Toxicity Study of a New Scale Inhibitor for Seawater Desalination

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Abstract

Maleic acid polymer scale inhibitor is a new domestic seawater desalination scale inhibitor. Guided by the Organization for Economic Cooperation and Development method, this study tested the acute oral toxicity, sub-chronic toxicity and genotoxicity of this new inhibitor. The LD_{50} obtained from the acute oral toxicity test was 6810 and 9260 mg/kg·BW for male and female rats, as well as 1/5, 1/10 and 1/20 LD_{50} were as the dose for sub-chronic toxicity test. It showed the weight of male rats with high dose was significantly lower than the control group during the exposure period (p<0.05), and the food consumption in the first 4 weeks was lower than the control group ($p_{\_week1}$ = 0.0261, $p_{\_week4}$ = 0.00222). The blood biochemical results showed the UREA in the medium- and high-dose groups were significantly higher than the control group ($p_{\_\text{female medium}}$ = 0.0047, $p_{\_high}$ = 0.0037; $p_{\_male\ medium}$= 0.0026, $p_{\_\text{high}}$<0.001), and increased as a dose dependence. Based on UREA results, the NOAEL and LOAEL were 1/20 LD_{50} and 1/10 LD_{50}, respectively (males: 340.5 and 170.25 mg/kg·BW, females: 436 and 231.5 mg/kg·BW). Comet assay in vitro and Mammalian Erythrocyte Micronucleus Test were jointly to judge genotoxicity. This inhibitor did not cause chromosome aberrations in mouse bone marrow cells. However, the tail moment of CHO cell in all groups (p<0.01) and the DNA% in tail in the 1/4 IC_{50} and IC_{50} groups were higher than the negative control (p<0.001) in comet assay, suggesting the potential DNA damage in CHO cell. The oral LD_{50} and the NOAEL and LOAEL obtained in this study provides a theoretical basis for further toxicity research and risk assessment.

1. Introduction

The reduction in the global availability of freshwater resources is present and unavoidable. Seawater desalination technology is an effective measure to overcome the shortage of water resources and constitutes a type of sustainable development. There are 19,744 desalination plants in the world, with a desalination capacity of approximately 99.7 million m^3/d. Data from the International Desalination Association show that these plants, located in 150 countries, are continuously providing water for 300 million people (Feria-Diaz et al., 2021). As a new type of water resource in coastal areas, the development and utilization of seawater desalination has gradually attracted the attention of the central and local governments in China. *Opinions on Accelerating the Development of the Seawater Desalination Industry* was issued by the General Office of the State Council in 2012 (General Office of the State Council of the People's Republic of China, 2012). Since 2020, the central and local governments have issued several policies and standards on desalination (General Office of Shandong Provincial People's Government, 2020; State Administration for Market Regulation and Standardization Administration, 2020a, 2020b, 2020c, 2020d). In June 2021, the *Action Plan for the Development of Seawater Desalination Utilization (2021–2025)* was jointly issued by the National Development and Reform Commission and the Ministry of Natural Resources. The development goals for seawater desalination and related policies have been put forward, proposing seawater desalination of more than 2.9 million t/d by 2025 (National Development and Reform Commission & The Ministry of Natural Resources, 2021). With an increasing focus on the seawater desalination industry, the process technology of seawater desalination has been rapidly progressing. Water with a high salt concentration is produced in the process of seawater desalination, and this water inevitably causes scaling, foam and other problems that affect the normal operation of machines and reduces their service life. Therefore, it is necessary to add treatment agents to the seawater desalination equipment to ensure its stable operation. Seawater desalination treatment agents include scale inhibitors, defoamers, bactericides and flocculants. The role of scale inhibitors is to prevent the deposition of insoluble salts, inhibit the buildup of dirt and prevent scale from forming on the surface of equipment. Scale inhibitors have been widely used in seawater desalination enterprises because they provide considerable economic and social benefits.

The development of water treatment chemicals in developed countries started early in the 1930s and has progressed rapidly since the 1980s. Several large manufacturers of scale inhibitors have controlled the international market for these chemicals (Lu, 2000). In China, water treatment agents have been developed since the 1970s with the introduction of modern water treatment technology, and research in this field has rapidly progressed in recent years (Jiao et al., 2021). However, the development of seawater desalination treatment agents in China cannot meet the needs of the seawater desalination industry at present, and the possible human health effects of seawater desalination treatment agents have not been assessed (Wu & Xu, 2020). The national marine public welfare industry scientific research special fund project, Research and Engineering Demonstration of Localization Technology of Seawater Desalination Water Treatment Agents (project no. 201505021), has carried out several studies on new domestically produced seawater desalination agents and has led to the development of four new seawater desalination agents, including three scale inhibitors and one defoamer. The project realized the large-scale production of new agents and formulated industrial standards. Based on this public welfare scientific research project, the present study conducted a toxicological investigation of one of the new scale inhibitors, a maleic acid polymer, to provide experimental data for human health and safety evaluation.

In China, the policy *Hygienic Safety Evaluation for Chemicals used in Drinking Water Treatment (GB/T 17218 – 1998)* requires toxicological evaluation of chemicals used in conventional water plants (State Administration for Market Regulation and Ministry of Health of the People's Republic of China, 1998). The evaluation methods require various assays, including genotoxicity testing and 90 day oral toxicity testing in rats. The toxicological data types and requirements for toxicity effect evaluation are described in NSF/ANSI 60-2016 Drinking Water Treatment Chemicals – Health Effects (US National Sanitation Foundation, 2016). The toxicity evaluation must include an in vivo micronucleus test and a 90 day subchronic oral toxicity test in rats. In addition, acute or short-term toxicity tests may be conducted as a supplemental test. This study completed a toxicity investigation of the maleic acid polymer scale inhibitor based on the requirements of these standards.

Maleic acid copolymer has good heat resistance and an excellent scale inhibition effect against $CaCO_3$, Ca_3 $(PO_4)_2$ and $CaSO_4$. It has a synergistic effect with organic phosphonic acid and multivalent metal chelating agents, and it is suitable for high-, medium- and low-temperature water systems. Maleic acid-ethylene glycol ester-acrylic acid terpolymer is synthesized by reacting maleic acid, ethylene glycol ester and acrylic acid. The maleic acid copolymer can strongly chelate Ca^{2+} and Mg^{2+} and can be used as a high-temperature-resistant water quality stabilizer. Maleic acid copolymer has a good synergistic effect with other water treatment agents. Through its effective combination with other water treatment agents, the technical problem of unreliable scale inhibition can be overcome to ensure the desired water yield of desalination equipment (Jia et al., 2019). The maleic acid polymer scale inhibitor was synthesized through copolymerization and compounding, and the production process is shown in the Supporting Information (SI).

2. Materials And Methods

2.1. Animals

A total of 120 6-week-old specific pathogen-free Wistar rats weighing 180–200 g and 50 specific pathogen-free Kunming mice weighing 25–30 g, half male and half female, were obtained from the Experimental Animal Center of the Academy of Military Medical Sciences (Beijing, China). Forty of the Wistar rats were used for acute oral toxicity testing and 80 for subchronic toxicity testing. The 50 Kunming mice were used for the mammalian erythrocyte micronucleus test. The animals were maintained in a temperature-controlled environment (23 ± 2 °C) with a 12-h light/dark cycle and with free access to food and water. The Committee of Laboratory Animal Welfare and Ethical Review, Institute of Environmental and Health-Related Product Safety, China CDC approved all of the animal procedures (approval documents provided in the Supporting Information). All of the animal experiments complied with the ARRIVE guidelines and were carried out in accordance with European Union directive 2010/63/EU for animal experiments.

2.2. Acute oral toxicity test

The acute oral toxicity test was based on Horn's method and was used to obtain the LD_{50} (Organization for Economic Cooperation and Development (OECD), 1987; US Environmental Protection Agency (USEPA), 1998. Four dose groups (1000, 2150, 4640 and 10,000 mg/kg) were established based on Horn's pre-experiment method. The new scale inhibitor was prepared in distilled water. Five male and five female rats in each group were administered a one-time oral dose, which was calculated as 0.1 mL/100 g body weight. The dose used in the formal experiment was selected based on the pre-experiment results. The number of animals in each group and the administration method in the formal experiment were the same as in the pre-experiment. After dose administration, the general condition, poisoning symptoms and mortality of the animals were observed for 2 weeks.

2.3. Subchronic oral toxicity test

2.3.1. Animal treatment

The experiment was based on the OECD guidelines for subchronic toxicity testing and pre-test results (OECD, 1998). The dose setting was based on the LD_{50} value obtained from the acute oral toxicity test. In the subchronic toxicity test, 1/5, 1/10 and 1/20 LD_{50} were set as the high, medium and low doses, respectively. Ten male and ten female rats in each group were exposed to maleic acid polymer scale inhibitor with a daily oral dose of 1 mL/100 g body weight for 90 days. Control rats were treated with the same amount of deionized water. During the exposure period, the rats were evaluated daily for behavioral activities and signs of diarrhea, dehydration and deterioration of their physical condition. The weight and food consumption of the animals were recorded weekly.

After the exposure period, the rats were fasted overnight. Under anesthesia with pentobarbital sodium, blood samples were taken via the abdominal aorta, and the rats were then euthanized by dislocation. Many organs and tissues (e.g. liver, kidney and spleen) were quickly dissected and carefully examined for any abnormalities. The organs were weighed and fixed with 10% formalin solution for histopathological examination. The blood samples were used for routine blood testing, and the serum obtained by centrifugation was used to determine blood biochemical indicators.

2.3.2. Biochemical examination

Blood biochemical indicators, including liver function-related indicators, renal function-related indicators and other important indicators, were assessed using a Toshiba 120 Automatic Biochemical Analyzer (Japan). The hepatic function-related indicators were alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, globulin, total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL) and alkaline phosphatase. The renal function-related indicators were urea nitrogen (UREA) and creatinine (CREA), and the other key indicators were blood glucose and total cholesterol. BioSino Bio-Technology & Science Inc. provided the blood biochemical test kits.

2.3.3. Hematology analysis

A Sysmex XP-100 automated hematological analyzer (Sysmex, Kobe, Japan) was used for the hematology analysis, which included red blood cell series, white blood cell series, platelet series and other indicators (details are provided in the Supporting Information).

2.3.4. Histopathological examination

The liver, spleen, kidney, stomach, duodenum, adrenal glands, bladder and testis/ovaries were removed from the euthanized rats and fixed in 4% neutral buffered formaldehyde. Thereafter, the organs and tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. The sections were examined under an Olympus BX43 optical microscope (Tokyo, Japan) by an experienced pathologist who was blinded to the sample identity. Images were acquired with an LV320 Digital Acquisition Device by an experienced pathologist.

2.3.5. Statistics

R was used to analyze the experimental data. The Shapiro–Wilk test was used to assess the distribution normality, and Bartlett's test was used to assess the homogeneity of variance. Dunnett's test was conducted if the data showed homoscedasticity and a normal distribution, and the Wilcoxon rank–sum test was used for pairwise comparison in the case of heteroscedasticity or a skewed distribution. In all groups, significant differences were identified at $p < 0.05$.

2.4. Genotoxicity test

In vivo and in vitro genotoxicity tests were carried out using mammalian erythrocyte micronucleus test and comet assay.

2.4.1. Mammalian erythrocyte micronucleus test

The micronucleus test of polychromatic erythrocytes in mouse bone marrow was conducted according to the OECD guidelines (OECD, 1997). The new scale inhibitor was prepared in distilled water and divided into three dose groups: 1000, 2000 and 5000 mg/kg body weight. The dose of mitomycin C in the positive control group was 1.0 mg/kg body weight (mitomycin C was obtained from Sigma–Aldrich Cheme GmbH, batch number 028K1815). Distilled water was used as the negative control. There were 10 mice in each group, 5 male and 5 female. The new scale inhibitor groups and the distilled water control group were administered the dose orally twice in 30 h, while the positive control group (mitomycin C) was administered the dose intraperitoneally twice in 30 h. 6

h after the second dose, all animals were euthanized by decapitation. The sternal bone marrow was collected, smeared with calf serum dilution and stained with Giemsa. Under a light microscope, 2,000 polychromatic erythrocytes were counted for each animal, and the micronucleus rate was calculated as the percentage per thousand polychromatic erythrocytes containing micronuclei. The results were analyzed by the Poisson distribution with the U-test.

2.4.2. Comet assay

Chinese hamster ovary (CHO) cells were used for the in vitro comet assay. The doses used in the comet assay were 0, 1/16 IC_{50}, 1/4 IC_{50} and IC_{50}, with $K_2Cr_2O_7$ (1 μg/mL) as the positive control. After dosing, the cells were placed in a cell incubator for 3 h. The CHO cells were then washed with PBS, digested with trypsin, centrifuged, and the density of CHO cells was adjusted to 1×10^5 cells/mL with HBSS buffer. A 30 μL aliquot of cell suspension and 300 μL of low melting point agarose (LMA) were fully mixed (cell suspension: LMA = 1: 10), and then 30 μL of the mixture was transferred to a glass slide and condensed. The prepared glass slide was immersed in a cold cracking solution in the dark at 4°C for 1 h. The slide was then placed in fresh electrophoresis solution for 30 min and then subjected to electrophoresis for 1 h (20°C, 25 V and 100 mA). After electrophoresis, the slide was rinsed with neutralization buffer, stained with SYBR Green I, and examined under a fluorescence microscope (Olympius BX41, Japan). One hundred cells in each dose group were randomly selected and analyzed with Comet Assay IV software. The Tail DNA content (Tail DNA%) and Tail Moment (TM) were respectively selected as single and composite indicators to evaluate the extent of DNA damage.

3. Results

3.1. Acute oral toxicity test

The LD_{50} of the maleic acid polymer scale inhibitor was obtained from the rat acute oral toxicity test using Horn's method, and the results are shown in **Table S1**. After dosing, the animals in the high-dose group showed intoxication symptoms such as excitement. According to Horn's method (OECD, 1987; USEPA OPPTS, 1998), the oral LD_{50} of the new scale inhibitor was 6810 mg/kg body weight for male rats and 9260 mg/kg body weight for female rats. The 95% confidence limit was 6360–13,500 mg/kg body weight.

Based on the LD_{50} results, the subchronic toxicity test doses were 1/5, 1/10 and 1/20 LD_{50} for the high-, medium- and low-dose groups and were 1362, 681 and 340.5 mg/kg body weight for male rats and 1852, 926 and 463 mg/kg body weight for female rats, respectively.

3.2. Subchronic oral toxicity test

3.2.1. Changes in body weight and food consumption of rats during the subchronic toxicity test

The weight and food consumption of rats were measured once per week during the 90 day exposure period. There was no significant effect on weight gain in female rats exposed to the maleic acid polymer scale inhibitor. However, male rats in the high-dose group had a significantly lower weight than those in the control group throughout the exposure period ($p < 0.05$) **(**Fig. 1A **and Table S2).**The food consumption of the female and male rats in the high-dose group decreased during the first 4 weeks of exposure **(**Fig. 1B**)**. Compared with the control group, at 1 week and 4 weeks, male rats in the high-dose group had significantly lower food consumption ($p_{\_week1} = 0.0261$, ($p_{\_week4} = 0.0222$). After 4 weeks of exposure, there were no significant differences in food consumption.

3.2.2. Blood biochemical indexes

Among the indexes related to liver function, the AST value of male rats in the high-dose group was significantly lower than that of the control group ($p < 0.001$). For female rats, the AST values in each dose group were significantly lower form those in the control group ($p_{\_\text{low}} = 0.00168$, $p_{\_\text{medium}} = 0.00032$, $p_{\_\text{high}} = 0.00147$). The TBIL values of male rats in the medium- and high-dose groups were significantly lower than in the control group ($p_{\_\text{medium}} = 0.0065$, $p_{\_\text{high}} = 0.0058$). The TBIL value of female rats in the high-dose group was also lower than that in the control group ($p = 0.014$) **(**Fig. 2A **and Table S3)**.

Among the indexes related to renal function, the UREA values of female and male rats showed dose-dependent increases. The UREA values of female and male rats in the medium- and high-dose groups were significantly different from those in the control group ($p_{\_ \text{female medium}}$ = 0.0047, $p_{\_\text{female high}}$ = 0.00037; $p_{\_ \text{male medium}}$ = 0.0026, $p_{\_ \text{male medium}}$ < 0.001). The CREA value in the high-dose males was significantly lower than that in the control group (p = 0.00081). In females, there were no significant differences between the dose groups and the control group (Fig. 2B **and Table S3)**.

Regarding the tested blood electrolytes, after long-term exposure to the maleic acid polymer scale inhibitor, there were no significant differences in blood K levels between any of the dose groups and the control group. The blood Ca levels of high-dose female rats (p = 0.0067) and each group of male rats ($p_{\_ \text{low}}$ = 0.0099, $p_{\_ \text{medium}}$ = 0.00047, $p_{\_ \text{high}}$ = 0.00017) were significantly higher than that of the control group. The blood P levels in the medium- and high-dose male rats were significantly higher than in the control group ($p_{\_ \text{medium}}$ = 0.0036, $p_{\_ \text{high}}$ = 0.0011). The blood P level in the female low-dose group was lower than in the control group (p < 0.001), while in the high-dose group it was higher than in the control group (p = 0.0207). The blood Cl level in male rats was not significantly different from the control in any of the dose groups, while the high-dose female group had a lower Cl level than the control group (p = 0.0105). The blood Na levels of female rats in all dose groups were lower than in the control group ($p_{\_ \text{low}}$ = 0.000462, $p_{\_ \text{medium},}$ $p_{\_ \text{high}}$ < 1× 10^4), while the blood Na level of male rats in the high-dose group was slightly lower than in the control group (p = 0.0164) (Fig. 3 **and Table S3)**.

3.2.3. Hematology analysis results

Immune-related blood cells were examined in the hematological analysis of rats exposed to the maleic acid polymer scale inhibitor. White blood cell (WBC), lymphocyte (Lymph), mid cell count (Mid), granulocyte and percent of granulocyte (Granand Gran%) of males in the high-dose group were higher than those in the control group, but Lymph% was lower than in the control group ($p_{\_ \text{WBC}}$ = 0.0017, $p_{\_ \text{Lymph}}$ = 0.0017, $p_{\_ \text{Mid}}$ = 0.033, $p_{\_ \text{Gran}}$ = 0.0017, $p_{\_ \text{Gran\%}}$ = 0.0019 and $p_{\_ \text{Lymph\%}}$ = 0.0246). There were higher red blood cell counts in the all treated group rats than in the control group, and there was a significant difference between the medium-dose group and control group (p = 0.0325). Hematocrit values in the medium- and high-dose male groups were higher than in the control group ($p_{\_ \text{medium}}$ = 0.0113, $p_{\_ \text{high}}$ = 0.0154). The mean corpuscular volume in the high-dose male group was significantly higher than that in the control group (p < 0.001), while the mean corpuscular hemoglobin concentration in the high-dose group was lower than that in the control group (p = 0.0096). The number of platelets and the plateletcrit value in the medium- and high-dose male groups were lower than those in the control group, and there was a significant difference between the high-dose group and control group for both values (p < 0.05). There was also a dose-dependent decreasing trend among the dose groups (**Table S4**).

In female rats, the hematological results showed that the maleic acid polymer scale inhibitor had no significant effect on blood cells after 90 days of exposure. There were significant differences between the low-dose group and the control group for Mid, Lymph%, Mid% and Gran% ($p_{\_ \text{Mid}}$ = 0.0433, $p_{\_ \text{Lymph\%}}$ = 0.0231 $p_{\_ \text{Mid\%}}$ = 0.029, $p_{\_ \text{Gran\%}}$ = 0.0495), but there were no significant differences in these values between the other dose groups and the control group **(Table S4)**.

3.2.4. Histopathology

The liver, spleen, kidneys, stomach, duodenum, adrenal glands, bladder, testicles/ovaries and other organs were pathologically examined. Two female rats in the control group had pathological changes in the liver, spleen and kidney, separately, and one female rats in high-dose group had pathological changes in the spleen and kidney, separately. One male rat in the control group had pathological changes in the liver, and in the high-dose group, two male rats had pathological changes in the liver, spleen and kidney, separately. No organ changes were detected in other male/female dose groups.The pathological changes observed in the control and high-dose groups mainly manifested as steatosis of the liver cells with infiltration of mononuclear cells, glomerular mesangial hyperplasia, and deposition of hemosiderin in the spleen (Table 1). These pathological changes were observed in the exposure group and the control group upon euthanasia of the animals and were present in small amounts. Furthermore, there were no significant differences within the groups; thus, it is thought that these were spontaneous changes in the animals that were unrelated to the new scale inhibitor. Typical pathological changes are shown in **Fig. S1**.

3.3. Genotoxicity test

3.3.1. Mammalian erythrocyte micronucleus test

A micronucleus test of polychromatic erythrocytes was conducted using mouse bone marrow. A total of 2,000 polychromatic erythrocytes were counted for each animal, and the micronucleus rate was calculated from the percentage of each 1,000 polychromatic erythrocytes containing a micronucleus. The experimental results were analyzed by the U test according to the Poisson distribution. The micronucleus rate of polychromatic erythrocytes in mouse bone marrow in each dose group was not significantly different than that of the negative control group ($p > 0.05$). There was a significant difference between the positive control group (mitomycin C) and the negative control group ($p < 0.01$), indicating that there was no distortion effect on polychromatic erythrocytes in the mouse bone marrow (Table 2).

3.3.2. Comet assay

The cytotoxicity pretest results showed that the IC_{50} of the maleic acid polymer scale inhibitor in CHO cells was 1.347 mg/mL (Wang L. Y. et al., 2018). The doses used in the comet assay were 0, 1/16 IC_{50}, 1/4 IC_{50} and IC_{50}. $K_2Cr_2O_7$ (1 μg/mL) was used as the positive control. The DNA in the tail (%) and the tail moment were used to assess DNA damage. There was no significant difference in the tail DNA content between the 1.347 mg/mL and 0.337 mg/mL dose groups (Table 3).

4. Discussion

Seawater desalination treatment agents are indispensable in the desalination process. However, few studies have investigated the biological toxicity of the chemical monomers used in these agents, and there is a particular lack of systematic toxicity studies. This toxicity study of a new domestically produced seawater desalination treatment agent provides a basis for risk assessment and its safe use.

The maleic acid polymer scale inhibitor examined in this study is a macromolecular polymer polymerized by maleic anhydride, sodium allylsulfonate, hydroxyethylidene diphosphate (HEDP) and acrylic acid. Maleic anhydride was found to be a skin irritant and corrosive to the eyes in New Zealand rabbits. An acute toxicity study found an oral LD_{50} of 400 mg/kg in rats and 465 mg/kg in mice (European Chemicals Bureau, 2016; Lewis, 2004). In a 90 day feeding trial, 100 mg/kg exposure to maleic anhydride resulted in kidney damage, and the no observed adverse effect level (NOAEL) was found to be 40 mg/kg. Respiratory exposure increased the number of peripheral white blood cells, slowed weight gain and reduced the phagocytic activity of neutrophils. No carcinogenic or teratogenic effects were observed (European Chemicals Bureau, 2016). Based on the results of experiments in which rats were fed with maleic anhydride for 2 years, the American Conference of Governmental Industrial Hygienists (ACGIH) listed it as a noncarcinogen (ACGIH, 2002). Sodium allylsulfonate is irritating to the eyes, respiratory tract and skin, and it was found to cause peripheral nerve injury in an occupational population (He & Zhang, 1985). HEDP is a low-toxicity chemical and is a commonly used water treatment agent (Franco & Ribeiro, 2020; Qingdao University of Science and Technology, 2005). The LD_{50} values of HEDP for mice and rats are 2.05 and 1.69 g/kg, respectively. The results of a 6 month rat feeding test showed that rats exposed to 160 and 400 mg/kg HEDP had bone and tooth growth retardation or stagnation and significantly increased urinary calcium excretion; furthermore, rickets-like bone, tooth and tissue decalcification were observed in the histopathology and X-ray examination. There were no apparent toxicity-related effects observed in rats exposed to low-dose HEDP (40 mg/kg and below) (Chen & Yang, 1983). Acrylic acid has low to moderate oral toxicity, and the oral LD_{50} in rats is 33.5–2500 mg/kg body weight (WHO, 1997). The results of a subchronic toxicity study showed that feeding rats with 150 and 375 mg/kg acrylic acid for 90 days resulted in gastrointestinal swelling with dyspnea, and rats in both groups died after prolonged feeding (European Chemicals Bureau, 2002). The ACGIH lists acrylic acid as a noncarcinogen (ACGIH, 2002). In a European Chemical Administration study on the genotoxicity of acrylic acid, rats were administered 0, 100, 333 or 1000 mg/kg acrylic acid by gavage, and peripheral blood was collected 6, 12 and 24 h later to test chromosome aberrations in bone marrow cells. The results showed that the mitotic activity of the rat bone marrow cells was not affected (European Chemicals Bureau, 2002). This study investigated the toxicity of a maleic acid polymer scale inhibitor. The LD_{50} in rats was obtained by conducting an acute oral toxicity test. The oral LD_{50} in male rats was 6810 mg/kg body weight, and in female rats was 9260 mg/kg body weight. The acute oral toxicity of the maleic acid polymer found in this study was significantly lower than that of the monomers found in previous studies.

The effects on rat liver and kidney were observed in a subchronic toxicity test. The liver function results showed that the AST values of the male and female rats were lower than those of the corresponding control groups. AST is an important clinical diagnostic indicator of liver function; in general, an increased AST indicates liver injury, but a decreased AST is not clinically significant (Wang X. H. & Lu, 2013). In this study, the AST values of all rats in each group were within the normal range of Wistar rats (Fan et al., 2010; Tajima, 1989). Taking into account the food consumption and body weight change results, the AST change seen in the rats may have been owing to the rats' diet, nutrition and metabolism. Therefore, the AST results indicate that the new scale inhibitor did not negatively impact the liver function of rats (Yang & Zhang, 2022).

The UREA values of the rats in all dose groups showed a dose-dependent increase. Compared with the corresponding control group, there was no significant difference in the UREA values of the male and female rats in the low-dose group; however, the medium- and high-dose groups were significantly different from the controls. These results were in line with the principles of the NOAEL and lowest observed adverse effect level (LOAEL) (OECD, 1998). Thus, the UREA value was used as a health effect in this study. The NOAEL and LOAEL values were 1/20 LD_{50} and 1/10 LD_{50}, respectively, that is, they were 340.5 and 170.25 mg/kg body weight for male rats and 436 and 231.5 mg/kg body weight for female rats.

We also found that the TBIL values of female rats in the high-dose group and of male rats in the medium- and high-dose groups were significantly lower than those of the control group. TBIL is a liver metabolite. It is an indirect bilirubin that is produced after the destruction of red blood cells; it enters the liver and is metabolized into direct bilirubin (Wang X. H. & Lu, 2013). TBIL is the sum of DBIL and IBIL. The DBIL and IBIL results indicated that the main reason for the decrease in TBIL was the decrease in IBIL. In clinical diagnosis, a decreased IBIL is often attributed to physiological reasons such as diet, anemia or other hepatobiliary diseases, but it should be judged in combination with other indicators. In this study, there was no obvious abnormality in the other hepatobiliary-related indicators. In male rats, the red blood cell analyses with obvious differences in the hematological parameters showed a dose-dependent upward trend. However, there were no obvious hematological changes in the female rats. TBIL and IBIL changed but were within the normal bilirubin range for Wistar rats. Therefore, the changes in bilirubin may have been caused by the diet of the rats. Considering the AST, food consumption and body weight results, we believe that the maleic acid polymer scale inhibitor had no significant effect on the liver function of rats. However, the subchronic experimental conditions such as the experimental duration and the diet may have affected nutrient absorption, leading to malnutrition in the rats.

We also analyzed changes in the main blood electrolytes. The blood Na levels were decreased, especially in female rats. Changes in blood electrolytes can be used to indicate the presence of disease. The decrease in blood Na may have been related to renal failure (Wang X. H. & Lu, 2013), which is consistent with the UREA results. The decreased blood Na may have also been related to gastrointestinal dysfunction. In this study, the blood Cl level in the high-dose female rats was also significantly decreased, which may also have been related to gastrointestinal dysfunction and innutrition (Wang X. H. & Lu, 2013). There were also clear changes in the blood Ca and P levels in the medium-dose and high-dose groups, showing an upward dose-dependent trend. The excessive consumption of vitamin D can promote Ca and P absorption in the small intestine, resulting in increased blood Ca and P (Wang X. H. & Lu, 2013). Taken together, the AST and blood electrolyte results suggest that long-term feeding with the macromolecular polymer may have caused gastrointestinal dysfunction in the rats.

In the hematological analysis, for female rats, only immune indicators were changed in the high-dose group, while the medium- and high-dose male rats showed changes in red blood cells, platelets and immune indicators. However, these changes were within the normal range of hematological indicators in Wistar rats of the same age, (Fan et al., 2010; Tajima, 1989); therefore, it was considered that there was no health impact.

To assess genotoxicity, both in vitro and in vivo assays were conducted in this study. After exposing the rats to the maleic acid polymer scale inhibitor, no chromosome aberrations in mouse bone marrow cells were observed. In contrast, the comet assay results showed that medium and high doses may have caused DNA damage in CHO cells. The in vivo metabolic system and the different genotoxicity testing methods can affect the determination of genotoxicity. Therefore, when evaluating safety, a combination of tests should be adopted to evaluate genotoxicity.

In addition to the two genotoxicity experiments, as part of the pre-experiment process, an Ames test was carried out in accordance with OECD Guidelines for Testing of Chemicals (No. 471, Adopted July 21, 1997). In the Ames test, countable revertant colonies were found in a dish exposed to 20 μg of the original solution of maleic acid polymer scale inhibitor, and the colony count was in the abnormal range. Repeated experiments showed background bacteria. After a 10-fold dilution, background bacteria still appeared in all dishes with revertant colonies, and the counts were in the abnormal range. The Ames test results were normal for spontaneous regression, the solvent control and the positive control group. However, the Ames test might not be suitable for assessing the genotoxicity of the new scale inhibitor because of the complexity of the macromolecular components of the maleic acid polymer scale inhibitor. Therefore, this part of the experiment is not described in detail in this paper. The genotoxicity assessment of seawater desalination treatment agents needs to consider the macromolecular structure and sample character. Appropriate research methods should to be further explored to find more suitable genotoxicity assays.

5. Conclusions

This study explore the acute toxicity, subchronic toxicity and genotoxicity of a new domestically produced maleic acid polymer scale inhibitor based on OECD guidelines. The oral LD_{50} and the NOAEL and LOAEL were obtained. The LD_{50} were 6810 and 9260 mg/kg·BW for male and female rats. Based on UREA results, the NOAEL and LOAEL were 1/20 LD_{50} and 1/10 LD_{50}, respectively (males: 340.5 and 170.25 mg/kg·BW, females: 436 and 231.5 mg/kg·BW). According to the results of comet assay, we judged that maleic acid polymer scale inhibitor may have potential genotoxicity in vitro. These data can be used as a basis for determining the separation point in subsequent safety evaluations and provide a research basis for the health risk assessment and the supervision of seawater desalination agents.

Declarations

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Contributions

Shaoping Zhang, Lixia Zhang and Jian Kong contributed to conceptualization; Chao Wang, Yuehan Long and Mengmeng Wang contributed to data curation; Lian Duan, Lu Kai and Yimin Li were involved in funding acquisition; Hongbin Yang and Lei Wei performed investigation; Wen Gu and Song Tang were involved in methodology; Chong Wang performed project administration; Song Tang and Chong Wang was involved in supervision; Lian Duan contributed to visualization and wrote the original draft; Lian Duan and Chong Wang wrote the review & editing. All the authors read and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Competing interests: The authors declare no conflicts of interest with respect to the authorship and/or publication of this article.

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tables

Table 1. Histopathology results of rats exposed to maleic acid polymer scale inhibitor. Values indicate the number of rats with pathological changes in the liver, spleen and kidney in the control and dosed groups. There were no pathological changes in the low- and medium-dose groups, and no pathological changes in other organs.

Organ	**Pathological Change**	Female（**n = 10**）				Male（**n = 10**）			
		Control	**Low**	**Medium**	**High**	**Control**	**Low**	**Medium**	**High**
Liver	Mononuclear cell infiltration	1	0	0	0	1	0	0	1
	Hepatocyte hypertrophy	0	0	0	0	0	0	0	1
Spleen	Hemosiderin deposition	1	0	0	1	0	0	0	1
Kidney	Mesangial hyperplasia	1	0	0	1	0	0	0	0

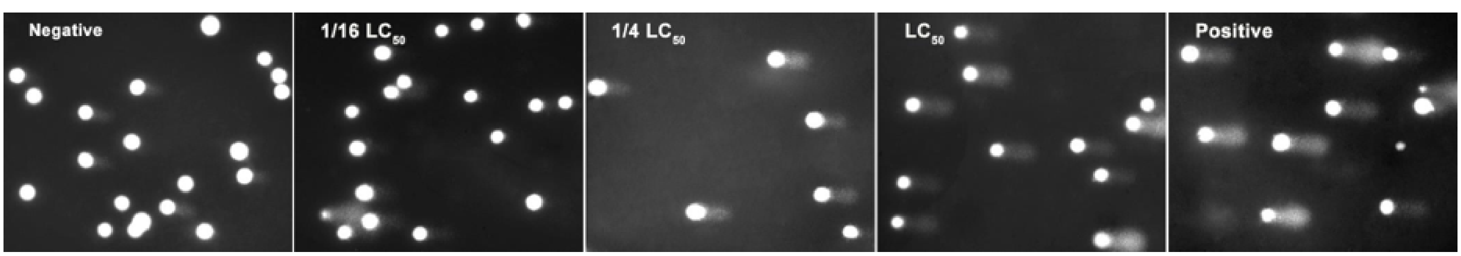
Table 2. Micronucleus test results of mouse bone marrow polychromatic erythrocytes. There was no teratogenic effect in mice exposed to different doses of maleic acid polymer scale inhibitor. 2000 polychromatic erythrocytes (PCEs) were counted for each animal, and the micronucleus rate was calculated as the percentage of polychromatic red blood cells containing micronuclei per thousand. The data are expressed as the mean ± SD per group (n = 10, 5 males and 5 females). The U-test was applied to the experimental results according to the Poisson distribution.

Groups	Dose	Female (n = 5)				Male (n = 5)			
	mg/kg	Total PCEs	PCEs with micronucleus	Micronucleus rate (‰)	P	Total PCEs	PCEs with micronucleus	Micronucleus rate (‰)	P
Maleic acid polymer scale inhibitor	1000	10,000	14	1.40 ± 0.84	>0.05	10,000	9	0.90 ± 0.84	>0.05
	2000	10,000	29	2.90 ± 1.30	>0.05	10,000	13	1.30 ± 0.89	>0.05
	5000	10,000	28	2.80 ± 1.14	>0.05	10,000	15	1.50 ± 1.41	>0.05
Negative control	—	10,000	14	1.40 ± 1.30	>0.05	10,000	14	1.40 ± 1.10	>0.05
Positive control (Mitomycin C)	1.0	10,000	185	18.5 ± 9.70	<0.01	10,000	175	17.5 ± 12.61	<0.01

Table 3. Comet assay results of CHO cells exposed to maleic acid polymer scale inhibitor with 1/16 IC50, 1/4 IC50 and IC50 (0.084, 0.337 and 1.347 mg/mL, respectively). The culture medium and $K_2Cr_2O_7$ were negative and positive controls, respectively. Use a fluorescent microscope with a photographic system (eyepiece 10 ×, Objective lens 20 ×, Green light excitation absorption filter 590 nm) to abtain the images of comet cells, as shown in the "Typical Images". 100 random cells in each group were analyzed using Comet Assay IV^{TM} software. All values are expressed as the mean ± SD. One-way ANOVA was used in the case of homoscedasticity, and the Brown–Forsythe test or Welch's test was used in the case of heteroscedasticity for statistical analysis comparing with the control group. Significance was determined at *p* < 0.001*** and *p* < 0.01**.

Groups		Dose (mg/mL)	DNA in Tail (%)	Tail Moment
Maleic acid polymer scale inhibitor	**1/16 IC_{50}**	0.084	4.99 ± 0.37	2.35 ± 0.10**
	1/4 IC_{50}	0.337	14.64 ± 1.14***	4.45 ± 0.39***
	IC_{50}	1.347	25.11 ± 1.47***	9.14 ± 0.63***
Negative control	**Culture medium**	—	5.27 ± 0.55	1.42 ± 0.16
Positive control	**$K_2Cr_2O_7$**	1 μg/mL	21.35 ± 1.01***	6.56 ± 0.33***

Typical Images. Typical examples of comet images of CHO cells in negative and positive control groups and treatment groups.



Figures

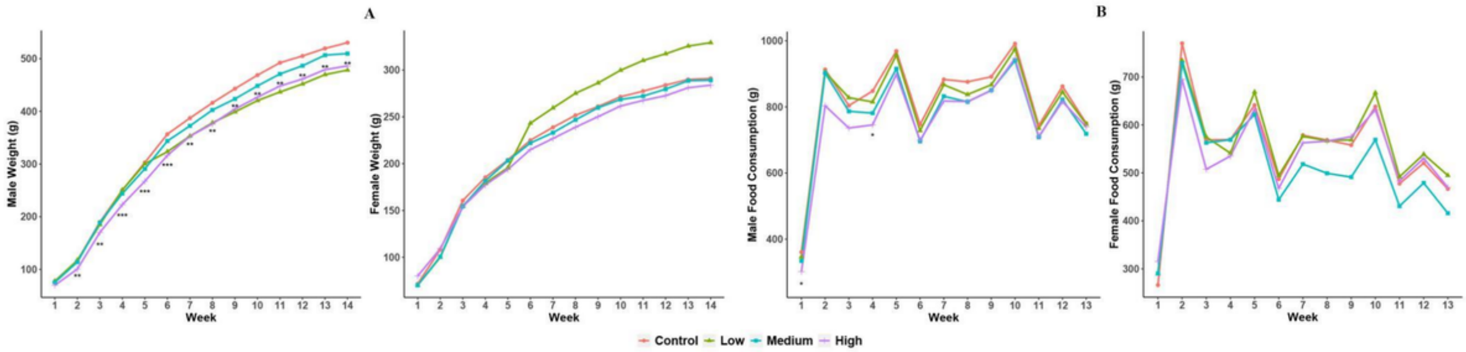


Figure 1

Changes in body weight and food consumption in male and female rats during 90 days of exposure to the maleic acid polymer scale inhibitor. Body weight and food consumption were measured every 2 weeks. A) Changes in body weight. B) Changes in food consumption. All values are expressed as the mean ± SD per group. (n = 20, 10 male and 10 female). The Shapiro–Wilk test was used to assess normality, and Bartlett's test was used to assess homogeneity of variance. Dunnett's test was used in the case of homoscedasticity and a normal distribution, and the Wilcoxon rank–sum test was used in the case of heteroscedasticity or a skewed distribution for statistical analysis comparing with the control group. Significance was determined at $p< 0.001$***, $p < 0.01$** and $p < 0.05$*.

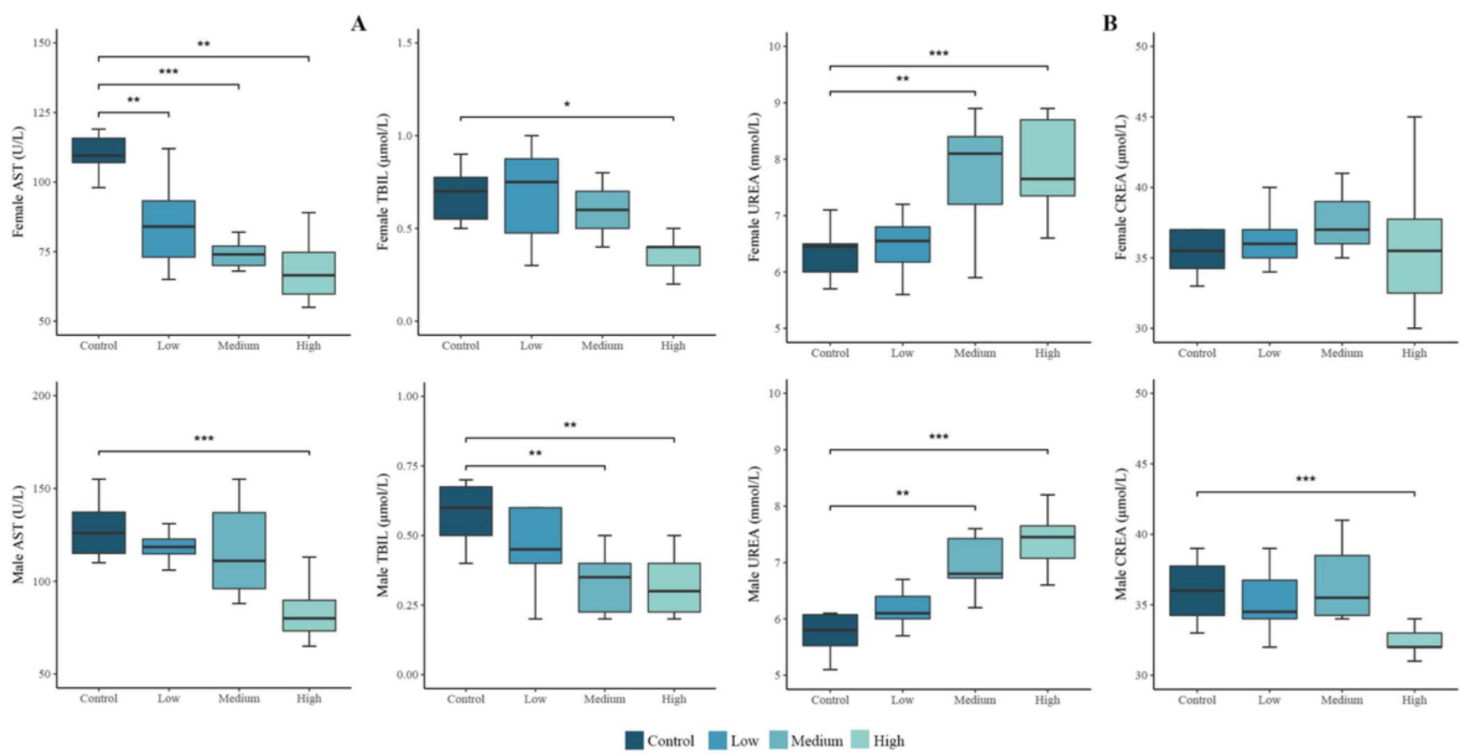


Figure 2

Changes in blood biochemical parameters of rats exposed for 90 days to different doses of the maleic acid polymer scale inhibitor. A) Changes in AST and TBIL, related to liver function. B) Changes in UREA and CREA, related to kidney function. All values are expressed as the mean ± SD per group (n = 20, 10 male and 10 female). The Shapiro–Wilk test was used to assess normality, and Bartlett's test was used to assess homogeneity of variance. Dunnett's test was used in the case of homoscedasticity and a normal distribution, and the Wilcoxon rank–sum test was used in the case of heteroscedasticity or a skewed distribution for statistical analysis comparing with the control group. Significance was determined at $p < 0.001$***, $p < 0.01$** and $p < 0.05$*.

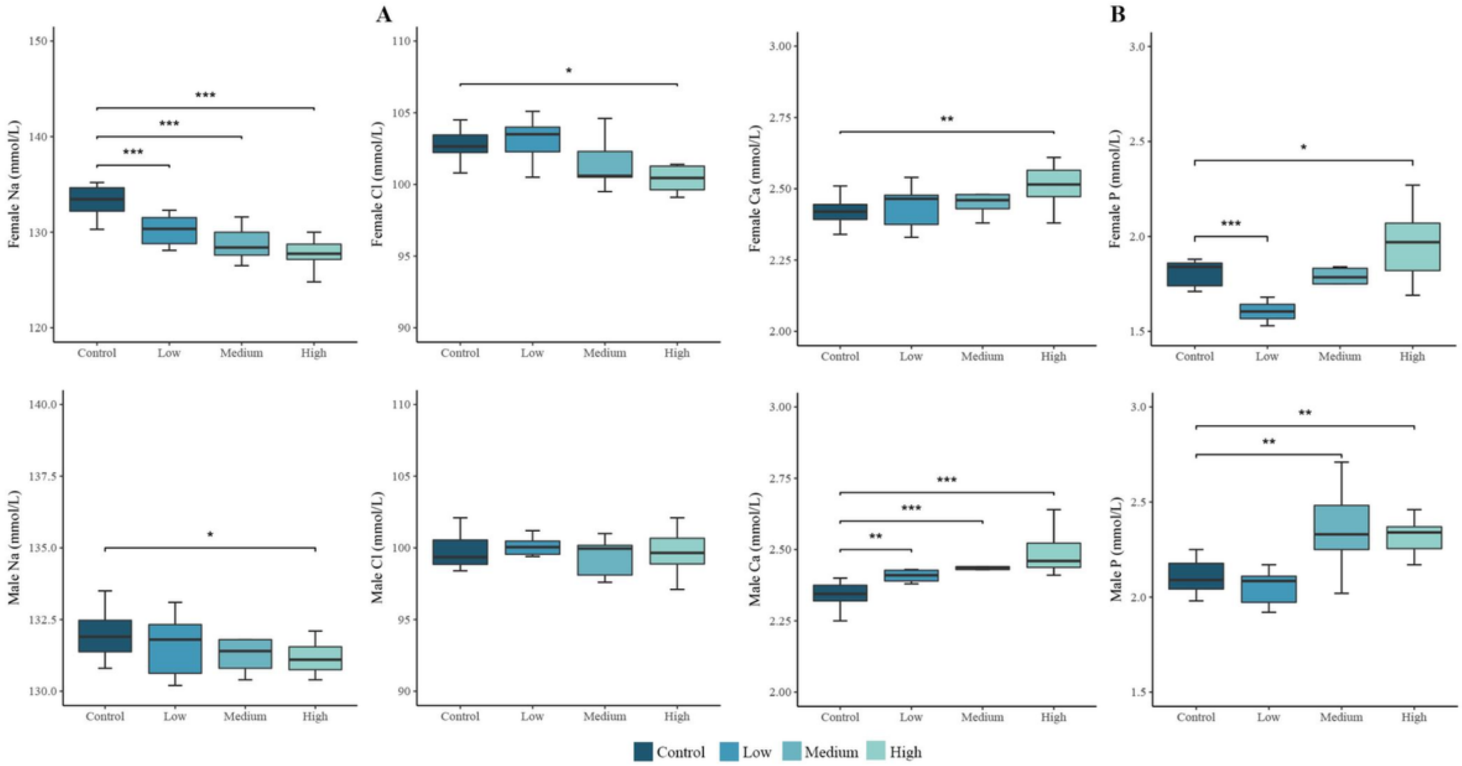


Figure 3

Changes in blood electrolytes in rats exposed for 90 days to different doses of the maleic acid polymer scale inhibitor. A) Changes in Na and Cl. B) Changes in Ca and P. All values are expressed as the mean ± SD per group (n = 20, 10 male and 10 female). The Shapiro–Wilk test was used to assess normality, and Bartlett's test was used to assess homogeneity of variance. Dunnett's test was used in the case of homoscedasticity and a normal distribution, and the Wilcoxon rank–sum test was used in the case of heteroscedasticity or a skewed distribution for statistical analysis comparing with the control group. Significance was determined at $p < 0.001$***, $p < 0.01$** and $p < 0.05$*.

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