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REVIEW

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Evaluation of adverse effects/events of genetically modified food consumption: a systematic review of animal and human studies

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Abstract

Objective: A systematic review of animal and human studies was conducted on genetically modified (GM) food consumption to assess its safety in terms of adverse effects/events to inform public concerns and future research.

Methods: Seven electronic databases were searched from January 1st 1983 till July 11th 2020 for in vivo, animal and human studies on the incidence of adverse effects/events of GM products consumption. Two authors independently identified eligible studies, assessed the study quality, and extracted data on the name of the periodical, author and affiliation, literature type, the theme of the study, publication year, funding, sample size, target population characteristics, type of the intervention/exposure, outcomes and outcome measures, and details of adverse effects/events. We used the Chi-square test to compare the adverse event reporting rates in articles funded by industry funding, government funding or unfunded articles.

Results: One crossover trial in humans and 203 animal studies from 179 articles met the inclusion criteria. The study quality was all assessed as being unclear or having a high risk of bias. Minor illnesses were reported in the human trial. Among the 204 studies, 59.46% of adverse events (22 of 37) were serious adverse events from 16 animal studies (7.84%). No significant differences were found in the adverse event reporting rates either between industry and government funding ($\chi^2 = 2.286$, $P = 0.131$), industry and non-industry funding ($\chi^2 = 1.761$, $P = 0.185$) or funded and non-funded articles ($\chi^2 = 0.491$, $P = 0.483$). We finally identified 21 GM food-related adverse events involving 7 GM events (NK603 × MON810 maize, GTS 40-3-2 soybean, NK603 maize, MON863 maize, MON810 maize, MON863 × MON810 × NK603 maize and GM Shanyou 63 rice), which had all been on regulatory approval in some countries/regions.

Conclusion: Serious adverse events of GM consumption include mortality, tumour or cancer, significant low fertility, decreased learning and reaction abilities, and some organ abnormalities. Further clinical trials and long-term cohort studies in human populations, especially on GM food-related adverse events and the corresponding GM events, are still warranted. It suggests the necessity of labelling GM food so that consumers can make their own choice.

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Introduction

Genetic modification is defined as introducing transgene(s) with desired traits into the recipient organism's genome by recombinant deoxyribonucleic acid (DNA) technology, and therefore it does not occur naturally [1–3]. Genetically modified (GM) crops are thought to address food security, sustainability and climate change solutions by improving crop yields, conserving biodiversity, providing a better environment in terms of the insect-resistant and herbicide-tolerant traits, reducing CO_2 emissions and helping alleviate poverty through uplifting the economic situation [4]. Insect-resistant and herbicide-tolerant traits were first introduced into four types of crop, canola, cotton, maize and soybeans, at the beginning of GM production [5]. At present, the mainstream characteristics of new crops still pursue higher-yielding, more nutritious, pest- and disease-resistant and climate-smart to meet future demand for a yield increase of major crops such as wheat, rice and corn, due to the growing population [6].

Since 1996, the first year of commercialization of GM crops, 70 countries had adopted GM crops until 2018, including 26 countries that cumulatively planted 2.5 billion hectares of GM crops and an additional 44 countries that imported GM crops. During the 27 years (1992 to 2018), 4349 approvals for 387 GM events from 27 GM crops were granted by 70 countries involving 2063 for food (when the direct consumers are mainly humans), 1461 for feed (the products only intended for animal consumption) use and 825 for environmental release or cultivation [4, 7]. The major agricultural product exporting countries like the U.S.A., Brazil and Argentina show over 90% adoption of biotech crops [4]. For GM animal products, biotech salmon, considered to be the first genetically engineered animal for human consumption, was approved by the United States Department of Agriculture and Food & Drug Administration in 2015 [8]. In addition, it is illegal to grow major GM food crops in China while there are substantial investments in biotechnology research and GM maize, soybeans, and canola are allowed to import and eat [9].

Genetically modified food, however, is an example of the controversial relation between the inherent uncertainty of the scientific approach and the need of consumers to use products resulting from scientific developments thought to be safe [10]. Significant health risks have not been reported in peer-reviewed studies on GM food safety/security, which may cause some publication bias [11] but with a few exceptions, like the most famous "Monarch Butterfly controversy" [12], "Pusztai case" [13] and the "Séralini case" [14]. Unexpected effects of GM crops were reported in these studies, occupying an important place in the pages of scientific journals. Nevertheless, the above controversies severely impacted the public image, leading to full or partial bans in 38 countries including the European Union [15].

The complexity of risk evaluation is shown in these conflicting results, and concerns about the citizen-consumers have been raised against GM food [10]. Of most concern, aroused from the controversial events and some research results, is the potential of carcinogenesis, teratogenesis [16], lethal effects and adverse influences on fertility. GM agriculture is now widely discussed in both positive and negative frames and currently serves as a hotbed of debate in the public and policymakers. Although there are some reports and evidence from human and animal studies on the potential health effects of GM food/feed, the evidence is not conclusive and public concerns have not been resolved.

We aimed to conduct a systematic review of animal and human studies on GM food consumption to assess its safety in terms of adverse effects/events to inform public concerns and future research.

Methods

This study was a systematic review of previously published studies, conducted and reported in adherence with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [17] guideline.

Search strategy

China National Knowledge Infrastructure (CNKI), Wanfang, VIP Database, Chinese Biomedical Database (SinoMed), PubMed, the Cochrane Library and Embase databases were searched from January, 1st, 1983 till July, 11th, 2020, using a predefined search strategy (Additional file 1: Appendix S1). Reference lists of retrieved articles were also searched.

Eligibility criteria

Based on the evidence pyramid proposed by the Medical Center of State University of New York in 2001, we determined the type of research we included in the study. For a comprehensive evaluation of the literature, all in vivo animal studies and human studies (cross-sectional studies, case reports, case series, case–control studies, case–crossover studies, cohort studies, controlled clinical trials, including randomized trials, quasi-randomized

trials and non-randomized trials) in multiple languages were included. Animal studies in all fields were included, that is, they could be clinical, agricultural and animal husbandry, veterinary medicine, life sciences, etc. Field studies were excluded.

The study population in animal studies was applied with inclusion criteria based on the categorization approach that highlights the actual use of them: laboratory animals and economical animals (livestock and aquatilia) were included, with no prespecified limitations on age, population, species/races, health status or others. Interventions/exposures of the genetically modified animal/plant/microorganism products included for animal/human ingestion referred to GM food, GM food ingredients and GM feed, regardless of their dosage or duration. The GM strain (line) and GM event were not limited. There was no restriction on whether controls were or were not included. The studies were excluded if they focused on the effects of GM food/feed on secondary or multilevel consumers in the food chain where GM food/feed was only consumed by primary consumers in the predator relationships. For instance, if non-GM fishes were fed with diet containing GM ingredients and then the fish was fed to the experimental cats, the study was excluded.

Outcomes focused on the incidence of adverse effects or adverse events in GM food/feed consumption, including primary outcomes on carcinogenesis, teratogenesis, lethal effect (all-cause mortality) and reproduction and secondary outcomes on other biomarkers were included. Toxicity studies of general toxicity studies (acute, subacute, sub-chronic, chronic and carcinogenicity toxicity studies) and specific toxicity studies (genotoxicity, reproductive and developmental toxicity, immunotoxicity and other toxicology studies) were included. Mortality in pups before weaning was considered as an outcome of reproductive toxicity but not as a lethal effect. Outcomes of adverse events in laboratory testing would not be included only when they could indicate tissue or organ toxicity. Outcomes of adverse events in breeding performance in animal husbandry studies, which focused on the economic benefits of the animal products, were included and these indicators were regarded as reproduction biomarkers in this research.

Outcomes of adverse events on growth performance, carcass traits, meat and fur production performance and meat quality for economic benefit evaluation of live stocks were excluded, of which the indicators included final body weight, weight gain, feed to gain ratio, half-eviscerated weight, eviscerated weight, percentage of eviscerated yield and muscle lean meat, sebum rate in some parts of the body, etc. Studies on the insecticidal effect of insect-resistant GM feed and outcomes of adverse events in gene fragments residual in the digestive tract were excluded. Besides, duplicate publications, studies with duplicate statistics, or references devoid of necessary information of participants, sample size, interventions/exposures or results were excluded.

Study selection and data extraction

Titles and abstracts of the retrieved articles were reviewed by 6 researchers in pair (C Shen, XC Yin, BY Jiao, J Peng, YZ Li, XH Cheng). 6 authors (C Shen, XC Yin, BY Jiao, JX Ren, J Li and XW Zhang) independently reviewed the full texts to identify the studies meeting eligibility criteria and then 8 researchers in pair (C Shen, XC Yin, BY Jiao, J Li, P Jia, XW Zhang, XH Cheng and JX Ren) independently extracted data from the included studies according to a predesignated extraction table. The discrepancies were resolved through consensus and if necessary, arbitrated by another author (JP Liu).

We extracted the name of the periodical, author and affiliation, literature type, the theme of the study, publication year, funding, sample size, target population characteristics, type of the intervention/exposure, outcomes and outcome measures. For those studies in which adverse effects/events occurred, details of interventions/exposures and control conditions (if any), dosage, duration, number of the generation, and the results were extracted.

Quality assessment

The methodological quality for animal studies was assessed, using criteria from the SYRCLE's risk of bias tool for animal studies. The quality of animal studies was categorized into low risk of bias, unclear risk of bias, or high risk of bias according to the risk for each important outcome within included studies, including the adequacy of generation of the sequence generation, baseline characteristics, allocation concealment, random housing, blinding (performance bias), random outcome assessment, blinding (detection bias), incomplete outcome data, selective outcome reporting, or other sources of bias. The judgment of other risk of bias was based on whether there were contamination (pooling drugs), inappropriate influence of funders, unit of analysis errors, design-specific risks of bias or new animals added to the control and experimental groups to replace drop-outs from the original population.

Statistical synthesis and analyses

Statistical analyses were carried out using Microsoft Excel 2016 and SPSS 20.0. The findings were reported mainly in two parts, characteristics of the included studies and detailed information on the studies in which adverse effects/events occurred. Initially, descriptive

statistics, frequencies, and percentages were calculated to summarize the data. Subsequently, studies that evaluated similar populations, interventions, controls (if any) and outcomes were pooled using a random-effects meta-analysis, and data from other studies were presented in tables and described in a narrative summary. The incidence of adverse events reported in articles funded by industry funding, government funding or unfunded articles were, respectively, counted and the Chi-square test was used for the comparisons.

Besides, we figured the incidence of serious adverse events (SAEs) by percentage. With reference to the Food and Drug Administration's definition [18], our study defined SAEs as death, life-threatening, hospitalization (initial or prolonged), disability or permanent change, disruption, impairment or damage in a body function or structure (including cancer or tumour), in physical activities or quality of life, congenital anomaly or birth defect in the newborn child or pups, infertility or significant low in the number of deliveries or live birth rate than the non-GM commercial, conventional or blank controls, and an event resulting in intervention/treatment to prevent permanent impairment, damage or to prevent one of the other outcomes.

Meanwhile, the adverse events which cannot be ruled out that it has nothing to do with GM food (hereinafter abbreviated as GM food-related adverse events) were identified and the percentages under each outcome were calculated.

Results

Description of studies

The flow diagram of the literature selection is shown in Fig. 1. A total of 9668 records were identified, including 9584 from the initial search through seven databases and 84 from other sources. After removal of duplicates and exclusion of references by reading titles and abstracts, 455 full-text articles were screened and 276 references were excluded with reasons (seen in the flow chart). Finally, 204 studies from 179 articles [19–197] (153

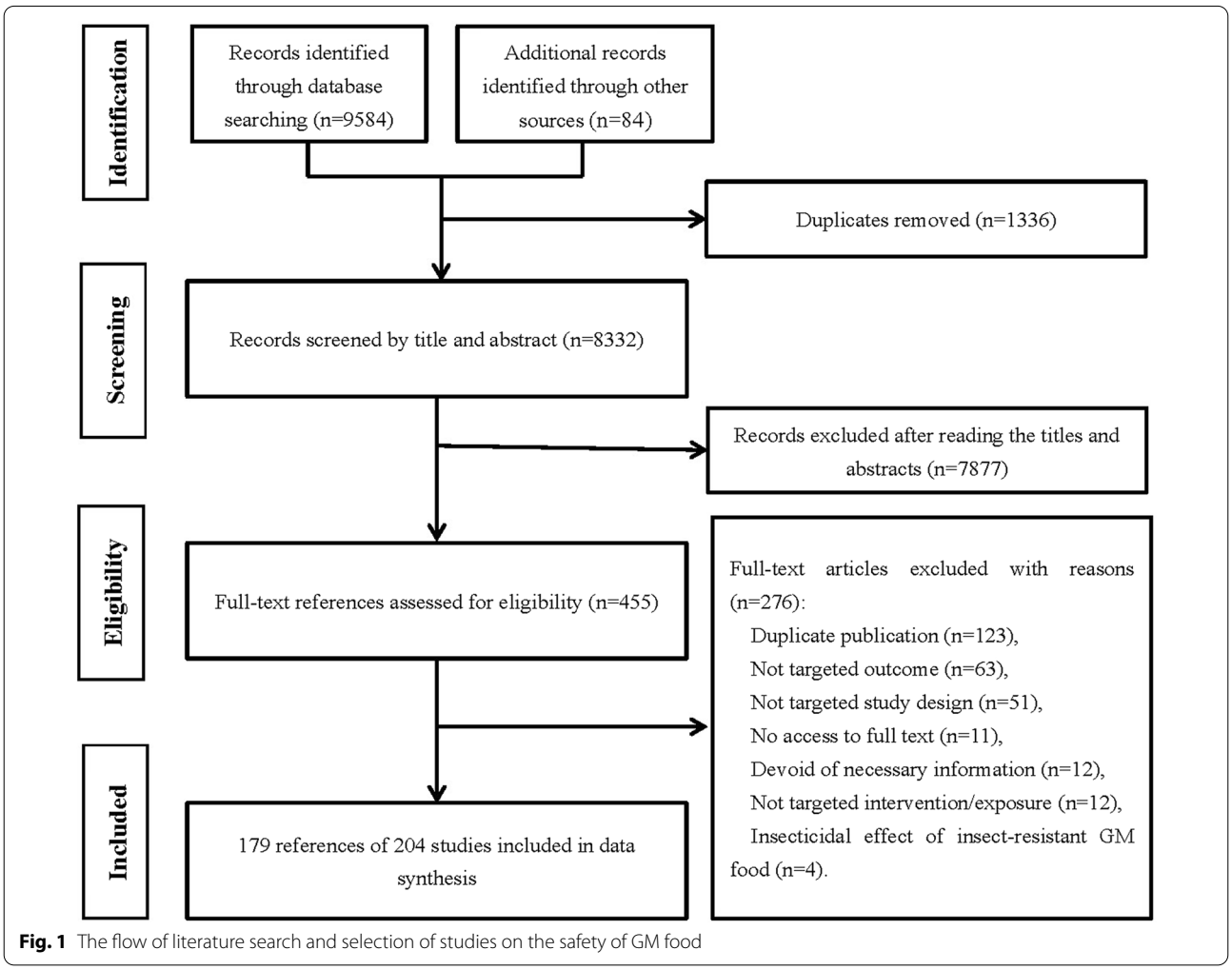

Fig. 1 The flow of literature search and selection of studies on the safety of GM food

journal articles, 22 dissertations, 3 conference proceedings and 1 unpublished report) were included in data synthesis, since there were more than one study conducted in each of the 2 included dissertations [107, 127], 11 journal articles [19, 33, 35, 63, 67, 88, 102, 118, 132, 172, 184] and 1 unpublished report [32]. The included studies were of 203 in vivo animal studies and 1 crossover trial [97] in humans.

Study characteristics

Of the 179 included articles, 94 were in English [19–112], 83 were published in Chinese [113–195], and 2 in Japanese [196, 197]. The earliest included reference dated back to 1998 [153] (shown in Fig. 2), after which the remaining articles were distributed from 2000 to 2020 (45 articles in the 2000s, while 131 in the 2010s and 2 in the 2020s). The year 2012 witnessed the largest volume of publication (n = 26 articles, 14.53%). For funding sources or sponsors (Additional file 1: Appendix S2), in addition to 57 articles not mentioning the funding/sponsor (hereinafter as non-funded articles), there were 116 articles (64.8% of the 179 articles) supported by 56 kinds of government funding from 12 countries/government organizations and, still, 9 articles (5.03%) by 10 kinds of industry/institute funding sources/sponsors from 4 countries (America, Australia, French and German). Among them, 3 articles [29, 62, 74] claimed to have been funded or sponsored by both government and industry. China had undertaken the most government/school-level funding projects (39 of 56 projects, 69.64%).

The periodicals that have published more than 5 included articles were *Food and Chemical Toxicology* (published 25 included articles), *EFSA Journal* (13), *Regulatory Toxicology and Pharmacology* (9), *Journal of Hygiene Research* (9) and *Chinese Journal of Food Hygiene* (8). 11 of 13 authors, who have published ten or more included studies, were from European Food Safety Authority and published 12 included articles as co-authors. They were Christina Tlustos (published 12 included articles), Claudia Bolognesi (12), Konrad Grob (12), Vittorio Silano (12), Andre Penninks (11), Gilles Riviere (11), Holger Zorn (11), Karl-Heinz Engel (11), Yi Liu (11), Natalia Kovalkovicova (10), Sirpa Karenlampi (10). In addition to the above 12 articles, the top 3 of the 11 authors who published five or more included studies was Yang Xiao-Guang (from Chinese Center for Disease Control and Prevention, published 11 included articles), Wang Jing (from Tianjin Centre for Disease Control and

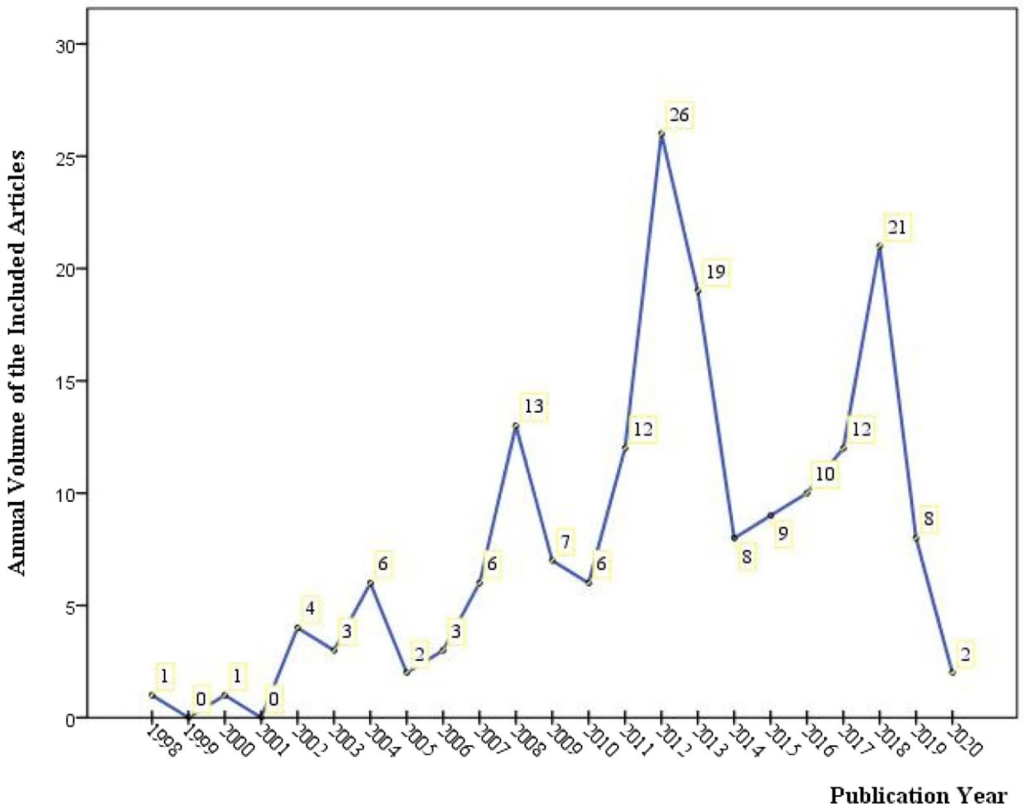

Fig. 2 The publications (number of articles) on the safety of GM food by year

Prevention, published 10 included articles) and Zhuo Qin (from Chinese Center for Disease Control and Prevention, published 7 included articles). The top 5 affiliations which published included articles were Chinese Center for Disease Control and Prevention (published 16 included articles), Tianjin Centre for Disease Control and Prevention (12), European Food Safety Authority (12), National Chung Hsing University (10), International Rice Research Institute (9).

Of the 204 included studies, one was a double-blind crossover trial ($n=36$) in humans and the others were all animal studies. Individual sample sizes of the total 54,392 study population ranged from 4 (cats) [153] to 21,000 (Atlantic salmon) [23]. The studies involved 14 different kinds of animals (see Table 1). Apart from the most commonly used rats/mice (in 160 studies, 78.82%), pigs and chicks were two of the most extensively studied animals (in 23 studies, 11.33%). For themes of the 178 included animal studies, 158 were on clinical and 20 were on agricultural and animal husbandry. For the ones on clinical, 117 were on general toxicity (8 on acute, 6 sub-acute, 84 sub-chronic, 16 chronic toxicity, and still 3 on both acute, sub-acute and sub-chronic toxicity), 35 on specific toxicity (15 on reproductive and developmental toxicity, 16 on immunotoxicity, 3 on teratogenic effect and 1 on mutagenicity), 3 on allergenicity, 1 on learning and memory ability, 1 on athletic ability and 1 on both sub-chronic toxicity and allergenicity.

For interventions/exposures, 31 kinds of GM food were identified, including 18 kinds of GM plant food, 7 kinds of GM animal food and 6 kinds of GM microorganism food. Each included study covered one intervention/exposure, except for one study, Chen [29], that involved two kinds of GM products (sweet pepper and tomato) modified with the same gene (coat protein gene of cucumber mosaic virus), respectively, in two experimental groups. Maize, rice and soybean were the three most popular kinds of GM plant food (taken 79.38%) in research while milk/milk powder and animal-derived protein occupied the top two in GM animal food (56.25%). As for GM microorganism products, 5 kinds of food/feed enzyme derived from 5 different kinds of GM fungi or bacteria as well as 1 kind of microorganism-derived protein were among included studies.

Table 1 Characteristics of the included studies on the safety of GM food

	Frequency (number of studies)	Proportion (%)
Types of animals	203	
Rat/mouse	160	78.82 [a]
Pig	13	6.40 [a]
Chick	10	4.93 [a]
Atlantic salmon	3	1.48 [a]
Drosophila melanogaster	3	1.48 [a]
Rabbit	3	1.48 [a]
Fish	3	1.48 [a]
Goat	2	0.99 [a]
Cat	1	0.49 [a]
Cynomolgus macaque	1	0.49 [a]
Frog	1	0.49 [a]
Monkey	1	0.49 [a]
Sheep	1	0.49 [a]
Tilapia	1	0.49 [a]
Types of intervention/exposure	205 [e]	
GM plant food/feed	160	78.05 [a]
Corn/maize	52	32.50 [b]
Rice/brown rice/paddy rice	42	26.25 [b]
Soybean/soybean meal	33	20.63 [b]
Tomato	7	4.38 [b]
Plant-derived protein	6	3.75
Papaya	5	3.13 [b]
Canola	3	1.88 [b]
Cottonseed	2	1.25 [b]
Mixed soy and corn	1	0.63 [b]
Alfalfa	1	0.63 [b]
Chilli	1	0.63 [b]
European black poplar Leaves	1	0.63 [b]
Poplar leaf	1	0.63 [b]
Potato	1	0.63 [b]
Seed oil from Camelina sativa	1	0.63 [b]
Soybean oil	1	0.63 [b]
Sweet pepper	1	0.63 [b]
Wheat	1	0.63 [b]
GM animal food	32	15.61 [a]
Milk/milk powder	12	37.50 [c]
Animal-derived protein	6	18.75 [c]
Beef/beef powder	4	12.50 [c]
Carp	4	12.50 [c]
Goat milk	2	6.25 [c]
Mutton	2	6.25 [c]
Pork	2	6.25 [c]
GM microorganism food	13	6.34 [a]
Food enzyme endo-1,4-b-xylanase	7	53.85 [d]
Food enzyme α-amylase	2	15.38 [d]
Food enzyme glucose oxidase	1	7.69 [d]

Methodological quality of the animal studies

According to our predefined quality assessment criteria, all of the studies were identified as being unclear or having a high risk of bias (Fig. 3). None of the studies were reported to blind researchers from knowing which intervention each animal received. None of the studies reported prior sample-size calculation, 31 studies (15.27%) described wrong randomization procedures

Table 1 (continued)

	Frequency (number of studies)	Proportion (%)
Food enzyme pullulanase	1	7.69 [d]
Food enzyme aqualysin 1	1	7.69 [d]
Microorganism-derived protein	1	7.69 [d]

[a] Percentage of the eligible studies

[b] Percentage of studies on GM plant food

[c] Percentage of studies on GM animal food

[d] Percentage of studies on GM microorganism food

[e] There was one study involving two kinds of GM food

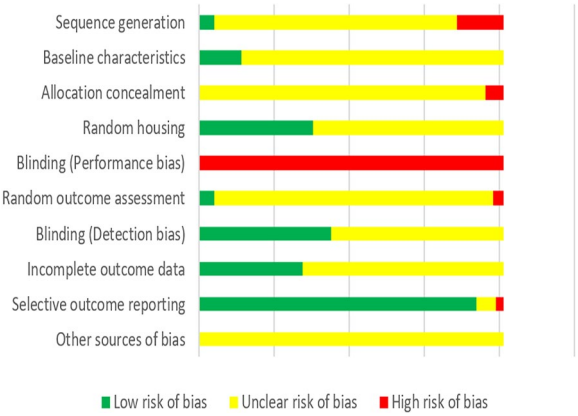

Fig. 3 Risk of bias of the included animal studies

or did not mention the method of "randomization", and 12 studies (5.91%) did not report adequate allocation concealment. 28 studies (13.79%) described that the groups were similar at baseline and 76 studies (37.44%) claimed that the housing conditions of animals from the various experimental groups were identical. 10 studies (4.93%) described randomly pick an animal during outcome assessment while 7 studies (3.45%) failed to select animals at random for outcome assessment. 88 studies (43.35%) completely used objective outcome indicators for outcome measurement. 185 studies (91.13%) reported consistent outcomes in the method and result sections while 5 studies did not, but none of the study protocols were available.

Incidence of adverse events/effects

No meta-analysis was conducted due to the significant heterogeneity of the primary studies. Among the 204 studies, a total of 29 studies (14.22%) from 23 articles reported 37 adverse events, involving 13 on mortality, 6 on reproductive toxicity, 3 on carcinogenesis and 15 on other biomarkers (including one human trial). It is worth noting that when, in one study, there were multiple aspects of adverse events on "other biomarkers", we recorded it as 1 adverse event. Then, 22 serious adverse events (59.46% of adverse events) were identified in 16 studies (7.84% of the included studies and 55.17% of the studies reporting adverse events, marked in the tables with double asterisks). The SAEs mainly rested on mortality (13 studies), tumour or cancer (3), significant low in the number of pup deliveries (2), decreased learning and reaction abilities (1), severe stomach inflammation (1), intestinal adenoma lesions (1), and other pathology abnormalities (1) as hypertrophies and hyperplasia in mammary glands and pituitary, liver congestions and necrosis as well as severe chronic progressive nephropathies.

The incidence of adverse events reporting in government funding, industry funding and non-funded articles were 10.34% (12 of 116), 33.33% (3 of 9) and 15.79% (9 of 57), respectively. When comparing the adverse event reporting rates using the Chi-square test, we found that there were no significant differences either between industry funding and government funding ($\chi^2 = 2.286$, $P = 0.131$), industry funding and non-industry funding ($\chi^2 = 1.761$, $P = 0.185$) or funded and non-funded articles ($\chi^2 = 0.491$, $P = 0.483$).

Incidence of adverse events/effects in human trial

As for the human trial [97], shown in Table 2, a randomized double-blind crossover design was conducted for acute consumption of two single breakfasts, with a 14-day washout period, containing either seed oil generated from transgenic *Camelina sativa* plants or commercially blended fish oil. 36 healthy people were randomly allocated into two groups and venous blood samples were collected after the postprandial session, 8 h after each meal. No follow-up was reported. No major adverse symptoms or health effects were reported but some unrelated minor illnesses for the 72 postprandial sessions from 36 participants, such as minor upper respiratory tract infections (2.78%), minor nose bleed (1.39%), pyelonephritis (1.39%) and headaches (8.33%).

Incidence of adverse events/effects in animal studies

For the 203 animal studies, 28 studies (13.79%) from 22 articles reported 36 adverse events, including 13 on mortality (Table 3, 36.11%), 6 on reproductive toxicity (Table 4, 16.67%), 3 on carcinogenesis (Table 5, 8.33%) and 14 on other biomarkers (Additional file 1: Appendix S3, 38.89%).

All causes of death were included in this analysis and 11 of the 13 studies claimed that the mortality was not significantly different between the groups or had nothing to do with GM food. One study (Ermakova [37]) reported higher pup mortality in the Roundup-Ready soya (40.3.2

Table 2 Adverse events reported in human studies

Study ID	Study design	Participant	Sample size (male/female)	Intervention	Dosage	Duration	Generation	Adverse event
West AL 2019 [97]	Double-blind crossover trial	Male younger participants (18 to 30 years) Female younger participants (18 to 30 years) Male older participants (50 to 65 years) Male older participants (50 to 65 years)	10 10 6 10	450 mg eicosapentaenoic plus decosahexaenoic from either 455 mg transgenic *Camelina sativa* seed oil (CSO) or 452 mg commercial blended fish oil (BFO) in test meal (a milkshake drink, and toast and jam)	455 mg CSO or 452 mg BFO in the test meal	2 single breakfast with at least 14 days apart	1	No specific oil-related minor illness and no test oil-related consequences. No major adverse symptoms or health effects. The minor illness was as follows: 1. 2 cases of minor upper respiratory tract infections (a cold and tonsillitis) out of the seventy-two postprandial sessions* (2 sessions for each of the 36 participants) 2. 1 case of minor nose bleed some hours after one postprandial session with a pre-existing non-clinical tendency towards this 3. 1 case of pyelonephritis between postprandial sessions which was fully resolved 4. 3 cases of headaches during both postprandial sessions

* Postprandial session means 8 h after each meal, during which venous blood samples were collected

Table 3 Mortality-related data of study population receiving GM food

Study ID	Participant (T/C/R)[a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
Carman JA 2013 [26]**	Yorkshire-cross piglets (isowean pig)	T:84	Mixed DK 42–88 RR YG PL corn and RR soy	Ad libitum	22.7 weeks	1	Mortality	T: 14%,
		C:84	Non-GM varieties of soy and corn					C: 13% (no difference)
Cyran N 2008 a [32]**	T1: F0 mice	36	33.0% of the transgenic corn (NK603 × MON810) (formulated in accordance with basal diet, similarly hereinafter)	Ad libitum	The duration of the F0 generation was not known	5	Mortality in parental performance (after weaning)	No differences were seen in the performance of the parental mice in all generations T2: 3 females of 24 pairs died before delivery for unknown reasons; C2: 1 female of 24 pairs died before delivery for unknown reasons; R2: 1 female of 24 pairs died before delivery for unknown reasons; C3: 1 female of 24 pairs died before delivery for unknown reasons;
	C1: F0 mice	36	33.0% isoline					
	R1: F0 mice	36	33.0% GM-free Austrian corn					
	T2: F1 mice	184	33.0% of the transgenic corn (NK603 x MON810) after weaning		For pups, the duration was 7 weeks (3 weeks suckling to the dam + 4 weeks consuming the test diet)			
	C2: F1 mice	185	33.0% isoline after weaning					
	R2: F1 mice	138	33.0% GM-free Austrian corn after weaning					
	T3: F2 mice	189	33.0% of the transgenic corn (NK603 x MON810) after weaning					
	C3: F2 mice	198	33.0% isoline after weaning					
	R3: F2 mice	194	33.0% GM-free Austrian corn after weaning					
	T4: F3 mice	208	33.0% of the transgenic corn (NK603 x MON810) after weaning					
	C4: F3 mice	202	33.0% isoline after weaning					
	R4: F3 mice	230	33.0% GM-free Austrian corn after weaning					
	T5: F4 mice	125	33.0% of the transgenic corn (NK603 x MON810) after weaning					
	C5: F4 mice	145	33.0% isoline after weaning					
	R5: F4 mice	199	33.0% GM-free Austrian corn after weaning					

Table 3 (continued)

Study ID	Participant (T/C/R)[a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
Cyran N 2008 b [32]**	T1: F1 mice	10	33.0% of the transgenic corn (NK603 x MON810) (formulated in accordance with basal diet, similarly hereinafter)	Ad libitum	22 months	1	1. Survive 2. Average life time of mice	1. 2 mice of each group were still alive after almost 22 months 2. 16.3 months in the ISO 15.7 months in the A REF 17.0 months in the GM group but was not significantly different
	C1: F1 mice	10	33.0% isoline					
	R1: F1 mice	10	33.0% GM-free Austrian corn					
Ermakova I 2005 [37] **	T1: F0 Wistar rats	6	T1: Roundup-Ready soya (40.3.2 line) (formulated in accordance with standard laboratory feed, similarly hereinafter)	T1/C1: before mating: 20 g soya flour for every cage (5–7 g flour for each rat) every day; upon delivery: the amount of soya supplement was increased by an additional g for every pup born	Not reported	2	1. Pup mortality (number of dead rats in F1/ number of rats born in F1)	1. T2: 25/45 (55.6%) C2: 3/33 (9%) R2: 3/44 (6.8%)
	C1: F0 Wistar rats	3	C1: traditional soya variety				2. Pup mortality from every female in T1 (number of pups died/number of newborn rats)	2. Female No.1: 7/11, 64% Female No.2: 4/8, 50% Female No.3: 6/13, 46% Female No.4: 8/13, 62%
	R1: F0 Wistar rats	6	R1: standard laboratory feed without any supplementation	Ad libitum				
	T2: F1 Wistar rats	45	T2: Roundup-Ready soya (40.3.2 line)	T2/C2: from 13–14 days of age: 2–3 g soya supplement for every pup				
	C2: F1 Wistar rats	33	C2: traditional soya variety					
	R2: F1 Wistar rats	44	R2: standard laboratory feed without any supplementation	Ad libitum				

Table 3 (continued)

Study ID	Participant (T/C/R)[a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
Hammond B 2006 [42]**	Sprague–Dawley rats	T1: male 20	Corn rootworm-protected corn event of approximately 11% w/w (formulated in accordance with basal diet, similarly hereinafter)	Ad libitum	90d	1	Mortality	T3 group: 1/20 (died on day 92, the cause of death was unknown because macroscopic or microscopic examination of tissues showed no unusual) C3 group: 1/20 (died on day 64, a broken maxilla was found at necropsy) 1/120 (1 male in a reference group died on day 88, the cause of death was not apparent) 2/120 (2 females in a reference group died at week 5 shortly after the interim blood collections)
		T2: female 20	MON 863 of 11% w/w					
		T3: male 20	MON 863 of 33% w/w					
		T4: female 20	MON 863 of 33% w/w					
		C1: male 20	Near-isogenic control of 11% w/w					
		C2: female 20	Near-isogenic control of 11% w/w					
		C3: male 20	Near-isogenic control of 33% w/w					
		C4: female 20	Near-isogenic control of 33% w/w					
		R1: male 20	Reference variety A of 33% w/w					
		R2: female 20	Reference variety A of 33% w/w					
		R3: male 20	Reference variety B of 33% w/w					
		R4: female 20	Reference variety B of 33% w/w					
		R5: male 20	Reference variety C of 33% w/w					
		R6: female 20	Reference variety C of 33% w/w					
		R7: male 20	Reference variety D of 33% w/w					
		R8: female 20	Reference variety D of 33% w/w					

Table 3 (continued)

Study ID	Participant (T/C/R)[a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
		R9: male 20	Reference variety E of 33% w/w					
		R10: female 20	Reference variety E of 33% w/w					
		R11: male 20	Reference variety F of 33% w/w					
		R12: female 20	Reference variety F of 33% w/w					
Naegeli H 2018 a [35]**	CD-1(ICR) mice	T1: 32 (16/16)	Cry1A.105 protein (in bicarbonate buffer solution)	At targeted nominal doses of 10 mg/kg bw per day	28d	1	Mortality	No test substance-related mortality was observed T3 group: 3/32 were found dead or were sacrificed as a consequence of gavage errors (1 male on day 3, 2 females on day 14 and 17) C group: 1/32 (1 male died on day 13 with an unestablished cause of death
		T2: 32 (16/16)	Cry1A.105 protein (in bicarbonate buffer solution)	At targeted nominal doses of 100 mg/kg bw per day				
		T3: 32 (16/16)	Cry1A.105 protein (in bicarbonate buffer solution)	At targeted nominal doses of 1000 mg/kg bw per day				
		C: 32 (16/16)	Bovine serum albumin	at a targeted nominal dose of 1000 mg/kg bw per day				
Naegeli H 2018 b [35]**	CD-1(ICR) mice	T1: 32 (16/16)	Cry2Ab2 protein (in bicarbonate buffer solution)	At targeted nominal doses of 10 mg/kg bw per day	28d	1	Mortality	No test substance-related mortality was observed T2 group: 1/32 (1 female died as a consequence of gavage errors) C group: 1/32 (1 male died as a consequence of gavage errors)
		T2: 32 (16/16)	Cry2Ab2 protein (in bicarbonate buffer solution)	At targeted nominal doses of 100 mg/kg bw per day				
		T3: 32 (16/16)	Cry2Ab2 protein (in bicarbonate buffer solution)	At targeted nominal doses of 1000 mg/kg bw per day				
		C: 32 (16/16)	Bovine serum albumin	at a targeted nominal dose of 1000 mg/kg bw per day				

Table 3 (continued)

Study ID	Participant (T/C/R)[a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
Qian ZY 2018 b [68]**	Wistar rats	T1: 40 (20/20)	7.5% transgenic DAS-44406–6 soybean (formulated in accordance with basal diet, similarly hereinafter)	Ad libitum	90d	1	Mortality	There were no test substance-related deaths, but two females in two GM groups were found dead before study termination. One female rat from the 7.5% DAS-44406–6 group was found dead on study day 52. The likely cause of death for this animal was consistent with acute trauma as microscopic findings included acute haemorrhage around the olfactory bulb of the brain, tongue, and salivary gland. One female in the 15% DAS-44406–6 group was found dead on study day 94. The cause of death was attributed to the jugular blood collection procedure as the animal died after phlebotomy and had macroscopic findings of dark red skeletal muscle on the ventral neck
		T2: 40 (20/20)	15% transgenic DAS-44406–6 soybean					
		T3: 40 (20/20)	30% transgenic DAS-44406–6 soybean					
		C1: 40 (20/20)	Standard basal diet (the main nutritional composition met with standard GB 14,924.3–2001 "Laboratory animals – Mice and rat formula feeds.")					
		C2: 40 (20/20)	7.5% near isoline soybean					
		C3: 40 (20/20)	15% near isoline soybean					
		C4: 40 (20/20)	30% near isoline soybean					
Sakamoto Y 2008 [196]**	F344 DuCrj rats	T1: male 50	30% Pioneer brand 90B72 GM soybean, (formulated in accordance with basal diet, similarly hereinafter)	Ad libitum	104 weeks	1	Survival rate	There was no significant difference between groups. The survival rates in each group were as follows 76%
		T2: female 50	30% Pioneer brand 90B72 GM Soybean					80%
		C1: male 50	30% 9071 Non-GM soybean					73%
		C2: female 50	30% 9071Non-GM soybean					70%
		C3: male 35	Commercial diet (CE-2)					80%
		C4: female 35	Commercial diet (CE-2)					74%

Table 3 (continued)

Study ID	Participant (T/C/R)[a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
Séralini GE 2014 [74]**	T1: male (virgin albino Sprague–Dawley rats, (similarly hereinafter) T2: female T3: male T4: female	20	Plain water and 11% of GM NK603 maize treated with Roundup	Ad libitum	2 years	1	1. Mortality by the end of the experiment	Control males: 30% (3 rats) Control females: 20% (2 rats) Males in GM groups with or without Roundup: 50% Females in GM groups with or without Roundup: 70%
		20	Plain water and 11% of GM NK603 maize treated with Roundup					
		20	Plain water and 11% of GM NK603 maize not treated with Roundup				2. Mortality before mean survival time	Females in all treated group and 3 male groups fed GMOs: mortality was 2–3 times more than controls and more rapid
		20	Plain water and 11% of GM NK603 maize not treated with Roundup					
	T5: male	20	Plain water and 22% of GM NK603 maize treated with Roundup					
	T6: female	20	Plain water and 22% of GM NK603 maize treated with Roundup					
	T7: male	20	Plain water and 22% of GM NK603 maize not treated with Roundup					
	T8: female	20	Plain water and 22% of GM NK603 maize not treated with Roundup					
	T9: male	20	Plain water and 33% of GM NK603 maize treated with Roundup					
	T10: female	20	Plain water and 33% of GM NK603 maize treated with Roundup					
	T11: male	20	Plain water and 33% of GM NK603 maize not treated with Roundup					
	T12: female	20	Plain water and 33% of GM NK603 maize not treated with Roundup					

Table 3 (continued)

Study ID	Participant (T/C/R)[a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
	C1: male	20	Plain water and standard diet from the closest isogenic non-transgenic maize control					
	C2: female	20	Plain water and standard diet from the closest isogenic non-transgenic maize control					
	C3: male	20	Control diet and water with 1.1*10–8% of R (the contaminating level of some regular tap waters)					
	C4: female	20	Control diet and water with 1.1*10–8% of R (the contaminating level of some regular tap waters)					
	C5: male	20	Control diet and water with 0.09% of R (US MRL of glyphosate in some GM feed)					
	C6: female	20	Control diet and water with 0.09% of R (US MRL of glyphosate in some GM feed)					
	C7: male	20	Control diet and water with 0.5% of R (half of the minimal agricultural working dilution)					
	C8: female	20	Control diet and water with 0.5% of R (half of the minimal agricultural working dilution)					

Table 3 (continued)

Study ID	Participant (T/C/R)[a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
Talyn B 2019 [88]**	3-day-old adult Drosophila melanogaster	T1: 278 (129/149)	Herbicide-tolerant w/ Roundup (standard medium made with genetically modified, herbicide-tolerant corn (NK603), which was sprayed with Roundup® Weather Max at the rate of 32 oz/acre)	Ad libitum	Until all flies in the vial had died	1	The lifespan of *Drosophila melanogaster*	A significant difference between organic (C2 group) and Roundup® Ready with Roundup® (T1 group) (Cox proportional hazards, risk ratio = 2.706, $p = 0.0073$), while other comparisons are not (remaining p-values range from 0.146 to 0.869)
		T2: 227 (112/115)	Herbicide-tolerant unsprayed (standard medium made with genetically modified, herbicide-tolerant corn (NK603) without Roundup®)					
		C1: 262 (138/124)	Non-GMO unsprayed (standard medium made with near-isogenic, NK603 progenitor strain into which the herbicide-tolerant construct had not been inserted)					
		C2: 271 (134/137)	Organic commercial (standard medium made with organic commercial corn)					
Tang XQ 2019 [156]**	Sprague–Dawley rats	T: 48 (24/24)	Transgenic rice T2A-1 with cry2A* gene (60.75% during the growth period and 66.75% during the maintenance period, formulated in accordance with AIN-93 diet)	Ad libitum	52 weeks	1	Mortality	There were no test substance-related deaths, but 1 female in the AIN-93 diet group and 1 male in the GM group were found dead at week 51, showing no obvious adverse symptoms before death and no obvious abnormality in the autopsy
		C: 48 (24/24)	Non-transgenic rice of parent 'Minghui 63' line (60.75% during the growth period and 66.75% during the maintenance period, formulated in accordance with AIN-93 diet)					
		R: 48 (24/24)	AIN-93 diet					

Table 3 (continued)

Study ID	Participant (T/C/R)[a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
Zhu H 2014 [193]**	Sprague–Dawley rats	T1: 20	T1: 9.41% of Bt-799 maize (formulated in accordance with AIN-93G purified diets, similarly hereinafter)	Ad libitum	13 weeks	1	Mortality	One rat in the T1 group died at the 11th week after the feeding, and no abnormalities or morphological changes were found by anatomic and pathological examination
		T2: 20	T2: 28. 23% of Bt-799 maize					
		T3: 20	T3: 84.68% of Bt-799 maize					
		C: 20	C: 84.68% of Zheng-58 maize					
		R: 20	R: AIN-93G purified diets					

[a] T/C/R refers to treatment group/control group/reference group

F0 refers to parental generation

F1–F4 refers to the generation of the filial generation

Bw refers to body weight

** refers to a serious adverse event

line) group compared with the controls. In Séralini [74], the general cause of death was large mammary tumours in females and other organ problems in males. Besides, rats in the Roundup-tolerant GM NK603 maize groups were 2–3 times more likely to die than controls, and more rapidly.

With respect to effects on reproduction, 5 animal feeding studies were reported to trigger reproductive toxicity but one study (Cisterna [31]) claimed to have no substantial impact on fertility. The reproductive toxicity manifested in the significant low in the number of deliveries, survival rate (from birth to weaning), litter weight, litter size and weight of some organs in the pups. For example, in Ermakova I 2005, the rats fed with Roundup-Ready soya had a 55.6% pup mortality rate during lactation periods compared to 9% in the control of traditional soya and 6.8% in the reference group. The pups kept dying during the lactation period while pups from the control group only died during the first week. Cyran N 2008 a and Cyran N 2008 c [32] were two rat feeding studies reported in one article, both given NK603 × MON810 maize. A multi-generation study was conducted as Cyran N 2008 a while Cyran N 2008 c did a continuous breeding study. Both of them indicated that fewer sum of pups was born and weaned in the GM groups. Pup losses, in Cyran N 2008 a, overall generations were about twice as many pups lost as compared to the control group (14.59% vs 7.4%) but was not significantly different and significantly lower litter weight was also reported in Cyran N 2008 c.

Three mouse/rat feeding studies reported triggering cancers/tumours when Tang [156] attributed the incidence of the tumour to the elder age of rats. Séralini 2014 (on Roundup-tolerant GM maize) found that females in the treatment groups almost always developed large mammary tumours more often than and controls. As for males, 4 times larger palpable tumours than controls were presented which emerged up to 600 days earlier. Cyran 2008 b [32] revealed a life term study where mice in the three groups were fed with transgenic maize NK603xMON810 (from 33.0% in the diet), control isoline diet and GM-free Austrian corn reference diet, respectively. The survival rate was not significantly different while cancer (leucosis) was the common cause of death.

GM food-related adverse events

Among the 37 adverse events reported, 16 of them claimed to have nothing to do with GM food, while the rest 21 (from 17 studies) did not, still leaving the question open. The GM food-related adverse events existed in mortality (2 studies), reproductive toxicity (5), carcinogenesis (2), and other biomarkers (12).

By gathering evidence, we identified 3 kinds of GM food associated with adverse events, GM soybean, GM

Table 4 Adverse events/effects—reproductive toxicity

Study ID	Participant (T/C/R) [a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
Carman JA 2013 [26]	Yorkshire-cross piglets (isowean pig)	T:84	T: GM corn containing 90% DK 42–88 RR YG PL (a triple stack of NK603, MON863 and MON810 genes) with the remainder being equal quantities of Pannar 5E-900RR (containing NK603), Pannar 4E-705RR/Bt (a double stack of NK603 and MON810) and Producers 5152 Roundup-Ready™ (containing NK603)	Libitum	22.7 weeks	1	Uterus weight	The medians of the non-GM-fed (now $N=33$) and GM-fed ($N=37$) groups after removing one extreme outlier: C: 0.084% of the body weight, T: 0.105% of the body weight ($p=0.025$)
		C:84	C: non-GM varieties of soy and corn					
Cisterna B 2008 [31]	Swiss mice	T:10	A standard diet containing 14% GM soybean obtained by the insertion of a bacterial gene conferring tolerance to glyphosate, the active ingredient of the herbicide Roundup	Ad libitum	Not reported	2	Labelling density over nucleoplasm and prenucleolar bodies in 2-cell and 4–8-cell embryos	The labelling was significantly lower in embryos from GM-fed mice than in controls in anti-polymerase II, anti-BrU and anti-hnRNP only at the 2-cell stage, and it was significantly lower in anti-CstF, anti-CFIm68 and polyadenylated RNA at both the 2-cell stage and the 4–8-cell stage GM soybean-containing diet does not affect mouse fertility, parturition time or litter health
		C:10	Identical diet but containing non-GM soybean					
Cyran N 2008 c [32]**	T1: F0 mice	48 (24 pairs)	33.0% of the transgenic corn (NK603 × MON810) (formulated in accordance with basal diet, similarly hereinafter)	Ad libitum	20 weeks	2	1. The number of pairs who delivered in the F0 generation/group	1. T2:23, T3: 23, T4: 22, T5: 20 ($P=0.05$); C2: 24, C3: 24, C4: 24, C5: 24
	C1: F0 mice	48 (24 pairs)	33.0% isoline				2. Average number of pups at birth/pair	2. T4: 9.68 ± 0.688 ($P=0.011$), T5: 8.21 ± 1.077 ($P=0.010$); C4: 11.92 ± 0.496, C5: 11.38 ± 0.462
	T2: 1st litter of T1	189	33.0% of the transgenic corn (NK603 × MON810) after weaning					

Table 4 (continued)

Study ID	Participant (T/C/R) [a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
	C2: 1st litter of C1	216	33.0% isoline after weaning				3. Sum of pups at birth/group	3. T2 + T3 + T4 + T5 (GM group): 844; C2 + C3 + C4 + C5 (ISO group): 1035
	T3: 2nd litter of T1	245	33.0% of the transgenic corn (NK603 × MON810) after weaning					
	C3: 2nd litter of C1	260	33.0% isoline after weaning				4. Sum of pups at weaning/group	4. T5: 173 (7.21 ± 0.985), C5: 235 (9.79 ± 0.525)
	T4: 3rd litter of T1	213	33.0% of the transgenic corn (NK603 × MON810) after weaning					
	C4: 3rd litter of C1	286	33.0% isoline after weaning				5. Sum of pup losses/group (birth to weaning)	5. T2: 2 (0.09 ± 0.060), T3: 19 (0.83 ± 0.375), T4: 2 (0.12 ± 0.081) * ($p = 0.025$), T5: 24 (1.00 ± 0.376) C2: 16 (0.67 ± 0.305), C3: 19 (0.79 ± 0.289), C4: 32 (1.33 ± 0.433), C5: 38 (1.58 ± 0.371)
	T5: 4th litter of T1	197	33.0% of the transgenic corn (NK603 × MON810) after weaning					
	C5: 4th litter of C1	273	33.0% isoline after weaning					
							6. Litter weights	6. The third litters: the average litter weights were statistically significantly lower in the GM group (T4) at birth and on the second day (at birth $p = 0.026$; 2d $p = 0.040$) as well as at weaning ($p = 0.031$); the fourth litter the average litter weight was significantly lower in the GM group (T4) ($p = 0.05$)

Table 4 (continued)

Study ID	Participant (T/C/R) [a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
Cyran N 2008 a [32]	T1: F0 mice	36	33.0% of the transgenic corn (NK603 x MON810) (formulated in accordance with basal diet, similarly hereinafter)	Ad libitum	The duration of the F0 generation was not known For pups, the duration was 7 weeks(3 weeks suckling to the dam + 4 weeks consuming the test diet)	5	1. Litter size	1. More litters with $n > 8$ were seen in the control groups compared to the GM groups
	C1: F0 mice	36	33.0% isoline				2. Sum of pups at birth/group (number of pups at birth/pair)	2. There were no statistically significant differences between groups: T1: 184 (10.22 ± 0.629), C1: 185 (10.28 ± 0.980), T2: 189 (7.88 ± 0.779), C2: 198 (8.25 ± 0.778), T3: 208 (8.92 ± 0.875), C3: 202 (8.42 ± 1.025), T4: 125 (5.68 ± 1.10), C4: 145 (6.59 ± 1.046),
	R1: F0 mice	36	33.0% GM-free Austrian corn					
	T2: F1 mice	184	33.0% of the transgenic corn (NK603 × MON810) after weaning					
	C2: F1 mice	185	33.0% isoline after weaning					
	R2: F1 mice	138	33.0% GM-free Austrian corn after weaning				3.Sum of pup losses from birth to weaning/group (pup losses/pair)	3. There were no statistically significant differences between groups: T1: 46 (2.61 ± 0.837), T2: 22 (1.00 ± 0.510), T3: 25 (2.95 ± 0.631), T4: 10 (0.71 ± 0.322) C1: 34 (2.06 ± 0.683), C2: 6 (0.26 ± 0.157), C3: 11 (0.58 ± 0.289), C4: 3 (0.19 ± 0.136) R1: 17 (1.06 ± 0.322), R2: 18 (0.78 ± 0.281), R3: 21 (0.91 ± 0.266), R4: 10 (0.50 ± 0.212)
	T3: F2 mice	189	33.0% of the transgenic corn (NK603 × MON810) after weaning					
	C3: F2 mice	198	33.0% isoline after weaning					
	R3: F2 mice	194	33.0% GM-free Austrian corn after weaning					
	T4: F3 mice	208	33.0% of the transgenic corn (NK603 × MON810) after weaning					
	C4: F3 mice	202	33.0% isoline after weaning					
	R4: F3 mice	230	33.0% GM-free Austrian corn after weaning					
	T5: F4 mice	125	33.0% of the transgenic corn (NK603 × MON810) after weaning					
	C5: F4 mouse	145	33.0% isoline after weaning					
	R5: F4 mouse	199	33.0% GM-free Austrian corn after weaning					

Table 4 (continued)

Study ID	Participant (T/C/R) [a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
Ermakova I 2005 [37]**	T1: F0 Wistar rats	6	T1: Roundup-Ready soya (40.3.2 line) (formulated in accordance with standard laboratory feed, similarly hereinafter)	T1/C1: before mating: 20 g soya flour for every cage (5–7 g flour for each rat) every day; upon delivery: the amount of soya supplement was increased by an additional g for every pup born	Not reported	2	1. Pup mortality during every lactation period (the number of dead rats)	1. 1st week: T2: 31.1% (14), C2: 9% (3), R2: 4.5% (2); 2nd week: T2: 13.4% (6), C2: 0% (0), R2: 2.3% (1); 3rd week: T2: 11.1% (14), C2: 0% (0), R2: 0% (0)
	C1: F0 Wistar rats	3	C1: traditional soya variety				2. Organ mass in small pups	2. The organs of small pups from the GM group were tiny in comparison with the same of other groups except for the brain mass
	R1: F0 Wistar rats	6	R1: standard laboratory feed without any supplementation	Ad libitum				
	T2: F1 Wistar rats	45	T2: Roundup-Ready soya (40.3.2 line)	T2/C2: from 13–14 days of age: 2–3 g soya supplement for every pup			3. Weight of pups	3. Birth weight of F1 rats: T2: 23.95 g ± 1.5 g C2: 30.03 g ± 1.1 g ($P < 0.005$) R2: 27.1 g ± 0.9 g ($P < 0.1$) the weight distribution of F1 rats by 2 weeks of age on different diets: 10–20 g: T2: 36%, C2: 6.7% ($P < 0.05$), R2: 6% ($P < 0.01$)
	C2: F1 Wistar rats	33	C2: traditional soya variety					
	R2: F1 Wistar rats	44	R2: standard laboratory feed without any supplementation	Ad libitum				
Tudisco R 2015 [69]	T1: F0 pluriparae goat	10	Oat hay and commercial concentrate containing 13% of GM (MON40-3–2) soybean meal	Ad libitum	120d	**2**	1. Body weight after 30 days in kids	1. Body weight at day 30 ($p < 0.05$): T3: 8.3 ± 0.7b, T4: 8.2 ± 0.6b C3: 9.5 ± 0.4a, C4: 9.4 ± 0.5a body weight at slaughtering ($p < 0.05$): T3: 10.3 ± 0.5b, T4: 10.1 ± 0.6b C3: 12.5 ± 0.4a, C4: 12.3 ± 0.5a
	T2: F0 pluriparae goat	10	Oat hay and commercial concentrate containing 20% of GM (MON40-3–2) soybean meal		120d			

Table 4 (continued)

Study ID	Participant (T/C/R) [a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
	C1: F0 pluriparae goat	10	Oat hay and commercial concentrate containing 13% of conventional soybean meal		120d		2. Height at the withers and chest width in kids	2. Height at withers ($p < 0.05$): T3: 45.7 ± 2.1b, T4: 46.0 ± 2.0b C3: 48.6 ± 2.6a, C4: 48.8 ± 2.4a chest width ($p < 0.05$): T3: 11.3 ± 1.0b, T4: 11.5 ± 1.1b C3: 12.6 ± 1.03a, C4: 12.7 ± 1.1a
	C2: F0 pluriparae goat	10	Oat hay and commercial concentrate containing 20% of conventional soybean meal		120d			
	T3: male kids of T1	10	Colostrum/milk from T1		60 ± 7d		3. Carcass weight in kids	3. Hot carcass weight ($p < 0.05$): T3: 6.4 ± 0.3b, T4: 6.2 ± 0.4b C3: 7.2 ± 0.4a, C4: 7.1 ± 0.5a Cold carcass weight ($p < 0.05$): T3: 6.2 ± 0.4b, T4: 6.0 ± 0.4b C3: 6.8 ± 0.6a, C4: 6.9 ± 0.7a
	T4: male kids of T2	10	Colostrum/milk from T2		60 ± 7d			
	C3: male kids of C1	10	Colostrum/milk from C1		60 ± 7d			
	C4: male kids of C2	10	Colostrum/milk from C1		60 ± 7d			

[*] T/C/R refers to treatment group/control group/reference group

F0 refers to parental generation

F1–F4 refer to the generation of the filial generation

[**] refers to a serious adverse event

Table 5 Adverse events/effects—carcinogenesis

Study ID	Participant (T/C/R) [a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
Cyran N 2008 b [32]**	T1: F1 mice	10	33.0% of the transgenic corn (NK603 x MON810) (formulated in accordance with basal diet, similarly hereinafter)	Ad libitum	22 months	1	1.Typical pathological findings	1. Cachexia, spleno- and hepatomegaly with diffuse or local infiltration with abnormal leukocytes
	C1: F1 mice	10	33.0% isoline				2.Incidence of cancer	2. 2 mice of each group were still alive after almost 22 months. The common causes of death were cancer (leucosis)
	R1: F1 mice	10	33.0% GM-free Austrian corn					
Séralini GE 2014 [74]**	T1: male (virgin albino Sprague–Dawley rats, (similarly hereinafter)	20	Plain water and 11% of GM NK603 maize treated with Roundup	Ad libitum	2 years	1	The number of pathological abnormalities of mammary tumours in females (the number of rats reached was in parentheses while 10 rats per group were analysed)	GMO 11% + R (T2 group): 10 (6) GMO 11% (T4 group): 15 (7) GMO 22% + R (T6 group): 11 (7) GMO 22% (T8 group): 10 (7) GMO 33% + R (T10 group): 13 (9) GMO 33% (T12 group): 15 (8) C2: 8 (5) C4: 20 (9) C6: 16 (10) C8: 12 (9)
	T2: female	20	Plain water and 11% of GM NK603 maize treated with Roundup					
	T3: male	20	Plain water and 11% of GM NK603 maize not treated with Roundup					
	T4: female	20	Plain water and 11% of GM NK603 maize not treated with Roundup					
	T5: male	20	Plain water and 22% of GM NK603 maize treated with Roundup					
	T6: female	20	Plain water and 22% of GM NK603 maize treated with Roundup					
	T7: male	20	Plain water and 22% of GM NK603 maize not treated with Roundup					
	T8: female	20	Plain water and 22% of GM NK603 maize not treated with Roundup					
	T9: male	20	Plain water and 33% of GM NK603 maize treated with Roundup					
	T10: female	20	Plain water and 33% of GM NK603 maize treated with Roundup					

Table 5 (continued)

Study ID	Participant (T/C/R) [a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
	T11: male	20	Plain water and 33% of GM NK603 maize not treated with Roundup					
	T12: female	20	Plain water and 33% of GM NK603 maize not treated with Roundup					
	C1: male	20	Plain water and standard diet from the closest isogenic non-transgenic maize control					
	C2: female	20	Plain water and standard diet from the closest isogenic non-transgenic maize control					
	C3: male	20	Control diet and water with 1.1*10–8% of R (the contaminating level of some regular tap waters)					
	C4: female	20	Control diet and water with 1.1*10–8% of R (the contaminating level of some regular tap waters)					
	C5: male	20	Control diet and water with 0.09% of R (US MRL of glyphosate in some GM feed)					
	C6: female	20	Control diet and water with 0.09% of R (US MRL of glyphosate in some GM feed)					
	C7: male	20	Control diet and water with 0.5% of R (half of the minimal agricultural working dilution)					
	C8: female	20	Control diet and water with 0.5% of R (half of the minimal agricultural working dilution)					

Table 5 (continued)

Study ID	Participant (T/C/R) [a]	Sample size (male/female)	Intervention/exposure	Dosage	Duration	Generation	Outcome	Result
Tang XQ 2019 [156]**	Sprague–Dawley rats	T: 48 (24/24)	Transgenic rice T2A-1 with cry2A* gene (60.75% during the growth period and 66.75% during the maintenance period, formulated in accordance with AIN-93 diet)	Ad libitum	52 weeks	1	Incidence of tumours	Only common spontaneous tumours in elderly SD rats were observed including 1 case of thyroid follicular adenoma, 1 case of renal tubular epithelial adenoma and 1 case of lipoma in the AIN-93 diet group, and 1 case of breast adenoma in the GM group
		C: 48 (24/24)	Non-transgenic rice of parent 'Minghui 63' line (60.75% during the growth period and 66.75% during the maintenance period, formulated in accordance with AIN-93 diet)					
		R: 48 (24/24)	AIN-93 diet					

[a] T/C/R refers to treatment group/control group/reference group

F0 refers to parental generation

F1–F4 refer to the generation of filial generation

** refers to a serious adverse event

maize as well as GM rice. For the 17 studies involved in the GM food-related adverse events, 4 studies were absent of information on the GM event of their test substance and the remainder concentrated on 7 GM events (3 studies on NK603 × MON810 maize, 2 on GTS 40-3-2 soybean, 2 on NK603 maize, 2 on MON863 maize, 2 on MON810 maize, 1 on maize mixed with MON863 × MON810 × NK603, NK603 × MON810 and NK603 and 1 on GM Shanyou 63 rice). When searching in the GM Approval Database on the ISAAA website, we found that all of the first 6 GM events listed, all developed by Monsanto Company, had been on regulatory approval for food, feed and cultivation in multiple countries/regions, including the European Union. GM -39 Shanyou 63 was developed in China and given approval for food, feed, and cultivation only by China in 2009.

Discussion

Summary of findings

We included 203 in vivo animal studies and 1 human trial, and all of the studies were identified as being unclear or having a high risk of bias. Overall, we reported two main findings. First, we identified 37 adverse events for GM food consumption while 22 of them (59.46%) were serious adverse events extracted from 16 animal studies (7.84%). SAEs were mortality, tumour or cancer, significantly low in the number of pup deliveries, decreased learning and reaction abilities, severe stomach inflammation, intestinal adenoma lesions, and other pathological abnormalities in the mammary glands, pituitary, liver and kidney.

Second, there were 21 GM food-related adverse events indicating that GM food may have effects on increased mortality (2 studies), reproductive toxicity (5 studies), which referred to significantly low fertility in parental generation and low survival rate, litter weight, litter size and weight of some organs in the pups, carcinogenesis (2 studies) and other biomarkers (12 studies). The effect-related GM food included 7 GM events (NK603 × MON810 maize, GTS 40-3-2 soybean, NK603 maize, MON863 maize, MON810 maize, MON863 × MON810 × NK603 maize and GM Shanyou 63 rice), which had all been on regulatory approval for food, feed and cultivation in some countries/regions.

Agreements and disagreements with other reviews

To our knowledge, there have been 3 previous systematic reviews (SRs) [198–200] and 6 conventional reviews [16, 201–205] addressing similar research questions on the unexpected effects of GM food consumption. Keshani et al. [198], searching in 4 English databases, included experimental studies on GM crops' potential effects on sperm parameters. The study finally included 7 rat feeding studies, which were all identified in our study, and indicated no harm to GM plants consumers. Edge et al. [199] addressed 30 review questions for including human studies, published in recent 20 years (1994–2014), on health effects of genetically engineered (GE) food crops, but found no human study on 25 questions. The remaining 5 questions, related to allergenicity and nutrient adequacy, were answered based on 21 human studies. The human studies were all excluded in our research because of no direct ingestion of GE food in the allergenicity assessment studies or no targeted outcomes in the nutrient assessment trial. To illustrate, the abovementioned nutrient assessment clinical trial evaluated the effect of carrots containing twofold higher calcium content on calcium absorption and we thought it was not on outcome related to adverse events/effects. The conclusion of the research also supported that there were no clear adverse health effects associated with the consumption of GE food. Moreover, Dunn et al. [200] included both human and animal studies for examining the allergenicity of GM organisms and finally found 34 human studies and 49 animal studies eligible. In addition to 32 human studies which involved human serum for IgE binding or inhibition studies and not direct consumption of GM product, the rest 2 [206, 207]studies were on actual ingestion of a GM food. However, they were not included in our research because of not targeted study type and unrelated outcomes. The conclusion agreed with the first two SRs that GM foods did not appear to be more allergenic than their conventional counterparts.

As for conventional reviews, Domingo showed special attention to the safety of GM food and published four literature reviews in 2000 [203], 2007 [204], 2011 [205] and 2016 [16]. Domingo searched two databases, PubMed and Scopus, to assess adverse/toxic effects of GM plants. In the latest updated review, he addressed the conclusion that GM soybeans, rice, corn/maize and wheat would be as safe as the parental species of these plants. However, our results may not be consistent with Domingo's conclusion: we focus on a summarization of adverse events for GM food consumption through a systematic search in 7 databases; we identified 37 adverse events, 22 serious adverse events and 21 GM food-related adverse events; GM maize, soybean and rice with some specific GM events were all related to GM food-related adverse events. In addition, Domingo found a notable advance of studies published in scientific journals by biotechnology companies. Coincidentally, we did a Chi-square test to compare the adverse event reporting rates and found no significant differences between industry funding, government funding and non-funded articles. Besides, our systematic review validated Domingo's findings that some GM plants were studied scarcely in recent years

including GM potatoes discussed in the controversy of Pusztai case.

Strengths and limitations

In this review, a systematic search of major databases was conducted to identify all available studies in all languages on the adverse effects/events of GM food consumption. To make the inclusion and data synthesis comprehensive, both in vivo human and animal studies in all fields were included, with no limitations on the type of participant, type of intervention/exposure or whether control was included. The terms used for searching, containing all kinds of names of GM food, were based on a basic search on the internet by the researchers and the list was perfected as much as possible. With respect to additional searching, we went through multifarious news which reported controversy of GM food and thus we identified several hot studies by following the clue. In order to trace the potential conflicts of interest, we performed a Chi-square test for comparing adverse events report rates in articles funded by industry funding, government funding or unfunded articles, but found no statistical significance. Nevertheless, it was hard to conduct a quantitative data synthesis for the effects of GM food consumption on the adverse events because of the significant heterogeneity of the primary studies.

There are several limitations in this review. The methodological quality of the included studies is generally poor, which indicates a high or unclear risk of bias resulting from insufficient reporting of methodological components in the studies. Methodological quality may not be fully reflected based solely on the reporting of the manuscript. There were unclear descriptions of randomization procedures and a lack of blinding in all of the studies, which may have created potential performance biases and detection biases, as researchers might have been aware of the effects of interventions. The ability to perform meta-analysis was limited because of the heterogeneity of the participants, interventions (GM food in various GM events), comparisons, feeding doses, administration time, other exposure factors, and the variance of composite outcome measures used in the 204 included studies. When we did the manual search, we found that related publications were retracted sometimes, under the name of inadequate experimental designs or statistical analysis. For example, Séralini 2012 was retracted by *Food and Chemical Toxicology*, but subsequently republished in another journal [14, 74]. This indicates that it was hard for us to find the original full-text papers of the retracted publications and articles provided by databases still have some unavoidable publication bias. The retraction on controversial researches may also cause the controversy for the public to doubt the reality of the studies published and to concern the safety of GM food. In addition, the lack of human studies is another key limitation of this research. As for the searching strategy, we did not include publication types as newspaper articles and comments. This was thought to be a limitation of this research because these sources may give us clues of related researches and can help us to do a manual search comprehensively. It is also an implication for future systematic reviews.

Implications for research

Future research should be conducted in humans, especially observational cohort studies. High-quality animal studies according to the ARRIVE reporting standard focusing on reproductive toxicity and carcinogenesis are still needed. Trials or studies should be registered prospectively, and be accessible. Furthermore, to address public concerns, future studies should focus on SAEs and GM food-related adverse events reported in this research such as NK603 maize, MON863 maize and MON810 maize. Meanwhile, some implications of findings still could be explored such as how GM food affects people's eating habits, labelling of GM food and public choice. Some of the included studies conducted an intergenerational or multigenerational evaluation of the safety of GM food, but only two studies (Cyran N 2008 a and Cyran N 2008 c) in one article reported adverse events related to fertility. The differences in the results may be due to different interventions/exposures (GM food in certain GM events), laboratory animals, intervention/exposure time, experiment environment, etc. Therefore, it is necessary for subsequent studies to start with intergenerational or multigenerational research to verify the safety of GM food in terms of study design.

Conclusion

Serious adverse events accounted for 59.46% of the total 37 identified adverse events of GM consumption, which include: mortality, tumour or cancer, significantly lower number of pup deliveries, decreased learning and reaction abilities, and organ abnormalities in the stomach, intestinal adenoma, mammary glands, pituitary, liver and kidney. The interventions/exposures in the adverse event related studies emphasized on GM soybean, maize and rice in specific GM events. Animal studies occupy the lowest hierarchy of evidence, and there are flaws in study design and is not convincing enough. The evidence on the effect of GM consumption on humans is still insufficient. Further clinical trials and long-term cohort studies in human populations, especially on GM food-related adverse events and the corresponding GM events, are still warranted. It is better to prove the safety before they are approved for food consumption and it also suggests

the necessity of labelling on GM food so that consumers can make their own choice.

Abbreviations

GM: Genetically modified; DNA: Deoxyribonucleic acid; CNKI: China National Knowledge Infrastructure; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; SAE: Serious adverse event; CSO: *Camelina sativa* Seed oil; BFO: Blended fish oil; bw: Body weight; SR: Systematic reviews; GE: Genetically engineered.

Supplementary Information

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Additional file 1: Appendix S1. Search strategy applied in English language databases. **Appendix S2.** Funding sources or sponsors. **Appendix S3.** Adverse events/effects—other biomarkers.

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Authors' contributions

All work was done by the authors. JPL and YTF conceived the study and revised the manuscript. CS contributed to data searching, screening and extraction, analysis of the data, drafted and revised the paper and approved the final version to be submitted. XCY, BYJ, JP, XHC, JXR, JL, XWZ, HDL, WBH and MF participated in identifying or screening the titles, abstracts and full-text screening and data extraction. XL, NR and JPL advised on the analysis of the data and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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