

# The environmental and occupational influence of pesticides on male fertility: A systematic review of human studies

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## Abstract

**Background:** The environment plays a key role in male infertility, changing the incidence in various populations, and pesticides are one of the most studied hazards. The use of the latter has never decreased, jeopardizing the safety of workers and the general population.

**Objective:** Our purpose was to summarize the results of studies discussing the association between pesticides and male fertility.

**Methods:** A comprehensive literature search was performed through MEDLINE via PubMed, Scopus, and Web of Science. Only human studies were considered. Semen parameters and DNA integrity were considered to evaluate the effect of pesticides on men.

**Results:** A total of 64 studies that investigated their impact in terms of semen parameters (51 studies) and chromatin and DNA integrity (25 studies) were included. The most frequently affected parameters were total sperm count, sperm motility, and sperm morphology, although a reduction in ejaculate volume and concentration occur in several cases. A tangible worsening of semen quality was associated with organochlorines and organophosphates. Furthermore, pesticide exposure, especially pyrethroids, was related to a higher DNA fragmentation index and chromosome aneuploidy in most articles.

**Conclusion:** The epidemiological evidence supports the association between pesticides and male fertility for workers and the exposed population in terms of semen quality, DNA fragmentation, and chromosome aneuploidy.

## KEYWORDS

chromosome aneuploidy, DNA integrity, male fertility, pesticides, semen parameters

## 1 | INTRODUCTION

According to the World Health Organization (WHO), infertility is a disease of the reproductive system defined by the failure to achieve

pregnancy after 12 months or more of regular unprotected sexual intercourse, affecting up to 15% of couples, and the etiology is attributable to men in one-third of the cases.<sup>1</sup> The environment plays a key role in male infertility, changing the incidence in various

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populations.<sup>2</sup> Hence, the identification of these exogenous factors is pivotal to reduce exposure and, consequently, improve semen quality. Doubtless, pesticides are one of the most studied risk factors. Pesticides prevent, destroy, or control harmful organisms (pests) or diseases; they protect plants or plant products during production, storage, and transport.<sup>3</sup> Their use has increased over the past 50 years as global arable land has increased. Although pesticides are essential for crop protection, food maintenance, and vector-borne disease prevention, they remain biocides with harmful effects on humans. Absorption can occur through different methods: ingestion, inhalation, dermal contact, and through the placenta.<sup>4</sup> Exposure can cause acute and chronic toxicity with respiratory, gastrointestinal, and neurological symptoms and affect male fertility. At the beginning of the 1980s, the harmful effect of dibromochloropropane (DBCP) was known,<sup>5</sup> and consequently, its use was banned by the Environmental Protection Agency and the WHO. However, there are no univocal indications of the danger of the other types of pesticides. Previously, Martenies and Perry<sup>6</sup> reported that pesticides affected semen quality, especially sperm concentration in overall pesticide classes. In 2013, Sengupta and Banerjee,<sup>7</sup> evaluating *in vitro* and *in vivo* studies, also reported the association of these substances with abortions, congenital malformations, and interference with the hypothalamic–pituitary–gonadal axis, in addition to worsening semen quality. Subsequently, two other reviews dealing with endocrine-disrupting chemicals (EDCs), including some types of pesticides, were published. Zamkowska et al.<sup>8</sup> showed consistency between semen parameter worsening and pyrethroids (PYRs) and organophosphates (OPs), while Rodprasert et al.<sup>9</sup> confirmed the effects of OPs and questioned those of organochlorines (OCs).

Seminal parameters are not the only factors used to assess male fertility. Indeed, after exposure to these EDCs, the nucleus of spermatocytes is affected with consequent DNA defragmentation and chromosome aneuploidy, which, in turn, may be markers of damage.

In this study, we aimed to perform a systematic review of the literature regarding the impact of pesticides on male semen quality and DNA integrity.

## 2 | MATERIALS AND METHODS

### 2.1 | Search design

A comprehensive literature search was performed between February 2 and March 3, 2021, through MEDLINE via PubMed, Scopus, and Web of Science. The following Medical Subject Headings were used: male infertility, male impairment, DNA damage, human spermatozoa, semen parameters, and genotoxic. These terms have been searched with pesticides and their most relevant groups (such as OCs, PYRs, OPs, or carbamates) and specific ones (such as atrazine or mancozeb). The search was further expanded by performing a manual search based on the references of the full text of the relevant papers.

### 2.2 | Identification of studies

Observational studies regarding the association of pesticide exposure and male infertility were selected. Papers were considered according to the PICOS (Patient Intervention Comparison Outcome Study type) model. P: general population or workers; I: exposure to pesticides; C: comparison with non-exposed men; O: alteration of semen parameters (ejaculate volume, sperm count, sperm concentration, total sperm motility, and sperm morphology) and DNA fragmentation or aneuploidy; S: observational study. No date limit was imposed on the search.

### 2.3 | Eligibility criteria

Papers were accepted based on the following criteria: (i) occupational or environmental exposure to pesticides; (ii) studies reporting semen parameters, DNA fragmentation index (DFI), and DNA aneuploidy; (iii) original articles; (iv) studies in English language; and (v) *in vivo* human studies.

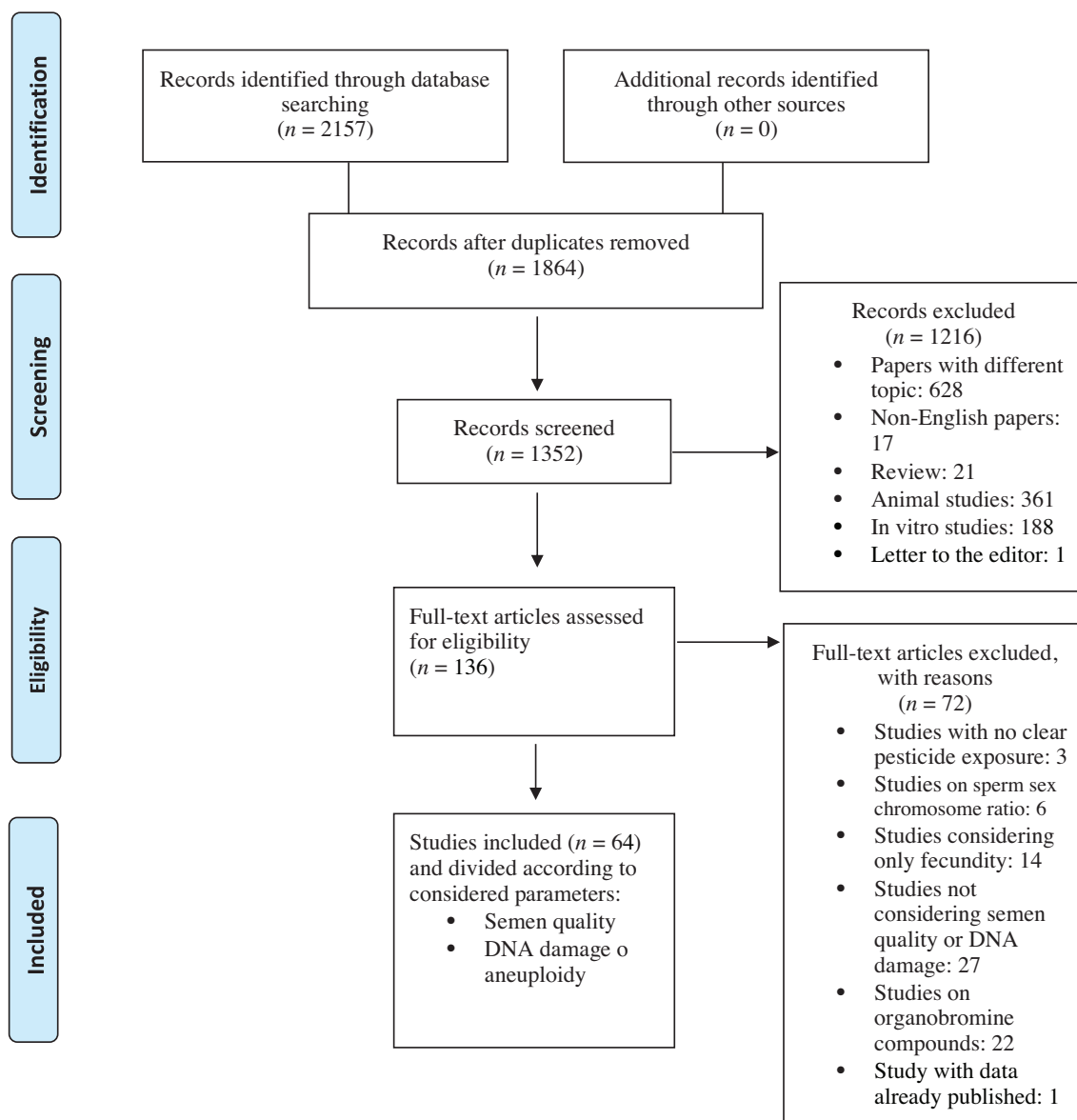
Articles relating only to epidemiological investigations, to other systems, or to heterogeneous occupational exposure with no multi-variable analysis were excluded. Studies focusing on sex chromosome ratio in spermatozoa as the only outcome of interest were also not accepted. Moreover, we excluded articles regarding organobromine compounds because of the already proven harmful consequences on male fertility.<sup>10</sup>

### 2.4 | Risk of bias assessment

The quality of individual studies was assessed following the guidelines established in the Office of Health Assessment and Translation (OHAT) Risk of Bias Rating Tool for Human and Animal Studies.<sup>11</sup> In studies where a control/reference group was not present, selection bias was not evaluable.

## 3 | RESULTS

The literature search retrieved 1864 papers. Two independent authors screened all retrieved records. Discrepancies were resolved by a third author. A total of 1352 studies were screened against the title and abstract. A total of 1216 articles were excluded for the following reasons: 628 articles had a different topic, 17 papers were not in English, 21 were reviews, 188 were *in vitro* studies, 361 were studies on animals, one was a letter to the editor, and one had data already published in another one by the same authors. The full text of the remaining 136 papers was further assessed for eligibility. In three articles, pesticide exposure was not clear, six studies investigated the sperm sex chromosome ratio, 14 considered only fecundity or time to pregnancy, 27 did not concern seminal parameters or DNA damage, one paper was



**FIGURE 1** PRISMA 2009 flow diagram

a duplicate, and 22 concerned organobromine compounds and were excluded. Finally, 64 studies were accepted and included in this review. Figure 1 shows the PRISMA flow diagram of the study.

### 3.1 | Quality assessment

Figure 2 shows the quality of evidence of the included studies according to the OHAT risk of bias rating tool. The examined studies exhibited an overall moderate risk of bias, with the remaining having a low risk. The most relevant parameters of this tool are the exposure characterization, which evaluates the methods' sensitivity to measure exposure, and the outcome characterization, which evaluates the methods' sensitivity to assess the outcomes. Regarding exposure characterization, in 45 out of 64 (70%) studies, serum/urine levels of pesticides or their

metabolites were measured, while years of exposure were calculated in only 14 out of 64 (22%), and in the remaining five articles (8%), people or workers were recruited in areas with high air pesticide concentrations. Regarding the outcome characterization, most studies described an appropriate method of semen collection and reported reproducibility of outcome measurement. The most common risk factor for bias was the risk of attrition/exclusion and confounding bias.

### 3.2 | Data extraction

The following data were extracted from the included studies: alterations in semen parameters (sperm motility, sperm morphology, sperm count, and semen volume) and DNA fragmentation and aneuploidy.

Table 1 shows the characteristics of the included studies.

	Exposure Characterization	Selection Bias	Other Sources of Bias	Outcome Characterization	Selective Reporting Bias	Confounding Bias	Attrition/ Exclusion Bias
Lerda 1991 [12]	+	+	++	++	++	-	+
Hauser 2003a [13]	+	NA	+	++	++	-	+
Dalvie 2004 [14]	++	+	++	++	++	+	+
Toft 2006 [15]	++	NA	++	+	++	-	-
Pant 2007 [16]	+	+	+	++	+	+	+
De Jager 2006 [17]	++	NA	++	++	++	+	-
Aneck-Hahn 2007 [18]	++	NA	+	++	+	+	-
Khan 2010 [19]	+	+	+	++	++	+	+
Pant 2014 [20]	+	+	+	+	++	-	+
Mumford 2014 [21]	++	NA	+	+	+	+	+
Bush 1986 [22]	+	NA	+	++	++	-	+
Rignell-Hydbom 2004 [23]	++	NA	++	++	++	+	+
Giwerzman 2007 [24]	+	+	+	++	++	-	+
Haugen 2011 [25]	++	+	++	++	++	+	-
Xia 2004 [26]	++	+	++	++	++	-	-
Lifeng 2006 [27]	++	+	+	++	+	-	-
Meeker 2008 [28]	++	+	+	+	++	++	+
Ji 2011 [29]	+	+	+	+	++	+	-
Radwan 2014 [30]	++	NA	+	+	++	+	-
Hu 2019 [31]	++	+	+	+	++	++	+
Bian 2004 [32]	++	+	++	+	++	-	-
Imai 2014 [33]	++	NA	+	+	++	++	+
Padungton 2000 [34]	++	+	+	++	++	+	-
Padungton 1999 [35]	++	+	+	+	++	-	+
Yucra 2006 [36]	+	+	+	++	++	+	-
Pérez-Herrera 2008 [37]	-	NA	+	+	++	+	-
Recio-Vega 2009 [38]	++	+	+	++	++	++	+
Yucra 2009 [39]	++	+	+	++	+	+	-
Hossain 2010 [40]	++	+	+	++	++	++	+
Melgarejo 2014 [41]	+	NA	+	++	++	+	-
Dziewirska 2018 [42]	+	+	+	+	+	+	-
Lwin 2018 [43]	++	+	+	+	-	-	-
Ghafouri-Khosrowshahi 2020 [44]	++	+	+	++	++	-	-
Sánchez-Peña 2004 [45]	++	NA	+	++	++	-	-
Multigner 2008 [46]	++	+	+	++	++	-	-
Wyrobek 1981 [47]	++	+	+	+	++	++	-
Xia 2005 [48]	+	+	+	++	++	-	-
Celik-Ozenci 2012 [49]	++	+	++	+	++	-	-
Swan 2003 [50]	++	+	+	+	++	+	-
Larsen 1998 [51]	++	+	+	++	++	-	-
Abell 2000 [52]	++	+	+	+	++	++	-
Oliva 2001 [53]	+	+	+	+	+	-	-
Kamijima 2004 [54]	++	+	++	++	++	-	-
Tuc 2007 [55]	+	+	+	+	++	-	-
Perry 2007 [56]	++	+	+	+	+	-	-
Miranda-Contreras 2013 [57]	++	+	+	++	++	-	-
Daoud 2017 [58]	+	+	+	++	+	-	-
Cremonese 2017 [59]	++	+	++	++	++	-	-
Tielemans 1998 [60]	+	+	+	+	+	+	-
Härkönen 1999 [61]	++	NA	+	+	+	-	-
De Fleurian 2009 [62]	+	+	+	++	++	++	-
De Jager 2009 [63]	++	NA	++	++	++	+	-
McAuliffe 2012 [64]	++	+	+	++	++	+	-
Perry 2016 [65]	++	+	+	++	++	+	-
Hauser 2003b [66]	+	NA	+	++	++	-	-
Rignell-Hydbom 2005 [67]	++	+	+	++	++	-	-
Spanò 2005 [68]	++	+	+	++	++	+	-
Stronati 2006 [69]	++	+	+	++	++	+	-
Young 2013 [70]	++	+	+	+	++	-	-
Jurewicz 2014 [71]	++	NA	-	-	++	+	-
Radwan 2015 [72]	++	NA	+	+	++	+	-
Recio 2001 [73]	++	NA	+	++	+	+	-
Figueroa 2019 [74]	++	+	+	++	++	+	-
Smith 2004 [75]	+	+	+	++	+	-	-

**FIGURE 2** Risk of bias assessment (Office of Health Assessment and Translation, OHAT). Several parameters are evaluated to assess the quality of the considered studies: exposure characterization (i.e., standards for exposure assessment), selection bias (i.e., similarity of the population in the two groups under comparison), other sources of bias (i.e., other biases derived from statistics or deviations from the protocol), outcome characterization (i.e., validity and reproducibility of methods for measuring outcomes), selective reporting bias (i.e., certification that primary and secondary outcomes have been reported), confounding bias (i.e., evaluation of the baseline characteristics risk factors, prognostic variables or co-occurring exposures in the reference population), and attrition/exclusion bias (i.e., data loss because of attrition or exclusion from analyses)

**TABLE 1** Studies concerning the effects of pesticides on semen parameters

First author (year)	Country of study	Pesticide	Study design/evaluation	Examined population, n	Controls/reference group	Reproductive effects
Lerda (1991) <sup>12</sup>	Argentina	2,4-D (OC)	Cross-sectional study	32 sprayers, 25 controls	Non-exposed men	Reduction of sperm concentration in sprayers
Hauser (2003a) <sup>13</sup>	United States of America	DDT (OC)	Cross-sectional study	29 men presenting for semen evaluation	None	Higher serum p,p'-DDE levels in men with sperm concentration, total motility, and morphology below standard
Dalvie (2004) <sup>14</sup>	South Africa	DDT (OC)	Cross-sectional study	27 sprayers, 27 controls	Non-exposed workers in the vicinity of the Department of Health Malaria Control Centre	Lower sperm count and normal morphology
Toft (2006) <sup>15</sup>	Greenland, Sweden (fisherman), Kharkiv (Ukraine), Warsaw (Poland)	DDT (OC)	Cross-sectional study	763 men	None	Sperm motility was inversely related to p,p'-DDE concentration
Pant (2007) <sup>16</sup>	India	HCH and DDT (OC)	Cross-sectional study	50 infertile men, 50 controls	Male volunteers with proven fertility whose partner had conceived spontaneously within 1 year	Association between seminal $\beta$ -HCH and p,p'-DDE levels and total sperm count and between $\gamma$ -HCH and total sperm motility
De Jager (2006) <sup>17</sup>	Mexico	DDT (OC)	Cross-sectional study	116 healthy men	None	Positive relationship between p,p'-DDE serum concentration and sperm percentage with abnormal tails
Aneck-Hahn (2007) <sup>18</sup>	South Africa	DDT (OC)	Cross-sectional study	311 healthy men	None	Negative relationship between mean CASA sperm motility, ejaculate volume and sperm count, and p,p'-DDE serum concentration
Khan (2010) <sup>19</sup>	India	HCH (OC)	Cross-sectional study	50 infertile men	50 fertile men	Negative association between total sperm count and $\gamma$ -HCH in asthenospermia patients and with $\beta$ -HCH and total HCH in oligo-asthenospermic patients
Pant (2014) <sup>20</sup>	India	Lindane and DDT (OC)	Cross-sectional study	193 men trying to conceive and 85 controls	Men with proven fertility	Negative association between seminal p,p'-DDE and lindane levels and sperm concentration and total motility
Mumford (2014) <sup>21</sup>	United States of America	HCH and DDT (OC)	Cross-sectional study	501 men of couples attending at infertility clinic	None	Positive relationship between DDT and sperm morphology and motility and between p,p'-DDE and motility
Bush (1986) <sup>22</sup>	United States of America	DDT (OC)	Cross-sectional study	170 men with fertility problems or undergoing vasectomy	None	No significant association between blood p,p'-DDE levels and total sperm motility

(Continues)

TABLE 1 (Continued)

First author (year)	Country of study	Pesticide	Study design/evaluation	Examined population, n	Controls/reference group	Reproductive effects
Rignell-Hydbom (2004) <sup>23</sup>	Sweden	DDT (OC)	Cross-sectional study	195 fishermen	None	No association between serum p,p'-DDE concentrations and semen volume or sperm motility
Giwercman (2007) <sup>24</sup>	Greenland, Sweden, Ukraine, Poland	DDT (OC)	Cross-sectional study	680 men from Greenland, Sweden, Ukraine, and Poland	Men with low serum p,p'-DDE levels	No relationship between androgen receptor gene CAG repeat length and susceptibility to OC concerning sperm concentration and total sperm count
Haugen (2011) <sup>25</sup>	Norway	DDT (OC)	Cross-sectional study	95 men from South Sweden with higher p,p'-DDE levels, 77 controls	77 men from North Sweden with lower p,p'-DDE levels	No significant association between CB-153 levels and sperm concentration, total count, and progressive motility
Xia (2004) <sup>26</sup>	China	Fenvalerate (PYR)	Cross-sectional study	12 factory workers, 12 internal, and 18 external controls	Healthy, young, non-smokers, and non-regular drinkers donors	Higher sperm abnormality percentage in exposed workers than external non-exposed subjects
Lifeng (2006) <sup>27</sup>	China	Fenvalerate (PYR)	Cross-sectional study	32 workers exposed to fenvalerate, 22 external, and 46 internal controls	Administrators in the office in the same pesticide factory and officers in a center for disease control in the urban district of the same city	Exposure to fenvalerate was significantly associated with lower sperm's progressive motility and total count
Meeker (2008) <sup>28</sup>	United States of America	Unspecified PYR	Cross-sectional study	207 men recruited from an infertility clinic	Men below the 50th percentile concentration of urinary PYR metabolite	Urinary TDCCA levels were inversely related to sperm concentration and total motility
Ji (2011) <sup>29</sup>	China	Unspecified PYR	Cross-sectional study	240 men recruited from an infertility clinic	Men with the lowest quartile of urinary 3-PBA levels	A trend in reduced levels of total sperm count and sperm concentration for higher 3-PBA levels
Radwan (2014) <sup>30</sup>	Poland	Unspecified PYR	Cross-sectional study	334 men recruited from an infertility clinic	None	Negative relationship between TDCCA and sperm concentration and positive relationship with sperm morphology
Hu (2019) <sup>31</sup>	China	Unspecified PYR	Cross-sectional study	346 volunteers seeking preconception assessment in a preconception care hospital	Men in the quartile with the lowest urinary 3-PBA levels	Negative associations between urinary 3-PBA levels and sperm normal morphology, and between urinary TDCCA concentration and total count (log-transformed)

(Continues)

TABLE 1 (Continued)

First author (year)	Country of study	Pesticide	Study design/evaluation	Examined population, n	Controls/reference group	Reproductive effects
Bian (2004) <sup>32</sup>	China	Fenvalerate (PYR)	Cross-sectional study	21 pesticides factory workers, 19 (external), and 23 (internal) controls	Men from the office area of the same factory (internal controls). Volunteers recruited from different departments at the same urban district (external controls)	No significant association
Imai (2014) <sup>33</sup>	Japan	Unspecified PYR	Cross-sectional study	322 healthy university students	None	No significant association between PYR insecticides exposure and semen quality
Padungtod (2000) <sup>34</sup>	China	Methylparathion, ethyl parathion, and methamidophos (OP)	Cross-sectional study	20 factories workers, 23 controls	Non-exposed farmers from the same factory	Decrease in sperm concentration and motility associated with the exposed group
Padungtod (1999) <sup>35</sup>	China	Unspecified OP	Cross-sectional study	20 Chinese pesticides factory workers, 22 controls	Non-exposed textile factory workers	PON1 determines the susceptibility to OP in terms of worsening of sperm concentration and normal morphology
Yucra (2006) <sup>36</sup>	Perú	Unspecified OP	Cross-sectional study	31 pesticide applicators, 80 non-exposed subjects	Exposes' male friends without any exposure	Reduction of semen volume, sperm rapid and progressive motility, and normal morphology in exposed men with respect to non-exposed
Pérez-Herrera (2008) <sup>37</sup>	Mexico	Unspecified OP	Cross-sectional study	54 farmworkers	None	Reduction in sperm normal morphology, semen volume, and total motility. Greater susceptibility in genotype PON1192RR
Recio-Vega (2009) <sup>38</sup>	Mexico	Methyl parathion, metamidophos, dimethoate, and diazinon (OP)	Longitudinal study	52 men from an agricultural community grouped according to urinary OP metabolite levels	Men in the lowest tertile of OP exposure based on a questionnaire	Decrease in semen volume and total sperm count in workers with higher exposure
Yucra (2009) <sup>39</sup>	Perú	Unspecified OP	Cross-sectional study	31 OP applicators, 31 controls	Male friend without any exposure to OP or agricultural activity	Negative association of urinary OP metabolites with seminal pH
Hossain (2010) <sup>40</sup>	Malaysia	Malathion and paraquat (OP)	Cross-sectional study	62 exposed farmers, 90 controls	Non-exposed farmers	Lower levels of semen volume, sperm concentration, and motility in exposed than non-exposed farmers
Melgarejo (2014) <sup>41</sup>	Spain	Unspecified OP	Cross-sectional study	116 men attending in an infertility clinic	None	Negative association of urinary OP metabolites with sperm concentration and total count

(Continues)

TABLE 1 (Continued)

First author (year)	Country of study	Pesticide	Study design/evaluation	Examined population, n	Controls/reference group	Reproductive effects
Dziewirska (2018) <sup>42</sup>	Poland	Unspecified OP	Cross-sectional study	315 men attended an infertility clinic	Men in the lowest percentile of the semen parameters distribution	Negative association between urinary TCPY concentration and total sperm motility
Lwin (2018) <sup>43</sup>	Myanmar	Unspecified OP	Cross-sectional study	100 groundnut farmers chronically exposed to OP pesticides	Same farmers in non-growing period	In the growing period, sperm motility, morphology, and count were reduced than the non-growing one
Ghafouri-Khosrowshahi (2019) <sup>44</sup>	Iran	Unspecified OP	Cross-sectional study	30 rural men, 30 controls	Urban men with no history of farmer or pesticide exposure	Lower levels in sperm count, total and progressive sperm motility in rural with respect to urban men
Sanchez-Pena (2004) <sup>45</sup>	Mexico	Methyl parathion, metamidophos, dimethoate, and diazinon (OP)	Cross-sectional study	33 farmworkers	None	No significant correlation between urinary OP metabolites and semen parameters
Multigner (2008) <sup>46</sup>	Guadeloupe	Unspecified OP	Cross-sectional study	42 banana plantation workers, 45 controls	Men working in non-agricultural sectors	No significant association
Wyrobek (1981) <sup>47</sup>	United States of America	CAR	Cross-sectional study	17 factory workers, 17 external controls	Unexposed, newly hired workers	Higher levels of sperm abnormalities in the exposed group with respect to unexposed
Xia (2005) <sup>48</sup>	China	CAR	Cross-sectional study	16 factory workers, 16 internal controls, and 16 external controls	Sperm donor from the same working area (external control). Workers in the same pesticide factory but far away from the pesticide workshop (internal control)	Higher levels in sperm normal morphology in exposed with respect to external controls
Dziewirska (2018) <sup>42</sup>	Poland	CAR	Cross-sectional study	315 men attended an infertility clinic	Men in the lowest percentile of the semen parameters distribution	Negative association between 1N and sperm normal morphology
Celik-Ozenci (2012) <sup>49</sup>	Turkey	Abamectin	Cross-sectional study	20 farmworkers, 20 external controls	Non-exposed men from the same region	Lower levels in sperm motility and higher levels of semen volume in exposed with respect to non-exposed men

(Continues)



TABLE 1 (Continued)

First author (year)	Country of study	Pesticide	Study design/evaluation	Examined population, n	Controls/reference group	Reproductive effects
Swan (2003) <sup>50</sup>	United States of America	Alachlor and Atrazine	Cross-sectional study	34 infertile men	52 fertile men whom the average sperm concentration was above the population median	Blood alachlor and atrazine levels were higher in infertile men and related to an increase in sperm abnormalities
Larsen (1998) <sup>51</sup>	Denmark	Insecticides, herbicides, and fungicides	Longitudinal study	161 sprayers, 87 controls	Farmers not spraying with pesticides	Decrease in the percentage of the normal sperm heads
Abell (2000) <sup>52</sup>	Denmark	Insecticides	Cross-sectional study	122 farmworkers: 13 with high, 64 with intermediate, and 44 with low exposure	Farmworkers ranked according to the level of dermal exposure estimated by work task exposure to pesticides and according to the duration of work	Lower percentage of normal morphology in higher exposed subgroup
Oliva (2001) <sup>53</sup>	Argentina	Unspecified pesticides	Cross-sectional study	225 male partners of couples having their first infertility consultation	Men who did not report any exposure and whose occupation did not expose them to any agent	Significant association between pesticide exposure and lower sperm concentration, count, and motility
Kamijima (2004) <sup>54</sup>	Japan	Insecticides	Cross-sectional study	18 sprayers, 18 controls	Age-matched students or medical doctors with no exposure	Significantly higher levels of slow progressive and non-progressive motility and lower levels of normal sperm morphology in sprayers with respect to controls
Tuc (2007) <sup>55</sup>	Vietnam	Unspecified pesticides	Case-control study	156 infertile rice farmers, 314 controls	Rice farmers with normal semen parameters	Significant higher probability of abnormal semen in cases living in less than 300 m from rice field, working over 10 years as a rice farmer, without pesticide training and without PPE when spraying, with respect to controls
Perry (2007) <sup>56</sup>	United States of America	PYRs and OPs	Cross-sectional study	18 randomly urine samples from the Department of Environmental Health	Participants with low OP and PYR exposure based on median values as cut off	Negative association between DETP levels and sperm concentration
Miranda-Contreras (2013) <sup>57</sup>	Venezuela	OP and CAR	Cross-sectional study	64 farmworkers, 35 controls	Healthy men living 90 km from the agricultural region not currently exposed to pesticides	Lower sperm viability and non-significant rapid and progressive sperm motility in exposed men

(Continues)

TABLE 1 (Continued)

First author (year)	Country of study	Pesticide	Study design/evaluation	Examined population, n	Controls/reference group	Reproductive effects
Daoud (2017) <sup>58</sup>	Tunisia	Insecticides, herbicides, and fungicides	Cross-sectional study	2122 men attending the Andrology laboratory	Unexposed (n = 847) subjects classified according to the reported information about their occupation	Negative association between pesticides and asthenozoospermia and necrozoospermia
Cremonese (2017) <sup>59</sup>	Brazil	Insecticides, herbicides, and fungicides	Cross-sectional study	99 rural and 36 controls	Urban healthy young men	Increase in sperm concentration and decrease in sperm motility and morphology in rural with respect to urban subjects
Tielemans (1998) <sup>60</sup>	Netherlands	Insecticides, herbicides, and fungicides	Case-control study	692 infertile men, 207 controls	Men with normal semen parameters	No significant association
Härkönen (1999) <sup>61</sup>	Denmark	Insecticides, herbicides, and fungicides	Cross-sectional study	32 farmers	None	No significant association
De Fleurian (2009) <sup>62</sup>	France	Unspecified pesticides	Case-control study	402 men consulting for couple infertility, 88 control	Men with normal semen parameters	No significant association between exposure to pesticides and infertility

Abbreviations: 3-PBA, 3-phenoxybenzoic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; 1N, 1-naphthol; CAR, carbaryl; CASA, computer-aided sperm analyzer; DDT, dichloro-diphenyl-trichloroethane; DETP, diethylthiophosphate; HCH, hexachlorocyclohexane; OC, organochlorine; OP, organophosphate pesticide; PON1, paraoxonase; p,p'-DDE, dichloro-diphenyldichloro-ethylene; PYR, pyrethroid; TCPY, 3,5,6-trichloro-2-pyridino; TDCCA, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid.

### 3.3 | Semen parameters

#### 3.3.1 | Organochlorine

Sperm motility, normal morphology, and vitality were significantly lower in sprayers exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) than in the control group.<sup>12</sup> Furthermore, among men waiting for semen evaluation in Massachusetts, dichloro-diphenyldichloroethylene (p,p'-DDE) concentrations tended to be higher in subjects with sperm concentration, total motility, and morphology below WHO threshold values.<sup>13</sup> Instead, according to Dalvie et al.,<sup>14</sup> dichlorodiphenyl-trichloroethane (DDT) was found to be associated with a lower semen count ( $p = 0.04$ ,  $r^2 = 0.05$ ), and the majority of vector-control workers (84%) had a percentage of "normal" sperm morphology below WHO criteria. Considering environmental and occupational exposures in different regions, sperm motility was the only parameter negatively associated with an increase in p,p'-DDE levels in Greenland and in combined data from Poland, Ukraine, and Sweden ( $\beta = -2.8$ , 95% confidence interval [CI]:  $-4.8$  to  $-0.7$  and  $\beta = -3.6$ , 95% CI:  $-5.6$  to  $-1.7$ , respectively).<sup>15</sup>

Recruiting infertile men from Krishna Medical Centre, Pant et al.<sup>16</sup> noted that the total sperm count was inversely related to seminal OC levels, mainly  $\beta$ -hexachlorocyclohexane ( $\beta$ -HCH) ( $r = -0.43$ ,  $p < 0.002$ ) and p,p'-DDE ( $r = -0.39$ ,  $p < 0.005$ ), as well as total sperm motility, mostly with  $\gamma$ -HCH ( $-0.29$ ,  $p < 0.05$ ). De Jager et al.,<sup>17</sup> who studied the general population in Chiapas (Mexico), showed that increasing p,p'-DDE serum levels were positively associated with the percentage of spermatozoa with morphological tail defects ( $\beta = -0.003$ ,  $p = 0.017$ ). Moreover, a non-significant negative relationship between DDE and the percentage of motile spermatozoa ( $\beta = -8.38$ ,  $p = 0.05$  for squared motility) was reported. Aneck-Hahn et al.<sup>18</sup> also reported the relationship between non-occupational exposure and sperm parameter alterations: lower values of mean computer-aided sperm analyzer (CASA) motility ( $\beta = -0.02$ ,  $p = 0.001$ ), squared root-transformed ejaculate volume ( $\beta = -0.0003$ ,  $p = 0.02$ ), and sperm count ( $\beta = -0.003$ ,  $p = 0.04$ ) were significantly associated with higher p,p'-DDE serum concentrations.

Y chromosome microdeletion has emerged as a new condition associated with male infertility. Khan et al.<sup>19</sup> evaluated a possible relationship with OC exposure. The authors reported that the incidence of the Yq deletion is high in azoospermic men with greater total and  $\beta$ -HCH levels (61.5%), corroborating the hypothesis of the mutagenic activity of the pesticide. Moreover,  $\gamma$ -HCH was negatively related to total sperm count ( $p < 0.001$ ) in patients with asthenospermia, as well as  $\beta$ -HCH ( $p = 0.03$ ) and total HCH ( $p = 0.04$ ) in those with oligo-asthenospermia.

Among male partners visiting the infertility unit in Lucknow (India), sperm concentration and motility decreased significantly from the lowest quartiles toward the highest p,p'-DDE and lindane levels ( $p < 0.001$ ).<sup>20</sup> Furthermore, evaluating data from the LIFE Cohort Study, which included 468 men attending infertility clinics in Michigan and Texas, DDT and  $\beta$ -HCH were associated with increased values in

sperm motility ( $\beta = 5.30$  and  $0.52$ ,  $p < 0.01$ , respectively) and normal morphology ( $\beta = 5.05$  and  $2.22$ ,  $p < 0.01$ , respectively).<sup>21</sup>

In a study recruiting men attending Albany Medical Center for fertility issues or vasectomy, p,p'-DDE concentration was not associated with sperm motility, the only seminal parameter considered.<sup>22</sup> Rignell-Hydbom et al.<sup>23</sup> also reported no relationship with p,p'-DDE serum concentration and sperm motility in Swedish fishermen. A gene-environment interaction has also been investigated. In any case, the length of the androgen receptor gene CAG repeats did not influence the impact of persistent organohalogen pollution (POP) on semen quality.<sup>24</sup> Haugen et al.<sup>25</sup> analyzed men living in Norway: no semen quality association with p,p'-DDE was highlighted, except for the low correlation found in men living in northern Norway ( $r = 0.25$ ,  $p = 0.03$ ); the p,p'-DDE concentration was higher in the southern area population than in the northern population.

#### 3.3.2 | Pyrethroids

Xia et al.<sup>26</sup> reported a higher percentage of sperm abnormalities among pesticide factory workers with respect to non-exposed subjects (mean % in exposed 22.2 vs. 15.9 in non-exposed,  $p = 0.024$ ), while sperm concentration, total count, and motility were comparable with controls. Significant associations were found with sperm progressive motility and count in fenvalerate-exposed workers in the pesticide plant with respect to those non-exposed (progression mean [SD] in exposed 1.8 [0.5] vs. 2.3 [0.5] in non-exposed,  $p < 0.05$ ; sperm count mean [range] in exposed 53.9 [2.0–233.2],  $p < 0.05$ ).<sup>27</sup>

Among men recruited from an andrology laboratory, in multivariable linear regression analysis, urinary pyrethroid metabolites were related to a reduction in semen quality.<sup>28</sup> Indeed, values above the 75th percentile of trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (TDCCA) with respect to <50th percentile values were associated with a reduction in sperm concentration ( $-34.2$  million spermatozoa/ml, 95% CI:  $-51.1$  to  $-5.7$ ) and total sperm motility ( $-10\%$ , 95% CI:  $-19$  to  $-1$ ), with  $p$ -values for trend  $\leq 0.05$ . In Chinese men attending an infertility clinic, urinary 3-phenoxybenzoic acid (3-PBA) levels and sperm concentration ( $\beta = -0.27$ , 95% CI:  $-0.41$  to  $-0.12$ ,  $p < 0.001$ ) were also inversely related.<sup>29</sup> Furthermore, a significant trend of decline in sperm concentration and total sperm count for increasing quartiles of metabolite concentration was reported. Multiple linear regression also showed not only a negative relationship between sperm concentration and TDCCA levels over the 50th percentile in urine ( $\beta = -0.33$ , 95% CI:  $-0.64$  to  $-0.02$ ,  $p = 0.04$ ) and between sperm abnormal morphology and TDCCA levels under and over the 50th percentile ( $\beta = 2.79$ , 95% CI:  $1.58$ – $5.00$ ,  $p = 0.01$  and  $\beta = 2.80$ , 95% CI:  $1.56$ – $5.04$ ,  $p = 0.02$ , respectively).<sup>30</sup>

A Chinese study on reproductive-age men in Shanghai revealed that men with the highest quartile of 3-PBA had a higher probability of below WHO reference sperm normal morphology with respect to men with the lowest quartile of 3-PBA (odds ratio [OR] = 3.08, 95% CI: 1.10–8.60).<sup>31</sup> Conversely, Bian et al.<sup>32</sup> found no significant differences

regarding sperm concentration and morphology between pesticide factory workers and the external group, whereas straightness motility was significantly lower. Moreover, no semen parameter was related to urinary 3-PBA concentration in a group of healthy Japanese university students.<sup>33</sup>

### 3.3.3 | Organophosphates

Padungtod et al.<sup>34</sup> assessed a reduction in sperm concentration and percent motility in factory workers exposed to OP pesticides than non-exposed workers ( $\beta_{\log \text{ sperm concentration}} = -0.6$ , 95% CI:  $-0.9$  to  $-0.2$ ;  $\beta_{\text{percent motility}} = -10.4$ , 95% CI:  $-19.2$  to  $-1.6$ , respectively). In addition, the same group investigated the interaction of human paraoxonase (PON1), which is responsible for OP deactivation, genotype with semen quality.<sup>35</sup> They reported that this enzyme, depending on the amino acid substitution at position 192, had different effects on semen parameters. In the presence of arginine, exposure to OPs was associated with a lower total sperm count ( $\chi^2 = 9.01$ ,  $p < 0.01$ ) and sperm normal morphology ( $\chi^2 = 4.18$ ,  $p < 0.05$ ) and percentage motility ( $p < 0.05$ ), while with glutamine homozygosity, even non-exposed subjects had a lower sperm concentration ( $\chi^2 = 4.90$ ,  $p < 0.05$ ) than the reference group (non-exposed to OP with arginine 192 homo/heterozygotes).

Ejaculate volume ( $\beta_{\text{regression}} = -0.60$ ,  $p_{\text{regression}} = 0.009$ ), rapid and progressive motility ( $\beta_{\text{regression}} = -0.22$ ,  $p_{\text{regression}} = 0.008$ ), and normal sperm morphology ( $\beta_{\text{regression}} = -9.74$ ,  $p_{\text{regression}} < 0.001$ ) were found to be lower in Peruvian pesticide sprayers than in non-exposed subjects.<sup>36</sup> In Mexican farmworkers with more than 18 years of work exposure to OPs, a high rate of abnormal morphology (100%), low ejaculate volume (46%), low sperm motility (30%), and viability (13%) were found.<sup>37</sup> The authors demonstrated that all cells of the spermatogenic cycle were sensitive to OP toxicity and confirmed the dose-effect relationships between sperm motility and viability and OP exposure in men with the homozygous PON1192RR genotype and, to a lesser extent, the heterozygous PON1Q192R genotype.

Urine metabolites of OP were positively related to a reduction in semen volume and sperm count in a dose-dependent manner among volunteers of an agricultural community.<sup>38</sup> This finding was confirmed by Yuca et al.,<sup>39</sup> who reported that urinary OP metabolites were negatively associated with seminal pH after application of Holm's test. Hossain et al.<sup>40</sup> confirmed that semen parameters were worse in farmers than in non-exposed men in Sabah, Malaysia, and Malathion, including semen volume, sperm concentration, motility, and normal morphology, despite a lower exposure.

Evaluating men attending infertility services in southern Spain, Melgarejo et al.<sup>41</sup> showed that sperm concentration and total count were significantly and inversely related to the concentrations of urinary metabolites. Dziewirska et al.<sup>42</sup> also demonstrated a negative association between urinary 3,5,6-trichloro-2-pyridinol (TCPY) concentration and a decrease in motility ( $p = 0.024$ ).

Lwin et al.<sup>43</sup> compared groundnut farmers in growing (June–October) and non-growing (November–April) seasons and found that

in the June–October period, there were significantly higher levels of sperm motility (Wilcoxon  $z$ -value =  $-2.838$ ,  $p = 0.005$ ) and significantly lower levels of morphology (Wilcoxon  $z$ -value =  $-4.338$ ,  $p \leq 0.001$ ) and sperm count (Wilcoxon  $z$ -value =  $-4.852$ ,  $p \leq 0.001$ ). In addition, sperm count and total and progressive motility were significantly lower in rural farmers than in urban men.<sup>44</sup> However, two studies did not report any seminal alteration after OP exposure. In the first study, no correlation between OP urinary metabolites and semen parameter alterations occurred in Mexican agricultural workers.<sup>45</sup> In the second study, Multigner et al.<sup>46</sup> showed no significant difference in sperm characteristics between banana plantation workers and men working in non-agricultural sectors.

### 3.3.4 | Carbamates

Three studies investigated the effect of carbamates. Wyrobek et al.<sup>47</sup> found a negative association between their exposure and sperm normal morphology. Even Xia et al.<sup>48</sup> reported sperm morphological abnormalities as the only altered parameter. In addition to the Carbaryl relationship with the percentile of spermatozoa with abnormal morphology ( $\beta = -7.25$ ), Dziewirska et al.<sup>42</sup> showed that also CASA parameter straight line velocity ( $\beta = 3.47$ ) was positively associated with its urinary metabolite 1-naphthol (1N) concentration.

### 3.3.5 | Fungicides

Only one article retrieved was on the effect of fungicides. Celik-Ozenci et al.<sup>49</sup> showed a significantly lower level of sperm motility in farmworkers exposed to abamectin than in non-exposed men.

### 3.3.6 | Herbicides

One paper regarding herbicides was included. In the first article, Swan et al.<sup>50</sup> reported that blood alachlor and atrazine concentrations were strictly related to poor semen quality ( $OR_{0.7 < \text{vs} < 0.15} (\mu\text{g/g creatinine}) = 30$ , 95% CI: 4.3, 210 and  $OR_{0.1 \leq \text{vs} < 0.1} (\mu\text{g/g creatinine}) = 11.3$ , 95% CI: 1.3, 98.9, respectively) and that at high levels of both pesticides, the sperm abnormality rate was higher. However, this association was imprecise, as indicated by the width of the CI, probably because of the two small samples examined.

### 3.3.7 | Mixture

In 13 studies, associations with mixtures of pesticides were analyzed.

In 1998, Larsen et al.<sup>51</sup> reported that the percentage of normal sperm heads was the only parameter affected in Danish farmers during the spraying season than the non-sprayer counterpart ( $p < 0.01$ ). Abell et al.<sup>52</sup> showed a significantly lower proportion of normal spermatozoa in greenhouse workers with a high level of dermal exposure

( $p = 0.02$ ). Moreover, using logistic regression analysis, a significant association was found between semen threshold values and pesticide exposure in men of couples seeking pregnancy, with estimated ORs of 3.0 ( $p = 0.015$ ) for sperm concentration ( $>1 \times 10^6/\text{ml}$  vs.  $<1 \times 10^6/\text{ml}$ ), 2.7 ( $p = 0.031$ ) for sperm count ( $<3 \times 10^6$  vs.  $>3 \times 10^6$ ), and 4.5 ( $p = 0.002$ ) for sperm motility ( $>50\%$  vs.  $\leq 50\%$ ).<sup>53</sup>

In OP and PYR sprayers, Kamijima et al.<sup>54</sup> reported significantly lower levels of sperm normal morphology and significantly higher levels of slow progressive and non-progressive motile spermatozoa in exposed with respect to non-exposed. In a case-control study investigating semen quality in rice farmers, sperm abnormalities were significantly associated with a distance of fewer than 300 m from household to rice fields and a duration of work over 10 years as a farmer (OR = 3.16, 95% CI: 1.97–5.05 and OR = 3.98, 95% CI: 2.20–7.21, respectively).<sup>55</sup> Furthermore, personal protective equipment absence was also reported to be associated with sperm morphology alterations (adjusted OR = 3.05, 95% CI: 1.92–4.85).

Perry et al.<sup>56</sup> evaluated 18 random urine samples and divided them according to low and high pesticide metabolite levels, discovering a significant crude difference in sperm concentration for diethylthiophosphate (DETP) (absolute difference =  $-1.0$ , 95% CI:  $-1.8$  to  $-0.2$ ,  $p < 0.05$ ). Instead, occupational exposure to OP and carbamates (CAR) is only associated with lower sperm concentration and live spermatozoa.<sup>57</sup> In a questionnaire-based study conducted on 2122 men attending an Andrology Laboratory in Tunisia, exposure to pesticides was significantly associated with asthenozoospermia (OR = 1.6, 95% CI: 1.0–2.4,  $p = 0.02$ ) and necrozoospermia exposure (OR = 2.6, 95% CI: 1.4–4.7,  $p = 0.001$ ).<sup>58</sup> Cremonese et al.<sup>59</sup> reported in 2017 a worsening of sperm motility ( $p = 0.01$ ) and morphology ( $p < 0.01$ ) in rural Brazilian men exposed to a mixture of fungicides in contrast to urban ones.

However, three studies reported no relationship between pesticides and semen parameters. Tielemans et al.<sup>60</sup> did not find any significant association in infertile men exposed to fungicides, herbicides, and insecticides. In a mixture of these toxicants, no relationship was shown to affect sperm concentration, the only examined parameter.<sup>61</sup> Similarly, men attending a Fertility Clinic underwent a self-reported occupational questionnaire, and exposure to pesticides was not significantly correlated with seminal quality (OR = 3.6, 95% CI: 0.8–15.8,  $p = 0.087$ ).<sup>62</sup>

### 3.4 | Chromatin and DNA integrity

Twenty-five articles investigated this relationship (Table 2).

#### 3.4.1 | Organochlorine

Nine studies regarded OC. Using the aniline blue staining method instead of the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, the percentage of Grade 3-stained samples (the least condensed spermatozoa) was positively associated with the

p,p'-DDE concentration, with an increase of 0.04% (95% CI: 0.001–0.070) per unit of the lipid-adjusted pesticide.<sup>17</sup> Giwercman et al.<sup>24</sup> demonstrated the susceptibility of DNA to OC based on the androgen receptor gene CAG repeat length ( $p = 0.01$ ). The group with higher (above the median) serum p,p'-DDE levels with respect to the group with lower levels presented a 43% (95% CI: 9.6–78) higher DFI for CAG  $<20$  and 38% (95% CI: 7.6–68,  $p = 0.01$ ) higher DFI for CAG 20/21.

De Jager et al.,<sup>63</sup> dividing subjects according to lipid-adjusted p,p'-DDE plasma concentration, noted an increasing trend in the log-transformed DFI coefficients as the serum level of this metabolite increased, with a significant difference between the lowest quartile group and third and fourth quartile groups ( $p < 0.05$ ). In sub-fertile couples, a higher serum p,p'-DDE concentration was associated with XX (incidence rate ratio [IRR] = 1.6, 95% CI: 1.4, 1.7,  $p < 0.001$ ), XY (IRR = 1.30, 95% CI: 1.23–1.38,  $p = 0.001$ ), and total (IRR = 1.27, 95% CI: 1.22–1.33,  $p < 0.001$ ) sex-chromosome disomy in men comparing the highest with the lowest quartile of p,p'-DDE distribution.<sup>64</sup> Perry et al.<sup>65</sup> have also demonstrated that men who were more exposed to DDT had increased rates of XX18 (IRR = 1.52, 95% CI: 1.35, 1.72), XY18 (IRR = 1.40, 95% CI: 1.30, 1.51), and total disomy (IRR = 1.32, 95% CI: 1.25, 1.35) compared with the highest with the lowest tertile of p,p'-DDE for adults in a cross-sectional evaluation.

However, in four studies, no relationship was highlighted. Hauser et al.<sup>66</sup> did not find any significant association between OCs and DNA damage. No significant difference in % DFI between the lowest exposed DDT group and the highest exposed DDT group using multivariable age-adjusted analysis occurred.<sup>67</sup> Moreover, a lack of association between DFI and p,p'-DDE was reported among European men.<sup>68</sup> However, through TUNEL assay, Stronati et al.<sup>69</sup> found no relationship between sperm DNA fragmentation and p,p'-DDE concentration ( $\beta = 0.0$ , 95% CI:  $-0.1$  to  $0.2$ ) using a linear regression model.

#### 3.4.2 | Pyrethroids

Seven studies focused on the relationship between PYR and DNA damage. In factory workers exposed to fenvalerate, Xia et al.<sup>26</sup> observed a significantly higher percentage of sex chromosome-bearing and disomic chromosome 18 sperm disomy ( $p < 0.01$ ) and nullisomy ( $p < 0.01$ ) than in control groups. Moreover, DNA fragmentation was significantly higher in exposed workers using the TUNEL assay, as well as the percentage of DNA in the tail ( $p = 0.044$  and  $0.024$ , respectively) and olive tail moment ( $p = 0.016$  and  $0.011$ , respectively) in Comet assay, than in internal and external non-exposed men.<sup>32</sup> Meeker et al.<sup>28</sup> reported a monotonic association between 3-PBA and % DNA in the comet tail ( $p$ -value for trend = 0.02), suggesting a dose-response effect. Among men recruited from an infertility clinic, a positive association between urinary 3-PBA levels and sperm DNA fragmentation ( $\beta = 0.27$ , 95% CI: 0.15–0.39,  $p < 0.001$ ) was reported.<sup>29</sup> Serum 3-PBA levels were also associated with a higher rate of YY18 disomy (IRR = 1.28, 95% CI: 1.15–1.42) and a lower rate of XY18 (IRR = 0.82, 95% CI: 0.77–0.87) and total (IRR = 0.93, 95% CI: 0.87–0.97) disomy.<sup>70</sup>

**TABLE 2** Studies concerning the effects of pesticides on chromatin and DNA integrity

First author (year)	Country of study	Pesticide	Study design/evaluation	Examined population, n	Controls/reference group	Reproductive effects
De Jager (2006) <sup>17</sup>	Mexico	DDT (OC)	Cross-sectional study	116 healthy men	None	Higher sperm DNA condensation with increasing levels of lipid-adjusted p,p'-DDE
Giwercman (2007) <sup>24</sup>	Greenland, Sweden, Ukraine, Poland	DDT (OC)	Cross-sectional study	680 men from Greenland, Sweden, Ukraine, and Poland	Men with low serum p,p'-DDE levels	Positive association between higher p,p'-DDE levels and DFI for CAG <20 and CAG 20/21
De Jager (2009) <sup>63</sup>	South Africa	DDT (OC)	Cross-sectional study	209 young men	None	Positive trend in the log-transformed %DFI coefficients as the increase in p,p'-DDE concentration
McAuliffe (2012) <sup>64</sup>	United States of America	DDT (OC)	Cross-sectional study	192 men from sub-fertile couples	Men in the lowest quartile of serum p,p'-DDE levels	Positive relationship between serum p,p'-DDE levels and rates of XX, XY, and total sex-chromosome disomy
Perry (2016) <sup>65</sup>	Faroe Islands	DDT (OC)	Cross-sectional evaluation (in adults) and prospective evaluations (prenatal and age 14 exposure)	90 men from the general population from three study groups: a group of men randomly selected from the population registry, a group of fertile men and a birth cohort	Men in the lowest tertile of the p,p'-DDE distribution	Positive association between p,p'-DDE concentration (the highest vs. the lowest tertile) and rates of XX18, XY18, and total disomy in adults (cross-sectional evaluation) and age 14 exposure (prospective evaluation); negative association between p,p'-DDE concentration (2nd vs. 1st tertile) and rates of XX18, XY18, and total disomy for prenatal exposure
Hauser (2003b) <sup>66</sup>	United States of America	HCH and DDT (OC)	Cross-sectional study	212 male partners of a sub-fertile couple	None	No significant association
Rignell-Hydbom (2005) <sup>67</sup>	Sweden	DDT (OC)	Cross-sectional study	176 fishermen	Men in the lowest serum p,p'-DDE quintile	No significant positive trend between p,p'-DDE concentration and DFI
Spanò (2005) <sup>68</sup>	Greenland, Sweden, Ukraine, Poland	DDT (OC)	Cross-sectional study	707 adult Swedish fishermen and males from Greenland, Ukraine, and Poland	Men in the lowest serum p,p'-DDE quintile	No relationship between p,p'-DDE and DFI
Stironati (2006) <sup>69</sup>	Greenland, Sweden, Ukraine, Poland	DDT (OC)	Cross-sectional study	652 adult males from Greenland, Sweden, Ukraine, and Poland	Men with low serum p,p'-DDE levels (p,p'-DDE 0-500 ng/g lipid)	No significant association between p,p'-DDE and DFI
Xia (2004) <sup>26</sup>	China	Fenvalerate (PYR)	Cross-sectional study	12 factory workers, 12 internal, and 18 external controls	Healthy, young, non-smokers and non-regular drinkers donors	Higher sperm abnormality in exposed workers

(Continues)

TABLE 2 (Continued)

First author (year)	Country of study	Pesticide	Study design/evaluation	Examined population, n	Controls/reference group	Reproductive effects
Bian (2004) <sup>32</sup>	China	Fenvalerate (PYR)	Cross-sectional study	21 pesticides factory workers, 19 external, and 23 internal controls	Men from the office area of the same factory (internal controls). Volunteers recruited from different departments in the same urban district (external controls)	Higher DFI, OTM, and DNA in tail were significantly associated with fenvalerate exposure
Meeker (2008) <sup>28</sup>	United States of America	Unspecified PYR	Cross-sectional study	207 men recruited from an infertility clinic	Men below the 50th percentile concentration of urinary pyrethroid metabolite	Linear relationship between 3-PBA levels and percent DNA in the comet tail
Ji (2011) <sup>29</sup>	China	Unspecified PYR	Cross-sectional study	240 men recruited from an infertility clinic	Men with the lowest quartile of urinary 3-PBA levels	Positive correlation between urinary 3-PBA level and sperm DNA fragmentation
Young (2013) <sup>70</sup>	United States of America	Unspecified PYR	Cross-sectional study	75 men recruited through an infertility clinic	Men with exposure levels below the limit of detection value	Linear relationship between 3-PBA concentration and YY18 disomy and between TDCCA and XX18, YY18, and total disomy, and negative association between 3-PBA concentration and XY18 and total disomy
Jurewicz (2014) <sup>71</sup>	Poland	Unspecified PYR	Cross-sectional study	286 men attending infertility clinics for diagnostic purposes	None	Positive association between over the median CDCCA and 3-PBA levels and medium and high %DFI, respectively
Radwan (2015) <sup>72</sup>	Poland	Unspecified PYR	Cross-sectional study	195 men attending an infertility clinic	None	Positive association between urinary TDCCA over the median levels and disomy of chromosomes XY and Z1 and between urinary 3-PBA levels (both above and below the median) and XY, Y, Z1, and total disomy
Recio (2001) <sup>73</sup>	Mexico	Unspecified OP	Cross-sectional study	6 men in total, 4 sprayers and 5 farmworkers during spraying season	None	Significant associations between DEP and sex null aneuploidy, and between DM1DTP and total aneuploidy
Sanchez-Pena (2004) <sup>45</sup>	Mexico	Methyl parathion, metamidophos, dimethoate and diazinon (OP)	Cross-sectional study	33 farmworkers	None	Positive association between urinary DETP concentrations and DFI

(Continues)

TABLE 2 (Continued)

First author (year)	Country of study	Pesticide	Study design/evaluation	Examined population, n	Controls/reference group	Reproductive effects
Pérez-Herrera (2008) <sup>37</sup>	Mexico	Unspecified OP	Cross-sectional Study	54 farmworkers	None	Positive relationship between OP exposure and DNA damage
Dziewirska (2018) <sup>42</sup>	Poland	Unspecified OP	Cross-sectional study	315 men attending an infertility clinic	Men in the lowest percentile of the semen parameters distribution	Greater TCPY urinary concentrations in men with the higher rate of the DNA damage
Xia (2004) <sup>48</sup>	China	CAR	Cross-sectional study	16 factory workers, 16 internal controls, and 16 external controls	Sperm donor from the same working area (external control). Workers in the same pesticide factory but far away from the pesticide workshop (internal control)	Higher frequencies of chromosome 18 and X/Y disomy and nullisomy
Dziewirska (2018) <sup>42</sup>	Poland	Unspecified CAR	Cross-sectional study	315 men attending an infertility clinic	Men in the lowest percentile of the semen parameters distribution	No association between urinary 1N concentration and DFI
Miranda-Contreras (2013) <sup>57</sup>	Venezuela	OP and CAR	Cross-sectional study	64 farmworkers, 35 controls	Healthy men, not currently exposed to pesticides, living 90 km from the agricultural areas	Higher rate of DFI in exposed group
Figueroa (2019) <sup>74</sup>	United States of America	OP and PYR	Cross-sectional study	159 men attending an infertility clinic	Men in the DAP lowest quartile with higher semen parameters	Inverse associations between total disomy and DMP increasing quartiles by concentrations of 3-PBA in the two lower quartiles
Härkönen (1999) <sup>61</sup>	Denmark	Insecticides, herbicides, and fungicides	Cross-sectional study	32 farmers	None	No significant association
Smith (2004) <sup>75</sup>	United States of America	Unspecified pesticide	Cross-sectional study	20 exposed men, 20 controls	Healthy males with no chronic disease and no chronic use of medication were recruited in Minnesota	No significant association

Abbreviations: 3-PBA, 3-phenoxybenzoic acid; 1N, 1-naphthol; CAR, carbaryl; CDCCA, cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid; DDT, dichloro-diphenyl-trichloroethane; DEP, diethylphosphate; DETP, diethylthiophosphate; DFI, DNA fragmentation index; DMPTP, dimethylthiophosphate; HCH, hexachlorocyclohexane; OC, organochlorine; OP, organophosphate pesticide; p,p'-DDE, dichloro-diphenyldichloro-ethylene; OTM, olive tail moment; PYR, pyrethroid; TCPY, 3,5,6-trichloro-2-pyridinol; TDCCA, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid.



Furthermore, a positive correlation between TDCCA concentration and XX18 (IRR = 1.30, 95% CI: 1.17–1.46), YY18 (IRR = 1.19, 95% CI: 1.06–1.34), and total (IRR = 1.09, 95% CI: 1.04–1.15) disomy was reported. Jurewicz et al.<sup>71</sup> also found a positive association between over the median cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (CDCCA) and 3-PBA levels and medium and high %DFI, respectively. Finally, Radwan et al.<sup>72</sup> reported that urine TDCCA over the median levels was positively related to XY disomy and disomy of chromosome 2. Furthermore, urinary 3-PBA concentration (both above and below the median) was directly related to XY disomy, Y disomy, disomy of chromosome 21, and total disomy.

### 3.4.3 | Organophosphates

Four studies evaluated the impact of OP on chromatin integrity. Recio et al.<sup>73</sup> reported that the frequency of total aneuploidies was slightly higher, relative risk (RR)<sub>DEP</sub> = 2.59 and RR<sub>DETP</sub> = 1.68, during the spraying season in nine men exposed to OP. Furthermore, the authors found a significant association between diethylphosphate (DEP) and sex null aneuploidy ( $\beta = 0.00022$ ,  $p = 0.0001$ ), as well as between dimethyldithiophosphate (DMDTP) and the rate of total aneuploidies ( $\beta = 0.00009$ ,  $p = 0.0001$ ). Sánchez-Peña et al.<sup>45</sup> discovered a positive relationship between urinary DETP concentrations and DFI ( $\beta = 0.48$ ,  $p = 0.03$ ). Furthermore, 75% of semen specimens were classified as having poor fertility potential (with DFI > 30%). Regarding the PON1Q192R genetic polymorphism, homozygote subjects for the 192R allele showed dose–effect relationships with OP exposure at the month of sampling ( $p = 0.025$ ).<sup>37</sup> Dziejirska et al.<sup>42</sup> also found that higher urinary TCPY levels in infertile men were significantly associated with a higher percentage of DFI.

### 3.4.4 | Carbamates

Two studies investigated the genotoxic effect of CAR. Xia et al.<sup>48</sup> showed a significantly higher rate of chromosome 18 and X/Y disomy and nullisomy in exposed groups ( $p < 0.01$  and  $< 0.05$ , respectively). However, no significant association was reported between urinary 1N levels and DFI.<sup>42</sup>

### 3.4.5 | Mixture

Two studies investigated the combination of CAR and OP together. Miranda-Contreras et al.<sup>57</sup> reported a higher rate of DFI in the exposed group ( $p < 0.0001$ ). The interaction between DAP metabolites and 3-PBA in adjusted models determined a varied correlation between their levels and total disomy, with the highest significant associations between the third exposure quartile of DETP and the second 3-PBA exposure quartile for an IRR = 2.31 (95% CI: 2.02, 2.64).<sup>74</sup> However, in workers with past occupational exposure to pesticides during the no growing season, no significant differences were reported in terms

of aneuploidy compared to the control group,<sup>61</sup> as reported by Smith et al.<sup>75</sup> for aneuploidy and diploidy frequencies for chromosomes 13, 21, X, and Y.

## 4 | DISCUSSION

In most studies included in this review, pesticides played a role in altering semen parameters. Indeed, in 40 of the 51 studies (78%), at least one parameter was affected negatively. The most frequently worsened parameters were sperm total count, motility, and morphology. In every case, we found high heterogeneity in the measures used to evaluate the association between each compound and semen parameters.

In most of the included studies, OCs were associated with altered semen parameters. The most frequent was sperm motility (in 10 out of the 12 studies reporting a significant association). It has been shown that OCs permit a massive entry of calcium ions into germ cells<sup>76</sup> with a consequent reduction in the mitochondrial membrane potential.<sup>77</sup> Instead, a reduction in semen count and volume was significantly associated with OCs in seven studies. This may be explained by the induction of apoptosis in Sertoli cells and germ cells, with increased caspases,<sup>78</sup> because of the increase in superoxide anion and hydrogen peroxide and the consequent oxidative stress.<sup>79</sup> Another emerging element is the dose-dependent relationship between DDT serum metabolites concentration and the decrease of the parameters.<sup>16–21</sup> However, some studies showed non-clear evidence on the correlation between OC and worsening of semen quality. Indeed, although stratifying cases according to p,p'-DDE serum concentrations, a negative trend of impaired semen parameters occurred,<sup>23</sup> and some papers did not report any relationship, showing that these pesticides are safe than other POPs, such as polychlorinated biphenyls (PCBs).<sup>22–25</sup> In any case, almost all human studies have a low level of evidence, and further studies with adequate quality are necessary for definitive evidence.

Regarding the effect of PYR on semen quality, more than half of the studies showed altered parameters. Sperm motility was affected in two studies, with low normal morphology and total count in three, while reduction of sperm concentration occurred in four out of eight studies. The reduction in sperm count and concentration may be attributable to the reduced size and number of cell layers in atrophic and distorted seminiferous tubules, as demonstrated in the F1 and F3 generations of female rats exposed during fetal gonadal sex determination.<sup>80</sup> Regarding sperm morphology, a considerable reduction in germ cells and impairments of the seminiferous tubules have been demonstrated in adult rats.<sup>81</sup>

OPs are among the most used and effective pesticides worldwide. They are excellent insecticides, although adverse effects can also occur in humans after exposure. OPs have also shown a worsening impact on fertility, altering the regular function of the blood–testis barrier in mice through oxidative stress,<sup>82</sup> forming covalent bonds with the zonula occludens-2 protein (ZO2).<sup>83</sup> The effect of OPs on semen quality emerged through a recent meta-analysis, where we demonstrated a decline in almost all parameters considered.<sup>84</sup> In 11 out of the 13 studies included in this review, sperm quality was significantly affected, and

the most frequently affected parameters were sperm total count (in three out of 11 studies), ejaculate volume and (in four out of 11 studies), sperm morphology and concentration (in five out of 11 studies), and motility (in seven out of 11 studies). Semen motility and morphology were the most frequently affected parameters. The phenomenon for the first one is associated with the generation of reactive oxygen species and, consequently, increased lipid peroxidation and a lower ratio of polyunsaturated/saturated fatty acids.<sup>85</sup> Regarding worsening sperm morphology rates, in male Wistar rats, OP exposure was related to abnormalities, such as lipid droplets, a higher number of mitochondria and apoptotic phenomena in germ cells (as confirmed by the altered expression of the BAX and Bcl-2 genes).<sup>86</sup> Moreover, within the seminiferous tubules, there are cell–cell contact loss and sloughing of epithelial cells, concomitant arrest of spermatogenesis, with a decrease in meiotic figures and elongating spermatids, reducing sperm total count and concentration in mature goats.<sup>87</sup>

Carbamates are derivatives of carbamic acid and are mainly used as insecticides. The singular parameter affected within all studies was sperm morphology. Indeed, under the microscope, a distorted organization of the seminiferous tubules in exposed mice can be observed, with a reduced number of spermatocytes and spermatids and the presence of cellular debris and necrotic cells.<sup>88</sup> Carbamates act directly on rat spermatogenesis, increasing abnormalities, such as detached heads, double heads, and coiled tails.<sup>89</sup>

In men exposed to a mixture of pesticides, establishing their likely effect was not possible because this may cause bias. Nevertheless, there are worthy considerations. First, there has been found a progressive worsening in semen quality based on the number of years exposed to those hazards.<sup>51,52</sup> Moreover, the impact on semen parameters was not evident during the spraying period, and therefore, semen samples should be collected later.<sup>61</sup>

According to the sixth edition of the WHO manual for the examination and processing of human semen, a sub-fertile condition is defined by values lower than the fifth percentile of the general population.<sup>90</sup> In studies recruiting workers or men with no fertility problems, sperm normal morphology<sup>14</sup> and ejaculate volume<sup>36</sup> were under the established threshold limit in only one study, while another study reported sperm motility<sup>31</sup> and total sperm count<sup>43,44</sup> lower than the fifth percentile. Furthermore, in the latest WHO manual, a “border-line zone” between pathological and normal values has been suggested.<sup>91</sup> Within that zone, sperm motility, morphology, count, and volume occurred in eight,<sup>14,15,18,26,27,32,34,44</sup> four,<sup>15,18,24,46</sup> three,<sup>14,27,39</sup> and three<sup>14,17,18</sup> studies, respectively. Therefore, a significant impact occurs, especially on sperm motility and normal morphology, as many studies have demonstrated, although semen parameters were under the WHO threshold limits in a few cases. In particular, OCs and OPs were found to be associated with semen quality, while others require further investigation.

In 25 studies documenting the effect of pesticides on chromatin condensation, most of them reported an association between exposure and DFI.

In studies regarding OC, the actual DNA damage is controversial. Only half of the studies showed an increase in DFI. Giwerzman

et al.<sup>24</sup> stated that this susceptibility was related to the length of the CAG sequences, a key site for androgen receptor function.<sup>92</sup> Only one study<sup>45</sup> found a DFI greater than 30%.<sup>93</sup> Therefore, further studies are needed to clarify this issue. Both studies evaluating aneuploidy documented a correlation between chromosomal disomy and blood p,p'-DDE levels. An interesting finding is that the susceptibility to semen parameter alterations was based on ethnicity. Messaros et al.<sup>94</sup> demonstrated how sperm motility influenced the GSTT1 genotype, while abnormal morphology influenced the CYP1A1 alleles. The phenomenon of allopatric speciation implies that an isolated population such as the Inuit from Greenland tends to be less prone to infertility, sperm alterations, and DNA integrity than others such as European men, even if there were no robust controls for each ethnicity group.

The studies investigating PYR exposure reported unequivocal results regarding DNA damage. Indeed, PYRs result in some increase in DFI. A linear relationship between the serum concentration of its metabolites and chromosome aneuploidy and DNA in the tail occurred. As reported by animal studies, this pesticide may determine nuclear morphological alterations, with the alteration of chromatin condensation and the arrangement of the cytoplasmic microtubules at the ultrastructural level.<sup>95</sup>

OPs, alone or in association with CARs, showed a harmful effect in exposed men, with a positive association between DFI and their urinary metabolites, as confirmed by DNA fragmentation higher than 30% in mouse studies. Indeed, in the cellular nucleus, OPs alter the methylation promoters of some genes by modifying their expression, such as NRF2 and OGG1 (fundamental for antioxidant action and DNA repair, respectively),<sup>96</sup> and reduce the chromatin condensation and integrity of DNA.<sup>97</sup> Moreover, farmworkers' susceptibility to chronic pesticide poisoning is influenced by genetic polymorphisms. Actually, the gene for PON1 plays a key role.<sup>98</sup> The latter is an enzyme against anticholinesterase toxicity, particularly with OPs. As already demonstrated by Pérez-Herrera et al.,<sup>37</sup> some polymorphisms of the PON1 gene determine reduced catalytic activity, and its exacerbation occurs depending on the intensity and years of occupational exposure.<sup>99</sup>

Only two studies explored the effect of CAR on DNA damage. Although a correlation of these pesticides with chromosomal disomy and aneuploidy was demonstrated,<sup>48</sup> no association with DFI occurred.<sup>42</sup>

In the literature, too few articles have reported the effects of pesticides on the ability to sire a pregnancy. Controversies emerged because only half of the studies confirmed the relationship. However, Cocco et al.<sup>100</sup> showed even a significant association between p,p'-DDE and stillbirths, while Sallmèn et al.<sup>101</sup> demonstrated that, despite exposure levels, farmworkers' fecundability is influenced by pesticides. In 2017, Smarr et al.<sup>102</sup> reported that not enough studies were published to affirm a general modification of human fecundity, except for some chemical hazards such as DBCP.

This review has some limitations to disclose. First, most studies were cross-sectional and a cause–effect relationship cannot be stated. Regarding the study design, a systematic error could be of concern. Selection bias could have occurred, as several studies selected the sample voluntarily. Exposure evaluation inaccuracies can occur in the

case of subject recall, thus biasing the results. Several studies adjusted the statistical models used for the analysis with different covariates or stratified the analysis to control for potential known confounders. However, the reported results of some studies were unadjusted, and several articles neglected to control for unmeasured confounders. As observation studies are prone to such bias, sensitivity analysis could be an opportunity to interpret the results. Second, several papers included a small sample size, which could have led to the inadequate statistical power of the study.

There are limitations linked to the considered studies. A comparison between studies is difficult because the exposures are measured differently, and various methods are applied to verify the association with the outcome. Indeed, the latter can be assessed through a logarithmic scale, partitioning with categories, threshold values, the logarithm of the odds or through the distribution of exposure level. The latter, then, also depends on the reference population and the study period, limiting the performance of a proper meta-analysis. Thus, studies involving several sites that share the same study protocol as that eligibility criteria, the definition of exposure and outcomes, would allow the specific assessments of each site and then obtain a global assessment (pooled analysis). Furthermore, there were no data about family and medical history in some articles, including previous/concomitant testicular disease, comorbidities, exposure time, and exposure dose, and the subjects would have probably been exposed to other confounding factors, such as alcohol, smoking, and others. Moreover, exposure levels were not standardized in each study, creating some disparity. Last, in studies regarding occupational exposure, no specified or uniform protective clothing was worn by workers.<sup>38</sup> Future research should focus on two main points: the effects of frequently applied pesticides but less considered in studies (such as glyphosate, herbicides, or carbamates) and the susceptibility to pesticides of various ethnic groups based on genetic polymorphisms.

## 5 | CONCLUSION

Occupational exposure to pesticides results in a harmful impact on human health, not only on workers but also in the general population, because of their ubiquitous persistence in different environmental media, their ability to bioaccumulate and their capacity for long-range atmospheric transport. In the present study, we summarized the consequences of pesticide exposure on human semen parameters, and we found a detrimental impact on sperm total count, motility, and normal morphology and damage to DNA integrity. These effects seem to be mainly correlated with exposure time rather than dose. However, current knowledge almost relies on cross-sectional evaluations, and future prospective studies with appropriate methodology to control for systematic uncertainty are mandatory to support these findings and to strengthen the evidence for causality.

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## CONFLICT OF INTEREST

The authors declare they have no conflicts of interest.

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## AUTHOR CONTRIBUTIONS

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## DATA AVAILABILITY STATEMENT

Data will be provided by the corresponding author upon reasonable request.

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