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Studies on the Sub-lethal Exposure of Camphor on the Hematology and Serum Biochemistry of Silver Carp

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ABSTRACT

Camphor is frequently used as medicine to treat variety of illnesses; it also has significant harmful effects on aquatic life. Many studies revealed camphor exposure can have an impact regarding the serum biochemical and hematological variables of fish. Toxicants including camphor cause the imbalance and abundance of reactive oxygen species (ROS), which subsequently promote sub-lethal toxicities when they are exposed in fish. The goal of current study was to evaluate how exposure to Camphor affects the hematological parameters and serum biochemistry in Silver Carp (Hypophthalmichthys molitrix). Three replicates were used to assess the 96 hours LC50 and lethal concentrations of camphor for silver carp in first phase of toxicity experiment. After lethal concentrated evaluated fish were subjected to sub-lethal concentrations for 35 days. At regular intervals (7, 14, 21, 28, and 35 days), fish samples were collected. Hematological parameters and serum biochemistry were recorded from different organs of fish after sub-lethal exposure to different concentration of camphor. Sub-lethal camphor exposure in Silver Carp fingerlings resulted in severe hematological and biochemical alterations. In exposed groups, RBC, hemoglobin, and hematocrit levels fell, particularly in T1, but MCV and WBC counts rose, suggesting an immunological response. Serum protein levels were highest in T1 but decreased in T2 and T3, while glucose levels decreased in all exposed groups, with T1 having the lowest levels. In T1, cholesterol was high but remained steady. These alterations point to camphor exposure-related toxicity and physiological stress.

INTRODUCTION

Camphor, a white crystalline compound with a strong scent and an unpleasant taste, is produced by the wood of plants belonging to the laurel family, such as Camphor laurel (Cinnamomum camphor). Two primary varieties of camphor are known. One of them is karpura, and it's made chemically. Its fragrant smoke is a common part of religious rituals and pujas in Indian temples. It dissolves in organic solvents such as chloroform, ether, alcohol, and almost no water at all. Turpentine oil is the beginning ingredient for the synthetic manufacture of camphor (Nirmal et al., 2003). After that, isononyl acetate is hydrolyzed to isoborneol, and then dehydrogenation turns it into camphor (Hamidpour et al., 2012).

A natural herbal remedy for numerous ailments, the second kind of camphor is derived from the Cinnamomum camphora and Cinnamomum agasthyamalayanum trees (Okamoto et al., 2011). Camphor, a naturally occurring chemical, has several uses in both traditional and alternative medicine. As an antimicrobial and anti-chill agent, it is used in herbal remedies. It is also a component in certain medications, a fragrance for home goods, and a source of aromatherapy for cosmetics. Both in the food industry and in the manufacturing of fragrance compounds, it plays an important role (Liu et al., 2006). In those with mental illness, cumulative doses of camphor can induce spasms. In addition to stimulating the central nervous system, it has stimulant effects on the respiratory, circulatory, and vascular systems.

The insect-repelling properties of camphor contributed to its high demand during World War II. Camphor has a long history of misuse as an inhalant and dietary supplement. When administered topically, its strong aroma and gentle anesthetic might fool people into thinking it's a real drug (Rivera and Barrueto, 2014). Anxieties, convulsing, autotrophs, anti-implantation, anti-estrogenic, and thyroid hormone activity reducers are all included in camphor chemical make-up. Additionally, camphor is a common component in topical analgesics and rubefacients used in contemporary medicine to alleviate mild aches and pains of the muscles (Shahabi et al., 2012).

The usage of camphor varies from one country to another. Many people use it for medicinal purposes, as a culinary flavoring ingredient, in aromatherapy, to alleviate pain, and for religious purposes. Chinese utilized camphor for its medicinal and circulatory effects, while the Japanese use it to make torches and even incorporate small amounts of it into pyrotechnics for a more intense display (Kumar et al., 2013). Camphoric fumes are a common method of cleaning the home. According to scientific research, it is safe for use in religious ceremonies in India and does not irritate the eyes. It is used in contemporary allopathic medicine. Intramuscular injections of medicines containing camphor alleviate the discomfort associated with breast engorgement (Snijders, 2018).

When it comes to toxicity, the two enantiomers are completely different. It is not so much the chemical's intrinsic toxicity as the chemical's widespread availability and safe therapeutic uses that pose the greatest threat to human health when it comes to camphor toxicity. Research has shown that certain amounts of camphor, when taken orally, can be fatal (Tabanca et al., 2013). Dangerous side effects, including kidney, brain, and gastrointestinal system blockages, can occur in adults who consume 2.0g of camphor or more. The skin and eyes might get irritated if camphor gets into them. There is currently no treatment for camphor poisoning, making it a very dangerous condition. Camphor is converted to alcohol camphoryl by oxygenation within an organism. It becomes water loving after being conjugated with glucuronic acid in the liver (Alam et al., 2019).

Previous research has shown that camphor also exhibited porphyrogenic activity in early cultures of liver cells and animal embryos (Chen et al., 2013). You may be exposed to camphor in three ways: by eating it, rubbing it on your skin, or breathing it in. One possible mechanism by which the cyclic terpene camphor crosses cell membranes rapidly is its high lipophilicity. It is absorbed through the skin, the lungs, the mucous membranes, and the digestive system. Once camphor is consumed, it is oxidized and conjugated in the liver before being excreted by the kidneys. The body excretes its active metabolites through urine after a period of storage in fat (Bronstein et al., 2012).

The immune system of fish exposed to camphor undergoes changes. Intestinal inflammation and the secretion of cytokines are two potential direct effects of ammonia stress on the immune system of fish. Camphor exposure can cause the release of immune suppressive chemicals into the bloodstream, which in turn triggers the production of peripheral lymphoid tissues. It dampens the immune response by reducing the quantity of lymphocytes and phagocytes. Multiple studies found that camphor caused tissue damage in fish and that total lymphocyte count (TLC) was achieved via immune system activation. Chronic exposure, on the other hand, damages the immune system and leads to a decline in white blood cells and lymphocytes (Yan et al., 2021).

Physiological, biochemical, and pathological changes in fish may be assessed with the use of hematological parameters, which are connected to the immunological state of fish. Because of their sensitivity to changes in their surrounding environment, biochemical measurements provide a more rapid reflection of fish health than other commonly used metrics (Atamanalp and Yanik, 2003). The levels of hematological markers in fish might go up or down when they're exposed to camphor. When fish are exposed to camphor, it can affect their hemoglobin (Hb), white blood cell (WBC), and red blood cell (RBC) numbers, among other hematological markers (Knudsen et al., 2013).

The relevance of silver carp (H. molitrix) in aquaculture and its susceptibility to environmental toxins made it the study chosen subject. Since silver carp are an important species in freshwater farming, any harmful effects of camphor on them may directly affect the productivity and health of the fish. It is also very sensitive to changes in water quality because of its function as a filter feeder, which makes it an excellent model for researching the effects of chemical pollutants like camphor on the environment. This is due to their desirable traits for aquaculture as well as their ability to invade and thrive in various environments (Molnár et al., 2021) The aim of this investigation is to ascertain the lethal concentration and LC50 of camphor for Silver Carp and evaluate its long-term impacts on the fish's serum biochemical and hematological markers.

METHODOLOGY

Acclimatization of fish

The study was conducted in the toxicological research lab department of Zoology, Wildlife and Fisheries at the University of Agriculture Faisalabad. For the experiment, the fish was sourced from the University of Agriculture Faisalabad fisheries research facility. In the study, fish fingerlings of uniform weight and length, all 90 days old, were acclimated in a controlled environment. They were placed in a glass tank containing 50 liters of de-chlorinated and aerated tap water. During acclimation and testing, the water quality parameters such as dissolved oxygen (DO) and pH were carefully monitored and maintained within optimal ranges for fish health and research validity.

Experimental design

The sub-lethal effects of camphor during acute toxicity were studied in 90-day-old Silver Carp fingerlings. Ten fish were introduced to each tank and divided into four groups: T0, T1, T2 and T3. While some experimental groups received varying doses of camphor, a control group T0 did not get any. During the acute phase, the fish were given camphor for 96 hours to find the LC50 and fatal doses. The entire trial for the equitable distribution of camphor was conducted with aeration provided to the tank. Time intervals of 24, 48, 72, and 96 hours were used to evaluate the results. Every day, the dead fish were removed.

Probit analysis:

Probit analysis was used to check fish mortality. Three concentrations of dioxin were used for the determination of LC50 as presented in table 3.1.

Table: 3.1 Camphor Sub-Lethal Concentration for Chronic Toxicity Studies

Treatment	Concentration	Exposure
Camphor	1/3rd	397.47 mg/L
	1/5th	238.482 mg/L
	1/7th	170.344 mg/L

Collection of blood samples

The fish caudal vein was used to draw blood samples. The collection technique was carried out using a 2ml plastic disposable syringe that had a 26-gauge needle to guarantee sterility. The syringe was coated with a solution containing 150-200ul of EDTA to prevent coagulation during the measurement of hematological parameters. To further evaluate serum biochemical characteristics, blood samples were also collected without anticoagulant. The blood plasma was separated from the other samples by centrifuging them at 10,000 rpm for 20 minutes.

Hematological analysis

Hematological parameters such as red blood cell count, the total white blood cell count, the hemoglobin concentration, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin were assessed using cell counter (medonic M- series) (Tanuja and Nivedita, 2014). The Biuret method was used to determine the serum protein concentration. The glucose oxidase-peroxidase technique was used to conduct the glucose assay. The endpoint technique and GPO-PAP assay were used to determine the triglyceride levels.

Statistical analysis

For statistical analysis, the records were subjected to ANOVA (analysis of variance and correlation) using the statistics software. The purpose of using Tukey's test was to compare variances. By employing the probit analysis method, we were able to determine the sub-lethal doses of camphor and the tolerance limits of Sliver Carp (Hamilton et al., 1977).

RESULTS

Acute toxicity evaluation

During a 96-hour acute toxicity study, the % mortality of Silver Carp against various camphor concentrations, starting at 0 mg/L and increasing up to 10000 mg/L was studied as presented in fig 3.1. Lethal concentrations for camphor are 2201.78+168.486 mg/L, whereas the LC_{50} was calculated as 1192.41+87.6395 mg/L.

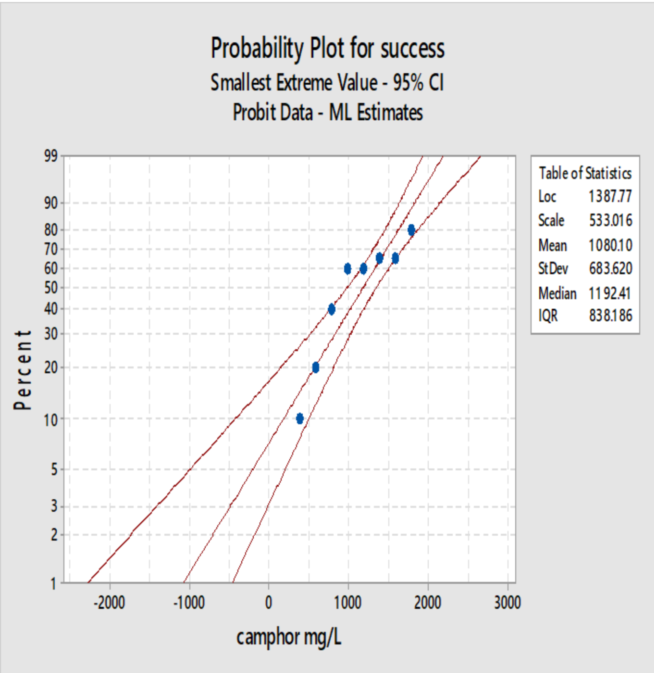

Fig: 3.1 Mean mortality of fish (*H. molitrix*) of test media at different concentrations of Dioxin

Chronic toxicity evaluation

Hematological parameters

Variety of hematological parameters for four distinct groups (T_0, T_1, T_2, and T_3) of fingerling Silver Carp subjected to camphor sub-lethal effects. T_0 (control) has the greatest RBC count, indicating normal red blood cell production. Exposure to camphor lowers RBC counts in both T_1 and T_2, with the greatest decline observed in T_1 as shown in graph 3.2. Although there has been some improvement, T_3 is still below the control. All exposed groups (T_1, T_2, T_3) have elevated WBC counts in comparison to the control after being exposed to camphor, with T_1 exhibiting the greatest rise as presented in fig 3.5. This may indicate an immunological reaction to the exposure to camphor. Fig 3.2 indicate that hemoglobin levels are highest in the control (T_0) and fall in the camphor-exposed groups, with T_1 being the lowest. This suggests a reduction in the ability to transport oxygen as a reaction to camphor.

Hematocrit readings exhibit a similar pattern to hemoglobin, with the exposed groups displaying lower percentages, particularly T_1, and the control group having the highest percentage as presented in fig 3.3. MCV rises in the groups exposed to camphor, suggesting that red blood cells in T_1, T_2, and T_3 are on average larger than those in T_0. T_1 had the lowest MCH, indicating a decrease in hemoglobin content per red blood cell in this cohort. Though they possess somewhat recovered, T_2 and T_3 are still less than the control.

Following chronic exposure to Camphor in Silver Carp there were significant variations observed in blood serum glucose levels. The exposed group of fish exhibited a decrease in glucose levels compared to the unexposed group as shown in fig 3.6. T_1 had the lowest level followed by T_2 and T_3. The serum protein levels differed across groups, with T_0 at 5.54 ± 0.30 g/dL, T_1 at 7.16 ± 0.85 g/dL (higher), and T_2 (4.16 ± 0.67 g/dL) and T3 (4.64 ± 0.44 g/dL) exhibiting lower levels. While the reduced levels in T_2 and T_3 point to possible inefficiencies in protein synthesis or increased protein catabolism as a result of camphor toxicity, the elevated protein level in T_1 might represent an acute phase response to stress or infection.

With T_0 at 96.42 ± 0.37 g/dL, T_1 at 106.86 ± 1.14 g/dL (significantly higher), T_2 at 94.24 ± 1.52 g/dL, and T_3 at 93.28 ± 1.26 g/dL, the blood cholesterol levels were comparatively steady among groups. Since increased cholesterol might happen in reaction to inflammation or cellular repair mechanisms, the rise in T_1 may be a reflection of alterations in lipid metabolism or a physiological stressor from camphor exposure.

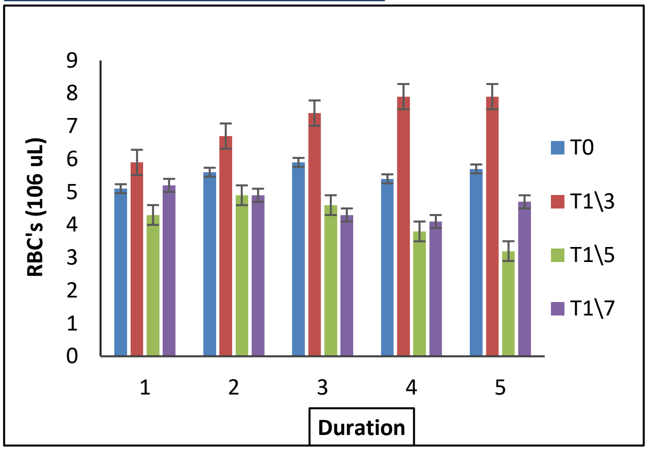

Fig 3.2 Graphical presentation of RBC after camphor exposure.

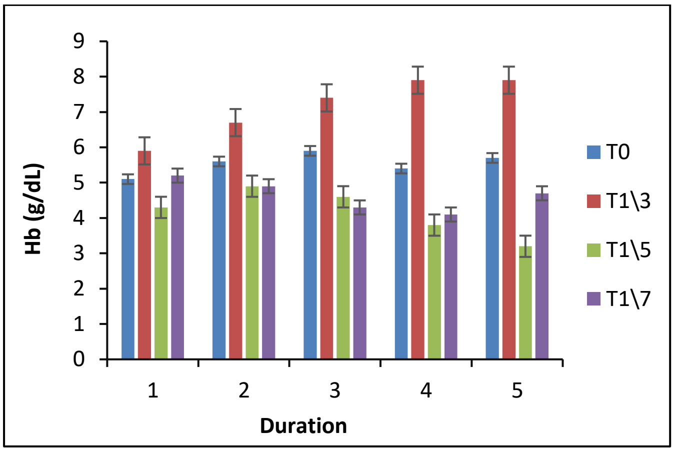

Fig 3.3 Graphical presentation of Hb after camphor exposure

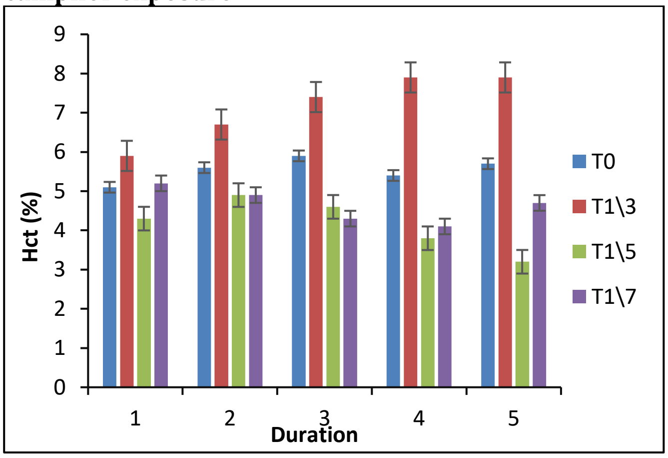

Fig 3.4 Graphical presentation of Hct after camphor exposure

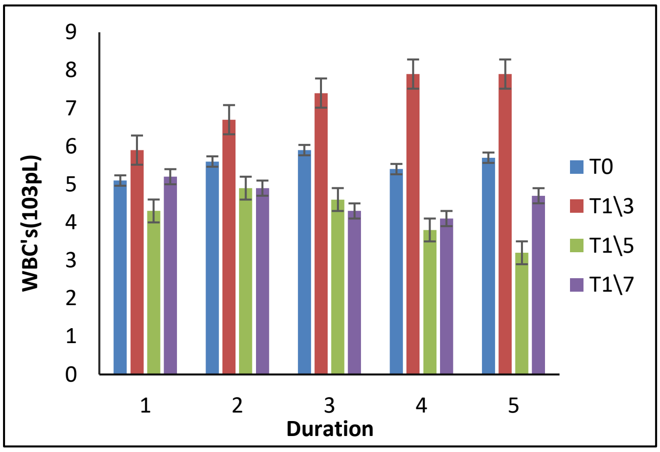

Fig 3.5 Graphical presentation of WBC after camphor exposure

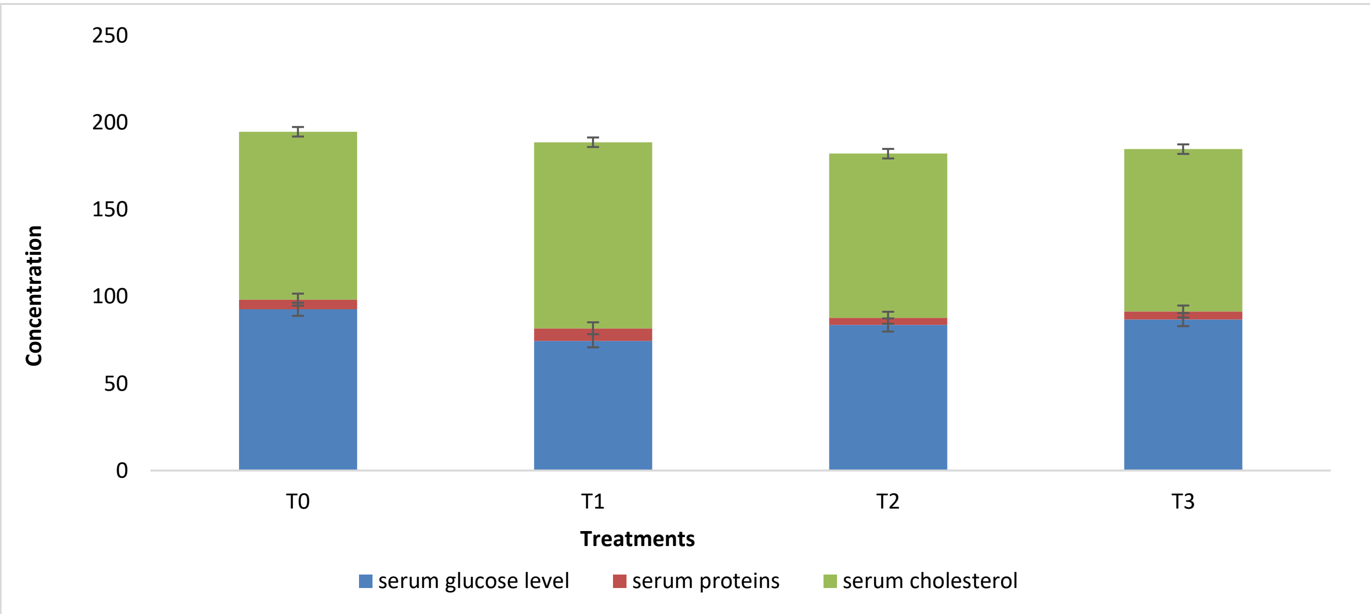

Fig. 3.6 Graphical presentation of Serum Biochemistry after camphor exposure

DISCUSSION

Camphor is the most potent, natural active ingredient with many applications in additional and modern medicine. The biological activity of camphor mainly showed anti-Bacterial, insecticide, anti-oxidation, anti-inflammatory, anti-microbial, and anti-viral effects (Hamidpour *et al.*, 2013). Camphor, however, is potentially dangerous when consumed and may result in epileptic attacks, anxiety and neuro-muscular hyperactivity (Salman *et al.*, 2012). Hematological framework is crucial especially when fish is in stress while assessing the effects of pollutants like camphor in a specific ecosystem, consequently serving as a major bio indicator. Numerous factors affect the hematological parameters including reproductive status of fish, sex, environmental conditions, stressors, age, temperature, ecological habitat, fish species and physicochemical parameters (Patra, *et al*., 2014).

The present research was conducted to study the Sub-lethal exposure of camphor on hematology and serum biochemistry of Silver Carp. During the present study, 96-hr LC_{50} of camphor was estimated as 1192.41+87.6395 mg/L. While the lethal concentration of camphor was calculated as 2201.78+168.486 mg/L during acute toxicity testing. Du *et al.* (2021) illustrated the acute toxic effects regarding camphor on Zebrafish. They found 587 µg/ml as 96-hr LC_{50} values of camphor on zebrafish. The present study was also following work of Du *et al,* which described that the 96-hr LC_{50} values of camphor is about 1192.41 mg/L in Silver Carp.

In this experiment, there was alternation in hematology and serum biochemistry in silver carp. This research shows similarities with findings of Araujo *et al.* Their research shows that exposure of the camphor on Senegalese sole (*Solea Senegalensis*) larvae at different levels of biological organization affect them. In this study, it was noted that there was significant increase in hematology and serum biochemistry in organs after 35 days of exposure. Changes were observed in the peripheral gills erythrocytes, such as enlarged lymphocytes, dead cells, cell fusion, binucleated cells, tear-shaped cells, dead cells, and abnormal cell structures and these same results were inferred by (Akhter *et al.,* 2020) in his studies.

Camphor had deleterious effects on the early life stages of silver carp, a relatively resilient fish species (Pervin *et al.,* 2020). A study conducted by Belair *et al.* 2001 on zebrafish, the lethal impact of Camphor on erythropoiesis, the process of red blood cell production was found. Previous research had shown that Camphor could cause mortality during the early stages of fish development. The study revealed that exposing Zebrafish to Camphor at an early stage and at a concentration higher g/kg resulted in reduced blood flow and a decrease in the number of circulating erythrocytes.

The effect of camphor on silver carp, as observed through serum biochemistry, can vary depending on factors like concentration, duration of exposure, and individual fish physiology. Additionally, camphor exposure might affect lipid profiles, leading to changes in cholesterol and triglyceride levels in the serum. Electrolyte balance could also be affected, possibly causing disruptions in ion concentrations such as calcium, potassium, and sodium (Hoseini *et al.,* 2018).

CONCLUSION

The results of the study showed that Silver Carp hematological and biochemical parameters were significantly affected by camphor exposure in a sub-lethal way. Higher doses proved deadly, however a 96-hour LC_{50} of 1192.41 mg/L was found in acute toxicity studies. Exposure to camphor resulted in decreased hemoglobin, glucose, and red blood cell counts; yet, there was an increase in serum protein and white blood cell counts, signifying an immunological response and stress. According to the findings, camphor alters physiological processes in fish, which may have wider ecological ramifications for aquatic systems that are subjected to these substances.

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