Technical Report

Environmental Safety Evaluation of Steelmaking Slag Applied to Coastal Environment

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Abstract

The use of steelmaking slag has started in marine environments and the safety issue of slag material has become a matter of concern. Our aim is to provide the safety information of the slag material. First, we evaluated the aquatic environmental toxicity of steelmaking slag material in acute toxicity tests using three types of freshwater organisms. We then evaluated the toxicity of the leachate from steelmaking slag material in acute toxicity tests using seven types of marine organisms and six types of fisheries resource organisms. Moreover, an experimental facility with mesocosm aquarium tanks, which integrated a tidal flat area and shallow area, was constructed in order to evaluate the environmental impact. Long-term operation over the five years from 2012 to 2017 was conducted to evaluate the long-term safety of slag materials. We observed the acute and chronic toxicity of seawater in the aquarium tanks by using three types of aquatic marine organisms such as bioluminescent bacteria, micro algae, and copepods. The results revealed that there was an extremely low effect of acute or chronic toxicity on organisms caused by slag material.

1. Introduction

Steelmaking slag products are not limited to land area applications, such as concrete aggregates, roadbed materials, easy pavement materials and other construction materials, soil conditioners for soft grounds, and agricultural fertilizers and soil conditioners. Recently, they have also been used as environmental materials for the remediation and improvement of sea area environments.

As utilization technologies in sea areas, steelmaking slag and artificial rock materials made from steelmaking slag are utilized as structural materials. Uses of steelmaking slag as functional materials are also proposed by taking advantage of steelmaking slag properties. Among such examples are mineral supply material as seaweed fertilizer and slag-improved soil. Technology development is under way to contribute to the creation of coastal area environments by utilizing these materials singly or in combination.

When we use steelmaking slag materials in actual environments, it is important for us to obtain large amounts of data on not only usefulness and stability but also on safety issues as per the effects on aquatic organisms and ecosystems in related environments.

The safety of steelmaking slag materials can be evaluated by methods to determine their hazardous components individually and by biological test methods to observe their hazardous effects on various organisms without identifying their individual components. The former methods include environmental standards and fishery water standards concerning the water quality of seawater in sea areas where the steelmaking slag materials are to be applied, and soil environment standards and water bottom sediment judgment standards concerning the hazardous components contained in or eluted from the steelmaking slag materials. When there is a possibility that fish, shellfish, or other marine organisms from such sea areas where steelmaking slag materials are used, the steelmaking slag materials are then subject to Japan's Food Sanitation Act and international food standards for their contents of hazardous substances.

Among the latter biological test methods are methods for selecting appropriate biological species according to the purpose of evaluation and assessing the acute and chronic toxicity of the selected species. For example, the United Nations recommendation "Globally Harmonized System of Classification and Labeling of Chemicals

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(GHS)" specifies health and environmental hazards as the hazard information to be contained in safety data sheets (SDSs) and presents the test guidelines (hereinafter abbreviated as TG) of the Organization for Economic Co-operation and Development (OECD). It is a major premise that materials must not be hazardous to health. We think it necessary to always pay attention to the hazardous environmental effects due to unexpected or secondary causes when the materials are used in the environment.

In the following sections, we describe the initiatives we have taken to evaluate the safety of steelmaking slag materials from the viewpoint of environmental hazards.

2. Environmental Impact Assessment by Biological Methods

The viewpoint of assessing the effects of materials on organisms at the respective levels of the food chain that comprises the ecosystem is considered basic to the assessment of the hazardous effects of materials on the natural environment. As shown in **Fig. 1**, the food chain can be considered at three levels: producers that produce food by photosynthesis, consumers that eat the food to grow organisms, and decomposers that decompose dead organisms and sustain the material cycle in a broad sense of the term. In the aquatic environment, for example, decomposers include bacteria and benthic organisms, producers include algae and phytoplankton, and consumers include fish, shellfish, crustaceans, and zooplankton.

2.1 Impact assessment using freshwater organisms

2.1.1 Test methods and results

In the above-mentioned GHS, the acute toxicity test using algae, crustaceans (*Daphnia*), and fish is proposed as the most basic and standard method for assessing hazards to the aquatic environment. Freshwater organisms have been studied as test organisms. This is probably because the acute toxicity test has been developed mainly in Europe and the United States where impact assessment is focused on freshwater areas such as rivers and lakes.

When the marine environment is considered, it is more logical to test the effects of materials using marine organisms rather than freshwater organisms. However, tests using marine organisms are not conducted with a general approach. We thus conducted the acute toxicity test, a standard method for assessing aquatic environment hazards, by using the above three types of freshwater organisms.

Steelmaking slag samples were crushed to 100 μm or less and added to pure water to a concentration of 100 mg/L to prepare test solutions. The slag sample is a fine powder but is a poorly water-soluble multi-component substance. It was dispersed, dissolved, and filtered. The obtained water-soluble fractions[1] were used for the biological test. Two steelmaking slags from different sources were evaluated. Given the possibility that the sample was not completely dissolved in the test solution, the toxicity test results were expressed in terms of the loading rate (sample weight/amount of test solution used for adjustment, mg/L).

(1) Algal growth inhibition test

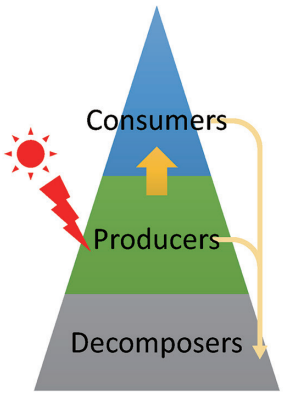
Based on the OECD TG201,[2] the algal growth inhibition test was conducted using the green alga *Pseudokirchneriella subcapitata*. The 1.8-fold serial dilutions of the test solution were used for the test. The effective loading rate that causes 50% reduction in the algal growth rate (ErL_{50}) was calculated based on the growth rate up to 72 hours after the start of exposure to the test solution. The results are shown in **Table 1**. The inhibition rate at the maximum test concentration was less than 50% for the two sets of samples. The ErL_{50} was thus judged to be greater than 100 mg/L. In addition, the loading rate of the test samples in which no significant growth inhibition was observed as compared to the control samples was 56 mg/L for one set of the test samples and 18 mg/L for the other. In the present test results, the 56 mg/L concentration was judged as the no observed effect loading rate (NOELRr). Also, morphological changes of cells (contraction, swelling, rupture, etc.) and aggregation of cells were not observed after 72 hours.

(2) *Daphnia magna* acute immobilization test

Based on the OECD TG202,[3] the acute immobilization test was conducted using *Daphnia magna*. A group of 20 *Daphnia magna* individuals per condition were exposed to the test solution. The inhibition rate was determined by the proportion of the individuals which lost motility 48 hours after the exposure. Immobilization was not observed even at the maximum concentration in both groups of samples. As shown in Table 1, therefore, the median effective loading rate (EL_{50}) was judged to be larger than 100 mg/L.

(3) Fish acute toxicity test

Based on the OECD TG203,[4] the fish acute toxicity test was conducted using *Oryzias latipes*. A group of 10 *Oryzias latipes* individuals per condition were exposed to the test solution. The mortali-



Biological classification	Freshwater organisms	Marine organisms	Fisheries resource organisms
Fish Shellfish Crustacean Zooplankton others	Japanese rice fish Daphnia	Mussel Marine rotifer Copepod Sea urchin	Red seabream Abalone Prawn
Algae Phytoplankton	Green algae	Marine diatom	Seaweed Marine diatom Red tide phytoplankton
Bacteria Benthos		Luminescent bacterium Polychaeta	

Fig. 1 Selection of toxicity test organisms based on ecological pyramid

Table 1 Acute aquatic toxicity tests for evaluation of ecological safety

Test name	Method	Test organism	Test period	Endpoint	Result #1	Result #2
Algal growth inhibition test	OECD TG201 (2009)	*Pseudokirchneriella subcapitata*	72 hours	ErL_{50}	>100 mg/L	>100 mg/L
				NOELRr	56 mg/L	18 mg/L
Daphnia sp. acute immobilization test	OECD TG202 (2004)	*Daphnia magna*	48 hours	EL_{50}	>100 mg/L	>100 mg/L
Fish, acute toxicity test	OECD TG203 (1992)	*Oryzias latipes*	96 hours	LL_{50}	>100 mg/L	>100 mg/L

ty rate was determined by the proportion of the individuals which were dead 96 hours after the exposure. Dead individuals were not observed even at the maximum concentration in both groups of samples. As shown in Table 1, therefore, the median lethal loading rate (LL_{50}) was judged to be greater than 100 mg/L.

2.1.2 Determination of aquatic toxicity based on test results

Acute aquatic toxicity is determined based on the results of the above three types of acute toxicity tests. These test results show that the median effect loading rate exceeded 100 mg/L in each test of the two types of slag samples. It can thus be judged that our steelmaking slag materials are not the acute aquatic toxic substances specified in the GHS.

Chronic toxicity (long-term toxicity) can be evaluated based on the no effect concentration in the algae growth test. Our steelmaking slag materials do not fall under the chronic aquatic toxic substances specified in the GHS. To strictly determine chronic aquatic toxicity, however, it is also necessary to make a comprehensive judgment including the results of the crustacean (*Daphnia*) reproduction test and of the early life stage toxicity test of fish.

2.2 Impact assessment using marine organisms

2.2.1 Viewpoint of ecotoxicity using marine organisms

Assuming application to the marine environment, we considered that the effects on marine organisms should be evaluated more directly. We thus selected and examined seven species of organisms as shown in Fig. 1. Test methods that should be referred to for each species were proposed. But we had to determine whether they were applicable to slag evaluation. If any effects of slag were observed, we had to determine whether the cause or reason originated from the test material or from the restrictions of the test method. Given these necessities, we took the initiative to optimize the test methods and other conditions. The acute toxicity tests we studied are: (1) 96-hour mortality of *Mytilus galloprovincialis*, (2) 24-hour mortality of *Brachionus plicatilis*, (3) 48-hour immobilization of *Tigriopus japonicus*, (4) egg fertilization inhibition of *Heliocidaris crassispina*, (5) 72-hour growth inhibition of *Phaeodactylum tricornutum*, (6) light emission inhibition of *Vibrio fischeri*, and (7) 96-hour mortality of *Hediste sp.* At the food chain levels, organisms (1) to (4) are assumed to be consumers, organism (5) is assumed to be a producer, and organisms (6) and (7) are assumed to be decomposers.

The test is based on the "Chemical substance test method for slag - Part 1: Elution amount test method (JIS K 0058-1: 2005)". Various steelmaking slags were used as samples without crushing. The solution eluted from each slag sample by using artificial seawater as a solvent at a solid-to-liquid ratio of 1:10 was prepared and used as the test solution. Although the details of the results are omitted in this report, the pH rise of the eluate from carbonated steelmaking slag was as small as about 9, and little acute toxicity was observed. The pH of the eluate from uncarbonated steelmaking slag increased to about 12, and hazardous effects were observed. Since the hazardous effects remained in some tests despite the neutralization of the pH, inhibiting factors were investigated. As the pH increased at the time of elution, the ion concentrations in seawater changed significantly (the Ca concentration increased and the Mg concentration decreased). The effects of these concentration changes were inferred.[5] It was thus found that the suitability of specific test methods must be fully considered when evaluating the hazards of the slag.

2.2.2 Viewpoint of impact assessment on fishery resource organisms

When the slag is applied in a fishery water area, it is necessary to check the safety of the slag on fishery resource organisms. In other words, it is necessary to assess the impact of the slag on useful fish as resources from a viewpoint different from the conventional viewpoint of ecotoxicity. On the advice of the Japan Fisheries Science and Technology Association, we conducted acute toxicity and other tests on the six species of fishery-related organisms shown in Fig. 1: (1) Red sea bream, *Pagrus major*, (2) Abalone, *Haliotis discus*, (3) Prawn, *Marsupenaeus japonicus*, (4) Seaweed, *Porphyra yezoensis*, (5) Marine diatom, *Skeletonema costatum*, and (6) Red tide phytoplankton, *Heterosigma akashiwo*. Specifically, we evaluated the Vivary™ series developed as fishing ground creation and regeneration materials and the slag-improved soil developed as fishing ground environment remediation material. These materials were observed to have no hazardous effects on the studied fishery-related organisms. They were eventually certified as useful and safe materials.[6]

3. Environmental Impact Assessment Using Mesocosm Tanks[7]

For the long-term use of slag products in the natural marine environment, it is important to continue to collect data on their long-term safety and chronic toxicity in organisms. In 2011, we opened a facility composed of two mesocosm aquarium tanks of the integral tidal flat-shallow type. After preliminary experiments, we built a tidal flat zone and shallow areas in each mesocosm tank. Dredged soil improved by slag (hereinafter referred to as the slag-improved soil) was used as bottom material in one mesocosm tank. Clams, *Ruditapes philippinarum*, were put into the tidal flat zone of each tank and eelgrass, *Zostera marina*, was transplanted into the shallow zone. We operated the mesocosm tanks for five years from 2012 to 2017 and observed changes in water quality and organisms in the tanks. We also assessed the acute and chronic impacts of tank seawater on organisms by using several species of marine organisms for the long-term safety assessment of the slag-improved soil.

3.1 Tank experiments[8]

The two mesocosm tanks of the integral tidal flat-shallow type used in the experiments were both fan-shaped, divided into a tidal flat zone and a shallow zone, and set as to the wave and tide conditions. The mesocosm tanks were installed indoors without windows. Artificial lighting was installed above each tank to simulate day and night. Each tank was equipped with a seawater cooler to prevent the seawater temperature from rising in summer. One of the two mesocosm tanks was used as a control site (or site A) where dredged soil was laid as the bottom material. The other was an experimental site (or site B) where slag-improved soil was laid as the bottom material.

Eelgrass was transplanted to the bottom material of the shallow zone. Clams were put into the tidal flat zone. The two tanks were installed at our research facility in Futtsu City, Chiba Prefecture. The seawater was pumped from the sea at the front of the research facility and was drawn into the tanks. The tanks were operated for five years from September 2012 to August 2017.

3.2 Aquatic organism tests

In November 2013, July 2014, and January 2015, the tank seawater and the influent seawater at that time were applied to biological toxicity tests.

(1) Marine luminescent bacteria test

The marine luminescent bacterium *Vibrio fischeri* was used in the test. This test provides results relatively highly correlated with those of other toxicity tests, produces the results in a short time, and is widely used as a simple toxicity screening method all over the world. We used a commercially available kit (Microtox 5000 toxicity test system of Strategic Diagnostics Inc., US) and performed the test according to the attached manual. The seawater from Tokyo Bay was sampled just before it entered the experimental tanks and was used as blank seawater for comparison.

The three tests produced similarly trending results. The test results of 2014 are shown in **Fig. 2**[7] as a typical example. The amount of luminescence tended to increase as the concentration increased in all of the blank seawater, site A, and site B samples. The luminescent bacterial solution used in the tests is a 2% sodium chloride solution, whereas the natural seawater contains calcium ions. The addition of the test seawater, therefore, is considered to have increased the calcium ion concentration and hence the amount of luminescence.[5] Since no concentration-dependent decrease in the amount of luminescence was observed in any of the test seawater samples, on the other hand, it was judged that the samples did not contain any components that may inhibit the activity of the luminescent bacterium.

(2) Algal growth inhibition test

The marine algal growth inhibition test was conducted using the diatom *Chaetoceros gracilis* and according to standard test methods.[9–11] The growth rate of *Chaetoceros gracilis* in the site A and B samples during 72-hour cultivation was calculated and compared with that in the blank seawater samples to determine the degree of growth inhibition.

The results of the three tests are shown in **Fig. 3**.[7] Logarithmic growth was observed in each test. The growth rates in the blank seawater samples were 1.79 ± 0.02, 1.54 ± 0.02, and 1.60 ± 0.05/day in 2013, 2014, and 2015, respectively. There were no significant differences among the blank seawater, site A, and site B samples in the 2013 and 2015 tests. In the 2014 test, on the other hand, significant differences (with a significant level of 5%) were observed between the blank seawater and site A samples and between the blank seawater and site B samples. By referring to the blank seawater samples, the growth inhibition rate was 4.3% in the site A samples and 6.4% in the site B samples. Since no significant difference was observed between the site A and B samples, however, it was judged that no algae growth inhibition due to the slag-improved soil was recognized.

(3) Copepoda reproduction inhibition test

Copepoda are positioned as consumers (zooplankton) between producers (algae) and higher-level consumers (fish, etc.) in the food chain and play ecologically important roles. Here, an acute toxicity test was first conducted according to the acute toxicity test[12] using the *Tigriopus japonicus* examined by the Fisheries Agency. A chronic toxicity test was then performed according to the *Tigriopus japonicus* chronic toxicity test method specified in the Fisheries Agency guidelines.[9]

Tigriopus japonicus hatches from a fertilized egg, becomes an early larva called nauplius, and after several molts, metamorphoses into an adult called a copepodid. In the Fisheries Agency guidelines,[9] acute toxicity is determined by lethality over a 24-hour or 48-hour test period. We conducted a 21-day exposure test to assess the toxicity effect. First, subchronic toxicity was determined using as indicators the lethality up to the 8th day from the start of the test and the metamorphosis from nauplii to copepodids. Also, until the 21st day thereafter, chronic toxicity was evaluated by taking as judgment items the number of days taken by the nauplius larvae to metamorphose to the copepodids, the number of days taken by female individuals to reach the first incubation and spawning after the start of the test, the breeding frequency, number of neonates per brood, and total number of neonates produced per female.

First, the results of the subchronic toxicity test are shown in **Table 2**.[7] Mortality was observed in 1 to 3 of the 24 individuals tested

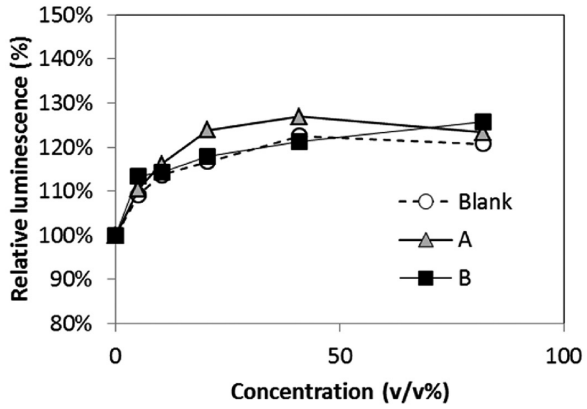


Fig. 2 Example of results of luminescent bacteria test[7]

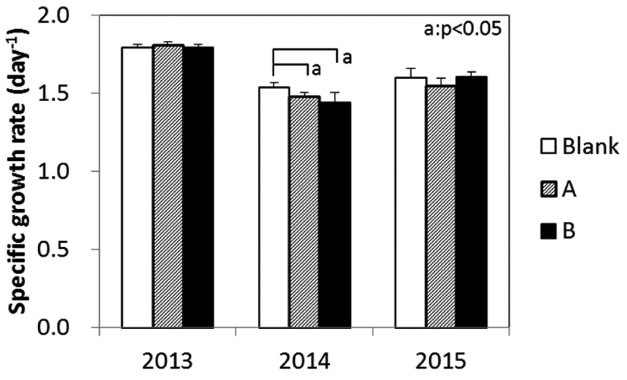


Fig. 3 Results of algal growth inhibition test[7]

Table 2 Results of sub-chronic toxicity test with marine copepods[7]

	8 days mortality (%)			8 days metamorphosis (%)		
Year	Blank	A	B	Blank	A	B
2013	0	0	2.9	100	100	100
2014	2.9	0	0	100	100	100
2015	2.9	8.6	0	100	100	100

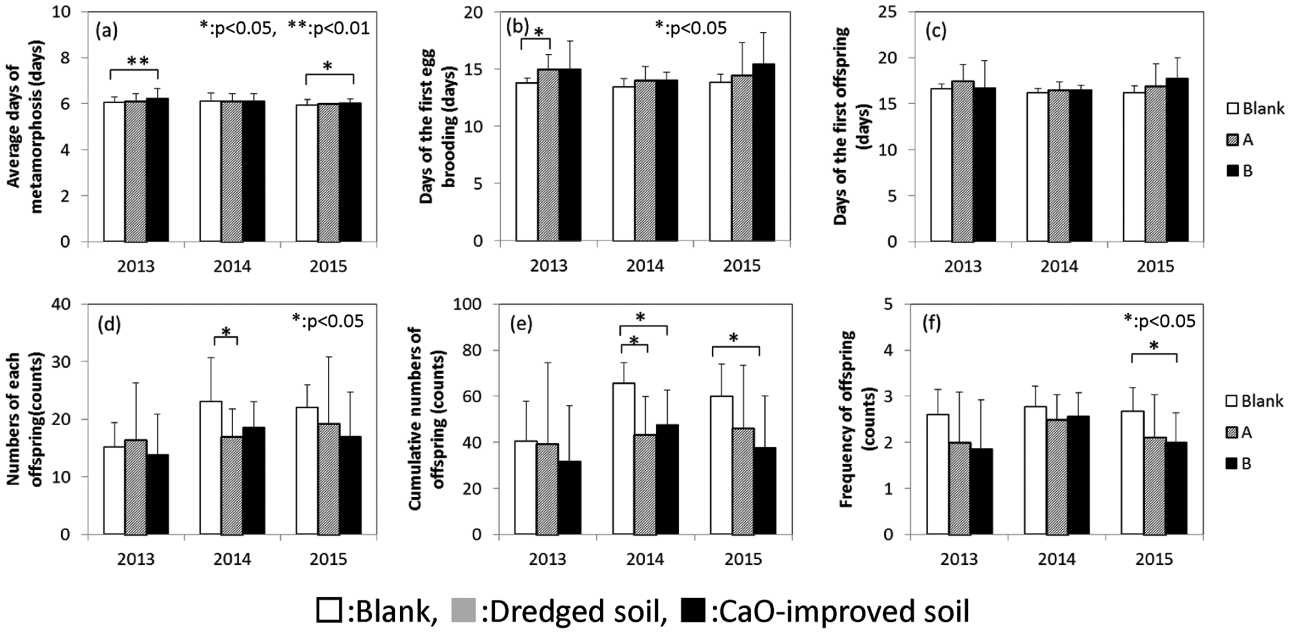


Fig. 4 Results of chronic toxicity test with marine copepods[7]

in the site B samples in 2013, the blank seawater samples in 2014, and the blank seawater and site A samples in 2015. On the other hand, the individuals that survived until the 8th day of the test all metamorphosed into adults in each sample.

Next, the results of the chronic toxicity test are shown in **Fig. 4**.[7] For the average number of days taken by larvae to metamorphose to adults (Fig. 4 (a)), significant delays were observed in the site B samples with respect to the blank seawater samples in 2013 and 2015 (significant levels of less than 1% and less than 5%, respectively). These results suggest the presence of hazardous components or the lack of useful components. Since the average number of days to metamorphosis was not different from that in the blank seawater samples tested during the same period, the effect was judged to be minor. For the average number of days to the first brood (Fig. 4 (b)), a significant delay (with a significant level of less than 5%) was observed between the blank seawater and site A samples in 2013, but no significant difference was observed between the blank seawater and site B samples. Regarding the average number of days to the first offspring (Fig. 4 (c)), no significant delay was observed in the site A and B samples in any of the years. As for the average fecundity per female individual (Fig. 4 (d)), a significant difference (with a significant level of less than 5%) was observed between the blank seawater and site A samples in 2014, but no significant difference was observed between the blank seawater samples and site B samples. Regarding the average cumulative fecundity per female individual (Fig. 4 (e)), a significant difference (with a significance level of less than 5%) was observed between the blank seawater and site A samples in 2014 and between the blank seawater and site A samples in 2015. No significant differences were observed between the site A and B samples in any of the years. For the average breeding frequency per female individual (Fig. 4 (f)), a significant difference (with a significant level of less than 5%) was observed between the blank seawater and site B samples in 2015. No significant differences were observed between the site A and B samples in any of the years.

According to the above results, significant differences were sometimes observed between the blank seawater and site B samples, but no significant differences were observed between the site A and B samples. It was thus judged that our steelmaking slag materials had no subchronic and chronic toxicity effects on Copepoda.

4. Conclusions

When using steelmaking slag products as fishery materials in the marine environment, we have continued efforts to appropriately evaluate the safety of the steelmaking slag materials from the viewpoint of environmental hazards. We first conducted acute toxicity tests using freshwater organisms to assess the aquatic environment hazards specified in the GHS. Assuming applications to the marine environment, we next performed acute toxicity tests using marine organisms and useful aquatic organisms. We then laid the slag-improved soil at the bottom of one of our two mesocosm tanks to simulate a marine environment, operated the mesocosm aquarium tanks for a long period of time, and conducted chronic toxicity tests using marine organisms. Our steelmaking slag materials have been observed to have no acute and chronic toxicity effects on aquatic and marine organisms. When the steelmaking slag materials are used in the marine environment, their aquatic environment hazards are judged to be minimal.

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