# Evaluation of heavy metal levels in serum of Wistar rats exposed to engine oil

Iyanda A.A.*[1 A,B,C,D,F] , Anetor J.I.[2 C,F], Anetor G.O.[3 C,F]

1. Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria.
2. Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria.
3. Department of Public Health, National Open University of Nigeria.

---

**A**- Conception and study design; **B -** Collection of data; **C -** Data analysis; **D -** Writing the paper;
**E**- Review article; **F** - Approval of the final version of the article; **G** - Other (please specify)

---

## ABSTRACT

---

**Purpose:** Data are available that indicate there is an elaborate elemental constitution of petroleum products, with identified elements contained in the many products being additive (e.g. Ca, Zn and P) as well as wear metals (e.g. Ag, Al, Ba, Cd, Cr, Cu, Fe, Mg, Mo, Na, Ni, Pb and Sn). In addition, incessant deliberate exposure of engine oil to both human beings and farm animals for therapeutic reason has been reported. Therefore, the objective of this study is to evaluate the levels of heavy metals in serum of engine oil-exposed rats.

**Materials and methods:** Thirty adult female rats were divided equally into 5 groups. The first and second groups were treated with engine oil by oral route (as contaminant of feed) at dosage levels of 0.5 and 1.0 mL/kg body weight respectively. The third and forth groups received the test agent through the dermal route at dosage levels of 0.5 and 1.0 mL/kg body weight while the fifth group served as the control. The duration of the study was 30 days, after which blood was obtained from each rat, centrifuged and the resultant serum used for the analysis of heavy metals by employing Atomic Absorption Spectrometry (AAS). Data were analyzed using analysis of variance (ANOVA), $p \leq 0.05$ was considered significant.

**Results:** Data obtained showed that there were significant differences in the levels of aluminium, silicon, cadmium, lead, arsenic, vanadium, and nickel.

**Conclusions:** These increases suggest that incessant exposure to engine oil may be dangerous and therefore constitute health hazard.

**Keywords:** Engine oil, oral and dermal routes, lead, cadmium, aluminum, silicon, vanadium, arsenic, nickel

---

DOI: 10.5604/01.3001.0012.8327

***Corresponding Author:**
Iyanda A.A; email: lapeiyanda@yahoo.com
Tel.: +2347039407465

Received: 29.09. 2018
Accepted: 26.11.2018
Progress in Health Sciences
Vol. 8(2) 2018 pp 94-98

## INTRODUCTION

Engine oil is obtained from crude oil or synthetic oil for lubrication of combustion engines [1,2]. It is known to inhibit corrosion, improve sealing, clean and cool the engine by conveying heat away from moving parts. The constituents of engine oil include a complex mixture of hydrocarbons that is between 80% and 90% by volume and performance enhancing additives that is between the range of 10% and 20% by volume [1,2]. The additives are usually metals that are incorporated to enhance the efficiency of the oil.

According to Chen and Jiang [3], fuel samples (petrol, diesel, engine oil) contain both mercury and lead. Virgin engine oil especially has been identified to contain iron, cadmium, chromium, lead, and other heavy metals, and that higher levels of these elements are found in used engine oils [4]. Data obtained from the study of Kim et al. [5] revealed a more elaborate elemental constitution of petroleum products, with identified elements contained in the products being additive (e.g., calcium, zinc and phosphorus) as well as wear metal elements (e.g. silver, aluminum, barium, cadmium, chromium, copper, iron, magnesium, molybdenum, sodium, nickel, lead and tin).

In addition, using wavelength-dispersive X-ray fluorescence spectrometry to measure elements of phosphorus, sulfur and calcium in 98 engine oil (different grades) samples from countries such as America, Germany and Japan, results revealed that there was a proportional relationship between zinc/phosphorus, sulfur/molybdenum and calcium/ magnesium as illustrated by the correlation between experimental data and additive contents [6].

Concentration levels of zinc, phosphorus, calcium, sulfur, magnesium and molybdenum in engine oil have been reported to be 0.09%-0.17%, 0.07%-0.15%, 0.13%-0.43%, 0.20%-0.47%, 0.04%-0.15% and 0.01%-0.05% respectively [6], of which the calcium, sulfur, phosphorus, and zinc were the most abundant elements of engine oil. Moreover, they also observed that magnesium and molybdenum were found to exist in high-quality level engine oil, whereas niobium, tungsten, thallium or sodium was identified in only engine oil.

The presence of these elements in engine oil especially raises the possibility that incessant exposure may result in their increase levels in exposed-animals, and since the harmful effects of many of them are well described, it becomes expedient that this study be carried out to estimate the serum concentrations of heavy metals in Wistar rats treated with two different doses of engine oil administered through different routes of exposure.

## MATERIALS AND METHODS

**Petroleum product**

Engine oil (AP motor oil) was purchased from a filling station located in Osogbo, Osun State, Nigeria in December, 2011.

**Experimental Animals**

This study was carried out in compliance with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research Institutes of Health (revised 1985). Adult female albino rats with an average weight of 218 g were obtained from the Animal House attached to the Department of Veterinary Physiology, University of Ibadan, Nigeria. The animals were left to acclimatize for two weeks before the commencement of the experiment. Animals were housed in cages at an ambient temperature of 24±3°C and a 12 h light, 12 h dark cycle. All the animals were given free access to their specific diets and water.

Thirty rats were divided into five groups comprising of 6 rats per group. The first and second groups were treated with engine oil by oral route (as a contaminant of feed) at dosage levels of 0.5 and 1.0 mL/kg body weight respectively. The third and fourth groups received the test agent through the dermal route at dosage levels of 0.5 and 1.0 mL/kg body weight while the fifth group served as the control. The treatment groups were exposed to this product for 30 days. Deliberate contamination of feed took place each morning shortly before the rats were supplied with the feed, in which engine oil was freshly and thoroughly mixed with feed. On the other hand, dermal exposure occurs each day through the application of engine oil to the neck region to prevent oral contact from self-grooming.

**Preparation of serum samples and heavy metal estimation**

On the 31$^{st}$ day the study was brought to an end, and blood samples were drawn from all the rats by retro-orbital bleeding. These were discharged into anticoagulant free bottles and centrifuged at 3000 g.

The resultant sera from the procedure were kept at -20 ºC until required for analysis. Serum levels of aluminium, silicon, cadmium, lead, arsenic, nickel and vanadium were carried out using the atomic absorption spectrometric method. Buck Scientific 205 Atomic Absorption supplied by Buck Scientific (East Norwalk, Connecticut, USA) was utilized for this purpose.

**Statistical analysis**

The mean values of the serum levels of the heavy metals for control and each of the treatment groups were compared using Student's *t*-test while control, dermal and oral routes were compared using analysis of variance (ANOVA). Value of $p \leq 0.05$ was considered significant.

## RESULTS

Administration of engine oil to Wistar rats through the oral route resulted in significant increases ($p<0.05$) in the levels Si, Pb, As, V, Ni, Cd and Al at 0.5 mL/kg body weight when compared with control. On the other hand, only V was significantly increased ($p<0.05$) in dermal exposed rats when compared with control (Table 1).

Also in Table 1 intergroup comparison of control, dermal and oral groups (using ANOVA) showed significant differences ($p<0.05$) for all estimated elements.

Results of 1.0 mL/kg body weight presented in Table 2 showed significant increases ($p<0.05$) for all elements determined, using not only Student's *t* - test but analysis of variance as well.

**Table 1.** Serum levels of select heavy metals in engine oil administered rats (0.5mL/kg dosage level)

| | Si (µg/L)** | Cd (mg/dl)** | Al (µg/dl)** | Ni (µg/L)** | V (ng/dL)** | Pb (µg/L)** | As (ng/dL)** |
|---|---|---|---|---|---|---|---|
| Controls | 0.030±0.006 | 0.056±0.010 | 0.072±0.015 | 0.044±0.009 | 1.28±0.081 | 0.078±0.005 | 0.064±0.011 |
| Oral route | 0.041±0.012* | 0.077±0.012* | 0.091±0.021* | 0.049±0.009* | 1.34±0.046* | 0.099±0.014* | 0.074±0.010* |
| Dermal **Route** | 0.032±0.009 | 0.061±0.017 | 0.078±0.020 | 0.047±0.006 | 1.80±0.054* | 0.082±0.010 | 0.066±0.006 |

Results are expressed as mean ± standard error of mean. *p <0.05 is significant when compared with control using Student's t test. **p <0.05 is significant when control, oral and dermal groups were compared using ANOVA.

**Table 2.** Serum levels of select heavy metals in engine oil administered rats (1.0 mL/kg dosage level)

| | Si (µg/L)** | Cd (mg/dl)** | Al (µg/dl)** | Ni (µg/L)** | V (ng/dL)** | Pb (µg/L)** | As (ng/dL)** |
|---|---|---|---|---|---|---|---|
| Controls | 0.030±0.006 | 0.056±0.010 | 0.072±0.015 | 0.044±0.009 | 1.28±0.081 | 0.078±0.005 | 0.064±0.011 |
| Oral route | 0.053±0.018* | 0.091±0.009* | 0.099±0.026* | 0.066±0.010* | 1.80±0.071* | 0.113±0.027* | 0.094±0.011* |
| Dermal **Route** | 0.036±0.013* | 0.067±0.013* | 0.084±0.018* | 0.052±0.013* | 1.98±0.062* | 0.088±0.016* | 0.069±0.014* |

Results are expressed as mean ± standard error of mean. *p <0.05 is significant when compared with control using Student's t test. **p <0.05 is significant when control, oral and dermal groups were compared using ANOVA.

## DISCUSSION

Normally for most newly introduced chemicals that humans or other living organisms may come in contact with, both the qualitative and quantitative evaluation of their harmful or toxic effects is essential to establish the potential hazard they pose to these animals [7]. On the other hand, some other chemicals that have been in use for decades may also have evaluation of their toxicity revisited. This becomes necessary in many parts of the world where such chemicals are used for a variety of purposes for which they are not meant and for that reason, their toxicity through different routes are sometimes re-evaluated. Many of petroleum products fall into this category since it has been discovered that they are commonly used for treatment of a wide range of

medical conditions by the impoverished people of the developing countries [8-11].

Aside the natural biogeochemical cycles, human activities play an important role in the mobilization, transformation, and transportation of metals in the environment. Mining, dredging, construction, and manufacturing have been reported as known processes that involve removal of metals from the locations in which they naturally occur, and may incorporate them into the human economic sphere. Thus increasing the potential for both human and animal exposure as it is revealed through the results of this study in which significant increases were recorded for the elements, in engine oil exposed rats.

The toxic effects of a particular xenobiotic may be exerted through a myriad of ways: a direct interaction or reaction between a chemical and the specific target molecule though possible, sometimes it may not be the basis of toxicity for many xenobiotics. Rather very many different foreign agents have been reported to adversely influence the biological (micro) environment, thereby leading to molecular, organellar, cellular, or organ dysfunction which may manifest as one form of deleterious effect or another [12]. For many of the toxic elements contained in petroleum products e.g. engine oil, their basic concept of toxicity may be more complex and in most cases involves several steps.

For instance, ingestion of arsenic containing substances causes gastrointestinal irritation, peripheral neuropathy, vascular lesions, anemia, and various skin diseases. In addition, skin cancer has been reported from its high-level oral exposure from this element [12-14]. This means that careful attention has to be paid to the issue of the use of engine oil for therapeutic purpose. In addition, many case reports of death in humans due to intentional or unintentional ingestion of high doses of arsenic compounds have been established [13]. While there is insufficient information that link hepatic, renal, and dermal or ocular effects to arsenic exposure through inhalation, ingestion of arsenic containing substances may lead to severe cardiovascular effects, but this was not assessed in these rats. Other effects like anemia and leukopenia, also have been reported following acute, intermediate, and chronic oral exposures [14-16]. Many other heavy metals present in engine oil and identified in serum of the test animals are known for their characteristic toxic manifestations [17].

After the toxicant like Hg, V, Si, or Al is delivered to its target or targets, the ultimate toxicant may combine with endogenous target molecules, thereby initiating perturbations in cell function and/or structure. Although the first response after xenobiotic-exposure involves initiation of repair mechanisms at the molecular, cellular, and/or tissue levels, others such as adaptive mechanisms that reduce delivery, boost repair capacity and/or compensate for dysfunction may occur. It is only after perturbations induced by the toxicant far exceed repair and adaptive capacity or when repair and adaptation are not effective or malfunctional, that toxicity occurs. The significant increases in the levels of these elements observed in the test groups compared with control may suggest that these adaptive mechanisms may be overwhelmed in Wistar rats, especially at 1.0 mL of engine oil /kg BW where all the elements were significantly increased.

The toxic effects of engine oil to mammals have been recognized in an earlier study [18] in which rats orally administered with engine oil at 1.0 mL/kg level of exposure featured the following abnormal histologic presentations (liver-diffuse vascular degeneration of hepatocytes and necrosis; kidney-protein casts in the lumen). In many instances, the degree of such toxicity is primarily determined by level and route of exposure as revealed by the results of this study. In which rats administered with a dose of 1.0 mL/kg had higher levels of Ni, Si, V, and As than those of 0.5 mL/kg level of exposure. Although when a product that is introduced to an animal is made up of many components as it is common with petroleum products, it may be difficult to postulate the individual role of each component in its possible toxic presentation.

The significant differences in the levels of many of the toxic metals estimated suggest that the level of exposure was sufficient to alter their serum levels in engine oil exposed rats. This also indicates that the significant increase in the serum level of Ni, Si, V, and As could have been one of the factors responsible for the earlier observation of tissue damage in Wistar rats treated with the same levels of engine oil. The significant differences in the levels of these heavy metals may be linked to their presence in the engine oil used for the study. It may also be appropriate to infer that this kind of presentation was one of the causes of tissue damage reported in an earlier study [18].

## CONCLUSION

Data obtained from this study indicate that exposure to engine oil may result in the significant presence of toxic metals in the serum of affected animals, a situation which may result in a myriad of abnormal clinical presentations.

Toxic metal-induced organ toxicity, for example, is well described in the literature.

## Conflict of interest

None declared.

## REFERENCES


1. Irwin RJ, Vanmouwrik M, Stepens L, Seese MD, Bashman W. Environmental Contaminants Encyclo-peadia. Natural Park Service, Water Resources Division, Fort Coflins, Colorado. 1997.
2. Collins C. Implementing phytoremediation of petroleum hydrocarbons. In: Willey N. (Ed). Hytoremediation Methods and Reviews (pp 99 – 108). Human Press Incorporated, New Jersey. 2007.
3. Chen F, Jiang S. Determination of Hg and Pb in fuels by inductively coupled plasma mass spectrometry using flow injection chemical vapour generation. Analytical Science 2009;25:1471-6.
4. Al-Ghouti MA, Al-Atoum L. Virgin and recycled engine oil differentiation: a spectroscopic study. J Environ Manage. 2009 Jan;90(1):187-95
5. Kim Y, Kim NY, Park SY, Lee DK, Lee JH. Classification and individualization of used engine oils using elemental composition and discriminant analysis. Forensic Sci Int 2013 Jul;230(1-3):58-67.
6. Wang H, Lin ZX, Wu BL. Concentration levels and sources analysis of additives element in engine oil. Guang Pu Xue Yu Guang Pu Fen Xi. 2013;33(9):2579-82.
7. Gregus Z. Mechanisms of toxicity. In: Casarett and Doull's Toxicology The Basic Science of Poisons (pp 45-107). Curtis D. Klaassen (Ed). McGraw-Hill Medical Publishing Division New York, 2008.
8. Orisakwe OE, Akumka DD, Afonne OJ, Gamanniel KS. Investigation into the pharmacological basis for folkloric use of Bonny Light Crude Oil in Nigeria. Indian J Pharmacol 2000;32:231-4.
9. Rostami K, Farzaneh E, Abolhassani H. Bilateral deep peroneal nerve paralysis following kerosene self-injection into external hemorrhoids. Case Rep Med 2010: 2010. pii: 850394
10. Ritchie G, Still K, Rossi J 3rd, Bekkedal M, Bobb A, Arfsten D. Biological and health effects of exposure to kerosene-based jet fuels and performance additives. J Toxicol Environ Health B Crit Rev. 2003 Jul-Aug;6(4):357-451.
11. Iyanda AA. Thirty days exposure to kerosene induces alteration in select serum trace element levels in rats. GARJMMS 2012;(8):208-13.
12. Liu J, Goyer RA, Waalkes MP. Toxic Effects of Metals In: Casarett and Doull's Toxicology The Basic Science of Poisons (pp 931-980). Curtis D. Klaassen (Ed). McGraw-Hill Medical Publishing Division New York, 2008.
13. Qian VC, Shi X. New perspectives in arsenic-induced cell signal transduction. J Inorg Biochem 2003;96:271–27.
14. Harris GK, Shi X. Signaling by carcinogenic metals and metal-induced reactive oxygen species. Mutat Res 2003 Dec;533(1-2):183–200.
15. Lemarie A, Morzadec C, Merino D, Micheau O, Fardel O, Vernhet L. Arsenic trioxide induces apoptosis of human monocytes during macrophagic differentiation through nuclear factor-kappaB-related survival pathway down-regulation. J Pharmacol Exp Ther. 2006 Jan;316(1):304–14.
16. Hughes MF, Kitchin KT.Arsenic, Oxidative Stress and Carcinogenesis Oxidative Stress, Disease and Cancer. London: Imperial College Press, 2006.
17. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metal toxicity and the environment. EXS 2012;101:133-64.
18. Iyanda AA, Iheakanwa CI, Aina OO. Histopathologic manifestations of wistar rats exposed to virgin engine oil. GERF Bull Biosci 2016;7(2):1-5.