Sub-Acute Toxicity of two Herbicides Halosulfuron-methyl and Clethodim on Biochemical and Hematological Parameters in Male Albino Rats

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Abstract: On male albino rats, this study has evaluated the toxicity of halosulfuron-methyl and clethodim on their hepatic, haematological, and oxidative effects. For a period of 28 days, the animals were given either halosulfuron-methyl (886 and 1773 mg/kg) or clethodim (163 and 326 mg/kg body weight) herbicides orally. As a control treatment, distilled water was used. Hematological and biochemical markers, as well as body weight gain and organ weight, were all assessed. In general, all treated rats' bodyweight gain and relative testes weight were considerably lower when compared to the control treatments. Furthermore, the relative weights of the liver, kidneys, lungs, spleen, brain, and heart were recorded at various rising levels. At the ending of the procedure, blood samples were taken. Hepatic toxicity was measured using the blood enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), whereas renal toxicity was measured using urea and creatinine. Red blood corpuscles (RBC), white blood cells (WBC) and hemoglobin were tested in the liver to detect oxidative damage and monitor changes in hematological parameters. Both herbicide treatments resulted in significant increases in the levels of liver enzymes (ALT and AST). In male albino rats, hematological parameters indicated considerable changes, with decreases in RBC and hemoglobin and a large rise in WBC. The findings suggest that even at subacute exposure, halosulfuron-methyl and clethodim might cause hematological parameter changes, which might be linked to the production of reactive oxygen species.

Keywords: Halosulfuron-methyl, Clethodim; hematological damage; herbicides

INTRODUCTION

Pollution has gotten a lot of coverage around the world. With increased human activity, it is becoming a major issue.Pesticides are thought to be heterogeneous whichare used to treat plant diseases and pests to increase the productivity of (pests or weeds) agriculture and production (Bolognesi, 2003). Pesticides are essential agricultural chemical materials, and their use has increased the yield of plants yield, resulting in lower crop costs. They do, however, persist in food products and the environment, posing health risks to both humans and animals (Dalsenter *et al.,* 1999). Controlling weeds is critical to increasing crop yields, Herbicides are used to combat weeds in commercial crop cultivation (Norsworthy *et al.,* 2012). Chemical weed control provides more efficient long-term weed control and higher vegetable yields, also herbicides hold promise for weed control that is timely, affordable, and reliable in areas where labour is scarce and costly (Adigun *et al.,* 2014). Herbicides are one of the main methods for controlling noxious weeds in agricultural and non-agricultural lands all over the world and it presented more than 60% of total pesticides used in agricultural sectors (Elalfy *et al.,* 2017). Clethodim is a systemic herbicide belong to that is quickly consumed and transported from treated vegetation to the plant's root system and growing portions, It has a role as a herbicide and an EC 6.4.1.2 (acetyl-CoA carboxylase) inhibitor (Turner, J. A., Pesticide manual, 2015). Clethodim is a herbicide that is commonly used in agriculture because it is highly effective, secure, and selective post-emergence herbicide that can be used to suppress annual and perennial grasses in large leaf crops like soybean, cotton, and sunflower, and the majority of vegetables (Yu *et al.,* 2007; Saini *et al.,* 2017; Villaverde *et al.,* 2018). Short-term toxicity of clethodim, the EPA stated; "There were no effects observed in oral toxicity studies including developmental toxicity studies in rats and rabbits that could be attributable to a single dose exposure (USEPA, 2010). Halosulfuron-methyl is a sulfonylurea herbicide that inhibits acetolactate synthase (ALS), an enzyme involved in the biosynthesis of branched chain amino acids such as valine, leucine, and isoleucine (Senseman, 2007; Duggleby *et al.,* 2008). It can be absorbed by both roots and leaves, preventing susceptible plants from growing (Soltani *et al.,* 2009). Halosulfuron-methyl is PPI, PRE, and POST herbicide against *Cyperus* spp., and many broadleaf weeds, such as redroot pigweed, velvetleaf, wild mustard, making it useful in a wide range of vegetable crops (Culpepper *et al.,* 2009). Like most other sulfonylurea herbicides, halosulfuron-methyl has low acute toxicity following oral, dermal or inhalational exposure. Its potential to cause eye irritation is rated as slight and it is not a skin irritant or skin sensitizer (USEPA, 2020). Thus, this research designed to evaluate the effects sub-lethal doses of clethodim and halosulfuron-methyl herbicides on biochemical and hematological parameters of male albino rats.

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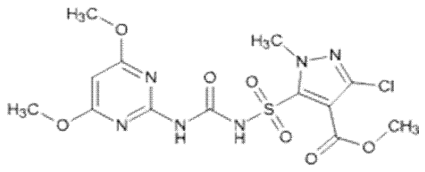
MATERIALS AND METHODS

Tested herbicides

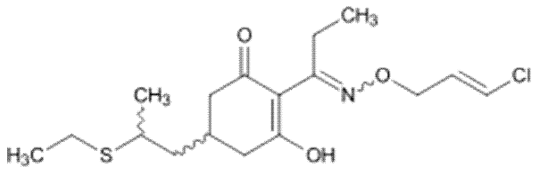
1-Halosulfuron-methy:

STRUCTURE:



2- Clethodim:

STRUCTURE:



Experimental design

The experiments were run out on male albino rats administered orally with sub-lethal doses of halosulfuron-methyl 75% WG (Inpul) and clethodim 12.5% EC (Select super), Which is used to control weeds, rice and maize crop, for four weeks to examine their toxicological qualities. These experiments were conducted in March 2020 at Damanhur University, faculty of Agriculture, department of Plant Protection, EL-Behera, Egypt.

Test animals

A Colony of mature male albino–rats Rattus norvegicus was obtained from Egyptian Organization, Vaccine and Biological Preparation, Helwan, Cairo. For acclimatization, rats were kept in a laboratory setting at 25.5°C and 65.5% Rh for two weeks before beginning the experiments. The food consisted of a commercial rabbit ration in pellet form or a pellet providing all of the nutritional requirements. It was obtained from the EL-Salam Dry Ration Factory in El-Marg, Cairo. Unless otherwise noted, rats were fed this food during the trial. Three male animals were kept together in suspended metal cages (35 x 25 x 20 cm) with a wire mesh bottom at laboratory condition and maintained on ad libitum diet and water.

Tested treatments

To investigate the toxicological effects of two herbicide formulations, halosulfuron-methyl 75 %WG (Inpul) and clethodim 12.5% EC (Select super) rats were given two sub-lethal dosages orally from each compound for four weeks (Table 1).

Table (1): The oral LD_{50} values of the two selected herbicides

Herbicide	Acute oral LD_{50} for rats value ($mg\ kg^{-1}$)	Sub lethal doses ($mgkg^{-1}$)	
		1/5 of LD_{50}	1/10 of LD_{50}
Halosulfuron-methyl	8866	1773	886
Clethodim	1630	326	163

Experimental procedures

For four weeks, rats were given sub-lethal dosages of potential toxicants orally on alternate days. After four weeks, three rats were sacrificed by capitation after being treated with each herbicide. Male rats weighing between 90 and 150 grams were chosen for the study. Herbicides were dissolved in distilled water in the proper proportions. A plastic syringe was linked to a curved stainless steel animal intubation needle with a spherical ball tip for oral dosage. The dosage was given to the animals, and they were checked on a daily basis. During the trial, rats were monitored daily for overt indications of toxicity and their weights were recorded to estimate body weight growth or loss from the start body weight, with the average determined weekly. Blood samples were drawn from each rat at the end of the study (4 weeks), and its liver, heart, brain, spleen, kidneys, lungs, and testes were dissected out, trimmed of superfluous fat, and weighted. The following formula was used to compute the relative organ weight per 100 g of rat body weight (Stevens, 1976):

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight}}{\text{Whole body weight}} \times 100$$

Hematological and Biochemical studies:

The purpose of this study was to illustrate the impact of tested toxicants on the blood images of rats. Finally, time interval blood samples from each slain rat were obtained and separated into two portions, the first was used to count red blood corpuscles (R.B.Cs) and white blood cells (W.B.Cs) as detailed by (Zahkouk, 1999), while the second portion was used to assess hemoglobin content as documented by Drabkin and Austin (1932). The second part was allowed to sit at room temperature for 10 minutes before being centrifuged for 15 minutes at 3000 rpm. The supernatant serum was taken and stored in the freezer until it was utilized to determine the activities of plasma aminotransferases (AST and ALT) according to method of Reitman and Frankel (1957). The level of creatinine and urea levels as assessed according to Bartles *et al*. (1972) and Fawcet and Soctt (1960). The results were presented as mean ± standard error of the mean. Differences between the groups were determined using one-way ANOVA, followed by

Duncan's test where appropriate, Sendecor and Cochran (1980). Significant differences were indicated by p-values ≤ 0.05 and 0.01.

RESULTS

Body weights obtained were drastically reduced after the dosage period, according to data in Table (2), at any time following the initial toxicant administration (*P= 0.05*) in male rats which were given sub-lethal doses of halosulfuron-methyl and clethodim orally for four weeks, compared to the control group. The degree of the reduction in body weight growth changed as the treatment period progressed, reaching its greatest values of reduction during the second week after the initial toxicant dose.

Table (2): Effect of different sub lethal doses of herbicides on body weight gain of male albino rats after oral administration for 4 weeks

Weeks after Administration /Sex	Body weight gain of control (gm)	Treatments							
		Halosulfuron-methyl				Clethodim			
		1/5 of LD_{50}		1/10 of LD_{50}		1/5 of LD_{50}		1/10 of LD_{50}	
		A	B	A	B	A	B	A	B
1	7	3	57.14	2	71.43	2.33	66.67	2.33	66.67
2	15	8	46.67	5.67	62.22	6.67	55.56	5.33	64.44
3	21	13.33	36.51	8.67	58.73	10.3	50.79	8.00	61.9
4	28	19	32.14	12.7	54.76	15.7	44.05	10.33	63.1
L.S.D			at 5%				at 1%		
Time			1.63				1.43		
Conc.			0.63				0.79		
Time X conc.			3.61				4.17		

A= The average of body weight gain (gm)
B= Percent reduction of weight corresponding to control

Treatments of rats with halosulfuron-methyl and clethodim led to a considerable reduction in body weight compared to control in a dose-dependent manner, as shown in Table (2). The highest levels of halosulfuron-methyl and clethodim significantly reduced rat weight growth from 28 g to 12.7 and 10.33 g, correspondingly, in comparison to the control.

In addition, the findings of relative organ weights of herbicide treatments and control rats are shown in Table (3). The tested herbicides generated a substantial rise in the relative weights of the liver, kidneys, spleen, lungs, brain, and heart when given orally, but a substantial decrease in the relative weights of the testes when given orally compared to those of the control group. Consequently, exposure led to a significant decrease in the absolute and relative weights (g) of the testes, as well as a substantial rise in the relative weights of the liver and kidney.

Effect on Haematological Parameters:

In treated rats, halosulfuron-methyl and clethodim generated a considerable decrease in RBC in a dose-dependent manner, as shown in Table (4). These medications, however, led to a considerable rise in WBC. These effects were more prominent in rats given greater doses of halosulfuron-methyl (326 mg a.i./kg/day) and clethodim (1773 mg a.i./kg/day), respectively. In treated rats, halosulfuron-methyl and clethodim generated a considerable decrease in RBC in a dose-dependent manner, as shown in Table (4). These treatments, however, led to a considerable rise in WBC.

Effect of tested herbicides on liver function parameters:

Serum enzymes are a valuable tool for determining the cause of illness. They detect system faults early enough to enable either forecasting or possible solutions. In reflections of this, blood enzymes and metabolic parameters in rats given daily oral sub-lethal doses of halosulfuron-methyl and clethodim for 28 days were measured. Table (4) demonstrates that halosulfuron-methyl and clethodim dramatically elevated AST and ALT activity in a dose-dependent manner. The highest doses of halosulfuron-methyl and clethodim elevated AST activity from 64 U/ml to 75.33 and 84 U/ml, respectively, and ALT activity from 33.67 U/ml to 44.33 and 54.33 U/ml, consecutively, in serum of halosulfuron-methyl and clethodim treated rats.

Effect on kidney function parameters:

Creatinine and uric acid levels in animal serum can be used to detect nephrotoxicity caused by exogenous substances early on. In live creatures, these measurements are utilized as a measure of kidney injury (Coles, 1986). In the serum of halosulfuron-methyl and clethodim treated rats, creatinine concentration (mg/dl) was dramatically raised and uric acid concentration (mg/dl) was dramatically elevated in a dose-dependent manner, as shown in Table (4). Thus, the highest doses of halosulfuron-methyl and clethodim elevated creatinine from 1.20 mg/dl in control to 2.70 and 2.29 mg/dl, respectively. On the contrary, these doses raised uric acid concentrations from 43.30 mg/dl in the comparison group into 59.60 and 49.80 mg/dl, correspondingly.

Table (3): Relative organs weights of male rats treated orally with sub-lethal doses of treatments for 4 weeks

Organs / Treatments	Liver ↑		Kidneys ↑		Spleen ↑		Lungs ↑		Brain ↑		Heart ↑		Testes ↓	
Does	1/5	1/10	1/5	1/10	1/5	1/10	1/5	1/10	1/5	1/10	1/5	1/10	1/5	1/10
Control	**3.28**		**0.89**		**0.17**		**0.73**		**1.08**		**0.35**		**1.52**	
Inpul 75%WG	**4.29**	**4.05**	**1.07**	**0.98**	**0.20**	**0.16**	**1.27**	**0.85**	**1.25**	**1.20**	**0.47**	**0.40**	**1.36**	**1.24**
Select super 12.5% EC	**4.01**	**3.48**	**1.10**	**0.91**	**0.24**	**0.19**	**1.25**	**1.11**	**1.42**	**1.20**	**0.53**	**0.46**	**1.24**	**1.19**
L.S.D at 5%	0.28		0.08		0.21		0.12		0.11		0.21		0.20	

(↑) Increase (↓) decrease relative to control

Table (4): Hematological parameters of male rats treated orally with sub lethal doses of Herbicides treated for 4 Weeks

Parameters	Counts of				Specific Activity								Concentration	
Treatments	**R. B. Cs** $10^6/mm^3$ ↓		**W. B. Cs** $10^3/mm^3$ ↑		**SGOT** U/ml ↑		**SGPT** U/ml ↑		**Creatinine** mg/dl ↑		**Urea** mg/dl ↑		**Hb** g/100ml ↓	
Does	1/5	1/10	1/5	1/10	1/5	1/10	1/5	1/10	1/5	1/10	1/5	1/10	1/5	1/10
Control	**6.42**		**4.85**		**64**		**33.67**		**1.20**		**43.30**		**18**	
Hhalosulfuron-methyl	**3.04**	**3.43**	**6.91**	**6.46**	**93.33**	**75.33**	**57.67**	**44.33**	**3.16**	**2.70**	**68.17**	**59.60**	**12.33**	**13**
Clethodim	**2.84**	**3.31**	**6.95**	**5.91**	**97.67**	**84**	**67.03**	**54.33**	**3.06**	**2.29**	**59.49**	**49.80**	**11**	**11.33**
L.S.D at 5%	0.87		0.52		11.78		6.31		1.05		4.97		3.21	

- (↑) Increase (↓) decrease relative to control.

DISCUSSION

Body weights

The decrease in body weight gain of treated rats compared to control may be due to the reduction of food intake as a result of poor palatability (taste) of the toxic diet. The elevation or decline in body weight is a measure of the toxicity of a substance (Lu, 1996). The loss of body weight in rats caused by pesticide exposure is a regular occurrence (Chung *et al.,* 2002; Shama *et al.,* 2005; Kalender *et al.,* 2006; Mansour and Mousa, 2010). In contrast, the body weight of rats given carbofuran increased significantly (Brkic *et al.,* 2008). The reduced body weight of treated rats might be related to either the herbcide's harmful effects or the diet's lower palatability (Paul, 2009). Decreases in body weight following exposure to the toxic chemicals were sensitive indicators of toxicity **(**Lee *et al.,* 2001; Teo *et al.,* 2002; Tayeb *et al.* 2010). The decrease in body weight growth of treated rats compared to untreated rats might be attributable to the treated rats' reduced food and water consumption.

Organ weight

The only overt sign of toxicity was decreased rat body weight gain and food consumption in male rats especially at high dose level. There were also changes in internal organs weights due to the enlargement of liver was might attributed to the accumulation of abnormal amount of fat (lipids) in the parenchymal cells of treated rats. The reduction of testes weight might be due to the reduction of the bioavailability of androgen, reduce the number of germ cells, inhibition of spermatogenesis and steroidogenic enzyme activity. Also, other organ system may be involved, including the brain; therefore, one could attribute the enlargement of the target organs to their increased activities. However, the increase in relative kidney weight could be due to edema and inflammation of kidney tissue. The higher studied doses of halosulfuron-methyl and clethodim increased absolute liver weight. These findings were consistent with those obtained by (Garg *et al.*, 1987; Zulalian, 1990; Shah *et al.,* 1994, 1997; US EPA, 1997; Hazarika and Sarkar 2001; TOXNET, 2003; Balkan and Akta 2005) to the two herbicides compared to corresponding controls. However, absolute kidney weight was lower in herbicide-treated rats than in control rats, that WHO (1996) stated that absolute kidney weight increased in males at 5000 mg/kg. However, Shah *et al.* (1998) mentioned that the chronic toxicity of pendimethalin was studied in adult male rats, pendimethalin had no significant effect on relative kidney weights. Organ and relative organ weights are significant factors for evaluating organ toxicity in toxicological studies (Timbrell, 2000; Crissman *et al.,* 2004). The formation of aberrant cells might explain the expansion of the liver and kidneys, as well as their weight gain, in pesticide-treated rats. The buildup of triglycerides was caused by a conflict between the rate of triglyceride synthesis and the rate of triglyceride release into the systemic circulation by parenchymal cells (Plaa, 1975).

Haematological Parameters

These effects were more prominent in rats given high sub lethal doses of halosulfuron-methyl (326 mg a.i./kg/day) and clethodim (1773 mg a.i. /kg/day), consecutively. It have been reported that the reduction in RBCs counts may be attributed to more than one factor. First, the failure to supply the circulation with cells from hematopoietic tissues which might have suffered from a destructive effect of the toxicant on these tissues. Second factor might be the possible destructive effect of toxicants to red blood cell membranes. In addition, the decrease in Hb values may be due to the reduction in the general food intake by rats of no extra iron. El-Bakary *et al.* (1995) found that hemoglobin concentrations are closely related to RBC count. According to Eissa and Zidan (2009), the inability to supply the blood circulation with cells from hemohepatic tissues, which plays a key role in the regeneration of RBCs, and hence hemoglobin concentration, might be attributable to pesticides. The rise in WBCs in herbicide-treated rats, on the other hand, might be due to the animal's defensive mechanism and immune system being activated, causing it to release cells that fight foreign invaders, demonstrating that halosulfuron-methyl and clethodim are hazardous to biological systems. Similar results on the effect of pesticides on hematological parameters were found by Marzouk *et al.* (2005), Eissa and Zidan (2009), Mossa and Abbassy (2012). In comparison to control rats, the results of this section of the study demonstrate that oral administration of sub-lethal dose of halosulfuron-methyl and clethodim to male rats caused hematotoxicity.

Liver function parameters

For the activities of amino transaminases are normally intracellular enzymes. (AST) increase in plasma is markedly myocardial infraction, while (ALT) increases in plasma are markedly liver cirrhosis due to hebatotoxicity causing permeability and leakage of lysosomal enzymes which enhanced the release of these enzymes. The findings presented above are similar to that reported by many authors (Shrivastava *et al.,* 1991; Adeniran *et al.,* 2006; Yousef *et al.,* 2006; Eissa and Zidan, 2009; Abbassy and Mossa, 2012) who mentioned pesticides induced considerable changes in various biochemical markers in the serum of pesticide-treated rats, and serum total proteins were elevated under the effect of pesticides (Soliman *et al.,* 1983). Farag *et al.* (1994) stated that the herbicides glyphosate and fluazifop-butyl, when given orally to adult albino rats, raised blood total protein and blood glucose levels. El-Sharkawy *et al.* (2011) discovered a dose-dependent rise in blood glucose, total protein, and cholesterol in fish exposed to 10% and 5% LC_{50} of Stomp Reg. On contrary, pesticides considerably lowered serum cholesterol concentration, according to Boudreau and Nadeau (1987), and Enan (1983). Also, Stomp 30 had no impact on total protein levels in treated rats, according to Shah *et al.* (1998).

Kidney function parameters

The change in uric acid and creatinine concentrations in the blood of treated rats might be due to a decrease in glomerular filtration in the kidney, as well as renal tubule malfunction (Hayes 1989; Walmsley and white, 1994).

CONCLUSION

Herbicide exposure leads to abnormal hematological and biochemical activities as well as induced Oxidative stress in rats, which in turn leads to observable toxicity. Treating rats with both herbicides for 28 days induced a significant decrease in RBC counts, and hemoglobin content. In contrast, the effects of both herbicides led to a significant increase in WBC counts. Additionally, tested herbicides exposure led to a significant increase in the production of transaminases, ALT and AST enzymes. The results also revealed a significant increase in serum creatinine levels in response to toxicity of tested compounds, which might be due to kidney function impairment by the herbicide that was used for this study. It is concluded that these herbicide effects may be attributed to induced Oxidative stress, which in turn led to the toxicity that was recorded in this work. The present study suggests that herbicides must be examined for their possible adverse effects on animals and humans before their application to agricultural fields. Therefore, further studies are needed for assessing the prevalence and severity of such damage from prolonged exposure to herbicides.

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السمية تحت الحادة لاثنين من مبيدات الحشائش الهالوسلفورون – ميثيل والكليثوديم على المقاييس البيوكيميائية والهيماتوليجية على ذكور الجرذان البيضاء

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تم دراسة التأثيرات السامة لمبيدي الحشائش الهالوسلفورون - ميثيل والكليثوديم بالجرعات المستخدمة على الكبد و الكلى و بعض مكونات الدم و ذلك على ذكور فئران التجارب البيضاء لمدة ٢٨ يوماً عن طريق الفم. حيث تم إعطاء الحيوانات جرعات من هالوسلفورون - ميثيل (٨٨٦ و١٧٧٣ ملجم/كجم من وزن الجسم) و الكليثوديم (١٦٣ و٣٢٦ ملجم/ كجم من وزن الجسم) والمساوية لـ١٠/١ و ٥/١ من الجرعة القاتلة للنصف لكل من المبيدين محل الدراسة يوما بعد يوم في حين تم إعطاء الماء المقطر للفئران في معاملة المقارنة (الكنترول). تم دراسة تأثير المبيدين المختبرين على بعض مكونات الدم وبعض الوظائف الحيوية للفئران المعاملة، بالإضافة إلي زيادة وزن الجسم ووزن الأعضاء. وبشكل عام كان معدل الزيادة في وزن الجسم والوزن النسبي للخصيتين لكل الفئران المعاملة أقل بشكل معنوي ذات دلالة إحصائية $p\leq$٠.٠٥ عند مقارنتها بمعاملة المقارنة، في حين أن الأوزان النسبية للكبد والكلى والرئتين والطحال والمخ والقلب على مستويات مختلفة من الارتفاع. ومن ناحية التأثير على بعض مكونات الدم في الفئران المعاملة أحدثت المعاملة بتلك المبيدات بالجرعات محل الدراسة تغيرات معنوية ذات دلالة إحصائية عند $p\leq$ ٠.٠٥، فتم حدوث خفض معنوي في عدد خلايا الدم الحمراء ونسبة الهيموجلوبين في حين حدثت زيادة معنوية في عدد كرات الدم البيضاء في الفئران المعاملة مقارنة بتلك الموجودة في معاملة المقارنة. في حين كان التأثير على وظائف الكبد ممثلة في دراسة نشاط الإنزيم الناقل لكلمن الامينواسبرتات (AST) والأمينو الانين (ALT)، في حين أن دراسة التأثير على وظائف الكلى كان ممثلا في تقدير مستويات الكرياتنين و حمض اليورك في سيرم دم الفئران المعاملة و أظهرت النتائج عن حدوث زيادة معنوية ذات دلالة إحصائية عند $p\leq$ ٠.٠٥ في مستويات إنزيمي الكبد (ALT, AST) ومستويات الكرياتنين و حمض اليورك وذلك مقارنة بمعاملة المقارنة (الكنترول) و كانت تلك الزيادة مرتبطة بالجرعة. وتشير النتائج إلى أنه حتى في التعرض تحت الحاد، قد يسبب الهالوسلفورون - الميثيل والكليثوديم تغييرات في بعض مكونات الدم و كذا حدوث تداخل ما بين المبيدات المختبرة بالجرعات المستخدمة ووظائف كل من الكبد و الكلى نتيجة التنشيط الحادث في الجهاز المناعي لدي الفئران لمحاربة المواد الدخيلة مما يؤكد أن مبيدى الهالوسلفورون - الميثيل والكليثوديم سموم للنظم الحيوية.