Original Article

The Effect of Increasing the Dose of Acetamiprid and Dichlorvos Pesticides on the Reproductive Performance of Laboratory Mice

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Abstract

Background: Pesticides are widely used around the world. However, these chemicals are being used more frequently and at increased doses in underdeveloped and developing countries. Although the hazard of pesticides has been studied in ecological fields, the effect of residual amounts of these compounds on the physiological processes of the body has always been debated.

Materials and Methods: In this experimental study, 45 greenhouse cucumber plants were sprayed with dichlorvos and acetamiprid pesticides in concentrations of twofold (acetamiprid 500 g/1000 L and dichlorvos 4 L/1000 L) and threefold of the recommended dose. After 24 h, the residual amount was obtained. To evaluate the residual effect of the mentioned pesticides, an equivalent of this residue was added to the drinking water of 105 mice.

Results: Pesticide residues were obtained for twofold and threefold concentrations of the recommended dose, 1.5 and 2.5 (mg/kg cucumber) for acetamiprid and 0.5 and 1 (mg/kg cucumber) for dichlorvos, respectively. Application of these chemicals at higher doses not only significantly reduced the body weight, food consumption, testosterone production, testicular germ cells and embryo numbers, but also increased the levels of follicle-stimulating hormone and luteinizing hormone in mice.

Conclusions: The emergence of biological disorders and reducing reproductive potential in male mice can be attributed to the addition of pesticides to their drinking water. Therefore, to reduce the hazards caused by insecticides, it is recommended to familiarize farmers with the harmful effects of overdose of pesticides and monitoring the residuals in agricultural products.

Keywords: Agrochemicals, crops, germ cells, hormones, mice, reproductive

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Introduction

Food security is becoming more fraught in many underdeveloped and developing countries. In April 2020, the UN World Food Program (WFP) warned that the number of people suffering "acute food insecurity" could be more than double this year, that is, it could rise up to 265 million. The importance of this issue is further highlighted by understanding and considering coronavirus disease 2019 (COVID-19) pandemic, which has restricted public access to food.[1] Therefore, food shortages can lead to the selection of methods that reduce the risk of crop harvesting. Improper use of pesticides is one of these methods. Not only do pesticides cause environmental pollution, such as water, air, and soil pollution, but also they cross the food chain by remaining in food and ultimately endangering human health.[2] Exposure to food containing pesticide residues is approximately five times greater than exposure to

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contaminated air or drinking water.[3] This is especially the case in underdeveloped and developing countries due to the lack of laws and regulations. In countries where agriculture is a major source of income, farmers are heavily dependent on pesticides to control crop losses. Excessive use of pesticides and harvesting of crops before the preharvest interval (PHI) of pesticides (the minimum amount of time between the last application of a pesticide and when the crop can be harvested, which is specified on the packaging of pesticides) are widely used.[1]

As one of the most consumed fruits in the world, cucumber is among the most contaminated fruits and vegetables with pesticides. Cucumber is covered with a natural protective layer of wax, and when pesticides are spread in this layer, they become harder to wash.[4]

Organophosphate and neonicotinoid pesticides are used to control a wide range of plant pests in both greenhouses and fields.[5,6] Neonicotinoids are a new class of insecticides that act as a selective nicotinic acetylcholine receptor (nAChR) agonist in the central nervous system of insects.[7] Acetamiprid, like other neonicotinoids, has the property of selecting the nAChR of insects over vertebrates and is less toxic to mammals.[8] But reports indicate that acetamiprid causes reproductive toxicity in various species.[9] Some nAChR subunits are effective in the reproductive system.[10] Organophosphorus pesticides are organic pesticides which are widely used in many fields, including agricultural sector. Dichlorvos is an organophosphate insecticide having nonsystemic, intrusive contact and respiratory (fume) and severe shock effect,[11] which acts by inhibiting the activity of the cholinesterase enzyme in the nervous system and has been reported to have an effect on male fertility.[12]

Since the research on the effect of acetamiprid and dichlorvos pesticides on male reproductive performance is scanty and most of the toxicity of these pesticides has been investigated based on the lethal dose, it is important to measure the residual of these pesticides in food in situations in which their use is often more than the recommended dose and is associated with noncompliance with the PHI of pesticides. Also, the study of the residual effect of pesticides on the reproductive performance of male mice gives a clearer picture of the consequences of this group of pesticides on humans. Therefore, in this study, the residues of acetamiprid and dichlorvos pesticides on greenhouse cucumber were measured at twofold and threefold concentrations of the recommended dose. Then, an equivalent of this residue was added to the drinking water of male BALB/c laboratory mice and its effect on reproductive function was investigated.

Materials and Methods

The experiments were performed in two parts. In the first part, the residues of dichlorvos and acetamiprid were detected in cucumber fruits 24 h after spraying, and in the second part, by using the obtained data, an equivalent amount of these residues on cucumber was mixed with the drinking water of laboratory mice and its effect on the reproductive performance of mice was investigated.

Cucumber plant cultivation

The plant used in this study to assess the pesticide residue was Sana cultivar of cucumber, which is widely consumed. The cucumber was cultivated in two stages of seedling production and its transfer to the main location. Both stages were performed inside a greenhouse with 20 × 2 × 2.5 (m) dimensions, with a day and night temperature of 25°C ± 5°C and 18°C ± 2°C, respectively, relative humidity of 50%–60%, and light conditions of 16 h of light and 8 h of darkness.

The arable land of the region was used for the generation of seedling and pots. After 45 days, the seedlings were ready for transfer to the main pots, which were made of polyvinyl chloride (PVC) with a height of 40 cm and lid diameter of 30 cm. The plants were irrigated once in every 2 days.

Determining the equivalent dose

The experimental units used to measure the residues of acetamiprid and dichlorvos in greenhouse cucumber included 45 pots of cucumber, which were considered in five treatments and three replications, and each repetition included three pots. Cucumber plants were sprayed with pesticides during the fruiting stage. Treatments for each agent included a concentration equal to twofold and threefold of the recommended dose as well as a control treatment (water). For spraying, SeeSa sprayer with a volume of 2.5 L was used. The first treatment was for the control group in which the experimental units were sprayed with water. The second and third treatments were for pots which were sprayed with twofold (500 g/1000 L) and threefold of the recommended dose of acetamiprid. The fourth and fifth treatments were for pots which were sprayed with a concentration equal to twofold (4 L/1000 L) and threefold of the recommended dose of dichlorvos.

Twenty-four hours after spraying the pots, one cucumber (three in each replication) was taken from each pot and placed in separate plastic bags, and after labeling, the samples were transferred to the laboratory. In the laboratory, all three cucumbers related to one replicate were mixed using a blender and the residues of pesticide in the sample were measured by the method of 17026 QuEChERS and using the gas chromatography–mass spectroscopy (GC–MS) device (Agilent). In this study, dichlorvos was utilized as an emulsifiable concentrate (EC) formulation, with a purity of 50% and PHI of 3 days, and acetamiprid was utilized as a water-soluble powder (WSP) formulation, with a purity of 20% and PHI of 14 days, and were obtained from Golsang Company, which is widely used in the region.

Reproductive performance experiments

In this section, the data of residual acetamiprid and dichlorvos pesticides in cucumber were used, which were obtained using the chromatographic device. The analysis results indicated that the residual pesticide was higher than the Codex standard, which was 0.5 and 1 ppm for dichlorvos and 1.5 and 2.5 ppm for acetamiprid in twofold and threefold of the recommended dose, respectively. Therefore, the same amount of pesticide was used in the drinking water of laboratory mice. For this purpose, 105 BALB/c mice (70 male and 35 female) were purchased from Royan Research Institute in Isfahan. To adapt the animals to the environment, they were placed in standard cages at 22°C ± 3°C, 12 h of light/dark, and 40 ± 10 humidity for 1 month. Food and water were provided to the mice on a daily basis.

For blood tests, 35 male mice were randomly divided into seven groups of five samples as follows:

Control group: No agent was added to the drinking water of this group.

Treatment group 1 (double dichlorvos): 0.5 ppm dichlorvos was added to the drinking water daily.

Treatment group 2 (double acetamiprid): 1.5 ppm of acetamiprid was added to the drinking water daily.

Treatment group 3 (double combined): Every other day, an equivalent dose of group 1 and 2 agents was added to the drinking water daily and alternately (one day 0.5 ppm of dichlorvos and the next day 1.5 ppm of acetamiprid).

Treatment group 4 (triple dichlorvos): 1.00 ppm dichlorvos was added to the drinking water daily.

Treatment group 5 (triple acetamiprid): 2.5 ppm acetamiprid was added to the drinking water daily.

Treatment group 6 (triple combined): Every other day, an equivalent dose of group 4 and 5 agents was added to the drinking water daily and alternately (one day 1.00 ppm of dichlorvos and the next day 2.5 ppm of acetamiprid).

In order to evaluate the fertility of male mice, a group of 35 male mice, which were treated with pesticides separately (according to the reproductive performance experiments), were mated with a group of 35 adult females. Female mice were examined daily (at the beginning of the day) to ensure mating, and after observing the vaginal plaque (white cap of vaginal opening), that day was considered as the zero day of pregnancy and from that day, the pregnant mice were kept in separate cages. On the 15^{th} day of pregnancy (the normal duration of pregnancy is 21 days), the mice were anesthetized and after dissection, the number of embryos in the two horns of the uterus was counted.

Measurement of some biological parameters

The weight of mice in the treatment groups was measured before adding the agents to the drinking water and was again measured at the end of the period (60 days). Also, during the experiment, the amount of food consumption by these treatment groups was measured.

Blood sampling

After drinking water mixed with pesticide was given to mice for 60 days, blood samples were taken from different groups of them. For this purpose, the mice were anesthetized, and then, the location of heart was determined by the tip of finger. After that, blood samples were taken directly from the animal's heart with a 5 mL syringe. All stages of maintenance and blood sampling of mice were performed in accordance with the rules approved by the ethics committee of the Islamic Azad University, Isfahan (Khorasgan) Branch, Iran, with the code IR.IAU.KHUISF.REC.1400.305.

For analysis of blood samples, enzyme-linked immunosorbent assay (ELISA) method, Monobind kit for follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and Diametra kit for testosterone (Tes) were used. For this purpose, first, all samples were prepared by centrifuging them at 3000 revolutions per minute (rpm) for 10 min. Then, the Stat Fax 2100 Microplate Reader device with a wavelength of 450–630 nm was used.

Also, testicular tissue was removed for evaluation of cell lines and was stained with hematoxylin and eosin. After microscope slide preparation, different cell lines were counted using a microscope.

Data analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software version 22. The difference between the groups was assessed by one-way analysis of variance (ANOVA), and Duncan's test at significance levels of $P < 0.05$ and $P < 0.01$ was employed to group the treatments.

Results

Table 1 shows the residual acetamiprid and dichlorvos according to the analysis of chromatography device, maximum residue limit (MRL), and the dose of agents added to drinking water of mice. As can be seen, in twofold and threefold doses of acetamiprid and dichlorvos, the residual agents are higher than the allowed level in greenhouse cucumber. The residual pesticide at twofold and threefold concentrations of the recommended dose in dichlorvos is 0.51 ± 0.005 and 1.16 ± 0.05, respectively, and in acetamiprid is 1.51 ± 0.005 and 2.51 ± 0.005, respectively. Therefore, the mentioned pesticide concentration was used in the drinking water of mice.

Figure 1 shows the amount of food consumption by the treated groups with dichlorvos and acetamiprid for 60 days, which indicated a significant difference between the treatments and the control group at the 99% confidence level.

Figure 2 shows the effect of different pesticide treatments on the weight of mice at the end of the experimental period. Comparison of the average weight change in mice showed that the control group had a significant difference compared to other treatments at 99% confidence level and the pesticides caused weight loss in mice.

Table 2 shows the effect of dichlorvos and acetamiprid at twofold and threefold concentrations of the recommended dose on reproductive hormones in mice ($P < 0.05$). Comparison of reproductive hormones indicated significant changes with increasing dose of pesticides.

Table 3 shows the results of comparing testicular germ cell number in mice treated with dichlorvos and acetamiprid at twofold and threefold concentrations of the recommended dose ($P < 0.05$). As can be seen, significant changes appeared in these cells with increase in the pesticide dose.

Figure 3 illustrates the effect of dichlorvos and acetamiprid at twofold and threefold concentrations of the recommended dose on mice fertility. The number of embryos decreased with increasing pesticide dose in the treatment groups compared to the control group. Also, the mixed group showed a significant difference compared to the other treatment groups (dichlorvos and acetamiprid) at threefold concentration of the recommended dose ($P < 0.05$).

Discussion

Farmers consider using a higher dose of pesticides, increasing spraying, or mixing different compounds as a general solution when faced with pest problems or to achieve a high-quality crop.[13] Therefore, in most cases, the residual pesticides on agricultural products reach the consumer as excess pesticide residues. According to the results of the first part of this study, the residues of acetamiprid and dichlorvos in greenhouse

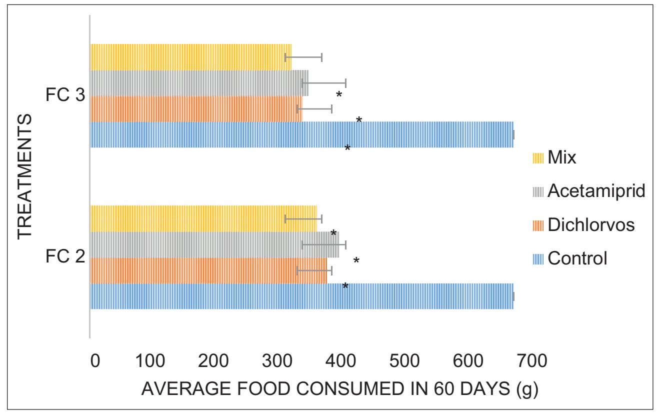

Figure 1: Comparing the amount of food consumption in groups treated with dichlorvos and acetamiprid at twofold and threefold concentrations of the recommended dose over 60 days. FC = food consumption 2: Twofold of the recommended dose 3: Threefold of the recommended dose *Significant difference with the control group (P < 0.01)

Table 1: Pesticide residues of acetamiprid and dichlorvos in greenhouse cucumber after 24 h according to chromatographic analysis, along with the MRL of each pesticide according to the Codex standard

Groups	Pesticide residues (ppm)±SD	MRL (ppm)	Dose of pesticides added to drinking water (ppm)
Treatment 1 Control: sprayed white water	0.01±0.00	-	-
Treatment 2 (Twofold the recommended dose (500 g/1000 L) of acetamiprid)	1.51±0.005	0.3	1.5
Treatment 3 (Threefold the recommended dose of acetamiprid)	2.51±0.005	0.3	2.5
Treatment 4 (Twofold the recommended dose (4 L/1000 L) of dichlorvos)	0.51±0.005	0.05	0.5
Treatment 5 (Threefold the recommended dose of dichlorvos)	1.16±0.05	0.05	1

Mean±SD. MRL=maximum residue limit

Table 2: The effect of pesticides on reproductive hormones in mice after 60 days of drinking water containing pesticide

Groups	FSH 2	LH 2	Tes 2	FSH 3	LH 3	Tes 3
Control	0.035±0.003	0.018±0.004	2.64±0.33	0.035±0.003	0.018±0.004	2.64±0.33
Dichlorvos	0.037±0.001	0.019±0.001	2.42±0.13	*0.042±0.001	*0.024±0.001	*1.86±0.05
Acetamiprid	0.036±0.001	0.019±0.004	2.52±0.25	*0.040±0.002	*0.023±0.0005	*2.04±0.13
Mix	0.038±0.005	0.020±0.005	2.34±0.11	**0.047±0.008	**0.028±0.0005	**1.52±0.16

FSH=Follicle-stimulating hormone (mIU/mL), LH=Luteinizing hormone (mIU/mL), SD=Standard deviation, Tes=Ttestosterone hormone (ng/mL). Mean±SD. 2: Twofold of the recommended dose. 3: Threefold of the recommended dose. *Significant difference with the control group ($P<0.05$). **Significant difference with the control group, treatment dichlorvos and acetamiprid ($P<0.05$)

Table 3: Calculation of the average number of testicular germ cells in mice after 60 days of drinking water containing the pesticide

Groups	S 2	SG 2	SC 2	S 3	SG 3	SC 3
Control	19.4±0.89	28.2±1.3	54.0±4.1	19.4±0.89	28.2±1.30	54.0±4.1
Dichlorvos	18.8±0.83	27.3±0.8	50.8±3.1	*16.4±0.54	*24.7±0.44	*49.2±0.83
Acetamiprid	19.1±0.74	28.0±1.0	53.0±4. 4	*17.0±1.73	*25.9±1.02	*50.4±0.54
Mix	18.4±0.54	26.6±0.4	48.0±1.2	**14.5±0.70	**22.1±0.74	**46.0±1.00

S=Stem cell, SC=Spermatocyte, SD=Standard deviation, SG=Spermatogonia. Mean±SD. 2: Twofold of the recommended dose. 3: Threefold of the recommended dose. *Significant difference with the control group ($P<0.05$). **Significant difference with the control group, treatment dichlorvos and acetamiprid ($P<0.05$)

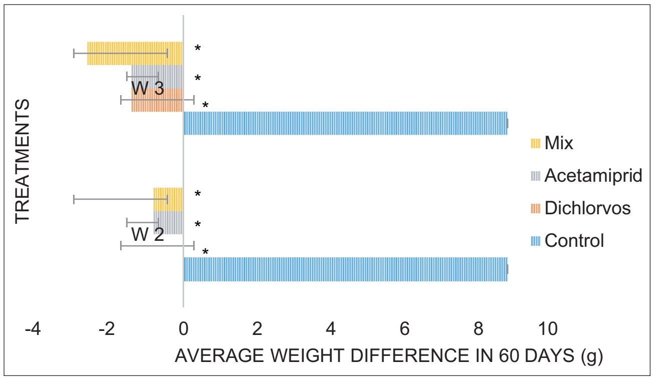

Figure 2: The effect of dichlorvos and acetamiprid at twofold and threefold concentrations of the recommended dose on mice weight loss in 60 days. W = weight 2: Twofold of the recommended dose 3: Threefold of the recommended dose *Significant difference with the control group ($P < 0.01$)

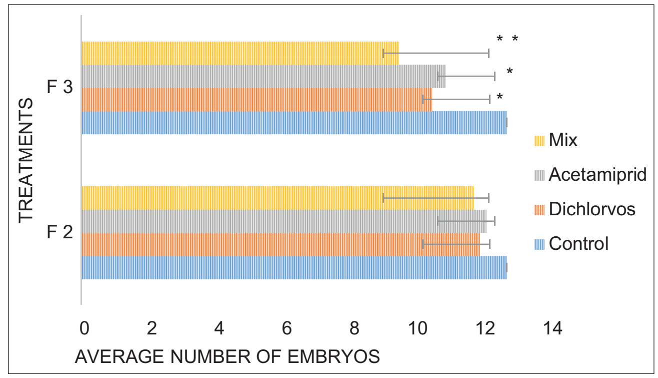

Figure 3: The effect of dichlorvos and acetamiprid (in twofold and threefold concentrations of the recommended dose) on the mice fertility after 60 days of drinking water containing pesticides. F = fertility 2: Twofold of the recommended dose 3: Threefold of the recommended dose *Significant difference with the control group ($P < 0.05$) **Significant difference with the control group, treatment dichlorvos and acetamiprid ($P < 0.05$)

cucumber after 24 h at twofold and threefold concentrations of the recommended dose were higher than the MRL set by Codex and the National Organization for Standardization [Table 1]. In this regard, the residual agents on 21% of the fruits and vegetables that were evaluated were higher than MRL.[14,15] Pesticide residues, which are used higher than the allowable limit in agricultural products of underdeveloped and developing countries, are more likely to appear due to increasing population and food needs, lack of training of producers and financial incentives, absence of regulatory bodies, and noncompliance with PHI.[1] For example, in a scientific research in Iran, cucumbers and tomatoes collected from vegetable and fruit fields were evaluated, and it was found that the residues of diazinon and carbaryl were higher than the allowable level, which were 0.37 and 0.72 µg/g for cucumber and tomato, respectively.[16]

Although the World Health Organization has a strong focus on food health, in most underdeveloped and developing countries, the laws on pesticide residues are less likely to be enforced. Since the residual effects of pesticides in food are not immediately apparent in humans and will be effective throughout life (from fertility to death and even after death with inherited consequences),[17] the effects of residual pesticides in food on health and the quality of life of some mammals such as mice can be examined as a human-like sample. Based on the results of the second part of this study, the effects of residual dichlorvos and acetamiprid at twofold and threefold concentrations of the recommended dose on mice caused body weight loss and lower food consumption compared to the control group [Figures 1 and 2]. Body weight loss and lower food consumption have also been observed by exposing mice to malathion[18,19] and various pesticides.[20] Nutritional indicators can be used as useful tools to improve understanding of the nutrition physiology and the effects of various substances (e.g. pesticides) in the body. Therefore, possible reasons for reducing the food consumption and body weight loss can be the destruction of lipids and proteins due to exposure to pesticides and oxidative stress.[21,22]

In this study, analysis of Tes, FSH, and LH showed that adding the remaining equivalent of dichlorvos and acetamiprid in greenhouse cucumber at twofold concentration of the recommended dose to the drinking water of laboratory mice increased FSH and LH levels and decreased Tes level, although statistically this reduction was not significant [Table 2]. Due to the different routes of entry of pesticides into the body, such as their presence in other products, the entry of higher levels of these pesticides into the body is inevitable. Therefore, with threefold concentration of the recommended dose of dichlorvos and acetamiprid, the trend of increasing FSH and LH levels as well as decreasing Tes became significant. Also, the mixed group indicated a significant difference with the other two treatment groups [Table 2]. These findings are consistent with the research of Bahrami *et al.*,[23] in which the mice were exposed to phthalate. In this regard, Bal *et al.*[24] conducted a study on the effect of neonicotinoid insecticides on male rats, which indicated that at low doses of insecticides, the decreasing trend of Tes was not significant and on increasing the dose of the insecticide, this process became more intense and significant. In support of this study, the research has illustrated that long-term exposure to pesticides leads to increase in their destructive effects (including the impact on reproductive performance).[25,26] Therefore, the decreasing trend of Tes production can be considered as a result of the damage of Leydig cells (Tes-secreting cells in the testicular tissue) and the increasing trend of FSH and LH production is a response to it.[27,28]

In this study, adding the residual equivalent of dichlorvos and acetamiprid at twofold concentrations of the recommended dose to the drinking water of mice reduced testicular germ cells (stem cell, spermatogonia, and spermatocyte) and by increasing the dose to three times, this decreasing trend became significant. Also, the mixed group showed a significant difference with the other two treatment groups (dichlorvos and acetamiprid) [Table 3]. In this regard, 40 adult male mice were exposed to dursban pesticide at doses of 15 and 30 mg/kg. By preparing tissue sections from the testis, different cell lines were counted, and the results indicated that the number of these cells in the experimental groups compared to the control group demonstrated a significant decrease.[29] In another study, 32 adult male mice were exposed to ethylhexyl to evaluate their reproductive system function. The results indicated that the number of spermatogonia and spermatocytes declined and also sperm motility decreased.[23]

Human fertility is a very sensitive process that can be influenced by many factors such as parental age, maternal condition, smoking, alcohol and coffee consumption, socioeconomic status, genetic parameters, hormonal imbalance, and using pesticides.[30] Adding the residual equivalent of dichlorvos and acetamiprid to cucumber at twofold concentration of the recommended dose to the drinking water of mice reduced the number of embryos, and by increasing the amount of pesticides to threefold concentration of the recommended dose, the results were significant. Also, the mixed group demonstrated a significant difference with the other two treatment groups [Figure 3]. In this regard, Gu *et al*.[31] studied the effect of neonicotinoid pesticide on male mice fertility. The results showed that the presence of chemical substances affected the fertilization process and zygotes, as well as the number of embryos. These results are consistent with recent studies on neonicotinoid pesticides that indicate impaired mammalian reproduction.[32,33]

Declining Tes production and increasing FSH and LH levels represent an initial testicular defect[22] due to the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which causes apoptosis[1] of Leydig cells.[34] By reducing testicular germ cells, it is inferred that pesticides decrease germinal cells by stopping mitosis cell division and inducing cell death. Given that these cells are essential in the process of spermatogenesis, the depletion of these cells affects cell lines such as spermatogonia.[35] Agricultural pesticides disrupt the process of spermatogenesis and damage the process of sperm production either by having a direct effect on the testicular tissue, atrophy of cells and their death, or by causing disturbances in the process of secretion of sex hormones.[36,37] Decreasing sperm motility, puberty disorder, and DNA damage are some of the reasons that lead to reduced male fertility.[25-39] Prolonged exposure to pesticides causes DNA strand breaks and crosslinks in mice sperm. Reduced transcription of defective transmitted genomes by sperm stops embryonic development. Chromatin abnormalities are associated with decreasing embryonic fission, which determines the importance of the association between chromatin damage and embryonic growth potential and reduces two-cell embryo and continues embryonic division.[25] Another factor in reducing the number of embryos is the free radical production of ROS, which is involved in stopping the meiosis growth of oocytes and in embryo growth retardation and cell death.[40]

1 The death of cells which occurs as a normal and controlled part of an organism's growth or development.

Conclusion

Improper and inadvertent use of pesticides (especially in underdeveloped and developing countries) is contrary to ecological principles and can be the source of several problems. Although the use of pesticides has increased agricultural production, residues of these agents enter human body together with food. Therefore, monitoring programs must be constantly implemented for the presence of pesticide residues in food to check the MRL and control the intake of these substances through the diet. According to the results of this study, the residual equivalents of dichlorvos and acetamiprid in cucumber, which were added to the drinking water of laboratory mice, not only caused biological changes (body weight loss and decreasing food consumption), but also reduced the Tes production, testicular germ cells, and embryo number and increased FSH and LH levels. Therefore, the addition of dichlorvos and acetamiprid pesticides to the drinking water of mice can be considered as a factor of biological disorders and reducing reproductive potential. Therefore, it is recommended to pay special attention to their consumption management.

Ethics statement

All ethical cases of working with laboratory animals in this study were performed according to the rules approved by the ethics committee with the identification code IR.IAU.KHUISF.REC.1400.305 at the Islamic Azad University, Isfahan (Khorasgan) branch, Iran.

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Conflicts of interest

There are no conflicts of interest.

References

1. Sarkara S, Dias J, Keeley J, Mohring N, Jansen K. The use of pesticides in developing countries and their impact on health and the right to food. Policy Department for External Relations Directorate General for External Policies of the Union PE 653.622 - January 2021.
2. Barraza D, Jansen K, Wesseling C, van Wendel de Joode B. Pesticide risk perceptions among bystanders of aerial spraying on bananas in Costa Rica. Enviro Res 2020;189:109877.
3. Juraske R, Mutel CL, Stoessel F, Hellweg S. Life cycle human toxicity assessment of pesticides: Comparing fruit and vegetable diets in Switzerland and the United States. Chemosphere 2009;77:939-45.
4. Environmental Working Group (EWG), EWG's 2021 Shopper's Guide to Pesticides in Produce™. Over 90 Percent of Non-Organic Citrus Fruits Contain Fungicides Linked to Cancer and Hormone Disruption. By EWG Science Team. MARCH 17, 2021.
5. Buszewski B, Bukowska M, Ligor M, Staneczko-Baranowska I. A holistic study of neonicotinoids neuroactive insecticides—properties, applications, occurrence, and analysis. Environ Sci Pollut Res Int 2019;26:34723–40.
6. Nguyen T, Rosello C, Bélanger R, Ratti C. Fate of residual pesticides in fruit and vegetable waste (FVW) processing. Foods 2020;9:1468. doi: 10.3390/foods9101468.
7. Han W, Tian Y, Shen X. Human exposure to neonicotinoid insecticides and the evaluation of their potential toxicity: An overview. Chemosphere 2018;192:59–65.
8. Bal R, Erdogan S, Theophilidis G, Baydas G, Naziroglu M. Assessing the effects of the neonicotinoid insecticide imidacloprid in the cholinergic

synapses of the stellate cells of the mouse cochlear nucleus using whole-cell patch-clamp recording. Neurotoxicology 2010;31:113–20.
9. Terayama H, Qu N, Endo H, Ito M, Tsukamoto H, Umemoto K. *et al*. Effect of acetamiprid on the immature murine testes. Int J Environ Health Res 2018;28:683–96.
10. Flora A, Schulz R, Benfante R, Battaglioli E, Terzano S, Clementi F, *et al*. Transcriptional regulation of the human a5 nicotinic receptor subunit gene in neuronal and nonneuronal tissues. Eur J Pharmacol 2000;393:85–95.
11. Cavari Y, Landau D, Sofer S, Leibson T, Lazar I. Organophosphate poisoning-induced acuterenal failure. Pediatr Emerg Care 2013;29:646-7.
12. Sengupta P, Banerjee R. Environmental toxins: Alarming impacts of pesticides on male fertility. Hum Exper Toxicol 2014;33:1017-39.
13. Thorburn C. The rise and demise of integrated pest management in Rice in Indonesia. Insects 2015;6:381-408.
14. Mustapha F, Jallow A, Dawood G, Awadh M, Albaho S, Vimala YD, *et al*. Monitoring of pesticide residues in commonly used fruits and vegetables in Kuwait. Int J Environ Res Public Health 2017;14:833.
15. Leupraserta L, Monmorab S, Puydechab M, Chapmana RS, Siriwong W, Taneepanichskula S. 2014. Innovative Pesticide Kit Model for Vegetable Farm Safety Surveillance Program. ICESD: February 19-21, Singapore.
16. Amrollahi H, Pazoki R, Imani S. Pesticide Multiresidue analysis in tomato and cucumber samples collected from fruit and vegetable markets in Tehran, Iran. Middle East J Rehabil Health Stud 2018:e64271. doi: 10.5812/mejrh. 64271.
17. Verger P, Al-Yousfi AB. The dilemma of pesticide residues in fruits and vegetables in the Eastern Mediterranean Region. East Mediterr Health J 2020;26:760–1.
18. Ibrahim Ali1 R, Abdelbasset Ibrahim M. Malathion induced testicular toxicity and oxidative damage in male mice: The protective effect of curcumin. Egypt J Forensic Sci 2018. doi: 10.1186/s41935-018-0099-x.
19. Slimen S, Saloua EF, Najoua GH. Oxidative stress and cytotoxic potential of anticholinesterase insecticide, Malathion in reproductive toxicology of male adolescent mice after acute exposure. Iran J Basic Med Sci 2014;17:522-30.
20. Wang M, Guckland A, Murfitt R, Ebeling M, Sprenger D, Foudoulakis M, *et al*. Relationship between magnitude of body weight effects and exposure duration in mammalian toxicology studies and implications for ecotoxicological risk assessment. Environ Sci Eur 2019. doi: 10.1186/s12302-019-0221-1.
21. H Mossa AT, Heikal TM. Omara EAA. Physiological and histopathological changes in the liver of male rats exposed to paracetamol and diazinon. Asian Pac J Trop Biomed 2012;2:S1683-90.
22. McPherson R, Pincus M. Henry's Clinical Diagnosis and Management by Laboratory Methods. Elsevier. 24th ed. 2021.
23. Bahramia N, Goudarzib M, Hosseinzadehc A, Sabbaghd S, Reitere RJ, Mehrzadic S. Evaluating the protective effects of melatonin on di (2-ethylhexyl) phthalateinduced testicular injury in adult mice. Biomed Pharmacother 2018;108;515–23.
24. Bal R, Türk G, Tuzcu M, Yýlmaz Ö, Kuloðlu T, Baydaþ G, *et al*. Effects of the neonicotinoid insecticide, clothianidin, on the reproductive organ system in adult male rats. Drug Chem Toxicol 2013;36:421-9.
25. D'Occhio MJ, Hengstberger KJ, Johnston SD. Biology of sperm chromatin structure and relationship to male fertility and embryonic survival. Anim Reprod Sci 2007;101:1–17.
26. Arıcan EY, Gökçeoğlu Kayalı D, Ulus Karaca B, Boran T, Öztürk N, Okyar A, *et al*. Reproductive effects of subchronic exposure to acetamiprid in male rats. Scientific reports natureresearch Sci Rep 2020;10:8985.
27. Araki A, Mitsui T, Goudarzi H, Nakajima T, Miyashita C, Itoh S, *et al*. Prenatal di (2-ethylhexyl) phthalate exposure and disruption of adrenal androgens and glucocorticoids levels in cord blood: The Hokkaido Study. Sci Total Environ 2017;581:297–304.
28. Mostofi FK, Spaander P, Gingor K. Cousensus on pathological clasihication of testicular germ cell tumors. Prog Clin Biol Res 1990;357:267-76.
29. Fattahi E, Jorsaraei SGA, Moghadamnia AA. Effects of dursban on sexual hormones and changes of testis tissue in mice. J Babol Univ Med Sci 2013;15:42-50.
30. Shojaei Saadi H, Abdollahi M. The importance of pesticides effects on human reproduction in farmers. Int J Pharmacol 2012;8:467-9.
31. Gu YH, Li Y, Huang XF, Zheng JF, Yang J, Diao H, *et al*. Reproductive effects of two neonicotinoid insecticides on mouse sperm function and early embryonic development in vitro. PLoS One 2013;8:e70112.
32. Kapoor U, Srivastava MK, Srivastava LP. Toxicological impact of technical imidacloprid on ovarian morphology, hormones and antioxidant enzymes in female rats. Food Chem Toxicol 2011;49:3086–9.
33. Ahmadi A. Evaluation of morphometrical and histomorphometrical changes of testes, fertility potential and sperm quality in mice treated with aflatoxin. J Shahid Sadoughi Univ Med Sci 2017;24:994-1003.
34. Erkekoglu P, Rachidi W, Yuzugullu OG, Giray B, Favier A, Ozturk M, *et al*. Evaluation of cytotoxicity and oxidative DNA damaging effects of di (2-ethylhexyl) - phthalate (DEHP) and mono (2 ethylhexyl)-phthalate (MEHP) on MA-10 Leydig cells and protection by selenium, Toxicol. Appl Pharmacol 2010;248:52–62.
35. Joshi SC, Mathur R, Gulati N. Testicular toxicity of chlorpyrifos (an organophosphate pesticide) in albino rat. Toxicol Ind Health 2017;23:439-44.
36. Mnif W, Ibn Hadj Hassine A, Bouaziz A, Bartegi A, Thomas O, *et al*. Effect of endocrine disruptor pesticides: A review. Int J Environ Res Public Health 2011;8:2265-303.
37. Mandal TK, Das NS. Testicular gametogenic and steroidogenic activities in chlorpyrifos insecticide-treated rats: A correlation study with testicular oxidative stress and role of antioxidant enzyme defence systems in Sprague-Dawley rats. Andrologia 2012;44:102-15.
38. Varnet P, Fulton N, Wallace C, Aitken RJ. Analysis of a plasma membrane redox system in rat epididymalspermatozoa. Biol Reprod 2001;65:1102-13.
39. Aitken RJ, West K, Buckingham D. Leukocytic infil tration into the human ejaculate and its association with semen quality oxidative stress and sperm function. J Androl 1994;15:343-52.
40. Shoukir Y, Chardonnens D, Campana A, Sakkas D. Blastocyst development from supernumery embryos after intracytoplasmic sperm injection. A paternal influence? Hum Reprod 1998;13:1632-7.