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Efeitos da exposição ao glifosato em parâmetros comportamentais de roedores: uma revisão sistemática.

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Resumo da Dissertação

Introdução: Os agrotóxicos são de suma preocupação global, existindo uma crescente comercialização mundial e aumento das informações na literatura sobre sua toxicidade e danos à saúde humana e ambiental. O glifosato (Roundup®) se destaca pela ampla utilização e comercialização, sobretudo na cultura da soja com as sementes geneticamente modificadas comercializadas pela Monsanto. Embora a toxicidade relatada na literatura seja baixa, há evidências sobre neurotoxicidade como alterações no sistema monoaminérgico, mudanças comportamentais e correlações com transtornos mentais e de desenvolvimento. Contudo, os estudos disponíveis não comparam os resultados nem caracterizam a qualidade das evidências geradas. **Objetivo:** Revisar sistematicamente os artigos disponíveis em bancos de dados sobre a exposição de roedores ao glifosato e sua relação com alterações comportamentais mais comuns, como ansiedade e depressão. **Materiais e métodos:** A estratégia de pesquisa foi definida através do método PICOT, e da questão de pesquisa: “Quais são as evidências disponíveis na literatura sobre a associação entre a exposição ao herbicida glifosato e efeitos neurocomportamentais?". Após a análise de título e resumos, os resultados da busca nas bases de dados foram tabulados em planilha do software Excel e comparados entre dois revisores independentes; em caso de ausência de consenso, um terceiro revisor foi consultado. Os artigos selecionados foram lidos na íntegra e verificados conforme os critérios de elegibilidade. O artigo foi redigido de acordo com o protocolo PRISMA e o risco de viés das metodologias dos estudos foi avaliada através da ferramenta de análise de viés SYRCLE. **Resultados:** A busca nas

bases de dados, de acordo com os filtros utilizados, gerou 2158 resultados no Pubmed, 311 no LILACS/Bireme BVS, 627 no Scopus e 0 na Scielo. Dos 16 artigos selecionados, acerca das desordens mentais avaliadas 56,25% dos autores avaliaram ansiedade, 43,75% avaliaram depressão, 43,75% avaliaram a memória e somente 1 artigo avaliou comportamentos relacionados a síndrome do espectro autista, como a interação social, devido a exposição de roedores ao glifosato. **Conclusões:** De acordo com os resultados encontrados nos estudos referentes às alterações nos parâmetros avaliados pelos testes comportamentais, conclui-se que a exposição ao glifosato está relacionada a indução de comportamentos do tipo ansioso e depressivo, alterações locomotoras, de memória e interação social.

Palavras-Chave: Glifosato; Neurotoxicidade; Comportamento, Modelo Animal.

Abstract

Introduction: Pesticides are of great global concern with a growing worldwide commercialization and increasing information in the literature about their toxicity and damage to human and environmental health. Glyphosate (Roundup®) stands out for its wide use and commercialization, especially in soybean cultivation with Monsanto genetically modified seeds. Although the toxicity reported in the literature is low, there is evidence of neurotoxicity such as changes in the monoaminergic system, behavioral changes, and correlations with mental and developmental disorders. However, the available studies do not compare the results or characterize the risk of bias of the methodologies used. **Objective:** To systematically review the articles available in databases on the exposure of rodents to glyphosate related to the most common behavioral changes, such as anxiety and depression. **Materials and methods:** The search strategy was defined using the PICOT method, and the research question: “What evidence is available in the literature on the association between exposure to the herbicide glyphosate and neurobehavioral effects?” After analyzing titles and abstracts, the search results from databases were tabulated in Excel datasheet and compared between two independent reviewers; a third reviewer was consulted if there was no consensus between the reviewers. The selected articles were read in full and verified according to the eligibility criteria. The article was written according to PRISMA protocol and the risk of bias of the methodologies was evaluated using the SYRCLE risk of bias tool. **Results:** The search in the databases, according to the filters used, generated 2,158 results in Pubmed, 311 in LILACS/Bireme BVS, 627 in Scopus, and 0 in Scielo. Of the 16 selected articles, between the evaluated behaviors, 56.25% of the authors evaluated anxiety, 43.75% evaluated

depression, both associated or not to other conditions. Furthermore, seven studies evaluated memory alterations through tests based on recognition and avoidance, resting only 1 article that analyzed behaviors related to autism spectrum syndrome, such as social interaction, due to the exposure of rodents to glyphosate. **Conclusions:** According to the results found in the studies regarding the alterations in the parameters evaluated by the behavioral tests, it is concluded that the exposure to glyphosate is related to the induction of anxious and depressive behaviors, locomotor alterations, memory, and changes in social interaction.

Keywords: Glyphosate; Neurotoxicity; Behavior; Animal model.

Lista de Abreviaturas:

ANVISA: Agência Nacional de Vigilância Sanitária

AOEL: Nível Aceitável de Exposição Ocupacional

CNS: Sistema Nervoso Central

DL50 - Dose letal 50 (dose necessária de uma substância para matar 50% da população em um teste).

FAO: *Food and Agriculture Organization of the United Nations*

IDA: Ingestão Diária Aceitável

NOAEL: Nível Sem Efeitos Adversos Observáveis

WHO: *World Health Organization*

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SUMÁRIO

1. REFERENCIAL TEÓRICO

Uma das maiores preocupações globais são os agrotóxicos devido a sua crescente comercialização no mundo e ao aumento de informações na literatura acerca da sua toxicidade e malefícios tanto para a saúde do ser humano, quanto para o meio ambiente. A preocupação surge quando a maioria dos pesticidas mundialmente disponíveis ainda possui componentes tóxicos em sua fórmula, sendo danoso não somente para o seu alvo, mas também para outros seres vivos. Ademais, estima-se que a produção de agrotóxicos aumentou em mais de 3 milhões de toneladas[1], sendo utilizados principalmente em países como Brasil, China, Estados Unidos e Argentina [2].

Os agrotóxicos são classificados atualmente de acordo com a *Food and Agricutural Organization* (FAO) como “Qualquer substância ou conjunto de substâncias químicas ou biológicas destinadas a repelir, destruir ou controlar qualquer praga ou regular o crescimento das plantas” [3]. Além disso, estão disponíveis no mercado através de formulações contendo solventes e aditivos em conjunto com o princípio ativo, ou até mesmo junto de compostos com o objetivo de potencializar a ação da substância principal [4].

Na última década, a quantidade de publicações associando a exposição aos agrotóxicos à patologias humanas disparou consideravelmente, pois devido a seus mecanismos serem direcionados para seres vivos com organismos totalmente diferentes dos seres humanos, a possibilidade de haver algum malefício para estes era praticamente ignorada[5]. Atualmente, devido às pesquisas, já se sabe que podem não só induzir imunotoxicidade e desregulação endócrina assim como atravessam a barreira hematoencefálica causando

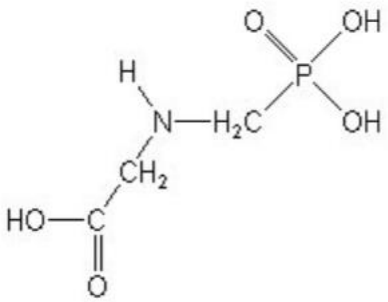
neurotoxicidade e transtornos de desenvolvimento e mentais como consequência [6].

1.2 Glifosato

O Glifosato é um herbicida não-seletivo, de ação sistêmica e pós-emergente [7]. Pertencente à classe dos organofosfatos e ao grupo químico das glicinas substituídas, ganhou atenção mundial recentemente devido à sua ampla comercialização e utilização em sementes geneticamente modificadas, principalmente em culturas de soja. O uso de glifosato mundialmente chegou a aumentar 15 vezes devido à implantação das sementes geneticamente modificadas pela Monsanto em 1996 [8].

A sua estrutura química (Figura 1) permite que atue como potente inibidor da atividade da 5-enolpiruvilshiquimato-3-fosfato sintase (EPSPS), levando ao bloqueio da via do ácido chiquímico, principal via de produção dos aminoácido aromáticos (fenilalanina, tirosina e triptofano), assim como à inibição da síntese de clorofila, estimulação da produção de etileno, redução da síntese de proteínas e por último, elevação da concentração do IAA [9].

Figura 1: Estrutura química do glifosato



*Fonte: ANVISA (2019)

Atualmente, é encontrado principalmente na formulação de sal de isopropilamina de glifosato, no entanto, pode ser encontrado também como sal de potássio de glifosato, sal de amônio de glifosato e sal de dimetilamina. Todas as formulações possuem classificação IV pela Agência Nacional de Vigilância Sanitária (ANVISA) e classificação baixa de acordo com a Organização Mundial da Saúde (WHO), enquanto a DL50 (Dose letal 50%) oral de glifosato puro em ratos é de 4230 mg/Kg e a NOAEL (Nível Sem Efeitos Adversos Observáveis) é de 1000 mg/Kg.

Um dos principais fabricantes de formulações com glifosato é a Monsanto, sendo estas popularmente conhecidas como Roundup®. Originalmente, o Roundup era constituído de 48% (m/v) de sal de isopropilamina de glifosato; atualmente, existem outros produtos da linha Roundup® com concentrações maiores. Além de formulações prontas de glifosato, a Monsanto comercializa também sementes geneticamente modificadas, as tornando mais resistentes à aplicação de agrotóxicos e consequentemente permitindo uma maior aplicação de glifosato sem danificar a plantação[10].

Apesar da baixa toxicidade deste pesticida, a literatura científica vem relatando malefícios para o organismo humano tanto do princípio ativo quando das formulações Roundup.[5]. Ademais, Defarge, N. (2018) demonstra em seu estudo que a toxicidade das formulações contendo glifosato é mais alta do glifosato em si, tanto para plantas quanto para células humanas, mesmo que o seu mecanismo de toxicidade ainda não seja bem conhecido.

Outra questão relevante é a toxicidade reprodutiva manifestada em vários ensaios *in vivo* e *in vitro*, em que diferentes pesquisadores sugerem que este herbicida é um provável desregulador endócrino [12–14]. Além de alterações no

sistema endócrino, Cattani e colaboradores (2017) relataram estresse oxidativo, disfunção astrocitária e comportamento depressivo[16].

A exposição ao glifosato pode ocorrer através de diversas formas, seja por resíduos na alimentação e na água potável, ou por meio da exposição ocupacional e ambiental, caracterizando um risco para a população em geral [17,18]. Atualmente, após reavaliação e considerando estudos internacionais, a ANVISA determinou a IDA (Ingestão Diária Aceitável) do Glifosato como 0,5 mg/kg/dia, e a AOEL (Nível Aceitável de Exposição Ocupacional) como 0,1 mg/kg/dia [19]. No entanto, ainda não está bem estabelecido o risco de exposição pela dieta, devido ao aumento de indivíduos portadores de intolerância à lactose, assim como de vegetarianos e veganos, classificando um risco principalmente para crianças, gestantes e lactantes, decorrente da ingestão de grandes quantidades de produtos derivados de soja em sua dieta, podendo ultrapassar a ingestão diária aceitável [20,21].

1.3 Neurotoxicidade do glifosato

Os mecanismos da neurotoxicidade da exposição ao glifosato ainda não são bem esclarecidos na literatura, assim como a sua relação com alterações comportamentais e o desenvolvimento de transtornos do desenvolvimento e mentais. Estudos recentes relacionam mecanismos neuroquímicos com a neurotoxicidade do pesticida, descrevendo excitotoxicidade glutamatérgica[22], além de decréscimo da atividade da acetilcolinesterase em eritrócitos *in vitro* [23]. Ainda, Martínez e colaboradores (2018) relataram em seu estudo o decréscimo de serotonina (5-HT), dopamina (DA) e norepinefrina (NE) no sistema nervoso

central (SNC), indicando que a exposição ao glifosato induz alterações significativas no sistema monoaminérgico[24].

1.3.1 Parâmetros comportamentais utilizados na avaliação da neurotoxicidade do glifosato

A neurotoxicidade do glifosato ainda não é bem estabelecida na literatura. Essa vem sendo estudada principalmente em modelos de roedores e peixes-zebra, que possuem similaridade com o sistema nervoso humano, além de modelos de testes bem estabelecidos para avaliação de alterações no comportamento[25,26]. As alterações comportamentais estudadas devido à neurotoxicidade do glifosato se concentram principalmente em comportamento tipo ansioso e tipo depressivo, alterações de memória e interação social.

Para a avaliação dos parâmetros comportamentais da neurotoxicidade em roedores foram encontrados na literatura vários testes, sendo os principais apresentados na Tabela 1. Cada teste avalia parâmetros diferentes que, ao se observar o comportamento apresentado pelo roedor, permite relacionar se este comportamento é natural ou manifesta alteração do padrão, sendo considerado patológico e passível de comparação com os humanos.

Tabela 1: Principais modelos de testes aplicados para avaliação de ansiedade, depressão, memória e interação social.

ANSIEDADE	DEPRESSÃO	MEMÓRIA	INTERAÇÃO SOCIAL
Campo aberto[27]	Nado forçado[28]	Reconhecimento de objetos[29]	Interação social[30]
Vocalização ultrassônica[27]	Suspensão de cauda[28]	Labirinto aquático de *Morris*[29]	"Preferência social (aversão social)"[30]
Transição claro-escuro[27]	Preferência por sacarose[28]	Labirinto radial[29]	
Interação presa-predador[27]	"Teste do *"splash"*"[28]	Esquiva ativa de duas vias[29]	
Ocultação defensiva condicionada[27]	Comportamento materno[28]	Alternância espontânea (Labirinto em Y)[29]	
Labirinto em cruz elevado[27]		Medo condicionado contextual[29]	
Labirinto em "T" elevado[27]		Esquiva passiva[29]	

Fonte: Autora. Os números representam as referências utilizadas.

A avaliação de um comportamento tipo ansioso em roedores procura reproduzir uma situação ambiental que gere aversão ou desconforto. Nos testes de campo aberto e labirintos está associada à avaliação do comportamento exploratório, sendo possível determinar também a normalidade da locomoção do animal[27].

A depressão é avaliada nos roedores a partir da observação de parâmetros como: desespero, apatia, anedonia, ansiedade e aversão social. Os testes mais comuns para avaliação destes parâmetros são: nado forçado, para avaliação de desamparo aprendido; preferência de sacarose, para avaliação de

anedonia; avaliação do *grooming* no "*Splash test*" e do comportamento materno, para verificação de apatia[28] .

Além da ansiedade e depressão, é possível realizar avaliações de interação social em roedores, seja pela interação do animal teste com roedores conhecidos e desconhecidos, ou com roedores desconhecidos e objetos. As respostas comportamentais relacionadas à interação social que indicam medo incluem fuga, evitação, congelamento, ameaça defensiva, ataque defensivo e avaliação de risco, porém o comportamento mais avaliado nos testes se refere ao tempo de interação com o animal desconhecido comparado ao conhecido ou a um objeto. Estes testes ainda podem auxiliar na predição de modelos de comportamento similar ao espectro autista[29].

A maioria dos testes de memória aplicados para verificar os efeitos de uma substância em roedores avaliam a memória de reconhecimento, seja de um espaço (memória espacial) ou objeto, restando outros que avaliam especificamente um comportamento de medo ou de evitação. Para uma avaliação completa dos efeitos comportamentais, geralmente é aplicada combinação de testes que determinem primeiramente a atividade locomotora. Na sequência são aplicados testes relacionados a comportamentos exploratórios e de aversão a novos objetos e animais, para investigar comportamentos ansiosos, depressivos ou até mesmo alterações de memória de acordo com o objetivo do estudo[30].

Por fim, para uma melhor avaliação da neurotoxicidade do glifosato é importante analisar os mecanismos de ação, assim como as respostas fisiológicas a estes mecanismos, caracterizando uma alteração no comportamento dos animais. A alteração do comportamento de roedores

expostos ao glifosato já é evidenciada por alguns artigos na literatura, porém, ainda não há um compilado dos dados para determinar possíveis convergências e robustez dos modelos, considerando a qualidade das metodologias aplicadas, e, assim, determinar quais os futuros passos necessários para qualificar as evidências que suportem a neurotoxicidade do glifosato, baseada em estudos conduzidos em modelo animal.

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3. OBJETIVOS

3.1. Objetivo geral

- Revisar a literatura de forma sistemática acerca dos efeitos da exposição ao glifosato no sistema nervoso por meio de alterações comportamentais em ratos.

3.2. Objetivos específicos

- Revisar as evidências sobre os efeitos da exposição ao glifosato no sistema nervoso por meio de alterações comportamentais em ratos.
- Relacionar as vias de exposição ao glifosato à neurotoxicidade gerada, caracterizando o tipo de dano (ocupacional, alimentar).
- Comparar as metodologias utilizadas pelos estudos e os resultados gerados para avaliar as alterações comportamentais devido à exposição ao glifosato.
- Avaliar o risco de viés metodológico de cada estudo.
- Estabelecer perspectivas futuras para o estudo dos efeitos neurotóxicos do glifosato em ratos de acordo com as evidências já disponíveis e os seus níveis.

4. ARTIGO CIENTÍFICO REDIGIDO EM INGLÊS

“Effects of glyphosate exposure on rodent behavioral parameters: a systematic review.”

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“Effects of glyphosate exposure on rodent behavioral parameters: a systematic review.”

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Abstract

Introduction: Pesticides are of great global concern with a growing worldwide commercialization and increasing information in the literature about their toxicity and damage to human and environmental health. Glyphosate (Roundup®) stands out for its wide use and commercialization, especially in soybean cultivation with Monsanto genetically modified seeds. Although the toxicity reported in the literature is low, there is evidence on neurotoxicity such as changes in the monoaminergic system, behavioral changes, and correlations with mental and developmental disorders. However, the available studies do not compare the results or characterize the risk of bias of the used methodologies. **Objective:** To systematically review the articles available in databases on the exposure of rodents to glyphosate related to the most common behavioral changes, such as anxiety and depression. **Materials and methods:** The research strategy was defined using the PICOT method, and the research question: "What evidence is available in the literature on the association between exposure to the herbicide glyphosate and neurobehavioral effects?" After analyzing the titles and abstracts, the search results from databases were tabulated in Excel datasheet and compared between two independent reviewers; a third reviewer was consulted if there was no consensus between the reviewers. The selected articles were read in full and verified according to the eligibility criteria. The article was written according to PRISMA protocol and the risk of bias of the methodologies was evaluated using the SYRCLE risk of bias tool. **Results:** The search in the databases, according to the filters used, generated 2,158 results in Pubmed, 311 in LILACS/Bireme BVS, 627 in Scopus, and 0 in Scielo. Of the 16 selected articles, between the evaluated behavior, 56.25% of the authors evaluated anxiety, 43.75% evaluated depression, both associated or not to other conditions. Furthermore, seven studies evaluated memory alteration through tests based on recognition and avoidance, resting

only 1 article that analyzed behaviors related to autism spectrum syndrome, such as social interaction, due to the exposure of rodents to glyphosate. **Conclusions:** According to the results found in the studies regarding the alterations in the parameters evaluated by the behavioral tests, it is concluded that exposure to glyphosate is related to the induction of anxious and depressive behaviors, locomotor alterations, memory, and social interaction.

Keywords: Glyphosate; neurotoxicity; behavior; animal model.

Introduction

Glyphosate is a non-selective and systemic herbicide used in forestry and mainly in agriculture, predominantly in corn, cotton, canola, soybean, sugar beet and alfalfa plantations, being also used in cereal, vegetable, and fruit plantations (United States Environmental Protection Agency (EPA) 2021; Galli and Montezuma 2005). Its mechanism of action in plants occurs through the inhibition of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) pathway, known as the shikimate pathway, due to the active ingredient of the formulation, which inhibits the synthesis of chlorophyll, also influencing the synthesis of phenylalanine, tyrosine, and tryptophan (Cole 1985). Although its systemic toxicity (Maddalon et al. 2021) is considered low (Oral LD50 >4 g kg^{-1} for rodents) and presenting a rapid degradation in the soil, its residue is currently found in water, food and contaminates those who apply it without proper equipment of security (Baer and Marcel 2014; Covaci 2014; Myers et al. 2016).

Exposure to glyphosate can occur due to occupational contact with the substance. However, humans are exposed daily through their feed, applications in gardens, and even in water for the treatment of aquatic herbs (Amarante Júnior et al. 2002). Human contact with glyphosate is documented in the literature as possibly harmful to health, due to evidence in humans and animals of reproductive toxicity and endocrine disruption (Dallegrave 2003; Dallegrave et al. 2007), immunotoxicity (Maddalon et al. 2021), and even increased risk of carcinogenicity (Zhang et al. 2019). Recently, scientific articles have drawn attention to the effects of exposure to glyphosate on the nervous system. Nonetheless, as toxicological studies have many biases, including the difficulty in tracking the causative agent of the pathology under study (Wandall, Hansson, and Rudén

2007), most researches focus on studying the effects of glyphosate on animals, and more specifically, on rodents.

The reduction in the expression of synaptic proteins in rats and changes in monoaminergic neurotransmitters after exposure to glyphosate have already been documented in the literature, showing the neurotoxic potential of glyphosate (Martínez et al. 2018; Luna et al. 2021). Other studies also suggest the influence of exposure to glyphosate on cognitive and behavioral parameters of rodents, showing exposure-related changes in behaviors such as anxiety, depression, and memory (Baier et al. 2017; Ait-Bali et al. 2020; Bali et al. 2019).

Since several studies show the effects of glyphosate on behavioral parameters related to disorders and cognitive disorders that currently plague the population, the present study aims to systematically review the evidence available in the literature to establish a causal link between the effects of exposure to glyphosate on the nervous system through behavioral changes in rodents, as well as to evaluate the risk of bias of the methodologies used to estimate the reliable data available in the literature.

Materials and Methods

A systematic review was conducted to analyze the evidence available in the literature about the exposure of rodents to glyphosate and its relationship with behavioral effects. Following the purpose of the review, a survey was systematically coordinated to answer the following question: What evidence are available in the literature about the association between exposure to the herbicide glyphosate and neurobehavioral effects? The research strategy was developed from the chosen research question using the PICOT method and the following Boolean operators: ((rats or mice) AND (pesticide OR

glyphosate OR herbicide) AND (behavior OR depression OR anxiety)) and the following databases were consulted for the selection of articles published between 2000 - 2021: Pubmed, LILACS/BIREME, SCIELO, and SCOPUS. The screening of articles according to the databases result was carried out in two stages and by two reviewers independently, first selecting articles that met the following selection (1 - 5) and exclusion criteria (6 - 10) by title, and secondly by the summary.

Selection criteria:

1. Articles from studies with rodents exposed to glyphosate.
2. Studies that have a control group.
3. Articles that contain behavioral assessments.
4. Original/primary study articles.
5. Articles in English, Spanish, or Portuguese.

Exclusion criteria:

6. Exposure to substances or pesticides other than glyphosate alone.
7. Studies with cells, humans or other animal species besides rats and mice (rodents).
8. Review articles and comments.
9. Absence of outcome (e.g., behavior, depression, anxiety, neurotoxicity).
10. Languages other than English, Spanish, or Portuguese.

The searched results in the databases were arranged in an Excel datasheet and when the reviewers did not reach an agreement, a third reviewer was called. After selection, the articles were read in full to extract the sample characterization, data, and outcome, named: title, species, strain, sex, age, route and agent of exposure, exposure

concentration, type of test (pre-natal, acute, subchronic, chronic), sample size, behavior evaluated, type of behavioral test applied, the period in which the test was applied, results, and conclusion.

The duplicate exclusion was performed immediately after data tabulation and the article was written by the PRISMA protocol. The articles were evaluated according to their methodology based on the method proposed by the SYRCLE group (SYRCLE RoB tool), to characterize the biases present in the studies methodologies and their quality (Hooijmans et al. 2014).

Results

The search in the databases, according to the used filters, generated 2,158 results in Pubmed, 311 in LILACS/Bireme BVS, 627 in Scopus, and 0 in Scielo. Of these, 89 were selected by titles and tabulated, of which only 37 were selected by abstracts to be read in full, and 21 duplicates were excluded remaining 16 articles for review (Figure 1).

[FIGURE 1]

Of the 16 selected articles, 56.25% used mice, and 43.75% used rats, and several strains of rats and mice were used by the authors, such as Wistar, Sprague-Dawley, Swiss, CF-1, BALB/c, and ddY. Regarding exposure characterization (Table 1), most articles used commercial formulations (75%) or the active ingredient glyphosate (18.75%), while only one article compared the two types of exposure between groups. Almost half of the articles (43.75%) used pre, peri, and postnatal exposures affecting the animals' development and maternal behavior, while 25% used acute exposures, 50% used

subchronic exposures, and 25% made chronic exposures. The exposure routes used were varied, with the predominance of oral (75%) and intraperitoneal (12.5%).

[TABLE 1]

Regarding the evaluated behaviors (Tables 2-6), 56.25% of the authors evaluated anxiety, 43.75% depression, 43.75% memory and only 1 article evaluated behaviors related to autism spectrum syndrome, such as social interaction, due to exposure of rodents to glyphosate. For the evaluation of cognition and behavior, several tests were used by the authors, predominating the Open Field (56.25%) for the evaluation of locomotion and characterization of an anxious-like behavior, Plus-Maze (50%) for the characterization of an anxious behavior type, lastly Novel Object Recognition (37.5%) for post-exposure memory assessment.

[TABLES 2-6]

The risk of bias evaluation of the studies methodologies showed the predominantly selection, performance, and detection biases common to experiments applied to rodents (Table 3). Furthermore, 93.75% of the studies showed a high risk of bias related to at least 5 questions evaluated by the SYRCLE method, demonstrating a low risk of bias for questions related only to attrition bias, reporting of results, and other biases (questions 8-10).

[TABLE 7]

Discussion

In agreement with the applied tests frequency by the authors presented above, the most observed behavior by the reviewed studies was a reduction in locomotor activity and an increase in anxious-like behavior in rodents exposed to glyphosate. The application of the motor locomotion and Open Field tests allowed the evaluation of general locomotor activity, exploration of the environment (Joaquim et al. 2013), and an initial screening for anxiety (Prut and Belzung 2003). In this case, most of the reviewed studies that opted for the application of Open Field revealed changes in groups exposed to glyphosate in several parameters such as increased time spent in the center, decreased distance traveled, longer time spent immobile, decreased mean speed of ambulation, increased thigmotaxis, reduced locomotion frequency, decreased number of squares crossed and the number of rearing and higher number of groomings (Ait-Bali et al. 2020; Ait Bali, Ba-Mhamed, and Bennis 2017; Aitbali et al. 2018; Baier et al. 2017; Joaquim et al. 2014; Coullery, Pacchioni, and Rosso 2020). The studies used different exposure concentrations from 35 mg/kg to 500 mg/kg in addition to using subchronic and chronic exposures, as well as peri and postnatal exposures to study the relationship between exposure in critical stages of development and behavioral changes. Only one study showed a significant difference with acute exposure, demonstrating a reduction in locomotion in the same way (Joaquim et al. 2013)

The parameters evaluated in the Open Field and evidenced as related to the reduction of locomotor activity have already been reported in the literature in studies with rats and mice exposed to 2,4-D pesticides and dichlorvos (Binukumar et al. 2010; Freitas et al. 2019). The results of locomotor activity observed in the reviewed studies can be correlated with the loss of dopaminergic neurons and reduction of dopamine receptors

(Gallo et al. 2015). Also Hernandez-Plata (2015) demonstrated in their study that the reduction of extracellular levels of DA (Dopamine) in the striatum is associated with hypoactivity in OP. In relation to locomotion reduction, the total distance reduction, frequency of locomotion, and the increasing of the immobility period can be observed in the analyzed studies, justifying the hypoactive behavior pointed out by the authors. Regarding to the reviewed studies which have applied the OP and locomotor activity tests, only two articles showed no significant difference between the exposed groups and the control group in the evaluated parameters, although the data presented in Bicca et al. (2021) showed anxiety and depression in other applied tests and Cattani et al. (2017) didn't find results of anxiety that lasted until 60 days, the rats showed depression behavior as well (Cattani et al. 2017; Bicca et al. 2021).

To complement the data obtained through the OP and confirm the change in anxious behavior generated in rodents due to exposure to glyphosate, some authors chose to perform the Plus-Maze test. Anxiety-like behavior was evidenced in the articles, even in contrast to hypoactivity in the OP, in altered parameters such as: decreased time in the open arm, time spent to entry in the open environment, increased anxiety index, decreased number of crossings and decreased number of center crosses (Ait Bali, Ba-Mhamed, and Bennis 2017; Bali et al. 2019; Aitbali et al. 2018; Baier et al. 2017; Bicca et al. 2021; Joaquim et al. 2013; 2014). The most evident parameter of decreased time spent in the open arms opposes to rodent's natural activity of exploring environments and indicates an anxious-like behavior due to glyphosate exposure (Ait Bali, Ba-Mhamed, and Bennis 2017; Ait-Bali et al. 2020).

Finally, only one article did not agree with the results evidenced by the majority (less time and frequency in the open arm), due to the increased percentage of time spent in open arms, even though this parameter was interpreted as an increase in anxiogenic-

like activity as well (Gallegos et al. 2018). This parameter observed in the EPM by Gallegos (2020), was found as well in rats exposed subchronically to Chlorpyrifos with the increased entries and time spent in the open arms in the elevated plus-maze test, indicating an increase in anxious-like behavior (Chen et al. 2011).

In addition to the reduced locomotor activity and anxiety-like behavior, an increase in depressive behavior was also observed. Depressive behavior was evidenced through increased immobility in the Tail Suspension test in chronically treated glyphosate mice (Ait Bali, Ba-Mhamed, and Bennis 2017; Aitbali et al. 2018; Joaquim et al. 2013), in the reduction of grooming in the Splash Test in mice exposed to glyphosate subchronically and chronically (Ait Bali, Ba-Mhamed, and Bennis 2017; Aitbali et al. 2018), and finally in the increased immobility and difference in activity in the Forced Swim test in rats and mice exposed to glyphosate subchronically and during pregnancy and lactation (Cattani et al. 2014; Bicca et al. 2021). The Sucrose Preference test was also used by two studies but showed no significant difference in the anhedonia parameter observed between groups, which does not put away the possibility of a depressive behavior since other authors have observed depression through other tests due to subchronic exposure and during pregnancy and lactation (Cattani et al. 2014; Bicca et al. 2021).

Exposure to glyphosate also generated memory alterations in subchronically and chronically exposed rats, in addition to being reported in studies that used peri and postnatal exposure affecting the development of the rodent's nervous system (Bali et al. 2019; Ait-Bali et al. 2020). The studies mostly applied the Novel Object Recognition test, showing lower time exploring the novel object and discrimination index. Finally, two studies also applied the Y-Maze test observing decreased spontaneous alternation and decreased latency in a study that used the Morris Water Maze to assess memory (Bali et

al. 2019; Ait-Bali et al. 2020; Baier et al. 2017; Coullery, Pacchioni, and Rosso 2020; Luna et al. 2021). The results corroborate the conclusion that exposure to glyphosate alters the memory of exposed rodents, whether at a low concentration of 50 mg or a high concentration of 500 mg/kg/day, questioning the extent to which human exposure to glyphosate is safe, subchronically and chronically as well as in children at a critical stage of their development.

The alterations in the sociability of rats were also observed by two studies that applied the Three-chamber Social Interaction test, which may be related to an autistic-like behavior induced in rodents exposed at critical stages of central nervous system development due to peri-exposure and postnatal (Ait-Bali et al. 2020; Pu et al. 2020). The relationship between exposure to glyphosate and the induction of autistic-like behavior has already been evidenced in a case-control study with humans, but due to biases related to toxicological studies applied to humans, further investigation is needed on this relationship to better establish the connection between cause and effect (Von Ehrenstein et al. 2019).

Evaluating the risk of bias of the reviewed studies, all the biases pointed out are common to animal experimentation and to the lack of an established protocol for studies in this area. Unlike human studies, animal studies are often not randomized at any stage and are frequently conducted by a small group of researchers, leaving no choice when it comes to blinding the study. Furthermore, the researchers must be trained since the beginning of the experiment and the rodents must be adapted to the presence of the researcher so this won't generate stressful situations that compromise the evaluation of behavior, making it even more difficult for many researchers to participate in a study with an animal model (O'Connor and Sargeant 2014). Therefore, it is concluded from the evaluation of the risk of bias of the methodologies that the results are reliable and related

to few biases inherent to how each animal experimentation is currently conducted, validating the results found on behavior change due to exposure to glyphosate. From this interpretation, future perspectives can be established regarding the creation of animal experimentation protocols to minimize common biases to this area, as it's already well established in studies with humans today.

According to the results found in the studies regarding the alterations in the parameters evaluated by the behavioral tests, we concluded that exposure to glyphosate is related to the induction of anxious and depressive behaviors, locomotor, memory, and social interaction alterations. Further studies are needed to establish the mechanism of action behind the observed changes, in addition to the comparison of groups exposed to technical glyphosate and the commercial formula to rule out the relationship between the adjuvants present in the commercial formulation and the behavior changes observed in the rodents.

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Declaration of interest statement

The authors declare no conflict of interest.

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Figure 1. Flowchart of search and selection

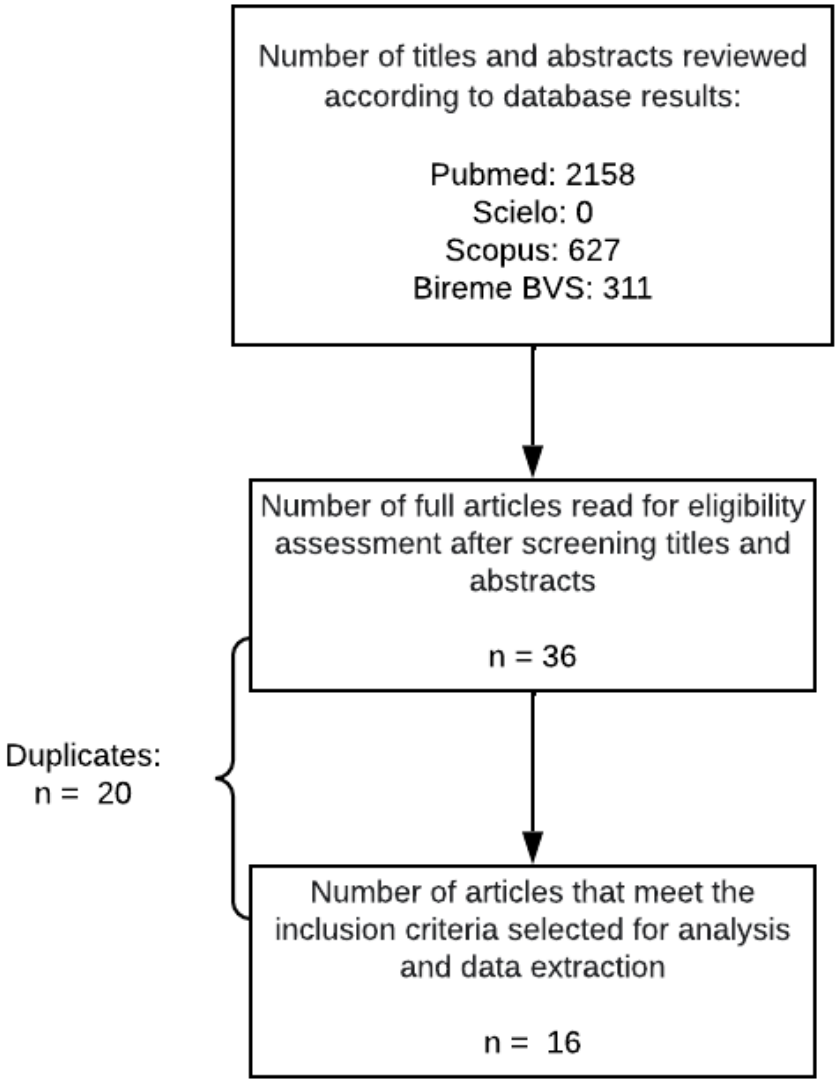

Table 1: Characterization of the studies.

SPECIE/STRAIN SEX/AGE	EXPOSURE ROUTE	EXPOSURE AGENT	DOSEGE/ CONCENTRATION	EXPOSURE PERIOD	SAMPLE SIZE (n)	BEHAVIOR TEST	REFERENCE
Mice (Swiss) 1 month (male)	Oral treatment (gavage)	Roundup® herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (NaCl 0,9%) 250 mg/kg/day 500mg/kg/day	Acute (1 day) (n=18) Subchronic (6 weeks) (n=18) Chronic (12 weeks) (n=18)	n=6/group	LOCOMOTOR ACTIVITY, ANXIETY AND DEPRESSION	(Ait Bali, 2017)
Mice (Swiss) 1 month (male)	Oral treatment (gavage)	Roundup® herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (NaCl 0,9%) 250 mg/kg/day 500mg/kg/day	Acute (1 day) (n=18) Subchronic (6 weeks) (n=18) Chronic (12 weeks) (n=18)	n=6/group	LOCOMOTOR ACTIVITY, ANXIETY AND DEPRESSION	(Ait Bali, 2018)
Mice (Swiss) 1 month (male)	Oral treatment (gavage)	Roundup® herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (NaCl 0,9%) 250 mg/kg/day 500mg/kg/day	Acute (1 day) (n=18) Subchronic (6 weeks) (n=18) Chronic (12 weeks) (n=18)	n=6/group	MEMORY	(Ait Bali, 2019)
Mice (Swiss) 3 months (female)	Oral treatment (gavage)	Roundup®r herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (tap water) 250 mg/kg/day 500mg/kg/day	Peri and post natal (G0 to postnatal day 21)	n=6 female/group (dams) n= 10/group (litters)	LOCOMOTOR ACTIVITY, ANXIETY, SOCIAL INTERACTION AND MEMORY	(Ait Bali, 2020)
Mice (CF-1) 65 days (male)	Intranasal	Glifloglex® (48 g of Gly isopropylamine salt/100 cm^3 product - equivalent to 35.6% w/v of Gly acid)	Control (NaCl 0,9%) 50 mg/kg/day	Subchronic (3 times per week during 4 weeks)	n = 10/group	LOCOMOTOR ACTIVITY, ANXIETY AND MEMORY	(Baier, 2017)

Continuation of Table 1

Mice (Swiss) 60 days (male)	Oral treatment (gavage)	Zamba® (n-phosphonomethyl glycine isopropylamine salt at 48% m/v–480 g/L, 36.5% m/v– 365 g/l of the equivalent acid)	Control (NaCl 0,9%) 50 mg/kg/day Other groups with Quercetin	Subchronic (daily during 30 days)	n = 10/group	LOCOMOTOR ACTIVITY AND ANXIETY	(Bicca, 2021)
Mice (BALB/c) 60 days old (male)	Oral treatment (Gavage)	Roundup® (480 g/L of GF, 648 g/L of isopropylamine salt)	Control (Water) 25 mg/kg 50 mg/kg 100 mg/kg	Acute (1 day)	n = 10 group (n=12/group control)	LOCOMOTOR ACTIVITY, ANXIETY AND DEPRESSION	(Joaquim, 2013)
			Control (0,9% NaCl) 100 mg/kg	Acute (1 day)	n = 9 - 16/group	LOCOMOTOR ACTIVITY, ANXIETY AND DEPRESSION	
Mice (BALB/c) 21 days (male and female)	Oral treatment (Gavage)	Roundup® (480 g/L of GF, 648 g/L of isopropylamine salt)	Control (Water) 50 mg/kg	Subchronic (PND23 to PND45)	n = 16 - 28/group	LOCOMOTOR ACTIVITY, ANXIETY AND DEPRESSION	(Joaquim, 2014)
Mice (ddY) 9-10 weeks old (female)	Oral exposure (drinking water)	Roundup® Maxload [48% (w/v) glyphosate (Nphosphonomethylglycine) potassium salt, 52% other ingredients	Water 0,98% Glyphosate	Peri and post-natal (GD5 to PND21)	Not informed	LOCOMOTOR ACTIVITY, MEMORY, ASR, SOCIAL INTERACTION, DEPRESSION	(Pu, 2020)

Continuation of Table 1

Rats (Wistar) Adults (female and male offspring)	Oral exposure (drinking water)	Roundup Original® (glyphosate 360 g/L)	Control (tap water) 1% of glyphosate (maternal exposure calculated in 70 mg/kg/day)	Peri-natal, post-natal and chronic exposure (Exposure in utero from GD5, continually up to PND1 to PND21 during lactation, and up to PND60 through tap water)	n = 4 dams/group n = 8 pups/mom After weaning (n=4 male/group)	LOCOMOTOR ACTIVITY AND DEPRESSION	(Cattani, 2017)
Rats (Wistar) 90–120 days (female)	Intraperitonial injection	Glyphosate® Sigma-Aldrich as a 40 % water solution of N-phosphonomethyl glycine-monoisopropylamine salt	Control (PBS solution) 24 mg/kg 35 mg/kg	Peri natal (GD8 to GD20 every 48h)	n=8 dams/group and 4 pups/group	LOCOMOTOR ACTIVITY AND MEMORY	(Coullery, 2020)
Rats (Sprague-Dawley) Adults (female)	Oral treatment (biscuits)	Glyphosate isopropylamine salt Dr Ehrenstorfer GmbH and Roundup® 3Plus (229 g L -1 glyphosate isopropylamine salt)	Control (Milli Q water) 5 mg/kg/day	Peri- natal and post-natal (GD10 to PND21)	n = 7 dams/group	Maternal Behavior	(Dechartes, 2019)
Rats (Wistar) 90–120 days (female)	Oral exposure (drinking water)	Glifloglex® (48 g of Gly isopropylamine salt/100 cm3 product - equivalent to 35.6% w/v of Gly acid)	Control (tap water) 0,65 g/L of Gly (100 mg/kg/day) 1.30 g/L of Gly (200 mg/kg/day)	Peri-natal and post-natal (GD0 to PND21)	n = 10 dams/group	SENSORIMOTOR DEVELOPMENT, LOCOMOTOR, EMOCIONAL ACTIVITY AND ANXIETY	(Gallegos, 2015)
Rats (Wistar) 90–120 days (female)	Oral exposure (drinking water)	Glifloglex® (48 g of Gly isopropylamine salt/100 cm3 product - equivalent to 35.6% w/v of Gly acid)	Control (tap water) 0,65 g/L of Gly (100 mg/kg/day) 1.30 g/L of Gly (200 mg/kg/day)	Peri-natal and post-natal (GD0 to PND21)	n = 10 dams/group	MEMORY	(Gallegos, 2018)

Continuation of Table 1

Rats (Sprague-Dawley) Adults (male)	Intraperitonial injection	Glyphosate® Sigma-Aldrich 40% water solution of N-phosphonomethyl glycine-monoisopropylamine salt	Control (0,9% NaCl) 50 mg/kg 100 mg/kg 150 mg/kg	Subchronic (3 injections/week/during 2 weeks)	n = 10/group	LOCOMOTOR ACTIVITY	(Hernandez-Plata, 2014)
Rats (Wistar) Newborn (male)	Subcutaneos	Sigma-Aldrich as a 40% water solution of N-phosphonomethyl glycine-monoisopropylamine salt	Control (Water) 35 mg/Kg 70 mg/Kg	Subchronic (PND 7 to PND 27, every 48 h)	n = 16/group	MEMORY	(Luna, 2021)

Tabel 2: Behavioral results about locomotion and anxiety evaluation.

SPECIE/STRAIN SEX/AGE	EXPOSURE ROUTE	EXPOSURE AGENT	DOSEGE/ CONCENTRATION	EXPOSURE PERIOD	BEHAVIOR TEST	RESULTS	REFERENCE
Mice (Swiss) 1 month (male)	Oral (gavage)	Roundup® herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (NaCl 0,9%) 250 mg/kg/day 500 mg/kg/day	Acute (1 day) (n=18) Subchronic (6 weeks) (n=18) Chronic (12 weeks) (n=18)	Open Field	Decrease in all parameters recorded during the Open Field in treated groups, especially the 500 mg/kg group. (Subchronic and Chronic)	Ait Bali, Y. (2017)
Mice (Swiss) 1 month (male)	Oral (gavage)	Roundup® herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (NaCl 0,9%) 250 mg/kg/day 500 mg/kg/day	Acute (1 day) (n=18) Subchronic (6 weeks) (n=18) Chronic (12 weeks) (n=18)	Open Field	Decreased time spent in the center in treated groups. (Subchronic and Chronic)	(Ait Bali, 2018)
Mice (Swiss) 3 months (female)	Oral (gavage)	Roundup® herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (tap water) 250 mg/kg/day 500 mg/kg/day	Peri and post natal (G0 to postnatal day 21)	Open Field	Decreased distance traveled compared to the control in group group of 500 mg/kg. Lower time in the center of the open field compared to the control in exposed groups, and significant diference between treated groups.	(Ait Bali, 2020)
Mice (CF-1) 65 days (male)	Intranasal	Glifloglex® (48 g of Gly isopropylamine salt/100 cm3 product - equivalent to 35.6% w/v of Gly acid)	Control (NaCl 0,9%) 50 mg/kg/day	Subchronic (3 times per week during 4 weeks)	Open Field	Lower total distance travelled treated mice compared to control mice. Longer time spent immobile and decreased mean speed of ambulation in treated mice. Increased thigmotaxis in treatd groups when compared to control mice during the first two 5 min intervals.	(Baier, 2017)

Continuation of Table 2

Mice (Swiss) 60 days (male)	Oral (gavage)	Zamba® (n-phosphonomethyl glycine isopropylamine salt at 48% m/v–480 g/L, 36.5% m/v– 365 g/l of the equivalent acid)	Control (NaCl 0,9%) 50 mg/kg/day Other groups with Quercetin	Subchronic (daily during 30 days)	Open Field	No significant diference.	(Bicca, 2021)
Mice (BALB/c) 60 days (male)	Oral (gavage)	Roundup® (480 g/L of GF, 648 g/L of isopropylamine salt)	Control (water) 25 mg/kg 50 mg/kg 100 mg/kg	Acute (1 day)	Open Field	Decreased locomotion frequency, when compared with the control group, in all doses in the first session (15 min after exposure), 50 mg/kg in the second session (30 min), and 25 mg/kg in the last session (120 min).	(Joaquim, 2013)
Mice (BALB/c) 60 days (male and female)	Oral (gavage)	Roundup® (480 g/L of GF, 648 g/L of isopropylamine salt)	Control (0,9% NaCl) 100 mg/kg	Acute (1 day)	Open Field	Reduced locomotion frequency in female mice exposed to 100 mg/kg.	(Joaquim, 2013)
Mice (BALB/c) 21 days (male and female)	Oral (gavage)	Roundup® (480 g/L of GF, 648 g/L of isopropylamine salt)	Control (water) 50 mg/kg	Subchronic (PND23 to PND45)	Open Field	Reduced locomotion of male mice compared to male control group in treated group.	(Joaquim, 2014)
Rats (Wistar) Adults (female and male offspring)	Oral (drinking water)	Roundup Original® (glyphosate 360 g/L)	Control (tap water) 1% of glyphosate (maternal exposure calculated in 70 mg/kg/day)	Peri-natal, post-natal and chronic exposure (Exposure in utero from GD5, continually up to PND1 to PND21 during lactation, and up to PND60 through tap water)	Open Field	No significant diference.	(Cattani, 2017)

Continuation of Table 2

Rats (Wistar) 90–120 days (female)	Oral (drinking water)	Glifloglex® (48 g of Gly isopropylamine salt/100 cm3 product - equivalent to 35.6% w/v of Gly acid)	Control (tap water) 0,65 g/L of Gly (100 mg/kg/day) 1.30 g/L of Gly (200 mg/kg/day)	Peri-natal and post-natal (GD0 to PND21)	Open Field	Decreased number of squares crossed, compared to the control group, in exposed female 45-day-old offpring treated with glyphosate. Decreased number of squares crossed and number of rearings, compared to the control group, in exposed female and male 90-day-old offspring. Higher number of grooming episodes, compared to the control group, only in exposed male 90-day-old offspring.	(Gallegos, 2015)
Rats (Wistar) 90–120 days (female)	Intraperitonial	Glyphosate® Sigma-Aldrich as a 40 % water solution ofN-phosphonomethyl glycine monoisopropylamine salt	Control (PBS solution) 24 mg/kg35 mg/kg	Peri natal (GD8 to GD20 every 48h)	Locomotor Activity	Reduced exploratory activity in higher dose treated group compared to control and lower-dose.	(Coullery, 2020)
Rats (Sprague-Dawley) Adults (male)	Intraperitonial	Glyphosate® Sigma-Aldrich 40% water solution of N-phosphonomethyl glycine-monoisopropylamine salt	Control (0,9% NaCl) 50 mg/kg 100 mg/kg 150 mg/kg	Subchronic (3 injections/week/during 2 weeks)	Locomotor Activity	Decreased exploratory activity in 100 or 150 mg/Kg treated groups before rats received injection 3, and this hypoactivity lasted throughout most of the experiment.	(Hernandez-Plata, 2014)
Mice (ddY) 9-10 weeks (female)	Oral (drinking water)	Roundup® Maxload [48% (w/v) glyphosate (Nphosphonomethylglycine) potassium salt, 52% other ingredients	Water 0,098% Glyphosate	Peri and post-natal (GD5 to PND21)	Locomotor Activity	No significant difference.	(Pu, 2020)

Tabel 3: Behavioral results about anxiety evaluation.

SPECIE/STRAIN SEX/AGE	EXPOSURE ROUTE	EXPOSURE AGENT	DOSEGE/ CONCENTRATION	EXPOSURE PERIOD	BEHAVIOR TEST	RESULT	REFERENCE
Mice (Swiss) 1 month (male)	Oral (gavage)	Roundupr herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (NaCl 0,9%) 250 mg/kg/day 500mg/kg/day	Acute (1 day) (n=18) Subchronic (6 weeks) (n=18) Chronic (12 weeks) (n=18)	Elevated Plus-Maze	Decreased time spent in the open arm (especially in 500 mg/kg) and increased anxiety index in treated groups.	Ait Bali, Y. (2017)
Mice (Swiss) 1 month (male)	Oral (gavage)	Roundupr herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (NaCl 0,9%) 250 mg/kg/day 500mg/kg/day	Acute (1 day) (n=18) Subchronic (6 weeks) (n=18) Chronic (12 weeks) (n=18)	Elevated Plus-Maze	Increased anxiety index (subchronic and chronic)	(Ait Bali, 2018)
Mice (Swiss) 3 months (female)	Oral (gavage)	Roundupr herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (tap water) 250 mg/kg/day 500mg/kg/day	Peri and post natal (G0 to postnatal day 21)	Elevated Plus-Maze	Lower ratio of time spent in the open arm and higher anxiety index in treated groups, compared to the control group	(Ait Bali, 2020)
Mice (CF-1) 65 days (male)	Intranasal	Glifloglex® (48 g of Gly isopropylamine salt/100 cm3 product - equivalent to 35.6% w/v of Gly acid)	Control (NaCl 0,9%) 50 mg/kg/day	Subchronic (3 times per week during 4 weeks)	Plus-Maze	Lower time spent in open arms and total number of arm entries, decreased percentage of entries to the open arms in treated mice comparted to control group.	(Baier, 2017)

Continuation of Table 3

Mice (Swiss) 60 days (male)	Oral (gavage)	Zamba® (n-phosphonomethyl glycine isopropylamine salt at 48% m/v–480 g/L, 36.5% m/v– 365 g/l of the equivalent acid)	Control (NaCl 0,9%) 50 mg/kg/day Other groups with Quercetin	Subchronic (daily during 30 days)	Elevated Plus-Maze	Avoided entry into the open environment in treated group, unlike the control group.	(Bicca, 2021)
Mice (BALB/c) 60 days old (female and male)	Oral (gavage)	Roundup (480 g/L of GF, 648 g/L of isopropylamine salt)	Control (0,9% NaCl) 100 mg/Kg	Acute (1 day)	Elevated Plus-Maze	Decreased number of entries in the closed arms and center crosses in male treated groups, when compared to control group. Higher levels of activity in center crosses in female mice compared to male mice.	(Joaquim, 2013)
Mice (BALB/c) 21 days (male and female)	Oral (gavage)	Roundup (480 g/L of GF, 648 g/L of isopropylamine salt)	Control (Water) 50 mg/Kg	Subchronic (PND23 to PND45)	Elevated Plus-Maze	Decreased number of crossings in male treated mice, compared to the control group.	(Joaquim, 2014)
Rats (Wistar) 90–120 days (female)	Oral (drinking water)	Glifloglex® (48 g of Gly isopropylamine salt/100 cm3 product - equivalent to 35.6% w/v of Gly acid)	Control (tap water) 0,65 g/L of Gly (100 mg/kg/day) 1.30 g/L of Gly (200 mg/kg/day)	Peri-natal and post-natal (GD0 to PND21)	Plus-Maze	Increased percentage of time spent in open arms, compared to control group, in treated female 45-day-old offspring. Higher time spent in open arms and higher percentage of entries to the open arms, compared to control group, in treated 90-day-old offspring.	(Gallegos, 2015)

Tabel 4: Behavioral results about depression evaluation.

SPECIE/STRAIN SEX/AGE	EXPOSURE ROUTE	EXPOSURE AGENT	DOSEGE/ CONCENTRATION	EXPOSURE PERIOD	BEHAVIOR TEST	RESULT	REFERENCE
Mice (Swiss) 1 month (male)	Oral (gavage)	Roundupr herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (NaCl 0,9%) 250 mg/kg/day 500mg/kg/day	Acute (1 day) (n=18) Subchronic (6 weeks) (n=18) Chronic (12 weeks) (n=18)	Tail suspension	Increased immobility time in chronicly treated group.	Ait Bali, Y. (2017)
Mice (Swiss) 1 month (male)	Oral (gavage)	Roundupr herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (NaCl 0,9%) 250 mg/kg/day 500mg/kg/day	Acute (1 day) (n=18) Subchronic (6 weeks) (n=18) Chronic (12 weeks) (n=18)	Tail suspension	Increased immobility time in chronicly treated group.	(Ait Bali, 2018)
Mice (BALB/c) 60 days old (female and male)	Oral (gavage)	Roundup (480 g/L of GF, 648 g/L of isopropylamine salt)	Control (0,9% NaCl) 100 mg/Kg	Acute (1 day)	Tail suspension	Increased immobility time in both male and female mice treated groups.	(Joaquim, 2013)
Mice (Swiss) 1 month (male)	Oral (gavage)	Roundupr herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (NaCl 0,9%) 250 mg/kg/day 500mg/kg/day	Acute (1 day) (n=18) Subchronic (6 weeks) (n=18) Chronic (12 weeks) (n=18)	Splash test	Decreased grooming time in subchronic and chronic treated groups.	Ait Bali, Y. (2017)

Continuation of Table 4

Mice (Swiss) 1 month (male)	Oral (gavage)	Roundupr herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (NaCl 0,9%) 250 mg/kg/day 500mg/kg/day	Acute (1 day) (n=18) Subchronic (6 weeks) (n=18) Chronic (12 weeks) (n=18)	Splash test	Decreased grooming time in subchronic and chronic treated groups.	Ait Bali, Y. (2018)
Rats (Wistar) Adults (female and male offspring)	Oral (drinking water)	Roundup Original® (glyphosate 360 g/L)	Control (tap water) 1% of glyphosate (maternal exposure calculated in 70 mg/Kg/day)	Peri-natal, post-natal and chronic exposure (Exposure in utero from GD5, continually up to PND1 to PND21 during lactation, and up to PND60 through tap water)	Forced Swim	Increased immobility time in forced swimming test in treated group, which was associated with decreased climbing time.	(Cattani, 2017)
Mice (Swiss) 60 days (male)	Oral (gavage)	Zamba® (n-phosphonomethyl glycine isopropylamine salt at 48% m/v–480 g/L, 36.5% m/v– 365 g/l of the equivalent acid)	Control (NaCl 0,9%) 50 mg/kg/day Other groups with Quercetin	Subchronic (daily during 30 days)	Forced Swim	Differed activity in treated group compared to control group.	(Bicca, 2021)
Mice (BALB/c) 21 days (male and female)	Oral (gavage)	Roundup (480 g/L of GF, 648 g/L of isopropylamine salt)	Control (Water) 50 mg/Kg	Subchronic (PND23 to PND45)	Forced Swim	No significant diference.	(Joaquim, 2014)

Continuation of Table 4

Mice (ddY) 9-10 weeks old (female)	Oral (drinking water)	Roundup® Maxload [48% (w/v) glyphosate (Nphosphonomethylglycine) potassium salt, 52% other ingredients	Water 0,098% Glyphosate	Peri and post-natal (GD5 to PND21)	Forced Swim	No significant diference.	(Pu, 2020)
Mice (Swiss) 60 days (male)	Oral (gavage)	Zamba® (n-phosphonomethyl glycine isopropylamine salt at 48% m/v–480 g/L, 36.5% m/v– 365 g/l of the equivalent acid)	Control (NaCl 0,9%) 50 mg/kg/day Other groups with Quercetin	Subchronic (daily during 30 days)	Sucrose Preference	No significant diference.	(Bicca, 2021)
Rats (Wistar) Adults (female and male offspring)	Oral (drinking water)	Roundup Original® (glyphosate 360 g/L)	Control (tap water) 1% of glyphosate (maternal exposure calculated in 70 mg/Kg/day)	Peri-natal, post-natal and chronic exposure (Exposure in utero from GD5, continually up to PND1 to PND21 during lactation, and up to PND60 through tap water)	Sucrose Preference	No significant diference.	(Cattani, 2017)
Mice (ddY) 9-10 weeks (female)	Oral (drinking water)	Roundup® Maxload [48% (w/v) glyphosate (Nphosphonomethylglycine) potassium salt, 52% other ingredients	Water 0,098% Glyphosate	Peri and post-natal (GD5 to PND21)	Grooming	Not informed.	(Pu, 2020)

Tabel 5: Behavioral results about memory evaluation.

SPECIE/STRAIN SEX/AGE	EXPOSURE ROUTE	EXPOSURE AGENT	DOSEGE/ CONCENTRATION	EXPOSURE PERIOD	BEHAVIOR TEST	RESULT	REFERENCE
Mice (Swiss) 1 month (male)	Oral (gavage)	Roundup herbicide (glyphosate concentration 360 g/l in the form of glyphosate isopropylamine salt 486 g/l)	Control (NaCl 0,9%) 250 mg/kg/day 500mg/kg/day	Acute (1 day) (n=18) Subchronic (6 weeks) (n=18) Chronic (12 weeks) (n=18)	Novel Object Recognition	Decreased ratio of time spent beside the novel object as well as the discrimination index, compared to control group. (Subchronic and chronic)	(Ait Bali, 2019)
Mice (Swiss) 3 months (female)	Oral (gavage)	Roundupr herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (tap water) 250 mg/kg/day 500mg/kg/day	Peri and post natal (G0 to postnatal day 21)	Novel Object Recognition	Lower time spent exploring the novel object and lower discrimination index, compared to control group, and significant diference between treated groups.	(Ait Bali, 2020)
Mice (CF-1) 65 days (male)	Intranasal	Glifloglex® (48 g of Gly isopropylamine salt/100 cm3 product - equivalent to 35.6% w/v of Gly acid)	Control (NaCl 0,9%) 50 mg/kg/day	Subchronic (3 times per week during 4 weeks)	Novel Object Recognition	Increased tendency of explore the novel object 6h after familiarization trial in control group and significant impairment of recognition in treated group.	(Baier, 2017)
Mice (ddY) 9-10 weeks old (female)	Oral (drinking water)	Roundup® Maxload [48% (w/v) glyphosate (N-phosphonomethylglycine) potassium salt, 52% other ingredients	Water 0,098% Glyphosate	Peri and post-natal (GD5 to PND21)	Novel Object Recognition	Exploratory preference was significant diferent between groups.	(Pu, 2020)

Continuation of Table 5

Rats (Wistar) 90–120 days (female)	Oral (drinking water)	Glifloglex® (48 g of Gly isopropylamine salt/100 cm3 product - equivalent to 35.6% w/v of Gly acid)	Peri-natal and post-natal (GD0 to PND21)	Control (tap water) 0,65 g/L of Gly (100 mg/kg/day) 1.30 g/L of Gly (200 mg/kg/day)	Novel Object Recognition	Diference between familiarization and test after 24h in male 0,65 g/L treated group.	(Gallegos, 2018)
Rats (Wistar) Newborn (male)	Subcutaneos injection	Sigma-Aldrich as a 40% water solution of N-phosphonomethyl glycine-monoisopropylamine salt	Subchronic (PND 7 to PND 27, every 48 h)	Control (Water) 35 mg/Kg 70 mg/Kg	Novel Object Recognition	Lower time exploring the novel object and discrimination index in treated groups, compared to control group.	(Luna, 2021)
Mice (Swiss) 1 month (male)	Oral (gavage)	Roundup herbicide (glyphosate concentration 360 g/l in the form of glyphosate isopropylamine salt 486 g/l)	Control (NaCl 0,9%) 250 mg/kg/day 500mg/kg/day	Acute (1 day) (n=18) Subchronic (6 weeks) (n=18) Chronic (12 weeks) (n=18)	Spontaneous alternation (Y-Maze)	Decreased spontaneous alternation in chronic group.	(Ait Bali, 2019)
Mice (Swiss) 3 months (female)	Oral (gavage)	Roundupr herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (tap water) 250 mg/kg/day 500mg/kg/day	Peri and post natal (G0 to postnatal day 21)	Spontaneous alternation (Y-Maze)	Lower spontaneous alternation in treated mice compared to control group, especially in 500 mg/kg than the 250 mg/kg.	(Ait Bali, 2020)

Continuation of Table 5

Rats (Wistar) 90–120 days (female)	Intraperitonial	Glyphosate Sigma-Aldrich as a 40 % water solution of N-phosphonomethyl glycine monoisopropylamine salt	Control (PBS solution) 24 mg/kg 35 mg/kg	Peri natal (GD8 to GD20 every 48h)	Morris water maze	No significant difference.	(Coullery, 2020)
Rats (Wistar) Newborn (male)	Subcutaneos injection	Sigma-Aldrich as a 40% water solution of N-phosphonomethyl glycine-monoisopropylamine salt	Subchronic (PND 7 to PND 27, every 48 h)	Control (Water) 35 mg/Kg 70 mg/Kg	Morris water maze	Longer time spent to locate the hidden platform in treated groups than controls on 3º and 4º days. Lower time spent in the target quadrant on trial day when the platform was removed, compared to control groups. Different time spent in the plataform quadrant in exposed groups compared to control.	(Luna, 2021)
Mice (Swiss) 3 months (female)	Oral (gavage)	Roundupr herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (tap water) 250 mg/kg/day 500mg/kg/day	Peri and post natal (G0 to postnatal day 21)	Passive Avoidance	Decreased latency after 2h in 500mg/Kg exposed groups and decreased latency 24h after the electrical shock administration in treated groups.	(Ait Bali, 2020)
Rats (Wistar) 90–120 days (female)	Intraperitonial	Glyphosate Sigma-Aldrich as a 40 % water solution of N-phosphonomethyl glycine monoisopropylamine salt	Control (PBS solution) 24 mg/kg 35 mg/kg	Peri natal (GD8 to GD20 every 48h)	Contextual fear conditioning	Lower freezing time in 35 mg/kg treated group during the test phase compared to control and 24 mg/Kg groups.	(Coullery, 2020)

Tabel 6: Behavioral results about interaction evaluation.

SPECIE/STRAIN SEX/AGE	EXPOSURE ROUTE	EXPOSURE AGENT	DOSEGE/ CONCENTRATION	EXPOSURE PERIOD	BEHAVIOR TEST	RESULT	REFERENCE
Mice (Swiss) 3 months (female)	Oral (gavage)	Roundupr herbicide (Gly concentration 360 g/l in the form of Gly isopropylamine salt 486 g/l)	Control (tap water) 250 mg/kg/day 500mg/kg/day	Peri and post natal (G0 to postnatal day 21)	Three-chambered sociability test	Lower time spent with the wire cup holding another conspecifc and longer time with the wire cup holding the object in treated mice, compared to the controls. Lower visit number of wire cup holding another conspecifc in 250 mg/Kg treated group, while both treated groups showed higher visit number of the wire cup holding the object compared to the controls.	(Ait Bali, 2020)
Mice (ddY) 9-10 weeks old (female)	Oral (drinking water)	Roundup® Maxload [48% (w/v) glyphosate (Nphosphonomethylglycine) potassium salt, 52% other ingredients	Water 0,098% Glyphosate	Peri and post-natal (GD5 to PND21)	Three-chamber Social Interaction	Social interaction deficits in juvenile offspring after maternal exposure.	(Pu, 2020)

Tabel 7: Risk of bias evaluation of studies methodologies according to the SYRCLE RoB Tool method.

	Selection bias			Performance bias		Detection bias		Attrition bias	Reporting bias	Other bias
	1	2	3	4	5	6	7	8	9	10
Aitbali, Y. (2017)	No	Yes	No	No	No	No	No	Yes	Yes	Yes
Aitbali, Y. (2018)	No	Yes	No	No	No	No	Yes	Yes	Yes	Yes
Aitbali, Y. (2019)	No	Yes	No	No	No	No	Yes	Yes	Yes	Yes
Aitbali, Y. (2020)	No	Yes	No	No	No	No	Yes	Yes	Yes	Yes
Baier, C. (2017)	No	Yes	Unclear	No	No	No	Unclear	Yes	Yes	Yes
Bicca, D. (2021)	No	Yes	Unclear	No	No	No	No	Yes	Yes	Yes
Cattani, D. (2017)	No	Yes	No	No	No	No	Yes	Yes	Yes	Yes
Coullery, R. (2020)	No	Yes	Unclear	No	No	No	No	Yes	Yes	Yes
Dechartes, J. (2019)	No	Yes	Unclear	No	No	No	No	Yes	Yes	Yes
Gallegos, C. (2016)	No	Yes	Unclear	No	No	No	No	Yes	Yes	Yes
Gallegos, C. (2018)	No	Yes	Unclear	No	No	No	No	Yes	Yes	Yes
Hernandez-Plata, I. (2015)	No	Yes	No	No	No	No	Yes	Unclear	Yes	Yes
Joaquim, A. (2013)	No	Yes	No	No	No	No	No	Yes	Yes	Yes
Joaquim, A. (2014)	No	Yes	No	No	No	No	No	Yes	Yes	Yes

Continuation of Table 7

Luna, S. (2021)	No	Yes	Unclear	No	No	No	No	Yes	Yes	Yes
Pu, Y. (2020)	No	No	No	No	No	No	No	Unclear	Unclear	Yes

5. CONCLUSÕES

De acordo com os objetivos propostos, conclui-se que as vias utilizadas para a exposição ao glifosato pelos estudos revisados caracterizam exposições de cunho alimentar, relacionado à população em geral, e respiratório, que se relaciona principalmente com uma exposição ocupacional devido à fabricação e aplicação do agrotóxico. As metodologias aplicadas pelos estudos permitiram concluir que a exposição ao glifosato está relacionada a alterações comportamentais como comportamento tipo ansioso e depressivo, alterações locomotoras, de memória e de interação social em ratos e camundongos. Além disso, é necessária melhor investigação acerca dos mecanismos de ação relacionados às alterações comportamentais, além da utilização de grupos expostos somente ao glifosato e ao formulado comercial em futuros estudos, para descartar a relação dos adjuvantes presentes na formulação comercial com as alterações de comportamento observadas nos ratos.

Os estudos avaliados demonstraram vieses comuns de seleção e alocação relacionados ao modo em que a experimentação animal é conduzida na área acadêmica atualmente. Portanto, para a minimização destes vieses, é necessária a criação de protocolos para a experimentação com roedores para que haja melhor randomização e cegamento dos ensaios.

6. CONSIDERAÇÕES FINAIS

A revisão sistemática foi conduzida com o intuito de compilar os resultados disponíveis acerca da neurotoxicidade do glifosato e a sua relação com os efeitos comportamentais em roedores, além de relacionar com o tempo, período e vias de exposição, e qualificar as metodologias utilizadas pelos estudos. De acordo com as expectativas, objetivos e os resultados encontrados no presente trabalho, foi possível relacionar de forma mais confiável a exposição ao glifosato e os efeitos comportamentais seja em fases do desenvolvimento ou na vida adulta dos roedores com diferentes vias e tempos de exposição. Visto que a maioria dos resultados encontrados concluem que houve alterações de comportamento devido à exposição ao produto comercial, é de extrema importância pesquisar e relacionar estas respostas fisiológicas com os mecanismos de ação para que se possa reverter esta toxicidade ou até mesmo encontrar meios de proteção. Ainda, como somente 1 artigo comparou o glifosato em grau técnico com o formulado comercial utilizado nas plantações, se faz necessário realizar futuros estudos que comparem estes dois produtos, de modo a isolar os efeitos relacionados somente ao glifosato dos efeitos de uma exposição a um formulado que possui a combinação do herbicida com demais adjuvantes, como surfactantes. Por meio dos resultados encontrados na avaliação da qualidade das metodologias aplicadas pelos autores, pode-se concluir que os protocolos utilizados atualmente para a pesquisa com roedores nesta área ainda carecem de estratégias para randomização e cegamento, garantindo menores vieses e maior confiança nos resultados gerados.