Answering Reviewers

Reviewer 1

Thank you very much for the observations, they were addressed in the manuscript and can be identified underlined in yellow.

1. **This review is well done. The issue of the influence of pesticides on the genesis and development of neurodegenerative diseases (AD, PD,...) has been discussed for many years, but a review on the effect on tauopathies (AD in this case) is scarce.**

   I suggest some small changes:

   - Abstract: " It is characterized by increased phosphorylation of Tau protein, beta-amyloid plaques and neurofibrils" = It is characterized, among other changes, by loss/dysfunction of cholinergic neurons, formation of amyloid plaques, and formation of neurofibrillary tangles (consequence of hyperphosphorylation of tau-protein). (or something similar)

The change was made as indicated. The abstract already with the changes is the following:

> "Alzheimer's disease (AD) is a progressive and neurodegenerative illness which results in alterations in cognitive development. It is characterized by loss/dysfunction of cholinergic neurons, and formation of amyloid plaques, and formation of neurofibrillary tangles, among other changes, due to hyperphosphorylation of tau-protein. Exposure to pesticides in humans occurs frequently due to contact with contaminated food, water, or particles. Organochlorines

(OCs), organophosphates (OPs), carbamates (Cs), pyrethroids (Ps) and neonicotinoids (Ns) are associated with the most diagnosed incidents of severe cognitive impairment. The aim of this study was to determine the effects of these pesticides on the phosphorylation of tau protein, and its cognitive implications in the development of AD. It was found that exposure to pesticides increased the phosphorylation of tau protein at sites Ser198, Ser199, Ser202, Thr205, Ser396 and Ser404. Contact with these chemicals altered the enzymatic activities of Cdk5, GSK-3Beta and PP2A. Moreover, it altered the expression of the MAPT gene, and changed levels of intracellular calcium. These changes affected tau protein phosphorylation and neuroinflammation, and also increased oxidative stress. In addition, the exposed subjects had poor level of performance in tests that involved evaluation of novelty, as test on verbal, non-verbal, spatial memory, attention, and problem-solving skills."

2. **This paragraph from page 7 (In AD, increased hyperphosphorylation of Tau forms aggregates in neuron cytoplasm and neurofibrillary tangles which are responsible for neurodegeneration) = In AD, increased hyperphosphorylation of Tau forms aggregates in neuron cytoplasm - forming the so-called neurofibrillary tangles- and neurotrophic neurites, which are responsible for neurodegeneration.**

The change was made as indicated, so the paragraph is indicated in the manuscript as:

" In AD, increased hyperphosphorylation of tau forms aggregates in neuronal cytoplasm, resulting in generation of the so-called

neurofibrillary tangles and neurotrophic neurites, which are responsible for neurodegeneration[30,33,39-40].”

3. **On page 6 "AD is characterized by depletion of cholinergic neurons, formation of neurofibrils, increase in levels of phosphorylated tau protein, formation of amyloid beta plaques, (reform this according to what is said above) and in some cases, early onset via mutations in the presenilin gene (delete this or make a new paragraph about different genetic AD).**

The change was made as indicated, so the paragraph is indicated in the manuscript as:

> “AD is characterized by loss of cholinergic neurons or dysfunctional cholinergic neurons, formation of amyloid plaques, and formation of neurofibrillary tangles, due to hyperphosphorylation of tau-protein[27-28]”

4. **Physiologically, the Tau protein is involved in myelination processes, regulation of glucose metabolism, rearrangement of microtubules, axonal transport, iron homeostasis, neurogenesis and processes related to learning and memory. Include original references for each topic.**

The change was made as indicated, so the paragraph is indicated in the manuscript as:

> “Physiologically, the tau protein is involved in myelination processes[29-30], regulation of glucose metabolism[31], rearrangement of microtubules[29], axonal transport[29], iron homeostasis[29], as well as neurogenesis and processes related to learning and memory[29-30]. However, exposure to pesticides may

affect the phosphorylation of tau protein and the formation of neurofibrils, resulting in morphological changes in CNS[32-33]."

5. **In the table: Specify as "type of study" [clinical/epidemiological studies in humans] [experimental studies] in two separate groups. In another section or table, the reviews need to be considered. It would be very important to record the exposure time of humans to pesticides. In "sample", identify the mouse model.**

The changes were made as indicated by the reviewer, additionally due to the suggestions of another reviewer, the tables were separated by type of pesticide (Tables 1 to 5) and a table was added only to describe the cognitive changes (Table 6), so that the changes suggested at this point will be reflected in each table. Regarding the exposure time and dose, the data indicated by the reviewer were added according to what was published in the reference articles (Tables 1 to 6).

6. **The bibliography should be reviewed. The references need to be shown in a homogeneous way. DOI can appear but PMDI is left over.**

The format of the references was homogenized. PMID is included for indications in Guidelines_for_Manuscript_Preparation_and_Submission-Review, and the PMCID data is deleted.

Reviewer 2

Thank you very much for the observations, they were addressed in the manuscript and can be identified underlined in green.

1. **The authors performed a well-designed, coherent and important review of the possible interaction between the pesticides and the phosphorylation of**

tau protein, with consequent interference in Alzheimer's disease. This is an innovative article as it analyzes the different classes of pesticides currently used. Checklist:
Title. Does the title reflect the main subject/hypothesis of the manuscript? Yes, I think this is an appropriate title.

Ok

2. **Abstract. Does the abstract summarize and reflect the work described in the manuscript? Yes, the abstract reflects the content of the manuscript.**

I confirm, however, due to suggestions from another reviewer, it was slightly modified. The final abstract is:

> "Alzheimer's disease (AD) is a progressive and neurodegenerative illness which results in alterations in cognitive development. It is characterized by loss/dysfunction of cholinergic neurons, and formation of amyloid plaques, and formation of neurofibrillary tangles, among other changes, due to hyperphosphorylation of tau-protein. Exposure to pesticides in humans occurs frequently due to contact with contaminated food, water, or particles. Organochlorines (OCs), organophosphates (OPs), carbamates (Cs), pyrethroids (Ps) and neonicotinoids (Ns) are associated with the most diagnosed incidents of severe cognitive impairment. The aim of this study was to determine the effects of these pesticides on the phosphorylation of tau protein, and its cognitive implications in the development of AD. It was found that exposure to pesticides increased the phosphorylation of tau protein at sites Ser198, Ser199, Ser202, Thr205, Ser396 and Ser404. Contact with these chemicals altered the

enzymatic activities of Cdk5, GSK-3Beta and PP2A. Moreover, it altered the expression of the MAPT gene, and changed levels of intracellular calcium. These changes affected tau protein phosphorylation and neuroinflammation, and also increased oxidative stress. In addition, the exposed subjects had poor level of performance in tests that involved evaluation of novelty, as test on verbal, non-verbal, spatial memory, attention, and problem-solving skills."

3. **Key Words. Do the key words reflect the focus of the manuscript? Yes, the words reflect the focus of the manuscript.**
   Ok

4. **Background. Does the manuscript adequately describe the background, present status and significance of the study? Yes, I think this is a nice background. It explains the context and the relevance of the article. I suggest a more detailed explanation of the effect that tau phosphorylation has on microtubule destabilization.**

   The change was made as indicated, so the paragraph is indicated in the manuscript as:

   "Tau protein, which is expressed in the distal extremity of the axon, controls the stability of microtubules. Hyperphosphorylation of tau protein stimulates the dissociation of microtubules, interrupts axonal extension, and enhances the aggregation of insoluble tau, leading to alterations in the synapse, and hence tauopathy[15-16]."

5. **Methods. Does the manuscript describe methods (e.g., experiments, data analysis, surveys, and clinical trials, etc.) in adequate detail? –**

   -

6. **Results. Are the research objectives achieved by the experiments used in this study? What are the contributions that the study has made for research progress in this field? –**

   -

7. **Discussion. Does the manuscript interpret the findings adequately and appropriately, highlighting the key points concisely, clearly and logically? Are the findings and their applicability/relevance to the literature stated in a clear and definite manner? Is the discussion accurate and does it discuss the paper's scientific significance and/or relevance to clinical practice sufficiently? The authors have done a great job in extracting and synthesizing the information of the studies, presented in the tables. However, I consider that this data should be analyzed in more detail in the manuscript to highlight the key points. The reduced number of studies for some classes of pesticides and the complexity of obtaining data for a specific class are mentioned as the limitations of the manuscript. It is true. However, I emphasize that one of the major limitations is the lack of large studies that assess the influence of pesticides on p-tau and correlate it directly with Alzheimer's.**

   Studies from each of the tables in the discussion for each type of pesticide were described to highlight the key points in greater detail as noted by the reviewer.

   The changes are:

1) " Table 1 shows 7 studies in which the effect of OCs on tau protein phosphorylation was determined. Two clinical studies reported that exposure to these pesticides may be associated with polymorphisms in microtubule associated protein tau (MAPT) and microtubule associated protein 1B gene (MAP1B) which are related to the formation of tau aggregates[46-47]. Studies have demonstrated that dichlorodiphenyltrichloroethan (DDT) exposure to an OC altered mitochondrial function, resulting in the formation of tau aggregates, with up-regulations in the expressions of proteins such as synaptosome-associated protein 25 kDa (Snap25), cytochrome C (Cytc), enolase A (Eno1), hemoglobin alpha chain (Hba1) and histone cluster 1 (H2bb), which are characteristic of AD[44,48-49]. Finally, Mir et al[50] has shown that exposure to 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) induced overexpression of GSK-3Beta, and hence tau phosphorylation. In all, 4 of the 7 studies described in Table 1 reported increases in tau phosphorylation[44,46,48,50]."

2) " Table 2 provides a breakdown of 18 studies on the effect of OPs on tau protein. The only report on cases and controls with OP exposure for more than 2 years showed higher levels of tau phosphorylation in exposed subjects[55]. On the other hand, eight out of eleven studies indicate that exposure to these pesticides increased tau phosphorylation through different mechanisms involving GSK-3Beta overexpression, increased Cdk5 activity, and decreased expression of PP2A, among other factors (Table 2). Six review studies described in Table 2 reported increases in tau phosphorylation related to greater Cdk5 activity,

with changes in regulatory proteins MAPT and MAP-2, and increased oxidative stress, among other changes."

3) "There are only a few studies on the effect of C pesticides on tau phosphorylation. It is important to highlight that there are no clinical or epidemiological studies on this topic, to date. Most of the studies analyzed in this review indicate that exposure to Cs led to hyperphosphorylation of tau[34,56,64-65]. Only two studies, reported otherwise[33,66]. Increased hyperphosphorylation may be mediated by increased GSK-3Beta activity and PP2A inhibition (Table 3). In a murine model, exposure to carbofuran, a C pesticides resulted in neuronal death at the cortex and hippocampus, as well as alterations in spatial memory and learning processes[67]. It is interesting to note that C pesticides are currently being used for their therapeutic potential as AchE inhibitors in different pathologies[66,68-69]. More details associated with the effect of exposure to C pesticides on tau protein are presented in Table 3…"

4) " Three out the few studies that have been so far published on the effect of Ps on tau protein, and one review, are shown in Table 4. Amongst the most relevant results reported are increased activity of GSK-3Beta[36,71], increased neuroinflammation[36,71] and decreased activity of PP2A[36]. In Table 4…"

5) " In a clinical case report on accidental ingestion of imidacloprid and thiamethoxam, the resultant increase in Ca2+ influx altered the kinase response[22]. Another mechanism involved activation

of the Wnt pathway, leading to apoptosis[73]. More details are shown in Table 5…"

8. **Illustrations and tables. Are the figures, diagrams, and tables sufficient, good quality and appropriately illustrative, with labeling of figures using arrows, asterisks, etc, and are the legends adequate and accurately reflective of the images/illustrations shown? Figure 1 is well designed and relevant. Authors should consider making a table for each of the pesticide classes rather than subdividing into 1a, 1b and 2a-2c. I would recommend an extra table summarizing the studies referenced in the topic "Pesticides and their cognitive implications", it would add value to this manuscript. All the legends seem accurate.**

   The suggestions indicated by the reviewer were made, which is why they are indicated as Tables 1, 2, 3, 4, 5 and 6 respectively. Additionally for each table due to suggestions from another reviewer, the type of study is added: clinical/epidemiological, experimental or review.

9. **Biostatistics. Does the manuscript meet the requirements of biostatistics? I think there was a miscalculation in the % of OCs, since the value gives me 57.14% and not the 62.5% mentioned.**

   This data was changed for OCs and the rest of the pesticides.

10. **Units. Does the manuscript meet the requirements of use of SI units? Yes**

    Ok

11. **References. Does the manuscript appropriately cite the latest, important and authoritative references in the Introduction and Discussion sections? Does the author self-cite, omit, incorrectly cite and/or over-cite references? The references are adequate, the latest and most important in this theme. There are some cases of omission, for example, in the 3rd line of the introduction when mentioning WHO data, etc. I recommend the authors review this aspect.**

    The correction was made as indicated by the reviewer.

12. **Quality of manuscript organization and presentation. Is the manuscript well, concisely and coherently organized and presented? Is the style, language and grammar accurate and appropriate? The manuscript is coherent and well organized. However, I would suggest splitting the topic "Pesticides and their impact on Tau protein" into several subtopics to help the reader (ex: an introduction to Tau and taupathies; and a topic for each of the pesticide classes). There are minor grammatical errors and language that could be more concise. There are very long sentences and constant repetition of words (ex: On the other hand)**

    The changes were made as noted by the reviewer, in the manuscript in green are underlined the sub-topics that were added, as well as the changes in the redaction.

13. **Research methods and reporting. Authors should have prepared their manuscripts according to BPG's standards for manuscript type and the appropriate topically-relevant category, as follows: (1) CARE Checklist (2013) - Case report; (2) CONSORT 2010 Statement - Clinical Trials study, Prospective study, Randomized Controlled trial, Randomized Clinical trial; (3) PRISMA 2009 Checklist - Evidence-Based Medicine, Systematic review, Meta-Analysis; (4) STROBE Statement - Case Control study, Observational**

study, Retrospective Cohort study; and (5) The ARRIVE Guidelines - Basic study. For (6) Letters to the Editor, the author(s) should have prepared the manuscript according to the appropriate research methods and reporting. Letters to the Editor will be critically evaluated and only letters with new important original or complementary information should be considered for publication. A Letter to the Editor that only recapitulates information published in the article(s) and states that more studies are needed is not acceptable? –

–

14. **Ethics statements. For all manuscripts involving human studies and/or animal experiments, author(s) must submit the related formal ethics documents that were reviewed and approved by their local ethical review committee. Did the manuscript meet the requirements of ethics? –**

    –

Answering Company editor-in-chief

Thank you very much for the observations, they were addressed in the manuscript and can be identified in the document.

1. **I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing**

requirements of the World Journal of Clinical Cases, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor. In order to respect and protect the author's intellectual property rights and prevent others from misappropriating figures without the author's authorization or abusing figures without indicating the source, we will indicate the author's copyright for figures originally generated by the author, and if the author has used a figure published elsewhere or that is copyrighted, the author needs to be authorized by the previous publisher or the copyright holder and/or indicate the reference source and copyrights. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is 'original', the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2023.

2. **If an author of a submission is re-using a figure or figures published elsewhere, or that is copyrighted, the author must provide documentation that the previous publisher or copyright holder has given permission for the figure to be re-published; and correctly indicating the reference source and copyrights. For example, "Figure 1 Histopathological examination by hematoxylin-eosin staining (200 ×). A: Control group; B: Model group; C: Pioglitazone hydrochloride group; D: Chinese herbal medicine group. Citation: Yang JM, Sun Y, Wang M, Zhang XL, Zhang SJ, Gao YS, Chen L, Wu MY, Zhou L, Zhou YM, Wang Y, Zheng FJ, Li YH. Regulatory effect of a Chinese herbal medicine formula on non-alcoholic fatty liver disease. World J Gastroenterol 2019; 25(34): 5105-5119. Copyright ©The Author(s) 2019. Published by Baishideng Publishing Group Inc[6]". And please cite**

the reference source in the references list. If the author fails to properly cite the published or copyrighted picture(s) or table(s) as described above, he/she will be subject to withdrawal of the article from BPG publications and may even be held liable.

Figures were designed de novo using BioRender tools. Attached at the end of this comment the website where you can consult the editable template (you just need to log in first).

I confirm that I have authorization from BioRender for the publication of the figure in research journals, for which I am attaching the license letter for publication issued by BioRender. On the other hand, according to the instructions by BioRender, it is indicated that I must include the following information as part of the Figure legend: "*All completed graphics must be accompanied by the following citation: "Created with BioRender.com".* So, the figure follows the guidelines indicated by the BioRender company. However, I have doubts about how to proceed in this regard, to comply with the journal guidelines but also comply with Biorender's guidelines, so I appreciate your guidance in this regard.

https://app.biorender.com/illustrations/644466d4f6e3f9d4931e1ce8

3. **Authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content.**

The changes indicated in the tables were made.