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Review - Human and Animal Health

Drosophila melanogaster as a Biotechnological Tool to Investigate the Close Connection Between Fatty Diseases and Pesticides

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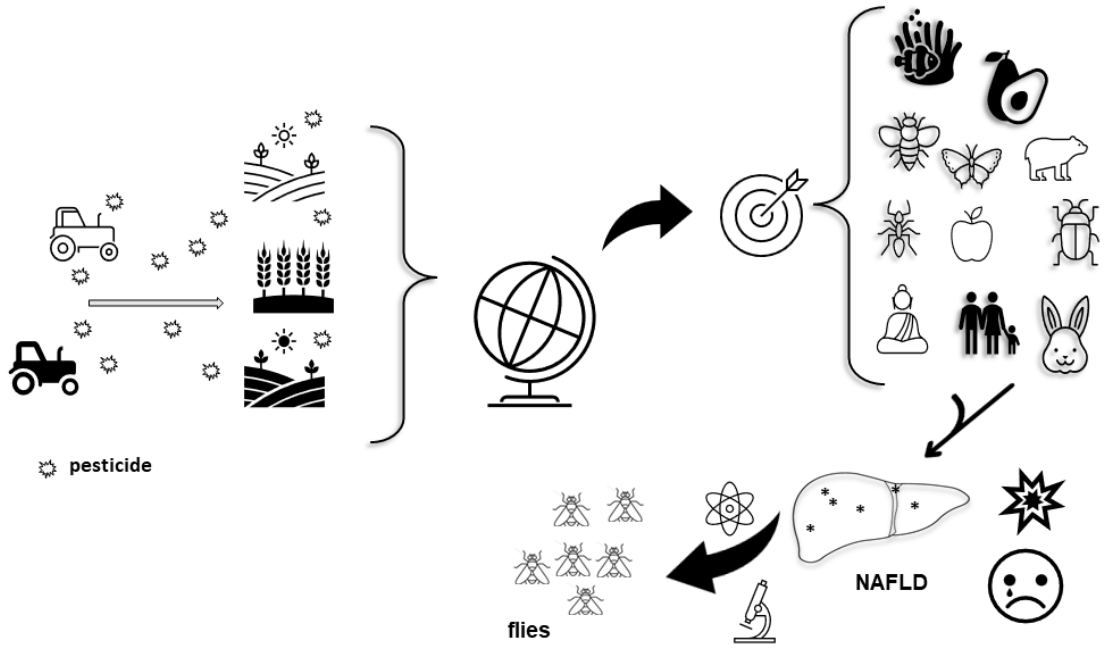
HIGHLIGHTS (MANDATORY)

- Nonalcoholic fatty liver disease as a global public health problem.
- Pesticides as poisoning elements to lipid metabolism and the liver homeostasis.
- *D. melanogaster* as biotechnological tool to detail molecular routes of fatty liver.

Abstract: Nonalcoholic fatty liver disease (NAFLD) is a public health problem developed by different etiologies, which induces metabolic dysfunctions and triglycerides accumulation in hepatocytes. This lipid accumulation can generate lipotoxicity, inflammation and the production of reactive oxygen species, collaborating with the progression of liver pathogenesis to more deleterious stage. Among the elements that initiate the establishment of liver diseases, pesticides should be considered. Worldwide, the use of agricultural chemicals to increase food production may accumulate in the environment, affecting non-target organisms. Thus, worldwide legislation must control pesticides use to preserve economies and lives. In this context, to address pesticide toxicity, the alternative animal model, *Drosophila melanogaster*, emerges as relevant biotechnological tool to investigate molecular connectors of toxic mechanisms in the establishment and development of NAFLD and liver diseases. In this review a comprehensive explanation about pesticides on human health and the use of *Drosophila melanogaster* as an alternative approach to defeat NAFLD will be presented.

Keywords: alternative animal model; biotechnology tools; NAFLD; pesticide; public health.

GRAPHICAL ABSTRACT



INTRODUCTION

Liver diseases are serious problems worldwide and account for approximately 2 million deaths per year and continue to increase [1-4]. They comprise a variety of diseases, which include metabolic disruptions, such as fat accumulation, hepatitis, organ fibrosis and/or cirrhosis and hepatocellular carcinoma [1,5,6]. Among them, nonalcoholic fatty liver disease (NAFLD) or hepatic steatosis is relevant. It begins when metabolic dysfunctions of the body lead to the accumulation of triglycerides in hepatocytes. Histologically, steatosis establishes when at least 5% of the total weight of the liver are lipids [2].

Steatosis has different nonalcoholic etiologies including obesity, type 2 diabetes, poor lifestyle, viral infections, genetics, and even environmental contaminants, which contribute to the development of this metabolic syndrome [2,5-8]. Because of this characteristic, the most accepted theory to explain the origin, development, and progression of nonalcoholic hepatic steatosis is the hypothesis of multiple hits [7,9]. This hypothesis takes into account the deleterious agents that disrupt organ homeostasis, favoring lipid accumulation and subsequent tissue disorganization. In addition, a negative aspect to consider is that at its initial steps, steatosis may not result in clinical manifestations; however, as the disease progresses, symptoms appear [10]. Nonalcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC) result from NAFLD progression to more deleterious stages [2,5,6,11]. Unfortunately, in our modern societies, NAFLD is currently considered an epidemiological problem affecting approximately 25% of the human population and should be carefully considered because of the lack of effective therapy to control or reverse this pathology. Currently, treatment strategies focus on weight loss and insulin resistance management through lifestyle changes, some medications and/or surgical procedures [2,5,12].

In fact, genetics largely contributes to the onset of steatosis; however, one point to consider is how public agencies and governments deal with health concerns and issues. Especially in poor and underdeveloped countries, where health systems are not accessible for everybody, the management of NAFLD has been considered a serious burden to public policy, because of the increasing number of people suffering from the disease [13]. In Latin America, for example, 30.5% of individuals present NAFLD. In addition, 61% of patients with NAFLD in South America also have NASH [5,14]. In Brazil, for example, although the incidence of NAFLD is not known, ultrasound evaluations estimate that approximately 18% of the population has hepatic steatosis [15]. Moreover, in that country, between 2001 and 2010, 853,571 hospital admissions were caused by liver diseases, 35% of which resulted from advanced clinical conditions [16], many of which had liver steatosis as their etiology. Other countries have their own characteristics correlated to the establishment and development of NAFLD, which depend on the population lifestyle and genetics [17]. In general, the mortality rates due to complications of NAFLD are ~ 15.44 per 1000 patients per year [2], and cardiovascular complications are the most common cause of death among patients with steatosis, especially among lean

patients. In addition, chronic kidney diseases, obstructive sleep apnea, and osteopenia are also related to NAFLD, affecting both adults and children, decreasing their quality of life [2,11,18,19]. Thus, considering the epidemiological characteristics of NAFLD and correlated implications, innovative therapeutics are needed.

Moreover, a critical aspect that should be considered as a real problem in several communities and countries is the large-scale use of environmental pollutants including pesticides, especially in countries economically dependent on the agricultural sector [20-22]. Several studies indicate that such agrochemicals have cumulative effects and act on non-target organisms even in human health [23-25]; however, there is still a lack of studies on the metabolic mechanisms of those compounds on cells that support the establishment of liver diseases, including NAFLD. Thus, such investigations are relevant to increase the understanding about the establishment and progression of NAFLD, as well as to develop innovative strategies to defeat the pathology.

Based on pointed observations, this study aims to present mechanistic aspects of nonalcoholic fatty liver disease and the worldwide problem of pesticides on liver health and their hepatotoxicity A solid and interconnected discussion will be presented about those themes, which indicate the need for society behavior changes. Moreover, the alternative animal model, *Drosophila melanogaster*, is presented as an alternative approach for the studies of the effects of toxic agents as pesticides in human diseases and metabolism [26,27], considering the limitation of human samples and the genetic similarities between humans and *Drosophilas*, which reinforces the use of this animal as an interesting tool in NAFLD research area.

MATERIAL AND METHODS

This study was elaborated based on literature search of full articles, reviews, short communications, and governmental information on the ISI Web of Science, Scopus and Pubmed databases with the keywords: “fatty liver”, “pesticides”, “lipid metabolism” and “Drosophila melanogaster”. The articles were analyzed, and relevant information found is presented in this study.

RESULTS: A REVISION OF THE LITERATURE

Lipid metabolism and its implications in the steatosis process

Lipids or fatty acids (FAs) in our diet are required to maintain cells and the entire body. In a cell, FAs are incorporated into the structure of membranes, they also can be stored, act as messenger molecules in cell signaling, or be used as an energy source due to their metabolism in oxidative reactions [28,29]. Those integrated reactions in a dynamic equilibrium maintain a healthy body; otherwise, the unbalanced equilibrium may result in pathogenesis [28]. Particularly, NAFLD begins with the intrahepatic accumulation of fat, mainly in the form of triglycerides (TGs), due to the imbalance absorption of free FAs that are incorporate through food ingestion, *de novo* lipogenesis (DNL) and decreased lipid oxidation [5,30]. After a meal, FA molecules are mostly absorbed from the circulation through fatty acid carrier proteins (FATPs). In addition, food ingestion stimulates metabolism and insulin release, which activates the glycolytic pathway and synthesis of lipogenic enzymes [31,32].

During the FA metabolism, citrate is produced as an intermediate molecule of oxidative reactions, which are metabolized by the ATP citrate lyase (ACLY), producing acetyl-CoA, which triggers the DNL. For that, the enzyme acetyl-CoA carboxylase (ACC) transforms acetyl-CoA into malonyl-CoA, whose levels are maintained in a dynamic equilibrium by malonyl-CoA decarboxylase (MLYCD or MCD), again producing acetyl-CoA (Figure 1). Moreover, increased concentrations of malonyl-CoA, allosterically inhibit carnitine palmitoyl transferase (CPT-1) in the outer membrane of the mitochondria, blocking the transport of fatty acids into the organelle, and favoring the FA esterification reactions, and synthesis of triglycerides [33,34]. On the other hand, low malonil-CoA concentrations favor the transport of FAs for beta-oxidation reactions.

During lipid biosynthesis, the fatty acid synthase (FASN) enzyme uses acetyl-CoA and malonyl-CoA molecules as substrate in chemical reactions to produce mainly palmitate, or palmitic acid (16:0), which can be metabolized to different FAs by intermediation of the stearyl-CoA desaturase 1 enzyme (SCD1) [35,36]. To be metabolized, the synthesized FAs must be activated by the long-chain acyl-Coa-synthetase (ACSL), which adds an acetyl-CoA group to the lipid molecules. Once activated, FAs can be (1) degraded in oxidative reactions, (2) stored and/or (3) used for membrane biosynthesis or cell signaling [35,36].

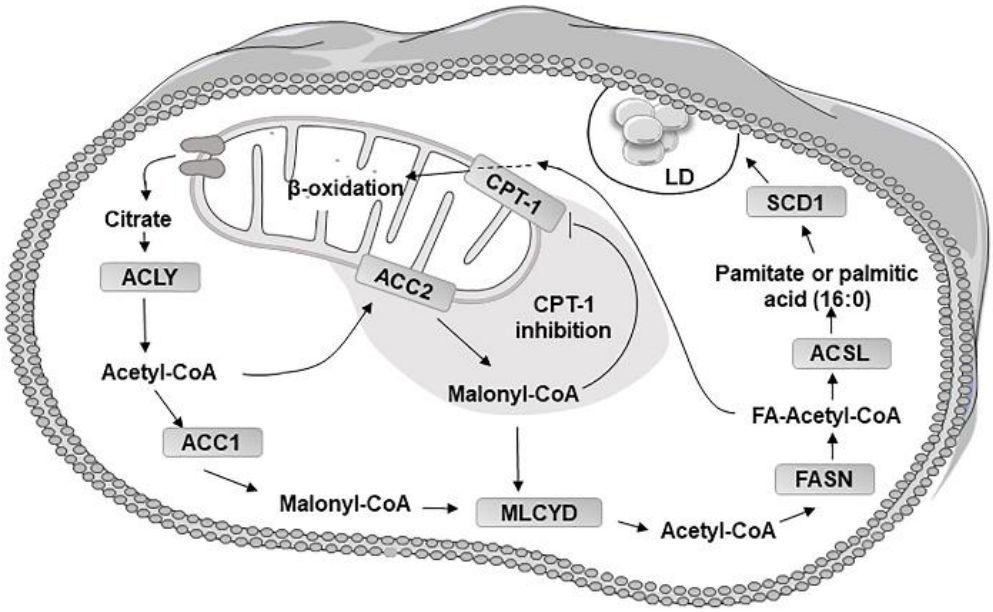


Figure 1. Schematic representation of lipid metabolic routes relevant to NAFLD establishment and progression

Moreover, other elements assist to strictly control lipid metabolism, and among them, transcription factors are relevant molecules in cellular signaling. The sterol regulatory binding protein 1c (SREBP1c), the carbohydrate response element binding protein (ChREBP), the peroxisome proliferator-activated receptors (PPARs), among others, play relevant functions in transcriptional control of important molecules in lipid metabolism and cell signaling network [29]. In addition, studies have demonstrated the effects of epigenetics and the intestinal microbiota in liver metabolism in the steatosis establishment [24,37,38], reinforcing the effects of the environment in controlling metabolism.

As previously described, deleterious stimuli disrupt hepatic metabolism and lipid accumulation in hepatocytes, which may cause NAFLD and lipotoxicity. Studies indicated that lipotoxicity correlates with the production of toxic lipid metabolites, such as ceramides, lysophosphatidylcholine, diacylglycerol and metabolites of cholesterol [39,40]. At the same time, lipotoxicity triggers the oxidative stress process and the production of reactive oxygen species (ROS), which alters mitochondrial function [41,42]. Mitochondrial dysfunction decreases ATP production and further increases the accumulation of toxic lipid intermediates, which accentuate the production of ROS and cell death mechanisms [32,40]. Together, metabolic changes favor the establishment of inflammatory conditions in the liver and the progression of pathogenesis to cirrhosis and even HCC [7,32,39,40,43].

Moreover, considering the deleterious stimuli that modify liver metabolism, several xenobiotics, including pesticides used in crops, are environmental pollutants that have been correlated with hepatotoxic capacities [41,44-46] as the development of fatty liver associated with toxic compounds (TAFLD) [44-49]. In addition, health screening to monitor people that work in agriculture fields, who are often exposed to toxic compounds, has not been shown to effectively detect TAFLD or the progression of pathogenesis. These results indicate that innovative ways to detect TAFLD and other hepatotoxicities should be developed. Moreover, directly or indirectly the entire population is exposed to agrochemicals, which may contribute directly to liver dysfunctions and diseases. The exposition and the consequences of that must be considered seriously.

Phytosanitary and its toxicity in lipid metabolism: an alarming situation to liver health

Phytosanitary products, also known as agrotoxics, pesticides, among others, are chemicals widely used in agriculture throughout the world for pest control to improve productivity of the agriculture commodities [20]. These substances are harmful to different classes of organisms and can be classified according to their target organism as insecticides, herbicides, and fungicides, for example [50]. However, many of these compounds accumulate in the environment and are toxic to non-target organisms [25,51,52] and, as previously mentioned, the large-scale use of the chemicals puts human health at risk [20,45,50,52,53]. Among the most used phytosanitary products organophosphates, organochlorinated, carbamates and pyrethroids takes relevant place [50]. Those compounds act on special metabolic routes inducing cellular damage and even death in affected organisms.

Organophosphates are pesticides whose esters are derived from phosphoric acid and promote neural hyper excitation by negatively modulating acetylcholinesterase activity, which cause cholinergic crises in target organisms [54]. Organochlorinated are organic compounds containing covalent bonded atoms of chlorine in its chemical structure. This class of pesticide is also very toxic to the non-target animals, even humans, causing a series of acute or chronic sequelae, such as neurological damage in contaminated organisms. In some countries, as in Brazil, because of their aggressiveness to the environment, the chemicals were substituted by other classes of pesticides [55]. Another widely used agrochemical is carbamate. This class of compounds is derived from carbamic acid and their structure consists mainly of an amide-ester bond. These agrochemicals have been used in various types of cultivars because of their rapid effect on target organisms [50]; however, adverse side effects of the chemical on non-target organisms have also been documented [56,57]. Finally, pyrethroids, which are the oldest class of pesticides, originally extracted from plants of the genus *Pyrethrum*, have still widely used around the world; and now these phytosanitary compounds are chemically synthesized. They reduce acetylcholinesterase activity, act on voltage-dependent Na+ channels, causing depolarization of nerve cells, neuronal excitation and death of the target organism [55]. These chemicals are frequently used to control insects and domestic pests, because of their lower environmental toxicity, due to their decomposition by sunlight. However, studies have demonstrated that these pesticides can alter the resistance of insects, creating an environmental imbalance, which is a serious concern [58-60].

Pesticide use started to scale up during the so-called “Green Revolution” which began in the 1960s supported by the technical development of agriculture machines, seeds, fertilizers and pesticides. This modernization in agriculture increased the production of seeds worldwide, and since then pesticides have been used on a large scale [61]. However, considering their ability to poison the environment and human health, many countries have tried control the total amount of pesticides used in crop fields, and even prohibit the use of some of them [62]. Table 1 lists the 10 best-selling pesticides in the world. Accordingly, the herbicide glyphosate [N-(phosphonomethyl) glycine], which belongs to the organophosphate group is the most widely used, followed by the 2,4-D and Mancozeb, despite the immense concerning about the unhealth effects of these chemical compounds to humans and other animals and to the environmental equilibrium [55]. Table 1 also suggests that it will be a long journey until the rational use of agrochemicals is achieved and the need to adopt laws that preserve the country’s economies and environmental and populations health. Moreover, comparing the use of pesticides in different countries, it is observed that economies dependent on agricultural activities employ large number of pesticides. The exacerbated consumption of pesticides has made Brazil one of the largest consumers of these compounds in the world [63]. In addition, Brazilian legislation has flexibility and favors the use of pesticides on large scale, which should be considered carefully, because they could compromise the equilibrium of the environment and the population’s health [23,24]. For humans, the main route of pesticides contamination is through the food chain [64]. Residues of these compounds can be found both in water and in food and are often routinely ingested without causing symptoms of acute intoxication. However, the frequent exposure to pesticides has been associated with adverse effects causing metabolic disorders, cancer, problems with fetal development, among others [20,45,46,53,64,65]. Reinforcing such observations, significant number of evidence indicate that the exposure to phytosanitary products is a serious risk factor for the development of NAFLD, since they disrupt homeostasis, alter the energetic metabolism and unbalanced the release of hormones that maintain the equilibrium [45,46,48,53,56,66].

Table 1. Ten best-selling active ingredients – 2020.

Top 10 Best-Selling Active Ingredients - 2020						
Unit of measurement: tons of active ingredients (AI)						
Ranking	**Active Ingredient**	**Classes (according to the nature of the organism to be fought)**	**Chemical group**	**Sales (ton. AI)**	**Banned countries (PAN, 2021)**	**Total bans for active ingredient**
1º	Glyphosate and its salts	Herbicide	Organophosphate	246.017,51	Luxembourg; Mexico; Vietnam	3
2º	2,4-D	Herbicide	Dinitrophenols	57.597,57	Mozambique; Norway; Vietnam	3
3º	Mancozeb	Acaricide/ Fungicide	Dithiocarbamates	50.526,87	EU*; Saudi Arabia; UK*	29
4º	Atrazine	Herbicide	Triazine	33.321,11	Cape Verde; Chad; Egypt; EU; Gambia; Mauritania; Morocco; Niger; Oman; State of Palestine; Senegal; Switzerland; Togo; UK; Uruguay	41
5º	Acephate	Acaricide/ Insecticide	Organophosphate	29.982,50	China; EU; Indonesia; Malaysia; Oman; State of Palestine; Serbia; Switzerland; UK	35
6º	Chlorothalonil	Fungicide	Isophthalonitrile	24.191,03	Colombia; EU; State of Palestine; Saudi Arabia; Switzerland; UK	32
7º	Malathion	Acaricide/ Insecticide	Organophosphate	15.702,11	EU; Indonesia; State of Palestine; Switzerland; Syria; UK	32
8º	Sulphur	Acaricide/ Fungicide	Inorganic	11.390,90	-	-
9º	Imidacloprid	Insecticide	Neonicotinoid	9.401,65	EU*; UK*	28
10º	Chlorpyrifos	Acaricide/ Insecticide	Organophosphate	8.864,88	EU*; Indonesia; Morocco; State of Palestine; Saudi Arabia; Sri Lanka; Switzerland; UK*; Vietnam	35

* = Not approved

Source: Adapted from AGROFIT and IBAMA / Consolidation of data provided by companies registering technical products, pesticides and the like, according to art. 41 of decree no. 4074/2002. Last updated data on 06/14/2021.

Considering the lipid metabolism of humans and animals, the phytosanitary products act through different mechanisms. Initially, they can change the pattern of lipid absorption through the intestine due to the induction of dysbiosis, the intestinal barrier and the metabolites released by bile secretion [53,56]. Pesticides can also interfere in the process of TG storage in the adipose tissues and liver and induce obesogenic effects in the organ [67,68], which may be connected to the development of insulin resistance, representing a higher risk for the development of type 2 diabetes and, consequently, NAFLD [53,69,70]. The ability of pesticides to unbalance lipid homeostasis is extensively reviewed in the literature. These substances can modulate the activation of molecular connectors of cellular signaling in lipid metabolism, increasing hepatic DNL and the accumulation of FAs in the liver [29,68]. Agrochemicals also activate molecules and metabolic pathways connected to the detoxification processes of xenobiotic agents. Among them, the activation of the nuclear receptor pregnane X (PXR) is observed [71]. Those transcription factors activate multiple genes involved in the metabolism of xenobiotics. PXR can also activate the peroxisome proliferator-activated receptor gamma (PPARγ), an important regulator of lipid metabolism, by favoring the hepatic uptake of FA molecules in the circulation and their accumulation within cells in lipid droplets [72]. In addition, positive PXR activation can decrease CPT1a enzyme levels, decreasing the β-oxidation process and resulting in greater accumulation of intracellular lipids [68].

Another xenobiotic detoxifying enzyme activated by pesticides is the cytochrome P450 complex, which induces ROS production [73,74] and is a relevant risk factor for the development and progression of NAFLD. Studies have indicated that the increased ROS production by pesticides is due to the reduction of the competence of the antioxidative system of the cell, since those compounds can alter the levels of antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) [41,69] This reduction in the antioxidant system changes the cellular signaling of adverse outcome pathways (AOP), which alter the activation pattern of receptors and transcription factors, further increasing the negative feedback of cell regulation against deleterious agents [75,76].

Therefore, the evaluation of cellular and molecular events that occur in cells exposed to the phytosanitary will help in the rational use of chemical compounds to ensure the quality of life of the population and the country's economy. This observation is relevant, considering that pesticides modulate gene expression in a negative way [77,78], which affects the fine-tuned regulation of the metabolism, contributing to the increasing number of steatosis and other liver diseases in modern societies [44,46]. Thus, investigating the molecular transactivation of cellular pathways to mitigate TAFLDs is relevant.

The alternative animal model *Drosophila melanogaster* as a biotechnological approach to defeat NAFLD-pesticide dependence

The liver is one of the main organs affected by drugs and other chemical compounds including pesticides. Considering its high metabolic rate, changes in its homeostasis caused by environmental compounds and other chemicals may lead liver to serious pathogenesis and even cancer. Over the last decades, technological advances have enabled the elucidation of metabolic routes and a large number of studies address the effects of environmental contaminants on human health and on the establishment of liver diseases. Some of them use *in silico analyses* [79], cell culture approaches [74,76,78] and animal models [80], considering the limitation of human samples. In addition, systematic reviews, and meta-analyses of toxic agents on liver health have been conducted over the last decade, demonstrating the relevance of this investigative research area. However, several of these models have high operating costs, delayed experimental standardization and ethical issues [27]. Therefore, alternative methodologies that allow systemic analysis of the effect of pesticides and toxic contaminants in a fast and practical manner are mandatory to our modern society.

Over the last years, alternative animal models have emerged in studies of toxic effects of chemicals, pesticides, and new products as nanomaterials [81,82]. In addition, those models have been contributing to mechanistic studies allowing a deep understanding of molecular and biochemical effects of the xenobiotics to targets and non-target animals. Among the innovative models, *Caenorhabditis elegans* [83,84], *Daphnia magna* [85,86] and *Danio rerio* [87] have been used and largely contributed to predictive toxicology area becoming a novel platform for a large-scale investigation of toxicants. Another alternative model that has emerged as a powerful tool in toxicological area is *Drosophila melanogaster*. This model has been studied since its introduction by Thomas H. Morgan at the beginning of century XX, and, in the last two decades, emphatic use of this animal in toxicology has been increasing [81,88] and among the studies, the toxicological effects of the pesticides have been explored.

Particularly, extensive literature presents results of physiological effects of the pesticides using *D. melanogaster* as an alternative animal model m. Table 2 summarizes many of those references found in the literature and some are presented and discussed in a sequence. As an example of those studies, Leão and coauthors [114] demonstrated that the exposure of *D. melanogaster* to the herbicide Palace® (a mixture of active 2,4-D and picloram ingredients) increased the mortality rate in adult flies in a dose-dependent manner. The authors also observed adverse effects on the development and behavior of flies, possibly related to mitochondrial dysfunction. In Mandi and coauthors [113], the insecticide acephate reduced the body weight of adult flies in a dose-dependent manner, caused changes in testicular structure, reduced the viability of germinative cells, increased the activity of enzymes correlated to oxidative stress, among other negative effects on the flies' homeostasis. In another study, Saraiva and coauthors [118] exposed flies to the fungicide mancozeb through the diet. The results demonstrated increased mortality rates and locomotor dysfunction in a time-dose-dependent manner. In addition, increased oxidative stress and changes in the activities of antioxidant enzymes, such as CAT, glutathione s-transferase (GSTs) and SOD were observed as a result of mancozeb activity in flies. Other studies point to relevant aspects of the toxicity mechanism, reinforcing the relevance of this alternative model for the biological sciences and health.

Table 2. *D. melanogaster* as an alternative animal for toxicity investigation in academy.

Year	Publication	Reference
2022	Rotenone mediated developmental toxicity in *Drosophila melanogaster*	[88]
2022	Characterization of a novel pesticide transporter and P-glycoprotein orthologues in *Drosophila melanogaster*	[89]
2022	Age-related tolerance to paraquat-induced parkinsonism in *Drosophila melanogaster*	[90]
2022	Potentiation of paraquat toxicity by inhibition of the antioxidant defenses and protective effect of the natural antioxidant, 4-hydroxyisopthalic acid in *Drosophila melanogaster*	[91]
2022	Herbicide Roundup shows toxic effects in nontarget organism *Drosophila*	[92]
2022	Protective capacity of carotenoid trans-astaxanthin in rotenone-induced toxicity in *Drosophila melanogaster*	[93]
2022	Cyromazine Effects the Reproduction of *Drosophila* by Decreasing the Number of Germ Cells in the Female Adult Ovary	[94]
2022	Low doses of the organic insecticide spinosad trigger lysosomal defects, elevated ROS, lipid dysregulation, and neurodegeneration in flies	[95]
2022	Short exposure to nitenpyram pesticide induces effects on reproduction, development and metabolic gene expression profiles in *Drosophila melanogaster* (Diptera: Drosophilidae)	[96]
2022	Using tissue specific P450 expression in *Drosophila melanogaster* larvae to understand the spatial distribution of pesticide metabolism in feeding assays	[97]
2021	An integrated host-microbiome response to atrazine exposure mediates toxicity in *Drosophila*	[98]
2021	Effects of some insecticides (deltamethrin and malathion) and lemongrass oil on fruit fly (*Drosophila melanogaster*)	[99]
2021	Chronic exposure to paraquat induces alpha-synuclein pathogenic modifications in *Drosophila*	[100]
2021	Pre-imaginal exposure to Oberon® disrupts fatty acid composition, cuticular hydrocarbon profile and sexual behavior in *Drosophila melanogaster* adults	[101]
2021	Transcriptomic identification and characterization of genes commonly responding to sublethal concentrations of six different insecticides in the common fruit fly, *Drosophila melanogaster*	[102]
2021	Protective effect of *Catharanthus roseus* plant extracts against endosulfan and its isomers induced impacts on non-targeted insect model, *Drosophila melanogaster* and live brain cell imaging	[103]
2021	Chlordane exposure causes developmental delay and metabolic disorders in *Drosophila melanogaster*	[104]
2021	Dietary behavior of *Drosophila melanogaster* fed with genetically-modified corn or Roundup®	[105]

Cont. Table 2

2021	Genetic basis of susceptibility to low-dose paraquat and variation between the sexes in *Drosophila melanogaster*	[106]
2021	Oxidative stress and decreased dopamine levels induced by imidacloprid exposure cause behavioral changes in a neurodevelopmental disorder model in *Drosophila melanogaster*	[107]
2021	Mancozeb impairs mitochondrial and bioenergetic activity in *Drosophila melanogaster*	[108]
2020	Exploring the multilevel hazards of thiamethoxam using *Drosophila melanogaster*	[109]
2020	Sublethal larval exposure to imidacloprid impacts adult behaviour in *Drosophila melanogaster*	[110]
2020	Low doses of the neonicotinoid insecticide imidacloprid induce ROS triggering neurological and metabolic impairments in *Drosophila*	[111]
2020	Exposure to Spectracide® causes behavioral deficits in *Drosophila melanogaster*: Insights from locomotor analysis and molecular modeling	[112]
2020	Potential risk of organophosphate exposure in male reproductive system of a non-target insect model *Drosophila melanogaster*	[113]
2019	Toxicological evaluation of the herbicide Palace® in Drosophila melanogaster	[114]
2019	Effect of herbicide glyphosate on *Drosophila melanogaster* fertility and lifespan	[115]
2019	Deleterious effects of neonicotinoid pesticides on *Drosophila melanogaster* immune pathways	[116]
2019	Atrazine or bisphenol A mediated negative modulation of mismatch repair gene, mlh1 leads to defective oogenesis and reduced female fertility in *Drosophila melanogaster*	[117]
2018	Exposure of *Drosophila melanogaster* to mancozeb induces oxidative damage and modulates Nrf2 and HSP70/83	[118]
2018	Azadirachtin effects on mating success, gametic abnormalities and progeny survival in *Drosophila melanogaster* (Diptera)	[119]
2018	Azadirachtin acting as a hazardous compound to induce multiple detrimental effects in *Drosophila melanogaster*	[120]
2017	Mutagenic, recombinogenic and carcinogenic potential of thiamethoxam insecticide and formulated product in somatic cells of *Drosophila melanogaster*	[121]
2017	Azadirachtin impact on mate choice, female sexual receptivity and male activity in *Drosophila melanogaster* (Diptera: Drosophilidae)	[122]
2017	Changes in neuronal signaling and cell stress response pathways are associated with a multigenic response of *Drosophila melanogaster* to DDT selection	[123]
2017	Monitoring the effects of a lepidopteran insecticide, flubendiamide, on the biology of a non-target dipteran insect, *Drosophila melanogaster*	[124]
2016	Atrazine exposure affects longevity, development time and body size in *Drosophila melanogaster*	[125]
2015	Fipronil induces apoptosis through caspase-dependent mitochondrial pathways in *Drosophila* S2 cells	[126]
2015	Study of the changes in life cycle parameters of *Drosophila melanogaster* exposed to fluorinated insecticide, cryolite	[127]
2014	Genotoxicity of dichlorvos in strains of *Drosophila melanogaster* defective in DNA repair	[128]
2014	Lethal and sublethal effects of imidacloprid, after chronic exposure, on the insect model *Drosophila melanogaster*	[129]
2014	Assessment of toxicity and potential risk of butene-fipronil using *Drosophila melanogaster*, in comparison to nine conventional insecticides	[130]
2014	Growth inhibition and differences in protein profiles in azadirachtin-treated *Drosophila melanogaster* larvae	[131]
2014	Azadirachtin blocks the calcium channel and modulates the cholinergic miniature synaptic current in the central nervous system of *Drosophila*	[132]

Cont. Table 2

2013	Acute exposure of *Drosophila melanogaster* to paraquat causes oxidative stress and mitochondrial dysfunction	[133]
2013	Paraquat-induced ultrastructural changes and DNA damage in the nervous system is mediated via oxidative-stress-induced cytotoxicity in *Drosophila melanogaster*	[134]
2011	Evaluation of toxicity and genotoxic effects of spinosad and deltamethrin in *Drosophila melanogaster* and *Bactrocera oleae*	[135]
2006	Evaluation of volatile low molecular weight insecticides using *Drosophila melanogaster* as a model	[136]
2005	Comparative toxic potential of market formulation of two organophosphate pesticides in transgenic *Drosophila melanogaster* (hsp70-lacZ)	[137]
2004	Evaluation of in vivo genotoxicity of cypermethrin in *Drosophila melanogaster* using the alkaline comet assay	[138]

Considering the pros and cons of alternative approaches to address functional and mechanistic aspects in biology, *Drosophila melanogaster* emerges as a powerful tool for the studies of human diseases and metabolism changes [27,139-143] induced by the effects of toxic agents as xenobiotics and pesticides [144]. This observation is pointed because most genes and metabolic pathways involved in liver diseases find their orthologs in *Drosophila* (~75% pathogenic-related human genes find their orthologs in fruit flies [27,145-149), despite the small complexity of the animal's genome, when compared to human [27]. In addition, the insect has a short life cycle and low maintenance cost [81,82]. In ~ 12 days a single mating pair generates dozens of offspring, which are useful to investigate developmental processes studies in larvae and adults. Moreover, adult flies have structures equivalent to mammalian organs, which are also useful for the study of different and human diseases as cardiac and neurological problems, renal and gastrointestinal diseases, metabolic diseases as diabetes and obesity and even cancer [148,149], among others. Thus, considering the metabolism, flies can be a useful biotechnological tool [145,146] for the study of toxicants induction of NAFLD.

Although the mammalian liver equivalent is not found in Drosophila, the fatty body (FB) performs liver functions [150], along with the action of oenocytes [151,152]. In *Drosophilas* the FB is a tissue that controls energy stocks in insects at all stages of development. Moreover, as a parallelism between human liver metabolism and the correlated insect metabolism, the presence of a powerful detoxification system in FB and oenocytes are present [153]. Further, parallel signaling pathways control lipid and sugar metabolism in response to the environment both in mammals and Drosophila [154]; Sanguesa and coauthors [155]. This is reinforced by the observation that in *Drosophila*, some classes of mammalian-like hormones are found, such as insulin-like peptides (ILPs), which are involved in the homeostasis of the energy metabolism of insects [156]. This group of molecules has amino acid sequences like human insulin and performs analogous functions in flies [156-158], controlling glucose and energetic metabolism as in mammals. More research should be done taking flies as an alternative approach in predictive toxicology to elucidate molecular mechanisms induced by the pesticides in human and animals' physiology, which can support the development of the effects of pesticides on fatty liver disease establishment and progression.

CONCLUSIONS

In recent decades, the uncontrolled use of pesticides has contributed to increasing rates of environmental contamination and deleterious effects to animal and human health. Thus, it is urgent to establish a rationale way to use pesticides in agriculture, for the safety of the economy and the quality of life of the environment and its habitants. Statistical analyses of regulatory agencies have demonstrated exacerbated consumption of phytosanitary products in some countries, while others have tried to strictly control pesticide use, by prohibiting their irresponsible commercialization. The world must achieve a correct protocol to educate the pesticide consumer to safeguard many countries' economies and the health of the planet.

As discussed above, pesticides may act on non-target organisms, considering their accumulation in the environment, which affects many cellular and molecular routes, breaking down cellular homeostasis. In humans, a sequence of metabolic changes and disruption of physiological routes are observed, contributing to the onset of NAFLD and reinforcing the toxicity of the environmental contaminants. In addition, the development of alternative protocols to safety evaluates the real toxicity of phytosanitary compounds, including a better description of the deleterious pathways, which may lead to liver pathologies, will be very useful to science and health. Thus, the alternative animal model, *Drosophila melanogaster*, represents a powerful and interesting biotechnological, which is a useful tool to systemically investigate the real toxic effects of several pesticides on the establishment and progression of fatty liver in the modern world.

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