Outcome of the first survey of atrazine in drinking water from Ijebu-North, South-West, Nigeria: Human health risk and neurotoxicological implications

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ABSTRACT

No recognised study has been conducted to monitor atrazine residue in drinking water from rural agricultural areas in Nigeria and its potential health implications. In this ecotoxicological study, we monitored atrazine residue in 69 hand-dug wells (HDW), 40 boreholes (BH) and 4 major streams from all the 6 communities (Ago-Iwoye, Ijebu-Igbo, Oru, Awa, Ilaporu and Mamu) in Ijebu North Local Government Area, Southwest Nigeria. Values of atrazine obtained were further used to evaluate the non-carcinogenic risk associated with ingestion and dermal contact in adults and children as well as neurotoxicity assessment. A total of 41 HDW, 22 BH and the 4 streams were tested positive for atrazine, which was higher in HDW than BH and stream. Ago-Iwoye recorded the highest concentration of 0.08 mg/L in its HDW while the lowest concentration of 0.01 mg/L was recorded in HDW from Oru. Hazard Index values associated with ingestion and dermal for children and adults were below the acceptable limit. Atrazine at 0.01, 0.03 and 0.04 mg/L concentrations appears to trigger defence mechanisms capable of protecting the structural integrity of the brain, but significant (p < 0.05) alterations in the oxidative stress parameters, acetylcholinesterase activity, membrane-bound ATPase enzymes, neurotransmitters as well as mild degenerative changes were observed in the brain of rats exposed to atrazine at 0.08 mg/L. Atrazine at 0.01, 0.03 and 0.04 mg/L concentrations found in drinking water from Ijebu-North may not pose any threat to brain function, but concern should be raised at 0.08 mg/L.

1. Introduction

The use of atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), a selective and organochlorine triazine herbicide, for the control of pre-and post-emergence broadleaf weeds on maize farms is on the increase worldwide. Nigeria is one of the largest producers of maize in Africa [1]. In order to maintain or improve the rate at which maize is produced in Nigeria, farmers depend on atrazine for the control of weeds. The increasing overdependence and use of atrazine on maize farms in Nigeria is now a major public health concern.

Of all the possible media through which humans can be exposed to atrazine, drinking water from contaminated groundwater or surface water source was noted to be the highest and most significant medium of exposure for children and adults [2]. Meanwhile, the Food Quality Protection Act of 1996 (FQPA) mandated the US EPA and other bodies to monitor dietary risks associated with exposure to atrazine from community water systems (CWS). This prompted the Environmental Protection Agency’s Office of Water (OW) to regulate atrazine under the Safe Drinking Water Act (SDWA). The agency later established a Maximum Contaminant Limit (MCL) of 3 µg/L for atrazine [3].

A rural hand-dug well survey conducted in Minnesota by the Minnesota Department of Agriculture (MDA) for atrazine recorded the highest concentration of 3.4 µg/L for the herbicide [4]. The U.S. EPA national survey of pesticides in drinking water wells found that atrazine was one of the most frequently detected pesticides in domestic water wells [5–7]. Surface water in 31 states and groundwater in 13 states had
earlier been tested positive for atrazine and almost 40% of surface water and 10% of groundwater samples were contaminated [8]. In addition, the Ontario Ministry of Environment earlier conducted a survey, among other surveys, for atrazine in Ontario farm wells and the herbicide was detected at levels or above 1 µg/L in 17% of 351 wells monitored [9]. This later prompted Canada to establish a maximum acceptable concentration (MAC) of 0.005 mg/L (5 µg/L) for atrazine and its metabolites in drinking water [10]. Up till now, no study has been conducted in rural agricultural areas in Nigeria to monitor atrazine in drinking water from wells and surface waters as well as its potential health implications.

Although the toxicological profiles of atrazine in experimental animal and human studies have been well documented [2,11]. Kroon et al. [12] reported that atrazine at nominal concentrations of 0.1, 0.5 and 5 µg/L has no effect on the endocrine and physiological parameters (including brain function parameter) of juvenile barramundi (Lates calcarifer) following a short-term acute exposure in a controlled laboratory experiment. However, atrazine at 0.01–10 µM was earlier observed to increase the expression of cytochrome P19A in the brain of juvenile zebrafish in a dose- and time-related manner [13].

It was recently stated by Stradtman and Freeman [14] that a study on atrazine as a neurotoxicant is limited at this time and has been advocated for consideration in human health risk assessments by the US EPA.

The brain is the most susceptible organ to oxidative damage from environmental toxicants due to its rich membrane polyunsaturated fatty acids, iron contents and low antioxidant profile [15]. Meanwhile, the available studies regarding the adverse effects of atrazine on neurotransmission suggest that the neurotoxicity of the herbicide is worth exploring, due to the potential for abnormal release of neurotransmitters and dysfunction to cause neuropsychiatric and neurodegenerative diseases [14,16]. In addition, the assessment of neurotoxicity of environmental toxicants is one of the health outcomes suggested by the Agency for Toxic Substances and Disease Registry (ATSDR) to be conducted during exposure [17,18].

Furthermore, ensuring sustainable access to safe drinking water is one of the major targets of the 2030 Agenda for Sustainable Development Goals (SDGs) [19], and achieving this target will majorly involve a significant improvement in rural areas of Sub-Saharan Africa which accounts for over 40% of the global population and without access to improved drinking water [20]. Out of 51% of Nigeria’s population living in rural areas, only 39–47% have access to improved water supply, while 72% of the 49% population in urban areas have access to potable water sources [21,22]. In addition, more than sixty-six (66) million people in Nigeria [ranked third after China (119 million) and India (97 million)] have been denied access to quality drinking water sources due to uncontrolled anthropogenic activities [20,23]. Therefore, the majority of Nigerians in rural communities, where there is a higher agricultural activity, still depend on streams, rivers and hand-dug wells for their drinking water. In the year 2007, the governing council of the Standards Organization of Nigeria established a maximum permissible level of 0.02 mg/L for 2, 4, 6 – trichlorophenol in drinking water [24], but provided no limits for the widely used atrazine herbicide.

We designed this study to (1) monitor loads of atrazine residues in water from the rural communities of Ijebu-North LG, Southwest Nigeria. (2) evaluate the potential human health risk of exposure to atrazine (3) carry out neurotoxicity of atrazine concentration in water from the study area.

2. Materials and methods

2.1. Study area

This ecotoxicological study was carried out in Ijebu-North LGA of Ogun State Nigeria. The local government comprises six communities including Ago-Iwoye, Ijebu-Igbo, Ori, Ilaporu, Awa and Mamu with a major farm village in each community. Agriculture is the economic mainstay of this LGA, which has a 2016 population projection of 390, 200 [25] and a total land area of 967 km². Ijebu North LGA is bounded by Oluoye LGA of Oyo State in the North, Ijebu East L.G in the west, Odogbolu and Ijebu Ode L.G. in the south and Ikeje L.G. in the east.

2.2. Water sample collection

Hand-dug well (HDW), borehole (BH) and stream waters from each community in Ijebu-North (Fig. 1) were collected in 20 mL amber glass bottles according to the procedures of the US Geological Survey [26]. Waters from HDW and BH were also collected from the state capital, Abeokuta urban area. This serves as the control location. The number of HDW, BH and stream sampled from the rural communities (study area) and urban area (control) is shown in Supplementary Table 1. In total, 69 HDW, 40 BH and 4 streams were sampled. The water sample was collected from the farm settlement and collection was maintained within at least a 400-meter distance from each sampling point throughout the community. The depths of all the HDW and the coordinates of each sampling point are shown in Supplementary Table 2a-g. The depth of all the HDWs sampled was less than 12 m. However, the depth of the borehole could not be measured. All the water samples were filtered at the point of collection and transported on ice to the laboratory for atrazine concentration analysis. Values obtained were used to evaluate the human health risk posed by the herbicide. The highest concentrations of atrazine observed in a water sample from each of the community was administered to experimental rat in a sub-chronic neurotoxicity study.

2.3. Determination of atrazine residue

The residue of atrazine was determined in the water sample using the analytical methods approved for drinking water monitoring of contaminants [27,28] with few modifications. A stock standard of atrazine was prepared by weighing 10 mg of analytical standard (Sigma-Aldrich, St. Louis, MO, USA) into a 100 mL flask, diluted to the mark with methanol and stored at –18°C in the dark. Solid-Phase Extraction (SPE) technique was employed with the C18 SPE cartridge, which was conditioned under gravity with 10 mL of methanol and 10 mL of deionised water. We added another 2 mL of deionised water in other to avoid dryness and closed the valve to the vacuum manifold. The configuration was completed by attaching a 75-mL solvent reservoir to the top of the C18 cartridge connected to the manifold. The water sample (200 mL, pH adjusted to 6) was transferred to the reservoir and pumped through the cartridge at a flow rate of 10 mL/min. The cartridge was later washed with 20 mL of deionised water. The cartridge was subjected to a vacuum system for 25 min to remove as much water as possible and then totally dried using a stream of nitrogen gas. The elution of the compound retained was done with 2 mL of methanol and the organic extract was completely dried with a rotary evaporator at 40°C and a stream of nitrogen gas. The final sample was reconstituted in acetone to a final volume of 1.0 mL ready for GC-MS analysis. The concentrated extracts were analysed for atrazine residues on a GC-MS. The procedures for the analysis followed US EPA Good Laboratory Practice Standards (40 CFR Part 79, 60, 1994). The gas chromatographic Model: 7890 A (GC) analysis was performed on Agilent Technologies interfaced with Mass Selective Detector Model: 5975 C (MSD). The oven temperature was programmed at an initial temperature of 110°C to hold for 2 min at 10°C per minute to the temperature of 180°C at 20°C per minute to the temperature of 250°C to hold for 8 min. The electron ionization was at a 70 eV with an ion source temperature of 230°C. The volume of the sample injected was 1 µL and the compound was separated on Agilent Technologies HP5MS column (30 m × 0.25 mm × 0.330 µm). The carrier gas used was Helium gas at 65 psi. The areas of the peaks were quantified and identification was done by comparing with the standard, which was used to prepare the calibration standard solutions.
2.4. Human health risk evaluations

We carried out non-carcinogenic human health risks for adults and children through ingestion and dermal routes in this study. The human health risk, which was computed as the average daily concentration of atrazine exposed to humans over time, was evaluated following standard protocol [29–33]. The average daily exposure concentration known as average daily intake (ADI) from ingestion and dermal contact was computed using the Eqs. (1 and 2) as below

\[ \text{ADI}_{\text{ingestion}} = \frac{\text{CW} \times \text{IRW} \times \text{EF} \times \text{ED} \times \text{AT}}{\text{BW} \times \text{AT}} \]  

(1)

\[ \text{ADI}_{\text{dermal}} = \frac{\text{CW} \times \text{SA} \times \text{KP} \times \text{ET} \times \text{EF} \times \text{ED} \times \text{CF} \times \text{BW} \times \text{AT}}{\text{BW} \times \text{AT}} \]  

(2)

\( \text{SA} \) is skin area (cm\(^2\)), \( \text{KP} \) is coefficient for dermal permeability (cm/ h), \( \text{ET} \) is exposure time (hrs/day), \( \text{EF} \) is exposure frequency (days/year), \( \text{ED} \) is exposure duration (years), \( \text{CF} \) is conversion factor (mg/kg), \( \text{BW} \) is body weight (kg), \( \text{AT} \) is the average exposure time (days) [29–33].

The non-carcinogenic hazard quotient (HQ) of atrazine exposure through ingestion and dermal contact was estimated using the standard US EPA reference dose (RfD) (0.035 mg/kg/day) shown in Eq. (3) below

\[ \text{HQ} = \frac{\text{ADI}}{\text{RfD}} \]  

(3)

The hazard index (HI) of atrazine exposure for adults and children was estimated through the summation of the HQ obtained for ingestion and dermal contact.

Fig. 1. Map of each community and Ijebu-North showing the sampling point.
2.5. Toxicity study

2.5.1. Experimental animal

A total of thirty (30) adult male albino rats (140 ± 10 g) were used for this study. The rats were initially acclimatized for one week under the laboratory conditions of 25 ± 5 °C and 65 ± 5 % relative humidity in a well-ventilated experimental section of the animal house of our institution before commencing the study. The rats were allowed free access to standard laboratory rat chow (Animal Care Ltd) and clean drinking water.

2.5.2. Study design

The 30 rats were randomized into 5 groups (four treatments and one control) of 6 rats per group. The four treatment groups were exposed to atrazine (PESTANAL, Sigma-Aldrich St. Louis, MO, USA: Purity- 98.2 %) at 0.01 mg/L, 0.03 mg/L, 0.04 mg/L and 0.08 mg/L concentrations while the control group was exposed to distilled water for 12 weeks. The concentrations were selected because they were the highest values of atrazine recorded in the water from each of the communities. The rats were handled in accordance with the regulations of the local ethics committee in the Animal Care Unit (ACU) of our Institution (OOU/SCIENG/EC/0003/140621) and the animal experiment was performed according to ethical guidelines of animal experimentation (regulation CEE 86/609).

2.5.3. Sample collection and preparation

At the end of the 12 weeks of exposure, the rats were sacrificed and the whole brain of the treated and control rats was excised after dissection and washed with saline solution. Portions of the brain excised were homogenized in 50 mM Tris-HCl (pH 7.4) containing 1.15 % KCl (10 %, w/v) and the obtained homogenates were centrifuged at 10,000g for 10 min at 4 °C. The resulting supernatants were divided into aliquots used for some enzymatic and non-enzymatic assays while the remaining portions of the brain tissue were subjected to histopathological examination.

2.5.4. Assessment of brain antioxidant system

Lipid peroxidation in the brain tissue was evaluated by measuring its end product, malondialdehyde (MDA), in a thiobarbituric acid reactive substance (TBARS) assay where 0.2 mL of 8.1 % (w/v) sodium dodecyl sulphate (SDS), 1.5 mL of 20 % (v/v) glacial acetic acid (pH3.5) and 1.5 mL of 0.8 % (w/v) thiobarbituric acid (TBA) were added to 0.1 mL brain homogenate as described in Okhawa et al. [34]. The activity of superoxide dismutase (SOD) was determined by monitoring the inhibition rate of reduction of nitroblue tetrazolium (NBT) by the enzyme [35]. A unit of the SOD denotes the amount of the enzyme required to produce 50 % inhibition of NBT reduction per minute. The concentration of reduced glutathione (GSH) was determined by employing an established protocol [36] which is based on the formation of 2-nitro-S-mercaptopentenzoic per mole of glutathione through the reduction of 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) by SH groups of glutathione. The activity of catalase (CAT) was determined by monitoring the disappearance of hydrogen peroxide at 240 nm [37]. A unit of CAT denotes the decrease of 1 μmol of hydrogen peroxide per minute. We adopted the protocol of Necleles et al. [38] for the determination of GPx activity using hydrogen peroxide and GSH as substrates. A unit of GPx represents 1 μg GSH consumed per minute. GST enzyme activity was determined by monitoring the thioether bond formed between GST and 1-chloro-2,4-dinitrobenzene (CDNB) spectrophotometrically at 340 nm wavelength as previously described [39]. All the enzyme activities were expressed as units per milligram of protein, which was determined according to Lowry et al. [40], and as modified [42,43]. The protocol is based on the incubation of 100 μl of brain aliquots with 5 μl of 2,7-dichloro-dihydro fluorescein diacetate (DCFH-DA) at 37 °C for 60 min. SpectraMax fluorescence plate reader was used to record the fluorescence emission of DCF emanating from the oxidation of DCFH-DA at 488 nm oxidation wavelength and 525 nm emission wavelength. Result expression was in nmoloes/DCF/g tissue/min.

2.5.6. Estimation of membrane-bound ATPase enzyme in the brain tissue

Activities of Ca²⁺/ATPase, Na⁺/K⁺ ATPase and Mg²⁺ ATPase in the brain homogenates of the rats were determined according to the protocols described in Afolabi et al. [44]; Dedeko et al. [45] and as modified in Owagboriaye et al. [42,43]. The assay involves the estimation of the amount of phosphorus liberated from the incubation mixture containing the homogenate, 5 mM of ATP, 60 mM of NaCl, 2 mM of MgCl₂, 20 mM of KCl, 2 Mm of CaCl₂ and protein enzyme. We incubated the tubes at 37 °C and 1 mL of cold 10 % (w/v) trichloroacetic acid (TCA) was added to inactivate the enzyme after 25 mins. The tubes were kept ice-cold for 20 mins and the precipitated proteins were removed by centrifugation technique. We also set up control through the addition of enzyme after TCA at the end of the incubation period. We adopted the established protocol of Lowry et al. [40] for protein estimation.

2.5.7. Estimation of dopamine and serotonin content in the brain

Dopamine and serotonin contents of the brain were determined with the high-performance liquid chromatographic (HPLC) technique described in Li et al. [46] and Wirbisky et al. [47] with little modifications. We carried out the chromatographic analysis using Agilent Technologies 1200 series HPLC with a fluorescence detector (Santa Clara, California, USA), which was connected to the Zorbax Eclipse XDB-C18 column (250 × 4.6 mm) (Thomas Scientific). It was run in isocratic mode with the mobile phase consisted a mixture of 0.08 M NaH₂PO₄, 10 % mannitol, 2 % acetonitrile, 2.0 mM OSA, 0.025 mM EDTA, and 0.2 mM TEA, in 13 mM purified water, pH 4.2. Separation was at 32 °C and maintained at a flow rate of 0.7 mL/min. The electrochemical potential of the HPLC detector was set at 250 mV. Dopamine and serotonin levels were quantified using standard curves obtained from each of the neurotransmitter standards.

2.5.8. Acetylcholinesterase activity estimation

Acetylcholinesterase activity was determined in the supernatant obtained from the brain samples as described in Ellman et al. [48] using acetylthiocholine iodide (30 μl final concentration) as substrate and 5, 5-dithiobis-2-nitrobenzoic acid (DTNB; 200 μl final concentration). Assay tubes were completed to 1 mL with sodium phosphate buffer (pH 8) as described by Cañadas et al. [49]. The enzyme activity was calculated relative to protein concentrations.

2.5.9. Histopathological examination of the brain

Sections of 4 μm thickness were taken serially with a rotary microtome and processed in alcohol-xylene series, stained with hematoxylin and eosin (H & E). The prepared slides were examined at × 100 and × 400 magnifications.

2.5.10. Statistical analysis

IBM Statistical Package (SPSS) version 20.0 [50] was used to analyse the data obtained. We compared the mean values with the Analysis of Variance (ANOVA) and the results were presented as Mean ± Standard Error of Mean (SEM). Student-Newman-Keuls (SNK) was used for the post hoc test and a Probability value less than 0.05 was considered to be statistically significant.
3. Results

3.1. Level of atrazine in water from Ijebu-North

Residues of atrazine in hand-dug wells, boreholes and streams water from the 6 communities in Ijebu-North and Abeokuta are shown in Table 1. A total of 41 hand-dug wells, 22 boreholes and all the 4 streams were tested positive for atrazine. Ago-Iwoye recorded the highest concentration of 0.08 mg/L in its hand-dug well water. This is followed by well water from Awa and Mamu (0.04 mg/L). Ijebu-Igbo and Oru recorded the highest atrazine concentrations of 0.03 and 0.01 mg/L respectively. As expected, no atrazine was detected in borehole water from Abeokuta, but in 3 wells at 0.001 mg/L concentration.

3.2. Human health risk evaluation

The estimated non-carcinogenic health risks through ingestion and dermal contact for children and adults according to the atrazine concentrations detected in each community are shown in Supplementary Tables 3, 4, 5, 6, 7 and 8. Ago-Iwoye recorded the highest HQ values of 0.146 and 0.063 for ingestion in children and adults, and HQ values of 0.082 and 0.045 for dermal contact in children and adults respectively. This was followed by the Awa community where the HQ values of 0.074 and 0.016 for dermal contact in children and adults, and HQ values of 0.040 and 0.023 for ingestion in children and adults respectively were observed. HI values in children ranged from 0.020 to 0.228 while it was between 0.012 and 0.143 in adults. The estimated cancer risks were all below 1E-06 for ingestion and dermal contact for children and adults according to the atrazine concentrations detected in each community.

3.3. Activities of antioxidant enzymes in the brain tissue

Table 2 shows the activities of antioxidant enzymes and concentration of GSH in the brain tissue of male albino rats exposed to atrazine concentrations in drinking water from Ijebu-North, Southwest Nigeria. Activities of SOD and catalase were observed to significantly (p < 0.01) increase in rats exposed to 0.03 mg/L concentration of atrazine compared to control. Activities of SOD and catalase recorded in rats exposed to 0.03 mg/L and 0.04 mg/mL concentration of atrazine were not significantly (p > 0.05) different. However, the activity of catalase in rats exposed to 0.03 mg/L concentration of atrazine was significantly (p < 0.01) increase with an increase in the concentration of atrazine exposure. Meanwhile, the level of lipid peroxidation was observed to insignificantly (p = 0.09) increase with an increase in the concentration of atrazine exposure.

Table 2: Activities of antioxidant enzymes in the brain tissue of male albino rat exposed to atrazine concentrations in drinking water from Ijebu-North, Southwestern Nigeria.

| Treatments       | SOD (U/mg Protein) | GSH (U/g Tissue) | CAT (U/mg Protein) | Gpx (U/mg Protein) |
|------------------|--------------------|------------------|--------------------|--------------------|
| Control          | 14.16 ± 0.96a      | 8.96 ± 1.11b     | 8.26 ± 0.73b       | 11.99 ± 0.78b      |
| 0.01 mg/L        | 16.80 ± 0.57ab     | 8.29 ± 0.80b     | 9.05 ± 0.84ab      | 11.50 ± 0.54ab     |
| 0.03 mg/L        | 19.85 ± 1.11ab     | 6.56 ± 0.64c     | 10.46 ± 0.50c      | 9.57 ± 0.36c       |
| 0.04 mg/L        | 19.04 ± 0.69ac     | 5.07 ± 0.71d     | 10.09 ± 0.54c      | 8.81 ± 0.82c       |
| 0.08 mg/L        | 15.18 ± 0.57bc     | 4.40 ± 0.47e     | 8.33 ± 0.40d       | 6.56 ± 0.72d       |

abc Mean values (± Standard deviation) in the same column having similar superscripts are not significantly different (p > 0.05); SOD – Superoxide dismutase; GPx - Glutathione peroxidase; GSH – Reduced glutathione

different. On the other hand, GPx activity and concentration of GSH were observed to significantly (p = 0.01) reduce with an increase in the concentrations of atrazine exposure.

3.4. Levels of reactive oxygen species (ROS) and lipid peroxidation in the brain tissue

The production of ROS and the level of lipid peroxidation in the brain tissue of male albino rats exposed to atrazine concentrations in drinking water from Ijebu-North, Southwest Nigeria are shown in Fig. 2. ROS production in the brain of the experimental rats was observed to increase with an increase in atrazine concentration. But the increase was only significant (p = 0.01) at 0.08 mg/L concentration of atrazine exposure. Meanwhile, the level of lipid peroxidation was observed to insignificantly (p = 0.09) increase with an increase in the concentration of atrazine exposure.

3.5. Activities of acetylcholinesterase and Glutathione-S-transferase in the brain tissue

The activities of acetylcholinesterase and glutathione-S-transferase in brain tissues of male albino rats exposed to atrazine concentrations in drinking water from Ijebu-North, Southwest Nigeria are represented

Table 1: Concentration of atrazine (mg/L) in water from the communities in Ijebu-North Local Government of Ogun State, Nigeria.

| Point | Abeokuta | Ijebu-Igbo | Oru | Ilaporo | Awa | Ago-Iwoye | Mamu |
|-------|----------|------------|-----|---------|-----|-----------|------|
| SP1   | SP1      | SP1        | SP1 | SP1     | SP1 | SP1       | SP1  |
| SP2   | SP2      | SP2        | SP2 | SP2     | SP2 | SP2       | SP2  |
| SP3   | SP3      | SP3        | SP3 | SP3     | SP3 | SP3       | SP3  |
| SP4   | SP4      | SP4        | SP4 | SP4     | SP4 | SP4       | SP4  |
| SP5   | SP5      | SP5        | SP5 | SP5     | SP5 | SP5       | SP5  |
| SP6   | SP6      | SP6        | SP6 | SP6     | SP6 | SP6       | SP6  |
| SP7   | SP7      | SP7        | SP7 | SP7     | SP7 | SP7       | SP7  |
| SP8   | SP8      | SP8        | SP8 | SP8     | SP8 | SP8       | SP8  |
| SP9   | SP9      | SP9        | SP9 | SP9     | SP9 | SP9       | SP9  |
| SP10  | SP10     | SP10       | SP10| SP10    | SP10| SP10      | SP10 |
| SP11  | SP11     | SP11       | SP11| SP11    | SP11| SP11      | SP11 |
| SP12  | SP12     | SP12       | SP12| SP12    | SP12| SP12      | SP12 |
| SP13  | SP13     | SP13       | SP13| SP13    | SP13| SP13      | SP13 |
| SP14  | SP14     | SP14       | SP14| SP14    | SP14| SP14      | SP14 |
| SP15  | SP15     | SP15       | SP15| SP15    | SP15| SP15      | SP15 |
| SP16  | SP16     | SP16       | SP16| SP16    | SP16| SP16      | SP16 |
| SP17  | SP17     | SP17       | SP17| SP17    | SP17| SP17      | SP17 |
| SP18  | SP18     | SP18       | SP18| SP18    | SP18| SP18      | SP18 |
| SP19  | SP19     | SP19       | SP19| SP19    | SP19| SP19      | SP19 |
| SP20  | SP20     | SP20       | SP20| SP20    | SP20| SP20      | SP20 |

SP-sampling point; ND- not detected; BDL- below detection limit, BH- borehole
Results showed a significant reduction (\(p = 0.01\)) in the acetylcholinesterase activity in the brain of the experimental rats with an increase in the atrazine exposure. However, there was no significant (\(p = 0.16\)) difference in the activity of acetylcholinesterase in the brain of rats exposed to 0.01 mg/L of atrazine (16.39 \(\pm\) 5.11) and 0.03 mg/L of atrazine (15.26 \(\pm\) 7.01).

GST activity was significantly (\(p = 0.01\)) increased (7.63 \(\pm\) 0.25) in the rats exposed to the 0.04 mg/L concentration of atrazine. This was followed by the rat group exposed to a 0.03 mg/L concentration of atrazine (7.01 \(\pm\) 0.33). However, there was no significant difference (\(p = 0.48\)) in GST activity observed in the rat groups exposed to 0.01 mg/L of atrazine (5.11 \(\pm\) 0.29) and 0.08 mg/L of atrazine (4.92 \(\pm\) 0.56).

The activities of ATPase enzymes in the brain tissue of male albino rats exposed to atrazine concentrations in drinking water from Ijebu-North, Southwest Nigeria are shown in Fig. 4. Activities of Mg\(^{2+}\) ATPase and Ca\(^{2+}\) ATPase in the brain of rats were observed to reduce with an increase in the concentration of atrazine exposure. However, the reduction was not significant (\(p > 0.05\)) compared to the control. In a similar trend, the activity of Na\(^+\)/K\(^+\) ATPase was observed to reduce with an increase in the concentrations of atrazine exposure. However, the reduction observed in the rat group exposed to atrazine at 0.08 mg/L was observed to be significant (\(p = 0.01\)) compared to other groups.

The levels of serotonin and dopamine observed in the brain tissue of male albino rats exposed to atrazine concentrations in drinking water from Ijebu-North, Southwest Nigeria are presented in Fig. 5. Serotonin level in the experimental rats was observed to reduce with an increase in the concentration of atrazine exposure. There was no significant difference in the level of serotonin between control (0.49 \(\pm\) 1.28) and rats exposed to 0.01 mg/L (0.48 \(\pm\) 1.26) as well as between those exposed to 0.03 mg/L (0.45 \(\pm\) 1.22) and 0.04 mg/L (0.43 \(\pm\) 1.26). However, serotonin was observed to significantly (\(p = 0.01\)) reduce (0.40 \(\pm\) 1.17) in rats exposed to atrazine at 0.08 mg/L. Meanwhile, the level of dopamine was observed to insignificantly (\(p = 0.24\)) reduce with an increase in the concentration of atrazine exposure.

Figs. 6 and 7 show the photomicrographs of the brain of rats exposed to atrazine concentrations in drinking water from Ijebu-North, Southwest Nigeria. The normal structural integrity of the brain with visible neurons was observed in the experimental rats when viewed at \(\times 100\) magnifications. except in rats exposed to atrazine at 0.08 mg/L where mild vacuolation of the neuronal cytoplasm seems to appear (Fig. 6). But this was confirmed when viewed at \(\times 400\) magnifications (Fig. 7).
4. Discussion

The concentrations of atrazine detected in water from Oru and Ilaporu communities were all below the MAC of 0.005 mg/L established by Canada and 0.003 mg/L established by the US EPA. Hand-dug well from Ijebu-Igbo contained atrazine concentration up to the MAC of US EPA. Meanwhile, well waters from Awa and Mamu communities both recorded atrazine concentration that is above the US EPA limit, but lower than the limit set by Canada. However, the concentration of atrazine above the US EPA and Canada limits was detected in sampling point 1 of the hand-dug well from the Ago-Iwoye community. This is an indication that hand-dug well waters from Awa, Mamu and Ago-Iwoye communities are highly polluted with atrazine herbicide. This could be attributed to the higher consumption of atrazine in the farm villages because a higher level of atrazine detected in some sites has been correlated with the higher herbicide use [51]. Meanwhile, variations and reductions in atrazine concentration detected in water were observed as we moved away from the farm villages of each community. This may be due to the geographical structure, depth of the hand-dug well as well as agricultural activities of the sampling area, which have been reported to influence the spatial distribution of atrazine [32,51]. The influence of depth on the concentration of atrazine detected in hand-dug well is a possibility. Since the concentration of atrazine in the boreholes was lower than that recorded in the well, it is expected that the depth of the boreholes will be greater than that of the hand-dug well.

The HQ values for non-carcinogenic effect through ingestion and dermal contact for children and adults were less than 1 in all of the community. This indicates that the water from each of the communities may be safe for domestic use. However, the HQ values through ingestion and dermal contact for children were higher than that for adults. Our
findings confirm Almasi et al. [32] and Dehghani et al. [33] who reported higher HQ values for ingestion and dermal contact in children than adults. HQ values recorded for children and adults through dermal contact in the communities show that dermal contact poses a higher risk for children than adults. In addition, Ago-Iwoye and Awa communities are more contaminated and pose the highest risk of water use. It is important to note that we did not evaluate the health risk arising from ingestion of atrazine through food such as corn, as this can also increase the health risk [52]. Thus, our findings in this study should be applied with caution.

Oxidative stress biomarkers (MDA), the concentration of GSH and antioxidant enzymes (CAT, SOD, GPx and GST) were studied in the brain of rats exposed to atrazine. Exposure to atrazine at concentrations found in water from Ijebu-North induced an increase in MDA level with an increase in atrazine concentration mostly at 0.08 mg/L. This might suggest oxidative stress at this concentration. SOD, an endogenous scavenger that dismutates superoxide radical, was observed to increase at 0.03 and 0.04 mg/L of atrazine exposure but fall at 0.08 mg/L concentration. The fall in SOD activity may be due to the inactivation of SOD by interaction with oxygen radicals [53]. Catalase is known to scavenge hydrogen peroxide at higher concentrations [54] and at lower concentrations by GPx [55]. In this study, the relative contributions of CAT and GPx in the decomposition of hydrogen peroxide are concentration-specific with CAT being more potent at 0.01, 0.03 and 0.04 mg/L of atrazine concentration but at 0.08 mg/L, while GPx is being important at the increasing concentration of atrazine. An increase in CAT activity observed in the brain of atrazine-exposed rats signifies that the enzyme is more responsive to the increased hydrogen peroxide concentration in the brain tissue, therefore, emphasizing its role in the control of cellular lipid peroxide concentration [56]. This is very important and significant, especially in the maintenance of cellular proliferation and differentiation in the brain with a high lipid content [53]. The decreased activity of GPx in the brain of the atrazine-exposed rats indicates its inability or reduced capacity to scavenge hydrogen peroxide generated in the tissue in response to atrazine exposure. Our finding is, in part, supported by Singh et al. [57] and Qian et al. [58] who found an increase in the activities of CAT and SOD as compensatory or adaptive mechanisms in response to oxidative stress induced by atrazine. In another study, CAT and SOD activities were observed to reduce and increase respectively in the brain of Wistar rats exposed to 120 mg/kg of atrazine for 16 days [59]. However, our finding partially contradicts Xing et al. [60] who reported concentration-dependent decreases in the activities of CAT, SOD and GPx in the brain of common carp following exposure to 4.28, 42.8 and 428 µg/L of atrazine.

The strengthening of the antioxidant defence in the brain of the atrazine-exposed rats seems to be accompanied by an increase in the GST activity, which is one of the primary detoxification enzymes. This indicates an increased ability to detoxify accumulated lipid peroxidation products during stress. In addition, an increase in the activity of GST in the brain in response to atrazine exposure is accompanied by a significant decrease in its GSH concentration suggesting a rise in conjugation reactions. While Kaushik and Kaur [53] noted that decreased GSH concentration may directly result from reduced synthesis or increased breakdown during stress conditions, Simmons et al. [61] and Alptekin et al. [54] attributed a decreased GSH concentration to increased lipid peroxidation. But here in this study, increased consumption of GSH in conjugation reactions due to increased GST seems to explain the reduced GSH concentration observed in the brain of the rats exposed to atrazine. A similar finding has been suggested by Kaushik and Kaur [53] and Slusser et al. [62] that a depleted GSH concentration could create conditions under which oxygen could damage membranes without the initial participation of O₂ radicals. Acetylcholinesterase catalyses the hydrolysis of acetylcholine (ACh) to choline and acetate [63]. The reduced acetylcholinesterase activity observed in the atrazine-exposed rats in this study may suggest possible alterations of the cholinergic system in the CNS. Our finding is consistent with Schmiedl et al. [64] who also observed a reduction in the acetylcholinesterase activity in the brain of zebrafish exposed to 1000 µg/L of atrazine.

Membrane-bound ATPase enzymes are good markers of membrane dysfunctions under oxidative stress conditions [45]. The enzymes supply energy at the expense of ATP through hydrolysis to regulate the permeability of the membrane and transportation of ions such as Ca²⁺, K⁺ and Na⁺ across the membrane [65]. This study showed for the first time that atrazine at 0.08 mg/L could reduce the activities of membrane-bound Ca²⁺/ATPase and Na⁺/K⁺-ATPase in the brain of exposed rats. These reductions could be attributed to the increased lipid peroxidation since Na⁺/K⁺-ATPase is lipid dependent and has a high affinity for the SH group and Mg²⁺ + -ATPase activity is sensitive to lipid peroxidation [66]. In addition, Miltonprabu et al. [66] noted that Na⁺/K⁺-ATPase inhibition could lead to a decrease in sodium efflux that disrupts membrane permeability, a condition that may lead to the leakage of Ca²⁺ ions into the cytoplasm and consequently reduce the Ca²⁺/ATPase activity in the membrane as previously suggested [42,43].

The vital role of serotonin in the body has been documented. As reported by Uppala et al. [67], a considerable amount of serotonin is required for regulating a stable mood and balancing excessive excitatory neurotransmitters in the brain. In the hypothalamus, serotonin also regulates sleep, body temperature, pain perception, blood pressure as well as hormonal activity. Therefore, a reduction in serotonin content in the brain of the exposed rats is an indication that atrazine, mostly at 0.08 mg/L, may be a major threat to the normal physiological processes of neurotransmission, which might disturb the mood of the exposed animal. Although only a few limited studies have been conducted on the impacts on serotonin following atrazine exposure [14], each of the few studies gives support that atrazine may interfere with the serotonergic system at doses near or below the current chronic dietary NOAEL at 1.8 mg/kg/day. Alterations in the serotonergic system were observed in zebrafish following atrazine exposure at 0.3, 3 and 30 µg/L [47]. In addition, a significant decrease in serotonin content in the brain of C57BL/6 mice exposed to atrazine at 1.4 mg/kg/day has been reported [68].

Dopamine is a neurotransmitter and its high concentration in the CNS has been linked to love and decision making, attention, increased mental activity, motivation and goal-directed behaviour [69,70]. However, a low level of dopamine or impaired dopamine function has been associated with depression in individuals [71]. Although in this study, there was no significant difference in the reduction of brain dopamine level with an increase in the concentration of atrazine exposure, the observed reduction might still suggest the potential of atrazine to induce depression in the exposed animal. Our finding is, in part, consistent with Li et al. [46,72] who found a decreased level of dopamine in Sprague Dawley rats following exposure to atrazine. However, the reduction was significant at higher doses of 100 or 200 mg/kg administered. A significant decrease in dopamine level was also reported in rats following exposure to higher atrazine concentrations of 0.1 or 10 µg/kg [73] and 25, or 50 mg/kg/day [74].

In this study, atrazine at 0.01 0.03 and 0.04 mg/L has no effect on the normal structure of the brain of the exposed rats. However, mild histopathological lesions were seen in the brain of rats exposed to atrazine at 0.08 mg/L. Although it is possible for the mild histopathological changes observed at 0.08 mg/L to influence the normal physiological activities of the rats, it is difficult to suggest neurotoxicity. In another study, normal brain structure with no visible lesion was observed in Wistar rats exposed to 120 mg/kg atrazine for 16 days [59]. Meanwhile, Xing et al. [60] observed no visible change in the brain of common carp exposed to atrazine at 4.28 µg/L, however, severe histopathological lesions were seen in the brain at 42.8 and 428 µg/L.

5. Conclusion

Our findings show the presence of atrazine residue in HDW, boreholes and stream waters from the communities in Ijebu-North LGA,
southwest Nigeria. The HI values for non-carcinogenic effect through ingestion and dermal contact for children and adults were below the acceptable limit for all the communities. But children from the LGA seem to be more at risk than adults. In addition, Ago-Iwoye and Awa communities were more contaminated and posed the highest risk of water use. Atrazine at environmentally relevant concentrations of 0.01, 0.03 and 0.04 mg/L was observed to induce antioxidant defence in the brain capable of protecting the tissue against oxidative damage, degenerative lesions and neurotoxicity. However, atrazine at 0.08 mg/L may call for concern in the exposed animal. As part of the requirements of FQPA on water quality assessment, this is the first study to monitor the level of atrazine in water from HDW, boreholes and streams of rural areas in Nigeria and could contribute to the establishment of MCL of atrazine in drinking water in Nigeria. This study could also contribute to the ongoing USDA Pesticide Data Program monitoring for pesticide residues in food and water. We recommend prompt intervention by the regulatory agencies and policymakers on the use of atrazine herbicide in rural agricultural areas of Ijebu-North southwest, Nigeria.

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Author Contributions

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Declaration of Competing Interest

The authors declare no conflict of interest in this study.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2022.06.012.

References

[1] A. Adeite, Maize Farming in Nigeria: Exciting Facts You Should Know. (2021) https://bambaraonga.com/maize-farming-in-nigeria-exciting-facts-you-should-know/ Accessed January 17th, 2022.
[2] US EPA, 2007. Archie document on Atrazine chemical summary for Toxicity and Exposure Assessment for Children’s Health 2007.
[3] US EPA, Interim Reregistration Eligibility Decision (IRED) for Atrazine, Cast (No. 0362) (2003).
[4] MHD, Human health assessment: atrazine report for the minnesota department of health, St. Paul, MN (2009).
[5] W.F. Ritter, Pesticide contamination of groundwater in the United States—a review, J. Environ. Sci. Health B 25 (1) (1990) 1–29.
[6] J.J. Quackenbush, et al. Design strategy for assessing multi-pathway exposure for children: the minnesota children’s pesticide exposure study (MNCPES), J. Expo. Anal. Environ. Epidemiol. 10 (2) (2000) 145–158.
[7] M.J. Focazio, et al., “The Chemical Quality of Self-Supplied Domestic Well Water in the United States.” http://health.gov. On the recommendations for minimizing domestic well contamination from herbicides, J. focazio_and_others,2006.pdf Ground Water Monit. Remid. 26 (3) (2006) 92–104.
[8] US EPA, Office of Drinking Water. Atrazine, in: Drinking Water Health Advisory: Pesticides, 43, Lewis Publishers, Chelsea, MI, 1986.
[9] Ontario Ministry of the Environment, Pesticides in Ontario drinking water — 1985. Toronto (1987).
[10] Health Canada, 1993. Guidelines for Canadian Drinking Water Quality Guideline Technical Document Atrazine 1993.
[11] WHO, Atrazine and Its Metabolites in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality (2011). WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.
[12] P.J. Kroon, S.E. Hook, D. Jones, S. Metcalfe, J.I. Osborn, Effects of atrazine on endocrinology and physiology in juvenile barramundi, lates calcarifer (Bloch), Environ. Toxicol. Chem. 33 (7) (2014) 1607–1614.
[13] M. Suzawa, H.A. Ingraham, The herbicide atrazine activates endocrine gene networks via non-steroidal ERα nuclear receptors in fish and mammalian cells, PLOS ONE 3 (2008), e2117.
[14] S.C. Stradtman, J.L. Freeman, Mechanisms of neurotoxicity associated with exposure to the herbicide atrazine, Toxics 9 (2021) 207, https://doi.org/10.3390/toxics9010027.
[15] T. Jayaraman, S. Kannappan, M.K. Rivichandran, C.V. Anuradha, Impact of Essential L. on ethanol-induced changes in rat brain and erythrocytes, Singap. Med. J. 49 (2008) 320–327.
[16] M. Sarter, J.P. Bruno, V. Parikh, Abnormal neurotransmitter release underlying behavioral and cognitive disorders: toward concepts of dynamic and function-specific dysregulation, Neuropsychopharmacology 32 (2007) 1452–1461.
[17] H.H. Schaumburg, P.S. Spencer, C. Joseph, J.C. Arezano, Monitoring potential neurotoxic effects of hazardous waste disposal, Environ. Health Perspect. 48 (1983) 61–64.
[18] B.L. Johnson, Impact of Hazardous Waste on Human Health, CRC press inc., New York, 1995, pp. 1–408. ISBN 0849364472.
[19] W.H.O. UNICEF Progress on Drinking Water, Sanitation and Hygiene. 2017 Update and SDG Baselines Geneva 2017. Available at: https://en.m.wikipedia.org/wiki/Sustainable_Development_Goals16/02/2018, 9:48 AM.
[20] T.O. Sogbanmu, S.O. Aitsegame, O.A. Olubamiji, J.O. Odiyo, Drinking water quality and human health risk evaluations in rural and urban areas of Ijebu-Lekki and Ibeju-Lekki local government areas, Lagos, Niger. Hum. Ecol. Risk Assess. Int. J. 26 (4) (2020) 1062–1075, https://doi.org/10.1080/10709368.2018.1554428.
[21] Federal Republic of Nigeria, National water supply and sanitation policy. Federal Ministry of Water Resources, first ed., Abuja, Nigeria, 2000.
[22] D.M. Akali, O.T. Ihebokan, J.A. Tuma, I.A. If电工, Provision of sustainable water supply system in Nigeria: a case study of Wannune-Benue State, World J. Environ. Eng. 2 (1) (2014) 1–5.
[23] W.H.O. UNICEF Progress on drinking water and sanitation 2012 Update 66 2012. (Available at) https://www.unicef.org/media/files/JMPreport2012.pdf.
[24] Standards Organization of Nigeria, UNICEFG. (2007) Retrieved 20 August, 2020, from http://www.unicef.org/nigeria/ng_publications_Nigerian_Standard_for_Drinking_Water_Quality.pdf.
[25] National Population Commission Nigeria: Administrative Division. States and Local Government Area 2016. (https://www.citypopulation.de/php/nigeria-admin.php) (Available at) https://www.unicef.org/nigeria/ng_publications_Nigerian_Standard_for_Drinking_Water_Quality.pdf.
[26] Technical Document Atrazine 1993.
[27] S. Huang, J.S. Tanton, Y. Lin, R.A. Yokley, Analytical method for the determination of atrazine and its dealkylated chlorotriazine metabolites in water using SPE sample preparation and GC-MSD analysis, J. Agric. Food Chem. 51 (2003) 7252–7256.
[28] EPA, Risk Assessment Guidance for Superfund, Volume I: Human Health Evaluation Manual (Part a). EPA/540/1–89/002 (1989).
[29] H. Canada Federal Contaminated Site Risk Assessment Fund, Canada: Part F: Guidance on Human Health Detailed Quantitative Risk Assessment for Chemicals (DQRChem). Health Canada Ottawa, ON, Canada 2010.
[30] M.D.N., 2006. Resources Departmental Missouri Risk-Based Corrective Action (MRBCA) Technical Guidance (Appendices), Table B-1, Lowest Default Target Levels, All Soil Types and All Pathways 2006.
[31] H. Almaz, T. Takasabu, N. Jusufzadeh, A.A. Babir, Y.T. Birgman, B. Cheraghian, A. Sakic, S. Jor, Spatial distribution, ecological and health risk assessment and source identification of atrazine in Shadegan international wetland, Iran, Mar. Pollut. Bull. 160 (2020), 111569.
[32] M. Dehghani, E. Gharechahi, S. Jafari, Z. Meoeini, Z. Derakhshan, M. Ferrante, G. O. Conti, Health risk assessment of exposure to atrazine in the soil of Shiraz farmlands, Iran, Environ. Res. 204 (2021), 112090.
[33] O. Okhawa, N. Ohishi, K. Yagi, Anzay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 5 (1979) 351–358.
