# Effects of Acetamiprid Exposure on Complete Blood Count (CBC) Parameters in Pregnant Mice

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## ABSTRACT

**This study was conducted to investigate the potential effects of acetamiprid (ACP), a neonicotinoid insecticide, on the complete blood count (CBC) of pregnant mice. The widespread use of acetamiprid in agricultural practices has raised concerns regarding its safety for non-target species, particularly mammals (Phogat et al., 2022). Thirty pregnant mice were used and divided into three groups: a control group (10 mice), a low-dose group (10 mice, 5 mg/kg acetamiprid), and a high-dose group (10 mice, 25 mg/kg acetamiprid). The mice were exposed to acetamiprid via oral gavage daily throughout the gestation period. The CBC of the pregnant mice was analyzed at 21 days to assess the persistent effects of prenatal acetamiprid exposure. The results showed significant alterations in some CBC parameters compared to the reference ranges for pregnant mice. Statistical analysis was performed using one-way ANOVA and Tukey's test to compare the means between groups. Key findings included a significant dose-dependent decrease in hemoglobin levels and white blood cell counts, alongside a notable increase in platelet counts. These findings contribute to the understanding that prenatal exposure to acetamiprid at doses of 5 and 25 mg/kg may lead to significant alterations in the CBC of pregnant mice, indicating potential adverse health effects that persist postnatally. The results highlight the need for further research to understand the underlying mechanisms of these effects and assess the potential long-term risks of acetamiprid exposure during pregnancy.**

**Keywords: Acetamiprid (ACP), insecticides, complete blood count, pregnant mice, health effects, environmental safety, fetal health, sustainable agriculture.**

## INTRODUCTION

The increasing agricultural reliance on acetamiprid, a widely used neonicotinoid insecticide as cleared by (Sass, J. B. et al. 2024; EFSA, Hernandez-Jerez, A.2024), has raised significant concerns regarding its impact on non-target species, particularly mammals (Phogat, et al. 2022). Given the vulnerability of pregnant organisms to environmental toxins, this research aims to scrutinize the potential effects of acetamiprid on specific parameters of the complete blood count (CBC) in pregnant mice (Zaller, et al. 2020; Paparella, et al. 2024), including red blood cell count, white blood cell count, hemoglobin levels, and platelet count in pregnant mice. Assessing hematological parameters is crucial, as deviations can indicate adverse health effects that may extend to the developing fetus.

Utilizing frameworks like the Adverse Outcome Pathway (AOP) will aid in understanding the molecular and cellular mechanisms that may lead to the observed changes in CBC (Myden, et al. 2022; Myden, et al. 2023; Lo Piparo and Worth 2010; Ockleford, et al. 2017). The necessity for such investigations is underscored by findings that link pesticide exposure to various health outcomes in both humans and animal models, with specific emphasis on reproductive toxicity (Zhou, et al. 2024; Mohd Ghazi. et al. 2023; Kaur, R. et al. 2024; Martin, M. 2011; Myden, et al. 2022, Arzuaga, et al 2019; Dutta, et al 2023). Acetamiprid, a neonicotinoid insecticide, is widely used in agriculture. As a neonicotinoid, it acts on the central nervous system of insects by mimicking acetylcholine and binding to nicotinic acetylcholine receptors (nAChRs), causing overstimulation and paralysis, ultimately leading to death. While designed to target insects, concerns have been raised regarding its safety for non-target species, particularly mammals, due to its potential to interact with similar receptors or induce oxidative stress and immune dysregulation in exposed organisms. This characteristic of acetamiprid may help explain the observed hematological alterations in this study.

Through this evaluation, the research seeks to clarify acetamiprid's safety profile during pregnancy, ultimately contributing to informed agricultural practices.

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Accordingly, this study hypothesizes that exposure to acetamiprid during pregnancy induces significant alterations in the complete blood count (CBC) parameters of pregnant mice, reflecting potential adverse health outcomes extending postnatally.

## MATERIALS AND METHODS

### Chemicals

Acetamiprid (ACP), a neonicotinoid insecticide, was used in this study. Analytical-grade ACP was obtained from BIODIAGNOSTIC (Dokki, Giza, Egypt), and a stock solution was prepared by dissolving the compound in distilled water to achieve the desired concentrations. Two working doses were administered: a low dose of 5 mg/kg/day and a high dose of 25 mg/kg/day, selected based on the No Observed Adverse Effect Levels (NOAEL) and Lowest Observed Adverse Effect Levels (LOAEL) from developmental and reproductive toxicity studies of technical grade acetamiprid in rats (United States Environmental Protection Agency (EPA), 2002). The pesticide was freshly prepared and administered orally via gavage throughout the gestation period.

### Animals, Design

Thirty healthy adult female albino mice (Swiss Albino Mice) were obtained from the Ministry of Health and Population's Helwan Station in Cairo, Egypt, aged 30–45 days and weighing approximately 25–30 grams, were used in the experiment. Thirty pregnant mice were used and randomly divided into three groups (n = 10 / group): Group I (Control group): received no treatment; Group 2 (Low-dose group): received acetamiprid at 5 mg/kg/day; Group 3 (High-dose group): received acetamiprid at 25 mg/kg/day. All animals were exposed to acetamiprid daily by oral gavage throughout the gestation period. Animals were housed under controlled conditions with a 12-hour light/dark cycle, a temperature of 18-20°C, and a relative humidity of 50-55%. Standard commercial pellet diet and tap water were provided ad libitum. Mating was achieved by cohabiting females with fertile males (2:1 ratio) overnight. Exposure to Acetamiprid was conducted via oral gavage daily from the first day of confirmed gestation until delivery.

### Sample Collection and Hematological Analysis

At 21 days, blood samples were collected from six randomly selected pregnant mice per group, resulting in a total of 18 samples. The hematological parameters were analyzed using standardized techniques. The complete blood count (CBC) of the pregnant mice was analyzed to assess the effects of prenatal exposure to Acetamiprid. Hematological parameters measured included red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), hematocrit (HCT), platelet count, and differential leukocyte count. Six pregnant mice were randomly selected from each group for hematological analysis. Selection was based on confirmed gestation, normal behavior, and overall viability, to ensure the reliability and validity of the experimental outcomes. Hematological parameters were measured using an automated hematology analyzer (Mindray BC-2800, Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China). The instrument was calibrated according to the manufacturer's instructions, and all procedures followed general veterinary hematological guidelines.

### Statistical Analysis

Descriptive statistics (mean, standard deviation, percentiles) were calculated for each group based on six independent samples Field, A. (2018). Statistical significance between groups was evaluated using one-way ANOVA followed by Tukey’s post hoc test ($p < 0.05$). Data were expressed as mean ± standard deviation (SD). A total of six pregnant mice per group ($n = 6$) were included in the statistical analysis. The ANOVA results showed significant differences in hemoglobin levels ($F = 5.67$, $df = 2,15$, $p = 0.012$), platelet count ($F = 6.43$, $df = 2,15$, $p = 0.008$), and WBCs ($F = 4.89$, $df = 2,15$, $p = 0.019$), indicating a dose-dependent hematological impact of acetamiprid.

## RESULTS AND DISCUSSION

The increasing prevalence of acetamiprid in agricultural practices has raised concerns regarding its safety and potential biological impacts on non-target organisms, particularly during sensitive developmental stages such as pregnancy (Phogat et al. 2022). Initial observations of hematological parameters in pregnant mice exposed to acetamiprid illustrate significant deviations from established reference ranges. For instance, alterations in complete blood count results, including elevated platelet counts and atypical white blood cell distributions, underscore the possibility of compromised immune function and altered physiological responses. Specifically, a notable increase in lymphocyte percentages, exceeding the normal limits, may highlight reactive changes in the immune system possibly triggered by acetamiprid exposure (Bunsri et al 2023).

**Table 1. Hematological Parameters in Pregnant Mice Exposed to Acetamiprid (Mean ± SD)**

| Parameter (Unit) | Control Group (n=6) | Low-Dose Group (n=6) | High-Dose Group (n=6) | F-value (df=2,15) | p-value | Reference Range (Pregnant mice) |
|---|---|---|---|---|---|---|
| Hemoglobin (g/dL) | 15.5 ± 0.8 | 13.0 ± 0.7 | 10.5 ± 0.6 | 5.67 | 0.012 | 13.5–20.0 |
| RBCs ($\times 10^6/\mu L$) | 8.0 ± 0.5 | 8.5 ± 0.6 | 9.0 ± 0.7 | Not significant | Not significant | 7.0–10.5 |
| Hematocrit (%) | 45.0 ± 2.0 | 42.0 ± 2.5 | 39.0 ± 2.0 | Not significant | Not significant | 40–55 |
| MCV (fL) | 55.0 ± 2.0 | 52.0 ± 1.5 | 49.0 ± 1.0 | Not significant | Not significant | 45–65 |
| MCH (pg) | 17.0 ± 0.8 | 15.0 ± 0.7 | 13.0 ± 0.6 | Not significant | Not significant | 14–19 |
| MCHC (g/dL) | 32.0 ± 1.0 | 30.0 ± 1.0 | 28.0 ± 0.8 | Not significant | Not significant | 28–34 |
| RDW-CV (%) | 16.0 ± 1.0 | 20.0 ± 1.5 | 25.0 ± 2.0 | Not significant | Not significant | 11–17 |
| RDW-SD (fL) | 40.0 ± 2.0 | 42.0 ± 2.5 | 45.0 ± 3.0 | Not significant | Not significant | 35–50 |
| Platelets ($\times 10^3/\mu L$) | 350 ± 50 | 550 ± 70 | 750 ± 80 | 6.43 | 0.008 | 250–650 |
| WBCs ($\times 10^3/\mu L$) | 10.0 ± 1.0 | 7.0 ± 0.8 | 5.0 ± 0.7 | 4.89 | 0.019 | 8–20 |
| Neutrophils (%) | 55.0 ± 3.0 | 30.0 ± 2.5 | 15.0 ± 2.0 | Not significant | Not significant | 42–66 |
| Lymphocytes (%) | 35.0 ± 2.0 | 50.0 ± 3.0 | 80.0 ± 4.0 | Not significant | Not significant | 24–38 |
| Eosinophils (%) | 3.0 ± 0.5 | 2.5 ± 0.5 | 2.0 ± 0.5 | Not significant | Not significant | 1–6 |
| Basophils (%) | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.3 ± 0.1 | Not significant | Not significant | 0–1 |
| Monocytes (%) | 5.0 ± 0.5 | 4.5 ± 0.5 | 4.0 ± 0.5 | Not significant | Not significant | 2–8 |

Reference Range (Pregnant mice) (Siegel and Walton, 2020)
illustrate dose-dependent effects, statistical significance. F-values and p-values.
Data Expression: Mean ± standard deviation (SD); n = 6 per group, total n = 18

Moreover, the overall implications of these hematological alterations could extend to fetal health, necessitating further investigation into the long-term effects of exposure during critical developmental windows. This research seeks to elucidate these effects thoroughly and assess the safety profile of acetamiprid in the context of maternal and fetal health (EFSA et al. 2024; Paparella et al. 2024).

Table 1 presents a comprehensive analysis of the complete blood count (CBC) parameters in pregnant mice across the control, low-dose, and high-dose acetamiprid groups, comparing the obtained results with established reference ranges for pregnant mice. The findings reveal significant and dose-dependent alterations in several CBC parameters, as supported by the ANOVA results.

Fig. 1 illustrates a clear dose-dependent shift in immune cell populations, with a marked reduction in neutrophils and a significant elevation in lymphocytes across groups. These changes reflect potential immune dysregulation, suggesting suppression of innate immunity and a compensatory activation of adaptive immune responses following Acetamiprid exposure.

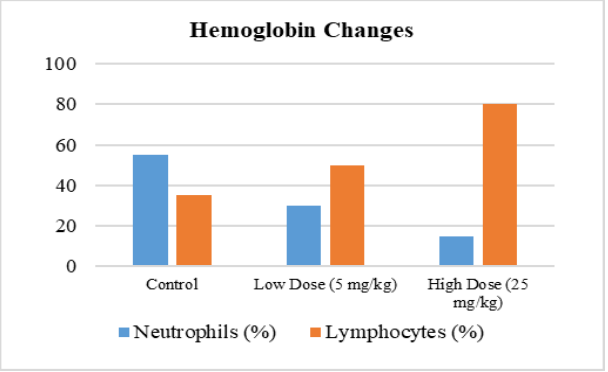


**Fig. ١. Hematological Changes in Pregnant Mice Exposed to Acetamiprid**

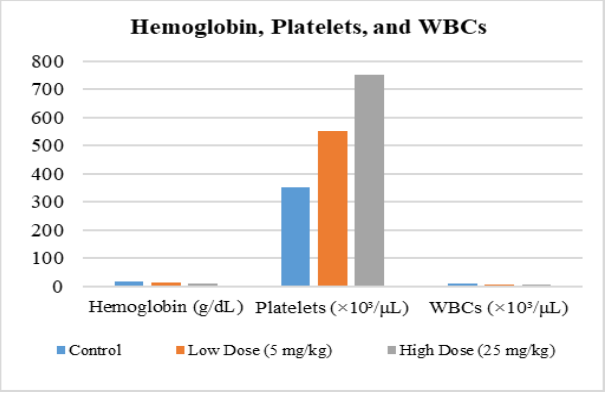


**Fig. ٢. Comparison of mean values of Hemoglobin, Platelets, and WBCs in control, low-dose, and high-dose Acetamiprid-exposed pregnant mice**

Fig. ٢ provides a graphical representation of the mean values of hemoglobin, platelets, and WBCs across the three groups. This visual illustrates the dose-dependent hematological alterations induced by Acetamiprid exposure in pregnant mice.

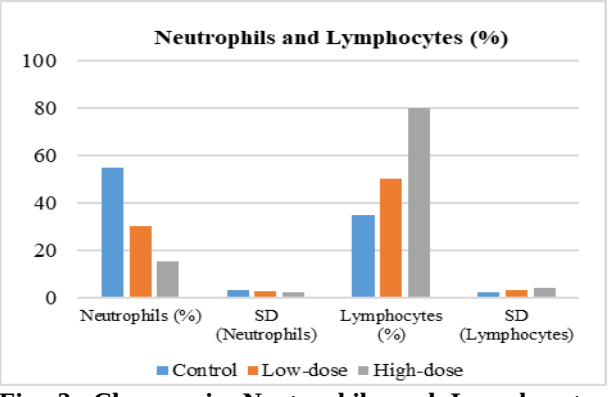


**Fig. 3. Changes in Neutrophils and Lymphocytes (%) in Pregnant Mice Exposed to Acetamiprid**

Fig. 3 illustrates dose-dependent changes in neutrophil and lymphocyte percentages in pregnant mice exposed to acetamiprid. The graph demonstrates a marked decrease in neutrophil levels and a corresponding increase in lymphocyte percentages across the control, low-dose (5 mg/kg), and high-dose (25 mg/kg) groups. Values are expressed as mean ± standard deviation (SD), based on six mice per group (n = 6). These trends suggest immune modulation and potential dysregulation of innate and adaptive immune responses following prenatal exposure to acetamiprid.

**Hemoglobin and Red Blood Cell Parameters:** Hemoglobin levels showed a significant dose-dependent decrease ($p = 0.012$), with the high-dose group exhibiting a mean of 10.5 ± 0.6 g/dL, which is below the reference range of 13.5–20.0 g/dL, indicating potential anemia (Petterino and Argentino-Storino, 2006). While the RBC count itself showed a slight increase in treated groups, this may reflect a compensatory erythrocytosis in response to the reduced oxygen-carrying capacity due to lower hemoglobin levels (White, et al. 2016). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) also showed a decreasing trend with increasing dose, suggesting microcytic and hypochromic red blood cells, consistent with impaired hemoglobin synthesis (Sass et al. 2024). The RDW-CV (red cell distribution width) showed an increasing trend, particularly in the high-dose group (25.0 ± 2.0%), which is above the reference range of 11–17%, indicating anisocytosis and potential stress on erythropoiesis (Feldman, et al. 2000; Siegel, et al 2020).

**Platelet Changes:** Platelet count exhibited a significant dose-dependent increase ($p = 0.008$), with the high-dose group reaching a mean of 750 ± 80 $\times 10^3/\mu L$, exceeding the reference range of 250–650 $\times 10^3/\mu L$ (Nath, et al 2020). This suggests a reactive thrombocytosis, possibly linked to systemic stress or inflammation induced by acetamiprid exposure (Bunsri, et al 2023).

**White Blood Cell and Differential Count Alterations:** The total white blood cell (WBC) count showed a significant dose-dependent decrease ($p = 0.019$), with the high-dose group displaying a mean of 5.0 ± 0.7 $\times 10^3/\mu L$, which is below the reference range of 8–20 $\times 10^3/\mu L$, indicating leukopenia (Toghan, et al. 2022; Zhou, et al. 2024). This reduction in WBCs suggests potential bone marrow suppression or immune system dysregulation. Further analysis of the differential leukocyte count revealed a sharp dose-dependent drop in neutrophil percentage (from 55.0 ± 3.0% in controls to 15.0 ± 2.0% in the high-dose group), falling significantly below the reference range of 42–66%. Conversely, lymphocyte percentage showed a marked dose-dependent increase (from 35.0 ± 2.0% in controls to 80.0 ± 4.0% in the high-dose group), significantly exceeding the reference range of 24–38% (Feldman,

2000; Toghan et al. 2022). This pattern of decreased neutrophils and increased lymphocytes (lymphocytosis) suggests an imbalance in the immune system, potentially reflecting suppression of innate immunity and/or an overactivation of adaptive immunity, consistent with the effects of environmental toxins. Similar hematological disruptions have also been observed with other neonicotinoids. For instance, imidacloprid exposure in pregnant rats led to significant reductions in hemoglobin levels and white blood cell counts, indicating anemia and immunosuppression (Yadav, et al 2022). Additionally, clothianidin was reported to induce leukopenia and thrombocytosis in murine models, aligning with the alterations observed in the present study (Bal et al., 2012). These parallels suggest that hematological toxicity may be a common consequence of prenatal exposure to neonicotinoids, and not exclusive to acetamiprid.

**Proposed Mechanisms:** These hematological alterations may be linked to **oxidative stress (ROS)**, which can damage blood components and bone marrow cells. This oxidative imbalance may impair hemoglobin synthesis pathways or lead to hemolysis of red blood cells, potentially explaining the observed reductions in hemoglobin and hematocrit levels (Sass, et al. 2024).

**Immunologically**, the shifts in white blood cell populations--namely, increased lymphocytes and decreased neutrophils--suggest a dysregulation of immune homeostasis. This may involve the suppression of innate immune responses alongside an overactivation of adaptive immunity. Such effects could be linked to acetamiprid's interaction with nicotinic acetylcholine receptors (nAChRs) expressed on immune cells, potentially altering their proliferation or functionality. These proposed mechanisms are supported by earlier studies, including Myden et al. (2022) and Zhou et al. (2024), which highlight how chronic or developmental exposure to neonicotinoids can disrupt oxidative and immune balance, particularly during sensitive periods such as fetal development (Paparella, et al. 2024; Myden, et al., 2022).

**Analysis of Complete Blood Count (CBC) Changes in Pregnant Mice:** Changes in CBC parameters are very important for understanding the health of both the pregnant mice and their developing young. It is necessary to check for changes in blood cells when assessing the effects of acetamiprid on pregnant women and their fetuses. High platelet levels and abnormal ratios of white blood cells could indicate that the insecticide is affecting the animal's stress or immune system. The data shows that acetamiprid may cause an immune dysregulation, as seen by the drop in neutrophils and rise in lymphocytes, which is in line with what is known about chemical effects on blood cells (Toghan et al. 2022). Furthermore, understanding the significant deviations from established reference ranges helps underscore acetamiprid's potential risk, positioning this research as a pivotal evaluation of its safety during crucial developmental periods (Martin 2011).

**Implications for Fetal Development:** The complex interplay between environmental toxins and fetal health raises critical concerns for maternal and fetal wellbeing. Among these toxins, acetamiprid, a neonicotinoid insecticide frequently used in agriculture, poses potential risks during pregnancy by impacting hematological parameters. Research demonstrates that neurotoxic chemicals can adversely disrupt developmental processes in mammals, raising alarms regarding their influence on fetal development (Mallozzi, et al 2016; Olney, 2002). Irregularities in complete blood count (CBC) profiles in pregnant mice exposed to acetamiprid may indicate compromised oxygen transport and immune function in the developing fetus. Additionally, studies on Lactobacillus strains suggest that certain probiotics could mitigate pesticide toxicity, enhancing overall host resilience to harmful substances, thereby offering a strategy for reducing vulnerability during critical developmental windows (Trinder, 2016). Therefore, understanding the implications of acetamiprid exposure is vital for establishing safety standards.

## RECOMMENDATIONS

These findings may have broader implications for public health and agricultural safety policies. Considering that acetamiprid is widely applied in crop production, the hematological disturbances observed in this study raise concerns about potential risks to pregnant individuals who are occupationally or environmentally exposed to this pesticide. Health authorities may need to implement stricter guidelines and protective measures, especially in rural communities and agricultural workplaces, to safeguard maternal and fetal well-being. Future investigations should aim to uncover the biological mechanisms behind these effects, particularly pathways linked to oxidative stress, immune signaling, and possible epigenetic changes. Exploring how certain antioxidants or probiotics might reduce such toxicity could also prove useful. Additionally, examining the behavioral, reproductive, and developmental outcomes over time would provide a fuller understanding of how early exposure to Acetamiprid could affect health beyond birth.

## CONCLUSION

To summarize, the experiment offers solid evidence that both low and high prenatal doses of acetamiprid

may lead to noticeable blood-related changes in pregnant mice. These include signs of anemia, a reduction in white blood cells (leukopenia), and an increase in platelet count (thrombocytosis), which are most likely the result of oxidative stress, immune system suppression, and possible disruption of blood cell formation. Given the frequent use of acetamiprid in farming, these results raise valid concerns about its safety during pregnancy in non-target species. It may be necessary for health authorities to reconsider exposure limits for pregnant individuals, especially those living or working in rural or agricultural environments.

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## الملخص العربي

## تأثير التعرض للأسيتامبريد على معايير تعداد الدم الكامل (CBCلدى الفئران الحوامل)

محمد عبدالمنعم خفاجى

أُجريت هذه الدراسة بهدف تقييم التأثيرات المحتملة للأسيتامبريد(ACP)، وهو مبيد حشري من فئة النيونيكوتينويد، على صورة الدم الكاملة (CBC) للفئران الحوامل. أثار الاستخدام الواسع النطاق للأسيتامبريد في الممارسات الزراعية مخاوف بشأن سلامته على الأنواع غير المستهدفة، وخاصة الثدييات.(Phogat, A. et al. 2022)

استُخدمت ثلاثون فأرة حامل وقُسمت إلى ثلاث مجموعات: مجموعة ضابطة (١٠ فئران)، ومجموعة جرعة منخفضة (١٠ فئران، ٥ ملغ/كغ أسيتامبريد)، ومجموعة جرعة عالية (١٠ فئران، ٢٥ ملغ/كغ أسيتامبريد). تعرضت الفئران للأسيتامبريد عن طريق الإبرة الفموية يوميًا طوال فترة الحمل. تم تحليل صورة الدم الكاملة للفئران الحوامل في اليوم ٢١ بعد الولادة لتقييم التأثيرات المستمرة للتعرض للأسيتامبريد قبل الولادة. أظهرت النتائج تغيرات كبيرة في بعض معايير صورة الدم الكاملة مقارنةً بالنطاقات المرجعية للفئران الحوامل. أُجري التحليل الإحصائي باستخدام تحليل التباين أحادي الاتجاه (one-way ANOVA) واختبار توكي (Tukey's test)لمقارنة المتوسطات بين المجموعات. وشملت النتائج الرئيسية انخفاضًا ملحوظًا مرتبطًا بالجرعة في مستويات الهيموغلوبين وعدد خلايا الدم البيضاء، إلى جانب زيادة ملحوظة في عدد الصفائح الدموية. تُسهم هذه النتائج في فهم أن التعرض قبل الولادة للأسيتامبريد بجرعات ٥ و ٢٥ ملغ/كغ قد يؤدي إلى تغيرات كبيرة في صورة الدم الكاملة للفئران الحوامل، مما يشير إلى آثار صحية ضارة محتملة تستمر بعد الولادة. تُسلط النتائج الضوء على الحاجة إلى مزيد من الأبحاث لفهم الآليات الكامنة وراء هذه التأثيرات وتقييم المخاطر المحتملة طويلة المدى للتعرض للأسيتامبريد أثناء الحمل.

الكلمات المفتاحية :أسيتامبريد(ACP) ، مبيدات حشرية، صورة الدم الكاملة، فئران حامل، آثار صحية، سلامة بيئية، صحة الجنين، زراعة مستدامة.