM, pH 4.4, 12–36°C	13 d	nr	nr	
Sandy loam, 0.91% OM, pH 5.5, 12–36°C,	110 d	nr	nr	

Silty clay loam, 0.91% OM, pH 6.4, 12–36°C	36 d	nr	nr	
Silty clay, 3.8% OM, pH 5.2, 30°C	38 d	nr	nr	(Dao et al., 1979)
Silty clay, 2.9% OM, pH 5.8, 30°C	37 d	nr	nr	
Fine silt loam, 2.9% OM, pH 6.3, 30°C	64 d	nr	nr	
Silt loam, 1.6% OM, pH 5.1–5.8, 22°C	37 d	nr	nr	(Hance, 1979)
Silt loam, 1.6% OM, pH 6.3–7.0, 22°C	37 d	nr	nr	
Silt loam, 1.6% OM, pH 7.7–7.9, 22°C	28 d	nr	nr	
Silt loam, 1.6% OM, pH 7.8–8.2, 22°C	27 d	nr	nr	
Silt loam, 4.0% OM, pH 4.6–5.2, 22°C	29 d	nr	nr	
Silt loam, 4.0% OM, pH 5.3–6.1, 22°C	32 d	nr	nr	
Silt loam, 4.0% OM, pH 6.3–7.2, 22°C	36 d	nr	nr	
Silt loam, 4.0% OM, pH 6.8–8.0, 22°C	40 d	nr	nr	
Sandy loam, 2.5% OM, pH 7.3, 22°C	71 d	nr	nr	(Moyer et al., 1972)
Loam, 12% OM, pH 7.6, 25°C Moisture content: 75% field capacity	140 d	nr	nr	
Transformation products				
HA	120 d	nr	nr	Aerobic soil studies conducted with silt loam soil (Tennessee)
DIA	33 d	nr	nr	
DEA	31 d	nr	nr	
Anaerobic soil biotransformation				
Loam soil flooded with water	159 d	nr	nr	Transformation products include DEA, DIA, DACT and HA.
Sandy loam soil	77 d	nr	nr	
Aerobic aquatic biotransformation				
Rhine river water/sediment system 77 days, 25°C	Water phase: > 400 d Whole system: NR	nr	NR	91% of applied atrazine remained in river water after 77 days. 59% of applied atrazine remained in pond water after 77 days. Transformation products not reported. (FBC, 1978).
Pond water/sediment system 77 days, 25°C	Water phase: 80 - 90 d Whole system: NR	nr	NR	
Anaerobic aquatic biotransformation				
Sandy clay water/sediment system	Water phase: 578 d Sediment phase: 330 d Whole system: 608 d	nr	NR	Radioactivity associated with parent atrazine at 12 months after treatment was 70% water and 4% sediment. Whole system transformation products include DEA, HA and DIA.

Mobility			
Process	**Soil type**	**K_{oc}**	**Comments**
Adsorption: atrazine	Sand (Wisconsin): 0.8% OM, pH 5.6, CEC: 1 meq/100g; Field moisture at 1/3 bar: 20.3%	90.9	K_{oc} values shown were considered by Health Canada in previous assessments (PACR 2007-05). Additional soil adsorption coefficient *K*d and K_{oc} values from the open literature are reported in the 2016 USEPA refined ecological risk assessment for atrazine (Table 13, page 66; PMRA# 274148). The soil *K*d values range from 0.17–91.8; corresponding Kfoc values range from 8.5–2571.
	Sandy loam (California) 3% OM, pH 6.1, CEC: 6 meq/100g; Field moisture at 1/3 bar: 30%	55	
	Silty loam (Mississippi) 2.1% OM, pH 7, CEC: 15 meq/100g; Field moisture at 1/3 bar: 20.1%	121	
	Clay loam (Maryland) 2.5% OM, pH 6.6, CEC: 14.7 meq/100g; Field moisture at 1/3 bar: 31%	135	
	Clay (Maryland): 4.8% OM, pH 5.9, 25% sand, 33% clay, 42% silt, CEC: 24.3 meq/100g	86.9	
	Sand (Maryland): 0.9% OM, pH 6.5, 96% sand, 2% clay, 2% silt, CEC: 1.8 meq/100g	38.5	
	Sandy loam (Maryland): 1.9% OM, pH 7.5, 63% sand, 20% clay, 17% silt, CEC: 6.1 meq/100g	70.4	
	Loam (California): 0.8% OM, pH 6.7, 44% sand, 47% clay, 9% silt, CEC: 4.3 meq/100g	155.3	
Adsorption: DACT	Sand (Wisconsin): 0.8% OM, pH 5.6, CEC: 1 meq/100g; Field moisture at 1/3 bar: 20.3%	23	
	Sandy loam (California) 3% OM, pH 6.1, CEC: 6 meq/100g; Field moisture at 1/3 bar: 30%	11.6	
	Silty loam (Mississippi) 2.1% OM, pH 7, CEC: 15 meq/100g; Field moisture at 1/3 bar: 20.1%	59.5	
	Clay loam (Maryland) 2.5% OM, pH 6.6, CEC: 14.7 meq/100g; Field moisture at 1/3 bar: 31%	53.3	
	Clay (Maryland): 4.8%	55.2	

	OM, pH 5.9, 25% sand, 33% clay, 42% silt, CEC: 24.3 meq/100g		
	Sand (Maryland): 0.9% OM, pH 6.5, 96% sand, 2% clay, 2% silt, CEC: 1.8 meq/100g	30.7	
	Sandy loam (Maryland): 1.9% OM, pH 7.5, 63% sand, 20% clay, 17% silt, CEC: 6.1 meq/100g	57.9	
	Loam (California): 0.8% OM, pH 6.7, 44% sand, 47% clay, 9% silt, CEC: 4.3 meq/100g	76.0	
Adsorption: DIA	Sand (Wisconsin):0.8% OM, pH 5.6, CEC: 1 meq/100g; Field moisture at 1/3 bar: 20.3%	47.9	
	Sandy loam (California) 3% OM, pH 6.1, CEC: 6 meq/100g; Field moisture at 1/3 bar: 30%	35.1	
	Silty loam (Mississippi) 2.1% OM, pH 7, CEC: 15 meq/100g; Field moisture at 1/3 bar: 20.1%	82.3	
	Clay loam (Maryland) 2.5% OM, pH 6.6, CEC: 14.7 meq/100g; Field moisture at 1/3 bar: 31%	76.3	
	Clay (Maryland): 4.8% OM, pH 5.9, 25% sand, 33% clay, 42% silt, CEC: 24.3 meq/100g	96.8	
	Sand (Maryland): 0.9% OM, pH 6.5, 96% sand, 2% clay, 2% silt, CEC: 1.8 meq/100g	30.4	
	Sandy loam (Maryland): 1.9% OM, pH 7.5, 63% sand, 20% clay, 17% silt, CEC: 6.1 meq/100g	45.2	
	Loam (California): 0.8% OM, pH 6.7, 44% sand, 47% clay, 9% silt, CEC: 4.3 meq/100g	58.1	
Adsorption: DEA	Sand (Wisconsin): 0.8% OM, pH 5.6, CEC: 1 meq/100g; Field moisture at 1/3 bar: 20.3%	24.7	
	Sandy loam (California) 3% OM, pH 6.1, CEC: 6	12.8	

	meq/100g; Field moisture at 1/3 bar: 30%		
	Silty loam (Mississippi) 2.1% OM, pH 7, CEC: 15 meq/100g; Field moisture at 1/3 bar: 20.1%	66.5	
	Clay loam (Maryland) 2.5% OM, pH 6.6, CEC: 14.7 meq/100g; Field moisture at 1/3 bar: 31%	64.2	
	Clay (Maryland): 4.8% OM, pH 5.9, 25% sand, 33% clay, 42% silt, CEC: 24.3 meq/100g	36.1	
	Sand (Maryland): 0.9% OM, pH 6.5, 96% sand, 2% clay, 2% silt, CEC: 1.8 meq/100g	12.2	
	Sandy loam (Maryland): 1.9% OM, pH 7.5, 63% sand, 20% clay, 17% silt, CEC: 6.1 meq/100g	31.8	
	Loam (California): 0.8% OM, pH 6.7, 44% sand, 47% clay, 9% silt, CEC: 4.3 meq/100g	44.9	
Adsorption: HA	Sand (Wisconsin): 0.8% OM, pH 5.6, CEC: 1 meq/100g; Field moisture at 1/3 bar: 20.3%	350	
	Sandy loam (California) 3% OM, pH 6.1, CEC: 6 meq/100g; Field moisture at 1/3 bar: 30%	360	
	Silty loam (Mississippi) 2.1% OM, pH 7, CEC: 15 meq/100g; Field moisture at 1/3 bar: 20.1%	680	
	Clay loam (Maryland) 2.5% OM, pH 6.6, CEC: 14.7 meq/100g; Field moisture at 1/3 bar: 31%	391	
	Clay (Maryland): 4.8% OM, pH 5.9, 25% sand, 33% clay, 42% silt, CEC: 24.3 meq/100g	13797	
	Sand (Maryland): 0.9% OM, pH 6.5, 96% sand, 2% clay, 2% silt, CEC: 1.8 meq/100g	374.2	
	Sandy loam (Maryland): 1.9% OM, pH 7.5, 63%	583.3	

	sand, 20% clay, 17% silt, CEC: 6.1 meq/100g		
	Loam (California): 0.8% OM, pH 6.7, 44% sand, 47% clay, 9% silt, CEC: 4.3 meq/100g	2572.9	
Soil column leaching (Information shown was considered by Health Canada in previous assessments (PACR 2007-05))	Aged soil column leaching ^{14}C atrazine was added to two soil columns (loamy sand and a silt loam soil) and aged 90 days prior to leaching. 88.4% and 96.2% of the initial radioactivity was retained in the loamy sand and silt loam, respectively. The majority of the radioactivity representing 56.8% and 64.4% in the loamy sand and silt loam, respectively, was detected in the top 10 cm. The leachate from both soils contained small amounts of atrazine (0.1%). The leachate from the loamy sand, contained DEA (2.9%) and DACT (1.1%). (Guth J.A., 1985 – PMRA# 1235041)		
	Un-aged soil column leaching ^{14}C atrazine was added to two soil columns (sand and a silt loam soil). The amount of radioactivity detected in the leachate was 1.2% and <0.1% of the applied ^{14}C in the sand and silt loam soils, respectively, indicating there was greater leaching in coarser-textured soil.		
	Un-aged soil column leaching ^{14}C atrazine was added to three soil columns (sand, loamy sand and sandy loam soil). The results indicated that most of the applied atrazine was retained in the top 0–5 cm of soil (59–100%). The amount of radioactivity detected in the leachate ranged from <0.02% to 0.18% of the applied ^{14}C.		
	The leaching of atrazine was reported in field studies. Atrazine was detected in tile drainage at a depth of 1.2–1.6 m at concentrations of 0.30–1.49 µg/L following an application of 2.8 kg a.i./ha to corn planted on a sandy loam soil in Canada (Muir and Baker, 1976, PMRA# 1404534). Following a period of heavy rainfall, atrazine was detected in tile drain water at 6 days after application. Over a 9-month period, approximately 0.15% of the applied atrazine was detected in tile drainage water. In a similar study, 0.13–0.22% of the applied atrazine was detected in tile drainage (Muir and Baker, 1978, PMRA# 1404535).		

Terrestrial field studies

Process	**$T_{1/2}$ or DT_{50}** (days)	**DT_{90}** (days)	**Kinetics (T_R or $T_{1/2slow}$)**	**Comments**
Hollandale, Minnesota (0–2.5 cm depth: loam – 6.2% OM; 5–10 cm depth: silt loam - 0.8% OM), pH 7.4–7.9.	58–99 days	279–694 days	Dissipation did not follow 1st order kinetics (an initial rapid dissipation phase was followed by a slower phase). Dissipation kinetic values were re-estimated using non-linear regression methods which significantly improved the fit to the original data. Atrazine was mobile as it leached to soil depths as great as 122 cm at approximately 1 year after application. Carryover to the following season ranged from 36 to 54%. The transformation of atrazine was slow as the maximum concentrations of the transformation products (DIA, DEA HA) were detected at approximately 1 year after application. Transformation products are first detected in soil at 450 days after the application of atrazine. Transformation products may persist in soil as subsequent detections can occur at 571–938 days after the first detection. PMRA# 1235065 and 1235066	

Khan and Saidak, 1981 (PMRA# 1235071) Research Station of Agriculture Canada in Ottawa, Canada. Atrazine was applied to a field planted to corn that previously received 20 consecutive annual applications at rates of 1.40–2.24 kg a.i./ha. It was reported that at 5 and 12 months after the final application, the concentration of atrazine in soil was 102 and 55 µg/g, respectively. The concentrations of the transformation products, DEA, HA, DEHA and DIHA, decreased between 5 and 12 months after treatment. From these limited data, the reviewer estimated that the DT_{50} for atrazine in soil was approximately 7 months. It was also reported that the annual rate of loss of atrazine during the years of application was approximately 85–90%, and there were no corresponding increases in the concentrations of transformation products. Thus, the annual carryover of atrazine was 10–15%. Atrazine residues were also detected in crops planted after the last application of atrazine (oats). As it was not possible to determine the dissipation pattern of atrazine, the study is considered to be of limited utility. The results, however, do indicate that long-term annual applications of atrazine to fields planted to corn result in the accumulation of residues which persist in soil beyond the final season of application. In addition, the study demonstrated that atrazine residues are absorbed by crops such as oats under field conditions.
(PMRA# 1235069) The study looked at the dissipation and mobility of atrazine in both aquatic and terrestrial environments when used under typical forestry conditions over 1 year (near Oregon city, Oregon). In soil, atrazine was detected only in the top 0–15 cm of soil throughout the study period. Only one transformation product, DEA, was detected in the 0–15 cm soil depth between 29 and 364 DAT. The estimated soil DT_{50} was 135 days, however, the dissipation data showed a poor correlation coefficient for first order kinetics (r^2 = 0.515). By excluding some of the outlier data, the correlation coefficient was improved (r^2 = 0.924) and the re-estimated DT_{50} was reported to be 87 days. Atrazine residues (parent, DIA and DEA) were detected in leaf litter throughout the study period. The DT_{50} of atrazine on leaf foliage was 13 days (r^2 = -0.92). Atrazine residues were not detected in stream water, sediment, caged fish or native fish throughout the study period.
Smith and Walker. 1989 (PMRA# 1495117) The persistence of atrazine was studied under laboratory and field conditions (heavy clay soil: 70% clay, 25% silt, 5% sand, OC 4.2%, 40% moisture content (wt/wt),and pH 7.7). 1.0 kg/ha of atrazine was applied to 4 replicate plots. A half-life for atrazine based on the terrestrial field data is not provided, however, a DT_{50} of 25-30 days is approximated by interpolation from the dissipation curve provided. At the end of the growing season, 32% of the applied atrazine remaining in soil. The dissipation of transformation products was not followed.
Khan et al., 1981 (PMRA# 1496120) The study examined the effects of time, method and type of application in corn on the degradation and persistence of atrazine. The subsequent uptake of residues by oat plants seeded in the treated field plots in the following spring was also investigated. The study was conducted in Woodlee, Ontario on Brookston clay soil (4.1% OM, pH 5.6). The DT_{50} for atrazine is reported to range from 56 to 69 days. Atrazine dissipation was shown to be faster in the first month than in the remaining 3 months. The percentage of atrazine residues remaining in soil at the end of first year ranged from 23 to 40%. Plots were plowed under at the end of the growing season (October, 1978). The percentage of residues measured in soil the following year (May 1979) ranged from 32 to 41%.
Frank et al., 1991 (PMRA# 1496119) The dissipation of atrazine and DEA was investigated following application to a clay loam soil (9% clay, 2.2–3.5% OM, pH 5.6–6.6). Atrazine formulation (Aatrex 480F) was applied to tilled soil on a single field (14 ha) on 3 separate occasions between 30 September 1986 and 10 May 1989. On 30 September 1986 atrazine was applied at 0.8 kg a.i./ha. Corn was planted the following year on 7 May 1986 and atrazine was applied again on 6 June 1987 at 2.4 kg a.i./ha. Atrazine declined rapidly over the summer months with a half-life disappearance of 37 and 64 days following the 1987 and 1989 applications, respectively. When the dissipation was extended to cover the winter months (22 months), the half-life increased to 125 days. Using the raw data provided in the study, the reviewer calculated a half-life of 113 days for the period from 6 June 1987 to 4 May 1989. However, the raw data reported in the study is total atrazine which is the combined measured concentrations of atrazine and DEA. The percentage of DEA of total atrazine ranged from 4.1 to as high as 37% in samples. Leaching was confirmed by the detection of atrazine in tile drainage water collected from 1.0-m depth and in groundwater samples beneath the field between a depth of 1.2 and 4.6 m.
Frank and Sirons 1985 (PMRA# 1235070) The purpose of the study was to identify initial rates of application of atrazine that would give carry-over residues below the phytotoxic threshold to susceptible crops grown next in rotation. The dissipation of atrazine and its metabolite, desethylatrazine (DEA), under field conditions was investigated in soil at three different application rates. The effect of charcoal addition to soil on the rate of dissipation was also investigated. Two soils located in

<table>
<tr><td colspan="2">Ontario were studied: clay loam soil (32% clay, 4.8% OM, pH 7.0) and loam soil (24% clay, 3.5% OM, pH 7.5).

Breakdown of atrazine was more rapid in 1st 5 months than in the later 7 months. Half-lives of 3.6, 3.0 and 3.5 months (108, 90 and 105 days) are reported for the clay loam soil based on concentration measured in the upper 0–6cm of soil for the three application rates tested, 1.1, 2.2, and 3.3 kg/ha respectively. However, the concentrations for atrazine residues include both atrazine and the metabolite desethyl-atrazine combined. It is not known whether the half-lives reported are based on the combined concentrations (atrazine + desethyl atrazine) or for atrazine alone. Using the combined concentrations reported for each of the application rates studies, the reviewer calculated similar half-lives of 110, 91 and 107 days for the three application rates tested (1.1, 2.2 and 3.3 kg/ha, respectively). The percentage of desethyl atrazine of total atrazine is not reported. The concentration of atrazine (and DEA) in clay loam soil in the following season (365 days after initial application was less than 10% of that measured at day 6 concentration; a measurement immediately after application (day 0) was not reported.</td></tr>
<tr><td colspan="2">The 2016 USEPA refined ecological risk assessment for atrazine reports field dissipation half-lives for atrazine ranging from 5.23 to 405 days in fallow and corn-planted soil; the degree of dissipation is stated as dependent on microbial-mediated degradation, runoff and leaching. Transformation products in the studies include HA, DEA and DIA. Concentrations of atrazine, DEA, HA and DIA were detected with soil depth in long-term dissipation studies.</td></tr>
<tr><th colspan="2">Aquatic field studies</th></tr>
<tr><td>Freshwater systems</td><td>The DT$_{50}$ for atrazine in lake enclosures was reported to be 150 days (Hamilton et al., 1989). The decreasing concentrations of atrazine in water was correlated with the levels of chlorophyll, O_2, dissolved organic carbon, and particulate organic carbon.</td></tr>
<tr><td>Swiss lakes</td><td>Atrazine in Swiss lakes (likely from atmospheric deposition) showed little or no dissipation. The observed decrease in atrazine concentration in these lakes was mainly from export with outflowing water (Buser, 1990).</td></tr>
<tr><td>Artificial streams</td><td>No significant accumulation of atrazine (< 1 µg/g) was observed in sediment after a 60-day atrazine-free period following an initial exposure to technical atrazine (49.5 µg/L) for 30 days. In the water column, atrazine disappeared to concentrations below 1 µg/L on day 3 of the 60-day depuration period. (Lynch et al., 1982).</td></tr>
<tr><td>Aerobic estuarine microcosms</td><td>Atrazine was eliminated from water and sediment with DT$_{50}$ values of 3-12 and 15-20 days, respectively. It was reported that this rapid removal of atrazine from these estuarine systems was largely attributed to enhanced hydrolysis to HA in water and on sediment. Although atrazine is stable to hydrolysis, the authors (Jones et al.,1982) postulated that the enhanced hydrolysis of atrazine may have been attributed to high concentrations of fulvic acid in the water which in laboratory studies was shown to accelerate the hydrolytic process (Khan, 1978).</td></tr>
<tr><td>Aerobic estuarine mesocosms</td><td>Cunningham et al.,1984 and Kemp et al., 1985 determined that the DT$_{50}$ of atrazine in estuarine mesocosms was 90-120 days.</td></tr>
<tr><td rowspan="2">Estuarine systems</td><td>The estimated DT$_{50}$ for atrazine in the Wye River was just under 30 days (Glotfelty et al., 1984); the Wye River is a tributary of Chesapeake Bay, it is a shallow well-mixed estuary surrounded by an agricultural watershed, a large portion of which is planted to corn.</td></tr>
<tr><td>A DT$_{50}$ of 30 days is reported in Ballantine et al., 1978.</td></tr>
</table>

nr = not reported; OM = organic matter content; SFO = single first order

Appendix VIII Risk quotients

Table 1 Screening level risk assessment for atrazine for earthworms using highest application rate (1500 g a.i./ha per year)

Exposure	Toxicity		EEC (mg a.i./kg soil)	RQ	LOC Exceeded
Acute	14-day LC_{50}	180.4 mg a.i./kg soil dw (*Eisenia foetida*)	0.67	0.007	No
Chronic	30-day LC_{50}	17 mg a.i./kg soil (*O. apuanicus*)	0.67	0.08	No

Table 2 Screening-level risk assessment of atrazine foliar applications for pollinators (Honey bee – *Apis mellifera*)

Crop (rate)	Exposure	Exposure to bee (µg a.i./bee/day)	Endpoint (µg a.i./bee)	RQ	LOC Exceeded[1]
Corn and switchgrass (1.5 kg a.i./ha)	Adult acute contact	3.6	>97	<0.04	No
	Larvae oral acute	18.2	33	0.55	Yes
	Larvae oral chronic	18.2	6	3.0	Yes
Sorghum (1.0 kg a.i./ha)	Adult acute contact	2.4	>97	0.03	No
	Larvae oral acute	12.1	33	0.37	No
	Larvae oral chronic	12.1	6	2.0	Yes

[1] Level of concern (LOC) = 0.4 for acute risk to pollinators and 1.0 for chronic risk to pollinators.

Table 3 Risk assessment (on-field and off-field) for atrazine on terrestrial plants

Acute Exposure	Endpoint	EEC (g a.i./ha)	RQ	LOC Exceeded
Screening level risk				
Vegetative vigour	HR_{10}: 22.4 g a.i./ha Number of species used: 33	Corn and switchgrass: 1500	67	Yes
		Sorghum: 1000	45	
Seedling emergence	14-day ER_{25} of 2.8 g a.i./ha for lettuce (based on reduced dry weight).	Corn and switchgrass: 1500	536	Yes
		Sorghum: 1000	357	
Potential risk from spray drift[1]				
Vegetative vigour	HR_{10}: 22.4 g a.i./ha Number of species used: 33	Corn and switchgrass: 90	4.0	Yes
		Sorghum: 60	2.7	
Seedling emergence	14-day ER_{25} of 2.8 g a.i./ha for lettuce (based on reduced dry weight).	Corn and switchgrass: 90	32	Yes
		Sorghum: 60	21	

HR_{10} = Hazardous concentration to 10% of species based on ER_{25} values. Numbers in parentheses indicate the lower and upper two-sided 90% confidence interval of HR_5.
FA = fraction of species affected. This value reflects the lower and upper two-sided 90% confidence level of the proportion of species expected to be affected at the HR_{10} value
[1]Off-field EECs were determined based on an ASAE “medium” droplet size; the maximum amount of spray that is expected to drift 1m downwind from the application site during spraying is 6%.

Table 4 Screening level risk assessment for atrazine for birds and mammals at the highest foliar application rate on corn and switchgrass (1500 g a.i./ha)

Exposure	Toxicity (mg a.i./kg bw/d)	Feeding guild (food item)	EDE (mg a.i./kg bw)	RQ	LOC Exceeded
Small bird (0.02 kg)					
Acute	78.3	Insectivore	122	**1.6**	Yes
Reproduction	7.9	Insectivore	122	**15**	Yes
Medium sized bird (0.1 kg)					
Acute	78.3	Insectivore	95	**1.2**	Yes
Reproduction	7.9	Insectivore	95	**12**	Yes
Large sized bird (1 kg)					
Acute	78.3	Herbivore (short grass)	62	0.8	No
Reproduction	7.9	Herbivore (short grass)	62	**7.8**	Yes
Small sized mammal (0.015 kg)					
Acute	133	Insectivore	70	0.5	No
Reproduction	4.0	Insectivore	70	**18**	Yes
Medium sized mammal (0.035 kg)					
Acute	133	Herbivore (short grass)	136	**1.02**	Yes
Reproduction	4.0	Herbivore (short grass)	136	**34**	Yes
Large sized mammal (1.0 kg)					
Acute	133	Herbivore (short grass)	73	0.6	No
Reproduction	4.0	Herbivore (short grass)	73	**18**	Yes

Table 5 Avian risk assessment using maximum and mean atrazine residue values based on the single application rate for sorghum (1000 g a.i.ha), and corn and switchgrass (1500 g a.i./ha).

			Maximum nomogram residues				Mean nomogram residues			
			On-field		Off field		On-field		Off field	
Exposure	**Toxicity (mg a.i./kg bw/d)**	**Food guild (food item)**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**
Sorghum – 1000 g a.i./ha										
Small Bird (0.02 kg)										
Acute	78.30	Insectivore	81	**1.0**	4.9	0.1	56	0.7	3.4	<0.1
	78.30	Granivore (grain and seeds)	13	0.2	0.8	<0.1	6.0	<0.1	0.4	<0.1
	78.30	Frugivore (fruit)	NR	NR	1.5	<0.1	NR	NR	0.7	<0.1
Reproduction	7.90	Insectivore	81	**10**	4.9	0.6	56	**7.1**	3.4	0.4
	7.90	Granivore (grain and seeds)	13	**1.6**	0.8	0.1	6.0	0.8	0.4	<0.1
	7.90	Frugivore (fruit)	NR	NR	1.5	0.2	NR	NR	0.7	<0.1
Medium sized bird (0.1 kg)										
Acute	78.30	Insectivore	64	0.8	3.8	<0.1	44	0.6	2.6	<0.1
	78.30	Granivore (grain and seeds)	9.8	0.1	0.6	<0.1	4.7	<0.1	0.3	<0.1
	78.30	Frugivore (fruit)	NR	NR	1.2	<0.1	NR	NR	0.6	<0.1
Reproduction	7.90	Insectivore	64	**8.0**	3.8	0.5	44	**5.6**	2.6	0.3
	7.90	Granivore (grain and seeds)	9.8	**1.2**	0.6	0.1	4.7	0.6	0.3	<0.1
	7.90	Frugivore (fruit)	NR	NR	1.2	0.1	NR	NR	0.6	<0.1
Large sized bird (1 kg)										
Acute	78.30	Insectivore	19	0.2	1.1	<0.1	13	0.2	0.8	<0.1
	78.30	Granivore (grain and seeds)	2.9	<0.1	0.2	<0.1	1.4	<0.1	0.08	<0.1
	78.30	Frugivore (fruit)	NR	NR	0.3	<0.1	NR	NR	0.2	<0.1
	78.30	Herbivore (short grass)	41	0.5	2.5	<0.1	15	0.2	0.9	<0.1
	78.30	Herbivore (long grass)	25	0.3	1.5	<0.1	8.2	0.1	0.5	<0.1
	78.30	Herbivore (Broadleaf plants)	38	0.5	2.3	<0.1	13	0.2	0.8	<0.1
Reproduction	7.90	Insectivore	19	**2.3**	1.1	0.1	13	**1.6**	0.77	0.1

Exposure	Toxicity (mg a.i./kg bw/d)	Food guild (food item)	Maximum nomogram residues				Mean nomogram residues			
			On-field		Off field		On-field		Off field	
			EDE (mg a.i./kg bw)	RQ	EDE (mg a.i./kg bw)	RQ	EDE (mg a.i./kg bw)	RQ	EDE (mg a.i./kg bw)	RQ
	7.90	Granivore (grain and seeds)	2.9	0.4	0.2	<0.1	1.4	0.2	0.08	<0.1
	7.90	Frugivore (fruit)	NR	NR	0.3	<0.1	NR	NR	0.16	<0.1
	7.90	Herbivore (short grass)	41	**5.2**	2.5	0.3	15	**1.8**	0.87	0.1
	7.90	Herbivore (long grass)	25	**3.2**	1.5	0.2	8.2	**1.0**	0.49	<0.1
	7.90	Herbivore (Broadleaf plants)	38	**4.8**	2.3	0.3	13	**1.6**	0.75	0.1
Corn and switchgrass – 1500 g a.i./ha										
Small bird (0.02 kg)										
Acute	78.30	Insectivore	122	**1.6**	7.3	0.1	84	**1.1**	5.1	<0.1
	78.30	Granivore (grain and seeds)	19	0.2	1.1	<0.1	9.0	0.1	0.5	<0.1
	78.30	Frugivore (fruit)	NR	NR	2.3	<0.1	NR	NR	1.1	<0.1
Reproduction	7.90	Insectivore	122	**16**	7.3	0.9	84	**11**	5.1	0.6
	7.90	Granivore (grain and seeds)	19	**2.4**	1.1	0.1	9.0	**1.1**	0.5	<0.1
	7.90	Frugivore (fruit)	NR	NR	2.3	0.3	NR	NR	1.1	0.1
Medium sized bird (0.1 kg)										
Acute	78.30	Insectivore	95	**1.2**	5.7	<0.1	66	0.8	4.0	<0.1
	78.30	Granivore (grain and seeds)	15	0.2	0.9	<0.1	7.0	<0.1	0.4	<0.1
	78.30	Frugivore (fruit)	NR	NR	1.8	<0.1	NR	NR	0.8	<0.1
Reproduction	7.90	Insectivore	95	**12**	5.7	0.7	66	**8.3**	4.0	0.5
	7.90	Granivore (grain and seeds)	15	**1.9**	0.9	0.1	7.0	0.9	0.4	<0.1
	7.90	Frugivore (fruit)	NR	NR	1.8	0.2	NR	NR	0.8	0.1
Large sized bird (1 kg)										
Acute	78.30	Insectivore	28	0.4	1.7	<0.1	19	0.3	1.2	<0.1
	78.30	Granivore (grain and seeds)	4.3	0.1	0.3	<0.1	2.1	<0.1	0.1	<0.1
	78.30	Frugivore (fruit)	NR	NR	0.5	<0.1	NR	NR	0.3	<0.1
	78.30	Herbivore (short grass)	62	0.8	3.7	<0.1	22	0.3	1.3	<0.1
	78.30	Herbivore (long grass)	38	0.5	2.3	<0.1	12	0.2	0.7	<0.1
	78.30	Herbivore (Broadleaf plants)	57	0.7	3.4	<0.1	19	0.2	1.1	<0.1

			Maximum nomogram residues				Mean nomogram residues			
			On-field		Off field		On-field		Off field	
Exposure	**Toxicity (mg a.i./kg bw/d)**	**Food guild (food item)**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**
Reproduction	7.90	Insectivore	28	**3.5**	1.7	0.2	19	**2.4**	1.2	0.2
	7.90	Granivore (grain and seeds)	4.3	0.5	0.3	<0.1	2.1	0.3	0.1	<0.1
	7.90	Frugivore (fruit)	NR	NR	0.5	0.1	NR	NR	0.3	<0.1
	7.90	Herbivore (short grass)	62	**7.8**	3.7	0.5	226	**2.8**	1.3	0.2
	7.90	Herbivore (long grass)	38	**4.8**	2.3	0.3	12	**1.6**	0.7	<0.1
	7.90	Herbivore (Broadleaf plants)	57	**7.2**	3.4	0.4	19	**2.4**	1.1	0.1

NR - The fruit guild category is not relevant with respect to on-field feeding for the registered use pattern (sorghum, corn and switchgrass).
Bolded values indicate that the RQ exceeds the LOC.

Table 6 Mammalian risk assessment using maximum and mean atrazine residue values based on the single application rate for sorghum and (1000 g a.i.ha), and corn and switchgrass (1500 g a.i./ha).

					Maximum nomogram residues			Mean nomogram residues		
			On-field		Off field		On-field		Off field	
Exposure	**Toxicity (mg a.i./kg bw/d)**	**Food guild (food item)**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**
Sorghum – 1000 g a.i./ha										
Small mammal (0.015 kg)										
Acute	133	Insectivore	47	0.4	2.8	<0.1	32	0.2	1.9	<0.1
	133	Granivore (grain and seeds)	7.3	<0.1	0.4	<0.1	3.5	<0.1	0.2	<0.1
	133	Frugivore (fruit)	NR	NR	0.9	<0.1	NR	NR	0.4	<0.1
Reproduction	4.00	Insectivore	47	**12**	2.8	0.7	32	**8.1**	1.9	0.5
	4.00	Granivore (grain and seeds)	7.3	**1.8**	0.4	0.1	3.5	0.9	0.2	<0.1

			Maximum nomogram residues				Mean nomogram residues			
			On-field		Off field		On-field		Off field	
Exposure	**Toxicity (mg a.i./kg bw/d)**	**Food guild (food item)**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**
	4.00	Frugivore (fruit)	NR	NR	0.9	0.2	NR	NR	0.4	0.1
Medium sized mammal (0.035 kg)										
Acute	133	Insectivore	41	0.3	2.5	<0.1	28	0.2	1.7	<0.1
	133	Granivore (grain and seeds)	6.4	<0.1	0.4	<0.1	3.0	<0.1	0.2	<0.1
	133	Frugivore (fruit)	NR	NR	0.8	<0.1	NR	NR	0.4	<0.1
	133	Herbivore (short grass)	91	0.7	5.5	<0.1	32	0.2	1.9	<0.1
	133	Herbivore (long grass)	55	0.4	3.3	<0.1	18	0.1	1.1	<0.1
	133	Herbivore (forage crops)	84	0.6	5.0	<0.1	28	0.2	1.7	<0.1
Reproduction	4.00	Insectivore	41	**10**	2.5	0.6	28	**7.1**	1.7	0.4
	4.00	Granivore (grain and seeds)	6.4	**1.6**	0.4	<0.1	3.0	0.8	0.2	<0.1
	4.00	Frugivore (fruit)	NR	NR	0.8	0.2	NR	NR	0.4	<0.1
	4.00	Herbivore (short grass)	91	**23**	5.5	**1.4**	32	**8.1**	1.9	0.5
	4.00	Herbivore (long grass)	55	**14**	3.3	0.8	18	**4.5**	1.1	0.3
	4.00	Herbivore (Broadleaf plants)	84	**21**	5.0	**1.3**	28	**6.9**	1.7	0.4
Large sized mammal (1 kg)										
Acute	133	Insectivore	22	**1.6**	1.3	<0.1	15	**1.1**	0.9	<0.1
	133	Granivore (grain and seeds)	3.4	0.3	0.2	<0.1	1.6	0.1	0.1	<0.1
	133	Frugivore (fruit)	NR	NR	0.4	<0.1	NR	NR	0.2	<0.1
	133	Herbivore (short grass)	49	**3.6**	2.9	<0.1	17	**1.3**	1.0	<0.1
	133	Herbivore (long grass)	30	**2.2**	1.8	<0.1	9.7	0.7	0.6	<0.1
	133	Herbivore (Broadleaf plants)	45	**3.4**	2.7	<0.1	15	**1.1**	0.9	<0.1
Reproduction	4.00	Insectivore	22	**5.5**	1.3	0.3	15	**3.8**	0.9	0.2
	4.00	Granivore (grain and seeds)	3.4	0.8	0.2	<0.1	1.6	0.4	0.1	<0.1
	4.00	Frugivore (fruit)	NR	NR	0.4	0.1	NR	NR	0.2	<0.1
	4.00	Herbivore (short grass)	49	**12**	2.9	0.7	17	**4.3**	1.0	0.3
	4.00	Herbivore (long grass)	30	**7.4**	1.8	0.4	9.7	**2.4**	0.6	0.1
	4.00	Herbivore (Broadleaf plants)	45	**11**	2.7	0.7	15	**3.7**	0.9	0.2

					Maximum nomogram residues				Mean nomogram residues	
			On-field		Off field		On-field		Off field	
Exposure	**Toxicity (mg a.i./kg bw/d)**	**Food guild (food item)**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**
Corn and switchgrass – 1500 g a.i./ha										
Small mammal (0.015 kg)										
Acute	133	Insectivore	70	0.5	4.2	<0.1	48	<0.1	2.9	<0.1
	133	Granivore (grain and seeds)	11	<0.1	0.7	<0.1	5.2	<0.1	0.3	<0.1
	133	Frugivore (fruit)	NR	NR	1.3	<0.1	NR	NR	0.6	<0.1
Reproduction	4.00	Insectivore	70	**18**	4.2	**1.05**	48	**12**	2.9	0.7
	4.00	Granivore (grain and seeds)	11	**2.7**	0.7	0.2	5.2	**1.3**	0.3	<0.1
	4.00	Frugivore (fruit)	NR	NR	1.3	0.3	NR	NR	0.6	0.2
Medium sized mammal (0.035 kg)										
Acute	133	Insectivore	62	0.4	3.7	<0.1	43	0.3	2.6	<0.1
	133	Granivore (grain and seeds)	10	<0.1	0.6	<0.1	4.5	<0.1	0.3	<0.1
	133	Frugivore (fruit)	NR	NR	1.1	<0.1	NR	NR	0.6	<0.1
	133	Herbivore (short grass)	136	1.0	8.2	<0.1	48	0.4	2.9	<0.1
	133	Herbivore (long grass)	83	0.6	5.0	<0.1	27	0.2	1.6	<0.1
	133	Herbivore (forage crops)	126	0.9	7.6	<0.1	42	0.3	2.5	<0.1
Reproduction	4.00	Insectivore	62	**15**	3.7	0.9	43	**11**	2.6	0.6
	4.00	Granivore (grain and seeds)	10	**2.4**	0.6	0.1	4.5	**1.1**	0.3	<0.1
	4.00	Frugivore (fruit)	NR	NR	1.1	0.3	NR	NR	0.6	0.1
	4.00	Herbivore (short grass)	136	**34**	8.2	**2.0**	48	**12**	2.9	0.7
	4.00	Herbivore (long grass)	83	**21**	5.0	**1.2**	27	**6.8**	1.6	0.4
	4.00	Herbivore (Broadleaf plants)	126	**32**	7.6	**1.9**	42	**10**	2.5	0.6
Acute	133	Insectivore	33	**2.5**	1.97	<0.1	23	**1.7**	1.4	<0.1
	133	Granivore (grain and seeds)	5.1	0.4	0.31	<0.1	2.4	0.2	0.2	<0.1
	133	Frugivore (fruit)	NR	NR	0.61	<0.1	NR	NR	0.3	<0.1
	133	Herbivore (short grass)	73	**5.5**	4.37	<0.1	26	**1.9**	1.6	<0.1

					Maximum nomogram residues			**Mean nomogram residues**		
			On-field		**Off field**		**On-field**		**Off field**	
Exposure	**Toxicity (mg a.i./kg bw/d)**	**Food guild (food item)**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**	**EDE (mg a.i./kg bw)**	**RQ**
	133	Herbivore (long grass)	44	**3.3**	2.67	<0.1	15	**1.1**	0.9	<0.1
	133	Herbivore (Broadleaf plants)	67	**5.1**	4.04	<0.1	22	**1.7**	1.3	<0.1
Reproduction	4.00	Insectivore	33	**8.2**	1.97	0.5	23	**5.7**	1.4	0.3
	4.00	Granivore (grain and seeds)	5.1	**1.3**	0.31	<0.1	2.4	0.6	0.2	<0.1
	4.00	Frugivore (fruit)	NR	NR	0.61	0.2	NR	NR	0.3	<0.1
	4.00	Herbivore (short grass)	73	**18**	4.37	**1.1**	26	**6.5**	1.6	0.4
	4.00	Herbivore (long grass)	44	**11**	2.67	0.7	15	**3.6**	0.9	0.2
	4.00	Herbivore (Broadleaf plants)	67	**17**	4.04	**1.01**	22	**5.6**	1.3	0.3

NR - The fruit guild category is not relevant with respect to on-field feeding for the registered use pattern (sorghum, corn and switchgrass).
Bolded values indicate that the RQ exceeds the LOC.

Table 7 Summary of screening level risk of atrazine to aquatic organisms exposed at the highest single foliar application rate for corn (1500 g a.i./ha)

Organism	Exposure	Species	Endpoint value (µg a.i./L)	Endpoint for RA[1] (µg a.i./L)	EEC[2] (µg a.i./L)	RQ	LOC Exceeded
Freshwater species							
Invertebrate	Acute	Midge (*Chironomus tentans*)	48h LC_{50} = 720	360	188	0.5	No
	Chronic	Scud (*Gammarus fasciatus*)	30 day NOEC = 60	60	188	3.1	Yes
Freshwater fish	Acute	African Catfish (*Clarias gariepinus*)	96h LC_{50} = 350	35	188	5.4	Yes
	Chronic	Brook trout (*Salvelinus frontinalis*)	44 week NOEC = 65	65	188	2.9	Yes
Amphibians	Acute	American bullfrog *(Rana catesbaeiana)*	96h LC_{50} = 410	41	1000	24	Yes
	Chronic	Black-spotted frog tadpoles *(Pelophylax nigromaculatus*)	20–25 d NOEC	8.0	1000	125	Yes
Freshwater algae	Acute	Chlorophycean green algae (*Chlorella vulgaris*)	96h EC_{50} = 4.3	2.2	188	85	Yes
Freshwater vascular plant	Acute	Waterweed (*Elodea Canadensis*)	14d EC_{50} = 4.6	2.3	188	82	Yes
Freshwater aquatic community	Chronic	Community-level effect	NOEC	20	188	9.4	Yes
Estuarine and marine species							
Marine Invertebrate	Acute	Opossum shrimp (*Neomysis integer*)	48h LC_{50} = 48	24	188	7.8	Yes
	Chronic	Copepod (*Amphiascus tenuiremis*)	41d NOEC < 3.5	<3.5	188	>54	Yes
Marine fish	Acute	Sheepshead Minnow (*Cyprinodon variegatus*)	96h LC_{50} = 2000	200	188	0.9	No
	Chronic	Atlantic salmon (*Salmo salar*)	21d NOEC = 8.5	8.5	188	22	Yes

Organism	Exposure	Species	Endpoint value (µg a.i./L)	Endpoint for RA[1] (µg a.i./L)	EEC[2] (µg a.i./L)	RQ	LOC Exceeded
Marine algae	Acute	Chlorophycean green algae (Ankistrodesmus sp.)	96 h EC_{50} = 11.9	5.9	188	32	Yes
Marine vascular plant	Acute	Pondweed (*Potamogeton perfoliatus*)	28 d EC_{50} = 30	1.215	188	13	Yes

1 - Endpoints used in the acute exposure risk assessment (RA) are derived by dividing the EC_{50} or LC_{50} from the appropriate laboratory study by a factor of two (2) for aquatic invertebrates and plants, and by a factor of ten (10) for fish and amphibians.
2 - EEC based on direct overspray of a 15 cm deep water body for amphibians and a 80 cm deep water body for all other aquatic organisms.

Table 8 Spray drift assessment for non-target aquatic organisms

Organism	Exposure	Species	Endpoint value (µg a.i./L)	Endpoint for RA[1] (µg a.i./L)	Use and aplication rate (g a.i./ha)	EEC Exposure from drift[2] (µg a.i./L)	RQ	LOC Exceeded
Freshwater species								
Invertebrate	Chronic	Scud (*Gammarus fasciatus*)	30 day NOEC = 60	60	Sorghum - 1000	7.5	0.1	No
					Corn - 1500	11.3	0.2	
Freshwater fish	Acute	African Catfish (*Clarias gariepinus*)	96h LC_{50} = 350	35	Sorghum - 1000	7.5	0.2	No
					Corn - 1500	11.3	0.3	
	Chronic	Brook trout (*Salvelinus frontinalis*)	44 week NOEC = 65	65	Sorghum - 1000	7.5	0.1	No
					Corn - 1500	11.3	0.2	
Amphibians	Acute	American bullfrog *(Rana catesbaeiana)*	96h LC_{50} = 410	41	Sorghum - 1000	40	0.9	No
					Corn - 1500	60	1.5	Yes
	Chronic	Black-spotted frog tadpoles *(Pelophylax nigromaculatus)*	20–25 d NOEC	8.0	Sorghum - 1000	40	5.0	Yes
					Corn - 1500	60	7.5	
Freshwater algae	Acute	Chlorophycean green algae (*Chlorella vulgaris*)	96h EC_{50} = 4.3	2.2	Sorghum - 1000	7.5	3.4	Yes
					Corn - 1500	11.3	5.1	
Freshwater vascular plant	Acute	HC_5 value (SSD of EC_{50} values, n = 8)	HC_5 = 18.7	18.7	Sorghum - 1000	7.5	0.4	No
					Corn - 1500	11.3	0.6	
Freshwater aquatic community	Chronic	Community level effect	NOEC	20	Sorghum - 1000	7.5	0.4	No
					Corn - 1500	11.3	0.6	

Organism	Exposure	Species	Endpoint value (µg a.i./L)	Endpoint for RA[1] (µg a.i./L)	Use and aplication rate (g a.i./ha)	EEC Exposure from drift[2] (µg a.i./L)	RQ	LOC Exceeded
Estuarine and marine species								
Marine Invertebrate	Acute	Opossum shrimp (*Neomysis integer*)	48h LC_{50} = 48	24	Sorghum - 1000	7.5	0.3	No
					Corn - 1500	11.3	0.5	
Marine fish	Chronic	Atlantic salmon (*Salmo salar*)	21d NOEC = 8.5	8.5	Sorghum - 1000	7.5	0.9	No
					Corn - 1500	11.3	1.3	Yes
Marine algae	Acute	Chlorophycean green algae (*Ankistrodesmus sp.*)	96 h EC_{50} = 11.9	5.9	Sorghum - 1000	7.5	1.3	Yes
					Corn - 1500	11.3	1.9	
Marine vascular plant	Acute	HC_5 value (SSD of EC_{50} values, n = 23)	HC_5 = 16.5	16.5	Sorghum - 1000	7.5	0.5	No
					Corn - 1500	11.3	0.7	No

1 - Endpoints used in the acute exposure risk assessment (RA) are derived by dividing the EC_{50} or LC_{50} from the appropriate laboratory study by a factor of two (2) for aquatic invertebrates and plants, and by a factor of ten (10) for fish and amphibians.
2 - EEC based on a 15 cm water body depth for amphibians and a 80 cm water depth for all other aquatic organisms.

Table 9 Refined risk assessment of atrazine for aquatic organisms from predicted run-off resulting from foliar applications (acute and chronic exceedances of the LOC for each crop and region shown only)

Organism	Exposure	Species	Endpoint reported (µg a.i./L)	Endpoint for RA[1] (µg a.i./L)	Use rates[2] (g a.i./ha)	Region	EECs[3] (µg a.i./L)	RQ
Freshwater invertebrates	Chronic	Scud (*Gammarus fasciatus*)	30-day NOEC = 60	60	1 × 1488 or 1020 + 480 g a.i./ha @ 15-d on corn	QC	80	1.3
						Atlantic	123	2.1
					1 × 1488 g a.i./ha on switchgrass	QC	67	1.1
						Atlantic	133	2.2
Freshwater fish	Acute	African Catfish (*Clarias gariepinus*)	96-h LC_{50} = 350	35	1 × 1488 or 1020 + 480 g a.i./ha @ 15-d on corn	AB	47	1.3
						MB	40	1.1
						ON	58	1.7
						QC	82	2.3
						Atlantic	128	3.7
					1 × 1488 g a.i./ha on switchgrass	AB	63	1.8
						ON	53	1.5
						QC	69	2.0

Organism	Exposure	Species	Endpoint reported (µg a.i./L)	Endpoint for RA[1] (µg a.i./L)	Use rates[2] (g a.i./ha)	Region	EECs[3] (µg a.i./L)	RQ
						Atlantic	135	3.9
	Chronic	Brook trout (*Salvelinus frontinalis*)	44-week NOEC = 65	65	1 × 1488 or 1020 + 480 g a.i./ha @ 15-d on corn	QC	70	1.1
						Atlantic	119	1.8
					1 × 1488 g a.i./ha on switchgrass	Atlantic	115	1.8
Amphibians	Acute	American bullfrog *(Rana catesbaeiana)*	96-h LC_{50} = 410	41	1 × 1488 or 1020 + 480 g a.i./ha @ 15-d on corn	AB	205	5.0
						MB	185	4.5
						ON	267	6.5
						QC	345	8.4
						Atlantic	591	14
					1 × 1008 g a.i/ha on sorghum	ON	133	3.2
						QC	114	2.8
					1 × 1488 g a.i./ha on switchgrass	AB	195	4.8
						MB	112	2.7
						ON	231	5.6
						QC	263	6.4
						Atlantic	612	15
	Chronic	Black-spotted frog tadpoles *(Pelophylax nigromaculatus*)	20- and 25-d NOEC	8.0	1 × 1488 or 1020 + 480 g a.i./ha @ 15-d on corn	BC	11	1.4
						AB	179	22
						MB	168	21
						ON	235	29
						QC	313	39
						Atlantic	515	64
					1 × 1008 g a.i/ha on sorghum	ON	119	15
						QC	108	14
					1 × 1488 g a.i./ha on switchgrass	AB	195	24
						MB	104	13
						ON	205	26
						QC	264	33
						Atlantic	591	74
Freshwater algae	Acute	Chlorophycean green algae (*Chlorella vulgaris*)	96-h EC_{50} = 4.3	2.2	1 × 1488 or 1020 + 480 g a.i./ha @ 15-d on corn	BC	2.5	1.1
						AB	47	21
						MB	40	18
						ON	58	26
						QC	82	37
						Atlantic	128	58
					1 × 1008 g a.i/ha on sorghum	ON	29	13
						QC	27	12
					1 × 1488 g a.i./ha on	AB	63	29
						MB	32	15

Organism	Exposure	Species	Endpoint reported (µg a.i./L)	Endpoint for RA[1] (µg a.i./L)	Use rates[2] (g a.i./ha)	Region	EECs[3] (µg a.i./L)	RQ
					switchgrass	ON	53	24
						QC	69	31
						Atlantic	135	61
Freshwater vascular plant	Acute	HC_5 value (SSD of EC_{50} values, n = 8)	HC_5 = 18.7	18.7	1 × 1488 or 1020 + 480 g a.i./ha @ 15-d on corn	AB	47	2.5
						MB	41	2.2
						ON	58	3.1
						QC	82	4.4
						Atlantic	129	6.9
					1 × 1008 g a.i/ha on sorghum	ON	29	1.6
						QC	27	1.4
					1 × 1488 g a.i./ha on switchgrass	AB	64	3.4
						MB	32	1.7
						ON	53	2.8
						QC	70	3.7
						Atlantic	136	7.3
Freshwater aquatic community	Chronic	Community level effect	NOEC	20	1 × 1488 or 1020 + 480 g a.i./ha @ 15-d on corn	AB	47	2.4
						MB	41	2.1
						ON	58	2.9
						QC	82	4.1
						Atlantic	129	6.5
					1 × 1008 g a.i/ha on sorghum	ON	29	1.5
						QC	27	1.4
					1 × 1488 g a.i./ha on switchgrass	AB	64	3.2
						MB	32	1.6
						ON	53	2.7
						QC	70	3.5
						Atlantic	136	6.8
Marine Invertebrate	Acute	Opossum shrimp (*Neomysis integer*)	48-h LC_{50} = 48	24	1 × 1488 or 1020 + 480 g a.i./ha @ 15-d on corn	QC	82	3.4
						Atlantic	128	5.3
					1 × 1008 g a.i/ha on sorghum	QC	27	1.1
					1 × 1488 g a.i./ha on switchgrass	QC	70	2.9
						Atlantic	136	5.7
Marine fish	Chronic	Atlantic salmon (*Salmo salar*)	21-d NOEC = 8.5	8.5	1 × 1488 or 1020 + 480 g a.i./ha @ 15-d on corn	QC	80	9.4
						Atlantic	123	15
					1 × 1008 g a.i/ha on sorghum	QC	26	3.1
					1 × 1488 g a.i./ha on	QC	67	7.9
						Atlantic	133	16

Organism	Exposure	Species	Endpoint reported (µg a.i./L)	Endpoint for RA[1] (µg a.i./L)	Use rates[2] (g a.i./ha)	Region	EECs[3] (µg a.i./L)	RQ
					switchgrass			
Marine algae	Acute	Chlorophycean green algae (Ankistrodesmus sp.)	96-h EC_{50} = 11.9	5.9	1 × 1488 or 1020 + 480 g a.i./ha @ 15-d on corn	QC	82	14
						Atlantic	128	22
					1 × 1008 g a.i/ha on sorghum	QC	27	4.6
					1 × 1488 g a.i./ha on switchgrass	QC	69	12
						Atlantic	135	23
Marine vascular plant	Acute	HC_5 value (SSD of EC_{50} values, n = 23)	HC_5 = 16.5	16.5	1 × 1488 or 1020 + 480 g a.i./ha @ 15-d on corn	QC	82	5.0
						Atlantic	128	7.8
					1 × 1008 g a.i/ha on sorghum	QC	27	1.6
					1 × 1488 g a.i./ha on switchgrass	QC	69	4.2
						Atlantic	135	8.2

[1] Endpoints used in the acute exposure risk assessment (RA) are derived by dividing the EC_{50}, LC_{50} from the appropriate laboratory study by a factor of two (2) for aquatic invertebrates and plants, and by a factor of ten for fish and amphibians. The HC_5 is the 5th percentile of the species sensitivity distribution for the LC_{50} or NOEC at 50% confidence intervals.
[2] Application rate represents the maximum single applications rates as indicated on labels.
[3] EEC based on a 15-cm water body depth for amphibians and a 80-cm water depth for all other aquatic organisms as determined by the Pesticide in Water Calculator (PWC) model (v.1.52).

Table 10 Atrazine in Canadian surface water relevant to the aquatic risk assessment between 2006 and 2019

Province	Sampling year	Number of samples	Number of detections	Maximum concentration[1] (µg/L)	Range of detection limits (µg/L)
AB	2006	817	1	0.036	0.005–0.025
	2007	765	0	0.0125	0.005–0.02502
	2008	362	0	0.0025	0.00186–0.005
	2009	145	2	0.012	0.00186–0.005
	2010	145	2	0.0111	0.00186–0.005
	2011	498	4	0.383	0.00186–0.05
	2012	563	4	0.528	0.005–0.05
	2013	670	6	0.154	0.00186–0.023
	2014	542	31	0.167	0.00186–0.0254
	2015	535	0	0.0122	0.00186–0.0245
	2016	348	3	0.087	0.002–0.025
	2017	132	0	0.00255	0.002–0.0051
	2018	128	1	0.0025	0.002–0.005

Province	Sampling year	Number of samples	Number of detections	Maximum concentration[1] (µg/L)	Range of detection limits (µg/L)
	2019	129	3	0.0025	0.002–0.005
BC	2006	29	29	0.0576	0.0001
	2007	20	14	0.156	0.000157–0.00112
	2008	39	20	0.0346	0.000272–0.00326
	2009	26	15	0.018	0.000845–0.00346
	2010	26	11	0.00756	0.000825–0.00508
	2012	4	2	0.00204	0.000781–0.00187
	2013	59	43	0.153	0.000336–0.0027
	2014	20	9	0.0157	0.000717–0.00116
	2015	1	0	0.000617	0.0001
	2016	8	8	0.00143	0.0001
MB	2006	273	21	0.147	0.00186–0.1
	2007	407	26	0.663	0.00576–0.1
	2008	476	32	0.511	0.00186–0.1
	2009	301	28	0.27	0.00186–0.1
	2010	262	18	0.56	0.00186–1
	2011	624	31	0.5	0.00186–1
	2012	394	25	0.237	0.00186–0.1
	2013	144	28	1.2	0.00186–0.1
	2014	101	30	0.4	0.00186–0.1
	2015	56	23	0.24	0.0002–1
	2016	82	31	1.04	0.0002–10
	2017	76	33	0.51	0.0002–0.1
	2018	59	28	1.22	0.1
	2019	33	33	0.947	NR
	NR	2	0	0.05	0.1
NB	2006	63	0	0.5	0.5–1
	2008	269	6	0.19	0.02–0.5
	2009	10	0	0.025	0.05
	2013	34	2	0.47	0.05
	2014	36	2	0.21	0.05–0.06
	2015	24	0	0.025	0.05
NFL	2013	2	0	0.025	0.05
NS	2008	32	0	0.04	0.02–0.08
	2009	23	10	0.33	0.05
	2013	34	2	0.08	0.05
	2014	26	6	0.07	0.05–0.06
	2015	30	8	0.13	0.05
	2016	18	14	0.0426	0.0051
ON	2006	283	138	6.63	0.00576–0.1

Province	Sampling year	Number of samples	Number of detections	Maximum concentration[1] (µg/L)	Range of detection limits (µg/L)
	2007	338	138	6.3	0.00034–0.1
	2008	382	76	4.82	0.00034–0.1
	2009	297	71	1.3	0.00186–0.1
	2010	153	69	3	0.00186–0.1
	2011	226	42	13	0.00186–0.1
	2012	135	74	11	0.00186–0.1
	2013	129	36	4.6	0.1
	2014	113	40	6.8	0.05–0.1
	2015	107	39	18	0.00186–0.1
	2016	141	32	2.4	0.006–0.07
	2017	133	23	13	0.05–0.07
	2018	137	20	3.8	0.07
	2019	141	20	18	0.07
	NR	2	0	0.035	0.07
PEI	2008	116	0	00.04	0.04–0.08
	2009	30	2	0.06	0.03–0.05
	2010	12	0	0.015	0.03
	2011	12	0	0.015	0.03
	2012	12	0	0.015	0.03
	2013	38	0	0.025	0.03–0.05
	2014	52	0	0.03	0.03–0.06
	2015	36	0	0.025	0.03–0.05
	2016	24	0	0.015	0.0051–0.03
	2017	45	0	0.015	0.03
	2018	54	0	0.015	0.025–0.03
QC	2006	407	296	9	0.02–0.03
	2007	289	256	6.7	0.01–0.03
	2008	257	233	8.5	0.01–0.02
	2009	176	174	7.2	0.02
	2010	256	204	10	0.02
	2011	220	193	11	0.02
	2012	310	222	11	0.01–0.02
	2013	408	252	37	0.01–0.02
	2014	303	281	13	0.01–0.02
	2015	318	309	8.5	0.01–0.02
	2016	231	217	15	0.01–0.02
	2017	330	179	7.9	0.004–0.1
	2018	348	221	1.1	0.01

Province	Sampling year	Number of samples	Number of detections	Maximum concentration[1] (µg/L)	Range of detection limits (µg/L)
SK	2006	22	1	0.00613	0.00576
	2007	31	0	0	0.00576
	2008	24	1	0.00262	0.00576
	2009	29	4	0.0155	0.00186
	2010	30	5	0.00625	0.00186
	2011	30	1	0.00297	0.00186
	2012	23	4	0.00374	0.00186
	2013	40	4	0.00394	0.00186
	2014	29	8	0.00692	0.00186
	2015	32	2	0.00601	0.00186–0.0051
	2016	21	1	0.00755	0.0002–0.0051
	2017	51	2	0.00545	0.0051
	2018	47	1	0.00715	0.0051
	2019	27	0	0.00255	0.0051
Canada	2009	104	70	0.0938	0.005
	2010	84	26	0.1655	0.005
Grand total	**-**	**17527**	**4633**	**37**	**-**

NR = not reported

[1]A value equal to half the limit of detection was assigned to non-detects.

Table 11 Comparison of water monitoring data results with the effects metrics for freshwater organisms

	Invertebrates		Fish		Aquatic vascular plants	Algae and aquatic vascular plants	Amphibians	
Exposure	**Acute**	**Chronic**	**Acute**	**Chronic**	**Laboratory**	**Mesocosm**	**Acute**	**Chronic**
Effects metric	48-h LC_{50} of 720 µg/L ÷ 2	30-d NOEC	96-h LC_{50} of 350 µg/L ÷ 10	44-week NOEC	7-d HC_5	NOEC, 12-d to 136-d tests, 8 studies, concentrations mainly based on nominal concentrations and not maintained	4-d LC_{50} of 410 µg/L ÷ 10	25-d NOEC, nominal concentrations, but daily renewal of concentrations over the duration of the study
Effects metric value (µg/L)	360	60	35	65	18.7	20	41	8
N samples with concentrations exceeding the effects metric	0	0	1	0	1	1	0	18
% of samples with concentrations exceeding the effects metric	0	0	0.01	0	0.01	0.01	0.00	0.10
N sites with detections exceeding effects metric	0	0	1	0	1	1	0	12
Risk quotient based on single maximum concentration measured (may not be appropriate for comparison with chronic effects metrics)	0.10	0.62	1.06	0.57	1.98	1.85	0.90	4.63

Table 12 Summary for sites with exceedances of the chronic effects metric for amphibians (25-d NOEC of 8 µg/L)

Site	Years of monitoring available at this site	Years exceeding compared to years of monitoring	Year exceedance was observed	Maximum 25-day (approx.) moving average, in µg/L	Timeframe for moving average (days)	Number of samples included in the average calculation	Maximum risk quotient using 25-d average	Maximum risk quotient calculated using single highest detect in the absence of a longer- term average
Ruisseau Rousse, QC	5	1	2011	1.123	24	8	0.14	
Rivière Chibouet, QC	13	4	2006	2.765	25	12	0.35	
			2008	2.951	25	12	0.37	
			2011	2.528	24	8	0.32	
			2013	2.659	24	8	0.33	
Rivière l'Adadie, QC	1	1	2013	9.665	21	4	1.21	
Ruisseau Déversant-du-Lac, QC	4	1	2016	2.291	24	8	0.29	
Rivière des Hurons, QC	13	2	2012	2.707	21	7	0.34	
			2013	2.38	23	7	0.30	
Rivière Saint-Régis, QC	13	1	2015	1.556	25	8	0.19	
Rivière Saint-Zéphirin, QC	13	2	2010	3.118	27	9	0.39	
			2014	2.86	26	6	0.36	
Otter Creek, ON	13	2	2011	not calculated	49 (too long to calculate)	would have been 2	not calculated	1.25
			2019	9.018	34	2	1.13	
Reynolds Creek, ON	13	1	2011	not calculated	49 (too long to calculate)	would have been 2	not calculated	1.63
McGregor Creek, ON	9	1	2012	5.71	28	2	0.71	
McKillop Drain, ON	8	1	2015	9.018	29	2	1.13	
Decker Creek, ON	9	1	2017	6.735	28	2	0.84	

Appendix IX Toxicity to terrestrial and aquatic organisms

Terrestrial organisms

Table 1 Atrazine toxicity endpoints for soil dwelling invertebrates

Species	Exposure	Toxicity value	Comments	Reference
Earthworm (*Eisenia fetida*)	Acute	14-d LC_{50} = 273–926 mg a.i./kg soil	Spiked soil study. Endpoints included mortality and body mass. Haque and Ebing 1983 (ECOTOX No. 40493)	USEPA 2016 review (PMRA# 3253945)
Earthworm (*Eisenia fetida*)	Acute	7-d LC_{50} = 204.8 mg a.i./kg soil 14-d LC_{50} = 180.4 mg a.i./kg soil	Artificial soil tests following OECD 207, 1984 (atrazine – 95% purity). Acute study results were also reported in Wang et al., 2016 – PMRA# 3194298.	Yu et al., 2019 (PMRA# 3194295)
Oligochaeta (*Enchytraeus cryptus*)	Chronic	Results based on technical: (mg a.i./kg soil) Avoidance test: EC_{10} = 14 EC_{50} = 101 Reproductive test (28 day): Survival EC_{10} and EC_{50} ≥ 400 Repro EC_{10} = 11; EC_{50} = 161 Full life cycle (46 days): Hatching EC_{10} = 11; EC_{50} = 208 Survival EC_{10} = 125; EC_{50} = 252 Repro EC_{10} = 95; EC_{50} = 236 Results based on formulation (Gesaprim): Avoidance test: EC_{10} = 11 EC_{50} = 148 Reproductive test (28 day): Survival and repro EC_{10} and EC_{50} ≥ 400 Full life cycle (46 days): Hatching –ND Survival EC_{10} = 378; ND Repro EC_{10} = 206; EC_{50} = 436	Conducted with nanoformulation of atrazine, atrazine technical and a commercial formulation (Gesaprim® 500 CG, 50% m/v atrazine a.i.) Atrazine is not registered in Canada in nano-encapsulated form; toxicity results shown are based on technical and formulated product (Gesaprim) only. ND = Not Determined	Gomes S. et al., 2019 (PMRA# 3194296)
Earthworm (*Aporrectodea caliginosa)*	Chronic	28-d LC_{50} = 381 mg a.i./kg soil	Spiked soil study. Endpoints included mortality and body mass. Mosleh et al., 2003 (Ecotox No. 77549)	USEPA 2016 review (PMRA# 3253945)
Springtails (Collembola: *Onychiurus apuanicus* and *Onychiums armatus*)	Chronic	30-d LC_{50} values: **17.2** mg a.i./kg soil (*O. apuanicus*) 20 mg a.i./kg soil (*O. armatus*) LOAEC = 2.5 - 20 mg a.i./kg soil (based on mortality)	Exposure occurred via treated soil. Mortality was 18% for *O. apuanicus* at 2.5 mg a.i./kg soil. Mortality was 51% for *O. armatus* at 20 mg a.i./kg soil. Lower concentrations were not tested. Control mortality was 0%. Mola et al., 1987 (PMRA# 3194293)	USEPA 2016 review (PMRA# 3253945)
Micro arthropods	Field study	NOAEC = 0.9 lb/acre (1.0 kg/ha)	Field application of 1 kg/ha; atrazine was not associated with adverse effects. Cortet et al., 2002 (Ecotox No. 75784)	USEPA 2016 review (PMRA# 3253945)
	Field	NOAEC = 2 kg/ha	Field study testing several species	

Species	Exposure	Toxicity value	Comments	Reference
	study	LOAEC = 6 kg/ha	of micro arthropods. It could not be determined if reduced abundance was caused by migration (repellency), by toxic effects, or both. Fratello et. al., 1985 (Ecotox No. 59428)	
Earthworm, wireworm, springtail	Field study	LOAEC = 8 lb/acre (9.0 kg/ha)	Field study examining the impacts of several herbicides on soil invertebrate populations. The endpoint measured was abundance of several species. Fox 1964 (Ecotox No. 36668)	

Table 2 Atrazine toxicity endpoints for pollinators

Species	Exposure	Toxicity value	Comments	Reference
Honey bee (*Apis mellifera*)	Acute contact	LD_{50} > 97 µg a.i./bee	5% mortality occurred at the highest dose tested (97 µg a.i./bee). MRID 00036935	USEPA 2016 review (PMRA# 3253945)
	Acute oral	72-h LD_{50} = 33 µg a.i./larva	Bee larvae were exposed to a single exposure of Aatrex 4L (atrazine formulation A8566A).	PMRA# 3242965
	Chronic oral	8 –d NOED = 6 µg a.i./larva (survival – larval and pupal) 22 -d NOED = 6 µg a.i./larva (emergence)	Bee larvae were exposed to repeated exposure of Aatrex 4L (atrazine formulation A8566A).	PMRA# 3242964

Table 3 Atrazine toxicity endpoints for beneficial and predatory arthropods

Species	Exposure	Toxicity value[1]	Comments	Reference
Ground Beetle (*Poecilus versicolor)*	Acute	NOAEC ≥ 8 lbs a.i./Acre (8.97 kg a.i./ha)	Spiked soil study; no effects occurred at any level tested. Kegel, 1989 (Ecotox No. 64007)	Reported in USEPA 2016 review (PMRA# 3253945)
Ground Beetle (*Poecilus cupreus*)	Acute	NOAEC ≥ 0.8 lbs a.i./Acre (0.9 kg a.i./ha)	Spiked soil study; no effects occurred at any level tested. Kegel, 1989 (Ecotox No. 64007)	
Ground Beetle (*Poecilus lepidus*)	Acute	NOAEC ≥ 0.8 lbs a.i./Acre (0.9 kg a.i./ha)	Spiked soil study; a 25% reduction in survival was observed at the highest level tested that was not statistically significant. Kegel, 1989 (Ecotox No. 64007)	
5 species of carabid beetles	Acute	NOAEC = 2 lbs a.i./Acre (2.24 kg a.i./ha)	Beetles were dipped in atrazine solution then placed in treated soil (2.24 kg a.i./Acre); a transient repellency effect occurred for 6 days after treatment. Brust, 1990 (Ecotox No. 70406)	
Rove beetle	Acute	NOAEC: The single level tested was intended to approximate practical field application rates (not reported).	Exposure occurred via sprayed sand; “no measurable effect” occurred. Samsoe-Petersen, 1995 (Ecotox No. 63490)	
Fruit flies Drosphilia	Not reported	NOAEC = 15 µg a.i./fly	No increased mortality occurred in groups exposed to atrazine alone relative to controls. Lichtenstein et al., 1973 (Ecotox No. 2939)	
mealworm beetles (*Tenebrio molitor*)	Acute	NOEC > 200 µg a.i./L	No observable effect on fecundity or larval body mass (NOEC > 200 µg a.i./L).	McCallum et al., 2013 (PMRA# 3262462)

Species	Exposure	Toxicity value[1]	Comments	Reference
Micro arthropods	Field study	NOAEC > 1 kg a.i./ha	Field application of 1 kg/ha; atrazine was not associated with adverse effects. Cortet et al., 2002 Ecotox No. 75784	Reported in USEPA 2016 review (PMRA# 3253945)
Micro arthropods	Field study	NOAEL = 2 kg/ha (1.05 ppm); LOAEC = 6 kg/ha (3.15ppm)	Field study testing several species of microarthopods. It could not be determined if reduced abundance was caused by migration (repellency), by toxic effects, or both. Fratello et. al., 1985 Ecotox No. 59428	Reported in USEPA 2016 review (PMRA# 3253945)
Arthropod populations	Field study	NOAEC > 1.12 kg a.i./ha	The effect of atrazine on various arthropod populations in St. Augustine grass was investigated following two applications at 1.12 kg a.i./ha (3-week interval). Atrazine applications (Aatrex 4L) had no significant short- or long-term effects (1- and 2-months) on population's densities of ants, chinch bugs, leafhoppers, planthoppers and spiders in St. Augustine grass. Exposure concentrations in the plots were not verified and there were no replicates of the treatment groups to validate the results.	Cherry and Rainbolt, 2009 (PMRA# 3253957)

1 – Endpoints in **bold** considered in the risk assessment.

Table 4 Summary of avian acute toxicity data for atrazine

Species	Product (% a.i.)	Endpoint (mg a.i./kg bw)	Comment	Reference
Northern bobwhite quail (*Colinus virginianus*)	Atrazine technical (purity not reported)	LD_{50} = 783	Conducted with 14-day old chicks and study only conducted for 8 days. Considered acceptable as no deaths occurred after the 4th day. MRID 0024721, Fink (1976).	PMRA# 1235079; Reported in USEPA 2016 review (PMRA# 3253945)
Mallard Duck (*Anas platyrhynchos*)	80 WP (76% a.i.)	LD_{50} > 2000	6-months old; 14-day test. Listed as supplemental by USEPA (only 3 birds; formulation); MRID 001600-00, Hudson et al., (1984).	Reported in USEPA 2016 review (PMRA# 3253945)
Ring-necked Pheasant (*Phasianus colchicus*)	80 WP (76% a.i.)	LD_{50} > 2000	3-months old; 14-day test. Listed as supplemental by USEPA (only 3 birds; formulation); MRID 001600-00, Hudson et al., (1984).	Reported in USEPA 2016 review (PMRA# 3253945)
Japanese quail (*Coturnix c. Japonica*)	Atrazine technical	LD_{50} = 4237	50-60 days old; 14-day test. Listed as supplemental by USEPA (species not native); MRID 00247-22, Sachsse and Ullman (1974).	Reported in USEPA 2016 review (PMRA# 3253945)

Table 5 Summary of avian acute toxicity data for DIA, HA and DEA

Species	Product (% a.i.)	Endpoint (mg a.i./kg bw)	Comment	Reference
Northern bobwhite quail (*Colinus virginianus*)	Deisopropyl atrazine (DIA)	LD_{50} > 2000	18-week-old chicks; 14-day test. USEPA study classification: Acceptable. MRID 465000-07, Stafford 2005a, PMRA# 2816896 (USEPA DER – PMRA# 2816897)	USEPA 2016 review (PMRA# 3253945)
Northern bobwhite quail (*Colinus virginianus*)	Hydroxy atrazine (HA); 97%	LD_{50} >2000	17-week-old chicks; 14-day test. USEPA study classification: Acceptable. MRID 465000-08, Stafford 2005b, PMRA# 2816894 (USEPA DER – PMRA# 2815895)	USEPA 2016 review (PMRA# 3253945)

Species	Product (% a.i.)	Endpoint (mg a.i./kg bw)	Comment	Reference
	Desethyl atrazine (DEA); 96%	LD_{50} = 768	16-week-old chicks; 14-day test. USEPA study classification: Acceptable. MRID 465000-09, Stafford 2005c, PMRA# 2816892 (USEPA DER – PMRA# 2816893).	

Table 6 Summary of subacute dietary avian toxicity data for atrazine

Species	% a.i.	5-day LC_{50} (mg a.i./kg diet)	Comment	Reference
Northern bobwhite quail (*Colinus virginianus*)	99	>5000	9-day-old chicks. No mortality observed. Classified as supplemental by the USEPA (no control raw data). MRID 000229-23, Hill et al., 1975.	Reported in USEPA 2016 review (PMRA# 3253945)
	technical	>10,000	Young adults. Classified as supplemental by the USEPA (adult birds and no raw data). Gulf South Gough and Shellenberger, 1972.	
	80% WP	5760	6-week-old chicks. Classified as supplemental by the USEPA (birds too old). MRID 000592-14, Beliles and Scott, 1965.	
Ring-necked Pheasant (*Phasianus colchicus*)	99%	>5000	10-day-old chicks. No mortalities observed. Classified as supplemental by the USEPA (no control raw data). MRID 000229-23, Hill et al., 1975.	
Japanese quail (*Coturnix c. Japonica*)	99	>5000 (7% mortality at 5000)	7-day-old chicks. 7% mortality at 5000. Classified as supplemental by the USEPA (no raw data and species not native).	
Mallard duck (*Anas platyrhynchos*)	99	>5000	10-day-old ducklings. 30% mortality at 5000. Classified as supplemental by the USEPA (no control raw data). MRID 000229-23, Hill et al., 1975.	
	80% WP	19560	Classified as acceptable for 80W formulation by the USEPA. MRID 000592-14, Beliles and Scott, 1965.	

Table 7 Summary of avian reproduction effects for atrazine

Species	% a.i.	NOEC/ LOEC (mg a.i./kg diet)	Comments	Reference
Northern bobwhite (*Colinus virginianus*) 20 weeks old	97.1	NOEC = 225 LOEC = 675	Based on reduced egg production, embryo viability and a reduction in weight gain in males. USEPA notes that the number of cracked eggs in the control was about three times the accepted threshold noted in OCSPP 850.2300 guideline. MRID 425471-02, Pedersen and Ducharme 1992.	USEPA 2016 review (PMRA# 3253945)
Mallard duck (*Anas platyrhynchos*) 20 weeks	97.1	NOEC = 75 LOEC = 225	Based on reduced number of eggs laid per pen. NOEL = 7.9 mg a.i./kg bw/day[2]	USEPA 2016 review (-PMRA# 3253945); Pedersen et al., 1992 (PMRA# 3242968)

Species	% a.i.	NOEC/ LOEC (mg a.i./kg diet)	Comments	Reference
Avian reproduction/Growth effects tests from open literature				
Male Japanese quail (*Coturnix c. Japonica*)	99.1	LOEC = 1000	Seven separate studies were conducted. Dietary concentrations ranged from 10 to 1000 ppm. Animals were approximately 6-week-old males. Endpoints evaluated: growth, liver effects, sexual maturation, and anti-estrogenic effects. Exposure duration was up to 4 weeks. In addition, studies using SC administration and silastic implants were also conducted that evaluated endpoints including growth, liver effects, testes weight, and circulating LH levels. Doses up to 10 mg/kg-bw were tested. At 1000 ppm, there was a reduction in growth rate and food intake and an elevation in testosterone levels, although the reduction in testosterone levels was not consistently observed across studies. Other statistically significant observations were considered spurious and not related to atrazine treatment. The study is classified as qualitative by the USEPA. Wilhelms et al., 2005.	USEPA 2016 review (PMRA# 3253945)
Female Japanese quail (*Coturnix c. Japonica*)	99.1	LOEC = 1000	Birds were exposed to dietary concentrations that ranged from 1 ppm to 1000 ppm. Endpoints evaluated: growth, food intake, liver, ovary, oviduct weight, plasma luteinizing hormone and estradiol levels. Exposure was up to 4 weeks. Growth, food intake, liver weight and circulating estradiol levels were significantly reduced in birds exposed to atrazine at 1000 ppm but not at lower levels. The study is classified as qualitative by the USEPA; a lower reproductive LOEC is reported for the Mallard duck (225). Wilhelms et al., 2006a.	
Japanese quail (*Coturnix c. Japonica*)	Purity not reported	NOEC = 1000	Birds were exposed to dietary concentrations that ranged from 0.001 to 1000 ppm. No effects on body weight, food intake, mortality, circulating corticosterone levels, or weights of liver, ovaries or oviducts. Wilhelms et al., 2006b.	
Japanese quail (*Coturnix c. Japonica*)	Formulated product (purity not reported)	NOEC = 10 (based on reduced body weight)	Male birds were orally dosed (crop tubing) at concentrations of 10–500 mg/kg bw for 45 days. Feed consumption was reduced at 100–500 ppm and body weight reduced at 25–500 ppm. At ≥ 50 ppm decreases in leukocyte counts are reported. No definitive reproductive endpoints, for the purpose of risk assessment, were measured. The exposure period is considered representative of sub-chronic dietary exposure; however, the dosing method (oral) is not considered relevant to sub-acute or sub chronic dietary toxicity testing. The study is classified as qualitative by the USEPA. A full citation for Hussain et al. (2011) was not found in the 2016 USEPA review. Hussain et al., 2011.	

[2] NOEL estimated using combined mean of male and female adult mallard body weights and feed consumption values from week 1 to week 20. Feed consumption was reported as combined value for male and female consumption (calculations provided in “ATR EAD Calculated Endpoints” – PMRA# 3256798).

Table 8 Toxicity of atrazine to mammals

Species	Purity/Group Size/Exposure	Toxicity endpoint	Result comments
Acute (parent)			
Rat (*Rattus norvegicus*)	Atrazine (purity not reported)	LD_{50} = 1869 mg a.i./kg bw	Listed in USEPA 2016 (PMRA# 2741498; study MRID 00024706).
Sprague-Dawley rat	Purity = 97.7%; 2-5.5 g/kg BW; 5/sex/group	LD_{50} = 3520 mg a.i./kg bw	Clinical signs of toxicity: piloerection, reduced activity and salivation Low toxicity
Oral toxicity - rat 5/sex/group	2150, 3100 mg/kg BW	LD_{50} > 3100 mg/kg bw	Low toxicity

Species	Purity/Group Size/Exposure	Toxicity endpoint	Result comments
Oral toxicity - rat	Nor reported	LD_{50} = 2850 mg/kg bw (both sexes combined)	Low toxicity
Oral toxicity - Tif. Mag mice	1670–6000 mg/kg BW	LD_{50} = 3992 (3557-4479) mg/kg bw (both sexes combined)	Signs: sedation, ataxia, diarrhoea, polyuria, ptosis, salivation, dyspnoea, curved body posture, ruffled hair. Low toxicity
Oral toxicity - HSD (ICR) % mice 15/group	0, 1332, 444 mg/kg b w 97.7% pure	LD_{50} > 1332 mg/kg BW	Signs: sedation, ataxia, body tremors, polyuria, ptosis, sensitivity to touch. Slight toxicity
Acute (transformation products)			
Acute Oral (gavage) SD rat	DACT Not reported	LD_{50} > 5050 mg/kg BW (♂) LD_{50} > 5550 mg/kg BW (♀)	Deaths up to twelve days post dosing was recorded. Signs: piloerection, reduced activity and salivation, up to day 15 post dosing Low toxicity
Acute oral toxicity Sprague-Dawley rat PMRA# N/A	DIA Purity = N/A	LD_{50} = 2290 (1880-2800) mg/kg (♂); LD_{50} = 810 (338-1940) mg/kg (♀)	Signs: piloerection, reduced activity and salivation. Moderate toxicity
Oral toxicity-Sprague-Dawley rat PMRA# N/A	DEA Purity = 95.7%	LD_{50} = 1890 (1440-2480) mg/kg (♂) LD_{50} = 600 (496-898) mg/kg (♀)	Signs: piloerection, reduced activity and salivation. Survivors normal by day 8 post dosing Moderate toxicity
Acute Oral Toxicity Sprague-Dawley rat PMRA# N/A	HA Purity = not stated 5/sex 5050 mg/kg bw	LD_{50} > 5050 mg/kg bw (♂/♀)	Low toxicity
Chronic (developmental/reproductive)			
2-generation reproductive toxicity (Diet) SD rats PMRA# 1233367, 1233368 Unpublished study Mainiero et al., 1987 Published study PMRA# 2816056, 2816783 DeSesso et al., 2014 (ATR2)	Purity = 97.6% 30/sex/group 0, 10, 50, 500 ppm (= 0, 0.72/0.82, 3.6/4.0, 36/41 mg/kg/d in P♂/♀)	Reported in USEPA 2016 (PMRA# 2741498; study MRID 4043136). Parental Toxicity: NOAEL = 3.6/4.0 mg/kg bw/d (♂/♀) 36/41 mg/kg bw/d: ↓ FC, ↓ BWG (in P and F1), ↓ BW (in P and F1 12–15% - started within the 1st week of pre-mating and persisted throughout the study) Offspring Toxicity: NOAEL = 4.0 mg/kg bw/d (♂/♀) 39/43 mg/kg bw/d: ↓ BWG, ↓ BW (8–10% in F1 and F2 males on PND 21 and F1 females) Reproductive Toxicity: NOAEL = 39.0 mg/kg bw/d The reproductive indices data were variable (for example, the fertility index in F1 control was 86%) Sperm parameters (counts, motility and morphology), estrous cycle length and periodicity, and ovarian follicle were not examined. Onsets of puberty were not examined.	

Table 9 Toxicity of atrazine to terrestrial plants - seedling emergence and vegetative vigour tests). Endpoints based on 21–28-day ER_{25} values.

Species/test	% a.i.	ER_{25} / NOAEC (g a.i./ha)	Comment	Reference
Seedling emergence				
Monocot - Corn (*Zea mays*)	97.7	>4483.4 / 4483.4	14-d study Reduction in dry weight. MRID 420414-03, Chetram 1989.	USEPA, 2020 (PMRA# 3292787)
Monocot - oat (*Avena sativa*)	97.7	4.48 / 2.80		
Monocot - onion (*Allium cepa*)	97.7	10.09 / 5.60		
Monocot - Ryegrass (*Lolium perenne*)	97.7	7.85 / 5.60		
Dicot - Carrot (*Daucus carota*)	97.7	3.36 / 2.80		
Dicot - soybean (*Glycine max*)	97.7	212.96 / 28.02		
Dicot - Lettuce (*Lactuca sativa*)	97.7	2.80 / 2.80		
Dicot - Cabbage (*Brassica oleracea alba*)	97.7	15.69 / 11.21		
Dicot - tomato (*Lycopersicon esculentum*)	97.7	38.11 / 11.21		
Dicot - cucumber (*Cucumis sativus*)	97.7	14.57 / 5.60		
Monocot - Corn (*Zea mays*)	43.3 (Atrazine SC)	>28 000 /28 000	Reduction in dry weight. Results based on standard 14-day exposure test (Test 1).	USEPA, 2020 (PMRA# 3292787); original study PMRA# 2816827
Monocot - oat (*Avena sativa*)	43.3 (Atrazine SC)	45 / 24		
Monocot - onion (*Allium cepa*)	43.3 (Atrazine SC)	>112 / 112		
Monocot - Ryegrass (*Lolium perenne*)	43.3 (Atrazine SC)	>112 / 112		
Dicot - Carrot (*Daucus carota*)	43.3 (Atrazine SC)	>55 / 55		
Dicot - soybean (*Glycine max*)	43.3 (Atrazine SC)	>493 / 493		
Dicot - Lettuce (*Lactuca sativa*)	43.3 (Atrazine SC)	>55 / 55		
Dicot - Cabbage (*Brassica oleracea alba*)	43.3 (Atrazine SC)	34 / 109		
Dicot - tomato (*Lycopersicon esculentum*)	43.3 (Atrazine SC)	59 / 53		
Dicot - cucumber (*Cucumis sativus*)	43.3 (Atrazine SC)	>112 / 112		
Monocot - Corn (*Zea mays*)	43.3 (Atrazine SC)	8600/28000	Reduction in dry weight. Results based on standard 14-day exposure test extended by an additional 14 days, for a total of 28 days (Test 2); extended time allowed for potential recovery from the initial application. Endpoint values shown do not include recovery phase.	
Monocot - oat (*Avena sativa*)	43.3 (Atrazine SC)	>53 / 53		
Monocot - onion (*Allium cepa*)	43.3 (Atrazine SC)	38 / 28		

Species/test	% a.i.	ER_{25} / NOAEC (g a.i./ha)	Comment	Reference
Monocot - Ryegrass (*Lolium perenne*)	43.3 (Atrazine SC)	>112 / 112		
Dicot - Carrot (*Daucus carota*)	43.3 (Atrazine SC)	>55 / 55		
Dicot - soybean (*Glycine max*)	43.3 (Atrazine SC)	>493 / 493		
Dicot - Lettuce (*Lactuca sativa*)	43.3 (Atrazine SC)	54 / 55		
Dicot - Cabbage (*Brassica oleracea alba*)	43.3 (Atrazine SC)	20 / 11		
Dicot - tomato (*Lycopersicon esculentum*)	43.3 (Atrazine SC)	168 / 106		
Dicot - cucumber (*Cucumis sativus*)	43.3 (Atrazine SC)	>112 / 5.3		
Vegetative vigour				
Monocot - Corn (*Zea mays*)	97.7	>4483.4 / >4483.4	Reduction in dry weight. MRID 420414-02, Chetram 1989.	USEPA, 2020 (PMRA# 3292787)
Monocot - oat (*Avena sativa*)	97.7	2690 / 2241.7		
Monocot - onion (*Allium cepa*)	97.7	683.7 / 560.4		
Monocot - Ryegrass (*Lolium perenne*)	97.7	>4483.4 / >4483.4		
Dicot - Carrot (*Daucus carota*)	97.7	1905.4 / 2241.7		
Dicot - soybean (*Glycine max*)	97.7	29.1 / 22.4		
Dicot - Lettuce (*Lactuca sativa*)	97.7	369.9 / 280.2		
Dicot - Cabbage (*Brassica oleracea alba*)	97.7	15.7 / 5.6		
Dicot - tomato (*Lycopersicon esculentum*)	97.7	807 / 560.4		
Dicot - cucumber (*Cucumis sativus)*	97.7	8.96 / 5.6		
Monocot - Corn (*Zea mays*)	43.3 (Atrazine SC)	>28000 / 11000	Reduction in dry weight. Results based on standard 21-day exposure test (Test 1).	USEPA, 2020) (PMRA# 3292787); original study PMRA# 2816828
Monocot - onion (*allium cepa*)	43.3 (Atrazine SC)	43 / <20		
Monocot - Ryegrass (*Lolium perenne*)	43.3 (Atrazine SC)	269 / 246		
Dicot - Carrot (*Daucus carota*)	43.3 (Atrazine SC)	61 / 22		
Dicot - soybean (*Glycine max*)	43.3 (Atrazine SC)	20 / 8		
Dicot - Lettuce (*Lactuca sativa*)	43.3 (Atrazine SC)	25 / 5		
Dicot - Cabbage (*Brassica oleracea alba*)	43.3 (Atrazine SC)	66 / 49		

Species/test	% a.i.	ER_{25} / NOAEC (g a.i./ha)	Comment	Reference
Dicot - tomato (*Lycopersicon esculentum*)	43.3 (Atrazine SC)	33 / 8		
Dicot - cucumber (*Cucumis sativus*)	43.3 (Atrazine SC)	17 / <5		
Monocot - Corn (*Zea mays*)	43.3 (Atrazine SC)	6000 / 1100	Reduction in dry weight: corn, onion, soybean, lettuce, cabbage, tomato. Survival: oat, ryegrass carrot, cucumber. Results based on standard 21-day exposure test extended by an additional 21 days, for a total of 42 days (Test 2); extended time allowed for potential recovery from the initial application.	
Monocot - oat (*Avena sativa*)	43.3 (Atrazine SC)	224 / 53		
Monocot - onion (*Allium cepa*)	43.3 (Atrazine SC)	112 / 103		
Monocot - Ryegrass (*Lolium perenne*)	43.3 (Atrazine SC)	2330 / 1090		
Dicot - Carrot (*Daucus carota*)	43.3 (Atrazine SC)	350 /246		
Dicot - soybean (*Glycine max*)	43.3 (Atrazine SC)	4.5 / 1.2		
Dicot - Lettuce (*Lactuca sativa*)	43.3 (Atrazine SC)	68 / 11		
Dicot - Cabbage (*Brassica oleracea alba*)	43.3 (Atrazine SC)	>247 / 247		
Dicot - tomato (*Lycopersicon esculentum*)	43.3 (Atrazine SC)	134 / 105		
Dicot - cucumber (*Cucumis sativus*)	43.3 (Atrazine SC)	146 / 53		

Table 10 Terrestrial plant growth toxicity endpoints for atrazine reported in open literature

Species	ER_{25} (g a.i./ha)	Comment
21- 28 day ER_{25} values from Boutin et al., 2010 – PMRA# 2743693		
English daisy (*B. perennis*)	90	Values are geomeaned based on individual ER_{25} values (reduction in biomass) derived from two types of experiments: 1) tests conducted using several ecotypes (originating from different areas of the world) for each plant species and 2) tests examining the effect of seasonal variation on the reproducibility of results.
Cornflower (*C. cyanus*)	245	
Common foxglove (*D. purpurea*)	197	
Elecampane (*I. helenium*)	618	
Self-heal (*P. vulgaris*)	542	
Curly dock (*R. crispus*)	44	
Black-eyed Susan (*R. hirta*)	172	
Canada goldenrod (*S. Canadensis*)	413	
American water horehound (*L. americanus*)	66	
White avens (*G. canadense*)	136	
Ox-eye daisy (*C. leucanthemum*)	124	
Wheat (*T. aestivum*)	511	
Lettuce (*L. sativa*)	24	
Tomato (*S. lycopersicon*)	23	

Species	ER_{25} (g a.i./ha)	Comment
21- 28 day ER_{25} values from Boutin et al., 2010 – PMRA# 2743693		
28 day ER_{25} values based on reduced dry weight from White and Boutin 2007, PMRA# 2482641		
Oats (*A. sativa*)	NR	Atrazine dose resulted in 100% mortality despite range finding test. The treatment levels used in the definitive test are not reported.
Corn (*Z. mays*)	Not tested	Definitive test was not conducted because of extreme insensitivity during screening tests as well as technical difficulties that result when spraying atrazine at high concentrations.
Common milkweed (*A. syriaca*)	NR	Atrazine dose resulted in 100% mortality despite range finding test. The treatment levels used in the definitive test are not reported.
Wheat (*T. astivum*)	148	
Strawberry (*F. ananassa*)	164	
Soybean (*G. max*)	165	
Sunflower (*H. annuus*)	72	
Lettuce (*L. sativa*)	40	
Radish (*R. sativus*)	177	
Tomato (*S. lycopersicon*)	55	
Canada bluegrass (*P. compressa*)	123	
Northern wheatgrass (*E. lanceolatus*)	217	
Big bluestem (*A. Gerardii*)	2162	
Thick-leaved strawberry (*F. virginana*)	20	
American vetch (*V. Americana*)	525	
Rough-leaved sunflower (*H. strumosus*)	100	
Tall blue spruce (*L. Canadensis*)	97	
Black nightshade (*S. nigrum*)	67	

NR – not reported.

Aquatic organisms

Table 11 Acute toxicity of atrazine to freshwater aquatic invertebrates

Organism	Exposure	Test substance	Endpoint value (µg a.i./L; measured/nominal)	Comments	Reference
Waterflea (*Daphnia magna*)	48 hr	Atrazine, 80 WP (79.6%)	48-h LC_{50} = 49 000 (measured)	Flow through test. Classified by USEPA as supplemental for formulation (EC_{50} higher than atrazine solubility).	Reported in USEPA 2016 review (PMRA# 3253945)
		Atrazine, 85.5%	48-h LC_{50} = 3500 (unknown)	Classified by USEPA as supplemental based on missing raw data.	
		Atrazine, purity not reported	26-h LC_{50} = 3600 (unknown)	Classified by USEPA as supplemental based on missing raw data, unknown a.i. purity and shorter 26-hour test duration.	
		Atrazine, 94%	48-h LC_{50} = 6900 (nominal)	Static test. Classified by USEPA as supplemental based on missing raw data.	
		Atrazine, 98.9%	48-h LC_{50} = 16 820 (nominal)	Followed USEPA method (2002, EPA-821-R-02-012). The acute test results for atrazine are the same as those reported in Schmidt et al., 2017 (PMRA# 3201379).	Sengupta et al., 2015 (PMRA# 3201381)

Organism	Exposure	Test substance	Endpoint value (µg a.i./L; measured/nominal)	Comments	Reference
		HA, 98%	48-h LC_{50} > 4100 (measured)	Static test. Classified as acceptable. Peither A., 2005, PMRA# 2816909 (USEPA DER – PMRA# 2816910).	Reported in USEPA 2016 review (PMRA# 3253945)
		DIA, purity not reported	48-h LC_{50} = 126 000 (measured dissolved)	Static test. Classified by USEPA as supplemental; the USEPA reports the 48-hour LC_{50} as >100 000 µg a.i./L. A 48 hour LC_{50} of 126 000 µg a.i./L is reported in the study (this value was extrapolated beyond the test range – highest test concentration was 100000 µg a.i./L. The study is considered acceptable by Health Canada (PMRA DER – 1893980).	
		DACT	48-h LC_{50} > 100 000 (measured dissolved)	Static test. Classified by USEPA as supplemental. Vial A., 1991, PMRA# 2816876.	
		DEA, 95.7%	48-h LC_{50} = 88 000 (measured, but unknown if used in calculation)	Static test. The study is considered acceptable by Health Canada (PMRA DER – 1892629).	PMRA# 1820752
Waterflea (*Ceriodaphnia dubia*)	48 hr	Atrazine, 97%	48-h LC_{50} > 4900 (measured)	Static test. No mortality observed. Classified as supplemental by USEPA (EC_{50} value not determined).	Reported in USEPA 2016 review (PMRA# 3253945)
Waterflea (*Ceriodaphnia dubia*)	48 hr	Atrazine, >99%	> 30 000 (measured)	Static 48-hour test. 57 mg/L $CaCO_3$. Classified as supplemental by USEPA based on missing raw data.	
Waterflea (*Daphnia pulex*)	Acute (duration not reported)	Atrazine, 15EC (40.8%)	36 500 (nominal) 46 500 (with sediment)	Classified as supplemental by USEPA based on formulation and EC_{50} exceeds water solubility and low water temperature (15°C).	
Amphipod (*Gammarus fasciatus*)	48 hr	Atrazine, 94%	5700 (nominal)	Classified as supplemental by USEPA based on missing raw data.	
Midge (*Chironomus tentans*)	48 hr	Atrazine, 94%	LC_{50} = 720 (nominal)	Static test. Classified as supplemental by USEPA based on missing raw data.	
Midge (*Chironomus tentans*)	10 days	Atrazine, 98.5%	Mortality: LC_{50} > 24 000 (measured; 37% mortality) NOAEC = 1,000 LOAEC = 24 000 Growth (dry weight): EC50 = 8300 (measured) NOAEC <3200 LOAEC = 3200	Flow-through 10-day test; water-spiked exposure. Classified as supplemental (does not fulfill any currently approved USEPA SEP guideline).	

Organism	Exposure	Test substance	Endpoint value (µg a.i./L; measured/nominal)	Comments	Reference
Midge (*Chironomus temtans*)	10 days	Atrazine, 98.5%	Mortality (measured conc): Sediment: NOAEC = 130 000, LOAEC = 270 000, Porewater: NOAEC = 26 000, LOAEC = 29 000 (14% mortality), LC50 >30 000 Growth: Dry Weight (measured conc) Sediment: NOAEC = 24 000, LOAEC = 60 000, Porewater: NOAEC = 4000, LOAEC = 21 500	Static-renewal – to maintain water quality 10-day test; sediment-spiked exposures. Classified as supplemental (does not fulfill any currently approved USEPA SEP guideline).	
Midge (*Chironomus riparius*)	Acute (duration not reported)	Atrazine, 85.5%	LC_{50} = 1000 (unknown)	Classified as supplemental by USEPA based on missing raw data.	
Midge (*Chironomus riparius*)	10 days	Purity not reported	LC_{50} > 33 000 (measured)	Static renewal (daily; 10-day test). Classified as supplemental by the USEPA (raw data are missing). The measured water concentration after 10 days was 18 900 µg/L.	
Scud (*Hyalella azteca*)	Acute (duration not reported)	Atrazine, 98%	LC_{50} = 1500 (measured)	≤ 7-d old. Static renewal. Classified as qualitative (raw data missing) by the USEPA.	
Scud juvenile (*Hyalella azteca*)	Acute (duration not reported)	Atrazine, 98.5%	LC_{50} = 14 700 (measured)	Juveniles. Flow-through test. Classified as supplemental by USEPA based on missing raw data.	
Amphipod (*Hyalella azteca*)	96 hr	DIA, 98%	LC_{50} = 7200 (measured)	Classified as qualitative (no raw data).	
Amphipod (*Hyalella azteca*)	96 hr	DEA, 98%	LC_{50} = 7200 (measured)	Classified as qualitative (no raw data).	
Scud (*Gammarus fasciatus*)	48 hr	Atrazine, 94%	LC_{50} = 5700 (nominal)	Static test. Classified as supplemental by USEPA based on missing raw data.	
Stonefly (nymph) (*Acroneuria* sp.)	Acute (duration not reported)	Atrazine, 98.5%	LC_{50} = 6700 (measured)	Flow-through test. 67.4 mg/L $CaCO_3$. Classified as supplemental by USEPA based on missing raw data.	
Shrimp (*Paratya australiensis*)	48 hr	Atrazine, 97%	9700–9900 (water only) 6500–6800 (water and sediment) (measured-initial)	Shrimp 1 to 1.5 cm. Static renewal 48-hour test. Endpoints based on initial measured concentrations. Classified by USEPA as qualitative (raw data missing, non-native spp.	
Scud juvenile (*Gammarus pulex*) Static-renewal - daily	10 days	Atrazine, 99%	LC_{50} = 14 900 (measured)	Static renewal (daily). Classified as supplemental by USEPA based on missing raw data.	
Cladoceran (*Pseudosida ramosa*)	Acute (duration not reported)	Atrazine, 99%	LC_{50} = 17 100 (mean of 20 trials) (nominal)	Static test. Based on nominal. Classified by USEPA as qualitative (raw data missing).	

Organism	Exposure	Test substance	Endpoint value (µg a.i./L; measured/nominal)	Comments	Reference
Waterflea (*Daphnia carinata*)	Acute (duration not reported)	Purity not reported	LC_{50} = 22 400–24 600 (water only) LC_{50} = 25 300–26 700 (water and sediment) (measured – initial)	Static test. Endpoints based on initial measured concentrations. Classified by USEPA as qualitative (raw data missing).	
Scud adult (*Diporeia* spp.)	Acute (duration not reported)	Atrazine, 89%	LC_{50} > 3000 (measured; unknown if used in calc)	Static renewal. Classified by USEPA as qualitative (raw data missing).	
Amphipod (*Diporeia* spp)	96 hr	DIA, 98%	96-hr LC_{50} >3000 (measured unknown if in calc)	Classified by USEPA as qualitative (raw data missing).	
Amphipod (*Diporeia* spp)	96 hr	DEA, 98%	96-hr LC_{50} >3000 (measured unknown if used in calc)	Classified by USEPA as qualitative (raw data missing).	
Juvenile signal crayfish (*Pacifastacus leniusculus*)	96 hr	Atrazine, 98.9%	96-hr LC_{50} =12 100 (measured unknown if used in calc)	Static renewal (48 hours). OECD guideline 203 followed.	Velisek et al., 2013 (PMRA# 3201383)
Leech (*Glossiphonia complanata*)	duration not reported	Atrazine, 99.2%	> 16 000 (measured)	Static renewal (weekly). Classified as supplemental by USEPA based on missing raw data.	Reported in USEPA 2016 review (PMRA# 3253945)
Leech (*Helobdella stagnalis*)	duration not reported	Atrazine, 99.2%	> 16 000 (measured)	Static renewal (weekly). Classified as supplemental by USEPA based on missing raw data.	
Snail (*Ancylus fluviatilis*)	duration not reported	Atrazine, 99.2%	>16 000 (measured)	Static renewal (weekly). Classified as supplemental by USEPA based on missing raw data.	
Mussel (glochidia and juveniles) (*Lampsilis siliquoidea*)	duration not reported	Atrazine, 98%	>30 000 (both life stages) (measured nominal)	Static test. Classified as qualitative by USEPA based on missing raw data.	
Mussel (glochidia and juveniles) (*Lampsilis siliquoidea*)	duration not reported	Atrazine, EC15 (40.8%)	>30 000 (both life stages) (measured, results based on nominal)	Static test. Classified as qualitative by USEPA based on missing raw data. USEPA reports that concentrations were measured but results based on nominal.	
Freshwater mussel (*Anodonta imbecillis*) juvenile and mature organisms	duration not reported	Atrazine, 97%	>60 000 (in both juvenile and mature *A. imbecillis*). (unknown)	Classified as qualitative by the USEPA based on no raw data, uncertainty about reported high concentrations.	
Freshwater mussel (*Utterbackia imbecillis*) Glochidia	duration not reported	Atrazine, EC15 (40.8%)	LC50 = 241 000 (unknown)	Static test. Classified as qualitative by USEPA based on testing of a formulated product at concentrations considerably higher than the solubility limit of atrazine.	

Table 12 Chronic toxicity of atrazine to freshwater aquatic invertebrates

Organism	Exposure	Test substance	Endpoint value (µg a.i./L; measured/nominal)	Comments	Reference
Scud (*Gammarus fasciatus*)	30-days / flow-through	Atrazine, 94%	NOEC = 60 LOEC = 140 (measured)	25% reduction in development to seventh instar. Classified as supplemental by the USEPA (used DMSO, no solvent control, 64–74% control survival, reproduction in 1 of 2 control replicates).	Reported in USEPA 2016 review (PMRA# 3253945)
Midge (*Chironomus tentans*)	38-days / flow-through	Atrazine, 94%	NOEC = 110 LOEC = 230 (measured)	Based on reduced adult emergence. Classified as supplemental by the USEPA (used DMSO, no solvent control).	
Waterflea (*Daphnia magna*)	21-days / flow-through	Atrazine, 94%	NOEC = 140 LOEC = 250 (measured)	54% reduction in F0 young/female ratio. Classified as supplemental by the USEPA (used DMSO, no solvent control, control survival 61%).	
Waterflea (*Daphnia magna*)	duration not reported	Atrazine, 94%	NOEC = 200 LOEC = 2000 (unknown)	Static renewal tests; 6 generations. 66% reduction in number of young in generations 4, 5, and 6. Classified as supplemental by the USEPA (methods and raw data are not reported).	
Waterflea (*Daphnia magna*)	21-days/ static renewal	Atrazine, (purity not reported)	NOEC ≥ 150 (nominal)	Static renewal (daily). Test conditions followed OECD guideline 211 (2012). Only one test concentration was used; a definitive NOEC could not be derived.	Religia P. et al., 2019 (PMRA# 3201377)
Waterflea (*Daphnia pulex*)	28-Day / static-renewal	Atrazine, 99.2%	NOEC = 1000 LOEC = 2000 (nominal)	Based on reduction in young/female. Classified as supplemental by the USEPA (no raw data for statistical analysis).	Reported in USEPA 2016 review (Appendix B: Supporting Ecological Toxicity Data – PMRA# 3253945)
Redclaw crayfish (*Cherax quadricarinatus*)	28-Day / static-renewal	Gesaprim, 90%	NOEC = 500 (nominal)	Static renewal (72h). Based on reduced somatic growth in juvenile females (lower weight gain and protein content in muscle) and an increased proportion of juveniles differentiated as females observed at the 2500 µg a.i./L (LOEC).	Mac Loughlin et al., 2016 (PMRA# 3201376)
Leech *(Helobdella stagnalis)*	40-Days / Static-Renewal (weekly)	Atrazine, 99.2%	NOEC <1000 LOEC = 1000 (measured)	65% reduction in percent hatch. Classified as supplemental by the USEPA (no raw data for statistical analysis).	Reported in USEPA 2016 review (PMRA# 3253945)
Waterflea (*Ceriodaphnia dubia*)	Two 7-Day static-renewal tests	Atrazine, >99%	NOEC = 2500 LOEC = 5000 (measured)	Based on reduction in mean total number of young per living female (3 broods). Classified as supplemental by the USEPA (no raw data for statistical analysis).	

Organism	Exposure	Test substance	Endpoint value (µg a.i./L; measured/nominal)	Comments	Reference
Waterflea (*Ceriodaphnia dubia*)	Two 4-Day static-renewal tests	Atrazine, >99%	NOEC = 5000 LOEC = 10 000 NOEC = 10 000 LOEC = 20 000 (measured)	Based on reduction in mean total number of young per living female (3 broods). Classified as supplemental by the USEPA (no raw data for statistical analysis).	
Green hydra (normal) (*Chlorohydra viridissima*)	21-Day Static test	Atrazine, purity not reported.	NOEC <5000 LOEC = 5000 (nominal)	Based on reduction in budding rates. Classified as supplemental by the USEPA (no raw data for statistical analysis).	
Freshwater Snail (*Ancylus fluviatilis*)	40 Days Static-Renewal weekly	Atrazine 99.2%	NOEC - NR LOEC - NR	Classified as supplemental by the USEPA (no raw data for statistical analysis).	
Leech (*Glossiphonia complanata*)	27-Days Static-Renewal weekly	Atrazine 99.2%	NOEC = 1,000 (reduced egg production) LOEC = 4,000 (mortality) (measured)	Classified as supplemental by the USEPA (no raw data for statistical analysis).	
Ancylus fluviatilis (river limpet), *Glossiphonia complanata* (leech), *Helobdella stagnalis* (leech)	40-days	Atrazine 99.2%	Effects observed at all concentrations (1000, 4000 and 16 000 µg/L) (unknown)	Based on food ingestion, growth, and egg production. Classified as qualitative by the USEPA. NOAEC was not achieved; no information was reported on experimental conditions (temperature, pH, dissolved oxygen); limited information on study design parameters.	
Numerous invertebrates including annelids, arthropods, and mollusks.	8-weeks	Atrazine, purity not reported.	No effects were observed at any concentration NOAEC = 670 (unknown)	Based on abundance of various taxa. Classified as invalid by the USEPA (Possible control contamination; unacceptable solvent; a solvent control, but no negative control was used).	
Freshwater snail *(Lymnaea palustris)*	12-week mesocosm study	Atrazine, 97.8%	No effects occurred at any concentration for mortality, growth, fecundity, and biochemical parameters glycogen content, polysaccharide hydrolysis. The NOAEC was 125 atrazine µg/L (nominal).	Endpoints evaluated included mortality, growth, fecundity, and biochemical parameters (glycogen content, polysaccharide hydrolysis. Classified as qualitative by the USEPA (replicate mesocosms were not used per concentration; concentrations were not analytically confirmed; no water/sediment quality data were provided.	
Mussel (1.475 mm) (*Lampsilis siliquoidea*)	21 –d static renewal (95% every 48 or 72 hours)	Atrazine, 98 and 40.8%	Tech EC_{50} = 10 100 Form EC_{50} = 3100 (measured)	Based on immobility of organisms, no effect on growth. Classified as qualitative by the USEPA (raw data reported water quality and control mortality was provided).	

Table 13 Effects of atrazine on freshwater fish

Organism	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Acute toxicity					
Rainbow trout (*Oncorhynchus mykiss*)	96 hr, static test	Atrazine, 98.8%	96-h LC_{50} = 5300 (nominal)	The USEPA classifies the study as acceptable; (water quality other than temperature not reported). Beliles & Scott, 1965	Reported in USEPA 2016 review (PMRA# 3253945)
Rainbow trout (*Oncorhynchus mykiss*)	96 hr, flow-through test	Atrazine, 40.8% (formulation)	96-h LC_{50} = 20 500 (nominal)	Classified as supplemental by the USEPA (no raw data). Howe et al,., 1998	
Rainbow trout (*Oncorhynchus mykiss*)	96 hr, static test	Atrazine, 43% (formulation)	96-h LC_{50} = 24 000 (nominal)	Mayer & Ellersieck, 1986	
Rainbow trout (*Oncorhynchus mykiss*)	96 hr, static test	HA, 98%	96-h LC_{50} > 3000 (measured dissolved)	Classified as acceptable by the USEPA. Peither, 2005a, PMRA# 2816902 (USEPA DER- PMRA# 2816903).	
Rainbow trout (*Oncorhynchus mykiss*)	96 hr, static test	DIA, (purity not reported)	96-h LC_{50} = 17 000 (measured dissolved)	Classified as supplemental by the USEPA. Vial, 1991a, PMRA# 2816875	
Rainbow trout (*Oncorhynchus mykiss*)	96 hr, static test	DIA, (98.9%)	96-h LC_{50} = 29 000 (mean measured)	USEPA DER – PMRA# 1903333.	PMRA# 1820759
Rainbow trout (*Oncorhynchus mykiss*)	96 hr, static test	DACT (purity not reported)	96-h LC_{50} > 100 000 (measured dissolved)	Classified as supplemental by the USEPA. Vial, 1991b, PMRA# 2816873	Reported in USEPA 2016 review (PMRA# 3253945)
Rainbow trout (*Oncorhynchus mykiss*)	96 hr, static test	DEA, 95.7%	96-h LC_{50} = 41 000 (mean measured)	DER PMRA# 1902323.	PMRA# 1820758
Brook trout (*Salvelinus tontinalis*)	96 hr, flow-through test	Atrazine, 94%	96-h LC_{50} = 6300 8-d LC_{50} = 4900 (8-day test) (unknown)	Classified as supplemental by the USEPA (52-gram fish and no raw data). Macek et al., 1976	Reported in USEPA 2016 review (PMRA# 3253945)
Bluegill sunfish (*Lepomis macrochirus*)	96 hr, flow-through test	Atrazine, 94%	96-h LC_{50} > 8000 7 d LC_{50} = 6700 (7-day test) (unknown)	Classified as supplemental by the USEPA (6.5-gram fish and no raw data). Macek et al., 1976	
Bluegill sunfish (*Lepomis macrochirus*)	96 hr static test	Atrazine, 98.8%	96-h LC_{50} = 24 000 (nominal)	Classified as acceptable by the USEPA. Beliles & Scott, 1965	
Bluegill sunfish (*Lepomis macrochirus*)	96 hr static test	Atrazine, 100%	96-h LC_{50} = 57 000 (endpoint exceeds the maximum solubility of atrazine in water at 25°C)	Classified as acceptable by the USEPA. Buccafusco, 1976	
Bluegill sunfish (*Lepomis macrochirus*)	96 hr static test	Atrazine, 43% (formulation)	96-h LC_{50} = 42 000 (unknown)	Classified as supplemental by the USEPA (no raw data). Mayer & Ellersieck, 1986	

Organism	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Bluegill sunfish (*Lepomis macrochirus*)	96 hr static test	Atrazine, 80% (formulation – 80WP)	96-h LC_{50} = 20 000 (nominal)	Classified as supplemental by the USEPA (limited raw data). Jones, 1962	
Bluegill sunfish (*Lepomis macrochirus*)	96 hr static test	HA, 98%	96-h LC_{50} > 3800 (measured dissolved)	Classified as acceptable by the USEPA. Peither, 2005b, PMRA# 2816900 (USEPA DER – PMRA# 2816901).	
Brown trout (*Salmo trutta*)	96 hr, Static-Renewal - daily	unknown	96-h LC_{50} = 27 000 (nominal)	Classified as supplemental by the USEPA (no raw data; slight aeration and purity unknown). Grande et al., 1994	
Fathead minnow (*Pimephales promelas*)	96 hr, 24 hr renewal test	Atrazine, 94%	96-h LC_{50} = 15 000 (nominal) 5 d LC_{50} = 15 000 (5-day test)	Classified as supplemental by the USEPA (no raw data). Macek et al., 1976	
Fathead minnow juvenile (*Pimephales promelas*)	96 hr, flow-through	Atrazine, 97.1%	96-h LC_{50} = 20 000 (measured)	Classified as acceptable by the USEPA. Dionne 1992 This data was published in Dionne et al., 2021 (PMRA# 3256767).	
Carp (*Cyprinus carpio*)	96 hr, semi-static test	Atrazine, 93.7%	96-h LC_{50} = 18 800 (nominal)	Classified as supplemental by the USEPA (no raw data). Neskovic et al., 1993	
Fish from the Nile River (*Chrysichthyes auratus)*	96 hr, static-renewal - daily	Atrazine, 96%	96-h LC_{50} = 6370 (unknown)	Classified as supplemental by the USEPA (non-native sp.; 26-gram fish; no raw data). Hussein et al., 1996	
Silver catfish (*Rhamdia quelen*)	96 hr	Formulation (Atrazine and simazine - 250 g/L both ais)	96-h LC_{50} =10 200 (atrazine only) 96-h LC_{50} =10 500 (atrazine and simizine) (nominal)	Classified as supplemental by the USEPA (nonnative sp., no raw data, unknown formulation used). Kreutz et al., 2008	
Zebrafish (*Brachydanio rerio*)	96 hr	Not reported	96-h LC_{50} = 37 000 (unknown)	Classified as supplemental by the USEPA (article unavailable). Korte & Greim 1981	
Zebrafish (*Brachydanio rerio*)	96 hr, static renewal	Atrazine, 95%	96-h LC_{50} = 34 190 (embryo) 96-h LC_{50} = 15 630 (larvae) 96-h LC_{50} = 6090 (juvenile) (nominal)	The study design followed OECD guidelines 203 and 236. The results based on atrazine alone are acceptable.	Wang et al., 2017a (PMRA# 3262555)
Goldfish (*Carassius auratus*)	96 hr, static test	Atrazine, 98.8%	96-h LC_{50} = 60 000 (nominal)	Classified as supplemental by the USEPA (not an acceptable species). Beliles & Scott 1965	Reported in USEPA 2016 review (PMRA# 3253945)
Black Bass - fry (*Micropterus salmoides*)	48 hr, static test	Atrazine, 80% (Formulation – 80WP)	48-h LC_{50} = 12 600 (nominal)	Classified as supplemental by the USEPA (48 hours; limited raw data). Jones, 1962	
Channel Catfish yolk sac (*Ictalurus punctatus*)	96 hr, static test	Atrazine, 80% (Formulation – 80WP)	96-h LC_{50} = 16 000 (nominal)	Classified as supplemental by the USEPA (limited raw data). Jones, 1962	

Organism	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Channel Catfish (*Ictalurus punctatus*)	96 hr, flow-through test	Atrazine, 40.8% (formulation)	96-h LC_{50} = 23 800 (nominal)	Classified as supplemental by the USEPA (no raw data). Howe et al., 1998	
guppy (*Poecilia reticulata*)	3 hours	Atrazine, 99.1%	AC_{50} = 0.065 (unknown)	No mortalities were reported in either test. The extent of the observed avoidance mechanism under environmental conditions, where fish would be exposed to a variety of chemical cues simultaneously is unclear. Qualitative.	Araújo et al., 2018 (PMRA# 3253944)
Nile tilapia larvae (*Oreochromis niloticus*)	96 hr, static test	Atrazine, 97%	LC_{50} = 17 870 (nominal)	The study design followed OECD guideline 203 (1992).	Chiste et al., 2020, (PMRA# 3253958)
Fingerlings and Juveniles of African Catfish, (*Clarias gariepinus*)	96 hr, static renewal	Atrazine (powdered formulation, purity not reported)	Fingerlings LC_{50} = 350 Juveniles: LC_{50} = 553 (nominal)	Sublethal effects included erratic swimming and gasping for air.	Doherty et al., 2019 (PMRA# 3256769)
Male juvenile Nile tilapia (*Oreochromis niloticus*)	96 hr	Nortox 500 SC, 50%	96-h LC_{50} = 5490 (unknown)	The details for the experimental test design for the preliminary assays are limited and a recognized standard test guideline is not cited. Qualitative.	Oliveira et al., 2018 (PMRA# 3262467)
Chronic: Early life stage (ELS), reproduction, life cycle toxicity data					
Rainbow trout embryo-larvae (*Oncorhynchus mykiss*)	ELS - 27 days flow-through	Atrazine, 80% (80WP)	LC_{50} = 660; 880 LC_{01} = 29; 77 (unknown)	Classified as supplemental by the USEPA (short test, no raw data for statistical analyses). Birge et al., 1979	Reported in USEPA 2016 review (PMRA# 3253945)
Rainbow trout embryo-larvae (*Oncorhynchus mykiss*)	ELS - 86 days flow-through	Atrazine (technical, purity not reported)	NOAEC = 410 LOAEC = 1100 (measured)	Based on delayed hatching, reduced wet and dry weight and mortality. Classified as supplemental by the USEPA (DMSO used as solvent, no raw data for statistical analyses). Whale et al., 1994	
Rainbow trout embryo-larvae (*Oncorhynchus mykiss*)	ELS – 90 days flow-through	DEA, 95.7%	NOAEC = 910 LOEC > 910 (measured)	No effects observed for the following parameters: Embryo viability, survival at hatch, normal larvae at hatch, larval survival, larval length and dry weight (DER PMRA# 1902460).	PMRA# 1820762
Rainbow trout embryo-larvae (*Oncorhynchus mykiss*)	ELS – 90 days flow-through	DIA, 99.1%	NOAEC = 2000 LOAEC > 2000 (measured)	No effects observed for the following parameters: Embryo viability, survival at hatch, normal larvae at hatch, larval survival, larval length and dry weight (DER PMRA# 1902904)	PMRA# 18207623
Channel catfish embryo-larvae (*Ictalurus punctatus*)	ELS - 8 days; flow-through	Atrazine, 80% (80WP)	LC_{50} 50 mg $CaCO_3$/L = 220 LC_{50} 200 mg $CaCO_3$/L = 230 (unknown)	16%, 47% and 86% of individuals exhibited terata at 420, 830 and 46,700 µg/L, respectively. Birge et al., 1979	Reported in USEPA 2016 review (PMRA# 3253945)
Zebrafish (*Brachydanio rerio*)	ELS - 35 days flow through	Atrazine, 98%	NOAEC = 300 LOAEC = 1300 (measured) 35-d LC_{50} = 890	Classified as supplemental by the USEPA (no raw data for statistical analyses). Gorge & Nagel, 1990	

Organism	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Japanese Medaka (*Oryzias latipes)*	30 day	Atrazine, 98%	NOAEC = 50 LOAEC > 50 (nominal)	Papoulias et al., 2014 (PMRA# 2863246). NOEC based on fecundity. USEPA DER – PMRA# 2863245	
Japanese Medaka (*Oryzias latipes)*	35 day	Atrazine, 97.5%	NOAEC = 52 LOAEC > 52 (measured)	Atrazine did not significantly alter fecundity or fertility at any treatment level. USEPA DER – PMRA# 2816823	Schneider et al., 2015 (PMRA# 2816822)
Japanese Medaka (*Oryzias latipes)*	29 day	Atrazine, 97.5%	NOAEC = 244 LOAEC > 244 (measured)	Atrazine did not significantly alter fecundity or fertility at any treatment level.	Schneider et al., 2017 (PMRA# 2816822)
Sockeye salmon (*Oncorhynchus nerka*)	Up to 165 days post fertilization Flow-through	AAtrex Liquid 480, 43.7%	NOEC ≥ 141 µg/L (hatch + emergence) NOEC < 15.8 µg/L (premature emergence, reduced fry weight) NOEC = 15.8 µg/L (whole body testosterone) (measured)	It is unclear whether small changes in growth and emergence timing, observed under controlled continuous exposure laboratory conditions, would result in population level effects to wild salmon. Qualitative.	Du Gas et al., 2017 (PMRA# 3256770)
Japanese Medaka (*Oryzias latipes)*	Embryonic exposure (8 hr–12 days)	Atrazine, ≤ 100%	NOAEC ≥ 50 (based on overt effects to overall reproductive health) (nominal)	No association can be made to such salient endpoints amenable for risk assessment. Qualitative.	Cleary et al., 2019 (PMRA# 3253959)
Zebrafish (*Danio rerio*)	Embronic exposure from 1–72 hours post fertilization	Atrazine, 98%	NOEC = 30 LOEC > 30 (nominal)	Embryonic atrazine exposure did not significantly alter reproductive function or offspring viability in adults up to the highest test concentration (NOEC ≥ 30 µg a.i./L). Qualitative	Wirbisky et al., 2016a (PMRA# 2863250)
Zebrafish (*Danio rerio*)	Embronic exposure from 1–72 hours post fertilization	Atrazine, 98%	NOEC = 30 LOEC > 30 (nominal)	No significant effects were observed at any treatment level based on the physical, hormonal and cellular endpoints measured (NOEC ≥ 30 µg a.i./L, the highest nominal test concentration). Qualitative.	Wirbisky et al., 2016b (PMRA# 2863252)
Zebrafish (*Danio rerio*)	Embronic exposure from 1–120 hours post fertilization	Atrazine (>97% purity), DACT (>99.5% purity), DIA (>97.5% purity) and DEA (>97% purity)	Hatchability (parent, DACT, DIA, DEA): NOEC = 300, LOEC >300 (nominal)	No statistically significant effects on hatchability were observed for atrazine or any of the transformation products at any test concentration. No toxicological effects on the development of larval zebrafish were observed below 100 µg/L with the exception of heart rate. Swim behaviour was observed for a short interval (10 minutes); the ability of fish to recover was not examined. Qualitative.	Liu et al., 2016 (PMRA# 3262459)

Organism	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Zebrafish (*Danio rerio*)	1, 3 and 10 days	Atrazine, DACT, DIA and DEA (purity not reported)	No consistent dose responses were observed	Although some differences were observed in the study (basal or swim stress induced cortisol levels), a consistent concentration response was not observed. Overall, the results suggest that atrazine and some of its transformation products (DEA, DIA and DACT) at the concentrations tested have minimal effects on the cortisol mediated stress response in adult zebrafish. Qualitative.	Van Der Kraak et al., 2015 (PMRA# 3262552)
Zebrafish (*Danio rerio*)	Embryonic exposure 96 hr	Atrazine (purity not reported)	NOEC ≥ 21600 (nominal)	Only one exposure concentration was tested. Qualitative.	Adeyemi et al., 2015 (PMRA# 3253941)
Brook trout (*Salvelinus frontinalis*)	44 weeks full life cycle, flow-through	Atrazine, 94%	NOEC = 65 LOEC = 120 (measured)	7.2 % reduction in mean body length; 16 % reduction in mean body weight. Classified as supplemental by the USEPA (used solvent dimethyl sulfoxide (DMSO), treated fish for disease after allocation to tanks, no solvent control). Macek et al., 1976	Reported in USEPA 2016 review (PMRA# 3253945)
Bluegill sunfish (*Lepomis macrochirus*)	6-18 months full life cycle, flow-through	Atrazine, 94%	NOEC = 95 LOEC = 500 (measured)	LOAEC based on loss of equilibrium in a 28-day test conducted at the same lab. Classified as supplemental by the USEPA (low survival for fry – F1 in the controls). Macek et al., 1976	
Fathead minnow (*Pimephales promelas*)	39 weeks full life cycle, flow-through	Atrazine, 97.1%	NOEC 150 LOEC = 250 (measured)	Based on reduced hatchability of embryos. Classified as supplemental by the USEPA (failed to identify a NOAEC). Cited as Dionne (1992) in 2016 USEPA review. This data was recently published in Dionne et al., 2021 (PMRA# 3256767).	
Fathead minnow (*Pimephales promelas*)	43 weeks full life cycle, static-renewal	Atrazine, 94%	NOEC = 210 LOEC = 870 (measured)	LOAEC based on 25% mortality in a 96-hour test conducted at the same lab. Classified as supplemental by the USEPA (high mortality in control adults). Macek et al., 1976	
Fathead minnow (*Pimephales promelas*)	28 day	Atrazine, 97.5%	NOEC = 105 LOEC > 105 (measured)	Atrazine did not significantly alter fecundity or fertility at any treatment level.	Schneider et al., 2017 (PMRA# 2816918)

Table 14 Effects of atrazine on freshwater algae

Note: The toxicity data for freshwater algae summarized in Table 14 reports the most sensitive EC_{50} endpoint value for each genus/species. The majority of the data cited in Table 14 originates from the 2020 USEPA BE for atrazine (Appendix 2-1, PMRA# 3292792) and/or the 2016 USEPA ecological risk assessment for atrazine (Appendix B: Supporting Ecological Toxicity Data – PMRA# 3253945), unless specified otherwise.

Genus and/or species	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Synedra acus	21 d	Atrazine, 99.8%	EC_{50} = 159.4 (chlorophyll *a* content)	Cited: Tang et al., 1997	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792)
Synedra radians	14 d	Atrazine, 99.8%	EC_{50} = 49.4 (chlorophyll *a* content)	Cited: Tang et al., 1997	
Cyclotella gamma	21 d	Atrazine, 99.8%	EC_{50} = 149 (chlorophyll *a* content)	Cited: Tang et al., 1997	
Cyclotella meneghiniana	14 d	Atrazine, 99.8%	EC_{50} = 180.4 (optical density)	Cited: Tang et al., 1997	
Staurastrum sebaldi	6 d	purity not reported	EC_{50} = 180.4 (abundance)	Cited: Berard et al., 2003	
Stigeoclonium tenue	1 d	purity not reported	EC_{50} = 127, 224	Effect metric not reported. Cited as Larsen et al., 1986. Tentative species Identification. The 2016 USEPA review (PMRA# 3253945) reported two individual endpoint values based on different media used. The USEPA classified the study as supplemental based on unavailability of raw data.	
Chlamydomonas reinhardtii	1 d	Atrazine technical (purity not reported)	EC_{50} = 19 - 48 (reduction in C-14 uptake)	Source cited as Larsen et al., 1986 in the USEPA 2020 BE. The 2016 USEPA review (PMRA# 3253945) reported three individual endpoint values based on different media used. The USEPA classified the study as supplemental based on unavailability of raw data.	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792
Chlamydomonas sp.	14 d	Atrazine, 99.8%	EC_{50} = 26.2 (optical density)	Cited: Tang et al., 1997	
Oophila sp.	14 d	Atrazine (purity not reported)	EC_{50} = 23.8 (photosystem II electron transport activity)	Cited: Baxter et al., 2015	
Chlorella fusca ssp. fusca	4 d	Atrazine, > 95%	EC50 = 68.2–76.9 (population growth rate)	Cited: Kottrikla et al., 1999	
Chlorella fusca var. vacuolata	1 d	Atrazine, 97.4%	EC_{50} = 46.9 (photosystem II electron transport activity)	Cited: Vallotton et al., 2008	
Chlorella pyrenoidosa	4 d	Atrazine, 38%	EC_{50} = 52.4 (population growth rate)	Cited: Maule et al., 2002	
Chlorella saccharophila	3 d	Atrazine, 98%	EC_{50} = 780 (population growth rate)	Cited: Carrasco and Sabater 1997	

Genus and/or species	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Chlorella sp.	21 d	Atrazine, 99.8%	EC_{50} = 46.8 (Population changes, general, chlorophyll *a* content)	Cited: Tang et al., 1997	
Chlorella vulgaris	4 d	Atrazine, 98%	EC_{50} = 4.3 (abundance)	Cited: Seguin et al., 2001	
Desmodesmus subspicatus	3 d	Atrazine, 100%	EC_{50} = 41–182 (Population growth rate)	Cited: Masojidek et al. 2011	
Raphidocelis subcapitata (formerly known as *Pseudokirchneriella subcapitata, Selenastrum capricornutum*)	4 d	Atrazine, 100%	IC_{50} = 26 (Abundance, population growth rate)	Cited: Caux et al. 1996	
	2 d	Atrazine, 97.5%	EC_{50} = 42.6 (Cell density) EC_{50} = 142 (Growth rate) EC_{50} = 59.5 (PSII quantum yield) (measured)	48-hour exposure period was followed by a 48-hour recovery test. No statistically significant reduction in growth rate or PSII quantum yield was detected 48 h after atrazine was removed from the test system. Effects at the highest test concentrations (250 µg/L was determined to be algistatic (reversible).	Brain et al., 2012 (PMRA# 2816820); submitted during DCI
	4 d	Atrazine, 96.2%	EC_{50} = 100 (Growth rate) EC_{50} = 44.8 (PSII quantum yield) (measured)	EC_{50} values are based on standard test conditions.	Baxter et al., 2016 (PMRA# 3253950)
Scenedesmus acutus	3 d	Atrazine, 98%	EC_{50} = 11 (population growth rate)	Cited: Carrasco and Sabater 1997	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792
Scenedesmus quadricauda	4 d	Atrazine, 38%	EC_{50} = 15.6 (population growth rate)	Cited: Ma et al., 2003	
	12–14 days	Atrazine, >95%	EC_{50} = 100 EC_{50} = 200 EC_{50} = 300 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classifies the study as supplemental (NOAEC and raw data unavailable).	Reported in USEPA 2016 review (PMRA# 3253945)
	12–14 days	DEA, >95%	EC_{50} =1200 EC_{50} 2000 EC_{50} =1800 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classifies the study as supplemental (NOAEC and raw data unavailable).	
	12–14 days	DIA, >95%	EC_{50} = 6900 EC_{50} = 6500 EC_{50} = 4000 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classifies the study as supplemental (NOAEC and raw data unavailable).	
	12–14 days	DACT, >95%	EC_{50} = 4600 EC_{50} = 10000 EC_{50} > 100 000 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classifies the study as supplemental (NOAEC and raw data unavailable).	
	12–14 days	HA, >95%	EC_{50} > 10000 EC_{50} > 10000 EC_{50} > 100 000 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classifies the study as supplemental (NOAEC and raw data unavailable).	

Genus and/or species	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
	3 d	DIA, (purity not reported)	EC_{50} = 1300 (nominal)	50% reduced cell density. The USEPA classifies the study as supplemental (study duration not sufficient to be classified as Tier II study).	
Scenedesmus subspicatus	1 d	Atrazine, 49.6%	EC_{50} = 12.4 (photosynthesis)	Cited: Zagorc-Koncan 1996	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792
	3 d	DIA, (purity not reported)	EC_{50} = 1300 (nominal)	50% reduced cell density. The USEPA classifies the study as supplemental (study duration not sufficient to be classified as Tier II study).	
Stichococcus bacillaris	3 d	Atrazine, 40%	EC_{50} = 1347 (population growth rate)	Cited: Rojickova et al. 1999	
Pediastrum sp.	21 d	Atrazine, 99.8%	EC_{50} = 28 (growth, optical density)	Cited: Tang et al., 1997	
Scenedesmus obliquus	1 d	Atrazine technical (purity not reported)	EC_{50} = 38–57 (reduction in 14-carbon uptake)	Cited: Larsen et al., 1986 The 2016 USEPA review (PMRA# 3253945) reports three individual endpoint values based on different media used. The USEPA classifies the study as supplemental based on unavailability of raw data.	
Scenedesmus sp.	4 d	Atrazine technical, purity not reported	EC_{50} = 169 (growth)	Cited: Fairchild et al., 1998	
Ankistrodesmus braunii	11 d	Atrazine, >95%	EC_{50} = 60 (abundance, growth)	Cited: Burrell et al., 1985 The USEPA 2016 review (PMRA# 3253945) classifies the study as supplemental based on unavailability of raw data.	
Ankistrodesmus sp.	1 d	Atrazine technical (purity not reported)	EC_{50} = 61–219 (reduction in 14-carbon uptake)	Cited: Larsen et al., 1986 The USEPA 2016 review (PMRA# 3253945) reports three individual endpoint values based on different media used (Taub and Dollar, algal assay - EC_{50} = 61, 72, 219, nominal). The USEPA classifies the study as supplemental based on unavailability of raw data.	
Chlamydomonas geitleri	3 d	purity not reported	EC_{50} = 151–604 (biomass, carbon fixation, population growth rate)	Cited: Francois and Robinson, 1990	
Chlamydomonas intermedia	6 d	purity not reported	EC_{50} = 34 (abundance)	Cited: Berard et al., 2003	
Chlamydomonas reinhardtii	10 d	purity not reported	EC_{50} = 10.2 (population growth rate)	Cited: Schafer et al., 1994	
Chlamydomonas sp.	14 d	Atrazine, 99.8%	EC_{50} = 26.2 (population growth rate, chlorophyll)	Cited: Tang et al., 1997	
Dunaliella tertiolecta	4 d	purity not reported	EC_{50} = 69.4 (Abundance)	Cited: Weiner et al., 2004	
Chlorella pyrenoidosa	5 d	purity not reported	EC_{50} = 282 (growth rate)	Cited: Parrish 1978	
	12–14 days	DEA, >95%	EC_{50} = 3200 EC_{50} = 7200 EC_{50} = 1800 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classifies the study as supplemental (NOAEC and raw data unavailable).	Reported in USEPA 2016 review (Appendix

Genus and/or species	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
	12–14 days	DIA, >95%	EC_{50} > 10 000 EC_{50} > 10 000 EC_{50} = 3600 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classifies the study as supplemental (NOAEC and raw data unavailable).	B: Supporting Ecological Toxicity Data - PMRA# 3253945)
	12–14 days	DACT, >95%	EC_{50} > 10 000 EC_{50} > 10 000 EC_{50} > 100 000 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classifies the study as supplemental (NOAEC and raw data unavailable).	
	12–14 days	HA, >95%	EC_{50} > 10 000 EC_{50} > 10 000 EC_{50} > 100 000 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classifies the study as supplemental (NOAEC and raw data unavailable).	
Chlorella sp.	21 d	Atrazine, 99.8%	EC_{50} = 46.8 (population growth rate, chlorophyll)	Cited: Tang et al., 1997	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792
Parachlorella kessleri	3 d	Purity not reported	EC_{50} = 693 (growth rate)	Cited: Rojikova et al., 1999	
Ulothrix subconstricta	1 d	Purity not reported	EC_{50} = 88 (reduction in 14-carbon uptake) (nominal)	Cited: Larsen et al., 1986 Tentative species Identification. The 2016 USEPA review (PMRA# 3253945) classified the study as supplemental based on unavailability of raw data.	
Tetrahymenidae pyriformis	1 d	Purity not reported	EC_{50} = 5.8 (reduction in survival)	Cited: Toth and Tomasovicova 1979 The 2016 USEPA review (PMRA# 3253945) classified this study as supplemental.	
Cryptomonas pyrenoidifera	6 d	Purity not reported	EC_{50} = 500 (population growth rate)	Cited: Kallqvist and Romstad 1994	
Microcystis sp.	3 d	Atrazine technical, purity not reported	EC_{50} = 90 (growth)	Cited: Fairchild et al., 1998	
Anabaena cylindrica	Not reported	Atrazine, 97%	EC_{50} = 37 (photosynthesis)	Cited: Stratton and Corke 1981. The USEPA classified the study as supplemental (raw data unavailable). The duration of exposure is not reported.	Reported in USEPA 2016 review (PMRA# 3253945)
	12–14 days	DEA, >95%	EC_{50} = 8500 EC_{50} = 5500 EC_{50} = 4800 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classified the study as supplemental (NOAEC and raw data unavailable).	Reported in USEPA 2016 review (PMRA# 3253945)
	12–14 days	DIA, >95%	EC_{50} >1000 EC_{50} >10 000 EC_{50} = 9300 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classified the study as supplemental (NOAEC and raw data unavailable).	
	12–14 days	DACT, >95%	EC_{50} > 10 000 EC_{50} > 10 000 EC_{50} > 100 000 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classified the study as supplemental (NOAEC and raw data unavailable).	

Genus and/or species	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
	12–14 days	HA, >95%	EC_{50} > 10 000 EC_{50} > 10 000 EC_{50} > 100 000 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classified the study as supplemental (NOAEC and raw data unavailable).	
Anabaena flos-aquae	2 d	Atrazine, 97.5%	EC_{50} = 56 (cell density) EC_{50} = 96 (Growth rate) EC_{50} = 87 (PSII quantum yield) (measured)		Brain et al., 2012 (PMRA# 2816820)
Anabaena inaequalis	12 d	Atrazine, >95%	EC_{50} = 30 (reduced cell count)	Cited: Stratton 1984. Stratton and Corke 1981 The USEPA classified the study as supplemental (raw data unavailable). The duration of exposure is not reported.	Reported in USEPA 2016 review (PMRA# 3253945)
	12–14 days	DEA, >95%	EC_{50} = 1000 EC_{50} = 4000 EC_{50} = 2500 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classified the study as supplemental (NOAEC and raw data unavailable).	
	12–14 days	DIA, >95%	EC_{50} = 2500 EC_{50} = 7000 EC_{50} = 9000 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classified the study as supplemental (NOAEC and raw data unavailable).	
	12–14 days	DACT, >95%	EC_{50} = 7000 EC_{50} > 10000 EC_{50} > 100000 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classified the study as supplemental (NOAEC and raw data unavailable).	
	12–14 days	HA, >95%	EC_{50} > 10000 EC_{50} > 10000 EC_{50} > 100000 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classified the study as supplemental (NOAEC and raw data unavailable).	
Anabaena sp	4 d	Atrazine technical, purity not reported	EC_{50} >3000 (growth)	Cited: Fairchild et al., 1998	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792
Anabaena variabilis	NR	Atrazine, 97%	EC_{50} = 100 (photosynthesis)	Cited: Stratton and Corke 1981 The USEPA classified the study as supplemental (raw data unavailable). The duration of exposure is not reported.	Reported in USEPA 2016 review (PMRA# 3253945)
	12–14 days	DEA, >95%	EC_{50} = 3500 EC_{50} = 7500 EC_{50} = 700 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classified the study as supplemental (NOAEC and raw data unavailable).	Reported in USEPA 2016 review (PMRA#

Genus and/or species	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
	12–14 days	DIA, >95%	EC_{50} = 5500 EC_{50} = 9200 EC_{50} = 4700 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classified the study as supplemental (NOAEC and raw data unavailable).	3253945)
	12–14 days	DACT, >95%	EC_{50} > 10000 EC_{50} > 10000 EC_{50} > 100000 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classified the study as supplemental (NOAEC and raw data unavailable).	
	12–14 days	HA, >95%	EC_{50} > 10000 EC_{50} > 10000 EC_{50} > 100000 (nominal)	50% reduction in cell count 50% reduction in growth rate 50% reduction in photosynthesis The USEPA classifies the study as supplemental (NOAEC and raw data unavailable).	
Euglena gracilis	7 d	Purity not reported	EC_{50} = 496 (photosynthesis)	Cited: Thuillier-Bruston et al. 1996	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792
Navicula pelliculosa	5 d	Atrazine, 97.1%	EC_{50} = 60 (growth)	Cited: Hughes 1986 The 2016 USEPA review (PMRA# 3253945) classified the study as supplemental based on unavailability of raw data. The EC_{50} was extrapolated; a NOAEC was not determined.	
	2 d	Atrazine, 97.5%	EC_{50} > 237 (Cell density) EC_{50} > 237 (Growth rate) EC50 = 123 (PSII quantum yield) (measured)	Effects at the highest test concentrations (250 µg/L) were determined to e reversible.	Brain et al., 2012 (PMRA# 2816820)
Porphyridium aerugineum	4 d	Purity not reported	EC_{50} = 215.7 (growth rate)	Cited: Boura-Halfon et al., 1997.	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792
36 freshwater algal strains	14 d	Atrazine, 99%	EC_{50} = 10 EC_{50} = 1000	The USEPA classified the study as supplemental (raw data unavailable).	Reported in USEPA 2016 review (PMRA# 3253945)

Table 15 Effects of atrazine on freshwater aquatic vascular plants

Note: The toxicity data for freshwater algae summarized in Table 15 reports the most sensitive EC_{50} endpoint value for each genus/species. The majority of the data cited in Table 15 originates from the 2020 USEPA BE for atrazine (Appendix 2-1, PMRA# 3292792) and/or the 2016 USEPA ecological risk assessment for atrazine (Appendix B: Supporting Ecological Toxicity Data - PMRA# 3253945), unless specified otherwise.

Organism	Exposure	Test Substance	Endpoint Value [1] (µg a.i./L)	Comments	Reference
Duckweed *Lemna gibba*	14-day	Atrazine, 97%	EC_{50} = 37 (measured)	50% reduction in growth. The USEPA classified the study as supplemental (NOAEC was not determined).	Reported in USEPA 2016 review (PMRA# 3253945)
Duckweed *Lemna minor*	7-day	Atrazine, >99%	EC_{50} = 40.5	Population growth rate Hu et al., 2017	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792
Duckweed *Lemna* sp.	96-hour static	Technical atrazine	EC_{50} = 92	Growth; Fairchild et al., 1998	Reported in USEPA 2016 review (PMRA# 3253945)
Lesser duckweed *Lemna aequinoctialis*	7-day	Atrazine, 100%	EC_{50} = 58	Photosystem II electron transport activity Park et al., 2017	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792)
Duckweed *Lemna perpusilla*	7-day	Atrazine, 100%	EC_{50} = 13 487	Population growth rate, Phewnil et al., 2012	
Broad Waterweed *Elodea canadensis*	14-day	Atrazine, 96%	EC_{50} = 4.6 (measured)	50% reduction in dry root biomass. McGregor et al., 2008. The USEPA classified the study as supplemental (no explanation provided).	Reported in USEPA 2016 review (PMRA# 3253945)
Waterweed *Elodea* sp.	14-day	Not reported	EC_{50} = 21	Wet weight	
Bearded stonewort *Chara caenscens*	1-day	Atrazsine, >99%	EC_{50} = 145.6	Chlorophyll: Kuster et al., 2007	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792)
Hydrilla *Hydrilla verticillata*	14-day	Atrazsine, 100%	EC_{50} = 110	Length; Hinman 1989	
Ceratophyllum sp.	14-day	Not reported	EC_{50} = 22	Wet weight	Reported in USEPA 2016 review (PMRA# 3253945)
Najas sp.	14-day	Not reported	EC_{50} = 24	Wet weight	
Eurasian Water-Milfoil *Myriophyllum spicatum*	4 weeks	Not reported	EC_{50} = 91	50% reduction in O_2 production. The USEPA classified the study as supplemental (raw data unavailable).	
Parrot Feather Watermilfoil *Myriophyllum aquaticum*	10-day	Atrazine, 98%	EC_{50} = 76.4	Biomass; Teodorovic et al., 2012	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792)
Water milfoil *Myriophyllum sibiricum*	14-day	Atrazine, 99%	IC_{50} = 2066 - 2118	Number of nodules/nodulated plant roots, Roshon 1997.	
Water celery *Vallisneria americana*	42-day	Atrazine, 100%	EC_{50} = 163	Growth, Forney et al., 1981.	

Organism	Exposure	Test Substance	Endpoint Value [1] (µg a.i./L)	Comments	Reference
Pondweed *Potamogeton perfoliatus*	4 weeks	Not reported	EC_{50} = 30	50% reduction in O_2 production. The USEPA classifies the study as supplemental (raw data unavailable).	Reported in USEPA 2016 review (PMRA# 3253945)
Sweetflag *Acorus americanus*	7-day	Atrazine, 100%	EC_{50} = 24 300	Biomass: Marecik et al., 2012	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792)
Catail *Typha augustifolia*			EC_{50} = 5240		
Catail *Typha latifolia*			EC_{50} = 8760		

Amphibian toxicity data

The acute lethality data cited in Table 16 originates from the 2016 USEPA ecological risk assessment for atrazine (Appendix B.2: Open Literature Review of Amphibians - PMRA# 3253947). No additional acute amphibian toxicity data was identified in the more recent 2020 USEPA BE for atrazine.

Table 16 Acute toxicity of atrazine to amphibians

Organism	Exposure	Test Substance	Endpoint Value (µg a.i./L)	Comments	Reference
American bullfrog *Rana catesbeiana*	4 day post hatch, flow-through	Atrazine formulation (wettable powder, 80%)	96-h LC_{50} = 410	The LC_{50} values were based on mortality as well as observed abnormalities expected to result in mortality under natural conditions.	Birge et al., 1980; (reported 2016 USEPA Appendix B.2 – PMRA# 3253947)
Leopard frog *Rana pipiens*			96-h LC_{50} = 7680		
Pickerel frog *Rana palustris*			96-h LC_{50} = 17 960		
American toad *Bufo americanus*			96-h LC_{50} > 48 000		
Leopard frog *Rana pipiens*	4 day, early and late stage, flow through	Atrazine 4L (40.8%)	96-h LC_{50} = 47 600 (early stage) 96-h LC_{50} = 14 500 (late stage)	Classified as qualitative by USEPA (2016) based on lack of control data, only LC_{50} values reported and testing above water solubility.	Howe et al., 1998; (reported 2016 USEPA Appendix B.2 – PMRA# 3253947)
American toad *Bufo americanus*			96-h LC_{50} = 26 500 (early stage) 96-h LC_{50} = 10 700 (late stage)		
African clawed frog *Xenopus laevis*	Adult, 4 day	Atrazine formulation (40.8%)	96-h LC_{50} = 40 800	Renewal period not reported. Classified as qualitative by USEPA (2016) based on limited water quality reported.	Morgan et al., 1996; (reported 2016 USEPA Appendix B.2 – PMRA# 3253947)

Organism	Exposure	Test Substance	Endpoint Value (µg a.i./L)	Comments	Reference
American bullfrog *Rana catesbeiana*	Tadpole, static	Atrazine technical (98%) Atrazine 500 (formulation - 48.5%)	96-h LC_{50} > 16 000 (technical) 96-h LC_{50} > 232 800 (formulation)	Classified as qualitative by USEPA (2016) based on lack of summary data (control mortality not reported).	Wan et al., 2006; (reported 2016 USEPA Appendix B.2 – PMRA# 3253947)
African clawed frog *Xenopus laevis*	Embryo, static	Atrazine technical (purity not reported)	96-h LC_{50} = 24 500–25 600	The EC_{50} values (teratogenic effects) for *X. laevis* and *X. tropicalis* range from 4100–4400 µg a.i./L and 2100–9300 µg a.i./L, respectively. The USEPA (20) states that the study provides relevant information on the acute effects of atrazine on amphibian development, but the test conditions in the assay tend not to be optimal for use in a risk assessment, especially in solvent choice and concentration (DMSO – 1%). The study was classified as valid.	Fort et al., 2004; (reported 2016 USEPA Appendix B.2 – PMRA# 3253947)
Western clawed frog *Xenopus tropicalis*			96-h LC_{50} = 19 400–27 500		

Chronic amphibian toxicity data

For the special review, an arbitrary threshold was used to focus on amphibian studies classified as acceptable (either from a qualitative or quantitative standpoint) reporting apical effect endpoints at or below 100 µg a.i./L; this threshold is consistent with several robust surface water monitoring data sets available for atrazine in Canada which show that atrazine concentrations do not exceed 100 µg a.i./L. Chronic effects reported across multiple studies are categorized into the following effect classes:

1) Survival - Table 17;
2) Effects on amphibian development (growth and metamorphosis) – Table 18;
3) Effects on Sexual Development (skewed sex ratio and gonadal effects) - Table 19.

A high degree of variability in the concentrations exists among reported chronic effect endpoints both within and across species; while several studies demonstrate effects in amphibians following atrazine exposure, other studies have not found any evidence of effects in the same species for the same endpoint.

The reason for the different responses to atrazine exposure may be due to differences in experimental design and experimental conditions (loading rate, water quality, species differences, source of test organisms and condition at the time of testing, absence or presence of stressors), and more importantly, the lack of knowledge of species-specific developmental windows.

There are no clear criteria for evaluating the quality and the results of non-standard studies from the open literature, especially when studies are set to assess chronic and endocrine effects. Furthermore, based on many uncertainties identified across amphibian chronic toxicity studies, the determination of a specific quantitative endpoint(s) suitable for the purpose of risk assessment is complicated and challenging. The environmental risk assessment considers any observed effect(s) that directly relate or result in a measurable wholistic effect endpoint (for example, mortality, reproduction, growth development, behavioural) that would potentially cause harm at the population level and at environmentally relevant concentrations. In consideration of all study evidence considered for the amphibian review, the most sensitive and salient apical endpoint that could be considered for the risk assessment is a NOEC of 8 µg a.i./L atrazine based on reduced growth in *P. nigromaculatus* tadpoles (Huang et al., 2020).

Table 17 Effect of atrazine on amphibian survival (chronic amphibian exposure studies)

Species	Developmental stage (NF or G)[1]	NOEC / LOEC values (µg a.i./L)	References
X. Laevis	NF stage 46–66	200 / >200 (highest test concentration)	Hayes 2002 (PMRA# 3284067)
	NF stage 46–66	100 / > 100 (highest test concentration)	Kloas 2009a (PMRA# 3284070)
	NF stage 46–66	320 / >320 (highest test concentration)	Sullivan and Spence 2003 (PMRA# 3293272)
	NF stage 47 – (58–62)	400 / >400 (highest test concentration)	Zaya et al., 2011 (PMRA# 3292202)
	NF stage 56	18 / >18 (single test concentration)	Tavera-Mendoza et al., 2002 a,b (PMRA# 2752414 and 2752413, respectively)
R. arenarum	Stage G25–42	100 / 1000	Brodeur et al., 2009 (PMRA# 3293278)
A. maculatum	Eggs (Harrison stage 10–17) – 76 days	50 / 100	Olivier and Moon 2010 (PMRA# 3292188)
Litoria raniformis	Stage G26–42	25 (single test concentration)	Choung et al., 2011 (PMRA# 3292194)
L. tasmaniensis	Stage G28–42	30 / >30	Spolyarich et al., 2010 (PMRA# 3292191)
B. americanus, P. triseriata	Stage G25–42	1.25 / > 1.25 (highest test concentration)	Williams and Semlitsch 2010 (PMRA# 3292193)
H. versicolor	Stage G25–42	1.25 / >1.25 (highest test concentration)	Williams and Semlitsch 2010 (PMRA# 3292193)
	Stage G25–42	20 / > 20 (single test concentration)	Boone and Bridges-Britton 2006 (PMRA# 2745191)
	Post hatch to metamorphosis	6.4 / >6.4 (single test concentration)	Relyea 2009 (PMRA# 3292186)
R. pipiens	Tadpoles to metamorphosis (initial stage not reported)	25 / >25 (highest test concentrations)	Detenbeck et al., 1996 (PMRA# 3292206)
	Post hatch to metamorphosis	6.4 / >6.4 (single test concentration)	Relyea 2009 (PMRA# 3292186)
	Stage G21–46	0.19 / > 0.19 (single test concentration)	Hayes et al., 2006 (PMRA# 3292209)
R. silvatica	Stage G23–26 to Stage G42–46	25 / > 25	Rohr and Crumrine 2005 (PMRA# 3292182)
R. clamitans	Tadpoles (stage unspecified) up to 4 weeks; survival results confounded from trematode infections	102 (single test concentration)	Rohr et al., 2008 (PMRA# 2752408)
R. palastris		102 (LOEC value, single test concentration)	
A. Blanchardi	Stage G25–46	100 / 200	Hoskins and Boone 2017 (PMRA# 3256783)
R. catesbiana	Tadpole stage (unspecified) to metamorphosis	20 (single test concentration)	DeNoyelles 1989 (reported 2016 USEPA Appendix B.2 – PMRA# 3253947)
A. barbouri	Embryo stage through metamorphosis	40 / 400	Rohr et al., 2004 (PMRA# 3292181)
	Exposure phase: Embryo stage through metamorphosis; long term survival 14 months post exposure	<4 / 4	Rohr et al., 2006 (PMRA# 1491556)
	Exposure phase: Embryo stage	400 / >400	Rohr and Palmer 2005 (PMRA# 3292184)

Species	Developmental stage (NF or G)[1]	NOEC / LOEC values (µg a.i./L)	References
	through metamorphosis; survival at 130 to 239 days post exposure		

1 – NF – Nieuwkoop and Faber staging scale; G – Gosner staging scale.

Table 18 Effects of atrazine on amphibian development (growth and metamorphosis)

Species	Developmental stage (NF or G)[1]	Developmental endpoint	NOEC/LOEC value (µg a.i./L)	References
X. laevis	NF Stage 47–58	Delay in metamorphic stage–	<100 / 100	Freeman and Rayburn 2005 (PMRA# 3293276)
	NF Stage 46–66	Time to metamorphosis, growth (body mass, SVL)	100 / >100	Kloas 2009a (PMRA# 3284070)
	NF stage 49 - 66	Time to metamorphosis	100 / >100	Oka et al., 2008 (PMRA# 3262466)
	NF Stage 46 - 66	Time to metamorphosis	320 / >320	Sullivan and Spence 2003 (PMRA# 3293272)
		Growth (mass at metamorphosis)	< 20 / 40	
	NF stage 47 – (58–62)	Growth (body mass)	25 / 200	Zaya et al., 2011 (PMRA# 3292202)
		Time to metamorphosis	200 / 400	
	NF stage 46–66	Growth (time to metamorphosis, mass, SVL)	200 / >200	Hayes 2002 (PMRA# 3284067)
	NF stage 47–66	Growth (body length, body weight, liver weight and hepatosomatic index -HSI of males)	97.7 / > 97.7	Sai et al., 2016 (PMRA# 3292213)
R. arenarum	Stage G38–39 to 42	LOEC based on slight accelerated time to reach metamorphosis (stage 42) based on statistical comparisons of EC_{50} values (time required for 50% of animals to reach stage 42). A NOEC for metamorphosis was not determined (ND).	ND / 100	Brodeur et al., 2009 (PMRA# 3293278)
L. raniformis	Stage G26–42	Time to metamorphosis, mass, SVL; (single test concentration)	25	Choung et al., 2011 (PMRA# 3292194)
A. maculatum	Eggs (Harrison stage 10–17) – 76 days	Hatching success	50 / 100	Olivier and Moon 2010 (PMRA# 3292188)
R. pipiens	Stage G21–46	Time to metamorphosis; (single test concentration)	0.19	Hayes et al., 2006 (PMRA# 3292209)
	Tadpoles to metamorphosis (intial stage not reported)	Time to metamorphosis	25 / > 25	Detenbeck et al., 1996 (PMRA# 3292206)
	Stage G24–42	Time to metamorphosis	<0.1 / 1.8	Langlois et al., 2010 (PMRA# 1849796)
	Stage G24–42	Growth (body weight, snout-vent length at metamorphosis)	1.8/ > 1.8	
	Post hatch to metamorphosis	Time to metamorphosis, growth (mass at metamorphosis); (single test concentration)	6.4	Relyea 2009 (PMRA# 3292186)
A. barbouri	Embryo stage through metamorphosis	Time to metamorphosis	4 / 40	Rohr et al., 2004 (PMRA# 3292181)
		Growth (body mass)	40 / 400	

Species	Developmental stage (NF or G)[1]	Developmental endpoint	NOEC/LOEC value (µg a.i./L)	References
L. tasmaniensis	Stage G28–42	Growth (total body length)	30 / >30	Spolyarich et al. 2010 (PMRA# 3292191)
H. versicolor	Stage G25–60	Time to metamorphosis	25.1 / >25.1	Storrs-Mendez and Semlitsch 2010 (PMRA# 3292187)
	Stage G25–42	Time to metamorphosis, growth (mass at metamorphosis); (single test concentration)	20	Boone and Bridges-Britton 2006 (PMRA# 2745191)
	Post hatch to metamorphosis	Time to metamorphosis, growth (mass at metamorphosis); (single test concentration)	6.4	Relyea 2009 (PMRA# 3292186)
	Stage G25–42	Growth (mass at metamorphosis); (single test concentration)	1.25	Williams and Semlitsch 2010 (PMRA# 3292193)
R. sphenocephala	Stage G25–60	Time to metamorphosis	30.4 / >30.4	Storrs and Semlitsch 2008 (PMRA# 3292185)
B. americanus			125 / >125	
	Stage G25–42	Growth (mass at metamorphosis); (single test concentration)	1.25	Williams and Semlitsch 2010 (PMRA# 3292193)
P. triseriata	Stage G25–42	Growth (mass at metamorphosis); (single test concentration)	1.25	Williams and Semlitsch 2010 (PMRA# 3292193)
R. catesbiana	Tadpole stage (unspecified) to metamorphosis	Growth (mass)	20 / >20	DeNoyelles 1989; reported in 2016 USEPA Appendix B.2 – PMRA# 3253947)
R. sylvatica	Stage G23–26 to Stage G42–46	Time to metamorphosis; (single test concentration applied twice – 2 week interval)	25	Rohr and Crumrine 2005 (PMRA# 3292182)
P. nigromaculatus	Stage G26 for 10, 15, 20 and 25 days	Growth (SVL, tadpole length, width and bodyweight)	8 / 16 (day 20 and 25 all growth parameters) 32 / >32 (day 10 bodyweight)	Huang et al., 2020 (PMRA# 3262453)
A. blanchardi	Stage G25 - 46	Time to metamorphosis, growth (body mass)	200 / >200	Hoskins and Boone 2017 (PMRA# 3256783)

1 – NF – Nieuwkoop and Faber stage; G – Gosner stage.

Table 19 Effects on atrazine on sexual development in amphibians (sex ratio, gonadal abnormalities)

Species	Developmental stage (NF or G)[1]	Developmental observation	NOEC/LOEC value (µg a.i./L)	References
Sex ratio				
X. laevis	NF stage 46–66	No effect up to highest test concentration.	100 / >100	Kloas 2009a (PMRA# 3284070)
	NF stage 49–66	Female skew at 10 µg a.i./L	1 / 10	Oka et al., 2008 (PMRA# 3262466)
	NF stage 47 – (58–62)	No effect up to highest test concentration.	400 / >400	Zaya et al., 2011 (PMRA# 3292202)

Species	Developmental stage (NF or G)[1]	Developmental observation	NOEC/LOEC value (µg a.i./L)	References
	NF stage 47–66	No effect up to highest test concentration.	97.7 / > 97.7	Sai et al., 2016 (PMRA# 3292213)
L. tasmaniensis	Stage G28–42	No effect up to highest test concentration.	≥30 / > 30	Spolyarich et al., 2010 (PMRA# 3292191)
H. versicolor	Stage G25–60	No effect observed at 2.81 µg a.i./L, but effect was observed at 0.92 and 25.1 µg a.i./L, highest test concentration)	<0.92 / 0.92	Storrs-Mendez and Semlitsch 2010 (PMRA# 3292187)
	Juveniles	Exposure stage and duration unspecified. No effect up to highest test concentration.	29.5 / >29.5	
R. sphenocephala	Stage G25–60	No effect up to highest test concentration.	30 / >30	
	Juveniles	Exposure stage and duration unspecified. No effect up to highest test concentration.	24.6 / > 24.6	
B. americanus	Stage G25–60	Female skew at 125 µg a.i./L	7.55 / 125	
	Juveniles	Exposure stage and duration unspecified.	<3 / 3	
R. pipiens	Stage G24–42	Female skew at 1.8 µg a.i./L	0.1 / 1.8	Langlois et al., 2010 (PMRA# 1849796)
Gonadal abnormalities				
X. laevis	NF stage 46–66	No gross or histological abnormalities reported up to highest test concentration.	100 / >100	Kloas 2009a (PMRA# 3284070)
	NF stage 49–66	Histological analysis: normal testes and ovaries observed up to highest test concentration.	100 / >100	Oka et al., 2008 (PMRA# 3262466)
	NF stage 47 – (58–62)	No gross gonadal abnormalities reported up to the highest test concentration. Gonadal histology was not conducted.	400 / > 400	Zaya et al., 2011 (PMRA# 3292202)
	NF stage 47 - 66	Male gonad weight, gonadosomatic index – GSI	9.7 / 97.7	Sai et al., 2016 (PMRA# 3292213)
		Histological analysis: testicular degeneration at all test concentrations, especially in froglets from the groups with 0.1 and 100 µg a.i./L.	<0.1 / 0.1	
	NF stage 56	Histological analysis: males - reduced testicular volume, number of spermatagonial cell nests and nursing cells; females - atretic oogonia, reduced primary oogonia, increased secondary oogonia). Single test concentration.	<18 / 18	Tavera-Mendez et al., 2002a, 2002b (PMRA# 2752414 and 2752413, respectively)
	NF stage 46–66	Histological analysis: increased testicular ova	0.01 / 0.1	Hayes 2002 (PMRA# 3284067)
		Histological analysis: reduced laryngeal muscle growth (reduction in the cross sectional area of the larynx in males).	0.8 / 1.0	
R. pipiens	Stage G24–42	Histological analysis: Testicular oocytes in male gonads of positive control (17β-estradiol treated males) but none were observed in controls, atrazine treatments or field collected samples.	1.8 / >1.8	Langlois et al., 2010 (PMRA# 1849796)
	48h post hatch to Stage G66	Gross morphology and histological analysis: underdeveloped testes with poorly structured closed lobules and low to absent germ cells. Presence of testicular oocytes.	<0.1 / 0.1	Hayes et al., 2003 (PMRA# 2750378)
	Stage G21–46	Histological analysis: Testicular oogenesis was not observed. Single test concentration.	0.19 / >0.19	Hayes et al., 2006 (PMRA# 3292209)
L. tasmaniensis	Stage G28–42	Gross morphology and histological analysis: No effect up to highest test concentration.	30 / > 30	Spolyarich et al., 2010 (PMRA# 3292191)
H. versicolor, R. sphenocephala, B. americanus	Stage G25 - 60	Gonadal histological analyses: Underdeveloped testes and testicular oocytes. Results were generally inconsistent across treatments, controls and positive controls among tests for all three species, and results were not analysed statistically.		Storrs-Mendez and Semlitsch 2010 (PMRA# 3292187)

1 – NF – Nieuwkoop and Faber staging scale; G – Gosner staging scale.

Table 20 Summary of freshwater aquatic microcosm, mesocosm and community-level studies

Note: All studies listed were considered and evaluated based on reporting in the 2003 USEPA RED for atrazine and the 2016 USEPA refined ecological assessment for atrazine, Giddings and Campana (2016) and studies available in the public literature. PMRA numbers are provided for studies that are available in the PMRA database only; full citations for studies (without PMRA numbers) are provided in the references. Bolded rows indicate the studies considered in the weight of evidence for the NOEC of 20 µg a.i./L used in the refined freshwater risk assessment.

Study parameters	Results and estimated toxicity endpoints (µg a.i./L)	Acceptability of study, and other comments	Reference/ PMRA#
Freshwater microcosm tested at 0, 0.5, 5, 50 µg a.i./L	Decreased net oxygen production: NOEC = 5 Inhibition or recovery in O_2 production was immediate when atrazine was added to or removed from the microcosms. Algal community structure was not affected.	Study is acceptable; however, immediacy of recovery or inhibition in dissolved oxygen indicates that transient effects are expected.	Brockway et al. 1984; 1404514
Freshwater microcosm (0.5 to 1-L) at 10g/L applied one time.	Plant biomass, midge survival and Daphnia mortality: NOEC > 10 Initial reduction in dissolved oxygen (DO) but recovery occurred at 6 weeks. NOEC = 10	Study is acceptable, however, the single application rate results in uncertainty in the NOEC for endpoints other than dissolved oxygen.	Huckins et al. 1986
Freshwater microcosm with bacteria, protozoa, algae, fungi, and small metazoans exposed to 0, 3.2, 10, 32, 110 and 337 µg a.i./L mean measured, for 21 days.	Decreased dissolved oxygen (DO) and uptake of Ca and Mg at study termination: NOEC = 10 Microbial population: NOEC = 110	Endpoints are acceptable. Quantitative.	Pratt et al. 1988; 1404537
Freshwater microcosms with macrophytes, algae, zooplankton and benthic invertebrates exposed to 10, 100 and 1000 µg a.i./L)	Macrophyte biomass, *Selenastrum* sp. dry wt., gross primary productivity with no recovery after 30 days: NOEC = 100	Study is acceptable. Quantitative.	Johnson 1986
Freshwater microcosm consisted of potted emergent plants (*Scirpus acutus* and *Typha latifolia*) exposed to 0, 10, 50, 100, 500 and 1500 µg a.i./L nominal).	Mean total height of *S. acutus* with no recovery observed during the 16-week study: NOEC = 100	There is uncertainty with the exact exposure concentration due to the lack of measurements; however, the NOEC can be considered in the risk assessment.	Langan and Hoagland 1996
Freshwater mesocosms with green algae, cladocerans, copepod nauplii, rotifers and bluegill exposed to 0, 15 and 153 µg a.i./L for 10 days and 0, 385 and 2,167 µg a.i./L (measured) for an additional 14 days.	Bluegill sunfish, primary productivity or algal cell density: NOEC = 153	The USEPA (2009) concluded this study was invalid for a number of reasons. See PMRA# 3301609 for details. PMRA agrees with their conclusion. The study cannot be used.	Hoagland et al. 1993
Freshwater mesocosms with plankton exposed to 0, 5, 10, 22, 68, 182 and 318 µg a.i./L (mean measured) for 63 days. Three treatments, 28 and 24 day intervals.	Dissolved oxygen. No recovery over study period. NOEC = 5 Copepod nauplii NOEC = 22 Phytoplankton cell density NOEC = 182	Only one replicate per level was used. USEPA ultimately excluded this study from further use in their risk assessment.	Juttner et al. 1995
Artificial ponds treated with one dose of atrazine at 20 and 500 µg a.i./L and monitored for 136 days.	**Phytoplankton biomass,primary productivity, phytoplankton species loss and successional changes and zooplankton biomass**	**Well conducted study. Obvious effects to phytoplankton and primary productivity at highest test concentration. The endpoint is acceptable.**	**deNoyelles et al., 1982**

Study parameters	Results and estimated toxicity endpoints (µg a.i./L)	Acceptability of study, and other comments	Reference/ PMRA#
	loss: NOEC = 20 **No effects observed in fish, NOEC >500**		
Artificial ponds treated with atrazine at 0, 20, 100 and 500 µg a.i./L; study duration was 16 weeks. Continuation of deNoyelles et al., (1982) ponds.	At 20 µg/L: 82% reduction in total insect emergence 89% reduction in non-predatory insect emergence 90% reduction in chironomid, *Labrundinia pilosella* 57% depression in nonpredatory insect species richness	Interpretation of this study is complicated when taking into consideration the introduction of grass carp into ponds and the effect this would have on macrophyte populations and subsequent indirect effects on insect production. The endpoint cannot be used, however, there is corroborating evidence for effects to macrophytes prior to the introduction of the grass carp (reported in Kettle et al. 1987)	Dewey, 1986 Denoyelles et al. 1989 (reported in USEPA 2016 PMRA# 2741498)
Artificial ponds treated with atrazine; two test concentrations at 20 and 500 µg a.i./L and monitored for 136 days. Concurrent with deNoyelles et al., (1982).	**60% reduction in macrophyte vegetation including elimination of *Potamogeton pusillus*, *P. nodosus*, *Najas quadalupensis*; dominated by *Chara globularis:* NOEC < 20** **90% reduction in macrophyte coverage 10 months after treatment.**	**This study was conducted prior to the introduction of grass carp (Dewey 1986 and Denoyelles et al. 1989); therefore, the effects observed on macrophytes in this study are considered to be relevant; however, the results described for the macrophytes are qualitative only (visual estimates verified by rake hauls given a score between 0 - empty and 4 - full).** **The study is acceptable: however, there is some uncertainty in the unbound NOEC.**	**Kettle et al. 1987**
Freshwater limnocorrals treated with atrazine two times with a six week interval. Measured concentrations were 80–140 µg a.i./L (first dose) with subsequent increases of 20–30% after the second dose.	Periphyton biomass; algal community structure, phytoplankton biomass and species composition zooplankton community structure changes NOEC < 80	Large mesocosms, highly realistic experiment. The study is acceptable: however, however, there is some uncertainty in the unbound NOEC.	Herman et al. 1986
Two artificial ponds, one pond was dosed with atrazine at 0, 100 and 300 µg a.i./L in 1985 and the other pond was dosed in 1987 at 0, 20, 100 µg a.i./L.	Reduced plant numbers, macrophyte and ohytoplankton species changes, zooplankton numbers: NOEC <100	A major deficiency with this study is the single replicate for each atrazine treatment. All results were based on observations of trends and no statistical analysis were conducted to decipher significant differences between treatments.	Neugebaur et al. 1990; 1404536
Lake enclosures , two test concentrations at 140 and 1560 µg a.i./L mean measured (1982 treatment, 56-day study). In 1983, treatment was at 80 µg a.i./L × 2 applications 35-day interval (223-day study).	Shift from a chlorophyte- to a diatom-dominated community, community productivity, reduced growth:.NOAEC < 80 µg a.i./L	Productivity recovery was observed after day 21. The study is acceptable; however, there is some uncertainty in the unbound NOEC.	Hamilton et al. 1987
Lake enclosures ; one test concentration at 100 µg a.i./L nominal; two pulses 35-day interval. 323-day study.	Phytoplankton species richness and community structure recovery noted after 77 days: NOEC <100	Studies are acceptable, however, the single treatment concentration results in uncertainty in the endpoint.	Hamilton et al. 1988, 1989

Study parameters	Results and estimated toxicity endpoints (µg a.i./L)	Acceptability of study, and other comments	Reference/ PMRA#
Natural streams, treated with 0, 2, 30, or 100 µg/L for 24 hr, followed by a second 24-hr atrazine pulse two weeks later. Observations made for 44-days.	NOEC >100 Decreases in biomass and cell density of periphyton were observed in both the controls and all treatments. There was no difference between any atrazine treatment.	Study is acceptable however, there is some uncertainty in the unbound NOEC.	Jurgensen and Hoagland 1990; 1404527
Artificial streams consisted of periphyton and benthic invertebrates exposed to a concentration of 5 µg/L on day 1 with dilution to 1 µg/L by day 7; 14-day observation	Functional or taxonomic composition of benthic invertebrate community chlorophyll *a* production: NOEC >5 Insect emergence: NOEC < 5	USEPA (2016) indicated no effects in this study. Short duration, study is acceptable; however, the single treatment concentration results in uncertainty in the NOEC.	Gruessner and Watzin 1996; 1404522
Artificial streams with periphyton exposed to atrazine at 0, 24 and 134 µg a.i./L; conducted at 10° and 25°C for 12-d.	**Periphyton biomass and chlorophyll *a*: NOEC < 24**	**The study is acceptable, however, there is some uncertainty in the unbound NOEC.**	**Krieger et al., 1988; 1404530**
Artificial streams with periphyton and macroinvertebrates exposed for 30 days to 25 µg a.i./L nominal with a 60-day interval; repeated 4 times in one year.	No effects on macroinvertebrate population structure, periphyton standing biomass or rates of primary productivity and community respiration: NOEC > 25	Study is acceptable; however, the single treatment concentration results in uncertainty in the endpoint.	Lynch et al., 1985
Artificial streams with periphyton exposed to atrazine at 100 µg a.i./L for 14-d followed by 21-d recovery. 43-day acclimatization period.	Algal biomass and total Chlorophyta biomass bacterial community structure: NOEC < 100 Community structure did not recover during the 21-d period.	Artificial streams, single concentration. Study is acceptable, however, the unbound NOEC results in uncertainty of the of the endpoint.	Hamala and Kollig 1985
Freshwater mesocosms treated at 0 and 50 µg/L (measured). Concentration declined to 20 µg a.i./L by 8 weeks after treatment. Four month study duration.	**LOEC: estimated to be between 20 and <50** **The macrophyte *Najas* sp. was nearly eliminated over a 4-month period and was replaced by the macroalga, *Chara* sp. Change in community structure.**	**Study is acceptable, however, the single treatment concentration results in uncertainty in the endpoint.**	**Fairchild et al. 1994; 1404520**
Freshwater cosms consisted of sediment, water, phytoplankton and macro- invertebrates exposed to 5 µg a.i./L for 7 weeks. Change in community structure was traced.	Plankton community structure: NOEC > 5	Study is acceptable, however, the single treatment concentration results in uncertainty in the endpoint.	Van den Brink 1995
Stream mesocosms; natural succession and planted macrophytes. Treatments consisted of a stepped exposure regime of four increasing concentrations: 15 µg/L (May 18-June I), 25 µg/L (June 2-July 15), 50 µg/L (July 16-August 17), and 75 µg/L nominal (August 18-September 4).	Gross Primary productivity and DO: NOEC <15: cattail biomass: NOEC = 50: *Elodea* biomass or stem length: NOEC > 75 Wild rice *(Zizania aquatica)* plants were visibly more senescent in treated wetlands than in control wetlands by August 12, the end of the 50 µg/L	Study is acceptable; however, it is impossible to determine if there was any recovery in primary productivity or DO at 15 µg/L because the treatment concentrations were increased monthly.	Detenbeck et al. 1996; 1404517

Study parameters	Results and estimated toxicity endpoints (µg a.i./L)	Acceptability of study, and other comments	Reference/ PMRA#
	exposure regime. Chlorophyll *a* content of wild rice leaves of plants collected from treated wetlands on August 25 was only *25%* that of plants from control mesocosms.		
Stream cosms, seeded with colonized stones (algae and bacteria) in upstream mixing boxes and, two weeks later, with kick-screen benthic samples (algae, bacteria, and invertebrates) from North Bosque River. Three 4-d pulses of atrazine with 7-d intervals between pulses. Pulses of 50 µg/L, 100 µg/L, and 150 µg/L were administered to give 60-d rolling average concentrations of 10 µg/L, 20 µg/L, and 30 µg/L. 60-day study.	Metaphyton and periphyton community structures were not affected at any treatment. NOEC >30	USEPA (2016, PMRA# 2741498) concluded this study was qualitative in nature. PMRA agrees with the analysis by the USEPA. Background levels of atrazine in the source water and continuous additions of nutrients likely confounded the results.	King et al. 2014 (reported in USEPA 2016 PMRA# 2741498) and King et al. 2016
Large plastic enclosures were filled with lake water and suspended in a lake for 20 days, treated at 1, 10 and 20 µg a.i./L (nominal).	There was no effect of atrazine on phytoplankton chlorophyll, photosynthesis, or species composition in any treatment. NOEC >20 µg a.i./L	Entirely closed cosms, no sediment, limiting the realism of the experiment. the unbound NOEC results in uncertainty.	Gustavson and Wangberg 1995
Artificial substrates placed into a 600 mL glass beaker with 500 mL of water, Atrazine exposures at 20 and 200 µg a.i./L, single dose. 168-h study duration.	Chlorophyll, carbon assimilation, bacterial abundance, and bacterial productivity: NOEC = 20 µg a.i./L Total protist taxa: NOEC <20 µg a.i./L.	Very small containers, not a true mesocosm study. Study not acceptable as a mesocosm study.	Downing et al. 2004
Five-week constant exposure to single concentration of approximately 70 µg a.i./L. Followed by an additional 5 months of sampling (173 DAT).	Inhibition of photosynthesis, phytoplankton abundance and species composition, macrophyte photosynthetic efficiency (days 2 and 5): NOEC <70 µg/L.	Studies are acceptable. The single test concentration results in uncertainty in the endpoint. Recovery was observed for some parameters.	Knauert et al. 2008, 2009, 2010
Outdoor tanks with sediment seeded with 1L of water from a nearby creek for colonization of microorganisms, algae, zooplankton, and snails. Natural aerial oviposition to populate insect species. Mean measured concentration of atrazine in cosms was 32 µg a.i./L over the 42 days. Pulse dose every 10 days.	**Adult insect abundance, benthic insect abundance, Chlorophyll was suppressed but recovered quickly: NOEC <32 µg a.i./L**	**Study was comprehensive with sampling of water quality, chlorophyll and higher trophic level species. The unbound endpoint results in some uncertainty in the endpoint.**	**Henry and Wesner 2018**
Freshwater microcosms, atrazine added at initial concentrations of 0, 1, 10, 30 and 100 µg/L.	**Mean concentrations at 1-h and 70-d were 0, 0.7, 7.4, 19.1, and 68.5 µg/L in the respective treatments. Macrophytes root weight and shoot weight: NOEC = 19.1 µg/L.**	**The study authors reported effects using nominal concentrations; however, PMRA used mean measured concentrations from 1-h and 70-d to determine the NOEC of 19.1 µg/L. This would be more indicative of the actual exposure concentration during the**	**Baxter et al. 2011 (reported in USEPA 2016 PMRA# 2741498)**

Study parameters	Results and estimated toxicity endpoints (µg a.i./L)	Acceptability of study, and other comments	Reference/ PMRA#
		course of the study. Study is acceptable.	
21-d study, artificial recirculating streams, single dose at 10, 100, 1000, 10,000 µg/L	Decrease of 40% in primary productivity at 10 µg/L, slight decrease in biovolume. Recovery: Primary productivity >21 days; biovolume <7 days	USEPA estimated the magnitude of changes from figures in the papers. Qualitative use only.	Kosinski 1984; Kosinski and Merkel 1984 (reported in USEPA 2016 PMRA# 2741498)
9 L water in plastic tubs, 30 day duration, 25 µg/L with additional 25 µg/L after 2--weeks	15.2% reduction in periphyton chlorophyll-a Recovery not reported. Authors assumed final concentration was 50 µg/L. NOEC cannot be determined.	Reviewed by the USEPA No. indication of recovery.	Rohr and Crumrine 2005 (reported in USEPA 2016 PMRA# 2741498)
800 L water held in plastic cattle tanks, single pulse at 117 µg/L, 4-weeks,	Phytoplankton chlorophyll-a and periphyton chlorophyll-a: at study termination. NOEC >117	Reviewed by the USEPA. Recovery not reported. The unbound endpoint results in some uncertainty in the endpoint. Qualitative use only.	Rohr et al. 2008
Atrazine at 6.4 µg/L as a single dose, 36-d study	No effect to phytoplankton, chlorophyll-a, periphyton biomass, and dissolved oxygen:NOEC >6.4	Reviewed by the USEPA. Recovery not reported. Qualitative use only. The unbound endpoint results in some uncertainty in the endpoint.	Relyea 2009 (reported in USEPA 2016 PMRA# 2741498)
40-d study, atrazine applied at 2 and 30 µg/L as a single application.	At 30 µg/L an increase in periphyton chlorophyll-a at study termination: NOEC >30	Reviewed by USEPA and they concluded that there were no negative effects. The unbound endpoint results in some uncertainty in the endpoint.	Sequin et al. 2001a (reported in USEPA 2016 PMRA# 2741498)
5000 L mesocosms, nominal 30 µg/L atrazine, one application. Monitored for 25 days.	**Chlorophyll-a, dry weight of phytoplankton and DO,changes in community structure and Bray-Curtis similarity index. DO recovered about day 12: NOEC <30**	**Reviewed by USEPA. No indication of measurements of atrazine. The unbound endpoint results in some uncertainty in the endpoint.**	**Seguin et al. 2002**
Anurans and endemic phytoplankton were exposed to a single concentration of atrazine at 207 µg/L in 1m^3 mesocosm for 25 days	chlorophyll: NOEC >207	Reviewed by USEPA. Phytoplankton measurements only conducted to determine affects to amphibians. Single treatment rate and no clear treatment effects limits the use of the endpoint in the risk assessment.	Boone and James 2003 (reported in USEPA 2016 PMRA# 2741498)
90 L water, introduced macrophytes and frogs. Atrazine added at 0, 20, 200 and 2000 µg/L as one time application. Measured concentrations. 42-day study	**DO recovered by day 10, then declined again to end of study, macrophyte biomass (recovery not determined), chlorophyll-*a*: NOEC = 20**	**This study was considered to be qualitative by the USEPA.**	**Diana et al. 2000**
Freshwater mesocosms treated at 0, 25, 50, 100 and 250 µg/L. 42 days study. Wet and dry root, wet and dry shoot weights in macrophytes were measured at day 14, 28 and 42 from 4 different macrophyte densities	DO: NOEC = 50. EC_{10} at day 42 ranged from 4.2–20.8 (wet root); 4.1–21 (dry root); 6.8–39 (wet shoot) 2.4–29 (dry shoot) in *Elodea canadensis.*	Study is acceptable. No data was provided for macrophytes from the controls, therefore, an empirical NOEC cannot be determined. The EC_{10}s calculated by the authors can	McGregor et al. 2008

Study parameters	Results and estimated toxicity endpoints (µg a.i./L)	Acceptability of study, and other comments	Reference/ PMRA#
and planting methods	EC_{10} at day 42 ranged from 21–23 (wet root); 2– 42 (dry root); 26–34 (wet shoot), 14–27 (dry shoot) in *Myriophyllum spicatum*	provide estimates of the NOEC, however, it is the opinion of PMRA that many of the lowest calculated EC_{10}s are extrapolated too far below the lowest test concentration (24.5 µg/L measured).	

Reference	Rationale for excluding from consideration
Kish 2006	Periphytometers were placed in a diverted stream. Periphytometers were a series of 250 mL Nalgene bottles. (NOEC = 9 µg/L). The study is not a higher tier mesocosm study.
Perschbacher et al. 2008	Primary productivity was reduced for two days and recovered by day 7 (NOEC of 11.3 µg/L). Chlorophyll production was reduced day 3 to day 7 NOEC = 11.3 µg/L. Study length was too short to determine recovery. No changes in zooplankton abundance at any concentration.
Berard et al. 1999a and 1999b Berard and Benninghoff 2001; Leboulanger et al. 2001; Seguin et al. 2001b	Small containers (5 L). Giddings and Campana reviewed these studies and determined a low reliability score of 0.5 (lowest of all studies they reviewed). Health Canada agrees this study is of little value because of the small structures of the system and the lack of complexity.
Muturi et al. 2017	Small containers (400 mL beakers with 200 mL solution). Treated at 20 mg/L, an unrealistic high concentration.
Mohammad et al. 2008 and 2010	Lemna sp., conducted according to OECD toxicity guidelines. Not a higher tier study. Recovery observed once removed from atrazine (7 to 10-days).
Teodorovic et al. 2012	Lemna sp., conducted according to OECD toxicity guidelines. Myriophyllum sp. study conducted with spiked sediment and water or just water. Not a higher tier study.
Moorhead and Kosinski 1986	Treatments were described as mg/kg, therefore are not useful for the aquatic RA.
Shimabukuro et al. 1970 and 1976	Studies conducted with corn. Not relevant for aquatic risk assessment.
Stay et al. 1985	This study was included in the 2011 endpoints used by the EPA to calculate their CELOC. However, the information is not included here because the lowest test concentration is 430 µg/L which is far higher than effects observed in many of the studies discussed above.

Table 21 Acute toxicity of atrazine to estuarine/marine invertebrates

Organism	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Copepod (*Acartia tonsa*)	96 hr, static renewal (daily), 22°C, salinity – 31 g/L	Atrazine, 70%	96-hour LC_{50} = 88.9 (measured)	12% control mortality. Classified as supplemental by the USEPA (raw data unavailable).	Reported in USEPA 2016 review (PMRA# 3253945)
	96 hr, static, salinity - 20 g/L; 20°C	Atrazine, 97.4%	96-hour LC_{50} = 94 (nominal)	Classified as supplemental by the USEPA (raw data unavailable).	
	96 hr, static renewal (daily), 22°C, salinity – 31-32 g/L	Atrazine, 70%	96-hour LC_{50} = 139 (measured)	Classified as supplemental by the USEPA (20% control mortality).	
	96 hr, flow-through test. Salinity 31-33 g/L; 20° C	Atrazine, 97.1%	96-hour LC_{50} = 4300 (measured)	17 days old. Classified as supplemental by the USEPA (cloudy with no 0.45 µM filter of undissolved material).	

Organism	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Copepod (*Eurytemora affinis*)	96 hr, static; 20 °C, salinity - 5, 15, 25g/L	Atrazine, 97.1%	96-hour LC_{50} = 500 (salinity 5 g/L) 96-hour LC_{50} = 2600 (salinity 15 g/L) 96-hour LC_{50} = 13 300 (salinity 25 g/L) (measured)	Nauplii < 24 hours old. Classified as supplemental by the USEPA (no raw data on mortality).	
	96 hr, static, salinity 2ppt, 18°C	Atrazine technical (purity not reported)	96-hour LC_{50} = 125 (nominal)	Classified as qualitative by the USEPA (raw data unavailable).	
Copepod (*Tigriopus brevicornis*)	96 hr, static, salinity 35 ppt, 20°C	Atrazine, 99%	96-hour LC_{50} = 121–153 (nominal)	Various life stages. Classified as qualitative by the USEPA (raw data unavailable).	
Copepod (*Tigriopus japonicas*)	96 hr, static renewal, 30 ppt, 25°C	Atrazine, 97.4%	96-hour LC_{50} > 20 000 (nominal)	Acute toxicity of atrazine was assessed at concentrations of 0 (control), 1, 5, 10 and 20 mg/L atrazine (97.4% purity) using ovigerous female adults over a 96 hour period.	Yoon et al., 2019 (PMRA# 3201404)
Mysid Shrimp (*Americamysis bahia*)	96 hr, flow-through test. Salinity 26 g/L; 22°C	Atrazine, 97.4%	96-hour LC_{50} = 1000 (Measured)	Classified as supplemental by the USEPA (raw data unavailable).	Reported in USEPA 2016 review (PMRA# 3253945)
Mysid Shrimp (*Americamysis bahia*	96 hr, flow-through test. Salinity 32 g/L; 25-26 °C	Atrazine, 97.1%	96-hour LC_{50} = 5400 (measured)	Classified as acceptable by the USEPA. This data was recently published in Brain et al., 2021 (PMRA# 3242966).	
Mysid shrimp (*Americamysis bahia*)	96 hr, static, 23 - 25°C, salinity – 19–20 g/L	Hydroxyatrazine, 97.1%	96-hour LC_{50} > 2000 (5% mortality) (measured)	Classified as acceptable by the USEPA. Sayers L.E., 2005, PMRA# 2816904 (USEPA DER – PMRA# 2816905).	
Brown Shrimp (*Penaeus aztecus)*	48 hr, flow-through test. Salinity 30 g/L; 27°C	Atrazine, 99.7%	48-hour LC_{50} = 1000 (nominal) Slope – none	Juvenile. Classified as supplemental by the USEPA (48 hr LC_{50} and no raw data).	
Pink Shrimp (*Penaeus duorarum*)	96 hr, static. Salinity 26 g/L; 22°C	Atrazine, 97.4%	96-hour LC_{50} = 6900 (nominal)	Classified as supplemental by the USEPA (raw data unavailable).	
Opossum shrimp (*Neomysis integer*)	48 hr, static renewal, 15°C.	Atrazine, 98–99%	48-hour LC_{50} = 48 (measured)	Juvenile (4–7 mm). Classified as qualitative by the USEPA (raw data unavailable).	
Copepod (*Acartia clausii*)	96 hr, static renewal (daily), 6–6.2°C, salinity – 31 g/L	Atrazine, 70%	96-hour LC_{50} = 7900 (nominal)	Classified as acceptable by the USEPA.	
Grass Shrimp (*Palaemonetes pugio*)	96 hr, static. Salinity 26 g/L; 22°C	Atrazine, 97.4%	96-hour LC_{50} = 9000 (nominal)	Classified as supplemental by the USEPA (raw data unavailable).	

Organism	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Eastern Oyster (*Crassostrea virginica*)	96 hr, flow-through test. Salinity 28 g/L; 28°C	Atrazine, 99.7%	96-hour EC_{50} > 1000 No effect (nominal)	Juvenile. Shell deposition. Classified as supplemental by the USEPA (EC_{50} not identified and no raw data).	
	96 hr, flow-through test. Salinity 11.8 g/L; 21°C	Atrazine, 80% (formulation, WP)	96-hour EC_{50} > 800 (nominal) No effect	Shell deposition. Classified as supplemental by the USEPA (EC_{50} not identified).	
	96 hr, flow-through test. Salinity 31–32 g/L; 20–21°C	Atrazine, 97.1%	96-hour EC_{50} > 17000 (measured) No effect	Shell deposition. Classified as acceptable by the USEPA. Cafarella M.A., 2005, PMRA# 2816889-unpublished version. This data was recently published in Brain et al., 2021 (PMRA# 3242966).	
	96 hr, flow-through test.	Hydroxyatrazine, 97.1%	96-hour EC_{50} > 2800 (measured)	Shell deposition. Classified as acceptable by the USEPA. Sayers L.E., 2005, PMRA# 2816907 (USEPA DER – PMRA# 2816908).	
Mud Crab (*Neopanope texana*) Static test	96 hr, static. Salinity and temperature unknown	Atrazine technical (purity not reported)	96-hour LC_{50} > 1,000 (nominal)	Classified as supplemental by the USEPA (LC_{50} exceeds water solubility).	
Pacific Oyster (*Crassostrea gigas*)	24-Hour Static-Renewal	Not reported	24-hour LC_{50} > 100 (nominal)	0.1–50% dead at 22 days 0.2–50% dead at 18 days Classified as supplemental by the USEPA (no 96-hour LC_{50} value).	
European Brown Shrimp (*Crangon crangon*)	48 hr, static. 15°C	Formulation WP, purity not reported	48-hour LC_{50} = 10 000 - 33,000 (nominal)	Classified as supplemental by the USEPA (only 48 hours and no raw data).	
European Cockle (*Cardium edule*)	48 hr, static. 15°C	Formulation (WP), purity not reported	48-hour LC_{50} > 100 000[a] (nominal)	Classified as supplemental by the USEPA (only 48 hours, LC_{50} exceeds water solubility and no raw data).	
Fiddler Crab (*Uca pugilator*)	96 hr, static test; 19°C, salinity - 30 g/L	Formulation, 80% (WP)	96-hour LC_{50} = 198 000[a] (nominal)	Classified as supplemental by the USEPA (LC_{50} exceeds water solubility).	
	96 hr, static test; 19°C, salinity - 30 g/L	Formulation (4-1-3-1, WDL), purity not reported	96-hour LC_{50} = 239 000[a] (nominal)	Classified as supplemental by the USEPA (LC_{50} exceeds water solubility).	

[a] Endpoint exceeds the water solubility of atrazine (33 000 µg a.i./L)

Table 22 Chronic toxicity of atrazine to estuarine/marine invertebrates

Organism	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Mysid shrimp (*Americamysis bahia*)	Flow-through test, salinity 20 g/L, 25±1°C	Atrazine, 97.4%	NOEC = 80 LOEC = 190 (measured)	37% reduction in adult survival at LOEC. Study duration unknown. Ward and Ballantine, 1985. Classified as supplemental by the USEPA (no raw data for statistical analyses).	Reported in USEPA 2016 review (PMRA# 3253945)
	28 days, flow-through test, salinity 19–21 g/L, 26±2°C	Atrazine, 97.1%	NOEC = 260 LOEC = 500 (measured)	9.8% reduction in male length. 11% reduction in male dry weight. 8.5% reduction in female dry weight. Classified as acceptable by the USEPA. Cafarella, 2006, PMRA# 2816884 – unpublished version. This data was recently published in Brain et al., 2021 (PMRA# 3242966).	
Copepod (*Eurytemora affinis*)	21 days, static renewal (every 3 days)	Atrazine technical (purity not reported)	10-day NOEC (survival) = 25 10-day LOEC = 49 30-day LOEC = 25	Delayed metamorphosis. Forget et al. 2005. Classified as qualitative by the USEPA (no raw data, limited data on test concentrations).	
Copepod (*Amphiascus tenuiremis*)	41-day static renewal (every 3 days). C1s (stage 1 copepodite juveniles) were exposed to mean measured concentrations of <LOD, 3.5. 30.3 and 246.6 µg a.i./L in the respective treatmernts.	Atrazine 98%	Decrease in viable offspring production per female (F1): NOEC <3.5, LOEC = 3.5; F0: NOEC = 30.3, LOEC = 246 Reproductive failure (F1): NOEC = 3.5, LOEC = 30.3 Copepod malformations (F1): NOEC <3.5, LOEC = 3.5 C1 survival to adulthood, developmental delays, sex ratios, development time and hatching success: NOEC >246.6, LOEC = 246.6	This study was classified as supplemental by the USEPA because a "negative control was not used; therefore, potential solvent effects could not be evaluated." According to the published paper a treatment containing a maximum of 0.02% volume/volume (v/v) acetone was used as a carrier control. The deficiency noted by the USEPA, therefore, is inaccurate. The NOEC value of < 3.5 µg a.i./L (based on decrease in viable offspring production per female - F1) is considered valid for the risk assessment.	Bejarano and Chandler 2003 (PMRA# 3268928)
Copepod nauplii (*Tigriopus japonicas*)	20 days, static renewal, 30 ppt, 25°C	Atrazine, 97.4%	NOEC = 10000	Developmental test: Newly hatched nauplii (<12h post hatch) were exposed to 0 (control), 1, 5, 10 and 20 mg/L atrazine for up to 20 days. Developmental effects observed: retardation in the growth and prolonged molting and metamorphosis.	Yoon et al., 2019 (PMRA# 3201404)

Organism	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Estuarine crab (*Neohelice granulata*)	32 days, static renewal, artificial seawater, 25°C	Gesaprim 90 WDG, 90%	NOEC = 2500 (nominal)	The effects of a 32-d exposure to atrazine (0, 2500, 5000 and 15 000 µg a.i./L) were evaluated in female ovigerous crabs both in terms of larvae hatching and ovarian re-maturation. Larval abnormalities observed included: hydropsy, hyperpigmented body, atrophy of spines and setae, and atrophy of eyes.	Alvarez et al., 2015 (PMRA# 3201387)
Estuarine crab (*Neohelice granulata*)	90 days, static renewal (weekly), artificial seawater, 23°C	Gesaprim 90 WDG, 90%	NOEC > 3000 (nominal)	Adult females were exposed to formulated atrazine (0, 30, 300 and 3000 µg a.i./L) during the 3-month pre-reproductive period. No effects (mortality, molting, spawning, weight gain) were observed up to the highest test concentration.	Silveyra et al., 2017 (PMRA# 3201382)
Opossum shrimp (*Neomysis integer*)	3 weeks (design not specified)	Atrazine, 98–99%	NOEC > 1 LOEC > 1 (measured)	<24 hours old. Noppe et al., 2007. Classified as supplemental by the USEPA (no raw data, missing test design information). The endpoint measured is undefined.	Reported in USEPA 2016 review (PMRA# 3253945)
Opossum shrimp (*Neomysis integer*)	Estimated NOEC = 3.8 (mortality). The USEPA (2016) derived an acute to chronic ratio of 12.5 for mysid shrimp based on an acute LC_{50} of 1000 µg/L and a chronic NOEC of 80 µg/L for (Americamysis bahia) and applied to the most sensitive endpoint of 48 µg/L for opossum shrimp (*neomysis integer*): 48/12.5 = 3.8 µg/L.				

Table 23 Toxicity of atrazine to estuarine/marine fish

Acute					
Organism	**Exposure**	**Test Substance**	**Endpoint Value (µg a.i./L)**	**Comments**	**Reference**
Sheepshead Minnow (*Cyprinodon variegatus*)	96 hr, static test, 20°C, salinity 25, 15, 5 g/L	Atrazine, 97.1%	96-hour LC_{50} = 2000 (Salinity 25 g/L) 96-hour LC_{50} = 2300 (Salinity 15 g/L) 96-hour LC_{50} = 16 200 (Salinity 5 g/L) (measured)	Larvae < 24-hours old. Classified as supplemental by the USEPA (no raw data on mortalities, fed fish during study).	Reported in USEPA 2016 review (PMRA# 3253945)
	96 hr, Flow-through test; 22–23°C, salinity - 31 g/L	Atrazine, 97.1%	96-hour LC_{50} = 13 400 (measured)	Classified as acceptable by the USEPA. This data was recently published in Brain et al., 2021 (PMRA# 3242967).	
	96 hr, Flow-through test	Atrazine, 97.4%	96-hour LC_{50} > 16000 (30% mortality, measured)	Classified as supplemental by the USEPA (no raw data).	

Organism	Exposure	Test Substance	Endpoint Value (µg a.i./L)	Comments	Reference
Acute					
Sheepshead Minnow (*Cyprinodon variegatus*)	96 hr, static test, 21–24°C, salinity - 32 ‰	Hydroxyatrazine (HA), 97.1%	96-hour LC_{50} > 1900 (no mortality measured)	Classified as acceptable by the USEPA. Biological results for the study were based on the mean-measured concentrations of Hydroxyatrazine, which remained constant at the limit of its water solubility throughout the duration of the tests. Sayers, 2005, PMRA# 2816898 (USEPA DER – PMRA# 2816899).	
Spot (*Leiostomus xanthurus*)	96 hr, static test; 22°C, salinity - 12 g/L	Atrazine, 97.4%	96-hour LC_{50} = 8500 (nominal)	Classified as supplemental by the USEPA (no raw data).	
Spot (juvenile) (*Leiostomus xanthurus*)	48 hr, Flow-through test; 28°C salinity - 29 g/L	Atrazine, 99.7%	48-hour LC_{50} > 1000 (nominal)	Classified as supplemental by the USEPA (48-hour test).	
Coho Salmon (*Oncorhynchus kisutch*)	144 hr, static-renewal (daily)	Atrazine, 40.8% (Formulation – AAtrex Liquid)	96-hour LC_{50} > 18000 (25% mortality, measured)	Classified as supplemental by the USEPA (no LC_{50} value and 12–17 months old).	
Chronic					
Sheepshead Minnow (*Cyprinodon variegatus*)	ELS (duration unknown, flow-through test; 30°C, salinity 13 g/L	Atrazine, 97.4%	NOEC = 1900 LOEC = 3400 (measured)	89% reduction in juvenile survival at LOEC. Classified as supplemental by the USEPA (no raw data for statistical analyses).	Reported in USEPA 2016 review (Appendix B: Supporting Ecological Toxicity Data - PMRA# 3253945)
	ELS-28 days, flow through test, 24–27°C, salinity 29–31 ‰	Atrazine, 97.1%	NOEC = 1100 LOEC = 2200 (measured)	The NOAEC and LOAEC values are based on growth (larval length and wet weight): 17% reduction in mean length; 46% reduction in mean wet weight at LOEC. Classified as acceptable by the USEPA. Cafarella, 2006, PMRA# 2816883 – unpublished version. This data was recently published in Brain et al., 2021 (PMRA# 3242967).	

Acute					
Organism	**Exposure**	**Test Substance**	**Endpoint Value (µg a.i./L)**	**Comments**	**Reference**
Juvenile grey mullet (*Liza ramada*)	9, 20, and 29 days	Not reported.	NOEC < 170 (survival at 20 and 29 days)	Exposure to control and single test concentration (170 µg/L) for 9, 20, and 29 days in static tests and for 11 days followed by 18 days of decontamination. Control mortality was a constant 10% throughout test. At 170 µg/L, 10, 25 and 60 percent mortality occurred following 9-, 20- and 29-day exposures, respectively. Biagianti-Risbourg and Bastide, 1995. The USEPA does not provide a classification for this study.	

Table 24 Toxicity of atrazine to Atlantic salmon (seawater challenge tests).

Organism	Exposure	Test Substance	Endpoint Value (µg a.i./L)	Comments	Reference
Atlantic salmon (*Salmo salar*)	5-day exposure in freshwater followed by 1–2.5-hour transition to seawater (33‰, no atrazine).	Atrazine, >99%	LC_{50} ~ 5.0 (NOEC = 0.5, nominal)	Smolt mortality. 15% and 43% mortality were observed within 24 hours after smolts were transferred to seawater at 1 and 5 µg a.i./L; the LC_{50} was approximated as 5.0 µg a.i./L (43% mortality) and the no observable effects endpoint was 0.5 µg a.i./L. The transition period from freshwater to seawater was relatively short 1–2.5 hours. Measured concentrations were highly variable (21–78% of nominal on last day of exposure). Raw data was not available.	Waring and Moore 2004; PMRA# 1493902
	81-day exposure in freshwater followed by 2-hour transition to seawater (25‰, no atrazine).	Atrazine, >99%	NOEC = 5.0 (nominal, highest test concentration)	Smolt mortality. No mortalities to smolts were observed (0.5 and 5.0 µg a.i./L). The transition period from freshwater to seawater was relatively short 2 hours. Mean measured concentrations were 54 and 72% of nominal, respectively). Raw data was not available. A slight reduction in migratory activity was observed at 5.0 µg a.i./L. However, no relationship was observed between migratory activity and any physiological parameters measured (for example, a transient reduction in gill Na^+K^+ - ATPase activity at 0.5 but not 5.0 µg a.i./L).	Moore 2007; PMRA# 2099051

Organism	Exposure	Test Substance	Endpoint Value (µg a.i./L)	Comments	Reference
	21-day exposure in freshwater followed by 24-hour transition to seawater and 3 months rearing in seawater	Atrazine, 98%	NOEC = 8.5 (based on mean measured concentration of the 10 µg/L nominal exposure group)	The NOEC is based on significant reduction in growth rate observed during the first month in seawater.	Nieves-Puigdoller et al. 2007; PMRA# 2118994
	4-day exposure in freshwater followed by transition to 50% seawater for two days and then 100% seawater for five more days.	Atrazine, 96.2%	NOEC > 100 (nominal)	No effect on survival, growth or iono-regulatory performance up to the highest test concentration.	Matsumoto and Van Der Kraak 2009; PMRA# 1777173

Table 25 Toxicity of atrazine to marine algae

Genus and/or species	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Prasinophyte (*Nephroselmis pyriformis)*	4 hours	Purity not reported	EC_{50} = 14.2	50% photo-inhibition. Classified as supplemental by the USEPA (no explanation provided).	Reported in USEPA 2016 review (PMRA# 3253945)
Ankistrodesmus sp.	4-day	Atrazine, 100%	EC_{50} = 11.9	Based on chlorophyll *a* concentration; Delorenzo et al., 2004.	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792)
Dunaliella tertiolecta	4-day	Atrazine, 100%	EC_{50} = 65	Based on chlorophyll *a* concentration; Delorenzo et al., 2004.	
Neogoniolithon fosliei	1-day	Atrazine, 95%	IC_{50} = 180	Photosynthesis, Negri et al., 2011.	
Pavlova sp.	4-day	Atrazine, 100%	EC_{50} = 96	Population growth rate, Pennington et al., 2001.	
Rhodomonas salina	3-day	Atrazine, 100%	EC_{50} = 165	Population growth rate, Debelius et al., 2008.	
Dinoflagelate (*Amphidinium operculatum)*	4-day	Purity not reported	EC_{50} = 17.9	50% reduction in total bio-volume. Classified as supplemental by the USEPA (no explanation provided).	Reported in USEPA 2016 review (PMRA# 3253945)
Cryptomonad (*Storeatula major)*	4-day	Atrazine, 100%	EC_{50} = 22.17	50% reduction in abundance. Classified as supplemental by the USEPA (no explanation provided).	
Tetraselmis chuii	3-day	Atrazine, 100%	EC_{50} = 20	50% reduction in population growth rate. Debelius et al., 2004	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792)

Genus and/or species	**Exposure**	**Test substance**	**Endpoint value (µg a.i./L)**	**Comments**	**Reference**
Chlamydomonas sp.	3-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 60 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (72 hours and endpoint); the USEPA's reasoning/explanation is unclear.	Reported in USEPA 2016 review (PMRA# 3253945)
Monochrysis lutheri	1 hour	Purity not reported	EC_{50} = 77	50% reduction in O_2 production. Classified as supplemental by the USEPA (short duration).	
Monochrysis lutheri	3-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 77 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (reasoning/explanation not provided).	
Porphyridium cruentum	3-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 79 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (reasoning/explanation not provided).	
Chlorophyceae Neochloris sp.	3-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 82 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (reasoning/explanation not provided).	
Cyclotella nana	3-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 84 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (reasoning/explanation not provided).	
Achnanthes brevipes	3-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 93 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (reasoning/explanation not provided).	
Isochrysis galbana	10-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 100 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (NOAEC unavailable).	
Chlorophyceae (*Chlorococcum* sp.)	10-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 100 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (NOAEC unavailable).	
(*Chlorophyceae Platymonas* sp.)	3-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 100 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (72 hours and endpoint); the USEPA's reasoning/explanation is unclear.	
Marine Bacillariophyceae (*Thalassiosira fluviatilis)*	3-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 110 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (72 hours and endpoint); the USEPA's reasoning/explanation is unclear.	
Marine Bacillariophyceae (*Stauroneis amphoroides)*	3-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 110 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (72 hours and endpoint); the USEPA's reasoning/explanation is unclear.	
Marine Algae (*Microcystis aeruginosa)*	5-day	Atrazine, 97.4%	EC_{50} = 129 (nominal) NOAEC = 65 (7% reduction in growth)	50% reduction in growth. Classified as supplemental by the USEPA (method and raw data unavailable).	
Marine Green Chlorophyceae (*Chlorella* sp.)	3-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 140 (nominal)	50% reduction in O_2 production Classified as supplemental by the USEPA (NOAEC unavailable).	
Marine green Chlorophyceae (*Dunaliella tertiolecta)*	5-day	Atrazine, 97%	EC_{50} = 180 (nominal)	50% reduction in growth. Classified as supplemental by the USEPA (NOAEC unavailable).	
Marine green Chlorophyceae (*Dunaliella tertiolecta)*	4-day static	Purity not reported	EC_{50} = 132	50% reduction in growth, Gaggi et al., 1995.	

Genus and/or species	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Marine green Chlorophyceae (*Dunaliella tertiolecta)*	10-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 300 (nominal)	50% reduction in cell growth.	
Marine green Chlorophyceae (*Dunaliella tertiolecta)*	10-day salinity 30 g/L	Atrazine, 76% (formulation 80WP)	EC_{50} = 400 (240 hours) EC_{50} = 600 (2 hours) (nominal)	50% reduction in cell growth. 50% reduction in O_2 production. Classified as supplemental by the USEPA (NOAEC unavailable).	
Marine Yellow-Green (*Nannochloropsis gaditana*)	3-day	Purity not reported	EC_{50} = 185	50% total fluorescence inhibition. Classified as supplemental by the USEPA (no reasoning/explanation provided).	
Marine Red – Rhodophyceae (*Porphyridium cruentum)*	5-day	Atrazine, 97.4%	EC_{50} = 308 (nominal)	50% reduction in growth. Classified as supplemental by the USEPA (NOAEC, method and raw data unavailable).	
Isochrysis galbana	10-day	Atrazine, 99.7%	EC_{50} = 100 (nominal)	Growth.	
Chrysophyceae *(Isochrysis galbana)*	5-day	Atrazine, 97.4%	EC_{50} = 22 (nominal)	50% reduction in growth. Classified as supplemental by the USEPA (no NOAEC, method and raw data unavailable).	
Isochrysis galbana	10-day salinity 30 g/L	Atrazine 76%, (formulation 80WP)	EC_{50} = 100 (240 hours) EC_{50} = 200 (2 hours) (nominal)	50% reduction in growth. 50% reduction in O_2 production. Classified as supplemental by the USEPA (NOAEC unavailable).	
Marine diatom (*Bellerochea polymorpha*)	2-day	Purity not reported	EC_{50} = 19.4	46 species tested. 50% reduction in population growth. Classified as supplemental by the USEPA (no explanation provided).	
Marine Diatom (*Skeletonema costatum)*	5-day	Atrazine, 97.4%	EC_{50} = 24 (nominal)	50% reduction in growth. Classified as supplemental by the USEPA (no NOAEC, method and raw data unavailable).	
Marine Diatom (*Skeletonema costatum)*	5-day	Atrazine, 97.1%	EC50 = 53 (measured)	50% reduction in growth.	
Marine Yellow Chlorophyceae *(Chlorococcum sp.)*	10-day salinity 30 g/L	Atrazine 76%, (formulation 80WP)	EC_{50} = 100 (240 hours) EC_{50} = 400 (2 hours) (nominal)	50% reduction in growth. 50% reduction in O_2 production. Classified as supplemental by the USEPA (NOAEC unavailable).	
Chlorococcum sp.	10-day	Atrazine, 99.7%	EC_{50} = 100 (nominal)	50% reduction in growth.	
Microcystis aeruginosa	10-day	Atrazine, 97.4%	EC_{50} = 129 (nominal)	50% reduction in growth.	
Marine yellow Chrysophyceae (*Phaeodactylum tricornutum)*	10-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 200 (nominal)	50% reduction in cell growth. Classified as supplemental by the USEPA (NOAEC unavailable).	
Marine yellow Chrysophyceae (*Phaeodactylum tricornutum)*	10-day salinity 30 g/L	Atrazine, 76% (formulation 80WP)	EC_{50} = 200 (240 hours) EC_{50} = 200 (2 hours) (nominal)	50% reduction in growth. 50% reduction in O_2 production. Classified as supplemental by the USEPA (NOAEC unavailable).	

Genus and/or species	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Marine Bacillariophyceae (*Nitzschia closterium)*	3-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 290 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (72 hours and endpoint); the USEPA's reasoning/explanation is unclear.	
Marine Bacillariophyceae *Nitzschia* (Ind. 684)	3-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 430 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (72 hours and endpoint); the USEPA's reasoning/explanation is unclear.	
Marine Bacillariophyceae (*Amphora exigua)*	3-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 300 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (72 hours and endpoint); the USEPA's reasoning/explanation is unclear.	
Marine Green - Chlorophyceae (*Kirchneria subcapitata)*	5-day	Atrazine, 97.4%	EC_{50} = 431 (nominal) NOAEC = 200	50% reduction in growth. 4% reduction in growth. Classified as supplemental by the USEPA (slight effect for NOAEC reported – NOAEC = 200 µg a.i./L, method and raw data unavailable).	
Marine Bacillariophyceae (*Navicula inserta)*	3-day salinity 30 g/L	Atrazine, 99.7%	EC_{50} = 460 (nominal)	50% reduction in O_2 production. Classified as supplemental by the USEPA (72 hours and endpoint); the USEPA's reasoning/explanation is unclear.	

Table 26 Toxicity of atrazine to marine vascular plants

Organism	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Estuarine/marine vascular plants (short term exposures 2 hours – 3 days)					
Pondweed *Potamogeton perfoliatu)*	2 hours	Purity not reported	EC_{50} = 77 (nominal)	50% reduction in O_2 evolution. Classified as supplemental by the USEPA supplemental (insufficient duration; raw data unavailable).	Reported in USEPA 2016 review (PMRA# 3253945)
Pondweed *Potamogeton perfoliatus*	2 hours	Purity not reported	EC_{50} = 80 (nominal)	50% reduction in O_2 production. Classified as supplemental in the 2016 USEPA review (insufficient duration; raw data unavailable).	
Common poolmat *Zannichellia palustris*	2 hours	Atrazine, 100%	EC_{50} = 91 (nominal)	50% reduction in O_2 evolution. Classified as supplemental in the 2016 USEPA review (insufficient duration; raw data unavailable). Value reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792) – Jones et al., 1984.	
Pondweed *Potamogeton perfoliatus*	2 hours	Purity not reported	LOEC = 100 (52 to 69% reduction in photosynthesis)	Classified as supplemental by the USEPA supplemental (raw data unavailable).	

Organism	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Widgeon-Grass *Ruppia maritima*	2 hours	Atrazine, 100%	EC_{50} = 102 (nominal)	50% reduction in O_2 evolution. Classified as supplemental in the 2016 USEPA review (insufficient duration; raw data unavailable). Value reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792) – Johnson et al., 1985.	
Eel grass *Halodule uninvervis*	1-day	Atrazine, 95%	IC_{50} = 16.6	Photosystem II (PSII) electron activity, Flores et al., 2013.	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792)
Widgeon-grass *Ruppia maritima*	2-hours	Atrazine, 100%	IC_{50} = 102	Photosynthesis, Jones et al., 1984.	
Eelgrass *Zostera muelleri*	3-days	Atrazine, 95%	IC_{50} = 16.6	Photosystem II (PSII) electron activity, Flores et al., 2013.	
Turtle grass *Thalassia testudinum*	4- hours	Atrazine, 99.7%	EC_{50} = 320	Photosynthesis, Walsh 1981, Walsh 1982.	
Estuarine/marine vascular plants (longer term exposures, ≥ 7 days)					
Pondweed *Potamogeton perfoliatu)*	28-day	Purity not reported	EC_{50} = 30	50% reduction in O_2 production. Classified as supplemental by the USEPA (raw data unavailable). Kemp et al., 1984.	Reported in USEPA 2016 review (PMRA# 3253945)
Pondweed *Potamogeton perfoliatus*	21 days	Purity not reported	EC_{50} = 50 (nominal)	50% reduction (the parameter measured is not reported). Classified as supplemental by the USEPA (raw data unavailable).	
Eelgrass *Zostera marina*	10 days	Purity not reported	EC_{50} = 69 EC_{25} = 50 (measured)	Leaf growth. 62% reduction in leaf growth at 80 µg a.i./L. Classified as supplemental by the USEPA (raw data unavailable).	
Wild celery *Vallisneria americana*	42 days	Purity not reported	EC_{50} = 163 (nominal)	50% reduction in shoot length. No difference was observed at 0, 3 and 6 parts/thousand salinity. Classified as supplemental by the USEPA (raw data unavailable).	
Wild celery *Vallisneria americana*	47 days	Purity not reported	12	~50% mortality.	
Seagrass *Halodule wrightii*	22–23 days	Atrazine 4L	30000 (measured)	46–58% reduction in above ground biomass. Classified as supplemental by the USEPA (raw data unavailable).	

Organism	Exposure	Test substance	Endpoint value (µg a.i./L)	Comments	Reference
Eurasian watermilfoil *Myriophyllum spicatum*	28-d	Atrazine, 100%	IC_{50} = 91	Biomass, Kemp et al., 1984.	Reported in USEPA BE 2020 (Appendix 2-1, PMRA# 3292792)
Smooth cordgrass *Spartina alternaflora*	21-d	Atrazine, >90%	EC_{50} = 2.3	Chlorosis, Scott 2011.	
Widgeon-grass *Ruppia maritima*	35-days	Atrazine, 100%	EC_{50} = 2500	Length, Johnson et al., 1995.	

Table 27 Summary of estuarine/marine aquatic microcosm and mesocosm field studies conducted with atrazine

Organism	Exposure	Endpoint value (µg a.i./L)	Comments	Reference
Estuarine/marine microcosms				
Estuarine microcosm: Wild celery *Vallisneria americana*	1 treatment: 4, 8,16, 32, and 64 µg a.i./L (nominal) 42 days	NOAEC < 4 µg a.i./L	No replicates were used. Should not be used for the marine risk assessment. Classified as supplemental in the 2016 USEPA refined ecological risk assessment.	MRID 450200-01 Cohn, 1985 S Reported in USEPA 2016 review (PMRA# 3253945)
Estuarine microcosm with macrophytes	Exposed to two concentrations for 4 weeks: Mean-measured concentrations in water were 130 µg a.i./L for the “low” treatment and 1200 µg a.i./L for the “high” treatment over a 4-week period	NOAEC < 130 (total macrophyte biomass)	Aquatic plants were planted and atrazine-treated sediments were added to 700-L microcosms. On Day 1.5, 93.4% of the total atrazine was dissolved in water. Reported in the 2007 PMRA monograph. Classified as supplemental in the 2016 USEPA refined ecological risk assessment.	(Cunningham et al., 1984) Reported in USEPA 2016 review (PMRA# 3253945)
Estuarine lab microcosm: two salt marsh diatom species (*Thalassiosira fluviatilis* and *Nitzschia sigma*)	7-day exposure: 22, 220 and 2200 µg a.i./L (nominal)	NOAEC = 100 (approximated from EC_1 = 100 µg a.i./L)	Reported in the 2007 PMRA monograph. Classified as supplemental in the 2016 USEPA refined ecological risk assessment.	(Plumley and Davis, 1980) Reported in USEPA 2016 review (PMRA# 3253945)
Estuarine field microcosm	108 days: single exposure of 0.4, 1.4, 4.5 and 45 lb a.i./acre (equivalent to 0.45, 1.6, 5.0 and 50.4 kg a/i/ha) (nominal)	NOAEC cannot be determined from this study.	Reported in the 2007 PMRA monograph. Classified as supplemental in the 2016 USEPA refined ecological risk assessment.	(Plumley and Davis, 1980) Reported in USEPA 2016 review (PMRA# 3253945)

Organism	Exposure	Endpoint value (µg a.i./L)	Comments	Reference
Estuarine microcosm *Spartina alterniflora* and *Juncus roemerianus*	5 weeks: 3 weekly applications followed by 2 weeks observation. Mean-measured concentration at approx. mid-point of Spartina test were 30, 280, and 3100 µg a.i./L and in the Juncus test were 30, 250, and 3800 µg a.i./L	***Spartina alterniflora:*** Growth: NOEC = 280 peroxidase activity: NOEC <30 *Juncus roemerianus* Chlorophyll a, chlr-a/chlor-b ration: NOEC = <3030, 250 and 3100 µg a.i./L (5 weeks): significant reduction in chlorophyll a (Chl-a) and Chl-a/Chl-b ratio in 250 and 3800 µg a.i./L (5 weeks): significant reduction in Chl-b Growth and oxidized lipids: NOEC = 250 250 µg a.i./L (1 year): partial recovery 3800 µg a.i./L (1 year): practically no survival	Classified as supplemental in the 2016 USEPA refined ecological risk assessment.	Lytle and Lytle, 1998 Reported in USEPA 2016 review (PMRA# 3253945)
Estuarine microcosm Algae: *Nannochloris oculata* and *Phaeodactylum tricornutum*	Duration not reported: 0, 50, and 100 µg a.i./L (nominal)	Both *Nannochloris oculata* and *Phaeodactylum tricornutum* were significantly (mostly at the 0.01 level) affected by changes in light, temperature, and atrazine concentration. NOAEC is undetermined. could not be determined because of interactive effects.	NOAEC could not be determined because of interactive effects.	Mayasich et al., 1986 Reported in USEPA 2016 review (PMRA# 3253945)
Estuarine microcosm Algae: *Nannochloris oculata* and *Phaeodactylum tricornutum*	Duration not reported: 0, 15, 30 and 50 µg a.i./L (nominal)	A significant effect on *N. oculata* growth rate is reported based on testing the two algae together. NOAEC could not be determined because of interactive effects.	Classified as supplemental in the 2016 USEPA refined ecological risk assessment.	Mayasich et al., 1987 Reported in USEPA 2016 review (PMRA# 3253945)
Estuarine microcosm Seagrass *Halodule wrightii*	22-23 days Single dose: Day 0: 30000 µg a.i./L (nominal) Day 22-23: 16400-17000 µg a.i./L (measured)	Initial # of ramets, above-ground biomass, average dry weight of ramets; NOAEC = >30 000 µg a.i./L	Examined the effect of atrazine and interactions of salinity (15, 25, 35 ppt), light intensity (115, 140, 173 uEm^{-2}s^{-1}), and cropping (either cut at 4-cm or 6cm). None of these environmental factors affected the response of the grass to atrazine. Classified as supplemental in the 2016 USEPA refined ecological risk assessment.	MRID 452051-01 Mitchell, 1987 Reported in USEPA 2016 review (PMRA# 3253945)
Estuarine microcosm *Zostera marina*	1, 10 and 100 µg/L, 4-week exposure in 3L beakers with sediment	N/A	No indication of atrazine measured concentrations; therefore, no way to confirm exposure. Not a true cosm study as beakers were ony 3 L. Not acceptable.	Gao et al. 2011 (PMRA# 3322622)

Organism	Exposure	Endpoint value (µg a.i./L)	Comments	Reference
Estuarine/marine mesocosms				
Marine Mesocosm inoculated with the diatoms *Thalassiosira punctigera*, *T. rotula*, *Nitzschia pungens* and *Skeletonema costatum* and a prymnesiophtye, *Phaeocystis globosa*.	15 days Nominal concentrations of 0 (open ocean), 0.12, 0.56, and 5.8 µg a.i./L	• Lower pH,dissolved organic nitrogen, primary production, particulate carbohydrates, chlorophyll: NOEC <0.12	Mesocosms (2 m^2) Classified as supplemental in the 2016 USEPA refined ecological risk assessment.	MRID 450200-21 Bester et al., 1995 Reported in USEPA 2016 review (PMRA# 3253945)
Salt marsh edaphic algae	Nominal applications of 0.45, 4.5, or 45 lb a.i./A	Carbon fixation NOAEC < 0.45 lb a.i./A (16 days) NOAEC = 4.5 lb a.i./A (42days)	Elaboration of Plumley et al., concerning the carbon uptake for algae in the top 0.5 cm of enclosure sediment. Carbon fixation was significantly reduced at the 0.45 and 4.5 lb a.i./A treatment levels for 16 days and at the 45 lb a.i./A treatment level for 42 days. Classified as supplemental in the 2016 USEPA refined ecological risk assessment.	MRID 450874-06 Plumley and Davis, 1980 Reported in USEPA 2016 review (PMRA# 3253945)
Field study Seagrass *Zostera capricorni*	10-hour exposure Atrazine doses: 0, 10, and 100 µg a.i./L at one application. % a.i. not reported.	NOAEC = >100 µg a.i./L (highest test concentration) Chlorophyll a , effective quantum yield (recovery to control values by end of 10-hour exposure period).	Classified as qualitative in the 2016 USEPA refined ecological risk assessment (no raw data provided, low number of replicates (2), relevance of fluorescence endpoints is of limited use in risk assessment.	Macinnis-Ng and Ralph, 2003 (EcoRef.# 72996) Reported in USEPA 2016 review (PMRA# 3253945)

Appendix X Estimated environmental concentrations

Introduction

The following sections summarize the screening level estimated level environmental concentrations (EECs) of atrazine in surface waters, and EECs resulting from water modelling for aquatic ecoscenarios.

Screening level EECS in surface waters

Table 1 Screening level EEC of atrazine in a body of water 80-cm and 15-cm deep after direct application rates of 1500 g a.i./ha (maximum single foliar application rate for corn).

Application rate (g a.i./ha)	No of applications (interval)	EEC (µg a.i./L) for 80-cm depth	EEC (µg a.i./L) for 15-cm depth
1500	1	188	1000

Modelling estimates

Application information and model inputs

Atrazine is registered in Canada for weed control on corn, sorghum and switchgrass. Only one yearly application is permitted on sorghum with a single maximum rate of 1008 g a.i./ha, applied either as pre-plant or pre-emergence by ground boom sprayer to soil. One yearly application is permitted on switchgrass with a single maximum single rate of 1488 g a.i./ha, applied either as pre-plant by ground boom sprayer to soil incorporated or irrigated to 5 cm, or as pre-emergence by ground boom sprayer to soil. Applications will not be made in subsequent years. One or two applications are permitted on corn, with single application rates ranging from 408 to 1488 g a.i./ha and yearly total of 1500 g a.i./ha. If weeds escape a pre-plant incorporation or pre-emergence application, an additional post-emergence application may be necessary. Up to two applications may be made if a pre-plant or pre-emergence application are made, but only one post-emergence may be made. The application methods for corn include pre-plant by ground boom sprayer to soil incorporated or irrigated to 5 cm, pre-emergence by ground boom sprayer to soil and irrigated to 5 cm, and post-emergence by ground boom sprayer to soil.

The ecological modelling was conducted with regional scenarios, regional application timing and, since modelling ground boom sprayer applications is more conservative than modelling soil incorporation methods for surface water bodies, all ecological modelling was performed using the soil surface application method. The main environmental fate parameters used in the models are summarized in Table 2.

Table 2 Ecological model inputs

Parameter	Value	Comment
Photolysis half-life (day) at 40° latitude	330	Longer of 3
Hydrolysis at pH 7	stable	One value
K_{oc} (L/kg)	54	20th percentile of 8
Soil half-life (day) at 25°C	233	One value
Aerobic aquatic half-life (day) at 20°C	136	Longer of 2
Anaerobic aquatic half-life (day) at 20°C	101	Longer of 2

Aquatic ecoscenario assessment

The EECs of atrazine from runoff into a receiving waterbody were simulated using the Pesticide in Water Calculator (PWC) version 1.52. The PWC model simulates pesticide runoff from a treated field into an adjacent body of water and the fate of a pesticide within it. Spray drift is not considered for this modelling. For the ecological risk assessment, EECs in water are calculated by modelling a 10 ha field adjacent to 1 ha water bodies of two different depths, 80 cm and 15 cm. The pore water EECs in a 80 cm wetland were also generated.

The PWC model calculates the amount of pesticide entering the water body and the subsequent degradation of the pesticide in the water and sediment. In ecological modelling, pesticide enters the water by runoff only, and deposition of pesticide on the water body due to spray drift is not included. The model is run for 50 years.

For each year of the simulation, PWC calculates peak (or daily maximum) and time-averaged concentrations. The time-averaged concentrations are calculated by averaging the peak concentrations over different time periods (24-hour, 96-hour, 21-day, 60-day, 90-day, and 1 year). The highest value of these averages for each calendar year is then calculated. The 90th percentiles of these yearly maxima are reported as the EECs for that period. In addition, the peak and 21-day average EECs in sediment pore water are generated by the model.

Several representative scenarios are selected for modelling different regions of Canada. The highest 90th percentile EECs of atrazine are listed in Table 3 for the 80 cm water body and in Table 4 for the 15 cm water body, respectively. These EECs cover all regions of Canada.

Table 3 Ecological water EECs (µg/L) of atrazine in 80-cm water body

Crop/region	Peak	1 d	96 hr	21 d	60 d	90 d	PW_pk	PW_21 d
Corn/BC	2.5	2.5	2.5	2.4	2.4	2.5	2.0	2.0
Corn/AB	47	47	47	45	46	43	30	31
Corn/MB	41	41	40	39	37	37	26	26
Corn/ON	58	58	58	56	51	48	34	34
Corn/QC	82	82	82	80	74	70	50	50
Corn/Atlantic	129	128	128	123	116	119	99	99
Corn-max	**130**	**129**	**129**	**124**	**117**	**120**	**100**	**100**
Sorghum/BC	NA	NA	NA	NA	NA	NA	NA	NA
Sorghum/Prairie	NA	NA	NA	NA	NA	NA	NA	NA
Sorghum/ON	29	29	29	27	25	23	14	14
Sorghum/QC	27	27	27	26	24	23	15	15
Sorghum/Atlantic	NA	NA	NA	NA	NA	NA	NA	NA
Sorghum-max	**29**	**29**	**29**	**27**	**25**	**23**	**15**	**15**
Switchgrass/BC	NA	NA	NA	NA	NA	NA	NA	NA
Switchgrass/AB	64	64	63	60	55	53	41	41
Switchgrass/MB	32	32	32	31	29	27	20	20
Switchgrass/ON	53	53	53	51	46	47	35	35
Switchgrass/QC	70	70	69	67	64	62	50	50
Switchgrass/Atlantic	136	136	135	133	124	115	93	93
Switchgrass-max	**136**	**136**	**135**	**133**	**124**	**115**	**93**	**93**

Table 4 Ecological water EECs (µg/L) of atrazine in 15-cm water body

Crop/region	Peak	1 d	96 hr	21 d	60 d	90 d
Corn/BC	13	13	12	11	8.1	7.2
Corn/AB	213	211	205	179	145	134
Corn/MB	193	191	185	168	131	114
Corn/ON	278	275	267	235	189	166
Corn/QC	353	349	345	313	260	233
Corn/Atlantic	610	604	591	515	412	370
Corn-max	**610**	**604**	**591**	**515**	**412**	**370**
Sorghum/BC	NA	NA	NA	NA	NA	NA
Sorghum/Prairie	NA	NA	NA	NA	NA	NA
Sorghum/ON	139	137	133	119	93	79
Sorghum/QC	118	117	114	108	89	77
Sorghum/Atlantic	NA	NA	NA	NA	NA	NA
Sorghum-max	**139**	**137**	**133**	**119**	**93**	**79**
Switchgrass/BC	NA	NA	NA	NA	NA	NA
Switchgrass/AB	195	195	195	195	206	187
Switchgrass/MB	116	115	112	104	95	93
Switchgrass/ON	237	234	231	205	173	172
Switchgrass/QC	268	265	263	264	244	222
Switchgrass/Atlantic	627	621	612	591	476	425
Switchgrass-max	**627**	**621**	**612**	**591**	**476**	**425**

Estimated concentration of atrazine in food sources potentially ingested by wild birds and mammals

The expected concentrations of atrazine in food sources potentially ingested by wild birds and mammals were determined according to a nomogram developed from the data of Hoerger and Kenaga (1972; PMRA# 1918526) and Kenaga (1973; PMRA# 1918527) and modified according to Fletcher et al. (1994; PMRA# 1918522). The nomogram provides expected pesticide residue concentrations on various food items immediately after foliar spray application. The nomogram provides maximum residue concentrations, which correspond to the 90th percentile of the residue values in the underlying nomogram dataset.

EECs were calculated based on the highest foliar application rate for corn use (1500 g a.i./ha). The EECs on various food sources based on the maximum Kenaga values for atrazine are provided in Table 5.

Table 5 Screening level EECs in vegetation and insects after a direct over-spray at the highest foliar application rate of atrazine on corn (1500 g a.i./ha).

Food item	Fresh / dry weight ratios	Corn (1500 g a.i./ha)	
		EEC (mg a.i./kg fw)[a]	EEC (mg a.i./kg dw)
short range grass	3.3[b]	321	1059
long grass	4.4[b]	147	647
Broadleaf plants	5.4[b]	182	980
Insects	3.9[c]	126	479
grain and seeds	3.8[c]	20	74
Fruit	7.6[c]	20	148

[a] Based on correlations reported in Hoerger and Kenaga (1972) and Kenaga (1973) and modified by Fletcher (1994)
[b] Fresh / dry weight ratios from Harris (1975)
[c] Fresh / dry weight ratios from Spector (1956)

If it is determined that a less conservative exposure scenario is necessary following the risk characterization, the mean residue values from the Kenaga nomogram could be considered. The application rates chosen to calculate the mean EECs cover the range of foliar application rates: the **minimum** single ground application for sorghum (1000 g a.i./ha) and the **maximum** single ground application for corn (1500 g a.i./ha). The EECs were determined for both on-field and off-field exposure. The off-field EECs were determined based on the percentage drift that is expected from the method of application (spray drift deposition of spray quality of ASAE medium for ground applications to sorghum and corn - 6%). The EECs on food sources based on the mean Kenaga values at the highest and lowest ground application rates for atrazine are provided in Table 6 (sorghum) and Table 7 (corn).

Table 6 Mean EECs in vegetation and insects after a direct over-spray at the minimum single crop application rate - sorghum (1000 g a.i./ha)

Food item	Fresh / dry weight ratios	Sorghum (1000 g a.i./ha)	
		EEC (mg a.i./kg fw)[a]	EEC (mg a.i./kg dw)
Mean residue concentrations – On field			
Short range grass	3.3[b]	76	251
Long grass	4.4[b]	32	141
Broadleaf plants	5.4[b]	40	216
Insects	3.8[c]	58	220
Grain and seeds	3.8[c]	6	24
Fruit	7.6[c]	6	47
Mean residue concentrations – Off field			
Short range grass	3.3[b]	4.6	15
Long grass	4.4[b]	1.9	8.5
Broadleaf plants	5.4[b]	2.4	13
Insects	3.8[c]	3.5	13
Grain and seeds	3.8[c]	0.4	1.4
Fruit	7.6[c]	0.4	2.8

[a] Based on correlations reported in Hoerger and Kenaga (1972) and Kenaga (1973) and modified by Fletcher (1994)
[b] Fresh / dry weight ratios from Harris (1975)
[c] Fresh / dry weight ratios from Spector (1956)

Table 7 Mean EECs in vegetation and insects after a direct over-spray at the minimum single crop application rate - corn (1500 g a.i./ha)

Food item	Fresh / dry weight ratios	Corn (1500 g a.i./ha)	
		EEC (mg a.i./kg fw)[a]	EEC (mg a.i./kg dw)
Mean residue concentrations – On field			
Short range grass	3.3[b]	114	376
Long grass	4.4[b]	48	211
Broadleaf plants	5.4[b]	60	324
Insects	3.8[c]	87	331
Grain and seeds	3.8[c]	9	35
Fruit	7.6[c]	9	71
Mean residue concentrations – Off field			
Short range grass	3.3[b]	6.8	23
Long grass	4.4[b]	2.9	13
Broadleaf plants	5.4[b]	3.6	19
Insects	3.8[c]	5.2	20
Grain and seeds	3.8[c]	0.6	2.1
Fruit	7.6[c]	0.6	4.2

[a] Based on correlations reported in Hoerger and Kenaga (1972) and Kenaga (1973) and modified by Fletcher (1994)
[b] Fresh / dry weight ratios from Harris (1975)
[c] Fresh / dry weight ratios from Spector (1956)

Appendix XI Species sensitivity distribution analyses

Health Canada reviewed registrant-submitted data, reviews conducted by other jurisdictions, and published literature studies for the risk assessment. Only those studies with reliable quantitative toxicity endpoints were considered for the SSDs (endpoints from acceptable or supplemental studies with endpoints deemed acceptable for quantitative use). Studies from which the USEPA drew endpoints that were used quantitatively in the draft Biological Evaluation of atrazine (USEPA, 2020; PMRA# 3292787, 3292792), were deemed acceptable for quantitative use in the SSD analysis.

Health Canada uses the software program ETX 2.2 to fit species sensitivity distribution (SSD) models to toxicity endpoint values. The software was produced by RIVM (Rijksinstituut voor Volksgezondheid en Milieu, The Netherlands). It fits lognormal models to datasets using maximum likelihood estimates. Several tests are included that assess normality of the data.

Sufficient laboratory toxicity data were available for terrestrial plants (vegetative vigour, seedling emergence), freshwater algae, vascular plants, fish, marine/estuarine algae and vascular plants to determine acute HC_5 values (the 5th percentile of the species sensitivity distribution (SSD) for the LC_{50}/EC_{50} at 50% confidence intervals). Summaries of the SSD analyses for each of these taxanomic groups follows.

Terrestrial plants (Vegetiative vigour and seedling emergence)

The following criteria were used to establish the terrestrial plant vegetative vigour and seedling emergence dataset selected for SSD analysis:

- Toxicity endpoints were limited to 25% effects levels (ER_{25} values) or median (50%) effects levels (ER_{50} values), and ER_{25} and ER_{50} values were not combined in the same SSDs.
- SSDs were also restricted to selected time ranges to avoid exposure duration influencing the characterization of species sensitivity in the model.
- The most sensitive of standard quantitative measures of effects in plants (were selected from each test (vegetative vigour: survival, biomass/weight and shoot length; seedling emergence: survival, dry weight and shoot length).
- If multiple toxicity endpoints for the same most sensitive measure of effects would be available for the same species from the same study, a geomean of these values was taken to represent the study.
- If a test organism was only identified to the genus level, it would be used in the SSD analysis, unless there were one or more endpoints for the same genus available, identified to the species level. In this case the value for the unidentified species would be excluded.
- If endpoints were available for the same species, but different ecoregion seed sources, Canadian seed sources would take precedence, and other endpoints for the same species from other regions would be omitted. North American seed sources would take precedence if Canadian seed sources were not available. Otherwise, other seed source endpoints would be considered in the analysis.
- If more than one study value was available for the same species, a geomean was taken of these values.

Vegetative vigour

Four SSD models were attempted, given the available data:

1) 21–28 d ER25s,
2) 21–28 d ER50s,
3) 42-d ER25s
4) 42-d ER50s.

Conventionally, the Environmental Assessment Directorate would potentially use, for terrestrial plants, the HC_5 of an SSD model fit to ER_{50} values, or the HC_{10} of an SSD model fit to ER_{25} values as an effects metric in risk assessment. HC_{10} estimates were calculated following Aldenberg and Jaworska (2000), as ETX 2.2 does not provide this estimate in the software's output.

Based on the results of the SSD analyses, the SSD models fit to 21-28 d ER_{25} and 42-d ER_{25} values had acceptable goodness-of-fit based on visual inspection, and acceptance of the null hypothesis at alpha = 0.05 (based on goodness-of-fit tests appropriate for the given samples sizes). The SSD model fit to 21–28 d ER_{25} values (n = 33) produced an HC_{10} of 22.4 g a.i./ha (with a two-sided 90% confidence interval of 11.3-37.7 g a.i./ha). The model fit to 42-d ER_{25} values (n = 10) resulted in an HC_{10} of 14.1 g a.i./ha (with a two-sided 90% confidence interval of 1.9–47.1 g a.i./ha).

The models fit to ER_{50} values (both 21–28 d and 42-d) did not produce acceptable model fit (the null hypothesis of normality was rejected at alpha = 0.5 for the Anderson-Darling test, and by visual inspection the models did not provide adequate fit).

Based on the results presented above, it is recommended that the HC_{10} from the model fit to 21-28 d ER_{25} values be considered as a potential effects metric in the risk assessment of atrazine for plants exposed in a vegetative vigour test (HC_{10} = 22.4 g a.i./ha).

This recommended value of 22.4 g a.i./ha falls below all available vegetative vigour study ER_{50} values, and as anticipated, generally falls below the vast majority of available ER_{25} values available for plants exposed to atrazine in vegetative vigour studies (only one ER_{25} falls below this value, soybean 42-d shoot dry weight ER_{25} = 4.5 g a.i./ha). The data used in the SSD analyses, are presented in Table 1 below. The SSD model is presented in Figure 1.

Table 1 21-28 d ER_{25}s for terrestrial plants exposed to atrazine in a vegetative vigour test used in SSD analysis

Species name or taxon	Monocot/ Dicot	Test ID	Test substance	Duration (d)	Measurement endpoint	Toxicity endpoint (g a.i./ha)	Primary reference	Species study value (g a.i./ha)	Species value for SSD (g a.i./ha)
American vetch (*V. Americana*)	Dicot	-	Aatrex Liquid 480	28	Dry weight	525	White and Boutin 2007 (PMRA# 2482641)	525	525
American water horehound (*L. americanus*)	Dicot	Fall	Aatrex Liquid 480 (480 g a.i./L)	28	Dry weight	36.5	Boutin et al. 2010 (PMRA# 2743693)	65.9442	65.9442
		Spring	Aatrex Liquid 480 (480 g a.i./L)	28	Dry weight	57.6			
		Winter	Aatrex Liquid 480 (480 g a.i./L)	28	Dry weight	136.4			
Big bluestem (*A. Gerardii*)	Monocot	-	Aatrex Liquid 480	28	Dry weight	2162	White and Boutin 2007 (PMRA# 2482641)	2162	2162
Black nightshade (*S. nigrum*)	Dicot	-	Aatrex Liquid 480	28	Dry weight	67	White and Boutin 2007 (PMRA# 2482641)	67	67
Black-eyed Susan (*R. hirta*)	Dicot	Fall	Aatrex Liquid 480 (480 g a.i./L)	28	Dry weight	5.29	Boutin et al. 2010 (PMRA# 2743693)	29.64324	29.64324
		Spring	Aatrex Liquid 480 (480 g a.i./L)	28	Dry weight	166.11			

Species name or taxon	Monocot/ Dicot	Test ID	Test substance	Duration (d)	Measurement endpoint	Toxicity endpoint (g a.i./ha)	Primary reference	Species study value (g a.i./ha)	Species value for SSD (g a.i./ha)
Cabbage (*Brassica oleracea alba*)	Dicot	Test 1	Atrazine SC (43.3)	21	Shoot Dry Weight	66	Unpublished report 2015 (PMRA# 2816828)	66	32.18176
		-	Technical grade active ingredient	21	Reduction in dry weight	15.6919	Unpublished report 1989 (MRID 42041402)	15.6919	
Canada bluegrass (*P. compressa*)	Monocot	-	Aatrex Liquid 480	28	Dry weight	123	White and Boutin 2007 (PMRA# 2482641)	123	123
Canada goldenrod (*S. Canadensis*)	Dicot	ON	Aatrex Liquid 480 (480 g a.i./L)	28	Biomass inhibition	413	Boutin et al. 2010 (PMRA# 2743693)	413	413
Carrot (*Daucus carota*)	Dicot	Test 1	Atrazine SC (43.3)	21	Shoot Dry Weight	60.5	Unpublished report 2015 (PMRA# 2816828)	60.5	339.5282
		-	Technical grade active ingredient	21	Reduction in dry weight	1905.445	Unpublished report 1989 (MRID 42041402)	1905.445	
Common foxglove (*D. purpurea*)	Dicot	North America East	Aatrex Liquid 480	28	Biomass inhibition	154.96	Boutin et al. 2010 (PMRA# 2743693)	169.2144	169.2144
Corn (*Zea mays*)	Monocot	Test 1	Atrazine SC (43.3)	21	Shoot Dry Weight	>28000	Unpublished report 2015 (PMRA# 2816828)	28000	28000
		Test 2	Atrazine SC (43.3)	21	Shoot Length	>28000			
Cornflower (*C. cyanus*)	Dicot	North America West	Aatrex Liquid 480 (480 g a.i./L)	28	Biomass inhibition	227.03	Boutin et al. 2010 (PMRA# 2743693)	227.03	227.03
Cucumber (*Cucumis sativus*)	Dicot	Test 1	Atrazine SC (43.3)	21	Shoot Dry Weight	16.8a	Unpublished report 2015 (PMRA# 2816828)	16.8	12.27364
		-	Technical grade active ingredient	21	Reduction in dry weight	8.9668	Unpublished report 1989 (MRID 42041402)	8.9668	

Species name or taxon	Monocot/ Dicot	Test ID	Test substance	Duration (d)	Measurement endpoint	Toxicity endpoint (g a.i./ha)	Primary reference	Species study value (g a.i./ha)	Species value for SSD (g a.i./ha)
Curly dock (*R. crispus*)	Dicot	North America East	Aatrex Liquid 480	28	Biomass inhibition	52.09	Boutin et al. 2010 (PMRA# 2743693)	52.09	52.09
Elecampane (*I. helenium*)	Dicot	North America East	Aatrex Liquid 480	28	Biomass inhibition	388.94	Boutin et al. 2010 (PMRA# 2743693)	618.3299	618.3299
			Aatrex Liquid 480	28	Biomass inhibition	983.01			
English daisy (*B. perennis*)	Dicot	North America West	Aatrex Liquid 480	28	Biomass inhibition	32.57	Boutin et al. 2010 (PMRA# 2743693)	32.57	32.57
Lettuce (*Lactuca sativa*)	Dicot	-	Aatrex Liquid 480	28	Dry weight	40	White and Boutin 2007 (PMRA# 2482641)	40	71.39791
		Test 1	Atrazine SC (43.3)	21	Shoot Dry Weight	24.6a	Unpublished report 2015 (PMRA# 2816828)	24.6	
		-	Technical grade active ingredient	21	Reduction in dry weight	369.8805	Unpublished report 1989 (MRID 42041402)	369.8805	
Northern wheatgrass (*E. lanceolatus*)	Monocot	-	Aatrex Liquid 480	28	Dry weight	217	White and Boutin 2007 (PMRA# 2482641)	217	217
Oat (*Avena sativa*)	Monocot	Test 2	Atrazine SC (43.3)	21	Shoot Length	200	Unpublished report 2015 (PMRA# 2816828)	264.5751	256.4479
		Test 1	Atrazine SC (43.3)	21	Shoot Length	350			
		-	Technical grade active ingredient	21	Reduction in dry weight	2690.04	Unpublished report 1989 (MRID 42041402)	2690.04	
		Spring	Aatrex Liquid 480	28	Dry weight	4	Boutin et al. 2010 (PMRA# 2743693)	23.69681	
		Winter	Aatrex Liquid 480	28	Dry weight	33.3			
		Summer	Aatrex Liquid 480	28	Dry weight	99.9			

Species name or taxon	Monocot/ Dicot	Test ID	Test substance	Duration (d)	Measurement endpoint	Toxicity endpoint (g a.i./ha)	Primary reference	Species study value (g a.i./ha)	Species value for SSD (g a.i./ha)
Onion (*Allium cepa*)	Monocot	Test 1	Atrazine SC (43.3)	21	Shoot Dry Weight	42.6a	Unpublished report 2015 (PMRA# 2816828)	42.6	170.6646
		-	Technical grade active ingredient	21	Reduction in dry weight	683.7185	Unpublished report 1989 (MRID 42041402)	683.7185	
Ox-eye daisy (*C. leucanthemum*)	Dicot	Fall	Aatrex Liquid 480	28	Dry weight	11.25	Boutin et al. 2010 (PMRA# 2743693)	124.2504	124.2504
		Spring	Aatrex Liquid 480	28	Dry weight	273.16			
		Winter	Aatrex Liquid 480	28	Dry weight	624.2			
Radish (*R. sativus*)	Dicot	-	Aatrex Liquid 480	28	Dry weight	177	White and Boutin 2007 (PMRA# 2482641)	177	177
Rough-leaved sunflower (*H. strumosus*)	Dicot	-	Aatrex Liquid 480	28	Dry weight	100	White and Boutin 2007 (PMRA# 2482641)	100	100
Ryegrass (*Lolium perenne*)	monocot	Test 1	Atrazine SC (43.3)	21	Shoot Dry Weight	269a	Unpublished report 2015 (PMRA# 2816828)	269	1098.196
Ryegrass (*Lolium perenne*)	monocot	-	Technical grade active ingredient	21	Reduction in dry weight	>4483.4	Unpublished report 1989 (MRID 42041402)	4483.4	
Self-heal (*P. vulgaris*)	Dicot	North America West	Aatrex Liquid 480	28	Biomass inhibition	83.53	Boutin et al. 2010 (PMRA# 2743693)	83.53	83.53
Soybean (*Glycine max*)	Dicot	-	Aatrex Liquid 480	28	Dry weight	165	White and Boutin 2007 (PMRA# 2482641)	165	45.96762
		Test 1	Atrazine SC (43.3)	21	Shoot Dry Weight	20.2a	Unpublished report 2015 (PMRA# 2816828)	20.2	
		-	Technical grade active ingredient	21	Reduction in dry weight		Unpublished report 1989 (MRID 42041402)	29.1421	
Strawberry (*F. ananassa*)	Dicot	-	Aatrex Liquid 480	28	Dry weight	164	White and Boutin 2007 (PMRA# 2482641)	164	164

Species name or taxon	Monocot/ Dicot	Test ID	Test substance	Duration (d)	Measurement endpoint	Toxicity endpoint (g a.i./ha)	Primary reference	Species study value (g a.i./ha)	Species value for SSD (g a.i./ha)
Sunflower (*H. annuus*)	Dicot	-	Aatrex Liquid 480	28	Dry weight	72	White and Boutin 2007 (PMRA# 2482641)	72	72
Tall blue spruce (*L. Canadensis*)	Dicot	-	Aatrex Liquid 480	28	Dry weight	97	White and Boutin 2007 (PMRA# 2482641)	97	97
Thick-leaved strawberry (*F. virginana*)	Dicot	-	Aatrex Liquid 480	28	Dry weight	20	White and Boutin 2007 (PMRA# 2482641)	20	20
Tomato (*Lycopersicon esculentum*)	Dicot	Test 1	Atrazine SC (43.3)	21	Shoot Dry Weight	32.5[a]	Unpublished report 2015 (PMRA# 2816828)	32.5	75.77024
		-	Technical grade active ingredient	21	Reduction in dry weight	807.012	Unpublished report 1989 (MRID 42041402)	807.012	
		Winter	Aatrex Liquid 480	28	Dry weight	5.2	Boutin et al. 2010 (PMRA# 2743693)	22.84907	
		Fall	Aatrex Liquid 480	28	Dry weight	100.4			
		-	Aatrex Liquid 480	28	Dry weight	55	White and Boutin 2007 (PMRA# 2482641)	55	
Wheat (*T. aestivum*)	Monocot	Winter	Aatrex Liquid 480	28	Dry weight	510.7	Boutin et al. 2010 (PMRA# 2743693)	813.5522	346.9953
		Spring	Aatrex Liquid 480	28	Dry weight	>1296			
		-	Aatrex Liquid 480	28	Dry weight	148	White and Boutin 2007 (PMRA# 2482641)	148	
White avens (*G. canadense*)	Dicot	Summer	Aatrex Liquid 480	28	Dry weight	88.3	Boutin et al. 2010 (PMRA# 2743693)	136.3023	136.3023
		Spring	Aatrex Liquid 480	28	Dry weight	210.4			

[a] As reported by USEPA (2020; PMRA# 3292787)

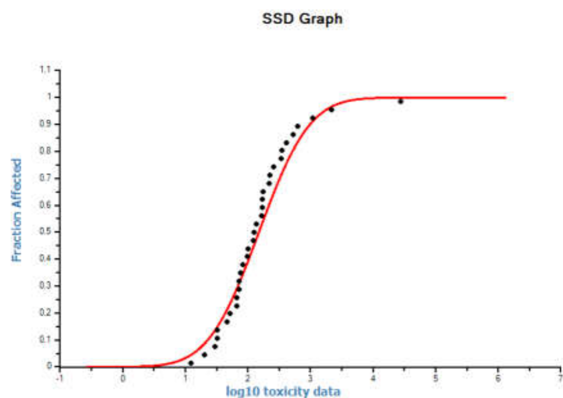

Figure 1 **Lognormal SSD model fit to 21- to 28-d ER_{25} values for terrestrial plants exposed to atrazine at a vegetative vigour lifestage**

Seedling emergence

An SSD model for seedling emergence study endpoints with data presented in Table 2. While corn seems insensitive to rates tested, other monocot species tested in the technical grade active ingredient study fall within the range of endpoints reported for dicot species from the same study.

Given the poor fit of the model to the terrestrial plant seedling emergence study toxicity data (rejection of the null hypothesis of normality at alpha = 0.05 for the Anderson-Darling test; most appropriate goodness-of-fit test for the small dataset; and visual inspection of the results shown in Figure 2), an SSD-based effects metric was NOT recommended for use in the risk assessment for terrestrial plants. In the absence of additional data, the lowest available and acceptable ER25 of 2.8 g a.i./ha for lettuce (14-d post emergence dry weight) was used in the risk assessment to establish an effects metric based on seedling emergence toxicity testing.

Table 2 **14-d dry weight ER25s for crops exposed to atrazine as technical grade active ingredients at a vegetative vigour stage (from an unpublished study from 1989, MRID 42041403; toxicity endpoints as cited by USEPA in the Draft Biological Evaluation for Atrazine, PMRA# 3292787)**

Species	Monocot/Dicot	ER25 (g a.i./ha)
Lettuce (*Lactuca sativa*)	Dicot	2.8
Carrot (*Daucus carota*)	Dicot	3.4
Oat (*Avena sativa*)	Monocot	4.5
Ryegrass (*Lolium perenne*)	Monocot	7.8
Onion *(allium cepa*)	Monocot	10.1
Cucumber (*Cucumis sativus*)	Dicot	14.6
Cabbage (*Brassica oleracea alba*)	Dicot	15.7
Tomato (*Lycopersicon esculentum*)	Dicot	38.1
Soybean (*Glycine max*)	Dicot	213
Corn (*Zea mays*)	Monocot	>4483.4

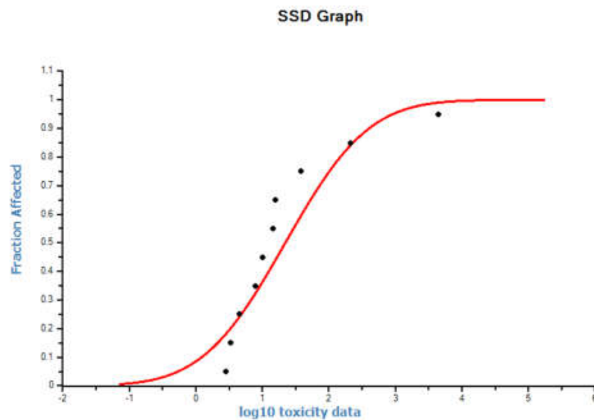

Figure 2 Species Sensitivity Distribution (SSD) model fit to 14-d ER25 toxicity endpoints for terrestrial plants exposed to atrazine prior to seedling emergence.

Freshwater algae species

The following criteria were used to establish the freshwater algae dataset selected for SSD analysis:

- Toxicity endpoints were restricted to endpoints considered apical (survival, growth or reproduction) or directly related to apical endpoints (for example, chlorophyll, photosynthesis, carbon fixation and oxygen production were all considered measures of effects that are directly related to growth, though not direct measures of growth themselves).
- Toxicity endpoints were restricted to estimated median effects level (EC_{50}s).
- Durations were restricted to 72–96 hours (3-4 days), which is consistent with standard algae toxicity study test durations (for example, OECD 201, OCSPP 850.4500, OCSPP 850.4550). The Primary SSD TT Evaluator noted: there did not appear to be an individual duration or range of durations that was consistently more or less sensitive than other durations for species with available data. This restriction on duration for the SSD analysis is consistent with the approach taken by USEPA (2020; PMRA# 3292792, 3292797)
- From studies deemed acceptable for consideration in the SSD analyses, the most sensitive measure of effects was selected (based on geomeans, if more than one toxicity endpoint was reported for the same measure of effects and duration).
- If there was more than one toxicity value per species (from different studies) those values were included in a geomean, representing a median effects level for that species in the SSD.
- If the test organism was only identified to the genus level, associated toxicity values were excluded if at least one other toxicity value was available from a study with species of the same genus that were identified to the species level.

A summary of toxicity data used in the SSDs on freshwater algae are presented in Table 3. All toxicity values were based on studies with the technical grade active ingredient or formulated product. Eighteen species representing four phyla (Chlorophyta, Euglenophyta, Rhodophyta and Cyanobacteria) are present in the SSD dataset, with the majority of species being green algae (Chlorophyta).

Given the poor fit of the model to the freshwater algae toxicity data, (rejection of the null hypothesis of normality at alpha = 0.05 for the Anderson-Darling test; most appropriate goodness-of-fit test for the small dataset; and visual inspection of the results shown in Figure 3) an SSD-based effects metric was NOT recommended for use in the risk assessment for freshwater algae. In the absence of additional data, the lowest available and acceptable 96 hour EC50/2 of 2.3 µg a.i./L for Chlorophycean green algae (*Chlorella vulgaris*, based on reduced abundance) was used in the risk assessment to establish an effects metric based on freshwater algae toxicity testing.

Table 3 72- and 96-hr EC50 values used in the Species Sensitivity Distribution (SSD) analysis for effects of atrazine on freshwater algae

Phylum	Class	Order	Family	Scientific name	Duration (d)	Measure of effects	Toxicity Endpoint (µg/L)	Reference	Species study value (µg/L)[a]	Species value used in SSD (µg/L)[b]
Chlorophyta	Chlorophyceae	Chlorococcales	Chlorococcaceae	*Oophila sp.*	4	Abundance	95	Baxter et al. 2014[c]	160.14	134.5
							172			
							175			
							230			
					4	Photosystem II electron transport activity	113	Baxter et al. 2015[c]	113.00	
			Oocystaceae	*Chlorella fusca ssp. fusca*	4	Population growth rate	68.2	Kotrikla et al. 1999[c]	72.42	69.14
							76.9			
				Chlorella fusca var. vacuolata	3	Population growth rate	66	Vallotton et al. 2008[c]	66	
				Chlorella pyrenoidosa	4	Population growth rate	55.1	Ma et al. 2001[c]	55.10	55.76
							52.44	Ma and Wang 2002[c]	52.44	
							60	Maule and Wright 1984[c]	60	
				Chlorella saccharophila	3	Population growth rate	780	Carrasco and Sabater 1997[c]	780	780
				Chlorella vulgaris	4	reduction in growth	121	Camuel et al. 2017	121	119.59
						reduction in growth	94	Fairchild et al. 1998	94	
						Growth	147	Gaggi et al 1995	147	

Phylum	Class	Order	Family	Scientific name	Duration (d)	Measure of effects	Toxicity Endpoint (µg/L)	Reference	Species study value (µg/L)[a]	Species value used in SSD (µg/L)[b]
						Population growth rate	157.02	Ma et al. 2002[c]	157.02	
						Abundance	4.3	Seguin et al. 2001[c]	27.20	
							172			
						Biomass	409.79	Shitanda et al. 2005[c]	409.79	
			Scenedesmaceae	*Desmodesmus subspicatus*	3	Population growth rate	41	Masojidek et al. 2011[c]	88.72	88.72
							192			
				Raphidocelis subcapitata	4	Population growth rate	200	Abdel-Hamid 1996[c]	206.46	105.12
							200			
							220			
					3	Chlorophyll	283	Abou-Waly 1991 (PMRA# 1404512)	283.00	
					4	Photosystem II electron transport activity	41.9	Baxter et al. 2016[c]	41.90	
					3	Population growth rate	92.9	Benhra et al. 1997[c]	123.55	
							164.3			
					4	Abundance	115	Berard et al. 2003[c]	115	
					4	Abundance, reduction in cell growth	26	Caux et al. 1996[c]	26	
					3	Population growth rate	81.4	Fai et al. 2007[c]	81.40	
					4	Abundance	65	Fairchild et al. 1994[c]	122.93	
							103			
							107			
							126			
							138			

Phylum	Class	Order	Family	Scientific name	Duration (d)	Measure of effects	Toxicity Endpoint (µg/L)	Reference	Species study value (µg/L)[a]	Species value used in SSD (µg/L)[b]
							277			
					4	Chlorophyll	117	Fairchild et al. 1998[c]	117.00	
					4	Chlorophyll A concentration	147	Gaggi et al. 1995[c]	147.00	
					4	Abundance	63.4	Garrett 2004[c]	81.33	
							76.4			
							86.1			
							89.9			
							94.9			
					3	Population changes, general	200	Kallqvist and Romstad 1994[c]	200	
					4	Abundance	41.8	Ma et al. 2006[c]	41.8	
					3	Population growth rate	164	Mayer et al. 1998[c]	164	
Chlorophyta	Chlorophyceae		Scenedesmaceae		3	Population growth rate	196	Perez et al. 2011[c]	196	
					4	Population growth rate	1600	Ralston-Hooper et al. 2009[c]	1600	
				Raphidocelis subcapitata	3	Population growth rate	41.16	Rojickova-Padrtova and Marsalek 1999[c]	41.16	
					3	Abundance	130	Sbrilli et al. 2005[c]	130	
					4	Abundance	118	Seguin et al. 2001[c]	118	
					4	Abundance	50	Versteeg	50	

Phylum	Class	Order	Family	Scientific name	Duration (d)	Measure of effects	Toxicity Endpoint (µg/L)	Reference	Species study value (µg/L)[a]	Species value used in SSD (µg/L)[b]
								1990[c]		
					4	Abundance	48.77	Weiner et al. 2004[c]	48.77	
					4	Reduction in cell growth	130	Unpublished report 1991 (PMRA# 1135768)	130	
					4	Growth, biomass estimated by fluorescence measurement	117	Fairchild et al. 1998[c]	117	
				Scenedesmus acutus	4	Abundance	56	Berard et al. 2003[c]	56	42.03
					3	Population growth rate	11	Carrasco and Sabater 1997[c]	11	
					4	Abundance	45	Seguin et al. 2001[c]	45	
				Scenedesmus acutus var. acutus	3	Photosynthesis	49.18	Chalifour et al. 2016[c]	49.18	
					3	Population growth rate	86	Liu et al. 2009[c]	86	
					4	Population growth rate	47.01	Ma 2002[c]	47.01	
				Scenedesmus quadricauda	4	Chlorophyll	169	Fairchild et al. 1998[c]	169	35.48
					4	Population growth rate	15.58	Ma et al. 2003[c]	15.58	
					3	Population	16.96	Rojickova-	16.96	

Phylum	Class	Order	Family	Scientific name	Duration (d)	Measure of effects	Toxicity Endpoint (µg/L)	Reference	Species study value (µg/L)[a]	Species value used in SSD (µg/L)[b]
						growth rate		Padrtova and Marsalek 1999[c]		
				Scenedesmus subspicatus	4	Abundance	21	Kirby and Sheahan 1994[c]	21	42.45
					3	Population growth rate	36.72	Rojickova-Padrtova and Marsalek 1999[c]	36.72	
					3	Population growth rate	99.2	Zagorc-Koncan J. 1996	99.20	
		Prasiolales	Prasiolaceae	Stichococcus bacillaris	3	Population growth rate	1347.16	Rojickova-Padrtova and Marsalek 1999[c]	1347.16	1347.16
		Volvocales	Chlamydomonadaceae	*Chlamydomonas geitleri*	3	Carbon fixation	150.98 198.43 235.09 273.91 289.01 370.97	Francois and Robinson 1990[c]	243.18	243.18
				Chlamydomonas reinhardtii	4	Population growth rate	49.82	Esperanza et al. 2016[c]	49.82	59.33
					4	Growth, chlorophyll	176	Fairchild et al. 1998	176	
					4	Abundance	56.08	Fernandez-Naveiraet al. 2016[c]	56.08	
					3	Population growth rate	29.32	Rojickova-Padrtova et	29.32	

Phylum	Class	Order	Family	Scientific name	Duration (d)	Measure of effects	Toxicity Endpoint (µg/L)	Reference	Species study value (µg/L)[a]	Species value used in SSD (µg/L)[b]
								al. 1999[c]		
					4	Population changes, general, population growth rate	51	Schafer et al. 1994[c]	51.00	
	Trebouxiophyceae	Chlorellales	Chlorellaceae	*Parachlorella kessleri*	3	Population growth rate	693.12	Rojickova-Padrtova and Marsalek 1999[a]	693.12	693.12
Cyanobacteria	Cyanophyceae	Nostocales	Nostocaceae	Anabaena flos-aquae	3	Reduction of chlorophyll (a) content compared with the control (fluorometrically)	56	Abou-Waly 1991 (PMRA# 1404512)	56	56
		Chroococcales	Microcystaceae	Microcystis sp.	3	Growth	90	Fairchild et al. 1998	90	90
Euglenophycota	Euglenophyceae	Euglenales	Euglenaceae	Euglena gracilis	3	Population growth rate	45000	Girling et al. 2000[c]	61481.70	61481.70
							84000			
Rhodophycota	Rhodophyceae	Porphyridiales	Porphyridiaceae	Porphyridium aerugineum	4	Population growth rate	215.68	Boura-Halfon et al. 1997[c]	215.68	215.68

[a] If multiple toxicity endpoints were reported from the same study for the same species, most sensitive measure of effects and duration, a geomean was taken of these toxicity endpoints to represent the results of that study for the species in the SSD analysis.
[b] If multiple toxicity endpoints were available for a single species from different studies, a geomean was taken of these values to represent the species in the SSD analysis.
[c] As cited by USEPA (2020; Appendix 2-1, PMRA# 3292792, Appendix 2-5, PMRA# 3292797)

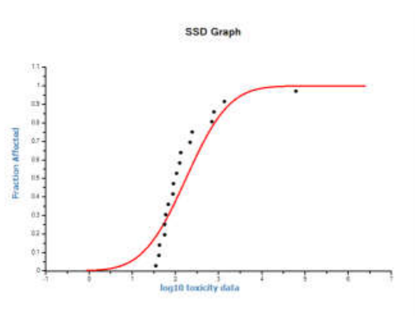

Figure 3 **Species Sensitivity Distribution (SSD) model fit to 72- and 96-hour EC50 toxicity endpoints for freshwater algae exposed to atrazine.**

Freshwater aquatic vascular plant

Only endpoints derived from 7-d exposures were used in the SSD analysis as this exposure period provided the highest number of endpoints. All studies that had been included in the USEPA Draft Biological Evaluation SSD for aquatic plants were considered acceptable, and the most sensitive endpoints from these studies were used in the current SSD. Geomeans were calculated to integrate data from multiple studies for a given species; and when multiple values were reported for a given measurement endpoint within a study. The endpoints selected for inclusion in the SSD are presented in table 4. The distribution generated from this dataset is shown graphically in Figure 4.

The SSD was used to estimate a hazardous concentration to 5% of species (HC_5), which theoretically is the concentration at which 95% of species do not have their acute median effects level (for example, EC_{50}) exceeded. The Species Sensitivity Distribution (SSD) was fitted for toxicity data from 8 freshwater vascular plants species. The toxicity data were tested for normality using the Anderson-Darling, the Komogorov-Smirnov and the Cramer von Mises tests. In the current assessment, these tests failed to detect departure from normality at a significance level of 0.05 and model fit is therefore considered to be acceptable. The calculated HC_5 for aquatic vascular plant EC_{50} is 18.72 (lower-upper confidence interval of 0.5294–118.7) µg a.i./L; this value was considered as the potential effects metric in the risk assessment of atrazine for freshwater aquatic vascular plants.

Table 4 **7-d EC_{50} values for freshwater vascular plants exposed to atrazine used in the SSD analysis**

***EC_{50}** **(µg a.i./L)**	**Test organism**
24300	*Acorus americanus*
13487	*Lemna perpusilla*
8760	*Typha latifolia*
5240	*Typha angustifolia*
225.6	*Myriophyllum aquaticum*, *Geomean 1 (n = 5)
200.37	*Lemna aequinoctialis*
101.2	*Lemna minor*, *Geomean 2 (n = 12)
69.3	*Lemna gibba*, *Geomean 3 (n = 5)

*EC_{50} (µg a.i./L)	Test organism
*EC_{50}: The endpoint operator (=, <, >) was not available from the data source.	
*Geomean 1 (n = 5) for *Myriophyllum aquaticum* was calculated from the following values: [135.1; 270.2; 386]; [170; 261]	
*Geomean 2 (n = 12) for *Lemna minor* was calculated from the following values: [86.3; 197.42]; [39.9; 79.9]; [61.71; 105.08; 125.23]; [61; 125]; 100; 100.9; 180	
*Geomean 3 (n = 5) for *Lemna gibba* was calculated from the following values: [32.1; 64.3]; 100; 89; 57	
Values in square brackets belong to a same study. A geomean of these values was calculated first.	

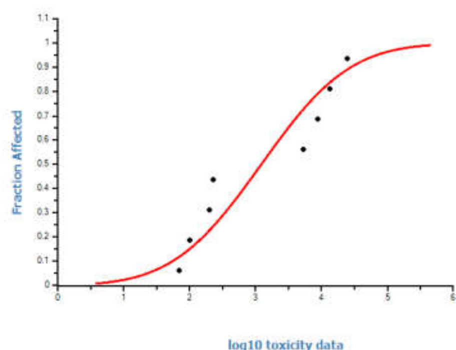

Figure 4 Species sensitivity distribution fit to vascular aquatic plant atrazine EC_{50}s (ETX 2.2)

Marine/estuarine aquatic vascular plants

The following criteria were used to establish the marine/estuarine aquatic vascular plants dataset selected for SSD analysis:

- Toxicity endpoints were restricted to endpoints considered apical (survival, growth or reproduction) or directly related to apical endpoints (for example, chlorophyll, photosynthesis, carbon fixation and oxygen production were all considered measures of effects that are directly related to growth, though not direct measures of growth themselves).
- Toxicity endpoints were restricted to estimated median effects level (EC_{50}s).
- Durations were restricted to 72–96 hours (3–4 days), which is consistent with standard algae toxicity study test durations (for example, OECD 201, OCSPP 850.4500, OCSPP 850.4550). This restriction on duration for the SSD analysis is consistent with the approach taken by USEPA (2020; PMRA# 3292797 and 3292792) and results in the highest number of endpoints included in the SSD analysis.
- From studies deemed acceptable for consideration in the SSD analyses, the most sensitive measure of effects was selected (based on geomeans, if more than one toxicity endpoint was reported for the same measure of effects and duration).
- If there was more than one toxicity value per species (from different studies) those values were included in a geomean, representing a median effects level for that species in the SSD.

- If the test organism was only identified to the genus level, associated toxicity values were excluded if at least one other toxicity value was available from a study with species of the same genus that were identified to the species level.

All studies that had been included in the USEPA BE SSD and presented in the USEPA refined assessment (2016) were considered acceptable, and the most sensitive endpoints for each species presented in these studies were used in the current SSD. Geomean values were calculated to integrate data from multiple studies for a given species; and when multiple values were reported for a given measurement endpoint within a given study.

The endpoints selected for inclusion in the SSD are presented in Table 5. The distribution generated from this dataset is shown graphically in Figure 5.

The SSD was used to estimate a hazardous concentration to 5% of species (HC_5), which theoretically is the concentration at which 95% of species do not have their acute median effects level (for example, EC_{50}) exceeded. The Species Sensitivity Distribution (SSD) was fitted to data from 23 marine non-vascular plants species. The toxicity data were tested for normality using the Anderson-Darling, the Komogorov-Smirnov and the Cramer von Mises tests. In the current assessment, these tests failed to detect departure from normality at a significance level of 0.1. The model fit is therefore considered to be acceptable. EC_{50} values ranged between 12 and 460 µg a.i./L, spanning 1.6 orders of magnitude. The least sensitive and most sensitive species were, respectively, *Navicula inserta* and *Ankistrodesmus sp*. The calculated HC_5 (lower-upper confidence interval) = 16.53 (8.55-26.31) µg a.i./L; this value was considered as the potential effects metric in the risk assessment of atrazine for marine/estuarine aquatic vascular plants.

Table 5 Toxicity data used in the marine/estuarine aquatic vascular plant SSDs for atrazine (censored values entered as non-censored in ETX 2.2).

*EC_{50} (µg a.i./L)	Test organism
460	*Navicula inserta*
430	*Bacillariophyceae Nitzschia* (Ind. 684)
300	Amphora *exigua*
290	*Nitzschia closterium*
185	*Nannochloropsis gaditana*
165	*Rhodomonas salina*
140	*Chlorella sp.*
123.10	*Geomean 1 (n = 2) for *Pavlova sp.*
110	*Stauroneis amphoroides*
110	*Thalassiosira fluviatilis*
100	*Platymonas sp.*
93	*Achnanthes brevipes*
84	*Cyclotella nana*
82	*Chlorophyceae Neochloris sp.*
79	*Porphyridium cruentum*
77	*Monochrysis lutheri*
67.66	*Geomean 2 (n=2) for *Dunaliella tertiolecta*
60	*Chlamydomonas sp.*

*EC_{50} (µg a.i./L)	Test organism
28.04	*Nephroselmis pyriformis*
22.17	*Storeatula major*
20	*Tetraselmis chuii*
17.19	*Amphidinium operculatum*
11.87	*Ankistrodesmus sp.*
*EC_{50}: The endpoint operator (=, <, >) was not available from the data source.	
*Geomean 1 (n = 2) for *Pavlova sp.* was calculated from the following values: 157.8578; 96	
*Geomean 2 (n = 2) for *Dunaliella tertiolecta* was calculated from the following values: 69; 66.35	

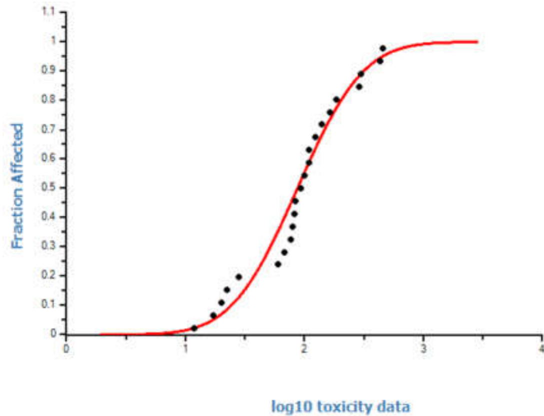

Figure 5 Species sensitivity distribution for marine algae toxicity EC_{50}s (ETX 2.2)

References

A. Information considered in the toxicology assessment

a. List of studies/Information submitted by registrant

PMRA Document Number	Reference
1123345	1991, 90 day oral toxicity study in rats (atrazine) (diaminochlorotriazine G-28273), DACO: 4.3.1
1137869	1993, addendum to report# MIN 892076 purity of test material used in the hydroxyatrazine, 13 week feeding study in dogs (atrazine) was 97.1 percent, DACO: 4.3.1
1150097	1994, diaminochlorotriazine (G28273): 90-day oral tox study in rats – grading system for opthalmoscopic examination – response to Health Canada, DACO: 4.3.1
1150098	1994, diaminochlorotriazine (G28273): 90-day oral tox study in rats – method of urine collection – response to Health Canada, DACO: 4.3.1
1150099	1994, diaminochlorotriazine (G28273): 90-day oral tox study in rats – definition of grading system for clinical pathology to Health Canada, DACO: 4.3.1
1199835	90 day oral tox – dog, DACO: 4.3.1
1199837	90 day dietary tox – rat, DACO: 4.3.1
1199838	90 day dietary tox – rat, DACO: 4.3.1
1199839	90 day dietary tox – rat, DACO: 4.3.1
1233361	1989, atrazine technical supplemental information for the chronic study in dogs (852008), DACO: 4.3.1
1233362	1990, (diaminochlorotriazine), 13/52 – week oral toxicity study in dogs (872151), DACO: 4.3.1
1234775	1989, hydroxyatrazine 90-day oral toxicity study in rats (882146), DACO: 4.3.1
1234776	1990, hydroxyatrazine 13-week feeding study in dogs (892076), DACO: 4.3.1
1234778	1988, G 30027 technical 14-day oral toxicity study in young rats (gavage) final report (871290), DACO: 4.3.1
1234780	1990, 14-day repeated dose oral toxicity/hormone study in female albino rats with atrazine and diaminochlorotriazine final report (483-268), DACO: 4.3.1
2945548	1994, 3-month oral toxicity study in rats, DACO: 4.3.1
2945549	1992, G 28279 technical – 90 day oral toxicity study in rats 3-month oral toxicity study in rats (administration in food), DACO: 4.3.1
2945550	1994, data evaluation record: G 28279 Technical – 90-day oral toxicity in rats; 3-month oral toxicity study in rats (administration in food), DACO: 4.3.1
1078579	1998, chronic (12-24 month) study in rats with atrazine technical, part 1 of 2, DACO: 4.4.1
1078580	1998, chronic (12-24 month) study in rats with atrazine technical, part 2 of 2, DACO: 4.4.1
1078581	2002, 52-week toxicity study of simazine, atrazine and DACT administered in the diet of female rats, DACO: 4.4.1

PMRA Document Number	Reference
1123335	1991, atrazine technical, chronic toxicity study in rats, study finalized, DACO: 4.4.1
1137874	1993, addendum: purity of test material + supplemental to 52 wk feeding study in dogs (MIN 852008) (atrazine), DACO: 4.4.1
1150100	1994, atrazine technical: chronic toxicity study in rats – response to Health Canada, DACO: 4.4.1
1150101	1994, atrazine (G30027): chronic toxicity study in rats – page 102 missing from the final report, response to Health Canada, DACO: 4.4.1
1150102	1994, atrazine (G30027): chronic toxicity study in rats – criteria used in grading histopathology lesions – response to Health Canada, DACO 4.4.1
1167680	1995, chapter 21 volume 24: one-year chronic toxicity study with atrazine technical in rats, preliminary report, DACO: 4.4.1
1167765	1995, 1-year chronic toxicity study with atrazine technical in rats, DACO: 4.4.1
1167774	1995, cont'd from roll#1550) chapter 22 volume 3,4: 1-year chronic toxicity study with atrazine technical in rats, DACO: 4.4.1
1233363	1988, atrazine technical: chronic toxicity study in rats (MIN 852214) pathology report, DACO: 4.4.1
1137873	1992, addendum: purity of test material + supplemental to 91 wk oral carcinogenicity study in mice (MIN 842120) (atrazine), DACO: 4.4.1, 4.4.2
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1203787	24 month chronic oral toxicity & oncogenicity study in rats utilizing atrazine tech. (410-1102), part 1-6, DACO: 4.4.1, 4.4.2
1203788	24 month chronic oral toxicity & oncogenicity study in rats utilizing atrazine tech. (410-1102), part 1-6, from roll 341 DACO: 4.4.1, 4.4.2
1203789	24 month chronic oral toxicity & oncogenicity study in rats utilizing atrazine tech. (410-1102), part 7-10, DACO: 4.4.1, 4.4.2
1203790	24 month chronic oral toxicity & oncogenicity study in rats utilizing atrazine tech. (410-1102), part 7-10, DACO: 4.4.1, 4.4.2
1203791	24 month chronic oral toxicity & oncogenicity study in rats utilizing atrazine tech. (410-1102), part 11-14B, DACO: 4.4.1, 4.4.2
1204001	24 month chronic oral toxicity & oncogenicity study in rats utilizing atrazine tech. (410-1102), part 11-14B (cont'd from roll 342), DACO: 4.4.1, 4.4.2
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1233357	(cont't from roll #853) atrazine technical 91-week oral carcinogenicity study in mice (MIN 842120), DACO: 4.4.1, 4.4.2
1234783	1987, atrazine technical 91-week oral carcinogenicity study in mice (MIN 842120) (cont't on roll #854), DACO: 4.4.1, 4.4.2
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1123318	1992, oncogenicity study in Sprague-Dawley rats with atrazine technical (HWA 483-277) final report (cont'd on roll#1147), study finalized, DACO: 4.4.2
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1180054	1996, chapter 23 volume 5&6: chronic (12/24 month) study in rats with atrazine technical supplement to EPA guideline no.83-1, final 12-month report, DACO: 4.4.5
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1233358	1987, atrazine technical 52-week oral feeding study in dogs (MIN 852008) (con't on roll# 855), DACO: 4.4.5
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1115084	1991, determination of hormone levels in Fischer-344 rats treated with atrazine technical (final report)… continued from PMRA#: 1115083, DACO: 4.5.12
1115085	1990, determination of hormone levels in Fischer-344 rats treated with atrazine technical (52 week interim report) (cont'd on roll# 1148), DACO: 4.5.12
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1135427	1991, determination of hormone levels in Sprague-Dawley rats treated with atrazine technical (final report) study finalized, DACO: 4.5.12
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1167669	1994, chapter 21 volume 14: rat mammary tumorigenesis: relevance of hormonal imbalance to dose selection, DACO: 4.5.12
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b. Additional information considered

i. Published information

Note: Only published studies that are cited in the PSRD are listed in the tables above and below;

a full list of published information considered in this special review is available upon request.

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ii. Unpublished information

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B. Information considered in the dietary assessment

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1180063	Chapter 23 Volume 16: Dispostion of Atrazine in Rhesus Monkey Following Intravenous Administration. DACO: 4.5.12.
1167662	Chapter 21 Volume 7: The In Vitro Percutaneous Absorption of Formulated [U-

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2863138, 2862339	Derivation of avian dermal LD50 values for dermal exposure models using in vitro percutaneous absorption of [14C]-atrazine through rat, mallard, and northern bobwhite full thickness skin: Supplemental Information. Methods and Detailed Results of Dermal Absorption Study. DACO: 9.9.
1234611	Dermal Absorption of 14C-Atrazine in The Rat (ABR-87098). Study Finalized: June 11, 1987. Authors; T. Murphy; B. Simoneaux. Study Director: T. Murphy; Issued By: R.A. Kahrs. Published By: Greensboro, North Carolina. DACO: 6.4.
1078585	Atrazine Impregnation on Bulk Dry Fertilizer and Mixing Atrazine with Liquid Bulk Fertilizer: A Description of the Processes and Occupational Risk Assessment. DACO: 5.14-1.
1180080	Chapter 24 Volume 6: Disposition of Atrazine in Rhesus Monkey Following Oral Administration (Final Report, Amendment 1, Contributing Report To MRID 44152113). DACO: 4.5.12.
2945595, 2549387	Determination of the Pharmacokinetics of Atrazine After Single Oral or Intravenous Doses to Adult Female Cynomolgus Monkeys - Final Report. DACO: 4.8-34.
1234614	Dermal Absorption Of 14C-Atrazine By Rats, Advanced Product Chemistry, S.C. Williams, G.J. Marco, Study Finalized: May 16, 1983 (M7-101-24A;ABR-83005). Published By: Ciba-Geigy Corporation, Greensboro, North Carolina. DACO: 6.4.

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2572745	Agricultural Handler Exposure Scenario Monograph: Open Pour Mixing and Loading of Liquid Formulations. DACO: 5.3, 5.4.
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3299950	Hui et al, 2011. Pharmacokinetics of [^{14}C]-atrazine in Rhesus Monkeys, Single Dose Intravenous and Oral Administration – Toxicological and Environmental Chemistry, Volume 93, Number 2, Pages 370 to 382. DACO: 4.5.9.
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Additional information considered

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