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Carcinogenicity and mutagenicity of malathion and its two analogues: a systematic review

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Abstract *Malathion has been widely used worldwide in arbovirus control programs. In 2015, it was classified by the International Agency for Research on Cancer (IARC) as a probable carcinogen to humans. This work aimed to systematize the evidence of the carcinogenic and mutagenic effects associated with the exposure of malathion and its analogs, malaoxon and isomalathion. The search was carried out in Toxline, PubMed and Scopus databases for original papers published from 1983 to 2015. In all, 73 papers were selected from a total of 273 eligible papers. The results of in vitro and in vivo studies showed mainly genetic and chromosomal damages caused by malathion. The epidemiological studies evidenced significant positive associations for thyroid, breast, and ovarian cancers in menopausal women. This evidence of the carcinogenic effect of malathion should be considered before its use in arbovirus control programs.*

Key words *Malathion, Carcinogenicity Tests, Mutagenicity Tests, Environmental Health*

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Introduction

Pesticides are chemical compounds used extensively in agriculture, for the chemical control of spontaneous species in urban environment and vectors, in public health campaigns[1] and are a danger to humans and nature[2]. Among the widely used is the group of organophosphates (OP), which are irreversible inhibitors of acetylcholinesterase (AChE), active in all animal groups that use acetylcholine as a neurotransmitter[3]. The consequent accumulation of this molecule in the body causes toxic effects on the different systems of exposed living beings, such as muscular, nervous, immune and endocrine alterations[4-7]. Among its possible chronic effects, characterized by late onset, are irreversible damages, such as paralysis and neoplasms[8].

In Brazil, with the exponential growth of the dengue epidemic in 2015, when more than 1 million cases were confirmed[9], and with the onset of Chikungunya fever (in 2014) and the Zika virus epidemic (in 2015), with consequences even more harmful to the population, a review of the National Aedes aegypti (transmitter mosquito) Control Program was requested, with the intensified use of larvicides and adulticides for this mosquito, going back to the 2014 guidelines regarding the use of UBV spraying with Malathion at 30% diluted in water for the whole national territory[10]. This was criticized by the Brazilian Association of Public Health, which issued a technical note warning of the dangers to health and the environment[11].

Malathion (diethyl (dimethoxythiophosphorylthio) succinate) is an OP used in various food crops for the control of unwanted species and is often used in insect control[12], as a commercial or technical quality product containing approximately 90-95% of the product in weight and may contain up to twelve impurities formed during manufacture and storage[13]. Among the relevant impurities are malaoxon and isomalathion, produced by oxidation[14] and the chemical or thermal isomerization of malathion, respectively[15].

Its mutagenic capacity and potential carcinogenic effect have been discussed[16-18]. However, despite widespread use, there are surprisingly few studies on the association of malathion and cancer, most of them in North America, others in Europe, while very few have been conducted in less industrialized countries where exposure is likely to be much higher[19]. Some authors point out their findings as something of concern since malathion has shown high levels of carcinogenic activity, as well as chemical properties that bring it closer to other notably carcinogenic substances such as aflatoxin and benzopyrene[20].

In the 1980s, malathion was considered and evaluated by the International Agency for Research on Cancer (IARC) Working Group as not classifiable as to its carcinogenicity to humans (Group 3)[21,22] because it concluded that there was insufficient evidence for the carcinogenicity of malathion or its metabolite malaoxon in experimental animals, and data for humans were not available at the time. However, in 2015, IARC published a new document, classifying the pesticide as a probable human carcinogen (Group 2A)[19].

In view of the widespread use of malathion as a pesticide worldwide, both in agriculture and public health, and in view of the risks that it can bring, this work aimed to systematize the evidence of the carcinogenic and mutagenic effects associated with the exposure of this organophosphate pesticide and its analogues (malaoxon and isomalathion).

Methods

Search data

A systematic review of the literature was performed by searching scientific papers published between 1983 and 2015. The year 1983 was chosen as the starting point of the research, since the IARC[21] monograph published in that year considered malathion as "not classifiable as to its carcinogenicity to humans (Group 3)", concluding that there was insufficient evidence for the carcinogenicity of malathion or its metabolite malaoxon in animal experiments, and there were no data regarding humans.

The search was performed in the electronic databases Scopus, PubMed and Toxline (in the latter the results of PubMed were excluded), and two command groups were employed. The first, consisting of terms related to the exposure of interest (malathion, malaoxon and isomalathion), and the second containing terms related to the outcome of interest (*cancer*, *carcinogenicity tests*, *carcinogens*, *neoplasms*, *mutagenesis*, *mutagenicity tests* and *mutagens*). A query was made in the Medical Subject Headings (MeSH) to select the descriptors/terms used. Boolean operator "OR" was used for the combination of the terms in each group, and "AND" operator was used for the combination between the groups.

Selection of papers

We selected original studies that showed results on the carcinogenic or mutagenic effect of malathion, malaoxon and isomalathion in living beings. Review papers, dissertations and theses identified in the search were excluded. English, Portuguese and Spanish manuscripts were considered.

The papers were selected by the researchers (authors of this paper) in two stages. In the first one, two researchers read separately the title and summary of the papers for the selection of those that should be the basis of the research. Those without an abstract or insufficient information to make a decision were kept into the next step. Cases of disagreement were resolved by a third researcher.

In the second step, all papers selected that met the inclusion/exclusion criteria and those with insufficient information for decision-making were analyzed in their entirety. As in the previous stage, the data of both reviewers were again confronted, and the differences were solved by the third reviewer.

The selected manuscripts were analyzed for the extraction of the following data: authors, year of publication, journal, study design, target population, country of study population (in epidemiological studies), exposure (malathion, malaoxon, isomalathion, mixed exposure) and main results identified regarding the carcinogenic and mutagenic effect of malathion, malaoxon and isomalathion.

Search was performed between July 4 and 12, 2016. Filters were used to select the languages and the period of publication of the manuscripts in the three databases.

Results and discussion

Search returned 178 results in Scopus, 147 in PubMed and 92 in Toxline, totaling 417 papers (Figure 1). A total of 142 duplications were identified, as well as two papers published in other languages (German and Chinese), leaving out 273 papers for eligibility evaluation.

After applying the inclusion and exclusion criteria and the two-step evaluation, 73 papers were included in this study, all in English. Of these, 24 correspond to *in vitro* experimental studies, including mutagenicity tests and cell cultures of both animals and human beings; 24 *in vivo* experimental studies, including mice, rats, hamsters, birds, frogs and flies; 25 epidemiological studies, including cohorts, case-controls and cross-sectional studies (two of them with *in vitro* experiments as well). The results found in the *in vitro* experimental studies that evaluated the carcinogenic or mutagenic effect of malathion and its analogs are summarized in Chart 1.

Three studies have shown significant increases in the DNA damage of rat peripheral blood lymphocytes following exposure to malathion[1,18,26]. Higher frequencies of chromosomal changes in bone marrow cells of mice in a dose-dependent manner to malathion were also observed[26]. Besides observing the significant reduction of cell viability and the significant increase of breaks in both single and double strand DNA, Ojha and Gupta[18] verified the formation of DPC, in time- and dose-dependent fashion after exposure of rats' lymphocytes to malathion both individually and mixed with two other organophosphates (Chlorpyrifos and Parathion-Methyl) when compared to the control. Since DPCs correspond to toxic lesions associated with the toxicity mechanism (s) of carcinogenic compounds[45], the study shows the carcinogenic effect of malathion. The research further concludes that malathion, along with the other pesticides of the study, should generate oxidative lesions of DNA base pairs, with a genotoxic potential to alter enzyme expression.

In cultures of human peripheral blood lymphocytes exposed to malathion, results showed dose-dependent increase of chromosomal changes and sister chromatid exchanges[37,39], as well as the alkylating effect of DNA-specific nucleotides[34]. No significant changes were observed in the DNA damage of these same cells[32] when malathion was evaluated in comparison to its two analogs, unlike malaoxon and isomalathion, which induced DNA damage in a dose-dependent fashion. The study by Blasiak et al.[32] was the only one to evaluate the three chemical agents in the same study and concluded that the damages caused to the DNA by malaoxon are more pronounced than those caused by isomalathion.

Josse et al.[23] evaluated the effects of malathion and isomalathion individually and combined on the human liver HepaRG cell line and showed that, although isomalathion was much more cytotoxic than malathion, both substances showed similar mutagenic effects in these hepatocytes. On the other hand, Błasiak and Trzeciak[33] claim that DNA damage to human lymphocytes is caused by isomalathion and not by its original compound.

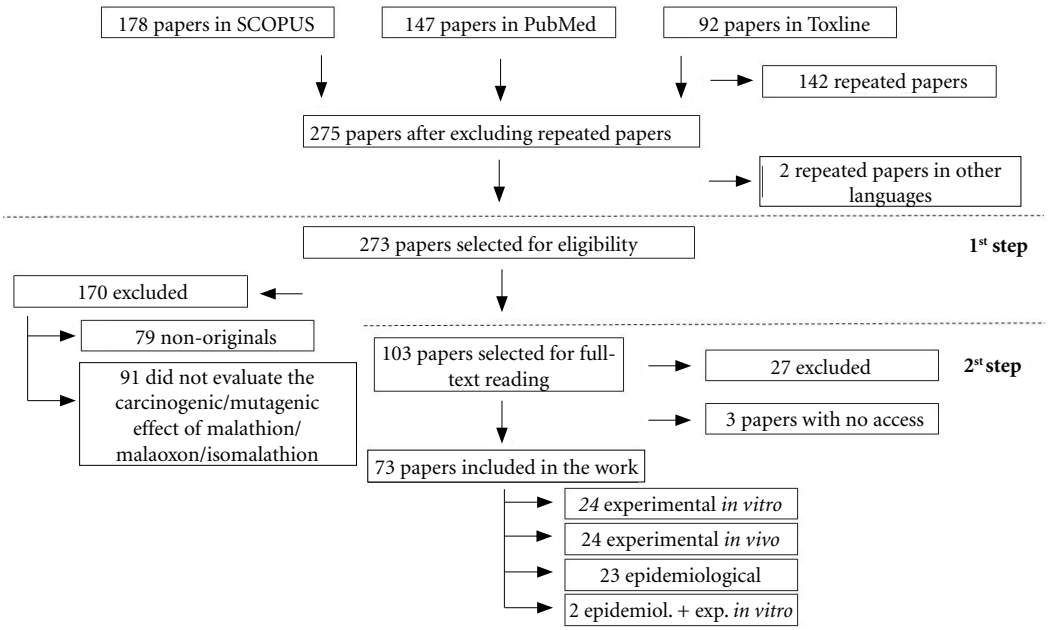

Figure 1. Flow chart illustrating the papers that were included and excluded in the systematic review, with reasons for exclusion.

Source: Elaborated by the authors.

The results are somewhat contradictory regarding malaoxon exposure. Blasiak and Kowalik[31] found that malaoxon promoted a significant increase in the level of DNA damage in human peripheral blood lymphocytes in the Comet Assay, and was higher with increasing dose, while in the human lineage choriocarcinoma cell (JAR) assay (model acceptable for human placental cells), malathion, and not the same agent, was found to be responsible for the cytotoxic and genotoxic effects in these cells *in vitro*. Authors argue that metabolites of pesticides with contradictory results regarding their carcinogenic potential should be studied, since they may represent the true carcinogens[46].

Malathion has also been evaluated in gene mutation tests that employ bacteria, such as the *Salmonella* lactam test (genotoxin detection method)[35], phage-MicroScreen (a miniaturized system that uses the induction of prophylaxis in *Escherichia coli* X as an indicator of genetic damage)[41] and the *Ames Salmonella* test[40]. In the three studies found, the results were negative for the mutagenic activity of malathion, and no mutagenic activities of malathion were observed in the last test before or after activation of the rat liver S9 fraction in the respective non-toxic doses (33 mg/L) and 90% toxic (1650mg/L). In a literature review, Flessel et al.[47] also observed the same results and concluded that malathion does not appear to induce timely mutations in DNA in bacterial systems.

Of the 24 *in vivo* experimental studies found in this systematic review, twenty performed the experiments on rats, mice or hamsters, while the others evaluated the effects of the pesticide on birds, frog and flies. Chart 2 shows the main results of these studies.

In 1984, Degraeve et al.[70] reported that Dynafos and Phosan Plus, insecticides containing malathion in their composition, did not induce chromosomal changes in bone marrow cells, spermatogonia or primary spermatocytes, nor alterations in the dominant lethal mutation assay, after intraperitoneal injection of rats of strain Q. However, the study has no data regarding impurities, solvents, emulsions and the like in these compounds, which shows its limitation.

In the same year, these authors exposed rats from the same strain to malathion at 99% purity

Chart 1. Synthesis of in vitro studies evaluating the association of exposure to malathion, isomalathion and malaoxon with the carcinogenic or mutagenic effects.

Authors, Year	Journal	Target Population	Exposure	Main Results Identified
Ojha e Gupta, 2015[18]	Hum Exp Toxicol	Wistar adult male albino rats (Rattus norvegicus)	Malathion, Chlorpyrifos, Methyl parathion (individually and mixed)	The 8h and 12h exposure of rat lymphocytes with 1/10 and 1/4 DL50 malathion (MLT) caused a significant ($p<0.05$) level of DNA damage (double DNA strand breaks, DSBs, and simple DNA strand breaks, SSBs). The formation of DNA-protein cross-links (DPC), in time and dose-dependent post exposure to the MLT, individually and mixed, was established in in vitro conditions as compared to the control.
Ojha e Srivastava, 2014[1]	Mutat Res Genet Toxicol Environ Mutagen	Wistar rats (peripheral blood lymphocytes)	Hydrogen peroxide, Chlorpyrifos, Methyl parathion and malathion (individually and mixed)	In vitro exposure of malathion rat lymphocytes individually or mixed for 2h and 4h caused a significant increase in DNA damage as evidenced by the Comet assay with dose-dependent increase of % of tail DNA, tail length and tail momentum. OPs induced oxidative stress in rat peripheral blood lymphocytes, responsible for DNA oxidation.
Josse et al., 2014[23]	Chem Biol Interact	Human hepatic HepaRG cell line	Malathion and isomalathion, individually and combined	Malathion and isomalathion alone or in combination induced the formation of micronuclei at low concentrations and had additive genotoxic effects when combined at 25 μM. Isomalathion individually or combined directly inhibited the activity of carboxylesterases (involved in malathion detoxification). Isomalathion was much more cytotoxic than malathion. Both compounds had comparable genotoxic effects on HepaRG hepatocytes at low concentrations.
Anjum e Malik, 2013[24]	Environ Toxicol Pharmacol	S. typhimurium strains TA97a, TA98, TA100, TA102 and TA104 and mutants of E. coli lineage K-12.	Malathion lindane, alpha-endosulfan, Chlorpyrifos, monocrotophos and dimethoate (mixed)	The test samples preferentially act on the base pair mutants GC (plotting) compared to those with AT base pairs at the mutation site and also initiate the inducible error-prone SOS response in the mutant E. coli water extracts. The mutagenic effect of the test samples would evidently represent a risk of neoplastic transformation in humans.
Galántai et al., 2011[25]	J Environ Sci Health B	Human lineage of choriocarcinoma cells (JAR) (model acceptable for human placenta cells)	Malathion and malaoxon	Malathion is responsible for the cytotoxic and genotoxic effects observed in vitro. The results suggest that malathion may exert cytotoxic and genotoxic effects on placental cells as well as other biological systems.
Moore et al., 2011[26]	Mutat Res Genet Toxicol Environ Mutagen	Bone marrow and peripheral blood cells from male rats of the Sprague-Dawley strain.	Malathion (98,2% of purity)	The frequencies of chromosomal changes in bone marrow cell assays increased with increasing dose of malathion. All doses of malathion induced a significant increase in mean percentages of DNA damage (from 42 ± 0.84 to 86.5 ± 0.57%) in peripheral blood lymphocytes treated with malathion compared to only 13.5 ± 0.2 % in control cells.

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Authors, Year	Journal	Target Population	Exposure	Main Results Identified
Jose et al., 2010[27]	Mar Environ Res	Penaeus monodon (giant tiger shrimp) hemocytes	Malathion (50%w/w, comercial preparation) and monocrotophos (organophosphates), cadmium chloride and mercury chloride (heavy metals)	All the pollutants tested induced DNA chain breaks and, thus, comets in the hemocytes (Comet Assay). Percentage of tailed cells was significantly higher ($P<0.05$) than the Control for the four pollutants, including malathion (> 60%). In this study, malathion proved to be genotoxic to the culture of P. monodon hemocytes.
Moore et al., 2010[28]	Environ Toxicol	Human hepatic carcinoma cells (HepG2)	Malathion (98,2% of purity)	Malathion induced a gradual increase of DNA damage in HepG2 cells with increasing concentration. After 48 hours of exposure, the mean percentages of DNA damage suggest that malathion acts as a genotoxic compound especially at the highest exposure level.
Calaf et al., 2009[29]	Int J Oncol	MCF-10F immortalized human breast epithelial cell lines	Malathion and ethyl parathion individually and combined with estrogen.	Malathion and ethyl parathion induced malignant transformation of breast cells through genomic instability in the p53 suppressor gene and the c-Ha-ras oncogene, considered to be fundamental in the cancer process.
Calaf e Roy, 2008[30]	Int J Mol Med	Human breast epithelial cells	Malathion, ethyl parathion and 17ß estradiol (individually and combined)	The estrogen combined with both malathion and ethyl parathion altered cell proliferation and induced cell transformation, as well as exhibited significant invasive abilities compared to the MCF-10F control cell line. Several genes were up-regulated by the effects of all treatments, such as cyclins, cyclin D1 and cyclin-dependent kinase 4, IGFBP3 and IGFBP5, and keratin 18. The c-Ha-ras oncogene was regulated by the effect of malathion alone and a combination of estrogen with both malathion and ethyl parathion. The DVL1 gene was up-regulated only with malathion alone and with the combination of ethyl parathion and estrogen. Expression of HSP 27, MCM2 and TP53 inducible protein 3 genes was up-regulated with malathion alone and with the combination of estrogen and malathion or ethyl parathion while TP53 (Li-Fraumeni syndrome) was regulated by estrogen alone and malathion alone. An induction of molecular changes indicative of transformation occurred.
Błasiak e Kowalik, 1999[31]	Pestic Biochem Physiol	Human peripheral blood lymphocytes	Malaoxon and sodium ascorbate	Malaoxon significantly increased the tail momentum (Comet Assay analysis) of lymphocytes in a dose-dependent manner. At the highest concentration of the chemical, that is, 200 μM, the increased tail momentum was seven times higher than the initial value (19.49 ± 3.40 μm versus 2.61 ± 0.22 μm, $P <0.001$). The higher concentration of malaoxon caused an increased fraction of lymphocytes with greater tail momentums of the comet in comparison with that of the control not exposed. These comets contain more DNA in their tails, which indicates a greater extent of DNA damage in these cells.

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Chart 1. Synthesis of in vitro studies evaluating the association of exposure to malathion, isomalathion and malaoxon with the carcinogenic or mutagenic effects.

Authors, Year	Journal	Target Population	Exposure	Main Results Identified
Błasiak et al., 1999[32]	Mutat Res	Human peripheral blood lymphocytes	Malathion, malaoxon and isomalathion (all with purity of at least 99.8%)	Malathion did not cause significant changes in lymphocyte comet length at the concentrations tested. Malaoxon, at its highest concentration (200 mM), produced severely damaged cells by promoting comets with almost all of the DNA in the tail. This resulted in comet lengths exceeding more than twice the comet length of control lymphocytes ($p < 0.001$). Isomalathion promoted an increased comet length, but the changes were not as marked as in the case of malaoxon - at the concentration of 200 mM, the increased comet length was 72% in comparison with control. Malaoxon and isomalathion introduced DNA damage in a dose-dependent manner. The effect induced by malaoxon was more pronounced than that caused by isomalathion. Comets resulting from lymphocytes exposed to malaoxon and isomalathion have longer tails, therefore, they contain more DNA in their tails than comets resulting from control lymphocytes and lymphocytes exposed to malathion.
Błasiak e Trzeciak, 1998[33]	Pol J Environ Stud	Human peripheral blood lymphocytes	Malathion and isomalathion (both with purity of at least 99.8%)	The comets resulting from exposure to malathion did not differ from those of controls. Regarding the average comet lengths for lymphocytes exposed for 1h to malathion and isomalathion, in comparison with the controls, it can be observed that the malathion, at the applied concentrations, did not have a significant effect on the migration of the DNA to the tail of the comet. On the other hand, isomalathion caused an increased comet length - in the concentration of 200 μM, the increase was 72% in comparison with the control. The increase was dose-dependent. Comets resulting from lymphocytes exposed to isomalathion had more DNA in the tail than those resulting from control lymphocytes and lymphocytes exposed to malathion. The data presented indicated that isomalathion, unlike malathion, can damage the DNA of human lymphocytes isolated from peripheral blood.
Pluth et al., 1998[34]	Mutat Res	Human peripheral blood lymphocytes	Malathion	Mutations at several base pair sites were frequent after exposure to malathion and were isolated from treated cells from at least two different individuals. Using a human hprt mutation database for comparison, the frequency of mutations at one of said base pair sites 134 was found to be significantly elevated in malathion treated cells ($p<0.0005$). Hprt mutations in malathion-treated cells arose preferentially in G: C base pairs, which is consistent with previous reports that malathion is an alkylating of guanine nucleotides.
Hour et al., 1998[35]	Mutagenesis	Salmonella typhimunum (JK3 and JK947) strains	14 pesticides, including malathion	Malathion, among seven other pesticides, was not mutagenic in the JK947 and JK3 strains in the Salmonella lactam test (genotoxin detection method).

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Chart 1. Synthesis of in vitro studies evaluating the association of exposure to malathion, isomalathion and malaoxon with the carcinogenic or mutagenic effects.

Authors, Year	Journal	Target Population	Exposure	Main Results Identified
Gupta et al., 1996[36]	Asian-Australas J Anim Sci	Goat peripheral blood lymphocytes	Malathion (MW 330) and parathion individually	A statistically significant increase ($p \leq 0.05$) in the frequency of chromosomal alterations of the lymphocytes treated with 100 µg/ml of malathion (9.5%) compared to control (5.5%) was observed. At high doses (150 and 200 µg/ml), the frequencies of induced changes were highly significant ($p \leq 0.01$). The incidence of chromosomal breaks, dicentric chromosomes and translocations were higher than the other types of alterations found.
Balaji e Sasikala, 1993[37]	Mutat Res	Human peripheral blood lymphocytes	Malathion	The results show that malathion causes a dose-dependent increase of chromosomal changes as well as - SCEs in human leukocyte cultures. A dose-dependent decrease in mitotic index was observed at all concentrations in this study. Thus, the results suggest that malathion is a mild mutagen and may cause genotoxicity in humans at higher concentrations.
Johnson, 1992[38]	Environ Toxicol Chem	Variants, isolated dark mutants, of the luminescent bacterium Photobacterium phosphoreum.	Progenotoxins, genotoxins, non-genotoxic (control group, including malathion) and solvents	Malathion and other 4 non-genotoxic substances (carbohydrate, di-2-ethylhexyl phthalate, simin and permethrin) did not show genotoxic or cytotoxic responses at test doses of ≤ 10 µg per tube (data not shown).
Garry et al., 1990[39]	Teratog Carcinog Mutagen	Human peripheral blood lymphocytes	Malathion, carbon tetrachloride, carbon disulfide, methyl bromide and chloropicrin	Statistically significant ($P < 0.05$) dose-dependent increases in SCEs were observed in human lymphocytes treated with short-term malathion (1/2 hour). Dose-dependent increases in chromosomal abnormalities with or without activation of the microsomal S-9 fraction (rat liver homogenate) were also observed. Dicentric and tetrarradial figures were found in high doses of the organophosphate.
Pednekaret al., 1987[40]	Environ Contam Toxicol	Strains of the tester Salmonella Typhimurium TA 97a, TA 98 and TA 100	Malathion and phosalone (organophosphates), endosulfan (organochlorine) and permethrin (pyrethroid)	No mutagenic activities of malathion were observed in the respective non-toxic (33 mg/L) and 90% toxic (1,650 mg/L) doses, either before or after the activation with S9 (post-mitochondrial) fraction of rat liver with three strains of the tester Salmonella Typhimurium TA 97a, TA 98 and TA 100, in the Ames Salmonella assay system, nor after its activation with rat cecal microbial extract in the same system.

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Chart 1. Synthesis of in vitro studies evaluating the association of exposure to malathion, isomalathion and malaoxon with the carcinogenic or mutagenic effects.

Authors, Year	Journal	Target Population	Exposure	Main Results Identified
Houk e DeMarini, 1987[41]	Mutat Res	Strains of Escherichia coli B/r	malathion (73,5% of purity), monuron, p,p'-DDT, mirex, lindane, nitrophen, chlordane, toxaphene, captan, and dichlorvos.	Malathion was not detected by the phage-MicroScreen induction assay (miniaturized system that uses prophage induction in Escherichia coli X as an indicator of genetic damage)
Wiaderkiewicz et al., 1986[42]	Acta Biochim Pol	Calf thymus DNA	Malathion, DDVP, Methyl parathion and methylbromphenvinphos	All the studied organophosphate insecticides react with the DNA in vitro causing methylation of nitrogenous bases. The methylation kinetics of purine bases in DNA by [14C] malathion showed that this process was a bit slow, reaching its maximum after 96 hours of the reaction. Longer incubation did not result in a significant increase in the number of alkylated bases (probably due to two competition processes: additional DNA alkylation and hydrolytic removal of alkylated purine bases from the polynucleotide chain). Methylbromphenvinphos appeared to be the most reactive organophosphate insecticide, followed by Methyl parathion and malathion.
Ma et al.1983[43]	Environ Mutagen	Plant stakes of the Tradescantia clone 03 and 4430	Malathion (Malathion 5, emulsifier, 55 % of active ingredient)	The genotoxicity of liquid malathion (malathion solution/deionized water) absorbed through the stem was very low and often masked by high toxicity, causing cell death in the stem, leaves and myocytes. Malathion spraying at 0.435% dosage in a closed chamber or in an open population of plants achieved negative responses. Malathion vapors in dosages of 0.15-0.25% induced significantly higher frequencies (0.05) of micronuclei above the controls and altered the nuclear structure to form nuclei of unequal size and multiple breaks in each of the four cells of a tetrad. It also caused nucleus degeneration, "nucleus protrusion" and inhibition of cell growth. Higher dosages (above 0.25%) were toxic.
Gilot-Delhalle et al., 1983[44]	Mutat Res	Yeast Schizosaccharomyces pombe (an isogenic mutant at the ade6 locus) and livers of male Q strain rats	12 organophosphate insecticides, including malathion (combined with trichlorfon)	The effects of 3 combinations of trichlorfon with each derivative of methyl, malathion, Methyl parathion and azinphos-methyl were investigated. The increased trichlorfon-induced mutation frequency alone corroborates the effects obtained in the first experiments (of mutagenicity in the ad6 forward-mutation test system of the yeast Schizosaccharomyces pombe) as well as the efficiency of the S9 microsomal liver fractions. The same conclusion can be reached for malathion and the other two organophosphates compounds that produced positive effects. In all three cases, a synergistic effect was found between trichlorfon mutagenicity and the second compound ($P<0.001$ in Xc2). This synergistic effect was greater for Methyl parathion than for malathion and greater for this latter compound than for azinphos-methyl.

Source: Prepared by authors.

Chart 2. Synthesis of in vivo experimental studies evaluating the association of exposure to malathion and / or its analogues to carcinogenic and / or mutagenic effects.

Authors, Year	Journal	Target Population	Exposure	Main Results Identified
Hussain et al. 2015[48]	Pak J Agri Sci	Japanese quail male (bird) (Coturnix japonica)	Malathion (95% technical degree)	The frequency of micronucleated and binucleated erythrocytes increased significantly in birds from groups D (60 days) to F (100 days) on all experimental days. The frequency of blebbed nucleated erythrocytes on days 34 and 51 in groups E (80 days) at G (120 days) increased significantly.
Omran e Omer, 2015[49]	Pathol Res Pract	Wistar female mice	Malathion (≥ 95%) and Alpha lipoic acid	Histological changes in the breast marked by fibrocystic alterations, atypical hyperplasia and malignant alterations, suggesting that chronic exposure to malathion may have long-term consequences for human health.
Selmi et al. 2015[50]	Toxicol Ind Health	Wistar male rats	Malathion (fyfanon 50 EC 500 g/L)	It induced oxidative stress evaluated by increased malondialdehyde content (MDA), reflecting lipoperoxidation, decreased thiol group content, and depletion of enzyme activities such as superoxide dismutase (SOD) and catalase (CAT), renal and hepatic antioxidant enzymes, and decreased significantly ($p<0.01$) the activities of these enzymes.
Calaf e Echiburú-Chau, 2012[51]	Oncol Rep	Female virgin rats of the Sprague-Dawley strain	Estrogen and malathion (individually and mixed)	Significant increased size ($p<0.05$) of the ducts in the proliferation phase (dsp/mm2) of the mammary gland, and in the number of epithelial layers in comparison with the controls. The increased proliferative ducts induced by the effect of malathion after 10 and 20 days coincided with increased expression of the mutant p53 protein. The combination of malathion and estrogen induced a greater cellular alteration in the mammary glands of the rat than estrogen or malathion alone.
Alfaro-Lira et al., 2012[52]	Int J Environ Res Public Health	Female virgin rats of the Sprague-Dawley strain	Malathion and 17β-estradiol (estrogen), individually and mixed	The combination of malathion and estrogen induced greater changes in the tubular section of the kidneys compared to any single substance. Malathion caused several types of damage to the control, such as a significant increase ($p<0.05$) in the degree of glomerular hypertrophy, signs of tubular damage and proliferation in the hilum region, and atypical proliferation in the cortical and hilar areas compared to control group. These abnormalities may be suggested as a sign of progressive malignancy.
Giri et al., 2012[53]	Aquat Toxicol	Indian skittering frog (Euflictis cyanophlyctis)	Malathion (50% EC comercial formula)	It induced the formation of micronuclei (MN) in erythrocytes of tadpoles in a concentration-dependent manner. The concentration-dependent increase in MN frequency in erythrocytes during the 96-hour study period after different concentrations of malathion clearly indicates the clastogenic potential of the pesticide in E. cyanophlyctis.
Giri et al., 2011[54]	Environ Mol Mutagen	Swiss albino mice	Malathion (95% of purity) and Fenvalerate	Malathion induces a dose-dependent increased frequency of micronucleated polychromatic erythrocytes (PCEs) in bone marrow cells of mice, which reinforces the clastogenic and/or aneugenic potential of malathion.

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Chart 2. Synthesis of in vivo experimental studies evaluating the association of exposure to malathion and / or its analogues to carcinogenic and / or mutagenic effects.

Authors, Year	Journal	Target Population	Exposure	Main Results Identified
Calaf e Garrido, 2011[55]	Int J Oncol	Female virgin rats of the Sprague-Dawley strain	Malathion and 17β-estradiol (estrogen), both individually and mixed	Progressive changes in the ducts of mammary cells were observed by the effect of malathion. Significantly increased ducts in size and number of cells per square millimeter and tumors as ductal carcinoma were produced. Treatment in combination of estrogen and malathion gave rise to tumors consisting of both proliferative and secretory lobes.
Bernhardt et al., 2011[56]	Int J Integr Biol	Swiss albino mice	Commercial class of Malathion (50%) and Withania somnifera L (as antigenotoxic agent).	Increased comet formation (Comet Assay) compared to control of peanut oil (p-value<0.001). DNA damage was caused by malathion in a dose-dependent manner.
Echiburú-Chau e Calaf, 2008[57]	Int J Oncol	Female virgin rats of the Sprague-Dawley strain	Malathion and 17ß-estradiol (individually and combined).	Numerous types of pre-neoplastic and neoplastic lesions have been found in the bronchiolar epithelium of rats such as hyperplasia, squamous metaplasia, carcinoma in situ and invasive carcinoma, suggesting a sign of progressive malignancy with greater possibility of lung cancer development.
R´eus et al., 2008[58]	J Agric Food Chem	Wistar adult male rats	Malathion	Increased DNA damage in whole blood of Wistar rats, notably at higher doses. Increased DNA damage was noted in the hippocampus.
Giria et al.,2002[59]	Mutat Res	Swiss albino mice	Malathion	Several types of chromosomal alterations, which consisted of chromatid and isochromatic types of gaps and breaks, double minutes (included between isochromatic breaks), swaps, and sister chromatid junctions (SCJ). Chromic-type breaks were found to be more frequent than others. Malathion induced a significantly higher frequency of changes ($p < 0.001$) for all three doses tested. The dose-response curve shows a linear increase in the frequency of changes with increasing doses ($r = 0.9734$, $p < 0.05$). All three acute doses of malathion induced a significantly higher frequency ($p < 0.001$) of sister chromatid exchange (SCE) compared to the control value. In addition, at the same doses, significant increases ($p < 0.02$, 0.001 and 0.001, respectively) were observed in the frequency of sperm head anomalies compared to the untreated control.
Cabello et al., 2001[60]	Environ Health Perspect	Female rats of the Sprague-Dawley strain	Eserin, parathion and malathion (cholinesterase inhibitors)	A significant increase ($p < 0.05$) in the size of the terminal end buds (TEBs) of the mammary gland, as well as the number of epithelial layers. These structures increased in size and development of mammary carcinomas was noted.
Amer et al., 1996[61]	J Appl Toxicol	Rats	Malathion (100% pure), Dursban, Sevin, Lannate, DMSO and DDT (insecticides)	Induction of chromosomal changes in rat spleen cells, whose percentage was highly statistically significant. Structural alterations were observed, such as chromatid and chromosomal gaps, but the former were the dominant forms.

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Chart 2. Synthesis of in vivo experimental studies evaluating the association of exposure to malathion and / or its analogues to carcinogenic and / or mutagenic effects.

Authors, Year	Journal	Target Population	Exposure	Main Results Identified
Fourernan et al., 1994[62]	Environ Mol Mutagen	*Male adults of Drosophila melanogaster*	70 chemical products, including malaoxon	Malaoxon was considered a mutagen, along with 15 other chemical products, in the sex-linked recessive lethal test (SLRL), SLRL assay.
Hoshiya et al., 1993[63]	Cancer Lett	Male F344 rats	Diethylnitrosamine (DEN), malathion, vinclozolin, S,S,S-tributylphosphorotrithioate (DEF), technazene, isoproturon and dichloran (individually and combined with DEN)	Malathion was positive in the analysis of glutathione S-transferase placental form (glutathione S-transferase placental, GST-P) positive focus, along with 4 other pesticides. The results suggest that malathion has tumor-promoting activity in the liver.
Hasegawa e Ito, 1992[64]	Fd Chem Toxic	F344 rats	94 chemical compounds, including malathion	Significant increase ($p < 0.05$) in the induction of GST-P (glutathione S-transferase placental form) positive focus compared to control levels of hepatocytes of rats.
Hoda e Sinha, 1990[65]	Internat J Vit Nutr Res.	*Mus musculus rats*	Malathion and Rogor (pesticides) and Vitamins B and C (protective effect)	Both pesticides significantly increased chromatid and chromosomal abnormalities in rat bone marrow cells.
Velázquez et al., 1987[66]	Environ Mutagen	*Drosophila melanogaster strains*	Malathion, 50% emulsifiable concentrate (50% of active ingredient, 50% of xylol and dispersants and emulsifiers)	The results of the sex chromosome loss (SCL), SCL test, after feeding and adult injection, the results of SCL and non-disjunction after treatment of third-stage larvae were negative, indicating that Malathion is also ineffective in producing total or partial losses of sex chromosomes and non-disjunction.
Alina Dzwonkowska e Henryk Hiibner, 1986[67]	Arch Toxicol	Syrian hamster (Mesocricetus auratus)	Malathion, demeton, dimethoate, dichlorvos, endosulfan, trichlorfon, carbaryl, lindane, methoxychlor and propoxur	Statistically significant increases ($p < 0.05$) in the number of cells with chromosomal alterations in the bone marrow of the malathion-treated Syrian hamsters.
Degraeve et al., 1985[68]	Environ Health Perspect	Male rats (Q strain)	Trichlorfon individually and combined with malathion or Methyl parathion or azinphos-methyl (all with 99% purity)	The frequency of chromosomal changes in bone marrow metaphases was not higher in treated male rats.

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Chart 2. Synthesis of in vivo experimental studies evaluating the association of exposure to malathion and / or its analogues to carcinogenic and / or mutagenic effects.

Authors, Year	Journal	Target Population	Exposure	Main Results Identified
Degraeve et al., 1984[69]	Arch Toxicol	Male rats (Q strain)	Malathion, dichlorvos, dimethoate, Methyl parathion and trichlorfon (all with 99% purity)	The percentage of chromosomal breaks in bone marrow cells did not reach 0.5%. A simple chromosomal exchange was observed in the group treated with malathion and the frequency of gaps was lower than the control level. The frequency of different types of chromosomal lesions was very low in spermatogonia. The number of chromosomal breaks observed in primary spermatocytes was not significantly increased. Some gaps (less than 0.1%) were detected, but translocations were not observed. In the dominant lethal mutation assay, for each treated group, the number of live embryos per litter was normal. The percentage of pre-implantation losses was not significantly increased and the frequency of post-implantation fetal mortality was lower than the control level.
Degraeve et al., 1984[70]	Fd Che Toxic	Rats (Q strain)	Luxan Tue-Taons, Metadipterex, Dynafos (155g of malathion, 60g of dichlorvos and 75g of carbaryl/ liter) and Phosan Plus (95g of dimethoate, 100g of malathion and 100g de methoxychlor/ liter) (comercial mix of insecticides)	No induction of chromosomal changes was observed in bone marrow cells, spermatogonia or primary spermatocytes of rats injected intraperitoneally with the substances. No evidence of potential genetic effects was obtained in the dominant lethal mutation assay.
Dulout et al., 1983[71]	Mutat Res	BALB/c strain rats	Malathion (95,5%)	The clastogenic effect of malathion was evidenced by the induction of sub-chromatid and chromatid alterations concerning the dose used in bone marrow cells of rats. The animals increased frequency of abnormal metaphases, gaps, breaks and chromatid exchanges in relation to the controls during the various time intervals, in a direct dose-response relationship, and the chromosomal damage induced was proportional to the dose of malathion.

Source: Prepared by authors.

by ingestion of water containing small amounts of the organophosphate (8 ppm, corresponding to the highest value allowed in Belgium for pesticide residues in fruits and vegetables) five days a week for seven consecutive weeks[69]. Again, they did not observe a significant increase in chromosomal breaks or gaps in the same cell types from the previous work, nor in the dominant lethal mutation assay. However, there is hardly any information on the analytical technique used to search for these changes. In 1985, the group evaluated the effects on rats injected with malathion (150 mg/kg) combined with trichlorfon (50 mg/kg), and also did not show cytogenetic effects (breaks, gaps, and chromatid alterations) of the combination[68].

However, of the total *in vivo* experimental studies found in this study, only four showed no positive association between mutagenic effects and malathion, all from the 1980s. The first three were those mentioned above, the fourth and last of them dating from 1987, which obtained negative results regarding the production of sexual chromosome losses and non-disjunction in strains of *Drosophila melanogaster* exposed to malathion with 50% of the active ingredient, a percentage that may have had a significant influence on the results found[66]. Since then, there have been a further 17 studies with positive results for carcinogenicity and genotoxicity of malathion, and one for malaoxon genotoxicity.

Honda and Sinha[65] showed a significant increase (at a level of 0.1%) in the chromatid alterations of bone marrow cells after exposure of *Mus musculus* rats. Swiss albino mice strains exposed to malathion evidenced bone marrow cell metaphases with various types of chromosomal alterations, and the pesticide-induced significantly higher frequency of alterations ($p<0.001$) in the three acute doses tested (2.5, 5 and 10mg/kg) than in control, besides increased frequencies of sister chromatid exchanges and sperm head anomalies[59]. Giri et al.[54] reported the dose-dependent increased frequency of micronucleated polychromatic erythrocytes in bone marrow cells of mice of the same species after *in vivo* exposure to malathion at 95% purity.

Regarding the research on the carcinogenicity of malathion in *in vivo* experiments, the first manuscript found dated back to 1992, in which the pesticide promoted a significant increase ($p<0.05$) in the induction of GST-P positive focus in hepatocytes of F344 mice compared to control[64]. The expression of the GST-P enzyme is usually low in fetal hepatocytes, quiescent adults or in regeneration, placenta, heart and other organs of male rats, however, hyperplastic nodules and chemically-induced liver tumors exhibit GST-P values of about 20 to 50 times and 10 to 30 times over, respectively, when compared to normal rat liver values[72]. Thus, the study suggests the possibility of malathion being a weak hepatocarcinogenic or hepatopromoting agent. On the other hand, the study by Hoshiya et al.[63], also with F344 mice, concludes and states that malathion has liver tumor promoting activity.

Studies have investigated the carcinogenic effects of malathion and estrogen in virgin female rats of the Sprague-Dawley strain, individually and in combination. Calaf and Garrido[55] observed progressive changes in the mammary cell ducts of rats treated with isolated malathion compared to control after 240 days of treatment. Besides the significantly increased size ($p<0.05$) of ducts in the mammary gland proliferation phase of rats treated with the pesticide, Calaf and Chau[51] found a growing expression of the mutant p53 tumor marker protein. Several types of preneoplasms in the bronchiolar epithelium of rats injected with malathion have also been found, besides carcinomas in situ[57]. In the renal tissue of malathion-exposed rats, results suggest abnormalities with signs of malignancy[52].

In most of the previously cited studies[51,52,57], treatment with the combination of malathion and estrogen-induced more cellular alterations than treatments with the substances alone. Thus, the combination of pesticides found in the environment, widely used in Latin America and many other countries, and an endogenous substance, such as estrogen, has the potential to induce deleterious effects on humans, such as breast cancer, for example[51]. Estrogen can also be found as a pollutant in surface and groundwater, and its presence in the environment can have severe toxicological and ecotoxicological repercussions since this substance is recognized as an endocrine disrupter, associated with premature puberty, infertility and congenital malformation[73-75].

The epidemiological studies found in this systematic review are summarized in Chart 3. The types of designs obtained were eleven control case studies, eight cohorts and six cross-sectional studies, where two of these also performed *in vitro* experiments with human peripheral blood lymphocytes.

Most studies (n = 16) investigated the correlations between exposure to malathion and the development of cancers. Of the 25 papers, 18 were conducted in North America (12 in the U.S.

and 6 in Canada), three in Europe, three in Asia and only one in Latin America (Chile). These findings corroborate the IARC manuscript, which states that very few studies on this approach have been conducted in less industrialized countries[19].

As to the evidence of genotoxicity investigated, in a cross-sectional study with male pesticide applicators, Andreotti et al.[76] found positive associations between the recent use of malathion and the shorter telomere relative length ($p = 0.03$). Telomere shortening is associated with several diseases, and most studies have reported associations between telomere length and increased risk of cancer[101,102].

In a prospective cohort with patients who attempted suicide by self-poisoning with drugs or insecticides, temporary but significant increases in peripheral blood leukocyte aneuploidy (6.3%, $p < 0.01$) and chromatid (5.3%, $p < 0.01$) and chromosomic (1.4%, $p < 0.01$) alteration rates after intoxication with malathion[97] were observed. Also, one of 14 people poisoned by organophosphate died.

Only one of the epidemiological manuscripts addressing the mutagenic effects of malathion and complex mix of pesticides including the organophosphate did not find a positive association[95]. This work was carried out both with a cross-sectional epidemiological study of workers exposed to pesticides and with *in vitro* experimentation of human peripheral blood lymphocytes also concluded that malathion has a relatively low potential to cause chromosomal damage *in vitro*, and the corresponding doses used in the experiment were much higher than those that professional applicators are likely to be exposed to *in vivo*.

On the other hand, a cross-sectional study with a worker occupationally exposed to pesticides (primarily malathion and phosphine) for more than five years, and an experimental *in vitro* study with human peripheral blood lymphocytes exposed to malathion revealed that the mutagenicity of malathion can be detected at a molecular level[96]. Molecular changes in the *hprt* assay were observed at doses of malathion that did not produce *in vitro* cytotoxicity (≤50 mg/ml) and at exposure levels experienced by the individual from which the *in vivo* mutant was retrieved. The authors state that alterations similar to those reflected in the *hprt* in this study may also occur in other *loci*, especially oncogene or tumor suppressor genes sites, and may play a role in inducing malignancies in individuals exposed to this or a similar agent.

In epidemiological studies where exposure to complex mixtures of pesticides or combinations including malathion was found, all results found positive associations with genotoxic effects[77,92,93,99,100] and carcinogenic effect[87]. Investigations of pesticides focused on the potential effects of these substances on an individual basis aim to facilitate the analysis and direct public policies. However, although multiple exposures hinder the assessment of the relationships between pesticides and their mutagenic or carcinogenic effect, they more accurately reflect how these compounds are used[87].

Among the manuscripts that sought to investigate correlations between malathion exposure and development of different types of cancers, five of them did not find significant positive associations in cases of non-Hodgkin's lymphoma (NHL)[79,91], multiple myeloma[82], prostate cancer[85,91], soft tissue sarcoma[86], combined lymphatic-hematopoietic cancers, leukemia, lung, colon and rectum, kidney and bladder cancer, and melanoma[91]. Another three found significant inverse associations between the use of organophosphate and the appearance of NHL[78,84] and pancreatic cancer[90].

The other half of them obtained positive associations at statistically significant levels for thyroid cancer[78], ovarian cancer in menopausal women[78], prostate cancer[80,88] and its aggressive type[83], breast cancer[81], NHL[94,98] and between body mass index and cancer colonization among men who used malathion[89].

In Chile, about 33 years after the first malathion spraying on the city of Arica, Cabello et al.[81] conducted a case-control study with women living in the city and women from Iquique, a control city where spraying had never occurred. The authors observed that those with the most extended exposure to malathion were 5.7 times more likely to be diagnosed with breast cancer, besides 30.5% of the cases of metastases found in the exposed group, compared to 16% in the never exposed group ($p < 0.05$). The study concluded that the increased breast cancer mortality rate in the city of Arica has a significant correlation with exposure to malathion sprayed for more than three decades.

Of all the studies included in this systematic review, only one was carried out in Brazil[58], which evidences the lack of research regarding the carcinogenic and genotoxic effects of malathion in the country. On the other hand, the use of pesticides, especially in developing countries, has grown over the years, making it necessary to

Chart 3. Synthesis of epidemiological studies that assessed the association of exposure to malathion and/or its analogues to carcinogenic and/or mutagenic effects.

Authors, Year	Journal	Study design	Target Population	Country	Exposure	Main Results Identified
Andreotti et. al., 2015[76]	PLoS ONE	Cross-sectional	Male workers applying pesticides	USA	Malathion	Significant associations were found between the recent use of malathion and shorter relative telomere length (p=0.03), suggesting that the length of the leukocyte telomeres may be affected by the recent (and cumulative) use of certain pesticides.
Arshad et al., 2015[77]	Saf Health Work	Cross-sectional	Workers in a pesticide industry	Pakistan	Complex pesticide mix, including malathion	The leukocyte count increased significantly with the increased period of exposure among exposed workers. In the Comet Assay, the mean tail length of the DNA estimated in the exposed blood cells of exposed workers was significantly higher than in control group individuals. Malathion was detected in 72% of blood samples. The linear fit curve showed a significant correlation (R2=0.91) of the concentration of malathion with the tail length of the comet. The linear correlation between the malathion residues in the blood and DNA damage is very alarming and highlights the risks involved in direct exposure during the production activity.
Lerro et al., 2015[78]	Occup Environ Med	Prospective cohort	Women spouses of pesticide applicators	USA	10 organophosphates, including malathion	The use of malathion was associated with a significantly increased risk of thyroid cancer (RR=2.04, 95% CI: 1.14-3.63). Significant interactions with the menopausal status of women and malathion for the risk of ovarian cancer (Pinteraction = 0.04) were also observed.
Alavanja et al., 2014[79]	PLoS ONE	Prospective cohort	Farmers and commercial applicators of pesticides	USA	Malathion (among 50 specific pesticides)	Showed high but not significant risks with respect to cases of non-Hodgkin's lymphoma (NHL) follicular B-cell subtype.
Koutros et al., 2013[80]	PLoS ONE	Case control	Licensed pesticide applicators in Iowa and North Carolina	USA	Malathion (among 50 pesticides)	Among men with two T alleles to rs2710647 in EH-domain binding protein 1 (EHBP1), the risk of prostate cancer in patients with low malathion use was 2.17 times those without use (95% CI: 0.91-5.14), and in those with high malathion use, it was 3.43 times those without use (95% CI: 1.44-8.15) (Pinteraction = 0.003).
Cabello et al., 2013[81]	Int J Morphol	Case control	Women living in Arica and Iquique	Chile	Malathion	Women with more exposure time to malathion were 5.7 times more likely to be diagnosed with breast cancer (OR=5.7, p<0.02). Metastases were found in 30.5% of the group exposed to malathion and only in 16% in the never exposed group (p<0.05), suggesting that the higher rate of mortality from breast cancer occurring in Arica has a significant correlation with the exposure to the sprayed malathion over the city for more than 30 years.

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Chart 3. Synthesis of epidemiological studies that assessed the association of exposure to malathion and/or its analogues to carcinogenic and/or mutagenic effects.

Authors, Year	Journal	Study design	Target Population	Country	Exposure	Main Results Identified
Kachuri et al., 2013[82]	Int J Cancer	Case control	Men residing in six Canadian provinces	Canada	Herbicides (2,4-D; Glyphosate and Mecoprop), insecticides (Methoxychlor; Malathion; Chlordane; DDT; Carbaryl and Lindane) and fungicides (Mercury dust; Captan and Formaldehyde)	The OR ratio adjusted for multiple myeloma concerning exposure to malathion: OR (95% CI) = 1.12 (0.71, 1.74). Excluding respondents by proxy: OR=1.28 (0.79, 2.07).
Koutros et al., 2012[83]		Coorte prospectiva	Agricultores e aplicadores comerciais de agrotóxicos	EUA	Malathion (entre 48 agrotóxicos)	Quatro inseticidas foram associados ao câncer de próstata agressivo, entre eles o malathion com RR para Q4 vs. não exposto = 1,43, IC 95%: 1,08; 1,88; Ptrend = 0,04).
Pahwa et al., 2012[84]	Am J Epidemiol	Prospective cohort	Farmers and commercial pesticides applicators	USA	Malathion (among 48 pesticides)	Four insecticides were associated with aggressive prostate cancer, among them malathion, with RR for Q4 vs. non-exposed = 1.43, 95% CI: 1.08; 1.88; Ptrend = 0.04).
Barry et al., 2011[85]	Int J Cancer	Case control	Men aged ≥ 19 years	Canada	[1,1'-(2,2,2-trichloroethylidene) bis[4-chlorobenzene]; 1,1,1-trichloro-2,2bis(4-chlorophenyl) ethane (DDT), malathion, (4-chloro-2-methylphenoxy) acetic acid (MCPA), Mecoprop and (2,4dichlorophenoxy) acetic acid (2,4-D)	Individuals with asthma, allergies or hay fever who reported malathion use, controversially, had a lower risk of NHL (OR=1.25, 95% CI: 0.69-2.26) than those with none of these conditions (OR=2.44, 95% CI: 1.65-3.61). Similar effects were observed for asthma and allergies evaluated individually.
Pahwa et al., 2011[86]		Caso controle	Homens residentes de seis províncias canadenses	Canadá	Malathion entre outros agrotóxicos (herbicidas, inseticidas, fungicidas)	Não foram encontradas associações significativas entre a incidência de Sarcoma de tecido mole (STS) e a frequência da exposição ao malathion.
Hohenadel et al., 2011[87]	Environ Health Perspect	Case control	White Pesticides Applicators from the Agricultural Health Study (AHS)	USA	Malathion (among 39 pesticides)	There was no positive association between malathion exposure and prostate cancer (low exposure: OR (95% CI) = 0.88 (0.69, 1.13). High exposure: OR (95% CI) = 80 (0.62, 1.04).

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Chart 3. Synthesis of epidemiological studies that assessed the association of exposure to malathion and/or its analogues to carcinogenic and/or mutagenic effects.

Authors, Year	Journal	Study design	Target Population	Country	Exposure	Main Results Identified
Band et al., 2011[88]	J Occup Environ Med	Case control	Men residing in six Canadian provinces	Canada	Malathion among other pesticides (Herbicides, insecticides, fungicides)	No significant associations were found between the incidence of soft tissue sarcoma (STS) and the frequency of exposure to malathion.
Andreotti et al., 2010[89]	Int J Environ Res Public Health	Case control	Men from six Canadian provinces	Canada	Malathion among other pesticides (Herbicides, insecticides, fungicides) - 36 combinations of pesticides	Increased risk of non-Hodgkin's lymphoma associated with malathion in combination with 2,4-D, Mecoprop, Carbaryl, Glyphosate, and DDT, where odds ratio was higher than the use of each pesticide individually.
Andreotti et al., 2008[90]	Prostate	Case control	Male patients with prostate cancer from the population-based British Columbia Cancer Registry (BCCR) and BC farmers from the occupational exposure matrix (JEM)	Canada	290 different chemical agents (of these, 180 pesticides, including malathion)	Exposure to malathion showed an excess risk to significant prostate cancer (OR=1.34, 95% CI: 1.01-1.78), and a dose-response relationship.
Bonner et al., 2007[91]	Cancer Causes Control	Cohort	Licensed private pesticide applicators and their spouses residing in Iowa and North Carolina, and commercial applicators residing in Iowa, all from the Agricultural Health Study (AHS)	USA	50 pesticides (including Malathion)	Significant positive associations between body mass index (BMI) and colon cancer among men who used malathion.
Zeljezic e Garaj-Vrhovac, 2002[92]	Int J Cancer	Case control	Pesticide applicators and spouses with pancreatic cancer, residing in Iowa and North Carolina of the Agricultural Health Study (AHS)	USA	50 pesticides (including Malathion), of which 24 pesticides examined for exposure always/never, and 13 pesticides for days of exposure in life time with weighted intensity.	The use (always) of malathion was significantly inversely associated with the risk of pancreatic cancer (OR=0.4, 95% CI 0.2-0.9).

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Chart 3. Synthesis of epidemiological studies that assessed the association of exposure to malathion and/or its analogues to carcinogenic and/or mutagenic effects.

Authors, Year	Journal	Study design	Target Population	Country	Exposure	Main Results Identified
Garaj-Vrhovac e Zeljezic, 2001[93]	Am J Epidemiol	Prospective cohort	Pesticide applicators and their spouses residing in Iowa and North Carolina of the Agricultural Health Study (AHS)	USA	Malathion among 50 pesticides	No conclusive evidence has been found that occupational exposure to malathion is associated with increased risk of cancers: combined lymphatic-hematopoietic cancers (multiple myeloma, leukemia, Hodgkin's lymphoma and NHL); leukemia; NHL; Lung, prostate, colon and rectum, kidney and bladder cancers; and melanoma.
McDuffie et al., 2001[94]	Chemosphere	Cohort	Workers employed in three different pesticide production units (the pesticide synthesis unit, the emulsion-concentrated production unit and the powder and liquid pesticide production unit)	Croatia	Complex pesticide mix (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid and malathion)	The mean value of sister chromatid exchanges (SCE) in the exposed group was significantly higher compared to the control group. The number of sister chromatid exchanges (HFCs) in the exposed group at all sampling periods was significantly higher compared to the control group. The results suggest that the increased number of SCEs found in exposed individuals is not the result of the cytotoxic action or epigenetic action of the pesticide mix, but rather the chronic occupational exposure to the pesticide mix.
Titenko-Holland et al., 1997[95]	Toxicology	*Cohort*	Workers employed in three different pesticide production units (the pesticides synthesis unit, the emulsion-concentrated production plant and the powder and liquid pesticides production unit)	Croatia	Complex pesticide mix (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid and malathion)	The exposed group showed an increased number of chromosomal and chromatid changes, regardless of the sampling time. The results of the first sampling series revealed dicentric chromosomes (n=17) and chromatid changes (n=4) in the exposed group, but none of them in the control group. In the second series of sampling, only dicentric (n=4) were observed in the exposed group, that is, the number reduced significantly after 8 months of non-exposure. A higher micronucleus (MN) frequency was observed in the exposed group compared to controls, regardless of sampling time. In the comet assay, the lymphocytes of the exposed workers expressed a greater DNA migration than the control.
Pluth et al., 1996[96]	Cancer Epide-miol Bio-markers Prev	*Case control*	Men living in six Canadian provinces	Canada	Herbicides, fungicides, fumigants and insecticides, including malathion	Malathion was the only organophosphate of individual exposure associated statistically with Non-Hodgkin's Lymphoma: ORa (95% CI) = 1.77 (1.28-2.46).
Czeizel, 1994[97]	Mutat Res	Cross-sectional/ In vitro experimental study	Human peripheral blood lymphocytes / Workers who were involved in the mosquito eradication program conducted by the California Department of Food and Agriculture – USA	USA	Malathion (95% of purity and 5% de impurity, including malaoxon)	A significant increase in micronucleated cells (47.5/1000 binucleated lymphocytes versus 16.0/1000 in the dimethyl sulfoxide control, p <0.001) was found on isolated lymphocytes at high dose levels (75-100 µg/ml), concomitantly with cytotoxicity and strong inhibition of proliferation (p<0.001). Many of the treated cells also had multiple micronuclei.

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Chart 3. Synthesis of epidemiological studies that assessed the association of exposure to malathion and/or its analogues to carcinogenic and/or mutagenic effects.

Authors, Year	Journal	Study design	Target Population	Country	Exposure	Main Results Identified
Cantor et al., 1992[98]	Cancer Res	Cross-sectional/ In vitro experimental study	Occupationally exposed worker. (> 5 years) / Human peripheral blood lymphocytes	USA	Malathion (batches with 1 to 3% contamination by isomalathion and malaoxon) and phosphine / Malathion	The mutant frequencies of the treated samples showed intra- and inter-individual variability and, in some cases, negligible increases on the controls. This study provided the first evidence of an association between exposure to malathion and specific mutations in human T lymphocytes.
Rupa et al., 1991[99]	Mutat Res	Prospective cohort	Self-intoxicating individuals (attempted suicide)	Hungary	Drugs and insecticides, including malathion	A temporary, but significant increase in peripheral blood leukocyte aneuploidy from the first samples collected 3 to 6 days after malathion intoxication (6.3%, $p<0.01$). Temporary increase in the rate of chromatid and chromosomal changes after malathion intoxication.
Rupa et al., 1989[100]	Cancer Res	Case control	Newly diagnosed men with non-Hodgkin's lymphoma and leukemia in the states of Minnesota and Iowa.	USA	23 specific insecticides used in animals (including malathion), 34 insecticides applied to crops (including malathion), 38 herbicides and 16 fungicides.	The first use before 1965 was associated with a higher risk (OR=1.8 / CI=1.0, 3.3) than those who had always handled (OR=1.3 / CI=0.9, 2.1), and was significant for the early reported use of malathion, as an animal insecticide. The OR for the management, blending or personal application of specific insecticides that could have been used in both animals and crops before 1965 shows a significantly elevated risk for malathion (OR=1.8, CI=1.1-3.1, 31 cases).
	Environ Mol Mutagen	Cross-sectional	Male pesticide applicators	India	Mix of DDT, BHC, endosulfan (35%), malathion, Methyl parathion, phosphamidon, monocrotophos, quinalphos, dimethoate, fenvalerate or cypermethrin	A statistically significant difference ($p<0.05$) was observed between the mean frequency of sister chromatid exchanges per cell in the control group (3.57) and the frequency of the exposed group (8.46). SCE frequency was also significantly higher in the exposed group at all exposure durations (1-10 years, 11-20 years and > 20 years).
	Mutat Res	Cross-sectional	Smokers exposed to pesticides, nonsmokers (control I) e non-exposed smokers (control II)	India	Mix of malathion, DDT, BHC, endosulfan, Methyl parathion, monocrotophos, quinalphos, dimethoate, phosphamidon, cypermethrin and fenvalerate	Changes in the chromatid type were higher in control II compared to control I. Changes in the isochromic type (gaps (0.04), breaks (0.02), fragments (0.13)) and dicentric (0, 52) in control II while they were absent in control I. In the pesticide-exposed population, the number of gaps and chromosomal breaks increased when compared to control II. A similar increase was observed in the fragments, deletions and dicentric in the exposed population compared to control II. Polyploids increased the duration of exposure to pesticides. The statistical analysis revealed a significant ($p<0.05$) increase in total chromosomal changes in pesticide-exposed smokers compared to non-pesticide-exposed smokers.

Source: Prepared by authors.

carry out further studies on occupational and environmental exposure to these pesticides[103].

The Brazilian Association of Collective Health (ABRASCO) has produced a Technical Note[11] warning about products such as malathion, among others, currently used in vector control of arboviruses, since the real damages caused to the environment and human health have not yet been adequately studied or revealed to vulnerable populations, including Public Health workers. Its harmful effects have been disregarded both in the aggravation of viruses and in the emergence of other pathologies such as allergies, immunotoxicity, cancer, hormonal disorders, neurotoxicity, among others[11].

Conclusion

This systematic returned results that evidenced the mutagenic effect of malathion used as a commercial formulation, that is, containing its analogs malaoxon and isomalathion, and its ability to promote changes in DNA *in vivo*. Thus, neoplastic processes can be triggered both in animals and in exposed humans once such changes can reach regions of oncogenes or tumor suppressors in the DNA.

The results of the *in vitro* studies in both animal and human cell cultures exposed to malathion showed DNA damage, chromosomal alterations, sister chromatid exchanges and micronuclei. *In vivo* experimental studies have shown sufficient evidence regarding the potential of the pesticide both in inducing genetic damage and inducing neoplasms in mammals. Epidemiological studies have shown statistically significant positive associations for thyroid, breast, and ovarian cancer in menopausal women.

The carcinogenic effect of this pesticide and its implications on the environment and humans should be considered, particularly in the context of arbovirus control.

Collaborations

PL Bastos participated in the collection and analysis of the data, in the design and final writing of the article. AFT Lima participated in the collection and analysis of data and review of the final text. AM Gurgel participated in the design of the article, in the analysis of the data and in the revision of the final text. IGD Gurgel participated in the data analysis, in the design, writing and final review of the article.

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