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Full Length Article

Copper and Zinc Toxicity Estimates for Juvenile Chinese Sturgeon *Acipenser sinensis* in the Yangtze River Estuary

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Abstract

Acute toxicity estimates of copper (Cu) and zinc (Zn) in juvenile Chinese sturgeon were studied in the Yangtze River estuary and using the static test method to assess Cu and Zn toxicity. The results showed that Cu was more severely toxic to Chinese sturgeon than Zn. The Cu LC_{50} after 24-, 48-, 72- and 96-h exposures to Chinese sturgeon were 0.0606, 0.0414, 0.0289 and 0.0217 $mg \cdot L^{-1}$, respectively, whereas these values were 3.590, 2.514, 1.759 and 1.200 $mg \cdot L^{-1}$ for Zn, respectively. Estimated safe concentrations of Cu and Zn were 0.00217 and 0.1200 $mg \cdot L^{-1}$, respectively. However, the Cu and Zn standards for fisheries water quality in China are 0.01 and 0.10 $mg \cdot L^{-1}$, respectively. The liver was the major accumulation site for Cu. Cu content in the liver of juveniles in the control group (no Cu) was the highest, followed by the gills, whereas the lowest content was detected in cartilage. The fastest accumulator of Cu was the liver, followed by the stomach, and cartilage and skin had the lowest contents. Superoxide dismutase (SOD) and catalase (CAT) activities in the liver tissues of juveniles were relatively low for the first 24 h, but the activities recovered gradually from 24 to 48 h and decreased after 48 h to levels lower than the contro1 group. These changes in SOD and CAT activities indicate that juvenile Chinese sturgeon were severely affected by Cu. These data show that Chinese sturgeon juveniles face the threat of Cu contamination and this alerts officials to the dangerous situation.

Keywords: Toxicity estimation; Antioxidant enzyme; Chinese sturgeon; Yangtze river estuary

Introduction

Rapid urbanization and industrial development in the past 10 years have caused environmental pollution. Heavy metal pollution is one of the main quality problems in many rivers, particularly for developing countries (Akoto *et al.,* 2008; Ahmad *et al.,* 2010). The concentrations of heavy metal ions in non-polluted rivers are generally very low. Heavy metals in rivers originate from natural and human activities and have contaminated many water ecosystems (Blais and Kalff, 1993; Karbassi *et al.*, 2008). Heavy metals are now recognized to be among the most relevant contaminants in the marine environment and their concentrations are increasing in some coastal and estuarine waters (Schuhmacher *et al.*, 1996; Garcia *et al.*, 2001).

Shanghai city and Jiangsu Province are the most developed regions in China, and many industrial and domestic sewage effluents are discharged into the Yangtze River estuary. Water quality investigations into the Yangtze River estuary indicate different degrees of heavy metal contamination in the coastal waters, including copper (Cu), zinc (Zn) and other pollutants (Li *et al.*, 2009). Heavy metals cause serious damage to the environment of the Yangtze River estuary in terms of habitat, biodiversity, fisheries resources, and water quality (An *et al.*, 2009). The average concentrations of Cu and Zn in the Yangtze River estuary from 2005 to 2008 were 0.01448 and 0.0275 $mg \cdot L^{-1}$, respectively (Zhuang *et al.*, 2009).

Heavy metals in the aquatic environment affect life in various ways, such as damage to the kidney and liver of fish, leading to weakness, poor appetite, and altered reproductive activity (Singh *et al.*, 2007). Many factors affect heavy metal accumulation in fish, such as the environment, habitat, and metabolic activities (Mustafa and Guluzar, 2003). The living environment is often considered more important because aquatic systems are complex and metallic contaminants are unevenly distributed.

Chinese sturgeon are included on the IUCN Red List of Threatened Species. As the life history of the Chinese sturgeon includes a transition from birth in freshwater to saltwater for maturity, juveniles concentrate in the Yangtze River estuary from May through September annually to develop osmoregulatory capacity (He *et al.*, 2009; Zhao *et al.*, 2015). The Chinese sturgeon population has declined drastically in recent years as a result of many activities, including heavy metal contamination as a contributing stressor restricting survival fitness (Zhuang *et al.*, 2016). The accumulation of heavy metals in sturgeon muscle may affect action potentials and the neuromuscular system (Hou *et al.*, 2011).

At present, research on the Chinese sturgeon breeding, domestication, growth, and nutrition is being actively carried out (Jiang, 1996; Du *et al.*, 2007). However, research and information on the toxic effects of heavy metals on Chinese sturgeon are fragmentary. Therefore, the present study was performed to assess Cu and Zn toxicity and reveal the effects of heavy metal pollution on juvenile Chinese sturgeon.

Materials and Methods

Acute Toxicity Estimate Experiment

Juvenile Chinese sturgeon were reared in 2 m diameter circular fiberglass tanks. Prior to conducting the experiments, the fish were acclimated for 30 days under ambient light and temperature in the laboratory. They were fed a commercial diet three times per day. No mortality of fish was noted during this period. After the 30-day acclimation, 270 active juveniles (wet body weight = 41.5 ± 4.0 g, total length = 24.2 ± 3.5 cm) were chosen for the experiment.

The sturgeon were not fed for 24 h prior to the Cu and Zn exposure experiment. The physicochemical parameters of temperature, pH, and dissolved oxygen (DO) were maintained at 20.0 ± 1.0°C, 7.0 ± 0.5, and 7.0 ± 0.5 $mg\cdot L^{-1}$, respectively. Tests were run at constant temperature with aeration to maintain DO levels at near saturation. In preliminary experiments a medium with 0.11 $mg\cdot L^{-1}$ Cu and 7.90 $mg\cdot L^{-1}$ Zn caused 100% mortality during a 24-h exposure, but no mortality occurred with exposure to 0.012 $mg\cdot L^{-1}$ Cu or 1.265 $mg\cdot L^{-1}$ Zn during 24 h of treatment.

Therefore, the Cu exposure concentrations were 0 (controls exposed to distilled water), 0.0083, 0.0120, 0.0173, 0.0250, 0.0360, 0.0530, 0.0760 and 0.1100 $mg\cdot L^{-1}$. The Zn exposure concentration were 0 (controls group), 0.8000, 1.2650, 2.0000, 3.1600, 5.0000 and 7.9100 $mg\cdot L^{-1}$. After acclimation to test conditions, acute static toxicity tests were carried out according to the biological hydrostatic test procedures. Water remained unchanged during the test; thus, the Cu and Zn solutions were not replenished. Those exposure concentrations referred to normal conditions at the beginning of the experiment. The test solutions were prepared from concentrated $CuSO_4\cdot 5H_2O$ and $ZnSO_4\cdot 7H_2O$ (analytical grade) stock solutions added to the aquaria.

Each test was replicated three times with six juveniles each. Fish were transferred randomly from the holding tanks to 300 L glass aquaria (dimensions: 80 × 80 × 60 cm) covered with plastic to reduce evaporative losses. Metal ion concentrations were verified at the beginning and at the end of the tests by analytical chemistry. Cu and Zn levels were not modified during the exposure in any of the experiments.

Mortality was recorded 24, 48, 72 and 96 h after adding the Cu and Zn solutions. The absence of response to a gentle mechanical stimulus was the criterion for death. The acute toxicity estimate experiments were terminated after 96 h. The LC_{50} values are expressed in mg metal per liter. Probit analysis was used to calculate the daily LC_{50} as described by the Spearman–Karber method (Hamilton *et al.*, 1978).

Distribution of Cu in the Body

Juveniles were cultured for 60 days in different tanks with Cu concentrations of 0 (control group), 0.40 μg/L (low dose), 0.89 μg/L (medium dose), and 2.00 μg/L (high dose). Test solutions were prepared from concentrated $CuSO_4\cdot 5H_2O$ (analytical grade) and the stock solutions were added to the aquaria. Each concentration group test was replicated three times with 12 fish each. The 48 active juveniles (wet body weight = 241.5 ± 13.7 g, total length = 35.2 ± 3.5 cm) were chosen for the experiment. They were fed a commercial diet twice daily. The physicochemical parameters of temperature, pH, and DO were maintained at 22.0 ± 1.0°C, 7.5 ± 0.5 and 8.0 ± 0.5 $mg\cdot L^{-1}$.

Sturgeon were not fed for 24 h prior to sampling. After 30 and 60 days, selected fish were anaesthetized with 100 mg/L MS-222 (C10H15NO5S; 3-aminobenzoic acid ethyl ester methanesulfonate) (Feng *et al.*, 2011). Then, tissues were sampled, including liver, gills, intestine and stomach, cartilaginous bone, dorsal bone plate, muscle, skin, and notochord. The Cu concentration was measured in different tissues by graphite furnace atomic absorption spectrometry.

The experimental data are expressed as mean + standard error (Mean + SE) (n = 6). The test data were analyzed by SPSS 13 statistical software (SPSS Inc., Chicago, IL, USA) using one way analysis of variance (ANOVA) and Duncan's multiple comparison test to identify differences among groups. A p-value < 0.05 was considered significant.

Liver Antioxidant Enzymes Activities

Juvenile Chinese sturgeon were reared in 2 m diameter circular fiberglass tanks. Seventy-two active juveniles (wet body weight = 40.6 ± 4.0 g, total length = 23.8 ± 3.5 cm) were chosen for the experiment. The fish were not fed during the experiment. The Cu concentrations tested were 0, 0.005, 0.0100 and 0.0150 mg/L. Test solutions were prepared from concentrated $CuSO_4\cdot 5H_2O$ (analytical grade) stock solutions added to the aquaria.

Sampling was carried out at 24, 48, 72 and 96 h. All tests were replicated three times, and six fish were randomly sampled in each group. The selected fish were first anaesthetized with 100 mg/L MS-222 and the liver tissue was sampled. Superoxide dismutase (SOD) and catalase (CAT) activities were determined with respective kits purchased from Nanjing Jiancheng Bioengineering Institute

(Jiangshu Province, China), according to the manufacturer's instructions. SOD and CAT activities were measured three times, and the average value of the experimental data for the repeated experiment was tested by *t*-test and ANOVA.

Results

Fish Symptoms after Exposure to Heavy Metals

No Chinese sturgeon died in the control group. The gills of fish in the control group were red and covered with mucus, whereas the gills turned black and secreted excess mucus in the Zn-exposed groups. The same symptoms were observed in the Cu-exposed group, except some light blue flocculence was attached to the gills. The livers of fish that had died were tumescent, and their body turned from red to white.

Different symptoms were observed in the beginning in fish exposed to the different Cu concentrations. An unusual phenomenon was observed at the highest concentrations after 4–6 h. The fish lost balance, started to swim in an unbalanced manner and the frequency of opercular flapping increased. Some individuals rolled over in the water, and started spinning in a corkscrew fashion up and down. This unusual phenomenon was also observed after 10–12 h exposure to the highest concentration of Cu.

Acute Toxicity of Cu and Zn to Juvenile Sturgeon

Juvenile Chinese sturgeon died at different times during exposure to the different concentrations of Cu and Zn (Table 1). No juvenile sturgeon died in the 0.0083 mg·L^{-1} Cu group, whereas all fish died in the 0.1100 mg·L^{-1} group. No fish died in the 0.8000 mg·L^{-1} Zn group before 24 h. However, mortalities were observed after 48-, 72-, and 96-h exposure. All juveniles died in the 7.9100 mg·L^{-1} Zn group. More sturgeon died as the concentrations of Cu and Zn were increased. Moreover, the more time the fish were exposed to the same concentration, the more fish died.

The LC_{50} values for the 24-, 48-, 72- and 96-h exposures to Cu were 0.0606, 0.0414, 0.0289 and 0.0217 mg·L^{-1}, respectively, whereas those for Zn were 3.590, 2.514, 1.759 and 1.200 mg·L^{-1}, respectively. Therefore, the estimated safe Cu and Zn concentrations were 0.00217 and 0.1200 mg·L^{-1}, respectively (Table 2).

Distribution of Cu in Different Tissues

Cu content was highest in the liver of control juveniles, followed by the gills, whereas the lowest content was detected in cartilage. Cu content of tissues showed different trends as Cu was increased. The fastest accumulator was the liver, followed by the stomach, whereas cartilage and skin had the lowest contents. The dorsal plate had some ability to accumulate Cu. These results show that the liver was the major accumulation site for Cu under different water conditions (Table 3).

Table 1: Acute toxicity of Cu^{2+} and Zn^{2+} to juvenile Chinese sturgeon (n = 6). Wet body weight of juvenile sturgeon were 41.5 ± 4.0 g, total length were 24.2 ± 3.5 cm. "–" denotes that all fish died

Metal	Concentration	Mortality (%)			
	(mg·L^{-1})	24 h	48 h	72 h	96 h
Cu^{2+}	0.0083	0	0	0	0
	0.0120	0	0	5.56	16.70
	0.0173	0	0	11.10	33.30
	0.0250	0	27.80	44.40	61.10
	0.0360	11.10	50.00	72.20	83.30
	0.0530	38.90	61.10	83.30	100
	0.0760	61.10	77.80	100	-
	0.1100	100	-	-	-
Zn^{2+}	0.8000	0	5.56	11.10	27.80
	1.2650	5.56	22.20	33.30	55.60
	2.0000	16.70	38.90	55.60	77.80
	3.1600	33.30	50.00	77.80	100
	5.0000	66.70	83.30	100	-
	7.9100	100	-	-	-
Control	0	0	0	0	0

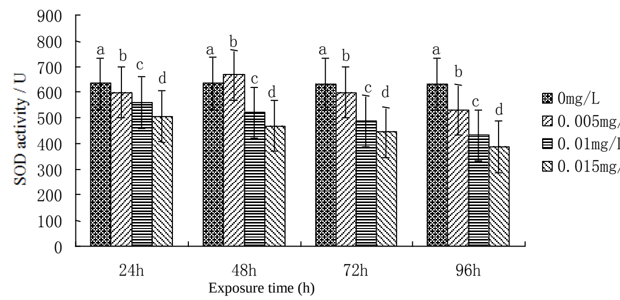

Fig. 1: Changes in superoxide dismutase (SOD) activity with exposure time in liver tissues of juvenile Chinese sturgeon (n = 6). Means with common letter did not differ ($P > 0.05$)

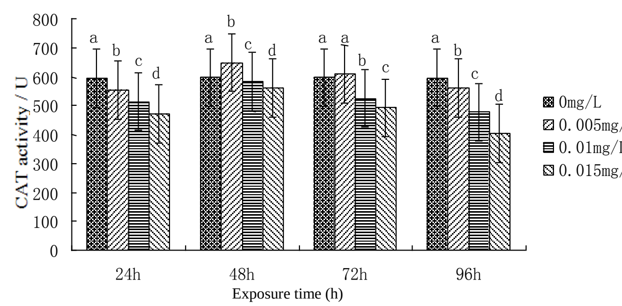

Fig. 2: Changes in catalase (CAT) activity with exposure time in liver tissue of juvenile Chinese sturgeon (n = 6). Means with common letter did not differ ($P > 0.05$)

Liver Antioxidant Enzyme Activities

SOD and CAT activities in the liver tissues of juveniles were relatively low during the first 24 h. SOD values were 599.275 ± 7.03, 560.45 ± 6.28 and 505.95 ± 9.71 U in the 0.005, 0.0100 and 0.0150 mg/L Cu groups, respectively.

Table 2: Linear regression analysis of Cu^{2+} and Zn^{2+} toxicity in juvenile Chinese sturgeon (n = 6). "y" signifies probit of mortality. "x" signifies logarithm of ion concentration

Metal	Test time/h	Regression equation	Correlation coefficient (R^2)	LC_{50} (mg·L^{-1})	95% confidence interval (mg·L^{-1})
Copper	24	y = 6.9484 x + 13.615	0.9277	0.0606	0.0489 - 0.0759
	48	y = 2.673 x + 8.754	0.9813	0.0414	0.0315 - 0.0545
	72	y = 3.767 x + 11.099	0.9503	0.0289	0.0224 - 0.0372
	96	y = 5.512 x + 14.26	0.9635	0.0217	0.0168 - 0.0280
Zinc	24	y = 4.5973 x + 2.6671	0.9186	3.590	2.679 - 4.811
	48	y = 2.8998 x + 3.7971	0.9701	2.514	1.792 - 3.528
	72	y = 4.1864 x + 4.0558	0.9569	1.759	1.283 - 2.412
	96	y = 4.6993 x + 4.7205	0.9506	1.200	0.899 - 1.603

Table 3: Accumulation of Cu (μg·g^{-1}dry weight) in different tissues of juvenile Chinese sturgeon (n = 6). Means with common letter did not differ (P > 0.05)

Tissue	Time	Control group	Low group	Medial group	High group
Liver	30	2.141±0.131^{a}	5.250±1.456^{b}	12.469±1.533^{c}	21.176±1.870^{e}
	60	2.314±0.162^{a}	6.643±1.637^{b}	18.798±1.781^{d}	29.281±3.199^{f}
Gills	30	1.199±0.0893^{a}	1.481±0.156^{b}	2.815±0.155^{d}	4.206±0.149^{f}
	60	1.262±0.0817^{a}	1.698±0.159^{c}	3.025±0.135^{e}	3.868±0.154^{g}
Intestine and stomach	30	0.121±0.0187^{a}	1.499±0.150^{b}	2.952±0.176^{d}	5.142±0.210^{f}
	60	0.123±0.0221^{a}	2.695±0.214^{c}	4.680±0.161^{e}	7.152±0.393^{g}
Cartilaginous bone	30	0.520±0.0407^{a}	0.593±0.0716^{a}	0.852±0.0842^{c}	1.606±0.153^{e}
	60	0.547±0.0456^{a}	0.713±0.109^{b}	1.113±0.100^{d}	2.002±0.125^{f}
Dorsal bone plate	30	0.963±0.0574^{a}	1.083±0.100^{ab}	1.180±0.0985^{bc}	1.240±0.124^{cd}
	60	1.057±0.129^{ab}	1.172±0.135^{bc}	1.295±0.146^{cd}	1.369±0.121^{d}
Muscle	30	0.840±0.0639^{a}	0.947±0.0998^{a}	1.295±0.120^{b}	1.896±0.229^{c}
	60	0.918±0.112^{a}	1.308±0.338^{b}	1.704±0.172^{c}	2.482±0.142^{d}
Skin	30	0.675±0.0940^{a}	0.735±0.0983^{a}	1.096±0.156^{b}	1.585±0.101^{d}
	60	0.710±0.0845^{a}	0.989±0.140^{b}	1.358±0.140^{c}	2.076±0.120^{e}
Notochord	30	0.625±0.0481^{a}	0.738±0.0766^{a}	1.003±0.122^{b}	1.270±0.0809^{d}
	60	0.649±0.0676^{a}	0.927±0.114^{b}	1.246±0.103^{c}	1.335±0.0870^{e}

Accordingly, CAT values were 553.67 ± 4.29, 513.15 ± 5.08 and 471.50 ± 5.36 U. However, the activities recovered gradually from 24 to 48 h, but decreased after 48 h, when they were lower than the contro1 group. The decrease was positively correlated with Cu concentration. These changes in SOD and CAT activities indicate that juvenile Chinese sturgeon were severely affected by Cu (Figs. 1 and 2).

Discussion

Cu and Zn are essential micronutrients for physiological processes, whereas many other metals, such as Cd, Cr, Pb, and Co, have no known physiological functions (Suthar and Singh, 2008). Metal ion binding sites are usually very complex and very specific to mono or divalent metals (Bertini *et al.*, 2001, Messerschmidt *et al.,* 2001). Cu and Zn make up many cellular enzymes and proteins. However, these elements are toxic, particularly at higher concentrations. Metals are non-degradable and accumulate in animals, damaging the nervous system and internal organs (Lee *et al.*, 2007). Therefore, research on metal contaminants is important owing to their potential toxicity to fish (Vinodhini and Narayanan, 2008).

Mucus is continuously sloughed in the gill to maintain normal functioning (Eddy and Fraser, 1982). However, the gills of Cu- and Zn-exposed fish that died turned black and secreted excess mucus. Death was probably due to excess mucous covering the gills as a result of Cu and Zn exposure. Uptake of Cu is known to cause acidosis reinforced by hypercapnia and accumulation of lactic acid, as it restricts respiratory exchange with internal tissues (Boitel and Truchot, 1989). The lethal effects of Zn, including cytological gill damage, is related to disrupted respiration and osmoregulation (Crespo, 1984).

Cu at normally low levels may contribute to the production of free radicals. Therefore, it may play a role regulating and inducing apoptosis. Conversely, Zn protected against oxidative damage, either directly or by inducing metallothionein. Redox activity appears to play a major role resolving the main effects of these trace metals (Jorge and Luisa, 1998). In the present study, mortalities were observed later in the low-concentration groups than in the high-concentration groups. The livers of Chinese sturgeon exposed to Cu and Zn were swollen and white, indicating heavy metal accumulation when concentrations exceed normal intracellular levels. Liver function was damaged to some extent by Cu and Zn.

The Cu and Zn standards for fisheries water quality in China are 0.01 and 0.10 mg·L^{-1}, respectively. Based on our results, Chinese sturgeon seem to be safe at 0.00183 mg·L^{-1} Cu, which is far below the water quality standards of China's fishing industry. The average Cu concentration in the Yangtze River estuary from 2005 to 2008 was 0.01448 $mg \cdot L^{-1}$, indicating that the Chinese sturgeon fry migrating in the Yangtze River estuary may face the threat of Cu contamination. This situation should arouse attention of the relevant departments.

The distribution and accumulation of heavy metals in aquatic animals differs, and different organs are selective for heavy metals. The present results showed that the liver, stomach, gills, and muscle had the highest to lowest Cu concentrations in Chinese sturgeon. A study on pelagic fish showed a low Cu muscle content, but the content was higher in the liver of benthic fish (Romeo *et al.*, 1999; Zauke and Savinov, 1999). Heavy metal concentrations have been studied in muscle, eggs, kidney, liver, gills, and fins of Nile tilapia. Cu accumulated the most in liver and eggs than in other tissues (Paulami *et al.*, 1999). Three commercially important fish (*Mullus barbatus*, *Merluccius merlliccius* and *Boops boops*) were studied in the Turkish Aegean Sea. As results, the accumulation of Cu in the muscle was low, and the accumulation of Cu in liver was higher than in other tissues and organs, indicating that a large amount of Cu accumulates in the liver (Zyadah and Chouikh, 1999). A study on Zilli fish showed that Cu accumulation was correlated with exposure concentrations in gills and liver. Cu seems to accumulate the most in the liver (Ay *et al.*, 1999). When carp (*Prochilodus. crofa*) were exposed to 0–29 μg/L Cu for 96 h, Cu was detected in the gills, intestine, kidney, and liver, but not in muscle. The accumulation was greatest in the liver, followed by the kidney and intestine. That study proposed that fish produce a metal binding protein that combines with the heavy metal for transport, distribution, or discharge (Mazaon and Fernandes, 1999).

Higher metal concentrations were usually found in liver tissue, while the lowest metal concentrations were detected in muscle tissues (Allen-Gil and Martynov, 1995). Lysosomal membrane phospholipids were oxidized and hydrolytic enzymes were released in the liver when Cu concentrations exceeded the threshold of the Chinese sturgeon juveniles, which can lead to liver damage in fish (Dai *et al.*, 1997). $CuSO_4$ is commonly applied to Chinese ponds to control algae. However, our results suggest that the effects of Cu on Chinese sturgeon should be further studied in ponds or tanks.

SOD activity increased significantly in juvenile sturgeon under the stress of a low Cu concentration, which has been reported previously (Beaumont and Newman, 1986). This appears to be a poisoning kind of phenomenon called the poison excited effect (Stebbing, 1982). Many studies have demonstrated the poison excited effect. Under high concentration Cu stress, SOD activity in the liver of juvenile sturgeon dropped significantly. Therefore, SOD activity decreased under Cu stress and activity against reactive oxygen species was also disrupted. This may be one reason that Cu affected Chinese sturgeon. Similarly, CAT activity also declined sharply during the 24-h treatment, but tended to increase from 24 to 48 h, and then decreased significantly. Damage to the liver of the sturgeon increased gradually with increasing exposure time.

Conclusion

The estimated safe Cu and Zn concentrations for juvenile Chinese sturgeon in the Yangtze River estuary were 0.00217 and 0.1200 $mg \cdot L^{-1}$, respectively. The average Cu and Zn concentrations in the Yangtze River estuary from 2005 to 2008 were 0.01448 and 0.0275 $mg \cdot L^{-1}$, respectively. This implies that Chinese sturgeon juveniles face the threat of Cu contamination. The liver was the major accumulation site for Cu, and the changes in liver SOD and CAT activities revealed the degree of damage caused by Cu. This situation should attract the attention of the relevant departments. The present results will help provide a measure of the dimensions of the problem.

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