

IMPACT OF HEAVY METAL EXPOSURE ON IMMUNOLOGICAL AND LIVER HEALTH IN PETROLEUM INDUSTRY WORKERS

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Abstract

This study investigates the health impact of prolonged exposure to heavy metals on workers in the petroleum industry. Forty participants were grouped into four categories: control, mechanic workers, filling station attendants, and artisan petroleum handlers. Blood samples were analyzed for the presence of heavy metals, immunological parameters, and liver function markers. The results showed that the highest concentrations of heavy metals were found in artisan petroleum handlers, followed by mechanic workers and filling station attendants, with the lowest levels observed in the control group. In terms of immune function, artisan petroleum workers exhibited the highest levels of immunoglobulins, while the control group displayed the lowest. Liver enzyme levels were significantly lower in the control group and progressively higher in the filling station attendants, mechanic workers, and artisan petroleum handlers. These findings highlight the potential risks posed by heavy metal exposure in the petroleum industry, particularly in terms of immunotoxicity and hepatotoxicity. The results underscore the need for improved health and safety regulations to protect workers from the detrimental effects of heavy metal accumulation.

Keywords: Heavy metals, Petroleum industry, Immunological health, Liver function, Occupational exposure

Introduction

Heavy metals are a group of elements that have long been a subject of concern due to their persistence in the environment and their potential toxicity to humans and wildlife. Unlike organic pollutants, which can degrade over time through natural processes such as biodegradation, heavy metals do not break down easily. Instead, they accumulate in the environment and in living organisms through processes such as bioaccumulation and biomagnification. This results in increasingly higher concentrations of these metals in the food chain, with top predators—including humans—bearing the brunt of exposure. This characteristic of heavy metals presents a unique challenge for public health, especially in industrial settings where exposure to these substances is

prevalent. Heavy metals such as lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), and chromium (Cr) are among the most commonly recognized pollutants in both the environment and occupational settings. The sources of heavy metal pollution are diverse, and they can be both natural and anthropogenic. Natural sources include volcanic eruptions, weathering of metal-bearing rocks, and forest fires. However, the majority of heavy metal pollution can be attributed to human activities. Mining, smelting, industrial production, and the use of metal-containing products such as batteries, pesticides, and fertilizers are primary contributors to the release of heavy metals into the environment (Jaishankar et al., 2014). These metals can enter soil, water, and air and are then absorbed by living organisms, leading to contamination of ecosystems.

Industries that involve the extraction, processing, and handling of heavy metals pose significant risks to workers due to direct and prolonged exposure to these substances. Among such industries, mining, metalworking, and petroleum refining are particularly notable. Workers in these fields are often exposed to heavy metals through inhalation of contaminated air, ingestion of polluted water or food, and dermal contact with contaminated equipment or surfaces. Petroleum refining, for instance, involves a range of processes that can release toxic metals such as vanadium, nickel, and lead into the environment. This exposure is compounded by the fact that these metals can persist in the human body, accumulating in various tissues and organs over time. The petroleum industry, while critical to global economic development by providing energy and raw materials for various products, presents considerable health hazards to its workers due to these persistent toxic exposures.

In petroleum refining, heavy metals are commonly released during the extraction, processing, and handling of petroleum products. The chemicals and by-products generated during these processes can contaminate the air, water, and soil around petroleum refineries, increasing the risk of exposure for workers. The metals commonly associated with the petroleum industry include lead, mercury, arsenic, and cadmium. These substances can enter the human body through several routes, including inhalation of contaminated air, dermal absorption from direct contact with contaminated surfaces, and ingestion of polluted food or water. As workers are regularly exposed to these metals, they may experience a variety of adverse health effects due to the accumulation of these substances in their tissues.

One of the primary organs affected by heavy metal exposure is the liver. The liver plays a central role in detoxifying harmful substances, including heavy metals. It metabolizes and excretes various toxic compounds, preventing them from causing systemic harm. However, chronic exposure to high levels of heavy metals can overwhelm the liver's detoxification capacity, leading to hepatotoxicity. Hepatotoxicity refers to liver damage resulting from the toxic effects of various substances, including heavy metals. Over time, exposure to metals like cadmium, mercury, and lead can impair liver function, leading to a range of conditions such as fatty liver disease, fibrosis, cirrhosis, and even liver cancer (Satarug et al., 2010).

Heavy metals also have a profound impact on the immune system. The immune system is crucial for defending the body against pathogens and maintaining overall health. However, exposure to heavy metals can disrupt the normal functioning of the immune system, leading to a variety of health issues. Metals such as lead, mercury, and cadmium can impair the activity of immune cells, including macrophages, lymphocytes, and neutrophils. This disruption can result in immunosuppression, which makes individuals more susceptible to infections and chronic inflammation. Additionally, heavy metal exposure has been linked to an increased risk of autoimmune disorders, where the body's immune system mistakenly attacks its own tissues. The immunotoxic effects of heavy metals are a major concern in occupational settings, where workers are exposed to these substances over extended periods. The interference of heavy metals with cellular signaling pathways and their ability to induce the production of reactive oxygen species (ROS) further exacerbate the damage to the immune system (Dietert & Piepenbrink, 2006).

Despite the growing body of research on heavy metals and their impact on health, there is still limited comprehensive information regarding the combined effects of heavy metal exposure on both immune and liver health in occupational settings, particularly in the petroleum industry. This gap in knowledge is concerning, as it limits the development of effective risk assessment strategies and safety guidelines for workers who are exposed to these toxic substances. The petroleum industry is a high-risk environment where workers are regularly exposed to a combination of heavy metals, and the long-term consequences of such exposure are still not fully understood. Furthermore, occupational health guidelines are often inadequate to address the specific risks posed by exposure to heavy metals in the petroleum industry. The cumulative effects of prolonged exposure to multiple heavy metals can have a compounded impact on workers' health, yet most studies have focused on the effects of individual metals in isolation. This lack of comprehensive research presents a significant challenge to developing effective safety regulations and workplace practices to protect workers.

Therefore, this manuscript aims to investigate the dual impact of prolonged exposure to heavy metals on the immune and liver health of workers in the petroleum industry. The primary goal is to understand the mechanisms through which heavy metals induce toxicity and how they affect immune and liver function. By bridging the knowledge gap on this topic, the study seeks to provide insights into the potential risks faced by workers in the petroleum industry and contribute to the development of improved health and safety regulations. With this research, we hope to increase awareness of the occupational hazards associated with heavy metal exposure and offer evidence-based recommendations for safeguarding the health of workers in this critical industry. Through better understanding and risk management, it is possible to reduce the adverse health effects of heavy metal exposure and improve the overall safety and well-being of workers in the petroleum sector.

MATERIALS AND METHOD Study Area

The study areas include; Mbodo Aluu in Ikwerre Local Government Area of Rivers State, Umuahia in Abia State, Nigeria, Choba, in Obio/Akpor Local Government Area of Rivers State. These are major communities in Rivers State and Abia. With Umuahia, in Abia State witnessing little or no oil and gas activities. While Choba and Aluu has major of such activities within Rivers State.

Ethical Considerations

Ethical clearance was obtained from the University of Port Harcourt ethical committee for permission to collect blood samples from subjects for the purpose of this study. Written consent was obtained also from participants and all data were kept anonymous and confidential.

Materials and Equipment

Equipment Measuring cylinder (Pyrex), Vacutainer bottles, Cover Slip, Centrifuge (Coslab: Cle-110), Pipette Spectrophotometer (Labomed: UVD-3500, USA), Incubator (Shel Lab: SM16, Chempet), Refrigerator (Labtob: LLR-170, Thailand), Timing device, Vacutainer Needle and Syringe., Test tubes (Pyrex)

Reagents All chemicals/reagents and analytical kits used for the analyses were of analytical grade. They include; IgD, IgA, IgM, IgE & IgG standards, Sample diluent, IgD, IgA, IgM, IgE & IgG antibody conjugated to enzyme, Substrate, stop solution, Microtiter plate coated with anti-human IgD, IgA, IgM, IgE & IgG antibody, Distilled water, Randox kits (Crumlin, Co. Antrim, United Kingdom)

Methods

Sampling Method Blood sample (5ml) was obtained through venipuncture with Vacutainer system and collected into tagged ethylene diamine tetra-acetic acid (EDTA) anti-coagulant and serum- separating tubes. The labeled tubes housing the blood samples were put in cooler boxes lined with ice pack at the point of blood collection. Personal protective equipment (PPE) as required were in use. **Heavy Metal Analysis** Sample preparation Wet digestion method: a 100ml of HNO₃, H₂SO₄, and HClO in the ratio of 40%:40%:20% were mixed together, 1g of sample was measured into conical flask, with 2mL of mixed acid added into each sample in the conical flask. It was thereafter digested/heated in a fume cupboard using hot plate until white fumes were seen. This solution was filtered into a 100ml volumetric flask and made up to mark with distilled water after cooling.

Atomic Adsorption Spectrophotometer (AAS) Procedure

Heavy metals (Lead, Cadmium, Chromium, Nickel and Zinc) were analyzed using AAS Model: S4=71096, with eight-socket hollow cathode lamps. The gases used in this instrument were acetylene and air.

Biochemical Parameters Assay

Alanine transaminase (ALT), Plasma Aspartate transaminase (AST), Plasma Alkaline Phosphatase Activity (ALP), and Plasma Gamma glutamyl transferase (GGT) activity were evaluated using the Randox Method while IgG, IgD, IgA, IgM & IgE were determined using Enzyme-Linked Immunosorbent Assay (ELISA).

Statistical Analysis

SPSS software version 23 was used. Mean values (M) $\pm$ SEM were calculated and One-Way ANOVA (Analysis of Variance) test was performed. Significance level was considered at 95 % confidence level ($p < 0.05$).

RESULTS Heavy Metals in Haem from Sampled Individuals

The heavy metal contents of blood samples obtained from sampled individuals differentially exposure to petroleum products is as shown in table 4.1. The result revealed Pb range from 0.0033 ± 0.001 in control to 0.8908 ± 0.125 , Cd 0.00061 in control to 0.019 , Cr 0.0044 to 1.982 , Ni 0.0016 to 0.218 and Zn 0.1 to 3.18 mg/L. minimum and maximum values for all heavy metals were obtained from Control (Umuahia) and APH (Artisan petroleum handlers in Mgbodo Aluu) respectively. The obtained results showed significant difference between the various groups and the control at $p < 0.05$.

Table 1: Heavy Metals in Haem from Sampled Individuals

Group	Pb (mg/l)	Cd (mg/l)	Cr (mg/l)	Ni (mg/l)	Zn (mg/l)
Control	0.0033 ± 0.00^a	0.00061 ± 0.00^a	0.0044 ± 0.0014^a	0.0016 ± 0.000^a	0.1003 ± 0.04^a
MW	0.3958 ± 0.08^b	0.0334 ± 0.005^b	0.7337 ± 0.0906^b	0.2049 ± 0.059^b	1.0267 ± 0.17^b
MAFL	0.2883 ± 0.10^b	0.0151 ± 0.005^c	0.9420 ± 0.274^b	0.1255 ± 0.044^b	1.8838 ± 0.23^b
APH	0.8908 ± 0.12^c	0.0193 ± 0.004^c	1.9820 ± 0.236^c	$.2182 \pm 0.0513^b$	3.1832 ± 0.47^c

Values are Mean $\pm$ SEM, means in the same column with same superscript alphabet are not significantly different while means in same column with different superscript alphabets are significantly different at $p \leq 0.05$, $n = 10$

Liver Function Indices in Haem from Sampled Individuals

The Liver function parameters assayed in blood samples obtained from sampled individuals differentially exposure to petroleum products is as shown in table 4.2. The result revealed ALP range from 45.03 to 182.02 , ALT 8.00 to 55.70 , AST 14.18 to 47.72 , GST 5.17 to 15.96 and GGT 11.82 to 49.30 U/L. minimum and maximum values for all hepatological indices were obtained from Control (Umuahia) and APH (Artisan petroleum handlers in Mgbodo Aluu) respectively. The obtained results showed significant difference between the various groups and the control at $p < 0.05$.

Table 2: Liver Function Indices in Haem from Sampled Individuals

Group	ALP	ALT	AST	GST	GGT
Control	45.03±3.73 ^a	8.00±1.33 ^a	14.18±0.96 ^a	5.17000.55 ^a	11.82±1.47 ^a
MW	175.43±6.06 ^b	55.10±4.17 ^b	41.99±2.42 ^b	13.42±1.31 ^b	44.88±3.43 ^b
MAFL	169.32±4.39 ^b	53.90±3.03 ^b	44.68±1.77 ^b	13.72±1.25 ^b	46.13±2.15 ^b
APH	182.02±20.02 ^b	55.70±6.44 ^b	47.72±2.32 ^c	15.96±1.35 ^b	49.30±3.51 ^b

Values are Mean±SEM, means in the same column with same superscript alphabet are not significantly different while means in same column with different superscript alphabets are significantly different at $p \leq 0.05$, $n=10$

Immunological Indices in Haem from Sampled Individuals

The Immunological Indices assayed in blood samples obtained from sampled individuals differentially exposed to petroleum products is as shown in table 4.3. The result revealed IgG range from 10.12 to 29.19, IgE 27.70 to 242.84 (MAFL), IgA 1.63 to 11.68 IgM 1.23 to 9.34 and IgD 34.60 to 259.66. Minimum and maximum values for IgG and IgE were obtained from Control (Umuahia) and MAFL (Filling Station in Aluu) respectively, while minimum and maximum values for IgA, IgM and IgD were obtained from Control (Umuahia) and APH (Artisan petroleum handlers in Mgbodo Aluu) respectively. The obtained results showed significant difference between the various groups and the control at $p < 0.05$.

Table 3: Immunological Indices in Haem from Sampled Individuals

Group	IgG	IgE	IgA	IgM	IgD
Control	10.12±0.78 ^a	27.70±8.84 ^a	1.63±0.24 ^a	1.23±0.17 ^a	34.60±4.17 ^a
MW	25.88±1.94 ^b	219.94±15.79 ^b	8.80±0.87 ^b	6.57±0.60 ^b	146.94±10.39 ^b

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MAFL	29.10±1.22 ^b	242.84±15.02 ^c	11.41±1.02 ^b	8.91±0.68 ^b	162.54±10.67 ^c
APH	29.16±1.94 ^b	237.08±15.05 ^c	11.68±0.90 ^b	9.34±0.72 ^b	259.66±97.69 ^d

Values are Mean±SEM, means in the same column with same superscript alphabet are not significantly different while means in same column with different superscript alphabets are significantly different at $p \leq 0.05$, $n=10$

DISCUSSION

Heavy metals are known as elements with a relatively high density when compared to water (Fergusson, 1990). With the postulation that heaviness and toxicity are inter-alia, metalloids such as arsenic are as well seen as heavy metals, as they are able to induce toxicity even at low concentrations (Duffus, 2002). Recently, environmental and ecological contamination/ pollution by these metals has been of high public health concern globally, as human exposure has risen dramatically due to an exponential rise from several industrial, domestic, agricultural, and technological applications, usages and contamination (Bradl, 2002).

International Agency for Research on Cancer (IARC, 1987) classified iron (Fe), zinc (Zn), copper (Cu) and nickel (Ni) amongst others as non-carcinogenic metals, with Pb, As, Cr and Cd being known as both carcinogenic and non-carcinogenic metals known for their widespread role in environmental pollution (Sanborn *et al.*, 2002). Neuropathy, cardiovascular deaths, slow growth development and respiratory illnesses are some prominent diseases associated with exposure to these metals (Turner and Hefzi 2010). The level of these metals obtained from different sample sites as shown in table 1. revealed notable difference when compared with known standards. The results of the heavy metal contents from blood samples of individuals exposed to various petroleum products pollution sources are shown in table 1. The result revealed Pb range from 0.0033 ± 0.001 in control to 0.8908 ± 0.125 , Cd 0.00061 to 0.019 , Cr 0.0044 to 1.982 , Ni 0.0016 to 0.218 and Zn 0.1 to 3.18 mg/L. minimum and maximum values for all heavy metals were obtained from Control (Umuahia) and APH (Artisan petroleum handlers in Mgbodo Aluu) respectively. The obtained results showed significant difference between the various groups and the control at $p < 0.05$. The values obtained were above WHO recommended values for all metals except Zn which were quite minimal compared to recommended standards. However, different heavy metals were shown to be spatially different when exposed to various heavy metal pollution sources. The result showed Pb levels were significantly different between MW and APH, and between MAFL and KPO. Cadmium levels were shown to be significantly different between MW and MAFL and between MW and APH. Chromium blood level was shown

to be significantly different between MW and MAFL and between MW and APH. The heme concentration of nickel (Ni), revealed a significantly different level of Ni between MW and MAFL. Zinc (Zn) levels were also shown to be significantly different between MW and MAFL, MW and APH and between MAFL and APH. All the groups were shown to be Significantly different when compared to the control at $P < 0.05$. Seregin and Ivaniov, (2001) reported that Pb has potentials to alter membrane permeability, water imbalance, inhibits enzyme activities, mineral nutrition disturbance with lots more other lethal effects at high Pb dosage. Report by Uaboi – Egbenni, (2010) also shows Its toxic effect within kidneys, liver etc. studies revealed that Cadmium even at Low ingestion is toxic and could on chronic exposure lead to; bone disorders, (Asia *et al.*, 2008; Dan-lin *et al.*, 2011). Dayan and Paine, (2001) reported that Cr(III) exposure could be associated with respiratory diseases, with evidenced wheezing, coughing etc. Several reports have shown Pb presence in the even in dust samples, Popoola *et al.*, (2012) reported Pb presence in classroom dusts of schools within Lagos State, Olua *et al.* (2018) also reported presence of heavy metals in dusts samples obtained from classrooms around high anthropogenic crude oil contaminated environment. It has also been reported that Pb may become toxic to plants and animals if their concentrations exceed certain levels (0.0035) as set by USEPA and WHO (Aydinalp and Marinova, 2009), as Pb has the potential to inhibit water imbalance, alter mineral nutrition, enzyme activities, hormonal status and membrane permeability alteration. Reports have shown that Pb at increased concentrations could inhibit cellular activities thus causing cell death (Seregin, and Ivaniov, 2001). It as well has a toxic destructive impact on the kidneys, central nervous system, reproductive system, liver etc with most severe effect being brain necrosis, these hence poses dangerous threat to our environment. (Ademoroti, 1996; Asia *et al.*, 2008; Uaboi – Egbenni, 2010). It's been reported that exposure to Pb can cause damage or reduce children's intelligence and academic performance. It also has potential to decrease hearing ability and children's sight and could cause memory loss and attention deficit disorders. (Sanborn *et al.*, 2002). The mean Cd levels seen is of high public health concern as Gough *et al.*, 1979; Adriano, 1986 and Aydinalp and Marinova, 2009; have independently reported that Cd does not have any known beneficial effects and could become toxic to plants and animals on bioaccumulation whereas Asia *et al.*, 2008 reported that Cd is toxic even if absorption by ingestion is low. Chronic exposure to elevated cadmium levels in food causes bone disorders, including osteoporosis and osteomalacia (Asia *et al.*, 2008). The EPA, (1971) accounted for the moderately toxicity of Cadmium to all organisms, with cumulative poisoning in animals concentrating at the liver, kidney, pancreas and thyroid of humans and other mammals (EPA, 1971). It's major route of entrance in humans is through gastro-intestinal tract by consumption of foods grown on contaminated soil, however smokers may receive a considerable part of their cadmium by inhaling cigarette smoke (Corbett *et al.*, 2002 and Iqbal, 2011). Cadmium levels on the average as observed in this study was above the average concentration in the earth's crust $0.2\mu\text{g/g}$ (Lewis, 2004), suggesting an anthropogenic Cd source in all sampled sites. Studies have shown that occupational exposure to Cr (III) via inhalation has been associated

with respiratory effects with an evidenced wheezing, coughing, etc. (Novey and Habib, 1983). Other observable symptoms include; hyperemia, asthma, chronic rhinitis, chronic bronchitis, chronic pharyngitis, tracheobronchitis, etc. (Dayan and Paine, 2001). Chromium absorbed via dermal contact could trigger immune response. Sensitized people will showcase allergic dermatitis when exposed to high chromium level (Polak, 2019). Localized vesicular lesions on points of contact or eczematous dermatitis could suggest sensitization (Lewis, 2004; ATSDR, 2020). Lung cancer risk analysis suggests potential excessive death risk from lung cancer amongst U.S. workers exposed to previous permissible limit for Cr(VI) ($52 \mu\text{g m}^{-3}$) (Braver and Infante, 1985). Recent studies also disclosed excessive mortal lung cancer risk from occupational Cr(VI) compounds' exposure (Gibb et al., 2020; Park et al., 2004). Activities of localized industries within sampled sites might be responsible for the Chromium levels reported in this study as the result showed that Cr is of the highest amount of all heavy metals assayed. The outcome of the plasma hepatospecific enzyme activities in sampled individuals as shown in Table 2 demonstrated significant alterations in activities of these indices as seen in individuals from various sampled locations. The result revealed ALP range from 45.03 to 182.02, ALT 8.00 to 55.70, AST 14.18 to 47.72, GST 5.17 to 15.96 and GGT 11.82 to 49.30 U/L. minimum and maximum values for all hepatological indices were obtained from Control (Umuahia) and APH (Artisan petroleum handlers in Mgbodo Aluu) respectively. The obtained results showed significant difference between the various groups compared to the control at $p < 0.05$. This study corroborates reports that cumulative exposures to crude oil related pollution in laboratory animals resulted in biochemical changes in the liver (Bhawan and Nagar, 2008). High ALT, AST values is suggestive of impairment of liver functions (Tomao *et al.*, 2002). Individuals exposed to petroleum product polluted environments presented highly significant levels of assayed enzymes compared to the control group obtained from Umuahia in Abia State. This is an indication of hepatocellular damage. Elevated levels of liver function markers are often found in blood circulation when the integrity of liver is compromised (Green and Flamm, 2002). The Immunological Indices assayed in blood samples obtained from sampled individuals differentially exposed to petroleum products shown in table 3 also revealed significantly elevated Immunoglobulins. This is suggestive of an immune response to invading or foreign antigens that might have been elicited by the presence of these heavy metals. The result revealed IgG range from 10.12 to 29.19, IgE 27.70 to 242.84, IgA 1.63 to 11.68 IgM 1.23 to 9.34 and IgD 34.60 to 259.66. Minimum and maximum values for IgG and IgE were obtained from Control (Umuahia) and MAFL (Filling Station in Aluu) respectively, while minimum and maximum values for IgA, IgM and IgD were obtained from Control (Umuahia) and APH (Artisan petroleum handlers in Mgbodo Aluu) respectively. The obtained results showed significant difference between the various groups and the control at $p < 0.05$. Immunoglobulins (Igs) are produced by B cells and contribute to the immune response via antigen binding and/or by mediating specific effector functions (Schroeder, 2010; Kaneko et al., 2000 and Griffin et al., (2000)). Both IgG, the most abundant Ig in the body, and IgM, the initial Ig expressed in response to an acute

infection, play critical roles in neutralizing toxins and other immunogens, whereas increased production of IgE specifically is closely associated with hypersensitivity and allergic responses (Kaneko et al., 2000). Hence, values obtained from this study is suggestive of hypersensitivity and allergic responses and is also indicative that the body treats these heavy metals as foreign bodies and or toxins. The results revealed significant difference between the various groups and the control at $p < 0.05$ degree of confidence. The IgA levels were shown to be significantly different between MW and MAFL, and between MW and APH. While IgM were significantly different between MW and MAFL, and between MW and APH. The findings suggest that individuals exposed to petroleum products, particularly artisan petroleum handlers, have significantly higher levels of heavy metals in their blood, accompanied by elevated liver function markers and immunological indices. This indicates both hepatotoxic and immunotoxic effects, with a strong association between exposure to heavy metals and adverse health outcomes in these populations. The significant differences observed between the exposed groups and the control emphasize the health risks associated with occupational and environmental exposure to petroleum products. This observation is consistent with the findings of Johnson et al. (2020), who reported that chronic exposure to lead resulted not only in immune system activation but also in subsequent immunosuppression, characterized by reduced T-cell counts and impaired immune responses.

CONCLUSION

The elevated levels of heavy metals, liver enzymes, and immunological parameters observed in this study suggest significant hepatotoxic and immunotoxic effects. These findings contribute to the growing body of evidence linking heavy metal exposure to serious health risks and underscore the need for preventive measures to protect vulnerable populations.

RECOMMENDATION

Future studies could employ longitudinal designs to better understand the temporal relationship between heavy metal exposure and health outcomes. Additionally, research could focus on the molecular mechanisms underlying the toxic effects observed.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

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