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Neurotoxic Effects of Pesticide Mixtures: A Systematic Review of the Experimental Literature

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Abstract

Studies on the effects of pesticide mixtures on the nervous system have not been reviewed. The aim of this article was to systematically review the literature on neurotoxicity induced by a mixture of pesticides. The review was limited to mammalian, neurobehavioural, neuropathological and neuropharmacological studies; *in vitro* and *in vivo* and other biochemical studies were considered. Inclusion criteria were developed a priori to ensure consistent assessment of the studies. Only studies published from January 2000 to December 2022 were included in the study. Limitations included the use of a single dose and/or test time, small sample sizes, limited data presentation and/or obviously toxic doses. Of the 2145 studies selected, only 7 were included, including 6 *in vivo* studies and 1 *in vitro* study. The studies differed in terms of species tested, pesticides, dosing scenarios and experimental designs. Two studies had assessed the evidence of an additive or synergistic effect of pesticides in co-exposure, but further long-term analyses should confirm this hypothesis. Other studies had less convincing results due to their exposure protocols. Taken together, despite the differences in quality between the studies, the pesticides studied and the exposure method, these studies require additional tests. Taken together, despite the differences in quality between the studies, the pesticides studied and the exposure method, these studies require additional tests.

Subject Areas

Neuroscience, Toxicology

Keywords

Pesticide Mixtures, Neurotoxicity, Chronic Exposure, Additive Effects, Synergistic Effects

1. Introduction

Pesticides are natural or synthetic substances that can control, attract, repel, kill or stop the development of living organisms (microbes, animals or vegetables) considered as harmful to agricultural production, to hygiene or public health, and veterinary health [1]. Pesticides have become one of the most important components of the world's agricultural system and are widely used to enhance crop yield and food production [2]. Due to their widespread use, human exposure to pesticides can occur via the dietary route following the ingestion of food products or water containing pesticide residues. Human exposure can also occur by the cutaneous route or by inhalation mainly in vicinity of agricultural or residential areas [3] [4]. Pesticide residues are still an important risk to human health even after foodstuffs transformation [5] [6].

In recent years, despite the many benefits of using pesticides in agriculture, their use has raised some public health concerns [7]. Published experimental approaches highlight the fact that pesticides and their metabolites are potential risk factors for many diseases in the human population [8] [9]. Therefore, several toxicological and ecotoxicological studies, including teratogenicity, neurotoxicity, nephrotoxicity, reproductive toxicity, and carcinogenicity [10]-[14] have reported serious effects of pesticides on human health. Damage to the central nervous system is one of the three main research areas into the health effects of pesticides, together with cancer and reproductive problems [15] [16]. With the increase in cases of pest resistance, professionals are increasingly turning to the combined use of a large number of pesticides simultaneously or sequentially in the integrated fight against vectors and pests [17]-[19]. Therefore, the adverse effects of pesticides on human health are often due to co-exposure to them [20]. It is then worth noting that the increase in risk of a substance individually taken must be globally different from the increase in risk of a xenobiotic taken in combination. However, a xenobiotic may not cause toxic consequences at certain concentrations, usually very low when tested individually, but is most likely to be harmful if it is part of a mixture [21]. Thus, it is possible that two or more chemicals may induce outcomes that are ignored when the toxicity of the xenobiotic is examined in isolation [22] [23]. Several experimental studies with mixtures show that simultaneous exposure to several potentially toxic substances can lead to additive, antagonistic or synergistic effects [23]-[25]. The frequent occurrence of synergies has led to the development of specific assessment strategies and regulatory approaches aimed at protecting against synergies in general [26]-[28].

Among the adverse effects attributed to pesticides are neurological disorders,

especially as epidemiological studies have shown an association between pesticides and the onset of several neurological disorders [29]-[32]. The increase in the number of people suffering from neurological disorders justifies the need to identify risk factors and develop strategies to prevent and treat these conditions [33]. However, there has been no systematic analysis of the literature on the risk of neurotoxic effects from pesticide mixtures. This scientific shortcoming was one of the main reasons for this study, in addition to the scientific task of systematically analyzing the literature on the neurotoxicity of pesticide mixtures.

2. Materials and Methods

2.1. Sources of Information

We conducted extensive and exhaustive literature searches in PubMed, Scopus, and Web of Science in December 2022. The filters used for the database searches were English and French. A temporal filter was applied and articles published between January 2000 and December 2022 were included in this review. The search formula was: ((“Pesticides” [MeSH]) OR (“Insecticides” [MeSH]) OR (“Herbicides” [MeSH])) AND ((“Neurobehavioral Manifestations” [MeSH]) OR (“Neurotoxicity Syndromes” [MeSH]) OR (“Brain” [MeSH]) OR (“Developmental Disabilities” [MeSH]) OR (“Memory” [MeSH]) OR (“Learning” [MeSH]) OR (“Depression” [MeSH])). MeSH terms were used in the PubMed literature search. In Scopus, an asterisk was used to replace any number of characters to expand the search, TITLE-ABS-KEY ((pesticides* OR herbicides* OR insecticides*) AND (Neurobehavioral Manifestations OR Neurotoxicity syndromes OR Brain* OR Developmental Disabilities OR Memory* OR Learning* OR Depression*)). The Web of Science search included a subject (title, abstract and keywords) with the formula (TS = (pesticides* OR herbicides* OR insecticides*)) AND (TS = (Neurobehavioral* Manifestations* OR Neurotoxicity* Syndromes* OR Brain* OR Developmental* Disabilities* OR Memory* OR Learning* OR Depression*)).

2.2. Review Protocol

Prior to the literature search, a detailed review protocol was prepared in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocol (PRISMA-P) [34].

2.3. Eligibility Criteria

The systematic review was initially structured using the acronym PICOS (Participants, Interventions, Comparators, Outcomes measures, Study design). The eligibility criteria for this systematic review were developed a priori to ensure consistent identification of studies on the neurotoxicity of pesticide mixtures. For greater efficiency, the criteria were applied in two stages, with the first-pass eligibility criteria based mainly on examination of titles and abstracts, and the second-pass criteria based on assessment of the full text of the article.

First-pass eligibility criteria:

Study type: *In vivo* studies in rodents and *in vitro* studies designed to assess cellular, biochemical, and/or molecular mechanisms with a focus on neurological or neuropathological endpoints of pesticide mixtures were considered for inclusion on a case-by-case basis. Studies with a reproductive or endocrine focus were not included (unless they included behavioral or neuropathological/pharmacological endpoints in parent or offspring animals), with the possible exception of thyroid hormone studies (with neurological and/or neuropathological correlates). Exploratory bioinformatic studies without neuropathology or neurological correlates were not included.

Exposure: studies using any route of exposure were included, with the exception of direct injection into the brain. For *in vivo* studies, the criteria used for experimental studies in rodents were applied. Studies using mixtures containing other chemicals in addition to pesticides were not included. Only non-acute multiple-dose studies were considered.

Only full-text articles were included; abstracts, conference proceedings or regulatory data abstracts/summaries were excluded. Only original research articles were considered, *i.e.*, not review articles, case studies, meta-analyses, editorials or commentaries.

Second round of full-text assessment: Second-round inclusion criteria

Studies that met the first-round criteria were assessed in more detail using the full-text article. Specific considerations for this second-round assessment, which are reflected throughout this review, are listed below:

Controls: The study should have a concurrent control group receiving the same vehicle as the treatment groups (if formulated, the formulation vehicle must be used). *In vivo*, vehicle studies with inherent neurological activity (e.g., dimethyl sulfoxide: DMSO and solvents) were considered to be of low quality. *In vitro*, studies with high vehicle concentrations (e.g., >0.5%) were considered to be of low quality. The performance, health, and response of the controls should be within normal values established in the laboratory or general literature.

Sample size: Studies should have sufficient animals per group or replicates (e.g., plates/wells for *in vitro*) per concentration, based on expert judgement and appropriate test guidelines, if available. Small sample sizes ($n \leq 3$/group, *in vivo*) or replicates ($n < 3$, *in vitro*) were considered to be pilot studies of low relevance. The sample size should be clearly reported for each parameter measured. For cell culture studies, the number of isolations for primary cells and the number of passages/flasks of frozen stocks of continuous passage cells should be reported.

- Statistics: Studies should report measures of central tendency (e.g., means, medians) for the groups as well as measures of variability. Statistical comparison with controls should be included. If the study is a developmental study, litter should be taken into account in the study design and statistics. A clear description and justification of the statistical approach should be provided. Studies with a lack of adequate statistical presentation and analysis were considered to be of poor quality.

Exposures: Studies should clearly state the dose(s) prepared and administered together with the method of exposure/application, including route, frequency, and duration. Studies involving dietary or drinking water intake of the test substance should assess consumption to verify the actual intake of the test substance. An unverified report of the dose administered was considered to be of poor quality. Any confounding exposure should be described and, if present, accounted for or discussed in the analysis. Appropriate routes include oral, dermal, inhalation, subcutaneous, intraperitoneal, and intranasal.

Toxicity: Neurotoxicity findings should be clearly described, including the nature, frequency, time of onset, severity, and duration of effects. Neurotoxicity findings associated with overt toxicity (e.g., weight loss, lethality, and cell death) are considered to be of limited relevance.

Test system: Studies should be adequately described. *In vivo*, this includes species, age, sex, state of health, life stage, source, and rearing. *In vitro*, this includes cell culture source, storage, passages, purity, composition, origin, negative and positive controls. Performance, health, and response of controls within normal values established in the literature.

Endpoints: Studies should have a full description of the test methods and results should be consistent with the endpoints discussed in the methods. They should be relevant to human health (functional, morphological and physiological in animals) and/or reasonably predictive of human neurotoxicity findings (e.g., *in vitro*, alternative species). Experimental procedures, including test duration and dosing, should be balanced between dose groups.

Dose: Doses associated with overt toxicity are considered to be of low quality.

Exclusion criteria were those that did not meet the previously defined PICOS characteristics.

2.4. Studies Selection and Data Collection

After duplicates were removed, a reviewer assessed the full list of results for eligibility. Where other relevant decisions needed to be made, these were discussed within the research team until consensus was reached. Any disagreements were resolved until a consensus was reached.

2.5. Risk of Bias in Individual Trials

"The SYRCLE tool for the assessment of risk of bias [35], which is based on the Cochrane Collaboration RoB tool [36] and adapted to aspects of bias in animal studies, was used to assess the methodological quality of the included animal studies.

SYRCLE includes 5 quality parameters: selection, power, detection, attrition, and reporting bias. It awards a maximum of 6 points for selection, 4 points for performance, 4 points for detection, 4 points for attrition, and 4 points for reporting (out of a total of 18 points). Consequently, the total quality index score is ranked as follows: 0 to 3, 4 to 6, 7 to 9, 10 to 12, 13 to 15, and 16 to 18, corresponding to Very Low (VL), Low (L), Medium Low (ML), Medium High (MH), High

(H), and Very High (VH), respectively.

2.6. Summary Measures and Analysis

We conducted a descriptive and critical review according to the above protocol. Our summary measures take the form of qualitative and quantitative interpretation, and narrative analysis.

3. Results

A flow chart illustrates the overall search strategy (Figure 1). The initial screening produced a total of 2145 studies, and after removal of duplicates and selection of articles by year, language and journal exclusion, a total of 1906 articles were selected. In addition, 1899 studies were excluded after title and abstract screening. Thus, the total number of studies included in this review was 7, including 6 *in vivo* studies and 1 *in vitro* study.

All preclinical studies on exposure to pesticide mixtures and neurotoxicity are summarized in Table 1.

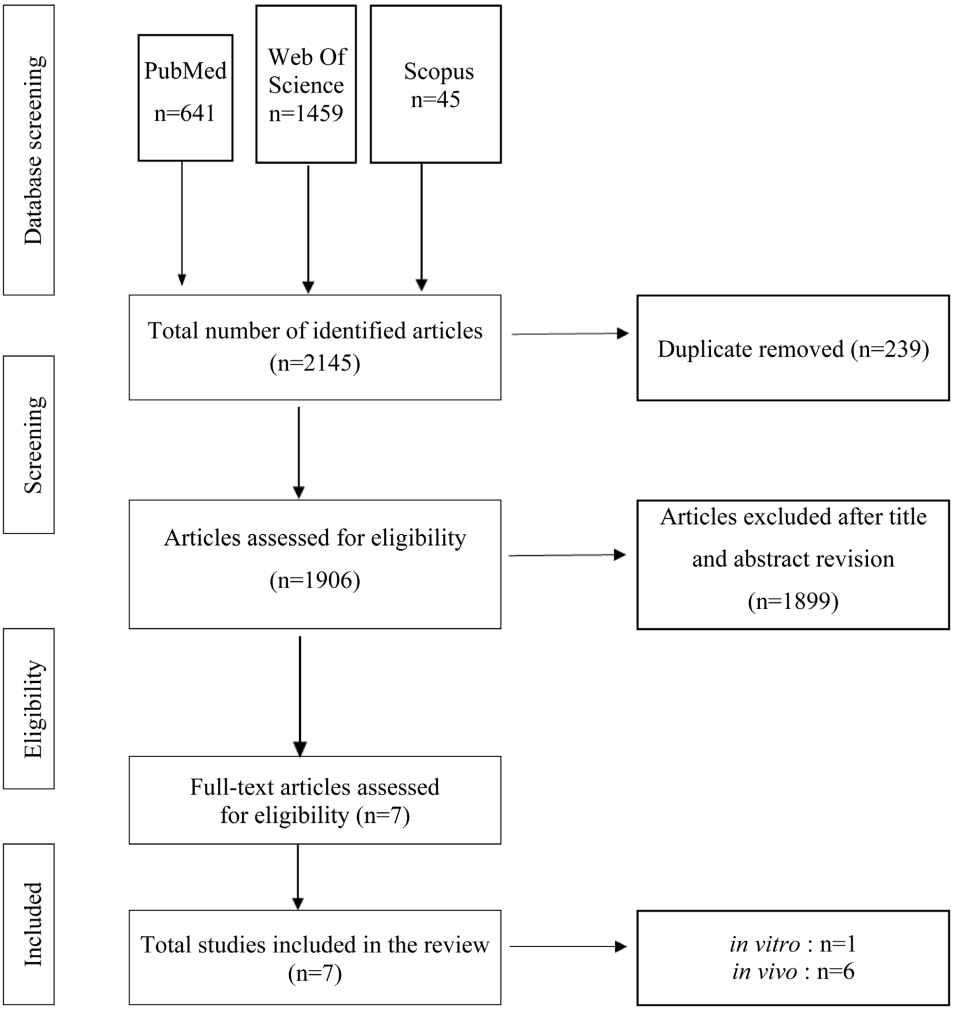

Figure 1. Flow chart of study selection.

Table 1. Neurotoxicity studies of pesticides mixture on animals.

Study, Year (Reference)	Strain/Age at Evaluation/Sex	Exposure Agent/Dose/Age/Route	Behavioral/ neuropathological/ pharmacological tests	Behavioral/Pharmacological/Physiological Outcomes	Quality Index
Kumar V *et al.* [37]	Adult male albino rats weighing 190 ± 10 g	Monocrotophos (MCP) (2.0 mg/kg,); Dichlorvos (DDVP) (2.0 mg/kg,); MCP (1 mg/kg) + DDVP (1 mg/kg) for 30 days Oral administration	Biochemical Assays in serum; Acetylcholinesterase activity, Dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT), Monoamine oxidase activity in brain	DDVP and MCP alone and in combination have modified the activity of acetylcholinesterase (AChE), DA, NE in rats	MH
Sharma P *et al.* [38]	Adult male and female Wistar rats	(Dichlorvos (8.8 mg/kg bw); Lindane (8.8 mg/kg bw); Dichlorvos + lindane (8.8 mg/kg bw each)) for 14 days; (Dichlorvos (8.8 mg/kg bw); Lindane (8.8 mg/kg bw); Dichlorvos + lindane (8.8 mg/kg bw each)) for 14 days followed by ginger juice (100 mg/kg bw) for 14 days Oral administration	Biochemical estimations (Lipid peroxidation (LPO), Reduced glutathione, Superoxide Dismutase, Catalase, Glutathione-S-Transferase, Glutathione peroxidase, Glutathione reductase, Quinone reductase) in brain	The oral exposure of Dichlorvos and Lindane causes a significant increase in oxidative stress in cerebral tissue of male and female Wistar rats as evident from increased level of LPO in groups Dichlorvos (D), Lindane (L) and D + L. The reduction in level of non-enzymatic and enzymatic antioxidants was more in group D + L as compared D and L	MH
Roede *et al.* [39]	SH-SY5Y human neuroblastoma cells	PQ (50 µM); MB (1.5 µM) et PQ (50 µM) + MB (1.5 µM) for 24 hours	GSH, Thioredoxin analysis	At 24 h, PQ þ MB caused a significantly more negative EhGSSG, *i.e.*, a more reducing redox potential	ML
Latuszynska *et al.* [40]	3-months-old female Wistar rats from 190 to 230 g.	27.8 mg/cm^2 of Chlorpyrifos and 2.7 mg/cm^2 of Cypermethrin dermally for a period of 1 and 4 weeks (4 h daily).	Brain acetylcholinesterase activity	Acetylcholinesterase (AChE) levels in the brain initially decreased after dermal exposure to a mixture of Chlorpyrifos and Cypermethrin and recovered to control levels 3 weeks after the end of the repair at one week and 4 weeks; slight histopathological changes in various areas of the brain to increase the density of cytoplasm in neurocytes in both experimental groups 3 weeks after exposure	L
Ma J *et al.* [41]	Virgin female (230 ± 20 g) and male (310 ± 20 g) Sprague-Dawley rats	Paraquat (PQ) (15 mg/kg) + Maneb (MB) (45 mg/kg); PQ (10 mg/kg) + MB (30 mg/kg) by gavaging (oral administration) twice a week from gestation to weaning	Dopamine dosage; Western blotting	The exhibition combined with PQ and MB during gestation and lactation alters expression of proteins associated with the formation and development of dopaminergic neurons in offspring. During pregnancy and lactation leads to an upregulation	L

				of protein expression Wnt5a and a negative regulation of protein expression Wnt1, Nuclear receptor related factor 1 (Nurr1) and Tyrosine Hydroxylase (TH)	
Forner-Piquer *et al.* [42]	Male and female C57BL/6j mice	Ziram (0.006), Thiophanate (0.08), Captan (0.1), Chlorpyrifos (0.01), Boscalid (0.04), and Thiacloprid (0.01); mg/kg BW/day from weaning and up to 14 months. Mouse perinatal exposure by gavaging (oral administration)	Open Field (OF); Y-maze; Light/Dark Transition test; Elevated-Plus-Maze (EPM); Social Interaction test; Rotarod test. Mouse brain histology	Perinatal dietary exposure to a cocktail of low-dose pesticides is associated with behavioral and neurophysiological changes in aging male mice with absence of major structural brain malformations	L
Klement W *et al.* [43]	C57BL/6j male and female mice	Ziram (0.006), Thiophanate (0.08), Captan (0.1), Chlorpyrifos (0.01), Boscalid (0.04), and Thiacloprid (0.01); mg/kg BW/day from weaning and up to 12 months by gavaging (oral administration)	Brain immunohistochemistry and quantifications; Open field (OF); Y-maze; Light/Dark Transition test; Elevated-Plus-Maze (EPM)	Installation of spatiotemporally confined hippocampal astrogliosis and perivascular pro-fibrotic changes as well as sex-specific changes behavioral changes caused after long-term but not short-term dietary exposure low-dose pesticides. Simultaneously, significant changes to the P450 device metabolic and detoxification pathways were triggered and endured by pesticides exposure	L

Several pesticide combinations were used in the rodent studies (6 out of 7; 85.6%). The most commonly used pesticide in the combinations was Chlorpyrifos [40] [42] [43]. The majority used exposure to a mixture of two pesticides (4 out of 6; 83.3%) [37] [38] [40] [41]. Two other studies used more than two pesticides, *i.e.*, six (Ziram, Thiophanate, Captan, Chlorpyrifos, Boscalid, and Thiacloprid) [37] [38] [43].

Among the studies, two used mice [38] [43] and four used rats [37] [38] [41]. Of all these studies, only one [37] used the cutaneous route of administration and the others used the oral route [38] [41] [43]. One used perinatal exposure (16.6%) [43], two used a gestational exposure protocol (33.3%) and three used adults (50%).

Regarding the *in vitro* study, Roede *et al.* (2011) [39] used SH-SY5Y human neuroblastoma cells exposed to the pesticides Paraquat (PQ) and Maneb (MB). Two studies assessed cognitive, anxiety or depressive behavior [38] [43]. In addition, five published articles assessed neuropharmacological parameters [37] [38] [41].

In terms of study quality, two of the seven studies were classified as MH (28.6%)

[37] [38], four as L (57.1%) [40]-[43] and the last as ML (14.3%) [39].

4. Discussion

Our review of the literature revealed that few studies have addressed the issue of neurotoxicity associated with exposure to pesticide mixtures. The studies reviewed show that the use of combinations of substances aims to reproduce realistic conditions, as human populations are simultaneously exposed to several pesticides through food and the environment. This co-exposure can lead, depending on chemical interactions, to amplified (synergy) or attenuated toxic effects compared to those expected based on data from individual substances [44]-[46].

Although the use of mice remains scientifically relevant, our analysis reveals a clear predominance of models using rats. This methodological choice is justified by the genetic and physiological proximity of rats to humans, particularly in terms of social behavior [39]. The majority of studies were conducted through oral exposure. Oral exposure remains the main route of contamination in the general population [4] [6] [47], while skin exposure mainly affects applicators and agricultural workers. The protocols examined reflect a diversity of exposure by incorporating different life stages (perinatal, gestational, or adult), highlighting that the risk potentially affects all age groups.

In vitro studies, although fewer in number, provide additional insight into the mechanisms of toxicity by offering precise control of experimental parameters and facilitating the exploration of specific biological pathways.

All of these studies followed specific neurotoxicity test guidelines [48], with endpoints limited to motor activity assessment, observational assessments and neuropathology. Although these tests represent only a few of the many neurological tests used in neurotoxicity studies, they are considered a broad screen, *i.e.*, tests that can be induced by chemicals acting by many different mechanisms and are therefore capable of detecting a range of neurotoxic chemicals [49] [50]. Motor activity is an apical behavior that reflects a number of underlying processes, including motor capacity, sensory function, emotional processing, and non-associative learning (habituation). Therefore, changes in motor activity may reflect changes in any of these functions as well as non-specific toxicity. Furthermore, it cannot be considered a specific measure of motor function per se, and changes cannot be directly and unambiguously related to changes in the function or structure of specific brain regions [51].

The studies labelled as L were characterized as following a protocol of exposure only to a mixture of pesticides, without exposure to individual pesticides. However, Ma *et al.* (2017) [41] evaluated a dose-dependent exposure to a mixture of PQ and MB over four weeks. At high doses, combined exposure to PQ and MB during pregnancy and lactation resulted in an alteration in Wnt1 protein expression and a non-significant decrease in dopamine. Latuszynska *et al.* (2003) [40], following dermal exposure of adult male Wistar rats to 27.8 mg/cm^2 of chlorpyrifos and 2.7 mg/cm^2 of cypermethrin, reported a decrease in brain acetylcholinesterase (AChE)

levels with recovery to control levels 3 weeks after exposure.

In their studies, Forner-Piquer *et al.* (2021) [42] and Klement *et al.* (2020) [43] exposed mice to a mixture of 6 pesticides for 12 months or more. Forner-Piquer *et al.* (2021) [42] reported behavioral and neurophysiological changes in ageing male mice after long-term perinatal dietary exposure (14 months), but no significant changes in young mice (2 - 3 months). In particular, there was an increase in spontaneous locomotor activity compared to the control and an increase in anxiety-type behavior when tested in the elevated maze. On the other hand, no major structural brain abnormalities were observed.

Discrete, time-dependent astrogliosis and sex-specific behavioral changes (in male mice) have been reported by Klement *et al.* (2020) [43]. In this study, the behavioral deficits reported here were not associated with other major deficits, suggesting that long-term exposure to dietary pesticides may be a permissive but not quite sufficient risk factor for permanent cognitive changes. Despite the behavioral and neurophysiological changes reported by Forner-Piquer *et al.* (2021) [42] and Klement *et al.* (2020) [43], the toxicological relevance of these effects is not sufficiently established. These findings therefore require further mechanistic and molecular analysis.

Roede *et al.* (2011) [39], in their ML-classified study, used SH-SY5Y human neuroblastoma cells and exposed them to PQ (50 μM); MB (1.5 μM), and PQ (50 μM) + MB (1.5 μM) for 24 hours. The authors reported that the potentiation of PQ neurotoxicity by MB was not due to an increase in oxidative stress. The results provide little evidence for synergy or potentiation of toxicity of one by the other.

Finally, the studies classified as MH were those of Kumar *et al.* (2018) [37] and Sharma *et al.* (2012) [38], which had used the co-exposure of Dichlorvos + Lindane, and Monocrotophos (MCP) + Dichlorvos (DDVP), respectively. In these studies, the effects of the pesticides alone and in combination on the central nervous system were assessed by measuring parameters of oxidative stress and neurotransmitters, a crucial assessment criterion that has been correlated with xenobiotic toxicity [52] [53].

Kumar *et al.* (2018) [37] exposed adult male albino rats to 2.0 mg/kg MCP, 2.0 mg/kg DDVP, and 1 mg/kg MCP + 1 mg/kg DDVP for 15 and 30 days. They observed a marked difference in serum AChE activity, and changes in brain Norepinephrine (NE) and Dopamine (DA) levels, which were almost similar to MCP alone in the combined DDVP + MCP group compared with the individual groups after 30 days of exposure. Levels of 5-hydroxytryptamine (5-HT) showed no change in activity during co-exposure. In addition, after 15 days of exposure, MCP alone had a more pronounced inhibition of serum AChE activity than DDVP, which was almost similar to the combined DDVP + MCP group. As for the levels of NE, DA and 5-HT, the combined exposure to these toxic substances did not result in more pronounced toxicity than their individual exposure, except in the case of norepinephrine. In contrast, the study by Sharma *et al.* (2012) [38] focused on the modification of oxidative stress factors. They performed oral exposure of Dichlorvos

(8.8 mg/kg), Lindane (8.8 mg/kg), and Dichlorvos + Lindane (8. 8 mg/kg each) for 14 days and found that the levels of Glutathione (GSH), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Glutathione Reductase (GR), Glutathione-S-Transferase (GST) and Catalase (CAT) decreased during exposure to Dichlorvos and Lindane in rats of both sexes and more significantly in the combination (Dichlorvos + Lindane), probably indicating an increased state of cellular oxidative stress in the exposed rodents. These statistical results can be considered as evidence of an additive or synergistic effect of pesticides in co-exposure, but further long-term analyses should confirm this hypothesis. This explains the classification of these studies as MH.

To further assess the weight of evidence for neurotoxicity, it may be advantageous to integrate behavioral and other neuropathological/neuropharmacological findings where study designs allow (same animals used). None of our seven studies investigated both behavioral and neuropathological/neuropharmacological endpoints. It should be emphasized that, despite the differences in quality between the studies, the pesticides studied and the exposure method, these studies require further investigation.

5. Conclusions

This review provides a synthesis of the available evidence from different sources assessing the association between exposure to pesticide mixtures and neurotoxicity. Our research revealed that the neurotoxicity of pesticide mixtures has been less well studied. The studies identified in the systematic literature review provided little clear evidence of neurobehavioral, neuropathological or neuropharmacological disturbances. In addition, many of the studies were methodologically weak and the results were inconsistent and/or of uncertain toxicological relevance. Researchers should increasingly focus on assessing the toxicity of low-dose mixtures of chemicals in general and pesticides in particular.

Future neurotoxicity studies assessing co-exposure to pesticides would be more useful for risk assessment purposes if they used relevant routes of exposure (e.g., oral and intraperitoneal), specified the test material used (e.g., technical glyphosate, saline form or formulation), included *in vivo* and *in vitro* studies, and used accurate test methods and solid scientific principles in neurological and neuropathological assessments.

Conflicts of Interest

The authors declare no conflicts of interest.

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