Ana Gagneten ORCID iD: 0000-0001-6402-2389
Pedro Carriquiriborde ORCID iD: 0000-0002-3152-9340

Atrazine characterization: An update on uses, monitoring, effects, and environmental impact, for the development of regulatory policies in Argentina

TITLE PAGE

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Running head
Agricultural atrazine environmental impacts in Argentina

Authors and affiliations:

Ana M. Gagneten[a]*, Luciana Regaldo[a,b], Pedro Carriquiriborde[c,b], Ulises Reno[a,b], Silvina V. Kergaravat[a,b], Mariana Butinof[d], Hernan Agostini[e], Melina Alvarez[e], Agustin Harte[e]

[a] Laboratorio de Ecotoxicología. Facultad de Humanidades y Ciencias. Universidad Nacional del Litoral. Ciudad Universitaria – Paraje El Pozo s/n (3000), Santa Fe, Argentina.

[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Argentina.

[c] Centro de Investigaciones del Medioambiente, Facultad de Ciencias Exactas, Universidad Nacional de la Plata-CONICET. Argentina.

[d] Facultad de Ciencias Médicas, Universidad Nacional de Córdoba. Argentina.

[e] Dirección Nacional de Sustancias y Productos Químicos, Secretaría de Control y Monitoreo Ambiental, Ministerio de Ambiente y Desarrollo Sostenible de la Nación (MAyDS). Argentina.

* Address correspondence to: Ana Maria Gagneten. amgagneten@gmail.com
ORCID. 0000-0001-6402-2389.

In accordance with the SETAC Code of Ethics, we confirm that

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AUTHOR CONTRIBUTION STATEMENT

A.M. Gagneten: Conceptualization; Methodology; Investigation; Formal analysis; Writing—original draft; Writing—review & editing; Supervision. **L. Regaldo**: Conceptualization; Methodology; Investigation; Formal analysis; Writing—original draft; Writing—review & editing. **P. Carriquiriborde**: Conceptualization; Methodology; Investigation; Formal analysis; Writing—original draft; Writing—review & editing. **U. Reno**: Conceptualization; Investigation; Formal analysis; Writing—original draft; Writing—review & editing. **S.V. Kergaravat**: Conceptualization; Investigation; Formal analysis; Writing—original draft; Writing—review & editing. **M. Butinof**: Conceptualization; Investigation; Formal analysis; Writing—original draft; Writing— review & editing. **M. Alvarez**: Conceptualization; Investigation; Formal analysis; Writing—original draft; Writing—review & editing; Project administration. **H. Agostini**: Conceptualization; Investigation; Formal analysis; Writing—original draft; Writing—review & editing; Project administration. **A. Harte**: Conceptualization; Investigation; Formal analysis; Writing—original draft; Writing—review & editing; Project administration.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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ABSTRACT

Atrazine (ATZ) is the third most widely used herbicide in Argentina (10 thousand t/year), and is approved for sugar cane, flax, corn, sorghum, and tea. An assessment of the ATZ environmental impacts was conducted at the request of the Ministry of Environment and

Sustainable Development of Argentina. A review of 541 national and international technical and scientific reports and a survey among agricultural technicians, applicators, and producers were carried out. The survey showed that 94% of ATZ applications are terrestrial and use diversion exists, mainly associated with soybean cultivation. ATZ was reported at high frequencies (50-100%) in surface and groundwater, sediments, and soils, sometimes exceeding permitted limits. Several sublethal effects induced by ATZ on invertebrate and vertebrate species were found, sometimes at concentrations lower than those in water quality guidelines (<3 µg L^{-1}) or the environmental concentrations found in Argentina. Available epidemiological or human health studies on local populations are extremely scarce. This assessment also demonstrated that herbicides are ubiquitous in the environment. The investigation highlights the need for further studies assessing the adverse effects of ATZ on local species, ecosystems, and human health. Therefore, the precautionary principle is recommended to promote better application standards and product traceability to reduce volumes entering the environment and to avoid use deviation. In addition, this work concluded that there is a need for reviewing the toxicological classification, establishing buffer zones for atrazine application, introducing specific management guidelines, and expanding local studies on toxicity, ecotoxicity, and human epidemiology for environmental and health risk assessments. This study could also serve as a preliminary risk evaluation for establishing a final regulatory action and considering ATZ inclusion in Annex III of the Rotterdam Convention. Finally, the requirements to consider its inclusion in Annex A (Elimination) or B (Restriction) of the Stockholm Convention were evaluated and discussed, and information on the potential of long-range transport was the only criterion with no information to consider.

Key Points

ATZ is ubiquitous in soil, surface and groundwater, atmosphere, drain water, sediments and biota, and has been detected in the 50% to 100% of surface water samples, sometimes at concentrations higher than the permitted limits. The lack of concentration guideline levels for ATZ in different environment matrices (only available for surface water), and the scarce information about ATZ and metabolite concentrations in food, biota, superficial water, groundwater, and air is highlighted. There is an urgent need for information about herbicide use statistics, epidemiological data on exposure, risk and health impacts, and environmental risk assessment, aiming to help decision analysis and develop regulatory policies. The possible inclusion of the ATZ in Annex A (elimination) or Annex B (restriction) of the Stockholm Convention, and Annex III of the Rotterdam Convention is discussed.

KEY WORDS: Argentina monitoring; Atrazine environmental impact and effects; decision analysis; Stockholm Convention; Rotterdam Convention

INTRODUCTION

The expansion of urban areas and the increase in the demand for food have led to the intensification of monocultures that are developed from technological packages characterized by the use of direct sowing, an increase in the use of agrochemicals and the constant development of new products (herbicides, insecticides, and fungicides), new crops with high yield potential, transgenic crops, and agricultural machinery adapted to large-scale production (Grillo et al., 2021).

Due to the high demand for food, Argentina has the highest crop production per capita in the world (FAO, 2019; Araneda-Cabrera et al., 2021). Oilseeds and cereals are the crops that occupy the most quantity of surface; both cover 68.9% of the total agricultural surface (33.2 Mha), with direct sowing being the main form of production, with 26,894,937.6 ha. The surface area treated with herbicide was 9,705,095.3 ha in 2018 (INDEC, 2021). Argentina is the country with the second highest use of herbicides per hectare in its production systems with regard to the use of herbicides per arable area (Gagneten et al., 2021). It was reported that herbicide was the third most used compound in the country, with an estimated quantity of 62 million kg for the 2013-2014 campaign (Alonso et al., 2018). Additionally, Gagneten et al. (2021) documented that the import of ATZ (technical grade and formulated substance) was 155,598,348.60 kg, which is three times greater than its export (57,418,382.80 kg) for the 2008-2019 period.

ATZ is a selective systemic herbicide that enters target organisms mainly through the roots, translocating through the xylem with the transpiration current and accumulating in the apical meristems and leaves. Its mechanism of action consists of blocking the electron transport of photosystem II during the Hill reaction, causing inhibition of carbohydrate synthesis, accumulation of carbon dioxide inside the leaf, peroxidation of lipids in membranes, destruction of chlorophyll, and reduction of carbon stock, which also leads to stomatal damage and inhibition of transpiration (Marchi et al., 2008). According to the structure and physical–chemical properties (Tables 1 and 2 in the Supporting Information), ATZ is slightly soluble in water and has relatively low volatility. Concerning environmental fate (Table 3 in the Supporting Information), ATZ is moderately persistent in soil and water (Aparicio et al., 2015). In Canada, it has been reported to travel long distances in the atmosphere (Waite et al., 2005). Moreover, ATZ is leachable, taking into account the GUS index (groundwater ubiquity score) (WHO, 1993).

Regarding its toxicity, ATZ is a substance that may cause organ damage and skin sensitization after prolonged or repeated exposure and may generate allergic skin reactions (Supelco, 2020). Atrazine was classified as noncarcinogenic for humans according to the USEPA (United States Environmental Protection Agency), NTP (National Toxicology Program), and IARC (International Agency for Research on Cancer) in 2020. However, there is information about carcinogenic effects in animals but insufficient studies in humans (PubChem, 2020). On the other hand, according to Regulation No. 1272/2008 of the European Parliament and Council, ATZ is set as Category 1, meaning that it is very toxic to aquatic organisms with long-lasting effects (Supelco, 2020). Environmentally, the main metabolites of ATZ degradation are desethylatrazine (DEA) and desisopropyl atrazine (DEIA), generated by microbial degradation, and hydroxyatrazine (HyA), generated by chemical degradation (Saavedra, 2012). In humans, DEA, DEIA, and HyA are the main metabolites reported (Joo et al., 2010), while didealquilatedatrazine (DIDEA), diaminochlorotriazine (DACT), and mercapturic acid conjugate have been reported in human urine samples (Panuwet et al., 2008), semen (Swan et al., 2003) and amniotic liquid (Bradman et al., 2003). In mammals, the herbicide is metabolized mainly to the major metabolite DACT (Komsky-Elbaz et al., 2019). The persistence and toxicity of degradation products are not well understood. and there are even contradictory studies. The USEPA (2006) and World Health Organization (WHO, 2011) have indicated that the toxicity of ATZ metabolites is similar to that of the individual herbicide.

The present paper (see Graphical Abstract in the Supporting Information) aims to review the available information on commercialization volumes, legal and illegal uses, environmental fate, and potential impacts on the human and ecosystem health of ATZ in Argentina and countries in which ATZ is still used. An analysis of the current regulatory framework and its inclusion in Annex A (elimination) or Annex B (restriction) of the Stockholm Convention and in Annex III of the Rotterdam Convention is discussed. This issue aims to contribute to helping decision analysis and developing regulatory policies.

Environmental behavior of ATZ considering its sources and reservoirs

The environmental fate of pesticides is the result of complex interactions between their physicochemical characteristics and environmental conditions. Figure 1 summarizes the possible destinations of ATZ after its application. The processes that can convert a pesticide into a contaminant are surface runoff, leaching, volatilization, and drift transport.

Sources and pathways to the environment from the production chain

Agricultural applications are the main entry of pesticides into the environment. Although there are no precise data on application volumes in Argentina, ATZ sales volumes exceed 10,000 tons per year. Pesticides can also enter the environment from uncontrolled releases in the production chain or due to inefficient management of the production system. The uncontrolled disposal of empty containers aggravates the problem since they are accumulated, thrown into landfills, and/or incinerated. It is estimated that in Argentina, approximately 20 million empty containers (approximately 17,000 tons of plastic) are generated in each agricultural campaign (or year). Another potential source of pollution is the industrial production of pesticides and applications in urban areas.

Atrazine in environmental matrices of Argentina

Table 4 in the Supporting Information shows the information collected from an analysis of 22 studies documenting ATZ in different environmental matrices in Argentina (surface freshwater, sediments, soil, groundwater and drinking water, rainwater, biota, and atrazine residues in food), two of which include Uruguay. There is approximately three times more information on ATZ concentrations in aquatic ecosystems than in terrestrial ecosystems. Information is available only regarding the concentrations of ATZ metabolites (DEA, DEIA, and HyA) in surface water, sediments, and soils in Buenos Aires Province. Little national information is available about ATZ concentrations in aquatic and terrestrial biota, corresponding to the bioconcentration of ATZ in fish from the Buenos Aires, Entre Ríos, and Uruguay aquatic systems. Regarding ATZ concentrations in the air, there are no Argentinian records.

Atrazine in surface freshwater

Figure 2 shows a map of Argentina, indicating the provinces in which information on ATZ and its metabolite concentrations in surface freshwater was collected, the frequency of detection (FD%), and the maximum concentrations (MCs) recorded.

In *Buenos Aires* Province, De Geronimo et al. (2014) recorded the highest frequency of detection (FD 100%) and the highest residue level in Azul. Banda Noriega et al. (2018) detected pesticides in the surface waters of seven streams. The MCs (2.3 µg L^{-1}) were recorded by Pérez et al. (2017) and by Corcoran et al. (2020). Pérez et al. (2021) evaluated the ecological risk of 30 pesticides in the Tapalqué stream basin and recorded ATZ in 100% of the samples (Figure 2). In *Santa Fe* Province, Regaldo et al. (2018) recorded ATZ in 75%-100% of surface freshwater stream samples. In four peri-urban aquatic systems of San Justo city, 100% detection was obtained, although the values did not exceed the Canadian and Argentine Environmental Quality Guidelines (CEQGs: 2 µg L^{-1} and AEQGs: 3 µg L^{-1}, respectively) for aquatic life protection (Méndez et al., 2019). In addition, in seven lotic systems in the central-southern part of the province (MC: 44 µg L^{-1}), ATZ concentrations exceeded the CEQGs (Canadian Council of Ministers of the Environment, 2008) in 40% of the samples (Frau et al., 2021). Andrade et al. (2020; 2022) analyzed ATZ concentrations in surface freshwaters after rain events in three sub-basins of the central region of Santa Fe Province (FD 67%-100%) and MC from 0.2 to 2.5 µg L^{-1}. Vera Candioti et al. (2021) recorded ATZ in the surface water of eight sites in the southern part of the same province, with FD up to 57% and a MC of 28 µg L^{-1}. In *Córdoba* Province, Bonansea et al. (2013) recorded a MC of 0.434 µg L^{-1}, while Corcoran et al. (2020) reported that the mean values were lower (up to 0.121 µg L^{-1}) in the Suquía River. In *Entre Ríos* Province, Van Opstal et al. (2022) analyzed the surface freshwater quality of seven streams in the Estacas Basin where the predominant productive system is agricultural livestock, with livestock under native forest (MC: 86 µg L^{-1}- FD 82%). Atrazine was detected in 73 % of the water samples from the Gualeguay Basin (Argentina) and was the most frequently detected pesticide, along with glyphosate and AMPA. Insecticide concentrations in water were above the recommended guidelines (Mac Loughlin et al. 2022). In *Tucumán* Province, De Geronimo et al. (2014) studied the impact of 29 pesticides on the quality of surface freshwater in the Mista Stream sub-basin. ATZ was the most frequently detected pesticide (FD 40%). At the same time, Portocarrero et al. (2016) studied concentrations of ATZ in surface freshwater of the Mista and Saladillo streams (FD 100%). As seen, as in the Pampa region, on the Tucuman depressed plain, a very high percentage of the surface freshwater samples presented concentrations of atrazine above the detection limit (LOD). In *Misiones* Province, De Gerónimo et al. (2014) studied the surface freshwater quality of the San Vicente microbasin, where ATZ was the most frequently detected pesticide (FD 80%), although the concentrations were below the limit of quantification (LOQ).

Upon comparison of the pesticide profiles of extensive- and horticultural-production systems in the country, atrazine can differentiate extensive agriculture from horticulture, emerging as a prime candidate to be used as a tracer of extensive agricultural contamination in the environment (Mac Loughlin et al. 2022).

Finally, it can be inferred that the most impacted surface freshwater is located in Santa Fe and Entre Ríos provinces. The MC of ATZ reached 44 μg L^{-1} and 86 μg L^{-1}, respectively, exceeding between 22 and 43 times the CEQG for the protection of aquatic life (Frau et al., 2021; Van Opstal et al., 2022).

Atrazine in sediments

There is less information available about the ATZ concentrations in sediments in Argentina. There are some records in the Crespo stream (Balcarce, Buenos Aires), where it was detected in 10% of the samples (MC: 2 μg g^{-1}) (Pérez et al., 2017); in the Carnaval stream (La Plata, Buenos Aires), detected in 80% of the samples (MC 32.7 μg g^{-1}) (Mac Loughlin et al., 2017); in the Suquía River (Córdoba), where the levels evaluated were below the LOD (Bonansea, 2015); and in the Tapalqué stream basin (Buenos Aires) (FD 33%), with a mean concentration of 1 μg g^{-1}. The HyA metabolite was detected in 100% of the samples (MC: 17.3 μg g^{-1}) (Pérez et al., 2021).

Atrazine in soil

ATZ was analyzed in different soils in a study carried out in Santa Fe, Córdoba, and Buenos Aires provinces between 2012 and 2014. The MCs were 7 μg kg^{-1} (FD 25%), 66 μg kg^{-1} (FD 47%) and 17 μg kg^{-1} (FD 23%), respectively (Alonso et al., 2018). In Buenos Aires, Pérez et al. (2021) found traces of ATZ at detectable, but not quantifiable, levels (LOD = 0.1 μg kg^{-1} and LOQ = 0.3 μg kg^{-1}) (FD 33%). In addition, the metabolites HyA (MC: 9.70 μg kg^{-1} - FD 100%), DEA and DEIA were found. In the same province, ATZ was measured at different depths (5-100 cm) in agricultural soils subjected to crop rotation (wheat–soybean–corn) and another to monoculture (soybean) (Caprile et al., 2019). In both types of systems, ATZ MCs were detected in the first 20 cm of soil depth. In the rotation system, the concentrations down to 5 and 20 cm soil depths were 2.92 and 2.88 µg kg^{-1}, and for the monoculture system, they were 1.90 and 2.28 µg kg^{-1}, respectively. In both systems, ATZ was found in all strata analyzed down to 100 cm soil depth. The findings of previous studies are available in the Addendum (Supporting Information, *Atrazine in soil*).

Atrazine in groundwater and in drinking water

In the Pampean region (Argentina), Banda Noriega et al. (2018) reported no detection of ATZ in 15 samples collected from wells in rural establishments. In contrast, Portocarrero et al. (2016) revealed ATZ concentrations in groundwater in the sugarcane sector of the province of Tucumán (FD 77% - MC: 0.5 µg L^{-1}). In southern Santa Fe Province, a recent study revealed the presence of ATZ in the groundwater of eight sites, with FD ranging from 29% to 75% and MC from 5.2 to 22.8 µg L^{-1} (Vera Candioti et al., 2021). Regarding drinking water, only one very recent study was found in Argentina (Mas et al., 2020) that measured pesticides in water from dams and cisterns for human consumption. The MCs of ATZ were 7.92 µg L^{-1} in cisterns and 0.8 µg L^{-1} in groundwater. There are no published studies that have directly measured the presence of the herbicide in tap water. For international information, please see the Addendum: *Atrazine in drinking water.* Considering the ubiquity and levels of ATZ detection in Argentina (Table 4, in the Supporting Information), it is possible that in regions where soils are highly permeable and poor in organic matter, sprayed pesticides find fewer obstacles in filtering down to the lower layers; in addition, the concentration of ATZ in groundwater could increase in periods of maximum applications and rainfall.

Atrazine in biota

Studies of ATZ accumulation in the native biota of Argentina are extremely scarce and are limited to different fish species. The MC of ATZ in fish was 895.4 µg kg^{-1} wet weight (w.w.)

and average values were 72 ± 120 µg kg^{-1} w.w. Brodeur et al. (2017) measured ATZ in *Jenynsia multidentata* collected at three sites in the Pergamino stream (Buenos Aires). The FD was very low (FD 0.9%), although the recorded concentration was 574.1 µg kg^{-1} w.w. In Entre Ríos Province, the ATZ concentration was evaluated in pacú (*Piaractus mesopotámicus*) raised in aquatic systems that receive water from agricultural soils by runoff and in aquaculture ponds that receive water from the Paraná River (Brodeur et al., 2021). In the first case, ATZ was detected in 100% of the samples, with concentrations between 70.4 and 105.6 µg kg^{-1} w.w. (86 ± 12 µg kg^{-1} w.w.). At the fish farming station, the FD was 30%, with concentrations of 16.9, 25.3, and 4.6 µg kg^{-1} w.w. (22 ± 5 µg kg^{-1} w.w.). Goncalves et al. (2020) measured ATZ in *Astyanax jacuhiensis* muscles from two sites in the Uruguay River. ATZ was detected at all sites and seasons, except for the autumn sampling at one of the sites (mean C: 0.24 ± 0.07 µg kg^{-1} w.w. and MC: 0.35 µg kg^{-1} w.w.). Another study analyzed pesticides in fish caught in Uruguay (mean C.: 1.6 µg kg^{-1} w.w. - FD: 16.1%) (Ernst et al., 2018). Surveys of pesticides in fish carried out by the Administrative Commission of the Uruguay River between 2012 and 2013, published in 2014, and between 2015 and 2016 (CARU, 2017) yielded different results. The MC of ATZ recorded in the first period was 0.6 µg kg^{-1} w.w. (FD 100%), while in the second period (FD 98% and 91% for 2015 and 2016, respectively), the MCs were much higher (in 2015: 895.4 µg kg^{-1} w.w. and in 2016: 864.5 µg kg^{-1} w.w.). No studies were found for Argentina that had measured ATZ in other groups of organisms. For findings at the international level, please see the Addendum: *Atrazine in biota.*

From the surveyed studies, it can be concluded that although the values have a great dispersion, the levels of accumulation in fish from some sites in Argentina are among the maximum reported worldwide in the aquatic biota. No studies were found about the accumulation of ATZ in terrestrial organisms exposed to field conditions under conventional use of the herbicide. This reveals an important information gap regarding the levels of accumulation in terrestrial organisms.

Atrazine residues in food

In Argentina, food control is carried out by the National Service of Food Health and Quality (SENASA) and the National Food Institute (INAL). SENASA establishes the tolerance or maximum residue limit (MRL) of pesticides in agricultural products and byproducts. Those products for which a MRL of the active ingredient has not been established must comply with the MRL approved by the *Codex Alimentarius*. If there is no MRL approved by the *Codex Alimentarius*, a default value of 0.01 mg kg^{-1} is established. For ATZ, the regulations only establish a MRL of 0.01 mg kg^{-1} for soybeans (consumption grain); 0.1 mg kg^{-1} for tea; 0.25 mg kg^{-1} for corn, sweet corn, and sorghum (grain for consumption) and sugar cane (fresh stem); and 15 mg kg^{-1} for corn and sorghum (forage) (SENASA, 2016). However, international organizations such as the European Food Safety Authority (EFSA) have recommended, according to the acceptable daily intake (ADI = 0.005 mg kg^{-1} of body weight) and the acute reference dose (ARfD = 0.025 mg kg^{-1}), reducing the MRL for cereals from 0.1 mg kg^{-1} to 0.05 mg kg^{-1} (an order of magnitude lower).

In Argentina, studies evaluating the concentration of ATZ in edible vegetables and/or animals are very scarce. In a study carried out in Buenos Aires Province (Mac Loughlin et al., 2018), ATZ was detected only in oranges at concentrations between 0.035 and 0.064 mg kg^{-1} w.w. In a similar study conducted in Canada, ATZ residues were detected only in lettuce (FD 33%) at concentrations between 0.00025 and 0.0075 mg kg^{-1} w.w. (Montiel-León et al., 2019a); that is, between one and two orders of magnitude lower than the concentrations detected in Argentina. The values found in Argentina exceed the 0.01 mg kg^{-1} established as permitted MRL in agricultural products not included in Annex I of the SENASA Resolution. These studies demonstrate that ATZ can be detected in animal products, although at concentrations well below the MRL.

No independent studies that evaluate ATZ residues in bovine meat have been found in Argentina. Values from other countries are available in the Addendum: *Atrazine residues in food.* ATZ residues in the muscle of freshwater fish of interest for human consumption have been evaluated by CARU in *Prochilodus lineatus* (sábalo) captured in the Uruguay River; the MC of ATZ was 0.123 mg kg^{-1} w.w. in 2015 and 0.864 mg kg^{-1} w.w. in 2016 (CARU, 2017). These values exceed the MRL established by SENASA.

According to this survey, it is evident that ATZ is detectable in food and water for human consumption, sometimes exceeding the MRL. On the other hand, it is also clear that the information available on food residues in Argentina is deficient; in general, it is not publicly accessible, and a greater number of pertinent studies should be carried out to enhance the food safety of the population.

Atrazine in rainwater

A study developed by Alonso et al. (2018) is the only one published to date addressing ATZ concentrations in wet precipitation (rain) in Argentina. The authors revealed that almost 20–30% of the dose sprayed on the fields does not reach the target field due to airborne drift and reported that ATZ was found in 80% of the rainwater samples on the Argentine pampa, at median-to-maximum concentrations of 0.22 to 26.9 $\mu g \cdot L^{-1}$. Conversely, many studies have been developed worldwide evaluating ATZ concentrations in the atmosphere in wet and dry precipitation and the gas phase (see international information in the Addendum: *Atrazine in* *air*). In summary, there is a lack of information on the ATZ concentrations in the air and wet deposition in Argentina and Latin America. The concentrations reported in rainwater in the only work available were comparable to those reported for other regions of the world.

Effects of ATZ on microalgae, aquatic and terrestrial invertebrates, alone and in mixture with other pesticides

Atrazine has been reported to be slightly to highly toxic to aquatic organisms (Sparling, 2016). Recently, Smith et al. (2021) affirmed that invertebrates in general are not highly sensitive to ATZ. However, a review of the literature provides abundant evidence that ATZ can affect the apical endpoints (survival, growth, development, and reproduction) of many invertebrate species present in aquatic and terrestrial environments at different concentrations, including environmentally relevant concentrations. Field studies are extremely scarce, and at the laboratory scale, the most studied groups are microalgae, cladocerans, copepods, freshwater shrimps, crabs, amphipods, chironomids, and mollusks. Among terrestrial invertebrates, studied groups include soil worms and insects. Of the total publications analyzed on the effect of ATZ on microalgae and terrestrial and aquatic invertebrates, 32% correspond to the United States, 16% to Brazil, only 6.8% to Argentina, and the rest to other countries.

Regarding microalgae, ATZ affects the growth and morphology of the cells and alters the electron transport chain of photosystem II, thus inhibiting photosynthesis, increasing intracellular calcium levels, and altering different biochemical markers. Among microcrustaceans, the main effects are an increase in antioxidant activities, embryonic and adult abnormalities, and an increase in the number of nonviable offspring. In an Argentine study, environmentally relevant concentrations (240 to 1,920 μg L^{-1} ATZ) produced subchronic effects, which have not been frequently investigated: through direct action, ATZ diminished survival and reduced the escape ability both in nauplii larvae and adult copepods of *Mesocyclops longisetus* via indirect action (Gutiérrez et al., 2013). In Argentinian studies carried out with the crab *Cherax quadricarinatus*, ATZ increased the proportion of females and unbalanced the endocrine system (Mac Loughlin et al., 2016) and decreased glycogen in the muscle and increased vitellogenin in the hepatopancreas of *Neohelice granulate* (Silveyra et al., 2017). Studies on the effects of ATZ on **terrestrial invertebrates** are extremely scarce. Recently, a review indicated that pesticides of all types pose a clear hazard to soil invertebrates. Negative effects are evident in both laboratory and field studies and in a wide variety of soil organisms and endpoints. ATZ and nano-ATZ formulations were tested on the annelid *Enchytraeus crypticus*; while all ATZ formulations affected hatching success, nano-ATZ did not produce avoidance (Gomes et al., 2019).

According to the national and international reviewed studies, several adverse effects of ATZ exposure alone or mixed with other pesticides on aquatic and terrestrial invertebrates at environmentally relevant concentrations have been reported in Argentina and other countries, including DNA damage, mortality, oxidative stress, and growth reduction. However, the majority of these studies utilized acute laboratory exposure, whereas in a real ecosystem, organisms are more likely to experience chronic exposure conditions to ATZ and life cycle trait and population- and community-level impairments. Surveys carried out in other countries are shown in the Addendum. Table 5 in the Supporting Information summarizes Argentinian and international surveys considering ATZ concentrations (alone and in mixture with other pesticides), time of exposure, taxa, species, and main effects on microalgae and aquatic and terrestrial invertebrates.

Adverse effects of atrazine on vertebrates (see *Addendum*)

Atrazine effects on native species

Several studies on native fish and amphibian species of Argentina are available in the literature. Atrazine acute lethal concentrations (96 h-LC_{50}) for the fish *Odontesthes bonariensis, Rhamdia quelen,* and *Piaractus mesopotamicus* were 5.2, 10.2, and 28.6 mg L^{-1}, respectively (Kreutz et al., 2008; López Aca et al., 2014; de Paiva et al., 2017). These values were among the sensitivities described for the Nearctic species included in a study by Huber (1993). In amphibians, the 96 h-LC_{50} estimated for *Rhinella arenarum* exposed to the active ingredient was 27.2 mg L^{-1} (Brodeur et al., 2009) and 19.7 and 36.4 mg L^{-1} for *Physalaemus cuvieri* and *Dendropsophus minutus*, respectively, exposed to commercial formulations (Gonçalves et al., 2017). Sublethal effects were reported for native fish. Biochemical and histological changes were induced by the herbicide in the gill of *Prochilodus lineatus* after 14 d of exposure to 10 µg L^{-1} and 25 µg L^{-1}, respectively (Paulino et al., 2012). General inhibition of the enzymes EROD, GST, SOD, CAT, GPx, and LPO in the liver, together with induction of DNA damage in the liver and blood, was observed in the same species exposed for 24 and 48 h to 2 and 10 µg L^{-1} (Santos and Martinez, 2012). In a similar study, several biochemical parameters in gill, liver, and blood were altered, and DNA damage was induced in the erythrocytes of *P. lineatus* exposed to 2 and 20 µg L^{-1} for 24 and 96 h (de Andrade et al., 2019). In the fish *R. quelen*, an increase in plasmatic cortisol levels was observed after 96 h of exposure to 16.6%, 33.3%, and 50% of the 96 h-LC_{50} (Cericato et al., 2008), but no differences in such a response with respect to controls were found when fish were challenged with adrenocorticotropic hormone (ACTH) (Cericato et al., 2009). Suppression of the innate immune response was found in *R. quelen* exposed to 10% of the 96 h-LC50 (Kreutz et al., 2012). Additionally, stimulation of serum antibody levels was found when fish were challenged against inactivated *Aeromonas hydrophila* (Kreutz et al., 2014). In other studies, *R. quelen* was exposed for 96 h to concentrations ranging from 2 to 100 µg L^{-1}, and changes in biochemical, physiological, and histological biomarkers were induced by the herbicide, but different responses were observed among different studies (Mela et al., 2013; Persch et al., 2018; Persch et al., 2017). In the fish *P. mesopotamicus* exposed to concentrations close to the LC50, hepatic and renal ultrastructural histological changes were observed, but no genotoxic effects were detected by the micronucleus test. In another study, fish were exposed for 30 d to 3.57 mg L^{-1}, and similar hepatic and renal ultrastructural histological alterations were found, but with an increase in the frequency of micronuclei (Delcorso et al., 2020). In the fish *Astyanax altiparanae*, oxidative stress, histological alterations, and dysregulation of thyroid hormones, but not of sexual hormones or cortisol alterations, were observed in fish subchronically exposed to ATZ concentrations ranging from 0.5 to 10 µg L^{-1} (Destro et al., 2021).

In native amphibians, sublethal nonmonotonic effects on growth and development were observed in tadpoles exposed to ATZ concentrations between 0.1 and 1000 µg L^{-1} (Brodeur et al., 2013; Brodeur et al., 2009). Teratogenic and neurotoxic effects were observed in *R. arenarum* embryos exposed for 24 h to 15 and 20 mg L^{-1}, respectively (Svartz et al., 2012). In tadpoles of *P. cuvieri*, NOEC and LOEC values of 24 and 48 mg L^{-1}, respectively, were reported for natatorium behavior (Wrubleswski et al., 2018). Sublethal effects were studied on the native reptile *Caiman latirostris*. Higher follicle development and lower testosterone levels were found in females born from eggs topically exposed to 200 µg L^{-1} ATZ and incubated at a female-forming temperature (Stoker et al., 2008). No studies were found assessing the potential adverse effects of ATZ on native bird species.

According to the national and international reviewed studies, it is possible to state that ATZ can induce adverse effects across all classes of vertebrates. Lethal effects were far from environmentally relevant concentrations, while sublethal responses were usually described at concentrations on the ppb order (i.e., µg L^{-1}), except for birds in which they were at ppm (i.e., mg kg^{-1}), usually found in agricultural ecosystems. The responses to nonspecific biological parameters (i.e., detoxifying and antioxidant enzymes, DNA damage, etc.) were usually unpredictable, but responses to gonad development, sex ratio, and amphibian metamorphosis were consistent and specific. For native species of Argentina, the assessed biological responses were usually linked to nonspecific responses, except for frog metamorphosis. However, it has generally been observed that the sensitivity was similar to that reported for Nearctic species.

Adverse effects of ATZ mixtures on vertebrates **(***see Addendum*).

The toxicity of mixtures of ATZ with other pesticides has been assessed since the 1990s. From the published studies, it is possible to state that the effects of ATZ mixtures with herbicides, insecticides, or other substances are complex and hard to predict. It should be assessed case by case.

Effects on human health associated with exposure to ATZ alone and in mixture with other pesticides

In Argentina, intoxication from pesticides is among the compulsorily notifiable diseases included in the National Health Monitoring System (SNVS); nevertheless, the information available on official sites reveals serious data gaps, a situation that has already been reported (Butinof et al., 2015; Franchini et al., 2016). Investigations on exposure to ATZ and the effects on health produced in the country have revealed chronic exposure to multiple pesticides used for agricultural purposes among populations of farmworkers and residents dwelling near cropland. Diaz et al. (2015) designed exposure intensity indices and others for accumulated exposure applied to a national scale, later used by Butinof et al. (2017), to assess labor exposure among workers who apply pesticides, identifying symptoms, self-reported tracer diseases, levels of plasma cholinesterase and biomarkers of genotoxicity associated with different levels of toxicity. Peralta et al. (2011) and Bernardi et al. (2015) studied genotoxicity biomarkers in communities adjacent to sprinkled fields; the simultaneous inquiry into multiple exposures has not made it possible to conclusively attribute the effects found to ATZ.

The international literature made it possible to identify several different effects (see Figure 3 and the Addendum), grouped by type of damage and the population affected. The reviewed effects include congenital malformations, carcinogenicity, endocrine disruption-hyperthyroidism, association between early menarche and in utero exposure, increase in weight, Parkinson’s disease, rheumatoid arthritis, and terminal kidney disease. Table 6 in the Supporting Information summarizes the impacts of ATZ on human health according to the literature reviewed.

Ecological Risk Assessment for ATZ

In Latin America, only risk assessments on aquatic ecosystems have been published for pesticides in general. One assessment conducted in Brazil showed that ATZ was one of the two more frequent pesticides in rural rivers (Severo et al., 2020). Two other assessments were performed within the Pampa Region of Argentina, and they found that the cumulative risk quotient for total pesticides usually exceeded the unit, and ATZ and its metabolites were among the pesticides that contributed most to the quotient (Iturburu et al., 2019; Pérez et al., 2021). From the governmental sector, in Argentina, a freshwater quality guideline for ATZ of 3 µg L^{-1} was developed by the Subsecretaría de Recursos Hídricos based on final chronic values established from micro- and mesocosm studies (SRHN, 2003). Similarly, a freshwater quality guideline of 1.8 µg L^{-1} was developed by Environmental Canada based on acute and chronic toxicity data using a safety factor of 0.1 (EC, 1999). A different approach was used in Europe, and ATZ was not included in Annex I of Directive 91/414/EEC because monitoring data were insufficient to demonstrate that concentrations of the herbicide and its metabolites will not exceed 0.1 µg L^{-1} in groundwater. The ecological risk assessment (ERA) was the tool selected by the USEPA for evaluating the potential impact of ATZ on the environment. A refined ERA conducted in 2016 concluded that in intensive agriculture areas, ATZ impacts the aquatic environment by affecting aquatic plant communities and posing a chronic risk for fish, amphibians, and invertebrates (Farruggia et al., 2016). Additionally, an ecological risk was identified for terrestrial mammal, bird, reptile, and plant communities. In particular, USEPA levels of concern for chronic risk exceeded between 22 and 198 times for vertebrates. Finally, the study stated that concentrations higher than 5 µg L^{-1} for several weeks will cause adverse reproductive effects in fish, and concentrations above 3.4 µg L^{-1} will impact primary production and aquatic plant community structure and function. In 2019, the USEPA used the results of the above-mentioned ERA to state an atrazine-proposed Interim Registration Review Decision, including actions for terrestrial and aquatic organisms such as spray drift reduction measurements for aerial and terrestrial applications and a stewardship program for run-off and spray drift (USEPA, 2019).

According to the mentioned ERA and the cited studies (also see Addendum: Ecological Risk Assessment for ATZ), it is possible to say that in intensive agriculture districts of Argentina and other Latin American countries where atrazine is still used, it is expected that ATZ poses an ecological risk, and therefore actions should be promoted by the government to ban or reduce its use and avoid potential adverse impacts.

In Argentina, regulations of pesticides for agricultural uses are on behalf of the SENASA (Servicio Nacional de Sanidad y Calidad Agroalimentaria). In 2012, a workshop organized by SETAC in Buenos Aires congregated members from academia, government, and industry to discuss ERA frameworks that can be implemented into the regulation of pesticides in Latin America (Carriquiriborde et al., 2014). Despite workshop conclusions showing that the international ERA framework would suit Latin America, it still has not been incorporated into SENASA regulation. Additionally, the workshop remarked on the need to consider regional particularities regarding species sensitivity, environmental conditions, agricultural practices, regulations enforcement, and human resource capabilities to conduct them. Consequently, the general recommendation for Argentina to adopt the ERA framework in the registration process of agricultural pesticides is renewed. A high-tier analysis would be more appropriate, considering the prolonged use of the herbicide in the country and the number of currently available studies assessing the environmental concentrations and adverse outcomes on local species. The probabilistic analysis, combining local data on exposure and effects with those from other worldwide agricultural regions, could be eligible for a quantitative risk assessment.

Current regulatory frameworks at the national and international level

The current Secretariat of Infrastructure and Water Policy (SIPH) established in 2003 the Water Quality Standard for drinking water (WQDW) for ATZ with a value ≤ 1.5 µg L^{-1} for surface or underground sources with conventional treatment or groundwater sources without treatment or with another disinfection technique. For this, the maximum tolerable intake established by the WHO of 0.5 μg kg (body mass) d^{-1} was considered. This value was obtained according to the following formula: WQDW ≤ TDI * BM * F/C. For that calculation, a body mass (BM) equal to 60 kg, a water consumption per person (C) equal to 2 L d^{-1}, and a tolerable daily intake (TDI) allocation factor for drinking water (F) equal to 0.1 were assumed (WHO, 1995). In our country, there is no other reference value for ATZ in any regional or national regulations. Regarding the tolerable daily intakes reported by the USEPA and the WHO, the values informed are equal to 35 μg kg^{-1} (body mass) d^{-1} and **0**.5 μg kg^{-1} (body mass) d^{-1}, considering a no observed adverse effect level (NOAEL) = 35 mg kg (body mass) d^{-1} and 0.5 mg kg (body mass) d^{-1}, respectively. In Canada, the maximum acceptable concentration for ATZ and its metabolites in drinking water is 5 μg L^{-1}, and the theoretical maximum daily intake of ATZ is estimated at 0.3 μg kg (body mass) d^{-1} for a Canadian adult (CCME, 1993). The SIPH established a chronic final value (FCV) for the protection of aquatic biota in unfiltered surface freshwater of ≤ 3 μg L^{-1}. This value is higher than that established by Canada, ≤ 2 μg L^{-1} (CCME, 2008), and lower than that established by Australia and New Zealand (fresh and seawater): ≤ 13 μg L^{-1} (Anzecc and Armcanz, 2000). There is no international reference level for ATZ in sediments. The maximum acceptable concentrations for ATZ in irrigation water are defined by the lowest concentrations calculated for the three irrigation scenarios considered: 0.13 μg L^{-1}, for Tr (annual effective irrigation rate) = 3500 m^3 ha^{-1}, 0.07 μg L^{-1}, for Tr = 7500 m^3 ha^{-1}, 0.04 μg L^{-1}, for Tr = 12000 m^3 ha^{-1}, and for Tr = 12000 m^3 ha^{-1}. At the international level, the Canadian and USEPA water quality guidelines for ATZ for the protection of agricultural uses mention a quality standard of 10 μg L^{-1} for irrigation water and 5 μg L^{-1} for livestock water (USEPA, 1977; CCME, 1989).

Control and restriction Multilateral Agreements

The Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides (entry into force: February 2004) was approved in Argentina by Law No. 25,278 in 2004. The import and export of chemicals included in the Rotterdam Convention are regulated by Resolution No. 110/2021. To date, the parties that have submitted a Final Regulatory Action (FRA) on ATZ are the EU (2005); the Sahel countries: Cape Verde, Chad, Gambia, Mauritania, Niger, Senegal, and Togo (2015); Uruguay (2018); and Turkey (2020). Thus far, no progress has been made on a definition of the status of ATZ and its possible inclusion in Annex III to the Convention. See Figure 4 for the chronological details of the presentations and decisions.

Regarding the Stockholm Convention on Persistent Organic Pollutants (POPs), we consider that this work has evidenced and demonstrated the fulfillment of several of the criteria established in Annex D, in particular: chemical identity, persistence, bioaccumulation and adverse effects. Further information on the potential for long-range environmental transport could be complemented to consider ATZ as a potential persistent organic pollutant.

States of use and prohibition in the world

ATZ is prohibited in the following EU countries: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain and Sweden (EU, 2004). In Australia, the industrial use of ATZ, as well as in-home gardens and commercial and nonagricultural lawns, is not allowed. In Africa, the Interstate Standing Committee for Drought Control in the Sahel (CILSS) decided to ban ATZ-based pesticides in 2015. No restrictions or bans have been found on the use of ATZ in Asia. In North America, ATZ is allowed in Canada, while in the U.S., the EPA has proposed risk mitigation by additional restrictions (USEPA, 2019). These restrictions are expected to reduce the risk of exposure of both listed and unlisted endangered species, whose habitats or distribution co-occur with the use of ATZ (Environmental Protection Agency (2020) Docket Number EPA-HQ-OPP-2013-0266. www.regulations.gov). For Latin America and the Caribbean, the application of ATZ is allowed with some restrictions, except for Uruguay, which has prohibited the import, registration, and renewal of ATZ-based medical devices (Resolution No. 104/016) (Figure 4). In 2020, the tariff reduction for one of the compounds used for the manufacture of ATZ, monoisopropylamine (MERCOSUR/CCM/DIR. No. 70/19), was approved in Argentina within the framework of the Resolution of the Common Market Group (CMG No. 08/08). These types of measures could cause a decrease in the production costs of ATZ, which would possibly cause an increase in the volumes applied.

Lessons and recommendations

Based on the review of ATZ, we recommend the following:

Regarding the entry of ATZ in the environment:

-Implement instruments and management actions to reduce the volumes of herbicide that enter the environment by reducing the annual sales volume; promote ATZ management alternatives tending to implement a lower level of use and/or its substitution (e.g., crop or product rotation); encourage training activities for health agents, agricultural producers and applicators; and stimulate education and responsible participation of the population.

Related to risks to the environment and human health:

-Increase monitoring and biomonitoring of ATZ and toxicity and ecotoxicity studies in the region; review its toxicological classification; establish strict management guidelines until its prohibition or use restrictions are decided; increase epidemiological g studies of ATZ in rural, urban and peri-urban areas including in the general population, in addition to the occupationally exposed population, given the several sources of potential environmental exposure identified in this study; and carry out an ecological and human health risk assessment for ATZ.

Based on the lack of data:

- Create a self-sufficient scientific-technical body for systematic data collection, sustained over time, at strategically defined points throughout the country and with public access, and create a network of national and regional reference institutions and laboratories for ATZ and other pesticide analyses, thus generating an updated network database, geo-referenced and available online. In particular, we highlight the relevance of strengthening the National Program for the Prevention and Control of Poisoning by ATZ and other pesticides. Promote international collaborative research networks (problem identification according to local contexts, data collection, analysis and dissemination), taking into account different national capacities, to strengthen the existing evidence on the impacts of this herbicide that is still used in many countries.

-Promote studies of ATZ concentrations in air and groundwater and its environmental behavior in the atmosphere and aquatic systems; manage and make available the data recorded in Argentina on the resistance of weeds to triazine; study the effects of ATZ in native species (bioconcentration, bioaccumulation, and biomagnification); study bioremediation technology for ATZ degradation; and analyze possible substitute molecules.

Regulations and control:

-Review and update national water quality guide levels for ATZ and generate guide levels for other matrices; promote compliance with Law 27,279/16 on the sound management of empty phytosanitary containers; promote public policies to establish strips of nonapplication of ATZ in urban and peri-urban areas and avoid deviations of ATZ use; and promote the establishment of a national law of minimum environmental estandards on the management of agrochemicals throughout the Argentine territory.

Finally, the authors analyzed Annex D of Law No. 26,011/05 and indicated the information – and the information gaps – available to discuss whether ATZ complies with the requirements and selection criteria to evaluate its possible inclusion in Annex A (elimination) or Annex B (restriction) of the Stockholm Convention. Further information on the potential for long-range transport is needed to consider its inclusion under this Convention.

Conclusions

ATZ is the third most used herbicide in our country, with more than 10 thousand tons per year that have entered and been distributed in different environmental compartments over several decades. Some deviations from permitted herbicide use were found, such as fallow land and soybean, potato, cotton, wheat, and sunflower crops. From the review on ATZ use and effects in Argentina and other countries, it can be concluded that it is moderately persistent and mobile in the atmosphere and in soils and leachable, which represents a contamination risk to groundwater. Additionally, it is a pseudopersistent pollutant in epicontinental waters because it exceeds the natural purification capacity of the environment. Consequently, ATZ has been detected in 50% to 100% of samples from Argentine basins; some of them had concentrations higher than the permitted limits. Concerning the effects, both ATZ and its metabolites can induce adverse effects on the biota: sublethal effects on many invertebrate and vertebrate species can affect development, growth, and reproduction at concentrations lower than the national guideline level established for aquatic biota protection (3 μg L^{-1}). There is enough evidence demonstrating a high possibility that environmental exposure levels can exceed the effect levels in aquatic and terrestrial media according to the evaluation of the ecological risk by the EPA. In Argentina, it is important to highlight the lack of concentration guide levels for ATZ in different environmental matrices (guiding levels only exist in water) and of ATZ and metabolite concentrations in food, biota, superficial water, groundwater, and air.

In addition, there is no information about herbicide-use statistics, epidemiological data on exposure, risk and health impacts, and environmental risk assessment that would permit decision analysis toward regulatory policies. Nevertheless, the abundant information on exposure and risks of ATZ contained in this report could serve as a good basis for deciding, based on an evaluation of ecological and human health risks, on a final regulatory action that could trigger the inclusion of ATZ in Annex III of the Rotterdam Convention.

List of captions

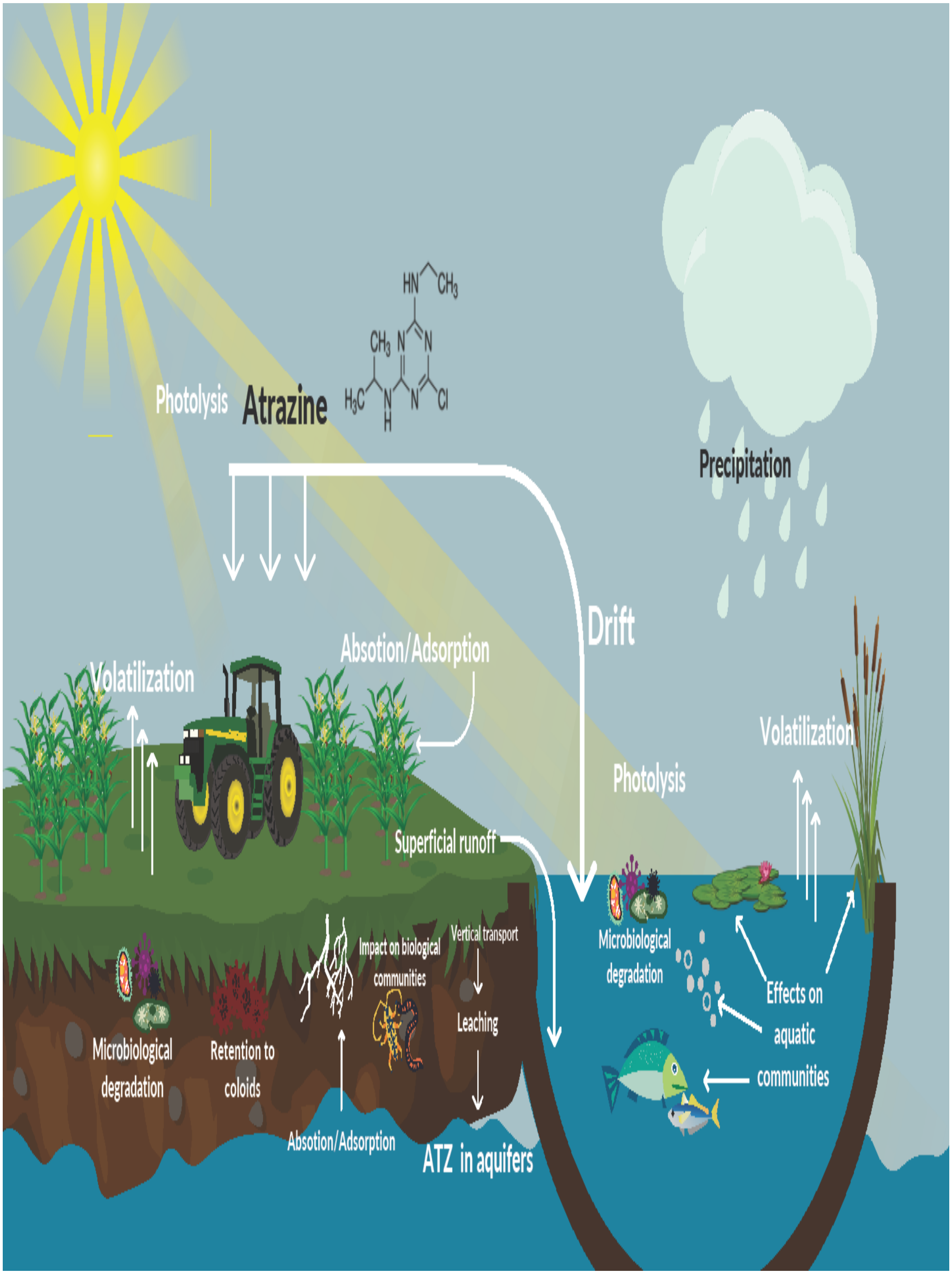

Figure 1: Routes of atrazine in the environment.

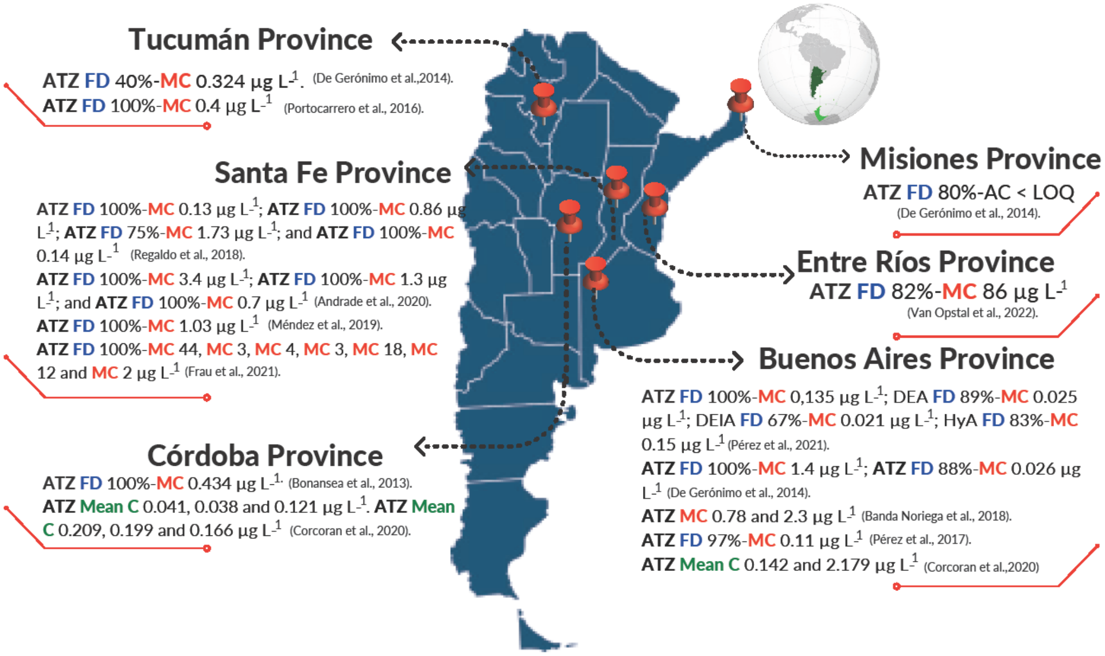

Figure 2: ATZ and metabolites (DEA, DEIA, and HyA) concentrations registered in surface freshwater of Argentine provinces. Frequency of Detection (FD%); Maximum Concentration (MC); Limits of Quantification (LOQ) Atrazine Concentration (AC); Mean Concentration (MeanC).

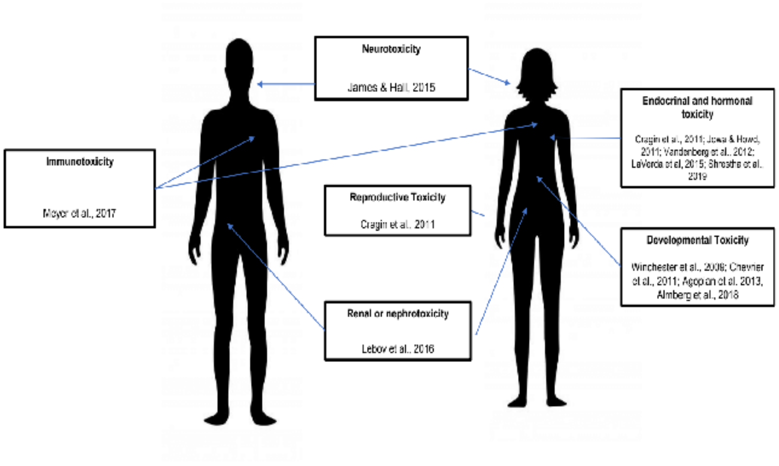

Figure 3: Effects on human health associated with exposure to atrazine, reported in the literature. Note: Studies that have not analyzed multiple exposures have been excluded, even though they mention ATZ in the pesticide mixture.

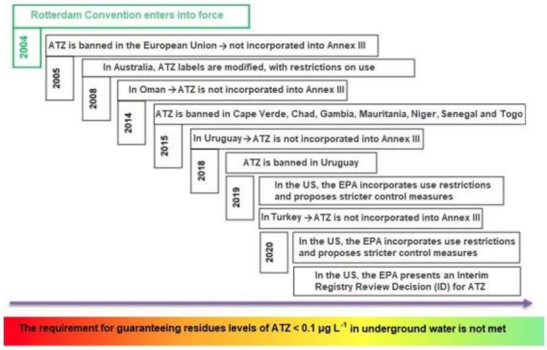

Figure 4: Chronological detail of the presentations and decisions policies regarding ATZ regulation in different countries.

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