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Effect of atrazine and cyhalothrin on the progesterone level of pregnant rabbits and dietary digestibility in growing rabbits

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Abstract
This research was conducted to evaluate the effect of two agrochemicals, atrazine and cyhalothrin on the progesterone level and reproductive performance of pregnant rabbits expose by inhalation or oral administration and the dietary digestibility of growing rabbits. The two chemicals are extensively used in agriculture for the protection of agricultural produce. Atrazine and cyhalothrin are linked with reproductive irregularities in animals and humans. Atrazine and cyhalothrin affect the normal function of the endocrine system which may lead to a negative reproductive system. Past research predominantly focuses on the disrupting of the estrogen and/or androgen receptor, but the hormonal function of animals may be disrupted in many different ways based on exposure. In this research, our focus was on the serum progesterone level of pregnant rabbits. The chemicals target the neuroendocrine framework made up of organs in the body that works together with the central nervous system to control and regulate reproductive functions. The most prominent of these is the hypothalamic-pituitary-gonadal (HPG) axis which is important to perpetuate appropriate reproductive function. When these get disrupted, it decreases the normal rate of progesterone secretion and functions.
Twelve New Zealand White rabbits between the age range of 10 to 11 months old and weighing ± 1.87 kg were used for the evaluation. Ten females and two males, the study was conducted using a Pretest- Posttest Experimental Design with one treatment group (5 females and one male for mating) and one control group (5 females and one male for mating). Five female rabbits at gestation day 14 were exposed to atrazine and cyhalothrin by inhalation at a different time for seven days at a dose and rate equivalent to that recommended for a maize field. The results showed a significant reduction in the serum progesterone of the exposed animals, thus resulting into series of reproductive disorders like spontaneous abortion, premature birth, birth abnormality, low daily weight gain, and loss of appetite in doe, and low dietary digestibility of growing rabbits ($P<0.05$). In the second phase of the experiment, five female rabbits at gestation day 14 were exposed to atrazine and cyhalothrin in their drinking water at a different time at a dose of 0.003 mg/L respectively. The results showed a significant effect of atrazine on the progesterone level ($P<0.05$). At the dose of 0.003 mg/L, cyhalothrin did not affect the progesterone level of the exposed rabbits, but when the dose was increased to 0.005 mg/L, seventy-five percent abortions were recorded.

Keywords: atrazine; cyhalothrin; reproductive effects; progesterone; dietary digestibility; rabbit

1. Introduction
Food and Agriculture Organization (FAO) has defined the term agrochemical as any material, a mixture of substances used to prevent, destroy or control pest, including human or animal disease vector, undesirable plants or animal species causing or prying with the production, processing, storage, transport or selling of agrarian products, wood and woody products or food for animals or substances that may be given to animals for the control of insects, arachnids or other parasites in or on their bodies. The term is usually referred to as substances designed for use as a plant development regulator, fumigant, drying agents, or agent for thinning fruit or avoiding the untimely dropping of fruits. Agrochemicals are also referred to as substances or the combination of substances added to agriculture produce before or after harvest to protect goods during storage and transport from spoilage. These substances can be categorized as "insecticides (insect killers), fungicides (fungal killers), herbicides (weed killers), rodenticides (rodent killers), repellent (substances used to discourage vermin from cultivated land) and fumigants (gaseous chemicals used to remove microbial planting). Although agrochemicals can improve the quality and quantity of agricultural produce, they are reported to cause reproductive effects in animals [1]. For instance, agrochemicals can cause endocrine disruptions and neurological interruptions, abnormal functions of the immune system, reproductive disorder, and developmental abnormalities [2]. Because of this, the toxicity of agrochemicals to a non-target organism is a cardinal burden of the world. Several agrochemicals initiate endocrine disruption by intruding with the productive functions, release, transport, metabolic action, or elimination of hormones [3].

1.2 Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1, 3, 5-triazine)
Atrazine is a popularly used agrochemical in many parts of the world for the control of pre and post-emergence weeds in crops like maize, sorghum, sugarcane, pineapple, and so on.
Jilin province in the north of China is an agricultural environment producing a wide variety of agricultural produce with a high rate of agrochemicals, and it risks the potential of environmental pollution because of the rate of chemical circulation.
Besides its persistence, widespread, and dissemination of

atrazine, it remains a concern because most studies have reported that atrazine is a potential endocrine disruptor in low doses in fish [4], amphibians [5], reptiles, and human cell lines [4], and at higher doses in reptiles [6], birds [7], and laboratory rodents [8]. Atrazine is active in amphibians, its effect is active even at a dose as low as $0.1x10^{-9}$ [9]. Even though some studies have reported that atrazine does not affect amphibians at certain laboratory conditions [10], in other reports, atrazine decreases testicular volume, germ cell, Sertoli cell numbers, and induces hermaphroditism in amphibian [11, 9], reduces testosterone [9]; and induces testicular oogenesis [12, 13]. Furthermore, demasculinization and feminization of amphibians are common in an agricultural environment where atrazine is the most used chemical [14] and directly related to atrazine toxicity in the wild [15, 16].

Research result also shows that atrazine exposures may result in birth defects. [17, 18] investigated the possibility of babies conceived during the period when agrochemicals in surface water are highest are at risk of birth defects.

Atrazine has also been found to revert the brain's pituitary operations, resulting in the obliteration of two hormones, luteinizing hormone and prolactin hormone [19].

1.2 Cyhalothrin

Cyhalothrin is broadly used to prevent various pests' infestation. It is used in the control of pests in the cotton field, fruit trees, and vegetable farms **[20]**.

Cyhalothrin does not only affect insects, but it is also toxic to mammals**.** The toxic signs include muscular trepidation, bedlam, and weakness of limbs, contortion, coma, and death. The respiratory disorder has been reported in animals after guzzling high doses of cyhalothrin, while its dermis contact in the facial area may cause illusory feelings of creeping or numbness.

Cyhalothrin is also a derma and eye irritant; its effects range from mild to serious skin irritation. Decreased feed intake, reduced body weight, and relative gonad weights loss have been noted in rabbits treated with cyhalothrin [21]**.**

Besides, hypothesized toxic effects of cyhalothrin, it reduces the number of implantation sites, number of doable fetuses and affects the daily weight gain of fetuses in rabbits treated with cyhalothrin reported by [22]**.**

A reduction in pregnancy rate, number of implantation sites, and the total number of recovered fetuses have also been reported in female animals treated with cyhalothrin during pregnancy and allowed mating with untreated male rabbits. The descendants of cyhalothrin exposed parents also showed toxic effects [23].

Cyhalothrin is extremely poisonous to cats which cannot withstand the therapeutic doses for dog**s [24].**

In this research, our focus is on the changes that occur in the progesterone level of pregnant rabbit due to exposure to atrazine, and cyhalothrin and how those changes affect the pregnancy, the unborn, as well the reproductive behavior of the rabbit and highlighting the results obtained by other research groups.

1.3 Research Objective

The general aim of this study was to;

1. Investigate the effects of atrazine and cyhalothrin exposure on the progesterone level of a pregnant rabbit as well as birth weight and daily weight gain of a newborn animal
2. Effect of atrazine and cyhalothrin on feed intake and dietary digestibility in pregnant and growing rabbits.

2. Materials and Methods

2.1 Effect of Atrazine and cyhalothrin Exposure on the progesterone level and reproductive Performance of Pregnant Rabbit by Inhalation and Oral Administration

2.1.1 Study Area

The research was carried out at the Jilin Agricultural University, Changchun, China.

The animals used and experimental procedures were approved by the committee on research of the Jilin Agricultural University and the experiment was conducted under the supervision of professor Zhongjun Liu of the department of Wild Production in Captivity, College of Animal Science and Technology, Jilin Agricultural University.

2.1.2 Chemicals

Atrazine: (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is a widely used herbicide in many countries of the world used for the control of broadleaf and grassy weeds in crops like maize, rice, sugar cane, etc (97% active ingredient). It was purchased from tianjiluyan bio-pharmaceutical co. LTD. Zip code: 30270 address: east of mahuan village, dagangzhongtang town, binhai new area, tianjin city Tel: 28036 19-20-83.

Cyhalothrin: Lambda-cyhalothrin is in EPA Toxicity Class II, where class I is the most toxic and Class IV is the least toxic. The product containing Class II toxicity must include the signal word "WARNING" on the label. While lambda-cyhalothrin may be potentially harmful to people or pets if swallowed, it poses little threat when used strictly according to the product. Label before applying pesticides. Cyhalothrin is used for repelling insects (4.5% active ingredient). Obtained from No.1, Gonhua Road, Huyuan Industrial Park, Tianjin Tel: 022-8371891583718916, Fax: 022-83718919 zip code 300384.

2.1.3 Management of Experimental Animals.

Five female rabbits were kept indoor as the control group, blood samples were collected at gestation day 14 and another sample after 14 days of parturition. Another group of five pregnant rabbits used as treatment group was kept near a maize field. Blood samples were collected before and after the application of atrazine to the maize field.

The five pregnant rabbits in the treatment group were kept under a tree, atrazine was sprayed around the cage, and blood samples were collected to compare the progesterone level with that of rabbits kept in the control group. The treatment group was again moved near the maize field when cyhalothrin was sprayed. Blood samples were collected and compared with that of the progesterone level of those kept in the control group.

The animals were managed intensively and housed one each in a specially constructed cage with gate. Facilities for drinking and feeding were provided. A total of 300g (pellet) feed and clean water was given to each animal per day during the period of the experiment.

2.1.4 Blood and Air Sample Collection

Blood sample collection was by cardiac puncher [28]

Table 1: Materials and manufacturer

Materials	Manufacturer
Steroid syringe (5ml) and needle (21)	Manufacturer: Changchun Minjian Medical Equipment CO., LTD Address: Economic Development zone, Helon Town, Nongan County(Sun Vegetable Association, Helon Town) Tel:0431-834306668 fax: 043183433232 Zip code 130216
Blood collection tube	No. 16, Zhanqian Road, Railway Station, Meiyan District, Taizhou City Tel:0523-8866855 fax:0523-886655
Centrifuge	A Shanghai meixiang Instrument Co., LTD no. 5428-166,Songjin Road, boashan district, Shanghai tel: 021-55966049 13564733908
SIMEN centaur xp	Siemens Healthcare Diagnostic (Shanghai) Co Ltd No.278Zhouzhu Road Pudong New AreaShanghai, China Tel. +864008105888
Ethyl alcohol	Tianjin Xinbote Chemical Industry 189 Xinda Road, Hebei District, Tianjin, China
Disposable gloves	North of changjin road, yuelai town, haimen city, Jiangsu province 0531-82665182: 0513-82855119 Website www--gmpst.com zip code 226134
Disposable plastic bag	Not available
Cotton	Winner Medical CO., Ltd Winner Industrial Park, No.660 Bulong Road, Long Hua New Dist. Shenzhen, Guangdong, 518108 China www.winnermedical.com Tel. +86-75528138888
Airflow meter	Chongqing Ruilibi Gas Equipment Limited Company Address: China, Chongqing District, 108 Shengdao
Electromagnetic air pump	Sensen Group Co., LTD Mr. SUN SUN, No.6179, Baima Street, Ma-Ao, Dinghai, China
Anesthetic agent	Tianjin Xinbote Chemical Industry 189 Xinda Road, Hebei District, Tianjin, China
Deionized water	Produced in the laboratory

2.1.5 Technical Route

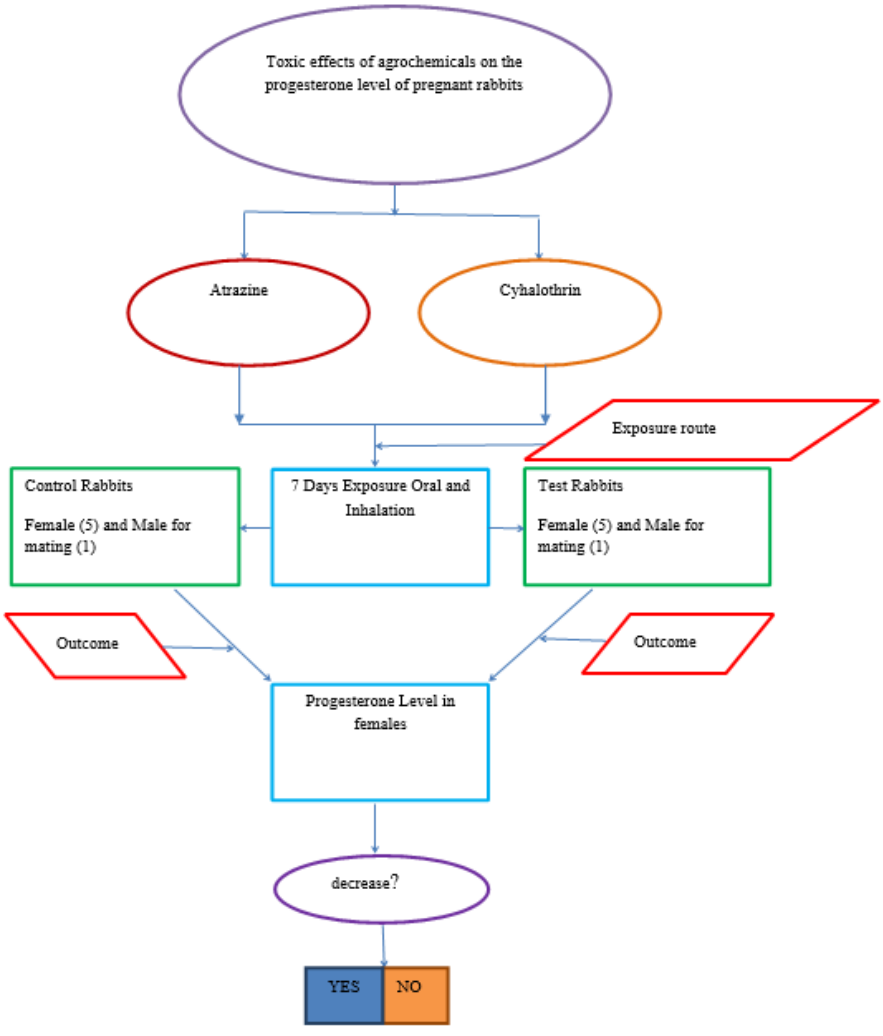

Fig 1: Workflow chart

2.2 Predicting and Measuring Environmental Air Concentration of Atrazine after Ground Application

The ability to detect a chemical concentration in the air is important in evaluating the effect of the chemical. In this test, an electromagnetic air pump was connected to an airflow meter. A tube was connected to the airflow and placed in one liter of deionized water which was used as a solvent medium to trap the air since atrazine does not dissolve in water. The samples were collected over three day period (day1 (12hrs), day2 (12hrs), and day3 (12hrs)) and analyzed using gas chromatography to get the concentration of atrazine in the air for the three days.

2.3 Effect of Atrazine and Cyhalothrin on Feed Intake and Dietary Digestibility in Growing and Pregnant Rabbits

Dry matter digestibility test was conducted to determine the digestibility ability of pregnant and growing rabbits.

2.3.1Animals, Materials and Management

Ten growing New Zealand White rabbits between the ages of 2.5 to 3 months, weighing ± 0.6 kg were kept in individual cages. A simple device with a piece of screen on the top and plastic under the screen was fixed under each cage for the separation of urine and feces. The experiment was conducted in two phases. During the first phase (six days), the animal was fed a commercial pellet feed ad libitum and given fresh water. During the second phase (six days), the animals were fed a commercial pellet ad libitum and given water containing 0.003 mg/L of atrazine. Daily feed intake of each rabbit was recorded and fecal samples were collected every morning of the trail. The same method was applied to cyhalothrin.

The feed and fecal samples were put in an oven at 100°C for 24 hours to determine the dry matter.

Dry matter digestibility (DMD) was calculated as follows:

$$\text{DMD (\%)} = \frac{\text{feed intake - faeces output}}{\text{feed intake}} \times 100$$

2.4 Statistical Analysis

Results were expressed as Mean ± SE. All data obtained from the experiment were analyzed via ANOVA followed by the Tukey post hoc comparison test. The statistical program used was GraphPad PRISM 6.01. P-Value (< 0.05) was considered statistically significant.

3. Result

3.1 Effects of Atrazine and Cyhalothrin on the Progesterone of Pregnant Rabbits by Inhalation

Table2. Listed the data of progesterone levels in pregnant rabbits kept near the maize field before and after spray of atrazine. Before exposure to atrazine, the serum progesterone level in this group of animals (8.838) was close to (or slightly higher than) that in rabbits kept indoor on the farm. After exposure to atrazine, serum progesterone reduced significantly ($p<0.05$).

Table 2: Progesterone level of pregnant rabbits before and after exposure to atrazine by inhalation

Rabbits	Progesterone level (ng/mL)	
	Control	Treatment
Mean±SE	8.838±0.65[a]	5.813±0.59[b]
P-value	0.0140	

Means pursued by different lowercase letters into a row in each treatment and parameter, as are significantly different $p < 0.05$ according to the Tukey test. ± standard error, *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.05$; ns, Non-significant

Table 3: Progesterone levels of pregnant rabbits before and after exposure to atrazine nearby

Rabbits	Progesterone level (ng/mL)	
	Control	Treatment
Mean ± SE	7.880±0.33[a]	2.592±1.56[b]
P-value	0.0108	

Means are pursued by different lowercase letters into a row in each treatment and parameter, as are significantly different $p < 0.05$ according to the Tukey test. ± standard error, *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.05$; ns, Non-significant

Table3.Compared the progesterone level of pregnant rabbit exposed to atrazine nearby. Atrazine was applied closed to the rabbit's cage to determine its effect on the progesterone level. As indicated by data in the table, the effect was statistically significant. The result also showed that rabbit exposed in an environment containing atrazine higher than 0.0110 mg/m^3, as indicated in table 8, its serum progesterone decline significantly.

Table 4: Progesterone levels of pregnant rabbits before and after exposure to cyhalothrin inhalation

Rabbits	Progesterone level (ng/mL)	
	Control	Treatment (0.003mg/L)
Mean±SE	7.880±0.33[a]	3.92 ± 0.58[b]
P-value	0.0001	

Means are pursued by different lowercase letters into a row in each treatment and parameter, as are significantly different $p < 0.05$ according to the Tukey test. ± standard error, *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.05$; ns, Non-significant

3.2 Effects of Atrazine and Cyhalothrin on the Progesterone of Pregnant Rabbits Administered in Drinking Water

Table 5: Progesterone levels of pregnant rabbits before and after exposure to cyhalothrin orally

Rabbits	Progesterone level(ng/mL)	
	Control	Treatment
Mean±SE	7.880±0.33[a]	7.625±0.45[a]
P-value	0.6968	

Means are pursued by different lowercase letters into a row in each treatment and parameter, as are significantly different $p < 0.05$ according to the Tukey test. ± standard error, *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.05$

Table 6: Progesterone levels of pregnant rabbits before and after exposure to atrazine orally

Rabbits	Progesterone level(ng/mL)	
	Control	Treatment
Mean±SE	7.880±0.33[a]	2.916±1.04[b]
P-value	0.0019	

Means are pursued by different lowercase letters into a row in each treatment and parameter, as are significantly different $p < 0.05$ according to the Tukey test. ± standard error, *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.05$; ns, Non-significant

Table 7. Showed the effect of cyhalothrin on the progesterone level of pregnant rabbits administers in the drinking water. Even though the data did not show a significant effect on the progesterone at cyhalothrin concentration of 0.003mg/L, there was a 75% abortion rate when cyhalothrin concentration was increased to 0.005 mg/L (Table5).

Table 7: Abortion rate of rabbits at different treatment level

	Inhalation			Oral		
	Atrazine(maizefiel)	Atrazine(in close proximity)	Cyholathrin (maizefield)	Atrazine	Cyholathrin at 0.003mg/L	Cyhalothrin at 0.05mg/L
Abortion rate%	0	0	50	0	0	75

Table 7 showed the different rate of abortion of rabbit at different level of exposure. Though atrazine affects the serum progesterone secretion and thus causing some reproductive problems, but did not terminate the pregnancy at any of the treatment level. As indicated in the table, cyhalothrin at different level of exposure cause termination.

3.3 Air Sample Collection Result

During the three days of monitoring, the atrazine concentration in the air being absorbed in the deionized water was 0.0110 to 0.181 mg/m^3 as indicated in (Table 4.6). The retention time for atrazine was 9.442 min as shown in figure 2

Table 8: Concentration of atrazine in the air after ground application

	Day1	Day2	Day3
Solvent(ml)	1L	1L	1L
Airflow(ml/h)	409.833	456.833	420.33
Total air volume	4918	5482	5044
Total atrazine absorbed	0.0778	0.0606	0.0915
Atrazine concentration in the air (mg/m^3)	0.0158	0.0110	0.0181

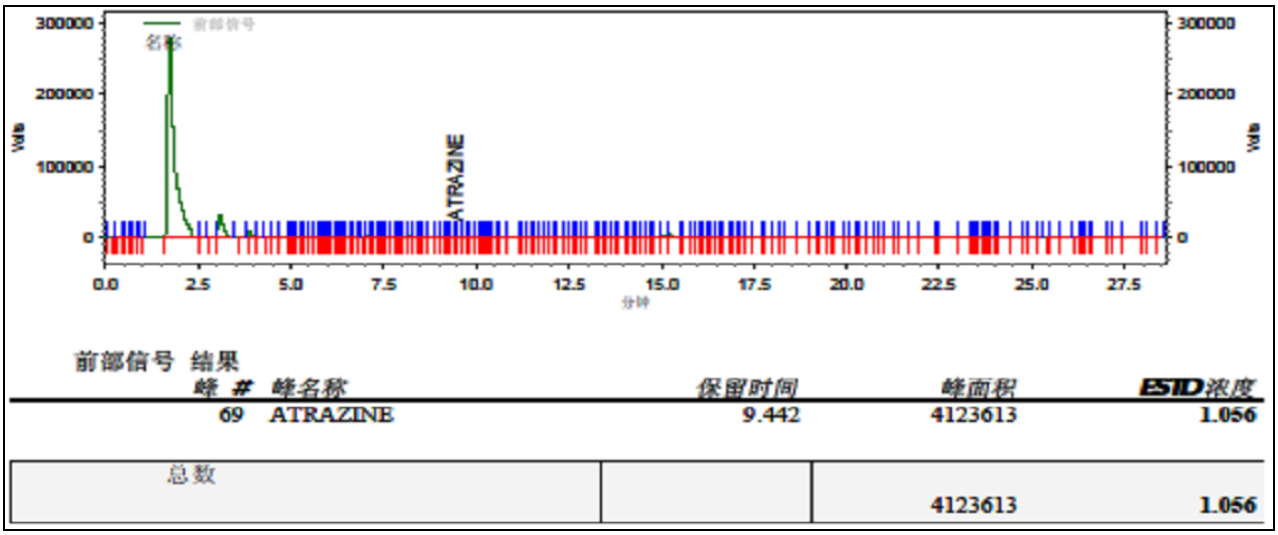

Fig 2: chromatogram of atrazine concentration in the air

3.4 Effect of Atrazine and Cyhalothrin on Feed Intake and Dietary Digestibility in Growing and Pregnant

Rabbits

The effects of atrazine and cyhalothrin on feed intake and dietary digestibility of growing rabbits were determined and based on results obtained as indicated by data in Table 9, there was a significant effect on feed intake and dietary digestibility of growing rabbits exposed to the chemicals ($p<0.01$).

Table 9: Comparing the effects of atrazine and cyhalothrin on feed intake and dietary digestibility in growing rabbits

Variable				
	Control	Atrazine	Cyhalothrin	Sig
	Mean±SE	Mean±SE	Mean±SE	
Daily feed intake	172.2±4.98a	163.2±5.70a	159.5±7.19a	0.323
Daily Faeces Output	70±3.56a	68±2.93a	84.12±3.42b	.0.003**
Digestibility	58±2.53a	57.7±1.89a	46.5±1.13b	0.0002***

Means pursued by different lowercase letters into a row in each treatment and parameter, as are significantly different $p < 0.05$ according to the Tukey test. ± standard error, *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.05$; ns, Non-significant

The effects of atrazine and cyhalothrin on feed intake and dietary digestibility of pregnant rabbits were determined and based on results obtained as indicated by data in table 10, there was no statistically significant effect on feed intake and dietary digestibility of pregnant rabbits exposed to the chemicals. However, the feces outputs were significantly increased in treatment groups.

Table 10: Compared the effects of atrazine and cyhalothrin on feed intake and dietary digestibility in pregnant rabbits

Variable				
	Control	**Atrazine**	**Cyhalothrin**	**Sig**
	Mean±SE	**Mean±SE**	**Mean±SE**	
Daily feed intake	172.2±4.98a	163.2±5.70a	159.5±7.19a	0.323
Daily Faeces Output	70±3.56a	68±2.93a	84.12±3.42b	.0.003**
Digestibility	58±2.53a	57.7±1.89a	46.5±1.13b	0.0002***

Means pursued by different lowercase letters into a row in each treatment and parameter, as are significantly different $p < 0.05$ according to the Tukey test. ± standard error, *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.05$; ns, Non-significant.

3.5 Effect of Atrazine and Cyhalothrin on the Birth Weight and Daily Weight Gain of Young Rabbits

To determine the effect of atrazine and cyhalothrin on the birth weight and daily weight gain of young rabbits, the babies of control rabbits were weighed on day one of birth and day on day 30 of growth. The same method was used for babies in the treatment group. As shown in table11, the birth weight and daily weight gain of babies in the group treated with atrazine and cyhalothrin were significantly lower than those in the control group ($p<0.01$).

Table 11: Comparison of atrazine and cyhalothrin effect on birth with and daily weight gain of the baby rabbit

Variable	Control	Atrazine	Cyhalothrin	Sig.[c]
	Mean±SE	Mean±SE	Mean±SE	
Birth weight day1(g)	70±0.00a	50±0.00b	50±0.00b	***
Birth weight day30(g)	520±0.01a	400±0.02b	420±0.01b	***

Means pursued by different lowercase letters into a row in each treatment and parameter, as are significantly different $p < 0.05$ according to the Tukey test. ± standard error, *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.05$; ns, Non-significant

4. General Discussion Conclusion, and Recommendation

4.1 General Discussion

Agrochemicals targeting the female reproductive system can cause a wide range of adverse reactions. Changes in sexual behavior, the initiation of puberty, cyclicity, fertility, gestation period, pregnancy and lactation, and premature menopause, among others are possible signs of female reproductive toxicity: all can disrupt a female reproduction. The mechanism of termination of gestation caused by atrazine and cyhalothrin is not clear; one possibility is that the chemicals affect the function of the corpus luteum resulting in the decline of serum progesterone secretion. This study was conducted to verify the speculation. The results obtained provide evidence of the potential effect of atrazine and cyhalothrin on the secretion of serum progesterone of exposed rabbits, thus leading to abnormalities in the reproductive processes of the animal as was similarly discussed by [26]. The results obtained support the hypothesis of this research. The study revealed the adverse effect of agrochemicals on non-target organisms, ranging from mal to severe toxicity. The findings showed the negative effects of atrazine and cyhalothrin on the progesterone level of pregnant rabbits in all treatment forms. These chemicals affect the normal function of the endocrine system which may lead to the negative reproductive system. Past studies focus mainly on interaction with the estrogen and/or androgen receptor, but exposure-based disruption of hormonal function can occur in many ways. Our focus was on the serum progesterone, atrazine and cyhalothrin generally target the neuroendocrine systems that are made up of organs throughout the body that works together with the central nervous system to regulate biological functions. Most important to perpetuate proper reproductive functions is the hypothalamic-pituitary-gonadal (HPG) axis. When these get disrupted, it decreases the normal rate of progesterone secretion and functions. Specifically, atrazine

and cyhalothrin affect the normal function of the corpus luteum resulting in the decline of the serum progesterone secretion thus leading to a series of reproductive disorders like spontaneous abortion, alteration of the length of gestation, premature birth, birth abnormality, etc. In one case, one exposed animal gave birth to three pulps four days before the due date, and later had five on the due date. In another case, the female animal died in the process of giving birth three days before the due date after being exposed orally. I am not sure if this happens as a result of the chemicals but further investigation is required to elucidate the possibility.

Progesterone is an endogenous progestogen steroid and sex hormone which is involved in several species' menstrual cycle, conception, and embryogenesis. It belongs to a group of steroid hormones called progestin and is the most progestogenic in the body. Progesterone has many important roles within the body; it is also a critical metabolic intermediate in the synthesis of other endogenous steroids, including the sex hormones and the corticosteroids, and plays an important role in brain function as a neurosteroid.

After ovulation occurs, the ovaries must start to produce progesterone needed by the uterus under normal conditions. Progesterone causes the uterine lining or endometrium to thicken to protect pregnancy when it functions properly.

The study result also showed that both cyhalothrin and atrazine significantly harmed the progesterone level of pregnant rabbits in all exposure method. Tables 2, 3, and 4 showed a statistically significant effect of the chemicals on the progesterone level of pregnant rabbits exposed by inhalation which resulted in reproductive disorders. The chemicals were applied at the dose and rate equivalent and recommended for a maize field. Similarly, many studies have reported the effects of these chemicals on various reproductive parameters in animals [27]. The mode of effects of atrazine and cyhalothrin defers but cannot be specifically distinguished.

We did an ambient air sample collection for three days to figure out the concentration of atrazine in the environment after ground application. During the three days of monitoring, the atrazine concentration in the air being absorbed in the deionized water as a solvent medium, was between 0.0110 to 0.181 mg/m^3 as indicated in (Table 8). The solvent was analyzed using gas chromatography. The retention time for atrazine was 9.442 minutes as shown in figure 2. This result indicates that rabbits kept in an environment with atrazine concentration greater than or equals to 0.0110 mg/m^3, its serum progesterone drops significantly.

Based on the results of dietary intake and digestibility trial, the chemicals reduced the feces output and digestibility of feed consume which is an indicated factor that affects the weight gain and growth performance of the growing rabbits. Even though the chemicals did not affect dietary digestibility in pregnant rabbits, we can, however, conclude that atrazine and cyhalothrin significantly affect the birth weight and the daily weight gain of the fetus of the pregnant rabbits as proven by the data in table 11. This result concurs with the many other results that showed that these chemicals affect up to the 3rd generation of an exposed animal [28].

Cyhalothrin starts to work instantly upon contact or ingestion, resulting in quick knock-down and killing of insects. Cyhalothrin destroys the insect central nervous system by serving asa high-power poison. The nerve cells of the insects get excited once poisoned, causing paralysis and eventual death.

During the first trimester of pregnancy, the nervous system is rapidly developing in young baby animals, so these chemicals must be kept from their surroundings to avoid any type of contact, orally, or by inhalation during this time.

Atrazine and cyhalothrin exposure can increase the chances of a miscarriage, preterm birth, young animal with birth abnormalities, or other problems after interfering with the secretion of the progesterone which should protect the pregnancy. These chemicals can also be passed from mother to their young through breast milk and can affect up to the 3rd generation of an exposed animal.

Studies like this allow broadening the knowledge about the toxic effects of atrazine and cyhalothrin on serum progesterone of rabbits and need to be continued. In this way, these chemicals will be used with caution and will provide a high level of cost-effective environmental protection.

4.2 Conclusion

Both chemicals had a significant effect on the serum progesterone level of the exposed animals in all treatment. It can be concluded that atrazine and cyhalothrin directly affect the corpus luteum of animals within the chemical's concentrated environment thus harming the serum progesterone secretion and resulting in a series of reproductive deficiencies. The user of agrochemical must take into consideration its adverse effects none target organism. Studies like this allow broadening the knowledge about the toxic effects of atrazine and cyhalothrin on serum progesterone of pregnant rabbits and need to be continued. In this way, these chemicals will be used with caution and will provide a high level of cost-effective environmental protection.

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Declaration of interest:

None

Reference

1. Damalas CA, Eleftherohorinos IG, Pesticide Exposure, Safety Issues. Risk Assessment Indicators. Int. J. Environ. Res. 2011; 1402-19.
2. Schug TT, Janesick A, Blumberg B, Heindel JJ. Journal of Steroid Biochemistry and Molecular Biology Endocrine disrupting chemicals and disease susceptibility. J Steroid Biochem. Mol. Biol. 2011; 127(3-5):204-15. Available from: http://dx.doi.org/10.1016/j.jsbmb.2011.08.007
3. Lushchak VI, Matviishyn TM, Husak VV, Storey JM. Review article: Pesticide toxicity : a mechanistic approach. Excli. 2018; 1101-36.
4. Suzawa M, Ingraham HA. The Herbicide Atrazine Activates Endocrine Gene Networks via Non-Steroidal NR5A Nuclear Receptors in Fish and Mammalian Cells. PLoS ONE, 2008, 3(5).
5. Arr Jaac, Entles Ang, Mith ERES, Oleman Walg, Rquidi Linaju, Huett KET, *et al*. RESPONSE OF Larval xenopus laevis to atrazine : assessment of growth, metamorphosis, and gonadal and laryngeal morphology. Environ toxicol chem. 2003; 22(2):396-

405.
6. Rey F, González M, Zayas MA, Stoker C, Durando M, Luque EH, *et al*. General and Comparative Endocrinology Prenatal exposure to pesticides disrupts testicular histoarchitecture and alters testosterone levels in male Caiman latirostris q. Gen Comp Endocrinol Gen. Comp. Endocrinol. 2009; 162(3):286-92. Available from: http://dx.doi.org/10.1016/j.ygcen.2009.03.032
7. Matsushita S, Yamashita J, Iwasawa T, Tomita T, Ikeda M. Effects of In Ovo Exposure to Imazalil and Atrazine on Sexual Differentiation in Chick Gonads. Poult. Sci. 2006; 85(9):1641-1647
8. Kniewald J. Testosterone metabolism in neuroendocrine organs in male rats under atrazine and influence.j. Steroid biochem. 1989; 33(1):141-6.
9. Hayes TB, Collins A, Lee M, Mendoza M, Noriega N, Stuart AA, *et al*. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proc. Natl. Acad. Sci. U.S.A. 2002; 99:5476-5480
10. Kloas W, Lutz I, Springer T, Krueger H, Wolf J, Holden L, *et al*. Does Atrazine Influence Larval Development and Sexual Differentiation in Xenopus laevis? Toxicol. Sci. 2009; 107(2):376-84.
11. Hayes TB, Stuart AAH, Mendoza M, Collins A, Noriega N, Vonk A, *et al*. Monograph Characterization of Atrazine-induced Gonadal Malformations in African Clawed Frogs { Xenopus laevis] and Comparisons with Effects of an Androgen Antagonist (Cyproterone Acetate) and Exogenous Estrogen (17 | 3-Estradiol): Support for the Dem.Environ.Health Perspect, 2006.
12. Evidence F, Hayes T, Haston K, Tsui M, Hoang A, Haeffele C, *et al*. Research | Articles Atrazine-Induced Hermaphroditism at 0. 1 ppb in American Leopard Frogs. Environ. Health Perspect. 2003; (4):568-575.
13. Rton Fro, Arr Jaac, Andy Ridh. Effects of nitrate and atrazine on larval development and sexual differentiation in the northern leopard frog rana pipiens. Environ toxicol chem. 2006; 25(1):65-71.
14. Taylor P, Preez LH Du, Solomon KR, Carr JA, Giesy JP, Gross TS, *et al*. Population structure of the African Clawed Frog (Xenopus laevis) in maize - growing areas with atrazine application versus non - maize - growing areas in South Africa. AFR J Herpetol, 2014, 37-41.
15. Reeder AL, Foley GL, Nichols DK, Hansen LG, Wikoff B, Faeh S, *et al*. Forms and Prevalence of Intersexuality and Effects of Environmental Contaminants on Sexuality in Cricket Frogs (Acris crepitans). Environ. Health Perspect. 1998; 106(5):261-66.
16. Reeder AL, Foley GL, Nichols DK, Hansen LG, Wikoff B, Faeh S, *et al*. Forms and prevalence of intersexuality and effects of environmental contaminants on sexuality in cricket frogs (Acris crepitans). *Environmental health perspectives*. 1998; *106*(5):261-266.
17. Winchester PD, Huskins J, Ying J. Agrichemicals in surface water and birth defects in the United States. Acta Paediatr Int J Paediatr. 2009; 98(4):664-9.
18. Larsen AE, Gaines SD, Deschênes O. Agricultural pesticide use and adverse birth outcomes in the San Joaquin Valley of California. Nat. Commun. 2017; 8(1):1-8.
19. Cooper RL. Atrazine Disrupts the Hypothalamic Control of Pituitary-Ovarian Function. Toxicol Sci. 2000; 53(2):297-307.
20. Saeed S, Ahmad M, Ahmad M, Kwon YJ. Insecticidal control of the mealybug Phenacoccus gossypiphilous (Hemiptera: Pseudococcidae), a new pest of cotton in Pakistan. Entomol. Res. 2007; 37:76-80.
21. Ullah Ms. Toxic effects of cypermethrin in female rabbits. Pak vet J. 2006; 26(4):193-196.
22. Elbetieha A, Da SI, Khamas W, Darmani H. E nvironmental C ontamination a n d T Evaluation of the Toxic Potentials of Cypermethrin Pesticide on Some Reproductive and Fertility Parameters in the Male Rats. Arch Environ CON TOX. 2001; (4)528:522–8.
23. Ahmad L, Khan A, Khan MZ. Pyrethroid-induced reproductive toxico-pathology in non-target species. Pak Vet J. 2012; 32(1):1-9.
24. LINNET PJ. Permethrin toxicosis in cats. Aust Vet J. 2008; 86(1-2):32-35.
25. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother. 2010; 1(2):87-93.
26. Campos-Pereira FD, Oliveira CA, Pigoso AA, Silva-Zacarin EC, Barbieri R, Spatti EF, *et al*. Early cytotoxic and genotoxic effects of atrazine on Wistar rat liver: a morphological, immunohistochemical, biochemical, and molecular study. *Ecotoxicology and environmental safety*. 2012; *78*:170-177.
27. Upholt WM, Kraybill HF. Ecological effects of pesticides. *Annals of the New York Academy of Sciences*. 1969; *160*(1):55-60.
28. McBirney M, King SE, Pappalardo M, Houser E, nkefer M, Nilsson E. *et al*. Atrazine induced epigenetic transgenerational inheritance of disease, lean phenotype and sperm epimutation pathology biomarkers. *PloS one*. 2017; *12*(9):e0184306.