

## Toxic Potency of Bonny Light Crude Oil on Littoral Organisms: A Case Study of *Sesarma Huzardii*

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### ABSTRACT

The effects of the oils and dispersants on marine life have been a subject of worldwide concern. It is for this reason that we set out to delimit the toxic potency of the water-soluble fraction (WSF) crude oil on the hairy mangrove crab *Sesarma huzardii*. In doing this, a total of 630 healthy sample of adult *Sesarma huzardii* was obtained from a Chicoco mud in the oil-prone Buguma creek, Rivers State (Nigeria) by handpicking and were transported to the laboratory in plastic containers where acclimation was done for 48 hours prior to analysis. The crabs were divided into six groups in a randomized complete block design and monitored for 28 days. The test organisms were treated to different concentrations of Bonny light crude oil with fixed physico-chemical properties in order to ascertain the response of *S. huzardii* to the lethal dose of the test sample. The physico-chemical indices measured for the WSF all exceeded the World Health Organization (WHO) permissible limit. pH ranged from 4.76 to 6.83, conductivity (329.2 to 518.1  $\mu\text{S}/\text{cm}$ ), DO (2.69 to 6.45 mg/L), TDS (146.5 to 216.1 mg/L) and temperature (26.35 to 28.32  $^{\circ}\text{C}$ ). The mortality rate was found to increase as the concentration of the test chemical was increased as well as the monitoring period/hours was increased in both samples. The overall physico-chemical properties of the WSF are expected to have acted synergistically on the test organism (*S. huzardii*), eliciting the quantal responses observed. The toxicity of the WSF points to the base constituent of the oil. Oil spills on water, near shore or onshore, should receive immediate contingency response because of the inherent toxicity of the soluble fractions and the associated danger of bioaccumulation, even at sub-lethal concentrations.

**Keywords:** Crude oil; Water-soluble fraction; Toxicity; Lethal concentration; Hairy mangrove crab

### Introduction

Crude oil contains hydrocarbon and hydrocarbon compounds. An average crude oil contains 50% naphthenic hydrocarbons, 25% aliphatic hydrocarbons, 18% aromatic hydrocarbons and 7% non-hydrocarbon compounds<sup>1</sup>. These components may

vary slightly in different crude oils. The aromatic hydrocarbon component consists of the monocyclic aromatic hydrocarbons (MAHs) and the polycyclic aromatic hydrocarbons (PAHs). The PAHs represent a large family of compounds, ranging from the two-ringed naphthalene's to the ten-ringed derivatives of

naphthalene. While the most toxic components of oil, the MAHs, are relatively water soluble, they evaporate quickly after oil is spilled. At the other end, the nonvolatile high molecular weight PAHs cannot effectively dissolve in water. Therefore, only intermediate-sized PAHs (such as acenaphthene, phenanthrene and fluoranthene) significantly influence the toxicity of oil to pelagic organisms in the water column. PAHs can lead to both carcinogenic and noncarcinogenic effects such as oxidative stress, suppression of the immune system and impairment of endocrine regulation and development<sup>2,3</sup>.

The toxic potency of crude oils derives its chemical composition and exposure concentration. The oil type, condition (fresh vs. weathered) and exposure regimen (continuous, pulsed, static renewal, etc.) should be taken into consideration. Although polar- and nonpolar compounds contribute to the toxicity of (weathered) crude oil, the water-soluble fraction (WSF) is dominated by polar compounds, which accounts for a large portion of the toxicity. Both the dissolved hydrocarbon and the suspended phase can be taken up by the organism and lead to potentially toxic effects<sup>4</sup>. Dissolved hydrocarbons distributed in the body via blood circulation can interfere with physiological functions, while the suspended phase can physically impact an organism by coating body surfaces or gills, impairing respiratory gas exchange<sup>5</sup>.

When oil spill occurs, primary responders need to immediately decide upon a course of action. Decisions to be made may involve ecological considerations and there is often a tradeoff between potentially lethal effects on different species and the potential impacts on natural resources. Ecological information such as the status of a population (endangered, threatened or common species), prevalent life stage of the species and the time of the year (spawning, nesting or migration) will also affect decision making. An understanding of the ecological consequences and toxicological impacts between variable habitats and species will help agencies predict impacts on the ecosystem and make better response decisions. Although each oil spill is unique and it is difficult to extrapolate toxicity data from the laboratory to the field, a thorough toxicological assessment prior to a spill will facilitate more effective decision making. For this reason, toxicity tests (such as bioassays) are carried out. The most common toxicological endpoints include acute effects such as mortality, narcosis and necrosis and chronic effects such as impacts on development, behaviour and reproduction<sup>6-8</sup>.

Generally, uptake of oil via diet is comparatively lower than from water. However, filter-feeding zooplankton and invertebrates ingest a large amount of oil by filtering the droplets. Species at higher levels of the aquatic food web (such as the hairy mangrove crab *Sesarma huzardii*) are at much greater risk of exposure and toxic effects posed by biomagnification, since they feed on organisms which may accumulate the toxic constituents of oil. Crabs are widespread in the oceans, need freshwater and are ground-living from mountain range, forests, desert and poles. Crabs' characters and ability to show several amazing features are always being eye-catching subjects concerning animal biology. Crabs are decapod crustaceans familiar to the people. About 4,500 species are recognized, some newly proposed and some subspecies and each country has its varieties. As the crab grows, the chitin compound, the crab exoskeleton, will be manufactured. Crabs have a definite front, a small tail, five legs for walking and three pairs of legs for their food capture and their fee-luring. Less or is it a sort of scar or

physical complications. Some factors include crab and gender species<sup>9</sup>. The present study seeks to determine the potential toxic effects of Nigeria's bonny Light crude oil on the littoral crab (*Sesarma huzardii*).

## Materials and Methods

### Test Organism

#### *Sesarma huzardii*:

The largest phylum in the kingdom Animalia is Arthropod (jointed legs), which includes crabs and contains over 42,000 species, accounting for more than 75% of all living things (Cannicil et al., 1995). Its ability to adapt to changing conditions in mangrove habitats is largely responsible for their diversity and abundance. They are one of the least overfished crustaceans among West African artisanal and trawler anglers. Due to their great export potential, oysters, prawns and shrimp are the target species in this region<sup>10,11</sup>. With a broad, roughly spherical upper carapace and a small, tucked-under belly, they live in freshwater, brackish and marine settings. The structure, colour and form of them differ amongst species. Their biology and overall morphology are extremely similar<sup>12</sup>.

Crabs are generally marine animals, while several kinds live in the littoral, supralittoral and even upcoast zones in freshwater and brackish water. They have been found as deep as 6000 meters below the ocean's surface and are common in many estuary settings where salinity and temperatures can vary significantly daily<sup>13</sup>. Numerous species habitually forage on land and some have evolved into semi-terrestrial status<sup>14</sup>. There is a wider variety of crab species in tropical and subtropical regions than in temperate and colder regions<sup>15</sup>. About 2 million tons of crabs are consumed annually, accounting for 20% of all marine crustaceans that are harvested and raised for food<sup>14</sup>. The mangrove crab (*Sesarmidae*) is the most common species of crab in mangrove wetlands. According to Cannicci et al, they live in the estuaries and lagoons below the drift and high tide marks<sup>9</sup>. They are amphibious in nature and inhabit the muddier, more humid intertidal areas of the mangroves<sup>12</sup>.

### Research design

The experimental design for the toxicity tests was 3 x 5 x 6 factor in a randomized complete block design, with six levels of treatment, observed at five intervals and in three. The acute toxicity was carried out using Adults of *Sesarma huzardii* (test organism) by exposing them to various lethal concentrations of the test chemicals (Crude oil wastewater or contaminant) in solution. Similarly, sub-lethal toxicity tests were conducted on the test organisms by exposing them to various sub-lethal concentrations of the test chemicals. The acute test was conducted using the static test procedure while the sub-lethal toxicity test was conducted using the renewable test procedure.

#### Acclimation of the test organism (*Sesarma huzardii*)

Acclimation was done according to laboratory conditions using a 150 litres capacity glass aquarium.

A total of 630 healthy samples of Adult *Sesarma huzardii* were obtained from a Chicoco mud where the crabs were collected by hand picking in hand gloves from the clean mangrove mudflat and transported in plastic containers to the laboratory. Acclimation was done according to laboratory conditions using a 150 litres capacity glass aquarium for 48hrs

and fed with sampling location mud rich organic matter until further analysis.

During acclimation, the tank was aerated continuously. The water in each glass tank was replaced with brackish water collected from the same station and stored in the laboratory. The water was replaced after 24 hours daily. Water soluble fraction (WSF) of Bonny Light crude oil was prepared following the method of Edema<sup>16</sup>. Preliminary test was first carried out to establish a range of concentration using a standard range finding method as recommended by manual of methods in aquatic environmental Research<sup>17,18</sup>.

### Test chemical

The test chemical (bonny light crude oil) was collected in a container from Shell Petroleum Development Company of Nigeria Limited (SPDC) in Port Harcourt and was stored under ambient conditions before usage in the laboratory.

### Range finding test

A preliminary test was carried out to establish a range of concentrations using a standard range-finding method. A preliminary test was carried out to establish ranges for the lethal concentration using six test concentrations and each triplicate with 10 juveniles of each crab per tank and was exposed for 24, 48, 72 and 96 hours during which mortality rate was estimated and the dead fish was removed to avoid contaminations.

### Definitive acute toxicity test

10 active crab samples were randomly selected and put in each of the test concentrations. Each treatment was in triplicates. Each treatment group was exposed for 96 hours during which mortality was determined at 24-, 48-, 72- and 96-hour periods and dead ones were removed immediately to avoid pollution. From the data, the concentration-response curves for mortality, the  $LC_{50}$ 's and the 95 per cent confidence intervals for test organisms at 24hr, 48hr, 72hr and 96hr in a static system were recorded.

### Mortality responses

The acute test was for 96 hours. The basic criterion for mortality was total lack of movement. They were confirmed dead if they remained immobile and showing no response after prodding with forceps. Death was confirmed by re-introducing dead organisms into fresh dilution water and observing them for response.

### Control mortality

Mortality values (M) were corrected for control mortality by Abbott's correction factor, using the formula  $M = 1 - Sc/So$ , where M is the mortality, Sx is the survival in the medium of concentration c and so is the survival in the control medium.

### Determination of the water quality of experimental water

The physicochemical parameters checked for the water quality were Temperature, pH, Conductivity, Dissolved Oxygen and Total Dissolved Solid. The Dissolved oxygen was measured with the Milwaukee Dissolved oxygen meter. The Temperature (measured in degree Celsius (°C)), Total Dissolved Solid (ppm) and Conductivity were measured in situ with a hand-held multimeter. The pH was measured using a hand-held pocket-sized pH meter (Milwaukee model pH600). The probe of the meter was inserted 15 cm into the experimental water and the

meter was switched on and allowed to stabilize for 10 minutes. The reading was recorded when the reading became stable.

### Preparation of Water-Soluble Fraction (WSF) of bonny light crude oil

The Bonny light crude oil used in the study was derived from the Shell Petroleum Development Company, in Port Harcourt, Rivers State. The water-soluble fraction (WSF) of the crude oil used was prepared using the method described in previous studies<sup>19</sup>. One part of Bonny light Crude Oil was added to 9 parts of distilled water (in a ratio of 10:1) in a bottle and mixed thoroughly with a rotatory magnetic stirrer for 20 hours at room temperature. The bottle was capped to minimize the evaporation of more volatile hydrocarbons. The stirring speed was adjusted so that the vortex was not extended more than 25% to the bottom of the container. Mixtures were allowed to rest for 12 hours to demarcate layers. Thereafter, a separating funnel was used to separate out the water-soluble fraction, which was corked as a stock solution in a 50cl capacity plastic container for use in the experiment.

### Selection of test organism for sub-lethal assay

Ten active and healthy juvenile crab specimens *Sesarma huzardii* have an average ( $\bar{x}$ SD) wet weight and length of 45.13 $\bar{x}$ 10.19 g and 4.2  $\pm$  1.92 cm respectively.

A relatively uniform size was picked randomly from the acclimation tanks and transferred into the different treatment units for 28 days to test for the toxicant. The treatments were in triplicates as well as the control.

The test was performed using a renewal method and the exposure medium was renewed every week to maintain toxicant strength and level of dissolved oxygen, minimizing changes due to metabolism by the crabs during this experiment.

## Results and Discussion

### Mortality Rate of *Sesarma huzardii*

The mortality data for *Sesarma huzardii* shows a similar pattern, with concentration-dependent mortality evident across the different exposure groups (**Table 1**). At 0 mg/L (control), survival rates remained 100% across all time points, again confirming that the chemicals had no effect at this concentration. At 5 mg/L, mortality was 20%, while at 3.025 mg/L, 100% mortality was recorded, reflecting the lethal effects of high toxicant exposure. These findings are in line with the results a similar test carried out on *Uca tangeri*, indicating that increasing concentrations of pollutants result in higher mortality rates across both species.

Exposure duration also significantly influenced mortality rates in *Sesarma huzardii*. As noted in the results, mortality rates increased over time, especially in the higher concentrations. The study emphasizes that conventional LC50 measures, which typically focus on lethal concentrations at fixed time points, might overlook the delayed effects of pollutants. Including exposure duration in survival models helps provide a more realistic and ecologically relevant picture of mortality, reflecting how species respond to longer-term chemical exposure<sup>20</sup>.

### pH

The pH values of the WSF exposed *Sesarma huzardii* was found to range from 4.76 to 6.83 as shown in (**Figure 1**). The

results showed that the water samples collected were slightly acidic. The pH values obtained in the water samples were found to be lower in values than the pH value obtained in the control water sample. The pH values obtained for the analysed water samples (concentration of 5 mg/l and the control water samples were found to be within the WHO permissible limit (6.5 to 8.5) for drinking water except for the water samples collected at other studied concentrations (10 mg/L, 15 mg/L, 20 mg/L and 25 mg/L) where the pH values were not within the WHO permissible limit. The acidity of the water samples was found to increase as the test concentrations was increased. The variation in the pH values could be activities of the crabs in the water samples. The variation in the pH values could be due to the differences in the release and composition of pollutants in the water. The variation in the pH values could be due to the differences in the release and composition of pollutants in the water. Studies have also shown that low pH in surface and groundwater could be due to natural geochemical and biochemical processes within the aquifers and also possibly due to the effect of dissolution of acid associated with discharges from the processing facilities<sup>21</sup>. Wang, et al. reported that metabolic activities of aquatic organisms are also dependent on the pH values<sup>22</sup>. The pH of a water body is very important because it has effect on the organisms living in the aquatic ecosystem<sup>23</sup>. According to Ramanathan, et al, pH is one of the vital environmental characteristics that decide the survival, metabolism, physiology and growth of aquatic organisms<sup>24</sup>. It is influenced by acidity of the bottom sediment and biological activities. High pH may result from high rate of photosynthesis by dense phytoplankton blooms. pH greater than 7 but less than 8.5 is ideal for biological productivity while pH < 4 is detrimental to aquatic life and pH is affected by total alkalinity, acidity, runoff from surrounding rocks and water discharges<sup>25-27</sup>.

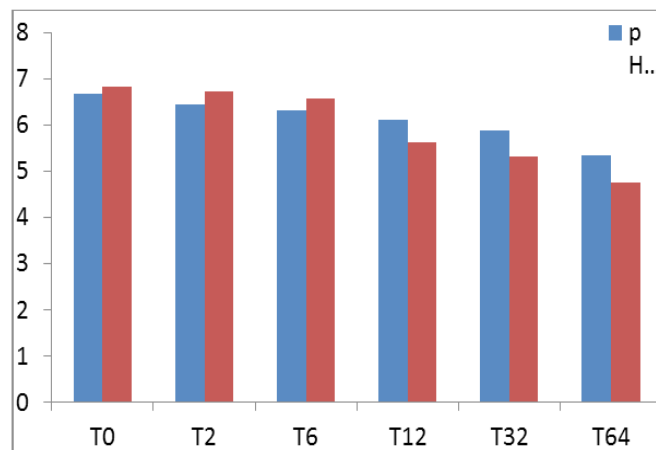
**Table 1:** Mortality Rate of *Sesarma huzardii* exposed to the toxicant.

Concentration	REP	24 h	48 h	72 h	96 h	Survival (%)	Mortality (%)
0	1	0	0	0	0	100	0
0	2	0	0	0	0	100	0
0	3	0	0	0	0	100	0
5	1	1	1	1	2	80	20
5	2	1	1	2	2	80	20
5	3	0	0	1	2	80	20
10	1	1	2	2	3	70	30
10	2	2	3	3	4	60	40
10	3	1	2	3	4	60	40
15	1	2	3	3	4	60	40
15	2	2	2	4	6	40	60
15	3	1	2	4	6	40	60
20	1	4	5	7	8	20	80
20	2	4	7	8	8	20	80
20	3	5	6	7	8	20	80
25	1	6	7	8	10	0	100
25	2	5	8	9	10	0	100
25	3	6	8	9	10	0	100

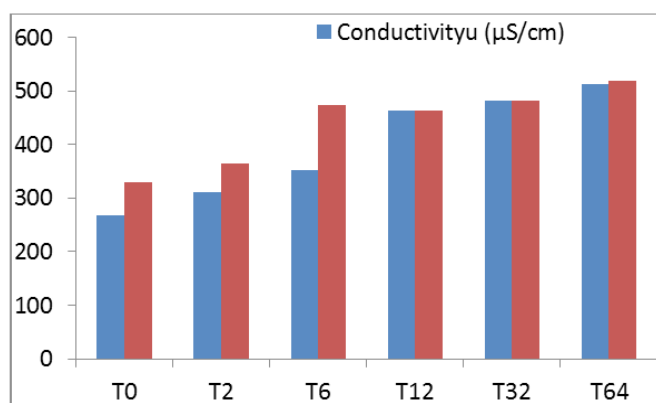
### Electrical conductivity

The conductivity values of the water samples exposed to *Sesarma huzardii* ranged from 329.2 to 518.1  $\mu\text{S}/\text{cm}$  as shown in (Figure 2). The conductivity values obtained in the analysed water samples were found to be below the WHO permissible

limit (500  $\mu\text{S}/\text{cm}$ ) in drinking water except for test concentration five where the conductivity value was found to exceed the WHO permissible limit. The electrical conductivity of water is an important parameter for determining the suitability of water for irrigation and is a useful indicator of the salinity or total salt content of effluents. The conductivity of receiving water is a function of the concentration of soluble ionic salts present in the effluent. Thus, an increase in the salinity of a receiving water body is a result of a high concentration of ionic salts in the effluent<sup>28</sup>. High conductivity value in test concentration five could be due to high number of ions in the water sample.



**Figure 1:** pH values of the analysed Water Samples.

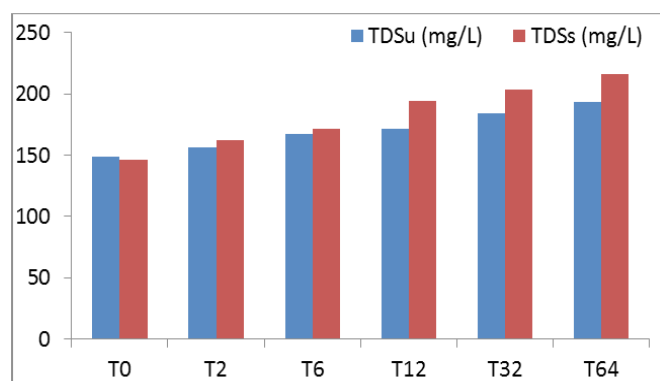


**Figure 2:** Electrical conductivity values of the analyzed Water Samples.

### Total Dissolved Solids (TDS)

The TDS values obtained in the water samples exposed to *Sesarma huzardii* ranged from 146.5 to 216.1 mg/L as shown in (Figure 3). In *Uca tangeri* exposed, the highest TDS concentration was obtained in test concentration five (193 mg/L), followed by test concentration four (183.97 mg/L) while the least concentration was found in the control water sample (148.7 mg/L). In *Sesarma huzardii* exposed water samples, the highest TDS concentration was obtained in test concentration five (216.1 mg/L), followed by test concentration four (203.6 mg/L) while the least concentration was found in the control water sample (146.5 mg/L) as seen in Figure 4. The TDS value obtained in the analysed water samples were found to be below the WHO permissible limit (500 mg/L) for TDS in drinking water. The highest TDS value was obtained in Increased TDS could be attributed to seepage of effluent discharges, agriculture and domestic wastes, surface run-off of water containing bicarbonates, chlorides, nitrate, sodium, potassium, calcium and

magnesium and these could result to hard water<sup>29</sup>. According to Karikari and Ansa-Asare, the suitability of water with TDS level less than 600 mg/L is considered to be good whereas water with TDS above 1200 mg/L becomes increasingly unsuitable<sup>30</sup>.



**Figure 3:** TDS values of the analyzed Water Samples.

### Dissolved Oxygen (DO)

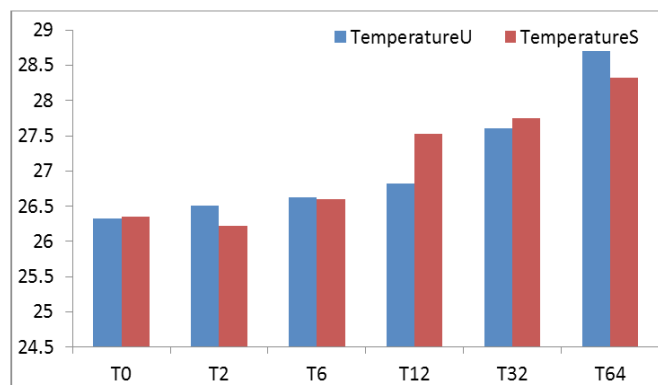
The DO values obtained for the exposed *Sesarma huzardii* ranged from 2.69 to 6.45 mg/L as shown in Figure 4. The results showed the DO values to be within the WHO permissible limit (4.0 mg/L) of DO in drinking water except for test concentration three to five where the DO values were not within the WHO permissible limit of DO in water. The lowest DO value was reported in test concentration five while the highest DO value was reported in the control water sample. Low DO could lead to death of the crabs as the survival of the organisms' survival could be very low. Low dissolved oxygen (DO) could primarily result from excessive algae growth caused by phosphorus. Nitrogen is another nutrient that could contribute to algae growth as the algae die and decompose, the process consumes dissolved oxygen. The DO values obtained in this study were found not to be close to the DO values reported in the study by Gallo-Corredor, et al, in the analysed water samples where they reported DO concentration ranging from 6.80 to 7.77 mg/L<sup>31</sup>. The implication of the DO values in the surface water of the sampling sites is that aquatic organisms will be well impacted. Organisms such as fish found in these environments are prone to contaminants especially PTMs in the water. These PTMs could bioaccumulate in the organisms which in turn can be transferred to human from the consumption of such organism (e.g. fish).

Further, low DO values are an indication of unsuitability of the water for the survival of aquatic organisms as it could lead to the death of aquatic organisms<sup>32</sup>. DO is an indication of the physical, chemical and biological processes that are taking place in water Wilén and Balmér<sup>33</sup>. It is an important parameter for investigating water quality because of its impact on organisms living in water. Too low level of DO could affect water quality and endanger aquatic life<sup>34</sup>.

### Temperature

The temperature of the water samples exposed to *Sesarma huzardii* ranged from 26.35 to 28.32 oC as shown in (Figure 4). The highest temperature values were obtained in test concentration five (28.7 and 28.32 oC), followed by test concentration four (with temperature values 27.6 and 27.75 oC) while the least temperature values were obtained in the control water samples (26.32 and 26.35 oC). the temperature values were found to increase as the concentrations of the test water were found to increase. Temperature value could indicate the

suitability of the water for the survival of the organisms during the test period as most aquatic organism could not survive warm environment<sup>31</sup>.



**Figure 4:** Temperature values of the analyzed Water Samples.

### Conclusion

The results of the water physicochemical parameters showed some of the parameters were found to exceed the World Health Organization (WHO) permissible limit of quality drinking water. This indicates the contamination of the water body from the exposure and such could result in the mortality of the exposed organisms. The results of the water physicochemical parameters in *S. huzardii* ranged: pH (4.76 to 6.83), conductivity (329.2 to 518.1  $\mu\text{S}/\text{cm}$ ), DO (2.69 to 6.45 mg/L), TDS (146.5 to 216.1 mg/L) and temperature (26.35 to 28.32 oC). The mortality rate was found to increase as the concentration of the test chemical was increased as well as the monitoring period/hours was increased. The toxicity of the WSF points to the base constituents of the test compound as seen in the altered physicochemistry of the organism's simulated habitat. In the event of any spill affecting the littoral ecosystem, expedient reclamation and contingencies are recommended to avoid acute lethality of such organisms. This would also prevent bioaccumulation of serious hazards in the human food chain.

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